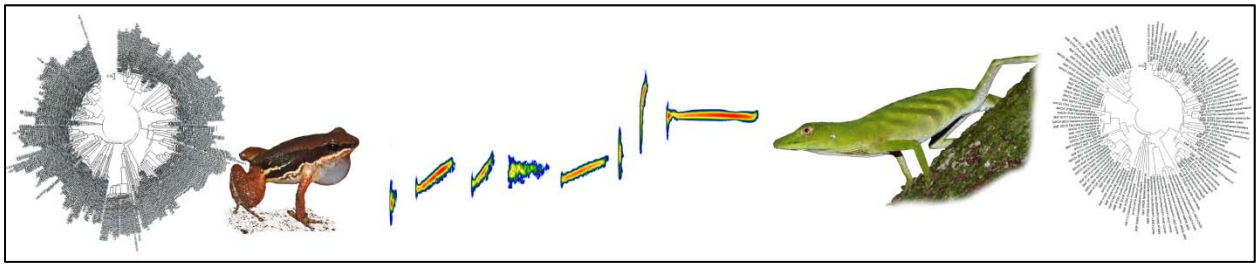
GOETHE UNIVERSITÄT FRANKFURT AM MAIN

TAXONOMY, DIVERSITY, AND BIOGEOGRAPHY OF THE HERPETOFAUNA OF EASTERN PANAMA.



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I would like to dedicate my dissertation

to my family

for all their motivation, love and support,

in particular to my parents papa Abel and mama Betty,

and my daughter Maia.

“The most fundamental question of taxonomy is not how to identify species, but rather how to delineate them” Benoît Dayrat, 2005.

Contents

Abstract

Panama, a small country between the major continents of North and South America, is one of the lesser studied regions in Central America, but is recognized for its mega-biodiversity. This is particularly true for Eastern Panama, which I am considering as the easternmost portion of the country, covering the area from the Chepo, which is also the beginning of the San Blas mountain range, towards east, up to the Darien Mountain range on the border with its neighboring country Colombia. In the lowland region I visited two physiographic areas: the Isthmian-Atlantic Moist Forests (IAMF) and the Chocó-Darién Moist Forests (CDMF). In the IAMF I worked at the localities of Río Mono, Wacuco, La Moneda, Arretí, Metetí, Filo del Tallo, and Laguna de Matusagaratí. In the CDMF I visited the localities of Cruce de Mono, Cana, Garachiné, Sambú, and Pavarandó. And I have worked in the highlands of Darién (DM), Majé (MM), Jingurudó-Sapo (JSM), Pirre (PM) and San Blas (SSM) in the highlands.

Before my research, 138 reptile and 104 amphibian species had been reported for EP. From 2008 to 2013, I collected specimens to evaluate the diversity of amphibians and reptiles for this region. I applied an integrative approach to evaluate the taxonomy, diversity, biogeography, and conservation of the herpetofauna of EP. I included analyses of morphometrics, molecular genetics (e.g. barcoding), biogeography, bioacoustics (in anurans), hemipenial morphology (in squamates), and ecology. This is the first regional evaluation of the biodiversity in EP applying integrative taxonomy. Aside from morphological and bioacoustic data, my work is based on the barcoding of 608 specimens, from which I obtained 16S mtDNA for 486 specimens and COI mtDNA for 455. In total I have got sequences for 69.2 %of the amphibian and 48.6 % of the reptile species present in EP. For the morphological analyses, I compared 1597 specimens, including my samples complemented by specimens obtained from various museums. The bioacoustic data were obtained from the analysis of 1504 calls of 27 species of frogs. Based on specimens collected in EP and according to external morphology, I could identify 65 species of amphibians and 72 reptiles, but after applying

an integrative approach these numbers increased to 79 amphibians and 88 reptiles described species within my collected specimens. Additionally, I uncovered 33 taxonomic units that could not be assigned to any described species until now, 22 of them represent confirmed candidate species (CCS), and 11 were classified as Unconfirmed candidate species (UCS). Thus, increasing the known species of amphibians by 14.4 % and of reptiles by 13 %. Currently, there are 156 reptiles and 119 amphibians known to occur in EP. Based on my results, I have initiated several projects to solve taxonomic uncertanties, including the species of the genera *Bolitoglossa, Diasporus, Dactyloa, Ecnomiohyla, Lepidoblepharis,* and the taxonomic status of the species *Pristimantis caryophyllaceus* and *Norops tropidogaster*.

Out of the 22 CCS I found, I described nine species new to science with type locality in EP, six amphibians and four reptiles. Among these is a new species of *Bolitoglossa* described from Cerro Chucantí, Cordillera de Majé, Provincia de Darién, Panama. Additionally, I include comments on the other species of congeneric salamanders known to occur in the region. Among the tink frogs, only *Diasporus quidditus* was known to occur in EP. During my field work I collected six additional species of this genus, four of which are new to science, plus two species new for this region. The new species can be differentiated from all congeners as follows: *Diasporus darienensis*, by having a reddish dorsal pattern with pale lines or blotches, a venter suffused with reddish colour, calls with dominant frequency (DF) of 3.34–3.81 kHz; *Diasporus majeensis* by having a reddish dorsal colour with brown or pale reticulations, black eye periphery, and calls with DF of 2.47–2.71 kHz; *Diasporus pequeno*, by having a brownish dorsal pattern with dark blotches, ventral areas translucent with dark speckles, Finger III with a small papillate ungual flap, and small sky-blue blotches, males with bright yellow vocal sac, and calls with DF of 3.44–3.48 kHz; *Diasporus sapo* by having a uniform red dorsal colour, and a sky blue eye periphery.

I also described one new species of *Dactyloa* (giant anole lizards) related to the former *D. chocorum.* I synonymized *D. chocorum* with *D. purpurescens*, and included information about the other species of the group from EP. The new species of *Dactyloa*

resembles *D. ibanezi, D. limon,* and *D. purpurescens* in external morphology but differs from these species in dewlap coloration, dorsal color pattern, morphometrics, and scalation. I discovered one species of the genus *Ecnomiohyla*, which exhibits significant genetic distances (16S mtDNA gene) and morphological differences to all known *Ecnomiohyla* species. Along with the description of the new *Ecnomiohyla* species, I provide detailed comparisons of morphological and molecular characters of almost all members of the genus in Lower Central America, as well as an identification key for the entire genus. Two new species of the genus *Lepidoblepharis* from EP were described. In the corresponding work, I include an analysis of *Lepidoblepharis* spp. in the region, including phylogeography and taxonomy. One of the new species, *Lepidoblepharis emberawoundule,* can be differentiated from most species in the genus by its small size and its low number of lamellae under the fourth toe and finger. The other species described from EP, *Lepidoblepharis rufigularis,* can be differentiated from all species in the genus by its small size and the reddish throat in males.

I examined the variation of morphology, genetics, and coloration in the *Pristimantis caryophyllaceus* complex from Panama, using different Molecular Operational Taxonomic Units (MOTUs). Phylogeny, ecology, and distributional information for this species shed light on the position and species delineation of *P. caryophyllaceus* and its congeners in Panama. I recognized two species of anoles (i.e., *Norops tropidogaster* [Hallowell 1854] and *N. gaigei* [Ruthven 1916]) related to what was formerly referred to as *Norops tropidogaster*. They clearly differ in hemipenal morphology, male dewlap, several pholidotic characteristics, and molecular genetics; subsequently, I resurrected *N. gaigei* from the synonymy with *N. tropidogaster*.

Panama is, and historically has been, very dynamic geologically. The current biogeographic patterns and consequently the origin of the herpetofauna in the area were tightly related with this geological past. The most important event is the Great American Biotic Interchange (GABI) that has been initiated in Panama during the Miocene-Pliocene, when species from North and from South America have migrated towards the respective other continent. I have applied a biogeographical analysis to one

amphibian and one reptile group, using them as models to evaluate the origin and biogeography of the herpetofauna in EP. What I have found is that indeed the geological process that took place in Panama during the closure of the isthmus and the connection of North and South America, coincide with dates of origin for certain groups of amphibians and reptiles in Panama. Furthermore, EP was not only a path or bridge used by the flora and fauna to colonize each continent, but also a place of speciation in situ for some amphibians and reptiles. Speciation events have occurred in the highlands of EP. The sea level fluctuation and isolation during the uplift of these mountains around the middle Miocene have promoted speciation several frogs, and have shaped the current distributional pattern and phylogeographic structure for many species of amphibians and reptiles in EP.

The isolation and speciation of several species in EP are reflected in the endemism of several species. The restricted distribution of these endemic species has contributed to increase the numbers of endangered species in the region. Based on my results, in EP there are 23 described endemic species which also inhabit other provinces within the country (11 amphibians, 12 reptiles), with most of them exclusive of EP (10 amphibians and 8 reptiles). Currently there are 29 species of the herpetofauna (14 amphibians, 15 reptiles) in the IUCN Red List of Threatened Species (Critically Endangered, Endangered or Vulnerable). However, 45 species (mostly recently described or reptiles) have not been evaluated by the IUCN specialists, and 36 are DD. According to the Environmental Vulnerability Score, another measure used specifically for amphibians and reptiles to evaluate the conservation status of species, in EP there are 108 species (50 amphibians, 58 reptiles) with a high vulnerability, 95 with medium (35, 60) and 36 (12 amphibians, 24 reptiles) with low vulnerability , and 35 (21 amphibians,14 reptiles) were not evaluated. I identified the main threats affecting the status of conservation of the herpetofauna in EP, among them: Chytridiomycosis, habitat fragmentation, habitat alteration, contamination, invasive species and climate change. A direct impact on the herpetofauna that recently has affected the populations of amphibians is chytridiomycosis. But more alarming is that there is evidence for amphibian decline linked to this infectious disease in EP, almost right after finishing the

field work for this thesis. Coupled with the chytridiomycosis, the impact of the habitat fragmentation or alteration on amphibians and reptiles has not been evaluated in EP yet, it must be assumed that it does affect amphibians and reptiles too. For example, every dry season protected areas are deforested by loggers, and in the buffer areas people set fires to open areas for cultivation. Those direct impacts on the environment can be silently affecting several populations of amphibians and reptiles. Therefore, monitoring projects are urgently needed to determine the status of amphibian and reptiles, and to suggest feasible conservation strategies that can guarantee the long term survival especially of endangered species.

Zusammenfassung

Panama, ein kleines Land zwischen den großen Kontinenten Nord- und Südamerika, ist eine der weniger untersuchten Regionen in Zentralamerika, beherbergt jedoch eine Mega-Biodiversität. Besonders der Osten Panamas (kurz EP für “Eastern Panama” – hier definiert als das Gebiet zwischen der Llano-Cartí-Straße und der kolumbianischen Grenze) ist ein wichtiger, aber wenig untersuchter Biodiversitäts-Hotspot. Zu Beginn meiner Studien waren 138 Reptilien- und 104 Amphibienarten für EP nachgewiesen. Zwischen 2008 und 2013 sammelte ich Belegexemplare, um die Diversität der Amphibien und Reptilien dieser Region zu evaluieren. Im Tiefland untersuchte ich zwei physiographische Regionen: Die Isthmisch-Atlantischen Feuchtwälder (IAMF für "Isthmian-Atlantic moist forests") und die Chocó-Darién-Feuchtwälder (CDMF für " Chocó-Darién moist forests"). Innerhalb der IAMF besuchte ich die Lokalitäten Río Mono, Wacuco, La Moneda, Arretí, Metetí, Filo del Tallo und Laguna de Matusagaratí. In den CDMF fand meine Feldarbeit an den Orten Cruce de Mono, Cana, Garachiné, Sambú und Pavarandó statt. Besonders intensiv bearbeitete ich alle wichtigen Gebirgszüge der Region, also die Serranías de Darién (DM), Majé (MM), Jingurudó-Sapo (JSM), Pirre (PM) und San Blas (SM). Ich verwendete eine integrativ-taxonomische Herangehensweise, um die Taxonomie, Diversität, Biogeografie und den Schutzstatus der Herpetofauna von EP zu evaluieren. Hierzu kombinierte ich morphometrische, molekulargenetische (z. B. Barcoding), biogeografische, bioakustische (bei Anuren), genitalmorphologische (bei Squamaten) und ökologische Analysen.

Diese Studie ist die erste regionale Evaluierung der Biodiversität in EP, die integrative Taxonomie verwendet. Neben morphologischen und bioakustischen Daten stützt sich diese Arbeit auf 486 16S- und 455 COI-Barcodes. Insgesamt liegen hiermit mtDNA-Sequenzen von 608 Individuen vor, die 69.2 % der Amphibien- und 48.6 % der Reptilienarten repräsentieren, die aus EP bekannt sind. Für die morphologischen Analysen habe ich 1597 Exemplare aus meiner eigenen sowie verschiedenen anderen Sammlungen verglichen. Die bioakustischen Daten entstammen den Analysen von

1504 Rufen von 27 Froscharten. Auf einer rein externmorphologischen Grundlage konnte ich unter den in EP gesammelten Exemplaren 65 Amphibien- und 72 Reptilienarten identifizieren, doch nach Anwendung der integrativen Herangehensweise wuchsen diese Zahlen auf 79 Amphibien- und 88 Reptilienarten an, die bereits beschrieben sind. Darüber hinaus fand ich 33 taxonomische Einheiten, die ich keiner bisher beschriebenen Art zuordnen konnte. Zweiundzwanzig dieser Linien repräsentieren Confirmed Candidate Species (CCS), die übrigen elf Einheiten klassifiziere ich als Unconfirmed Candidate Species (UCS). Somit wird der Artenreichtum der Amphibien um 14.4 % und derjenige der Reptilien um 13 % erhöht. Insgsamt sind nun also 119 Amphibien- und 156 Reptilienarten aus EP bekannt.. Auf der Grundlage meiner Ergebnisse initiierte ich mehrere Projekte zur Klärung taxonomischer Unsicherheiten, etwa für die Gattungen *Bolitoglossa, Diasporus, Dactyloa, Ecnomiohyla* und *Lepidoblepharis,* sowie den taxonomischen Status von *Pristimantis caryophyllaceus* und *Norops tropidogaster*.

Aus dem Kreise der 22 vorgefundenen CCS sind die wissenschaftlichen Erstbeschreibungen für neun Arten (sechs Amphibien und drei Reptilien) mit Typuslokalitäten in EP bereits publiziert. Zu diesen gehört eine neue *Bolitoglossa*-Art vom Cerro Chucantí, Cordillera de Majé, Provincia de Darién, Panama, , die sich in ihrer Färbung und ihren Körperproportionen von allen panamaischen Mitgliedern der Gattung unterscheidet. Das entsprechende Kapitel beinhaltet auch Kommentare zu anderen Arten dieser Gattung, die aus der Region nachgewiesen wurden. Bisher war mit *Diasporus quidditus* nur eine Art der so genannten Tink-Frösche aus EP bekannt. Während meiner Feldarbeit konnte ich sechs weitere Arten sammeln, von denen zwei Erstnachweise für die Region und die anderen vier bisher unbeschriebene Arten darstellen. Diese vier neuen Arten lassen sich wie folgt von den anderen Vertretern der Gattung differenzieren: *Diasporus darienensis* hat einen rötlichen Rücken mit einem Muster heller Linien oder Flecken, den Bauch mit Rottönen durchsetzt und ruft mit einer Dominanzfrequenz (DF) von 3.34–3.81 kHz. *Diasporus majeensis,* der nur aus der Serranía de Majé bekannt ist, hat einen rötlichen Rücken mit braunem oder hellem Netzmuster, eine schwarze Augenperipherie und ruft mit einer DF von 2.47–2.71 kHz.

Diasporus pequeno hat eine bräunliche Oberseite mit dunklen Flecken, dunkle Sprenkel und kleine himmelblaue Flecken auf den durchscheinenden ventralen Oberflächen, eine kleine papillate Protuberanz an der Spitze von Finger III, einen leuchtend gelbe Schallblase bei Männchen und ruft mit einer DF von 3.44–3.48 kHz. *Diasporus sapo* hat eine einheitlich rote Rückenfärbung, eine himmelblaue Augenperipherie und ist nur aus dem Sapo-Jingurudo-Höhenzug bekannt. Darüber hinaus beschreibe ich eine neue Art Riesenanolis der Gattung *Dactyloa*, die eng mit der traditionell anerkannten Art *D. chocorum* verwandt ist. Letztere synonymisiere ich mit *D. purpurescens* und kommentiere die übrigen in EP vorkommenden Arten der Gattung. Die neue *Dactyloa*-Art ähnelt äußerlich ihren nahen Verwandten *D. ibanezi, D. limon* und *D. purpurescens,* unterscheidet sich aber von diesen in der Färbung der Kehlfahne, dem Zeichnungsmuster der Flanken, Körperproportionen und Beschuppung. Weiterhin entdeckte ich eine neue Art der Gattung *Ecnomiohyla*, die signifikante genetische Distanzen (16S mtDNA) und morphologische Unterschiede zu allen bekannten *Ecnomiohyla*-Arten aufweist. Ihre Beschreibung wird von detaillierten Vergleichen der morphologischen wie molekularen Merkmale fast aller Gattungsmitglieder aus dem südlichen Zentralamerika sowie einem Bestimmungsschlüssel für die gesamte Gattung ergänzt. Darüber hinaus beschrieb ich drei neue Arten der Zwerggecko-Gattung *Lepidoblepharis,* von denen zwei in EP vorkommen und nur von dort bekannt sind. Eine dieser beiden neuen Arten, *Lepidoblepharis emberawoundule,* kann von allen anderen Gattungsmitgliedern durch seine kleine Größe, die niedrige Zahl von subdigitaler Lamellen unter dem vierten Zeh und die Konfiguration des ventralen und subfemoralen Escutcheons der Männchen unterschieden werden. Die andere neue Art aus EP, *Lepidoblepharis rufigularis,* unterscheidet sich von allen übrigen Vertretern der Gattung durch seine kleine Größe, die rötliche Kehlfärbung bei Männchen und die Konfiguration des ventralen Escutcheons. Auch in dieser Arbeit präsentiere ich phylogeografische und morphologische Analysen der übrigen in der Region vorkommenden Gattungsmitglieder. Ausgehend von Molecular Operational Taxonomic Units (MOTUs) untersuche ich die morphologische, genetische und farbliche Variabilität des *Pristimantis caryophyllaceus*-Komplexes in Panama. Phylogenetische, ökologische und

biogeografische Daten für dieses nominelle Taxon ermöglichen ein besseres Verständnis der Position und Artabgrenzung von *P. caryophyllaceus* und seinen Gattungsgenossen in Panama. In einer weiteren Studie erkenne ich zwei Anolis-Arten (*Norops tropidogaster* [Hallowell 1854] und *N. gaigei* [Ruthven 1916]) an, die in Panama bisher als *N. tropidogaster* angesprochen wurden. Aufgrund deutlicher Unterschiede in ihrer Hemipenismorphologie, Kehlfahnenfärbung, Beschuppung und Genetik revalidiere ich *N. gaigei* als eigenständige Art.

Panama hatte, und hat noch, eine hohe geologische Dynamik. Die heutigen biogeografischen Muster und dementsprechend auch der Ursprung der Herpetofauna dieser Region sind eng mit ihrer geologischen Geschichte verknüpft. Der herausragendste Vorgang war der Große Amerikanische Faunen- und Florenaustausch, der in Panama im Miozän-Pliozän begann, als Biota aus Nord- und Südamerika sich in Richtung des jeweils anderen Kontinents ausbreiteten. Ich habe biogeografische Analysen für je eine Amphibien- und eine Reptiliengruppe durchgeführt und nutze diese als Modelle, um Herkunft und Biogeografie der Herpetofauna von EP zu evaluieren. Meine Ergebnisse zeigen, dass die geologischen Prozesse, die im Bereich des heutigen Panama während der Schließung des panamaischen Isthmus und der Etablierung der mittelamerikanischen Landbrücke abliefen, zeitlich mit der Entstehung bestimmter Amphibien- und Reptiliengruppen zusammenfallen. Darüber hinaus war EP nicht nur ein Ausbreitungskorridor für Flora und Fauna, sondern, besonders im Bereich seiner Gebirge, auch ein Schauplatz für in situ-Artbildungsprozesse. Die Meeresspiegelschwankungen und Isolationsmechanismen während der Hebung dieser Höhenzüge um das mittlere Miozän haben die Speziation mancher Frösche gefördert und entscheidend zum den heutigen Verbreitungsmustern und phylogeografischen Strukturen vieler Amphibien- und Reptilienarten in EP beigetragen.

Die Isolation und Artentstehung in EP äußern sich auch im Endemismus mehrerer Arten. Die begrenzten Verbreitungsgebiete dieser Endemiten tragen zur Erhöhung der Anzahl bedrohter Arten in der Region bei. Laut meinen Ergebnissen sind

innerhalb der Herpetofauna von EP 23 beschriebene Arten (11 Amphibien, 12 Reptilien) endemisch für Panama, wobei die meisten (10 Amphibien, 8 Reptilien) ausschließlich in EP vorkommen. Derzeit werden in der offiziellen Roten Liste der IUCN ganze 29 Arten der Herpetofauna (14 Amphibien, 15 Reptilien) einer der „Gefährdet“-Kategorien ("gefährdet", "stark gefährdet" oder "vom Aussterben bedroht") zugeordnet. Allerdings wurden 45 Arten (größtenteils erst kürzlich beschriebene Arten und Reptilien) bisher noch nicht von den Spezialisten der IUCN bewertet, während weitere 36 aufgrund ungenügender Datengrundlage noch nicht eingestuft werden konnten. Anhand der Environmental Vulnerability Score, eines speziell für Herpetofauna entwickelten Maßes für die Gefährdung von Arten, lassen sich in EP 108 Arten (50 Amphibien, 58 Reptilien) mit hoher, 95 Arten (35, 60) mit mittlerer und 36 Arten (12, 24) mit geringer Gefährdung identifizieren, wobei 35 Arten (21, 14) nicht evaluiert wurden. Als die hauptsächlichen Gefährdungsfaktoren der Herpetofauna von EP identifiziere ich unter anderem Chytridiomykose, Habitatverlust und -degradation, Umweltverschmutzung, invasive Arten und den Klimawandel. Chytridiomykose hat durch den Befall von Amphibien einen direkten Einfluss auf die Herpetofauna, der kurz nach Beendigung meiner Feldarbeit auch mit Populationsrückgängen von Amphibien in EP in Verbindung gebracht werden konnte. Auch wenn in diesem Zusammenhang der Einfluss von Habitatfragmentierung und -degradation auf Amphibien und Reptilien in EP bisher nicht untersucht wurde, ist angesichts der alarmierenden Situation in der Region davon auszugehen, dass deren Populationen auch hierdurch in Mitleidenschaft gezogen werden. Beispielsweise werden in jeder Trockenzeit selbst Wälder in Schutzgebieten zur Holzgewinnung gerodet, während in den Pufferzonen Brandrodung zur landwirtschaftlichen Erschließung von Flächen betrieben wird. Sowohl diese direkten Umwelteinflüsse als auch jene, die bisher noch nicht evaluiert wurden, können mehr oder weniger offensichtlich auf Amphibien- und Reptilienpopulationen einwirken. Deshalb besteht ein dringender Bedarf an Monitoring-Projekten zur Feststellung des Populationsstatus diverser Arten sowie zur Erarbeitung gangbarer Schutzstrategien, um ein langfristiges Überleben besonder der bedrohten Arten zu ermöglichen.

Resumen

Panamá, un país pequeño entre los grandes continentes de América del Norte y del Sur, es uno de los menos estudiados de las regiones de América Central, pero es reconocido por su mega-biodiversidad. En particular, el Este de Panamá, un área que estoy considerando como la parte más oriental del país, que cubre el área de la Chepo, que es también el comienzo de la cordillera de San Blas, hacia el este, hasta el rango de la serranía de Darien en la frontera con el vecino país de Colombia. En la región de tierras bajas visité dos zonas fisiográficas; los bosques húmedos del Istmo-Atlántico (IAMF) y los bosques húmedos del Chocó-Darién (CDMF). En el IAMF las localidades de: Río Mono, Guacuco, La Moneda, Arretí, Metetí, Filo del Tallo, y Laguna de Matusagaratí. En el CDMF visité las localidades de Cruce de Mono, Cana, Garachiné, Sambú y Pavarandó. Y en las tierras altas las serranías de Darién (DM), Majé (MM), Jingurudó-Sap (HSM), Pierre (PM) y San Blas (SSM).

Antes de mi investigación, 138 especies de reptiles y 104 de anfibios habían sido reportados para EP. De 2008 a 2013, he colectado muestras para evaluar la diversidad de anfibios y reptiles de esta región. El trabajo de campo se llevó a cabo en sus principales cordilleras, es decir, Darién, Jingurudó, Maje, Pirre, Sapo y San Blas. He aplicado un enfoque integral para evaluar la taxonomía, la diversidad, la biogeografía y conservación de la herpetofauna de EP. Incluí análisis de morfometría, genética molecular (e.g. códigos de barras), biogeografía, Bboacústica (en anuros), ecología y morfología de hemipenes (en los reptiles). Esta es la primera evaluación regional de la biodiversidad en EP aplicando taxonomía integradora. Aparte de los datos morfológicos y bioacústicos, mi trabajo se basa en el código de barras de 608 especímenes, que obtuve de 486 ejemplares para el 16S mtDNA y 455 para COI mtDNA. En total tengo secuencias para el 69,2% de los anfibios y el 48,6% de la especies de reptiles presentes en EP. Para los análisis morfológicos, comparé 1597 especímenes, incluyendo mis muestras, complementadas con muestras obtenidas de diversos museos. Los datos de bioacústica se obtuvieron del análisis de 1504 llamadas de 27 especies de ranas. Sobre la base de las muestras recogidas en el EP y a la

morfología externa, pude identificar 65 especies de anfibios y 72 reptiles, pero después de aplicar a un enfoque integrador aumentó a 79 anfibios y 88 reptiles con especies descritas dentro de las muestras colectadas. Además, he descubierto 33 unidades taxonómicas que no pudieron ser asignados a cualquiera de las especies descritas hasta ahora, 22 de ellos representan especies confirmadas candidatas a nuevas especies (CCS), y 11 fueron clasificadas como especies candidatas no confirmados (UCS). Por lo tanto, hay un aumento de las especies conocidas de anfibios en un 14,4% y en un 13% de los reptiles. En la actualidad, hay 156 reptiles y 119 anfibios que se encuentran en el EP. Con base en los resultados, he iniciado varios proyectos para resolver los problemas taxonómicos de la región, incluiyendo las especies de los géneros *Bolitoglossa*, *Diasporus*, *Dactyloa*, *Ecnomiohyla*, *Lepidoblepharis*, y la situación taxonómica de la especie *Pristimantis caryophyllaceus* y *Norops tropidogaster*.

De los 22 CCS que he encontrado, he descrito nueve especies nuevas para la ciencia con la localidad tipo en EP, seis anfibios y cuatro reptiles. Entre estas, una nueva especie de *Bolitoglossa* de Cerro Chucantí, Cordillera de Maje, Provincia de Darién, Panamá. Además, se incluyen comentarios sobre las otras especies de salamandras congenéricas que se encuentran en la región. Entre las ranas martillo, solamente *Diasporus quidditus* se suponía estaba presente en EP. Durante mi trabajo de campo he colectado seis especies adicionales de este género, cuatro de las cuales son nuevas para la ciencia, además de dos especies nuevas para esta región. Las nueva especies se pueden diferenciar de la siguiente manera: *Diasporus dairenensis*, por tener diseño dorsal rojizo con líneas o manchas pálidas; vientre teñido de color rojizo, el canto tiene frecuencia dominante (DF) entre 3,34 a 3,81 kHz; *Diasporus majeensis* es de color dorsal rojizo con reticulaciones de color marrón o pálidas, la periferia de los ojos es negra, la especie está restringidas a la cordillera de Maje y elñ canto tiene DF entre 2,47 a 2,71 kHz; *Diasporus pequeno*, tiene diseño dorsal pardo con manchas oscuras, las zonas ventrales translúcidas con manchas oscuras, tercer dedo con una solapa pequeña ungueal y papilada, machos con saco vocal brillante de color amarillo, el canto tiene DF entre 3,44 a 3,48 kHz; *Diasporus sapo* tiene el color

dorsal rojo uniforme, periferia del ojo es color cielo azul, esta especie está restringida a la serrranía Sapo-Jingurudo.

Por otro lado, se describe una nueva especie de *Dactyloa* (lagartijas gigantes) relacionados con la antigua *D. chocorum*. He sinonimizado *D. chocorum* con *D. purpurescens*, e incluyó información sobre otras especies del grupo de EP. El nuevo *Dactyloa* se asemeja a *D. ibanezi, D. limon*, y *D. purpurascens* en la morfología externa, pero se diferencia de estas especies en la coloración de la papada, patrón de coloración dorsal, morfometría y escamación. Descubrí una especie del género *Ecnomiohyla*, que exhibe distancias genéticas significativas (16S genes mtDNA) y se diferencia morfológicamente a todas las especies *Ecnomiohyla* conocidas. Junto con la descripción de la nueva especie de *Ecnomiohyla*, se ofrece una comparación detallada de los caracteres morfológicos y moleculares de casi todos los miembros del género en América Central, así como una clave de identificación para el género entero. Se describen dos nuevas especies del género *Lepidoblepharis* presentes en EP. En el apendice correspondiente, incluyo en el análisis de *Lepidoblepharis* spp. en la región, incluyendo filogeografía y la taxonomía. Entre las especies descritas de EP, *Lepidoblepharis emberawoundule* puede diferenciarse de muchas especies en el género por su pequeño tamaño y su bajo número de laminillas bajo el cuarto dedo del pie y mano, *Lepidoblepharis rufigularis* puede diferenciarse de todas las especies del género por su pequeño tamaño, la garganta de color rojizo en los machos.

He examinado la variación de la morfología, la genética y la coloración en el complejo *Pristimantis caryophyllaceus* de Panamá, utilizando diferentes unidades taxonómicas operacionales moleculares (MOTU). La filogenia, la ecología y la información sobre la distribución de esta especie arrojan luz sobre la posición y delimitación de *P. caryophyllaceus* y sus congéneres en Panamá. He reconocido dos especies de anolis (*Norops tropidogaster* [Hallowell 1854] y *N. gaigei* [Ruthven 1916]) en relación con lo que lo que anteriormente se conocía como *Norops tropidogaster*. Als dos especies se diferencian claramente en la morfología de hemipenes, la papada

masculina, varias características folidoticas, y por genética molecular. Despues del análisis resucité a *N. gaigei* de la sinonimia con *N. tropidogaster*.

Panamá históricamente ha sido, muy dinámico geológicamente. Los actuales patrones biogeográficos y el origen de la herpetofauna en la zona estan estrechamente relacionados con el pasado geológico. El evento más importante es el Gran Intercambio Biótico Americano (GABI), que se inicio en Panamá durante el Mioceno-Plioceno, cuando las especies de Norte y de América del Sur migraron hacia un continente o el otro. Se aplico un análisis biogeográfico a un género de anfibio y uno de reptil, utilizandolos como modelos para evaluar el origen y la biogeografía de la herpetofauna en EP. Lo que he encontrado es que el proceso geológico que tuvo lugar en Panamá durante el cierre del istmo y la conexión de América del Norte y del Sur, coincide con las edades de origen para determinados grupos de anfibios y reptiles en Panamá. Más aún, EP, no sólo es un camino o un puente utilizado por la flora y la fauna para colonizar cada continente, sino un lugar de especiación *in situ* para algunos anfibios y reptiles. Hay eventos de especiación que se han producido en las tierras altas de EP. La fluctuación del nivel del mar y el aislamiento durante el levantamiento de las montañas durante el Mioceno medio han promovido la especiación de varias ranas, y han dado forma al patrón de distribución actual y la estructura filogeográfica para muchas especies de anfibios y reptiles en el EP.

El aislamiento y la especiación en EP se refleja en el endemismo de varias especies. La distribución restringida de especies endémicas ha contribuido a aumentar el número de especies en peligro de extinción en la región. Con base en los resultados en EP hay 23 especies endémicas descrita, que también habitan otras provincias del país (11 anfibios, 12 reptiles), con la mayoría de ellos exclusivos de EP (10 anfibios y reptiles 8). Actualmente hay 29 especies de la herpetofauna (15 anfibios, 14 reptiles) 15 en la lista roja de especies amenazadas de la UICN (en peligro crítico, en peligro o vulnerables). Sin embargo, 45 especies (en su mayoría recientemente descrito, o reptiles) no han sido evaluadas por los especialistas de la UICN, y 36 son DD. .De Acuerdo a la puntuación de la vulnerabilidad ambiental, otra medida utilizada

específicamente para anfibios y reptiles para evaluar el estado de conservación de las especies, en EP hay 108 especies (50 anfibios, 58 reptiles) con una alta vulnerabilidad, 95 con medio (35, 60) y 36 con baja vulnerabilidad (12 anfibios, 24 reptiles), y 35 (21 anfibios, 14 reptiles) no han sido evaluados. Identifiqué las principales amenazas que afectan el estado de conservación de la herpetofauna en la EP, entre ellos: la quitridiomicosis, la fragmentación del hábitat, la alteración del hábitat, la contaminación, las especies invasoras y el cambio climático. Un impacto directo sobre la herpetofauna que recientemente ha afectado a las poblaciones de anfibios es la quitridiomicosis. Pero lo más alarmante es que casi justo después de terminar el trabajo de campo de esta tesis, se encontro evidencia de disminución de anfibios vinculadas a esta enfermedad infecciosa en EP. Junto con la quitridiomicosis, el impacto de la fragmentación del hábitat o la alteración de los anfibios y reptiles no ha sido evaluado en EP. Por ejemplo, todas las áreas protegidas durante la estación seca son deforestadas por los madereros, y en las zonas de amortiguamiento la gente provocan incendios para abrir áreas para el cultivo. Estos impactos directos sobre el medio ambiente pueden ser silenciosos y afecta a varias poblaciones de anfibios y reptiles. Por eso se necesitan con urgencia proyectos de monitoreo para determinar el estado de los anfibios y reptiles, y sugerir posibles estrategias de conservación que puedan garantizar la supervivencia a largo plazo, especialmente de las especies en peligro de extinción.

1. INTRODUCTION

1.1. Motivation and preface to this study

In 2006 the Senckenberg Research Institute and Natural History Museum (SMF) initiated a comprehensive project in Panama "The Herpetofauna of Panama." In the same year, I joined this project with a three months scholarship to study the biodiversity of amphibians and reptiles from western Panama funded by the DAAD. During my short stay in Frankfurt, along with my advisor and other colleagues, I accomplished the publication of several papers, including descriptions of five species new to science, and other noteworthy records. Subsequent in 2009, two German students, Andreas Hertz and Sebastian Lotzkat, started their PhD research on the herpetofauna of western Panama. In 2011, and after getting my master degree at Los Andes University in Colombia (2008–2009), I was awarded a PhD scholarship from the Panamanian government to study the biodiversity of amphibians and reptiles in eastern Panama (EP). My PhD work presented herein, and those by Hertz (on amphibians) and Lotzkat (on reptiles) of western Panama, complement one another to cover all of Panama's herpetofauna, which Dr. Gunther Köhler started in 2006.

The basis of this research deals directly with taxonomy, but this is just the bedrock for a more comprehensive analysis of the herpetofauna of EP. In recent years, several new technologies have been appearing which can be well applied to taxonomy. For example, molecular genetics has had an unprecedented impact on the scientific world in the last decades. Recent approaches attempted to combine modern and classical taxonomic techniques to improve the delimination of species. The interest is not just in describing new species, but also how to delineate (clearly distinguish) them from other closely related or morphologically similar species. Controversial discussions have arisen, for example, how to differentiate among species, and which traits are diagnostically useful to delineate them. Not surprisingly, in the past, and even nowadays, many described species that have been treated as "good species" (well defined taxonomic lineages), but without a well-supported diagnosis, neither

morphologically or ecologically, are now treated as synonymsof other nominal species, after acquisition of more evidence, such as new comparative material from individuals in life to museum specimens, and the application of molecular genetics. Furthermore, still many undescribed species are wearing names of other species; this is particularly the case with cryptic species, i.e. species that are actually composed of several yet undescribed species. In trying to solve the multitude of uncertanties in taxonomy and avoid over- and underestimation of actual biodiversity, taxonomists are applying increasingly an integrative approach, using a broad array of methods and including various lines of evidence. Thus, in this work, I am dealing with these uncertainites and solve various taxonomic problems in different species groups of amphibians and reptiles from EP.

Taking into account the information I have collected using the integrative approach, and the complexity of the geology in the Eastern Panamanian region, I conducted a phylogeography analysis for two groups of frogs and one of reptiles. I also provide an updated check list of the herpetofauna for the region, including a general distributional map according geographical regions for the species present in EP. During the course of this research, I have coauthored eight research articles, five of which are already published. Furthermore, I have published several news notes for the local press in Panama. Additionally, I have presented the results at five international congresses, visited museums in Colombia, and have borrowed numerous specimens for comparison from museums in the United States. Finally, I have published a book on the conservation status of the endangered amphibian species of the region supported by non-governmental authorities in Panama.

1.2 Inside eastern Panama: physiography.

Eastern Panama is not a political division, but is the easternmost part of the country and it comprises an important biogeographical unit, the Chocoan region. Within this area, one can find five mountain ranges: the Darién, Jingurudó-Sapo, Maje, Pirre, and San Blas (Fig. 1.2.1); in the lowlands the landscape is dominated by the drainages of the biggest rivers in the region: the Balsas, Chucunaque, Sambú, and Tuira rivers

(Fig. 1.2.1). Within the lowlands there are other geographical features, which are small mountain ranges that usually do not exceed 500 m a.s.l., those are the Filo del Tallo-Canglón in middle of Darién, and the Bagre in the southeastern portion of Darién beside the Sambú river (Fig. 1.2.1).

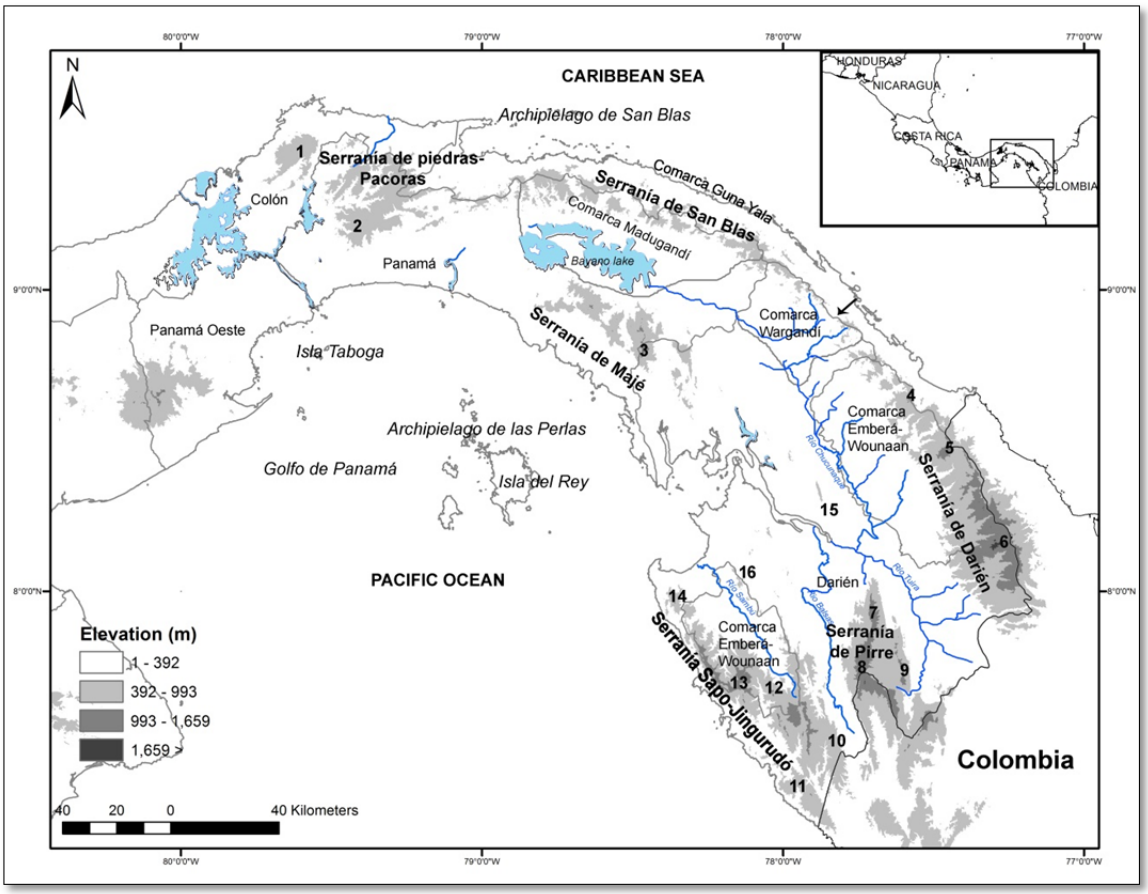

Figure 1.2.1. Topography of Eastern Panama. Numbers show the main mounts and mountain ranges that are not named in the map. Main mounts: 1. Cerro Bruja, 2. Jefe, 3. Cerro Chucantí, 4. Cerro Bell, 5. Cerro Armila, 6. Cerro Tacarcuna, 7. Cerro Pirre, 8. Alturas del Nique, 9. Cerro Setetule, 10. Altos del Espavé, 12. Cerro Bailarín, 13. Cerro Piña, 14. Cerro Sapo; Mountain ranges or Serranías: 11. Serranía de Juradó, 15. Filo del Tallo, 16. Bagré. Blue lines are the main rivers in EP. Layer source: GIS Laboratory, Smithsonian Tropical Research Institute, Panama, 2015.

Breder (1946) made a general description of the Chucunaque river banks, but the situation in this area has changed a lot since then. Whereas in the 1940's the river bank was edged by dense jungle, nowadays it is a very disturbed area. Detailed descriptions of the main mountains have been given by Myers (1969); since then, the forest has not

changed significantly. More recently, Samudio (2001) described the cloud forest of Panama, including a summary for the cloud forest of eastern Panama. What follows is a general description of the physiography of the main mountain ranges and the lowlands in EP:

The Serranía de Darién: This mountain range is almost undifferentiated from the Serranía de San Blas (Samudio 2001). However, an interpretation of the cutoff between these two mountain ranges could be made based on the relief of the area. Thereby, all areas with tributary rivers running towards the Chucunaque River could be considered as part of the Serranía de Darién; the northwestern end coincides almost with the political division at the level of the Panamá-Darién provinces (9.081° N, 78.053° W). From there the areas with rivers running to the northwest toward the Cañazas River belong to the Serranía de San Blas (see splitting point, arrow in Fig 1.2.1). Then the Serranía de Darién is the longest mountain range in EP, *ca*. 160 km long. The highest elevations are in the extreme southeast on the border with Colombia, with the maximum elevation reached by the Cerro Tacarcuna (1,879 m), followed by Cerro Armila (1421 m), Cerro Tanela (1415 m), Cerro Mali (1,410 m), Cerro Gandí (1170 m), Cerro Bell (1046 m), and Cerro Sasardí (610 m; Fig. 1.2.1).

This mountain range is part of the Eastern Panamanian montane forests (Fund 2014). Accordingly, in this ecoregion the precipitation ranges between 4,000 and 5,000 mm, and the temperature between 20–27 °C (Samudio 2001). The life zones in this area are: the Lowland Moist Forest (0–500 m elev.), Premontane Moist Forest (500–1000 m elev.) and a small area of the Lower Montane Wet Forest above 1500 m elev. around the Cerro Tacarcuna (Holdridge 1967; Fig. 1.2.2). In this region, rainfall occurs between April and December (Paya meteorological station, 500 m elev. http://www.hidromet.com.pa/, accesed on 19/09/2015).

The Serranía de Jingurudó-Sapo: This is a complex of mountains connected by elevations above 500 m. The Serranía de Sapo runs along the Pacific coast from north (from Garachiné) to south (to Jaqué), spanning a distance of *ca*. 56 km. The highest point is Cerro Piña (1,580), with an isolated mountain in the north of the mountain

range, the Cerro Sapo (1,080 m). The vegetation at Cerro Sapo was described in detail by Myers (1969). The Serranía de Sapo is connected to the Serranía de Jingurudó behind the Cerro Piña (to the east). The Serranía de Jingurudó, called also Jaqué-Imamadó Divide by Myers (1969), lacks cartographic and structural definition (Myers et al. 2007). It is surrounded by the Serranía de Sapo, Jaqué-Imamado River, and the Balsas River, with elevations reaching around 1,400 m. At the south end of the Serranía de Jingurudó is the Serranía de Juradó (Fig. 1.2.1 #1), which is a small mountain range on the border with Colombia, and reaches elevations of around 1000 m. This is a largely unexplored area.

These mountain ranges are part of the Eastern Panamanian montane forests (Fund 2014). In this ecoregion the annual precipitation ranges between 3,000 and 4,000 mm, and the temperature between 20 and 27 °C, although Myers (1969) recorded temperatures of of 22 to 35° C in the lowlands, whereas the observed range was 17.5 to 21° C in the cloud forest (850–960 m). The life zones in this area are: Lowland Wet and Moist Forest (0–500 m elev.), and Premontane Wet and Moist Forest (Holdridge 1996; Fig. 1.2.2). In this region, rainfall occurs mostly between April to December (Manené and Piña meteorological stations, http://www.hidromet.com.pa/, accessed on 19/09/2015).

The Serranía de Majé: Called the Serranía de Cañazas by Myers (1969), it is an isolated mountain range, part of the Baudo-Maje geological unit (see 1.5 section). It is *ca.* 47 km long, and separated by the Serranía de San Blas by a *ca.* 30 km hiatus across the valleys of the Ríos Chepo and Chucunaque. To the south, it is separated *ca*. 20 km from the Pacific Ocean; to the west, it is separated from the Serranía de Piedras Pacoras by *ca*. 70 km and to the east it is separated from the highlands of Pirre – Jingurudó-Sapo by *ca*. 110 km (Angehr & Christian 2000). The highest elevation is the Chucantí mountain (1,489 m, 8.8046° N, 78.4595° W). This mountain range is part of the Eastern Panamanian montane forests (Fund 2014). There is no climatic information for this área (Samudio 2001), but according to the ecoregion, the precipitation is expected to range between 3,000 and 4,000 mm, and the temperature between 20 and

27 °C. The life zones in this area are: Lowland Moist Forest (0–500 m elev.), Premontane Moist Forest (500–1000 m elev.) and a small area of the Premontane Wet Forest above 1000 m elev. at Cerro Chucantí (Holdridge 1967; Fig. 1.2.2). In this region, rainfall occurs mostly between April to December (Rio Maje meteorological station, 70 m elev. http://www.hidromet.com.pa/, accessed on 19/09/2015). Previous to my study, the Serranía de Majé had not been surveyed herpetologically (Myers 1969; Samudio 2001). In 1996, however, it was visited by an ornithological expedition to the highest point of the mountain range, the Cerro Chucantí (Angehr & Christian 2000).

Serranía de Pirre: This serranía is in the south-central portion of Darién, between the Serranias de Jingurudó-Sapo and Darién (Fig. 1.2.1), and is part of the Baudo-Maje geological unit (see 1.5 seccion). It is a ridge *ca*. 40 km long, that intrudes in part into Colombia. The ridge runs almost in its entire length above 1,200 m of elevation, with the higest points at Aturas de Nique (1,700 m) and Cerro Pirre (1,444). In the southeastern portion, there is the Valley of Cana, an old abandoned gold mine. Cana was the most populated area in Darien during 1665–1728 and from the 1890s through 1907. Now the vegetation in the area is in restoration, and it is one of the most conserved lowland areas in Darién. It is 45 km away from the last outpost of Boca de Cupe, and currently is accessible only on foot, althought years ago a field station was located there that received tourists and scientists (inactive since 2010). At that time, there was also an airport for small airplanes. To the east of Cana, there is another ridge, Cerro Setetule (Fig. 1.2.1, # 9); it is a small mountain with elevations reaching around 1,000 m. it is another unexplored area in eastern Panama, and no herpetological information have been collected from this mountain.

The Serrania de Pirre is part of the Eastern Panamanian montane forests (Fund 2014). Accordingly, in this ecoregion, the precipitation ranges between 3,000 and 4,000 mm, and the temperature between 20 and 27 °C. The life zones in this area are: Lowland Wet and Moist Forest (0–500 m elev.), and Premontane Wet and Moist Forest (Holdridge 1967; Fig. 1.2.2). In this region, rainfall occurs mostly between April and

December (Manené meteorological station, http://www.hidromet.com.pa/, accesed on 19/09/2015).

Lowlands: Most of the EP zone lies in the lowlands; in this region, Lowland Moist Forest is predominant. Almost all areas around the Panamerican Highway are modified for agriculture and pasture. While in the 1940's Breder (1946) said "*The Rio Chucunaque and its tributaries run through unbroken, virgin jungle for nearly their entire lengths*," in the 1980's the construction of the Pan American Highway in Darien opened a forest gap, along with the promotion of colonization programs undertaken by the government, brought as a result one of the biggest negative impacts to the forests of the region (Heckadon-Moreno 2009). Nowadays, most river banks away from the main roads are devastated, and only the most remote lowland areas remain untouched.

The longest river in the country is the Chucunaque (231 km in length), followed by the Tuira (230 km), the Bayano (206 km), the Balsas (*ca.* 80 km), and the Sambu River (*ca*. 70 km). Most of these rivers and their tributaries are populated by Embera, Wounaan, and Gunas indigenous peoples, and negroids, most of them in the Tuira bank and in the river mouth of the Tuira-Chucunaque. All the big rivers in EP are navigable, allowing transportation for long distances. Most people use a dugout canoe as the main transportation vehicle; they call it a "piragua."

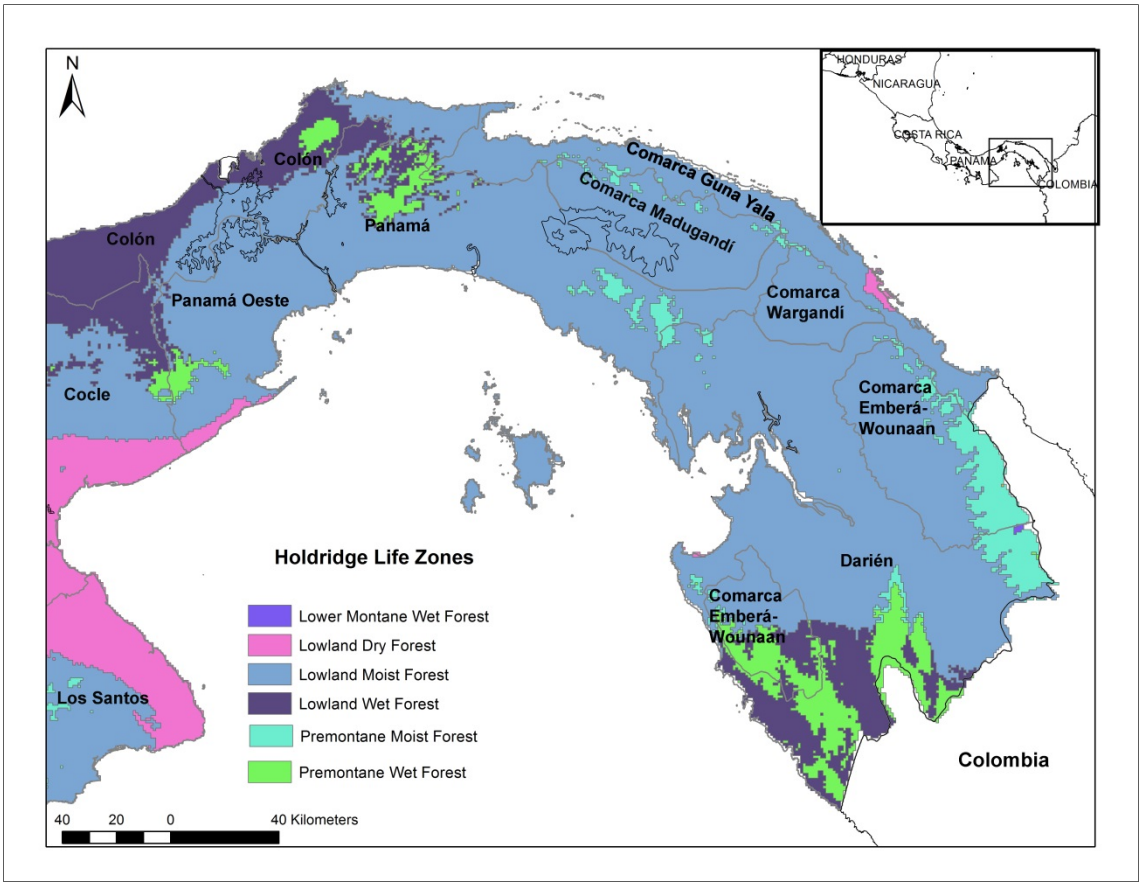

Figure 1.2.2. Life Zones after Holdridge (1967) in Eastern Panama. Layer source: GIS Laboratory, Smithsonian Tropical Research Institute, Panama, 2015.

1.3 Inside eastern Panama: Politics.

Politically, EP comprises the following subdivisions (from northwest to east): the Chepo district in the Panamá province, the indigenous reserves or comarcas of Guna Yala, Wargandí, Marragandí, and Embera-Wounaan, and the Darién province (Fig. 1.3.1). Although the Guna Yala, Wargandí, and Marragandí are in different political subdivisions, they come from the same ethnicity, called Dule or Tule (meaning "people", in the Guna language); moreover, in the easternmost border of EP are the indigenous towns of Púcuro and Paya (in the foothills of the Serranía del Darien), which are also Dule, but do not belong to any reserve. To the north and southeast of Darién is the Embera-Wounaan comarca (Cémaco and Sambú areas); the villages of this comarca are located mainly along the Chucunaque, Tuira, and Sambú rivers, and comprise two

ethnicities, the Embera and the Wounaan. EP is diverse in racial terms; apart from the three indigenous groups (Dule, Embera, and Wounaan) there are the Colonos (mestizos) and the negroids. The Colonos are Latin people from the western provinces who came to Darién to open the agricultural border in the 1970's and earlier, and are located mostly along the Panamerican Highway, with most of them near Metetí, which currently seems to be the largest city of Darién (7,976 hab.). The official capital of Darién, however, is La Palma (4,205 inh.), located on the east side of the Tuira-Chucunaque river mouth at the coast. La Palma, Yaviza (in the Chucunaque river), El Real de Santa María, Yape, Boca de Cupe, Chepigana, Jaque and Garachiné, among others are occupied mostly by negroids. In total, the population in the area is approximately 108,411 inhabitants. It occupies an area of approximately 23,553.0 km^2, with the largest portion being Darién with an area of 11,892.5 km^2 (www.contraloria.gob.pa/inec).

In EP, most of the infrastructure is poorly constructed, and the level of poverty is high, relative to other regions in the country (ANAM, 2011a). The access to all regions is difficult, the main road is the Panamerican Highway, which is not in good condition. Few others are paved; most are unpaved and many can be accessed only in the dry season. Otherwise, one must use horses or walk long distances to reach the villages. Other principal transportation is aquatic; the big rivers are navigable, and many people use "piraguas" (dugout canoes) for mobility.

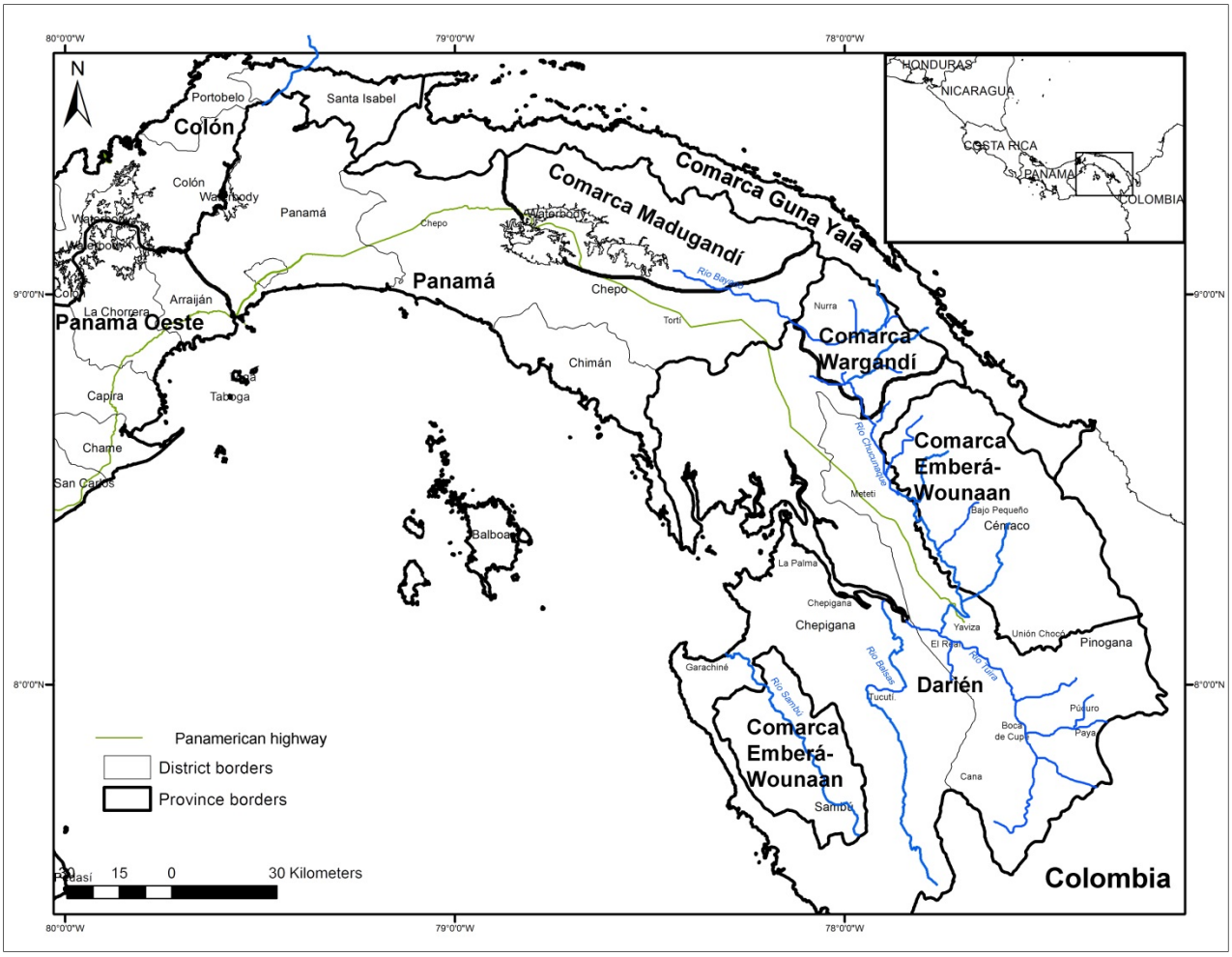

Figure 1.3.1. Eastern Panama, province and district subdivisions, and major roads and rivers.

1.4 Geological history of eastern Panama

Eastern Panama is named as an important biodiversity hotspot due to its great variety of habitat, high endemism, and rapid habitat loss (Parker et al., 2004; http://www.biodiversityhotspots.org; accessed: 05/06/2011). Coincidently, it is a geologically complex part of the Choco biogeographical region (Duque-Caro, 1990a). In EP, the Choco block (called Panama Block by Farris et al., 2011; Montes 2012a) is divided into three geographical units: in the northeastern portion the Dabeiba arch (San Blas and Darién Massif), in the middle portion the Chucunaque basin drainage (Chucunaque and Tuira basin), and in the southwestern portion the Baudo arch (Maje

Pirre, Jingurudó and Sapo). Although there is no doubt that these mountains and the Chucunaque basin completed their uplift during the middle Pliocene (Duque-Caro, 1990; Coates and Obando, 1996; Coates et al., 2004), the beginning of such events seems to be happened earlier.

The geological activity in the region began during the late Cretaceous–Eocene, induced from the interaction of the Caribbean, Cocos, Nazca, and South American plates (Coates et al., 2004). During that epoch, and distant from South America in deep sea, rocks of the main massifs of Maje, Sapo-Jingurudó, Darién and San Blas were established, later integrating with the Panama arc (see Montes *et al*., 2011b, Fig. 1). Around 20–25 Ma (millions of years ago), a sequence of geological events drove the Panama arc formations and the subsequent collision with the south (Coates et ., 2004; Farris et al., 2011; Montes et al., 2012a-b). Already by the early Miocene, the San Blas range was emergent above sea level, forming the end of a North American Peninsula leaving a narrow seaway between North and South America (Kirby & MacFadden 2005), and by 15 Ma the gap between the continents disappeared (Fig. 1.4.1; Montes et al., 2012b). Yet by 8.6 Ma, much of the Darien region was emergent, but by ca. 7–6 Ma a eustatic sea-level rise occurred near the top of the Chucunaque basin (Coates et al., 2004), although still there is evidence that water exchange occurred in Central America after 15 Ma and even more recently around 3.5 Ma (Duque-Caro, 1999a; Coates et al., 2004). Recent studies, however, have found another explanation; indeed in Central America the water exchange (although not permanent) has been continuous earlier than 15 Ma, not as was thought before through the Atrato Seaway (between the junction of EP and Northern South America (Duque-Caro, 1990b; Coates et al., 2004; Kirby et al. 2008), but by the west of the Canal Basin (see Fig. 1: Montes et al. 2015), thus supporting a more recent biotic interchange between North and South Americataking place in EP.

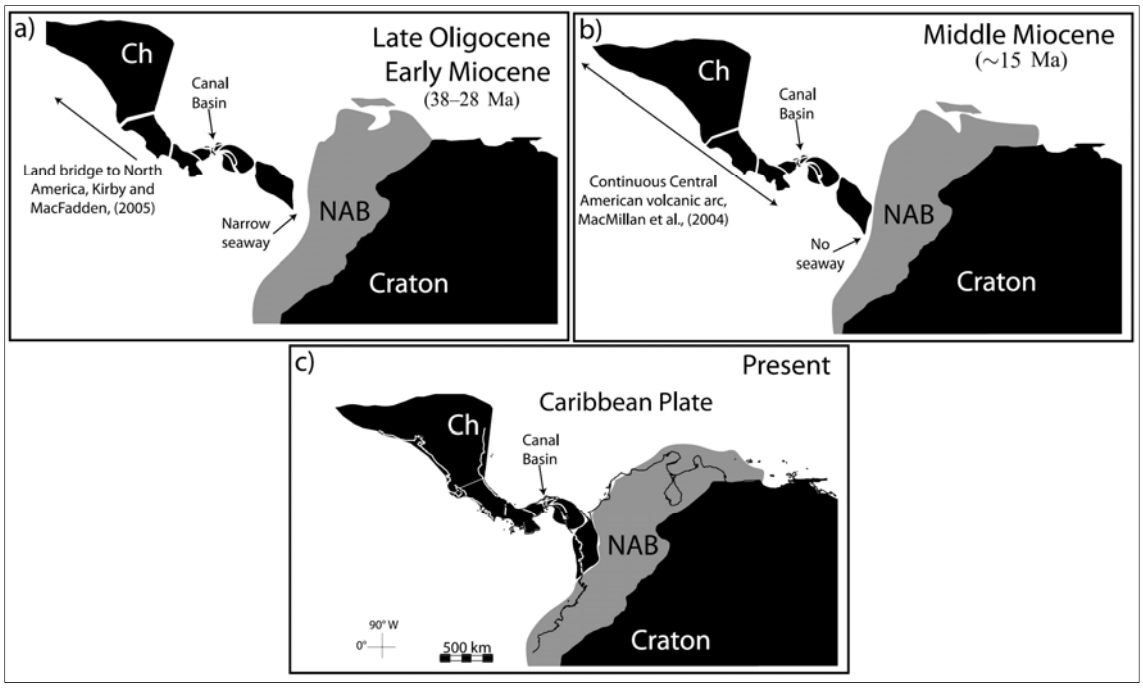

Figure 1.4.1. Graphic reconstruction of the most recent hypothesis for the closure of the Panamanian isthmus from the Oligocene to the present. (a) from late Oligocene to early Miocene, the gap was narrow. (b) at the time of seaway closure (middle Miocene), the Campanian to Eocene belt was exhumed; and (c) Present configuration. Ch: Chortis block, NAB: northern Andean blocks. From: Montes et al., 2012 a.

1.5 The role of eastern Panama in the Great American Biotic Interchange (GABI).

The connection between the Panama arc and South America promoted the most important biological dispersal between the South American (SA) and North American (NA) continents, the so-called Great American Biotic Interchange (GABI; Marshal et al., 1982; Savage 1982; Webb 1996). Evidence of the GABI in the fossil record is scarce in the Neotropics, but during recent excavations at the Panama Canal Basin, a finding of a considerable number of fossils has given new insights into the biogeographical history of the region. The fossil record suggests that the first herps crossing the Panamanian isthmus were turtles (Cadena et al., 2012), boas (Head et al., 2012), and caimans (Hasting et al., 2013) during the early Miocene, primarily from South America. At this time (around 20 Ma), these reptiles crossed the narrow seaway (100 to 200 km wide

marine strait) that was still present at the Atrato basin dividing NA and SA. Not surprisingly, these animals were capable of crossing the seaway, for example tortoises (e.g. Gerlach et al., 2006), caimans (Hasting et al., 2013), and even a snake, that has arrived to Panama not later than 19.3 Ma (Head et al., 2012). Dispersion across the seaway by boas could have been possible either by swimming or by rafting, although these species have no salt glands, and the degree of resistance to dehydration and salt water tolerance are unknown (Hart et al., 2012). Still, incongruences between the timing of the closures of the isthmus with the capability of dispersion for some species remain inexplicable. In frogs, there are no fossil records in the region; therefore, most of the evidence about the dispersion of these animals is is derived from phylogenetic data on recent species.

The land bridge formed by the completion of the Panamanian isthmus allowed the colonization of new environments, diversification, and extinctions (e.g., by competition) of animals from both continents (Marshal *et al.,* 1982; Pinto *et al.,* 2012; Savage 1982). There are several hypotheses about when the faunal dispersal occurred, but there is no fixed date, and it seems that the timing has varied among different organisms. Among vertebrates, bird species have dispersed very early, most of them before 5 Ma, with the majority of species having dispersed around 3 Ma (Cody *et al.,* 2010; Weir et al., 2009), with exceptions of hummingbirds that colonized NA from SA earlier (McGuire *et al.,* 2014). Most of the mammals crossed the isthmian bridge prior to 10 Ma (Cody *et al.,* 2010; Marshal et al., 1982). Delays (of nearly 10 Ma) in the GABI after the formation of the landbridge between NA-SA (mainly birds and mammals) might be unrelated to seaway closure and instead may be linked to Plio-Pleistocene global climatic transitions (Montes et al. 2015, and references therein). Contrary to most birds and mammals, many amphibians and reptiles have crossed the isthmus earlier, during the Miocene and established their populations either in North or South America (Farris et al., 2011; Montes et al., 2012a-b), among them: salamanders (Elmer *et al.,* 2013), many frogs (Moen *et al.,* 2009; Pinto *et al.,* 2012; Santos *et al.,* 2009), snakes (Daza et al., 2010); the genus *Gonatodes* (Gamble et al., 2008a), and the genus *Marisora* (Hedges & Conn*,* 2012). Earlier events occurred with caecilians during the beginning of

the Cenozoic (Zhang & Wake 2009) and with *Lepidoblepharis* during the Eocene (Gamble et al., 2008b). During the Miocene (~15.0 Ma), the route used by the terrestrial fauna was the Baudo Pathway (Fig. 1.5.1). Explanations for some species that colonized SA or NA prior to the Panama land bridge completion are: that species colonized new continents by a hypothesized previous land connection between Nuclear Central America and South America during the Paleocene, or by a proto-Antillean land bridge (Savage 1982, 2002), or by fortuitous island-hopping, or by rafting (Wang *et al.,* 2008). Presently, however, a reliable hypothesis about this matter is still unavailable.

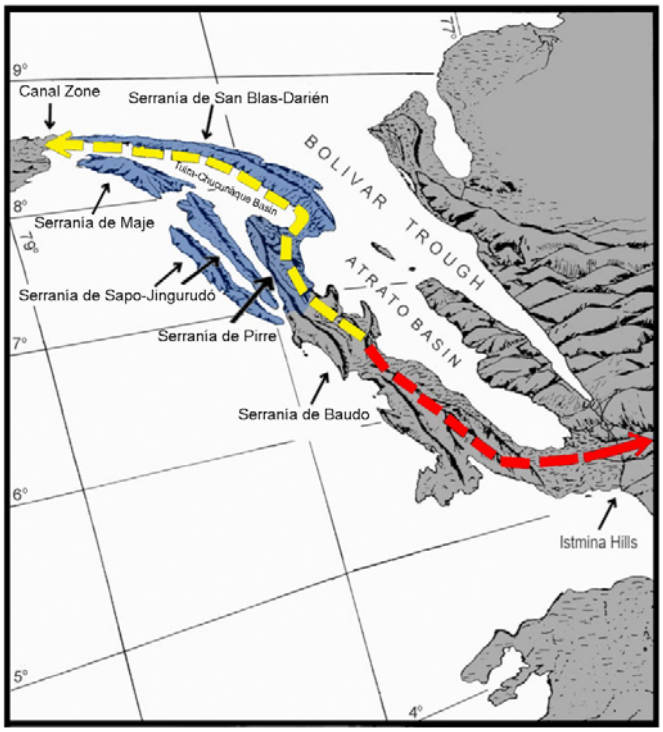

Figure 1.5.1. The potential pathway that was used by terrestrial vertebrates during the GABI in the late Miocene ~15.0 Ma, via the Baudo Pathway between the Serranías de San Blas-Darién and Baudo, to the South (red arrow) and to the North (Yellow arrow). The blue shaded area corresponds to the current area visited for this study. From Campbell et al. (2000), modified by Porthero et al. (2014), and slightly modified in this study.

1.6. Origin, composition and endemism of the eastern Panamanian Herpetofauna.

In Eastern Panama (EP) most amphibian and reptile species have their centers of distribution and differentiation either in South America or tropical Mesoamerica. Forty-six percent of the genera in EP have South American, 38 % tropical Mesoamerican, and a few species and/or genera North American origins (Savage 1982, 2002; Faivovich 2005; Hedges et al., 2008; Jaramillo et al. 2010). As most of EP is part of the biogeographical Chocoan unit, the herpetofauna from western Ecuador and northwestern Colombia shares much of its herpetofauna with EP (Lynch et al. 1997). Based on the physiographic classification used by Campbell (1999) and Jaramillo et al. (2010), there are three regions in EP: the Pacific lowlands (CP) from west-central Costa Rica through Panama, the Caribbean lowlands (NP) from eastern Nicaragua through Panama, and the eastern Panama highlands (EPH), including the Serranías of Darién, Jingurudó-Sapo, Maje, Pirre, and San Blas. Most of the lowlands in EP are more influenced by the South American rather than the Nuclear Central American fauna (Savage 1982; Lynch et al. 1997). The inclusion of all EP either in NP and/or CP would be erroneous, For example, the part of the CP in EP is more similar to the NP (in Campbell 1999; see also Fig. 1: Crawford et al. 2007), than to the rest of the CP, towards the west of the Panama Canal area on the Pacific coast. Then, because of this incongruence, here I am using the ecoregions of the world (Crawford et al. 2007; http://www.eoearth.org/ accessed on 21/Sept/2015). Thus being the CP of Campbell (1999) in EP, the same as the ecoregion Isthmian-Atlantic moist forest (Hogan & Fund 2014a), whereas most of the NP of EP (in Campbell 1999) and the border with Colombia is related to the Chocó-Darién moist forest ecoregion (Hogan & Fund 2014b), not dealt with by Campbell (1999) or Jaramillo et al. (2010).

Our knowledge of herpetofaunal diversity in Panama has changed significantly during the last several decades. In the 1970's (Myers 1972) 143 amphibians and 214 reptiles were known to occur in EP; in the 1990's these numbers had risen to 171 amphibians and 228 reptiles, whereas during the early 2000's it remained relatively stable (176 amphibians and 229 reptiles, Ibáñez et al. 2001), but in the last two decades, it has increased to 199 amphibians and 248 reptiles in 2010 (Jaramillo et al. 2010), and to 215 amphibians (Batista et al., 2014a; Hertz 2015) and 268 reptiles

(Lotzkat 2014; Batista et al. 2015a-b) at the present. This diversity is represented by 14 and 30 families of amphibians and reptiles, respectively. Among the amphibians, the most diverse family is the Hylidae (24 sp), followed by the Craugastoridae (16 sp) and the Centrolenidae (13 sp); the families with fewest species are the Microhylidae (2 sp), Pipidae (1 sp) and Ranidae (1 sp). Among the reptiles, the most species-rich families are the Dipsadidae (41 sp), the Colubridae (26 sp), and the Dactyloidae (17 sp); the families with fewest species are the turtles, the Hoplocercidae (1 sp), the Tropidophiidae (1 sp), the Alligatoridae (1 sp), and the Crocodylidae (1 sp). It is difficult to evaluate species richness within political regions in the country, because most species accounts given for the country lack geographic specification (Myers 1972, Ibañez et al. 2001) and the one available (Young et al. 1999) is too old. Although of small scale, the maps shown in Köhler (2008 & 2011), can provide general data about the current diversity in the EP region, along with some selected literature (Batista et al., 2014b-c, 2015; Hertz 2015; Lotzkat 2014). In total, it currently is evident that there are 96 amphibians and 151 reptiles species present in EP.

"*The eastern Panamanian highlands are interesting areas of endemism clearly in need of much further study*" (Myers et al. 2007). Indeed, in recent years several species of amphibian and reptiles have been described (Myers et al. 2012; Batista et al, 2014a-b; Batista et al. 2015), with others on the way to being described. There are ten amphibian and seven reptile endemics restricted to EP, among them the amphibians *Oscaecilia elongata, Bolitoglossa cuna, B. chucantiensis, Ecnomiohyla thysanota, Atelopus certus, Colostethus latinasus, Anomaloglossus isthminus, A. astralogaster, Pristimantis pirrensis*, and *Pipa myersi* and the reptiles *Norops triumphalis*, *Diploglossus montisilvestris, Ptychoglossus myersi, Lepidoblepharis emberawoundule, L. rufigularis, Atractus darienensis, A. hostilitractus,* and *Coniophanes joanae*. One amphibian (*Atelopus limosus*) and four reptiles (*Dipsas nicholsi, Dactyloa kunayalae, Dipsas viguieri,* and *Geophis tectus*) are endemics that occur in EP but also in other areas of Panama. Most of the endemics are restrictect to a serranía, or an isolated mount, probably due to lack of sufficient survey. Some species have not been reported since the species description (e.g. *Oscaecilia elongata, Pipa myersi, Diploglossus*

montisilvestris, Atractus darienensis, and *Coniophanes joanae*). In EP there are several serranías and isolated mounts that have not been explored by any herpetologist. Thus, it is expected that, as more fieldwork is undertaken, there will be an increase in species numbers.

1.7 Taxonomy and diversity research in eastern Panama

Panama, a little country between the continents of North and South Americas, is recognized for its mega-biodiversity (Wilson et al., 2010). Panama's complex geological history has promoted the formation of an impressive variety of ecosystems, allowing the existence and diversification of species originating from both continents. The list of 215 amphibians and 268 reptile species currently known from Panama reflects this amazing biodiversity (Jaramillo et al., 2010; Köhler, 2008, 2011).

Since the arrival of the first naturalist on the Panamanian isthmus at the end of the eighteenth century, many scientists have been attracted by this country's amazing biodiversity. Several herpetological expeditions to the territory have been undertaken since that time, with major contributions by E. R. Dunn, C. W. Myers, and more recently by Gunther Köhler. Nevertheless, most works on the herpetofauna had been carried out in Central Panama (Ibáñez et al., 2001). In the past two decades, important works have contributed to the knowledge about these animals, with a focus on taxonomy (Ibáñez et al., 1999; Ibáñez et al., 2001; Auth 1994; Young et al., 1999; Jaramillo et al., 2010). Recently, expeditions to remote areas in the western and eastern portions of the country have initiated a new era of herpetological research in Panama (Crawford et al.,. 2010; Hertz et al., 2012; Köhler et al., 2007, 2008; Lotzkat et al., 2010; Wang et al., 2008). Much work, however, remains to be done in many areas.

The first historical mention of the herpetofauna of EP was in 1526, when Oviedo (1526) wrote about the relationships among amphibians, reptiles, and humans. Later on, the first known scientific collection in the region was made by Enrico Festa, who visited the lowlands of the Tuira drainage on June 1895 (Peracca 1896a; Heckadon-Moreno 2006). Festa reported 39 species, 7 amphibians and 32 reptiles, and collected a new species, *Ptychoglossus festae* described by Peracca (1896b). On April 1912,

Edward A. Goldman delved into the depths of the Darién forest, going up to the remote Cana mine, near the Colombian border. Goldman was not a herpetologist, but a naturalist who collected some material that later served as reference (Heckadon-Moreno 1998), even for the description of a new species, *Atelopus glyphus* Dunn 1923, from Río Limón, Darién. Subsequently, on March 1922, Thomas Barbour and Williams Brooks (Barbour & Brooks, 1923) went to Cerro Sapo, where they discovered the harlequin frog *Atelopus certus* Barbour 1923. Other important contributions from that epoch were made by Emmett Dunn, who described several species from the region (Dunn 1931, 1933; Dunn & Bailey 1939). Several other herpetologists have contributed to the knowledge of the EP herpetofauna; among these are Charles Breder, with his work on biodiversity and natural history (Breder 1946), and Harold Heatwole, on conservation and populations (Heatwole 1966; Heatwole & Sexton 1966). Without a doubt, however, Charles Myers and Williams Duellman, who carried out intensive expeditions in Darién, were the herpetologists who have contributed the most relevant research in the region, with the publication of several papers, including new species descriptions (e.g., Duellman 1966; Myers 1969, 1972, 1982; Williams & Duellman 1967), up to the present time (Myers 2003, 2012). Other contributions came from Linda Trueb (Trueb 1984), John Lynch (Lynch 2001; Myers and Lynch 1997), Dennis Harris (Harris 1994), and Roberto Ibáñez, Cesar Jaramillo, and Andrew Crawford (Crawford et al., 2010; Ibañez & Crawford 2004).

In Eastern Panama (EP) the known herpetofauna amounts to 92 amphibians and 149 reptiles (AmphibiaWeb, 2014; Frost, 2014; Jaramillo et al., 2010; Köhler, 2008, 2011; Uetz & Hošek 2014), with 75 % of the amphibian and the 31 % of the reptile genera having their geographic origin in South America. Nevertheless, among the reptiles, a major portion of species has affinities to Mesoamerica (54 %; Savage, 1982, 2002). The diversity of ecosystems within this region makes it an interesting place to study its fauna, especially in unexplored places and those that certain herpetologists visited many decades ago (Schmidt, 1933; Dunn & Bailey 1939; Breder 1946; Myers 1969; Williams and Duellman 1967). Many endemics and rare species in this region have unknown conservation status. Now scientists are starting to pay attention to

conservation issues and, together with national authorities, have emphasized the urgency of surveys to determine conservation strategies for the herpetofauna in Panama, with emphasis in EP (Ibáñez et al., 2001; ANAM 2011b; Jaramillo et al., 2010).

1.8 Integrative taxonomy.

When working taxonomically with species of a particular lineage, one usually has to consider two relevant issues: the species delimitation that separates the taxa within a lineage and any evidence of isolation among the species. These issues have been discussed extensively to find a consensus among the different species concepts (Sites and Marshall 2003, de Queiroz 2007, Wiens 2007, Padial et al. 2010a-b, Hart 2011). A widely accepted view defines a species as a group of separately evolving meta-populational lineages, the so called General Lineage Concept (GLC; de Queiroz 1998, 2005), which has received a consensus among many evolutionary biologists (Padial and De la Riva 2010, Padial et al. 2010, Hart 2011, Zapata and Jiménez 2012). Along with the recently proposed Evolutionary Species Concept (ESC), new methods for testing species boundaries (e.g., integrative approach) have emerged, facilitating the work of recognizing and describing species. Combining different disciplines to evaluate species richness appears the most robust approach to obtain more balanced results and to draw realistic conclusions about the status of the biodiversity for a particular region or a specific biogeographic area.

The integrative taxonomical approach (Vieites et al., 2009), combining mtDNA barcoding, morphology, biacoustic (in anurans), pholidosis (in reptiles), ecology, and biogeography (Jansen et al., 2011; Aguilar et al., 2013; Glaw et al., 2010; Lotzkat et al., 2013), currently is one of the strongest methods for identifying species and to solve the taxonomic uncertainties of cryptic taxa inherent to many species complexes, which usually can't be solved through classic taxonomy (Padial et al., 2010). Vieites et al. (2009) proposed three categories to assess the taxonomic status of genealogical lineages when using an integrative approach: (1) confirmed candidate species (CCS), (2) unconfirmed candidate species (UCS) and (3) deep conspecific lineages (DCL) (for

details see Vieites et al., 2009). The application of these categories has been successfully used to solve taxonomic problems and suggest new classifications by means of deliminating species boundaries.

During the last decades, DNA barcoding has been used as an important technique for identifying species of fauna and flora on the basis of genetic information (Fouquet et al., 2007; Francis et al., 2010; Bruni et al., 2012; Che et al., 2012; Bhargava and Sharma, 2013), as well as for monitoring biodiversity and environmental changes (Crawford et al., 2010; Hausmann et al., 2011). The DNA sequences obtained can also serve to roughly assess phylogenetic relationships of the barcoded taxa (Neigel et al., 2007; Crawford et al., 2013). Currently, barcoding of mtDNA sequences is being increasingly applied across a multitude of organisms (see: http://www.barcodeoflife.org/) to facilitate the estimation and documentation of Earth's biodiversity. For amphibians and reptiles, the Cold Code project was initiated as a global initiative to DNA barcode 'cold blooded' terrestrial vertebrates (Murphy *et al.* 2013). Its ultimate goal is to evaluate and preserve the biodiversity all over the world. With the help of this new method, the number of described species per year is constantly increasing, in both amphibians and reptiles (Glaw & Köhler 1998; Köhler et al. 2005; Uetz & Hošek, 2014). However, integrative taxonomy, combining barcoding with taxonomy, bioacoustics, and biogeography, has not been applied for amphibians and reptiles in Panama yet; thus, it is my aim is to use an integrative approach to classify lineages eligible as candidate species, and to promote future studies on the herpetofauna biodiversity in Eastern Panama.

1.9. Aims of my dissertation.

Taxonomy: Identify taxonomic problems among amphibians and reptiles, applying an integrative taxonomy approach. Revise and clarify taxonomic problems of the amphibians of the genera *Bolitoglossa, Diasporus,* and *Ecnomiohyla,* and the reptiles of the genera *Dactyloa* and *Lepidoblepharis,* and also of the species *Pristimantis caryophyllaceus* and *Norops tropidogaster*

Biogeography: Explore the phylogenetic relationships associated with the distributional patterns and geological events to propose evolutionary scenarios for the origin of the herpetofauna of EP and its relationships with the closure of the Panamanian Isthmus. To do so, I will use as models the genera *Diasporus, Lepidoblepharis,* and the species *Pristimantis caryophyllaceus.*

Diversity: Update the checklist of the amphibians and reptiles of EP.

2. MATERIAL AND METHODS

2.1. Definition of the study area

In this work, I am considering EP as the easternmost part of the country, covering the area from the Chepo district situated roughly between 7°56" and 9°09" north and 77°57" and 78°50" west, which is also the beginning of the San Blas mountain range, towards east, up to the Darien Mountain range on the border with the neighbor country of Colombia.

Recently (2011−2013), I made several research expeditions to Eastern Panama, visiting the Chucunaque and Tuira basins in the lowlands (EPLL: Eastern Panama Lowlands), and the Darién (DM), Majé (MM), Jingurudó-Sapo (JSM), Pirre (PM) and San Blas (SSM) Serranías in the highlands (Fig. 2.1.1); a detailed description of the regions visited in this study is given as follows:

2.1.1 Eastern Panama Lowlands.

I visited two physiographic regions; the Isthmian-Atlantic moist forests (IAMF) and the Chocó-Darién moist forests (CDMF). In the IAMF are the localities of: Río Mono, Wacuco, La Moneda, Arretí, Metetí, Filo del Tallo, and Laguna de Matusagaratí (Fig. 2.1.1). In the CDMF are the localities of Cruce de Mono, Cana, Garachiné, Sambú, and Pavarandó.

Río Mono at Bayano (Fig. 2.1.1): This was my first stop on the way to Eastern Panama. This is not the Río Mono mentioned in several papers by Myers and Duellman, which is a tributary of the Río Tuira. The locality I visited is 5 km to the east from the Bayano Lake's bridge. At this place, I made two brief stops; on 02 August 2011 on the way to Ambroya, Majé, I spend a couple of hours during the night. The Río Mono is beside the Panamerican Highway, thus I stopped the car few meters before the bridge, and searched 200 m downstream and 100 m upstream. The gallery forest is a secondary forest; the dominant vegetation there are bushes, cecropias (*Cecropia* spp.) and balsa trees (*Ochroma pyramidale*), and in the surrondings the most common plant is the cuipo (*Cavanillesia platanifolia*). Months later, on a second time, I walked in the

creek few meters (upstream) during the daytime.

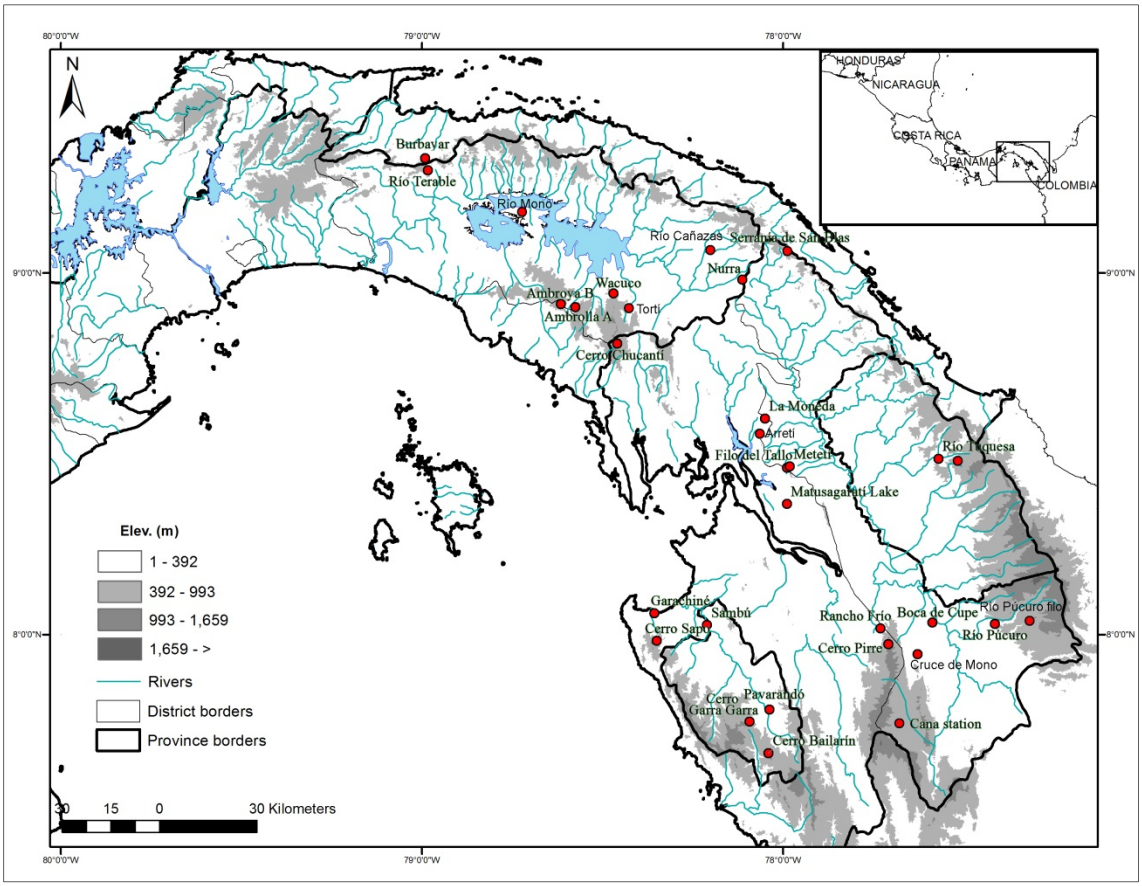

Figure 2.1.1. Research localities in the study area.

Wacuco (Fig. 2.1.1): This place is in the foodhills of the Serranía de Majé, it is alongside of the Panamerican Highway, a few kms before Tortí. This is an agricultural farm (in the flat areas) with a protected area on the hills; it is managed by the priest Fr. Wally "Padre Pablo" Kasuboski. On November 29 2012, Padre Pablo allowed Konrad Mebert and me to go into the preserve with his worker Arquimedes Batista, who guided us during the night to a trail going to the water supply for the farm.

Tortí: Konrad Mebert and I spent one night along the Río Tortí. It is located a few meters behind Tortí town. We searched along the river (upstream, approximately 200 m) and along a path (approx. 400 m) going up to a hill. Both areas were very disturbed; it was a secondary growth forest with lot of bushes.

La Moneda: Since the beginning of my project, I was accepted kindly to spend some

nights camping at the farm of the Faustino and Hermelinda family. Thus, in the following I made several stops there when returning from more distant places in EP. La Moneda is a small village beside the Panamerican Highway (*c*. 13 km to Metetí). In the Fausto's farm was an old secondary forest patch, but surrounded by crops.

Arretí: It is the end of the Filo del Tallo hill. The entrance is in La Moneda, turning right (coming from Panama city) from the Panamerican Highway. I spent one night searching on the hill; the access is walking to the south from the Arreti village. I climbed the hill up to 320 m elev. On the hill, the forest is pristine.

Filo del Tallo: About 3.5 km south from Metetí, beside the road is the entrance to the Balsal trail at the Filo del Tallo reserve. It is a trail (approx. 1 km long) in the Jungle. Here is the water supply for the Metetí town. I was there several times.

Laguna de Matusagaratí: It is a wetland area (*c*. 49,249 ha). It has several entrances; I entered from the entrance at Río Iglesias, along the road to Puerto Kimba, turning to the left coming from Metetí ca. 4.6 km by car up to the village of Aguas Calientes. I spent one night searching along the border of the lagoon. The vegetation around the lagoon is a pasture, and inside the lagoon is a mixture of plants, with bushes, big trees, and grasses. Currently, the Laguna de Matusagaratí is under threat; it has been drained to make crop and agricultural areas.

Rancho Frío: It is the main field station inside the Darien National Park (DNP). The access is from Yaviza town (the end of the Panamerican Highway); from there, one must take a dugout at the Río Chucunaque. In about one hour, the boat arrives either at Mercadeo along the Río Tuira or El Real de Santamaría along the Río Pirre. Then one takes a car to the Pirre village; from there one walks one hour to the Rancho Frío field station. On the way to the station, there is a buffer area of old growth secondary forest; at the station all is pristine forest, and it is located beside the Río Perresénico. At Rancho Frío, there are basic accommodations, with rooms for visitors, a kitchen, toilet, and shower. This is the starting point to go to Cerro Pirre. To visit this area, I contacted Isaac Pizarro. He is the guide who knows the region best.

Cana and Cruce de Mono: This locality is inside Darien National Park. To go there, one must take a dugout at Yaviza up to Boca de Cupe (approx. 5 hrs.). From Boca de Cupe,

Cana is 42.5 km away, and walking is the only option to get there. I went there with my helper Yorlis Cáceres and my guide Mario “Urrutia” Cuñapa. Mario worked at Cana when the station was under the management of the ANCON Expedition. The trail to Cana is the former railway used when the gold mine was active. Just before the trip to Cana, I received the permit to visit Cana from Marco Gandasegui, Executive Vice-President of ANCON Expedition (at that time). My plan was to climb to the Pirre ridge from Cana and explore the area. When I arrived at Cana, however, the maintenance person was told by Segundo Sugasti (via satellite phone), that I was not allowed to climb to the hill, and I just was allowed to walk in the suroundings of the station. It was very disappointing after such long walk from Boca de Cupe. Thus, in Cana I spent only two nights searching along the Setegantí and Chimenea trails. On the way back, I stopped one night beside the Río Paca (14 km from Cana). Cruce de Mono is about half way to Cana; I stopped one night there on the way to Cana and one night on the way back. Most of the vegetation along the trail was old growth secondary forest. In Cana there is a small airport that some years ago received charter flights. Since 2012, the station has been closed for security reasons due to the potential presence of Colombian guerrillas in the surroundings.

Garachiné: It is located in the southeastern part of Darién on the coast of the Pacific Ocean. I visited Garachiné several times, since it is the starting point to go to Cerro Sapo and the Serranía de Jingurudó. To get there, I took a boat in Puerto Kimba (*ca*. 16 km from Metetí); the boat took about 2 hrs. to reach Garachiné. The boat departure was every Monday, Wednesday, and Friday. Once I was delayed by the SENAFRONT’s security protocols, and I missed the boat. I had to spend two nights in La Palma waiting for the next boat. Garachiné is a lowland area; most of the vegetation in the surrondings is secondary forest or pasture.

Sambú and Río Indio: As with Garachiné, the boat departure is from Puerto Kimba. Sambu is populated by mostly negroids and Colonos, and Puerto Indio is occupied by Embera Indians; these communities are in the same area, but are separated by the Río Indio. The area is lowland; most of the vegetation in the surroundings is secondary forest or pasture. Usually each year, the flooding of the Río Sambú affects these towns,

and once affected my trip as well. I was planning to go to the Bagre área, but the day I arrived to Sambú, the surroundings of Río Sambú were flooded over the night. I waited for two days for the river to go down in level, but it never happened. Then I moved to Garachine, but the rainy weather continued for three days more. During that trip, I had to return without any success.

Figure 2.1.2. Some lowland localities visited during the course of this study. A) Lowland

forest at La Moneda; B) Laguna de Matusagaratí; C) Cana Field station; D) Yorlis Cáceres, behind observe the Cerro Setetule, photo taken at Cana field station; E) Sambú village, a day before flooding; F) Sambu village during flooding; G) Filo del Tallo reserve, photo taken from the road Meteti-Puero Quimba; H) Pavarandó village.

Pavarandó: In Sambú I meet the comarcal authorities Mr. Tino Quintana (Cacique General del área de Sambú), Daniel Berrugate (secretary) and Laciro Caibera (the authority or "Noco" of Pavarandó). After talking with them, I obtained a permit to go to Pavarando. I visited Pavarando twice, I had a good relationship with Laciro, and he arranged everything for my trip to Pavarando. In my first trip to Pavarando, I was accompanied by Milan Vesely. From Puerto Indio, we took a dugout in the Río Sambú, after 5 hour traveling by boat we arrived at Pavarando, a small but very nice village. We spent one night there in a "tambo," a small, elevated cottage; we searched the surroundings of the village that night, the day after we began the trekking to Cerro Bailarín. In my second visit to Pavarandó, I was alone. Laciro took the Piragua and drove from Puerto Indio to Pavarandó. In this trip I searched around the village the first night before to continue my journey to Cerro Garra Garra.

2.1.2 Eastern Panama Highlands.

Serranía de Darién: This is one of the most inaccessible areas in EP. There are no roads, and the easiest way is using the river, although by Piragua the rivers reach only the lowland areas (approx. 90 m a.s.l.). Then to get to the highlands with elevations above 1000 m, one must walk long distances, at least two days. Because this Serranía is located at the border between Panama and Colombia, there is regular presence of the "Colombian Guerrillas" or "narcos". These are armed groups, with revolutionary and/or narcotraffic interests, what makes the access to this area even more difficult . I took risks, and traveled alone to this area, with Indian people as guides, to try to get as far as I could.

Between 3 and 12 of June 2012 I went to the Río Púcuro, and between the 4 to 12 of November 2012, to Río Tuquesa. The village of Púcuro is the last village to access the Alto Tuira region. From here one can access Cerro Tacarcuna and its surroundings. I spent two days in this village, just waiting to get the approval of the SENAFRONT to

enter the area, at the beginning they wanted me to go to the forest during the day and return during the dusk, that was impossible to me, as I wanted to go much farther than around of the village. Finally I got the approval to go with three guides along the Pucuro River. We began walking along a trail in a ridge beside the river. After three hours the main guide Anselmo Caicedo decided to avoid a potential trail used by the Guerrilleros or Narcos, and then we decided to walk along the river to avoid letting tracks. After all day walking we set the camp at the river side and spent the first night there. That night I searched along the Río Pucuro and some other streams near the camp. Next day early morning we began the walk, and few minutes later we found plastic bags written “made in Colombia”, some meters later we found an abandoned base camp of the Guerrillas. We walked all day along the river and around 15:00 hrs. we stopped and set another camp beside the river. The next day and for safety we decide to change our route and built a new trail along a ridge at the other side of the river. We climbed and set another camp at 830 m.a.s.l. That day we ran out of water, and my guides started to cut vines to obtain fresh water spilled from the vines, they collected enough water that we could make rice and good enough for the next walk. As in the other camps I searched during the night around the camp. Next day we climbed up to 1043 m a.s.l. and set the last camp there (for two days), this day it did not rain as well, and we tried to get water from the bromeliads, we filter with dry cloths and drank it, finally around 04:00 hrs the rain fell and we could collect enough water for the next days. In this camp we stopped walking. The guides told me that we had reached a mountain and the ridge ended, and there was no chance to continue. But later on, checking a topographic map and Google Earth, indeed we must walked down, and below was a connection to another ridge that could take us to Cerro Tacarcuna (10 km away).

The trip to Río Tuquesa was less complicated. I traveled to the Bajo Pequeno village, and two guides took me to the Pechito Parado mountain (858 m a.s.l.), an isolated area surrounded by the Ríos Tuquesa and Tupisa.

Serranía de Majé: I visited three places in this mountain range. At Ambroya I went to Cerro Ambroya (Fig. 2.1.1 Ambroya B; 3–8 August 2011) and to the La Javillosa area (Fig. 2.1.1 Ambroya A; 26–29 September 2012), and the third place was Cerro Chucantí

(Fig. 2.1.1; 1–8 December 2012). To reach Ambroya, I turned right from the Panamerican Highway in Loma Bonita 25 km before Tortí, then drive for about 1 hr. to Ambroya, the road is unpaved and in rainy season there are some difficult parts, you need a 4x4 car. In ambroya I met Hugo Martinez who was my guide for both trips near Ambroya. To Cerro Chucantí I contacted Guido Berguido (http://advantagepanama.com/), the manager and owner of the Cucanti Private Reserve. To get to Chucantí, I drove from Tortí to Río Pavo, from there I rode a horse for around four hours up to the reserve. I spent two nights searching in the reserve, before climbing to the top of the hill. From the reserve (800 m a.s.l.) it is two hours to the ridge of the hill very close to the top. There I set a camping place for three nights. In this tour I was accompanied by Konrad Mebert.

Serranía de Jingurudó-Sapo: In these mountain ranges, I went twice to each place. To Cerro Sapo from 3 to 7 December 2011, and 25 to 30 August 2012, and to Jingurudó to Cerro Bailarín from 20 to 30 September 2011 and to Cerro Garra Garra from 13 to 18 November 2012. The access to Cerro Sapo is from the Garachiné village; arriving there I walked by around four hours to the first camp at 200 m a.s.l. spending one night there. The second day I moved to the second camp at around 850 m a.s.l. and stayed there one more night. The third day I walked to the top of the mountain at 1,168 m a.s.l. The walk to the top is very difficult, especially in the areas above 1000 m elevation. The first time I went there, there was no open trail to the top from the second camp. My guide Gustavo Dojiramá had to make his best to open a little path between and under roots and bushes. In the top there are no water sources available, and then I had to pray for rain every day. Without rain is almost impossible or very difficult to be more than one day in the top. For the second trip I used the same camps for the same duration of days.

Cerro Bailarín is a mountain in the Serranía de Jingurudó. To access to this place I went with Milan Vesely and two guides by boat from Pavarandó along the Río Sambú, up to the mouth of Aldo creek, to start walking. Before to reach the Cerro Bailarín we set three camps in the way, in the last one we spent two nights. To Cerro Garra Garra (you can see it from Pavarando) I walked with two guides for five hours to the first camp.

Although I tried to reach the Cerro Garra Garra, the guides when we were there lost the trail; they took a wrong one which did not lead us to the mount. Instead, I spent three nights bellow the Cerro Garra Garra at 653 m a.s.l. when I expected to climb up to around 1000 m a.s.l.

Figure 2.1.2. Some highland localities visited during this study. A) Serranía del Darién, view from north ridge beside Rio Pucuro; B) Cloud fores at Serranía del Darién, ridge 1043 m a.s.l. at Rio Pucuro; C) Forest, Serranía de San Blas approx. 500 m a.s.l.; D) Cloud forest at Serranía de Pirre (photo by K. Mebert); E) Cloud forest at Serranía de Sapo approx.. 950 m a.s.l.; F) Top of Cerro Sapo; G) Cloud forest at Cerro Bailarín

(Photo by M. Vesely); H) Cerro Chucantí, view from Río Pavo.

Serranía de Pirre: I visited this mountain range twice, the first time during the 08 to 16 August 2011, and the second time from 04 to 12 December 2012. To get to this place I took a Piragua at Yaviza, traveling one hour to the harbors of El Real or Mercadeo, in the Río Tuira. From there I took a car to Pirre 1, to start walking for one hour to the Rancho Fío field station, the main station in the Darien National Park. I stayed one night in this station. The following days began my hiking to the Pirre ridge. I set a first camp at the Mirador dos (600 m a.s.l.), near the Perresenico creek, this night I searched along the creek and a trail towards the Pirre ridge. In the first trip I set two camps along the ridge at elevations between 1100 to 1250 m. In the second trip I traveled with Konrad Mebert and stayed four days in the ridge, in one of the nights we hike to the highest point around, the Cerro Pirre, *ca*. 10 km from the Rancho Fío field station.

Serranía de San Blas: In this mountain range I visited four places, Burbayar, Río Terable, Alto Cañazas arriba and Nurra. In Burbayar (right beside the main road of El Llano-Cartí) I stayed three nights from the 26 to 28 November 2012. Mr. Iñaki Ruíz the owner of this private reserve has built a cottage for visitor scientists, and has allowed me to stay in this place several times. During my trip to Burbayar (on the Caribbean slope of the Serranía) I visited for one night the Terable River, which is in the pacific side of the Serranía de San Blas; Konrad Mebert joined me at this trip.

On the 24 September 2012, Milan Vesely and I tried to reach the Serranía de San Blas going to the village of Alto Cañazas (Fig. 2.1.1, in the Comarca Madugandí), in the Río Cañazas. After a long way through the river, in the afternoon we arrived to the village, we had to wait until the evening for a special meeting (they call congreso) with all the community to discuss if we could have the permit to explore the mountains behind the village. After two hour listening them, discussing in the Guna language, they decided to forbid us the access to the mountain, we complain but they said: there (in the jungle) are monsters and nobody from this village is going there, we said ok, we are going alone, but almost angry they replied and told us that they had arranged the boat for us and that we must to return the next morning and pay them for the cost of the travel down to the road. After this disappointed trip, we tried another way, through the Nurra

village, a bit toward the South, but in another Comarca (Comarca Wargandí), we still scared from the previous experience we took risks and we ride horses for six hours until we reached the Nurra community. There again we had to wait for a “Congreso”, we were skeptics that we could get the permit. In Nurra the people is more civilized than in Alto Cañazas, maybe because that, we finally got the permit to go to the Serranía along with two more guides. We pay a contribution to the community and the salary for the guides. We spent a week in this Serranía, walking up to the continental divide; we had no permit to continue to the Guna Yala Comarca. In Nurra, after two hours walking along the Río Chucunaque, one can find pristine forest, a good place to seek for herps.

2.2. Methods and Morphological studies

For candidate species and their delimination, I followed the integrative approach of Vieites *et al.* (2009). In evaluating species boundaries among amphibians and reptiles found in EP, I followed the Unified Species Concept of de Queiroz (2007). As lines of evidence for species delimitation, I applied a phenotypic criterion, in particular external morphology, including diagnosable traits for coloration, morphometrics, and pholidosis, which are suitable to differentiate among species within a group or family of closely related taxa, and a criterion for reproductive isolation, such as genetic distinctness of mtDNA-genes (16S, COI), hemipenis morphology and bioacoustics. Every family and/or group was evaluated separately to avoid overgeneralizing of diagnostic characters between different groups.

Morphological nomenclature, measurements, and diagnosis are detailed for salamanders in Appendix I, for *Diasporus* in Appendix II, for *Ecnomiohyla* in Appendix III, for *Pristimantis caryophyllaceus* in appendix IV, for *Norops tropidogaster* in Appendix V, for *Dactyloa* in Appendix VI and for *Lepidoblepharis* in Appendix VII. Bioacustic methods, and molecular laboratory and phylogenetic inference are detailed in the Appendix II (methods).

3. RESULTS

The results of this study are based on published papers, which are shown in seven appendixes. Appendix I, I present an overview on the species of the genus *Bolitoglossa* genus in EP, with the description of a new species for science. In Appendix II, I contribute new information by describing the advertisement call of *P. caryophyllaceus* from EP for the first time, and provide essential information on the ecology, biogeography, and morphology; according to the information I collected, I am including a discussion on species delimitation, using as a model the species complex *P. caryophyllaceus*. In Appendix III, I review the taxonomic status of the genus *Diasporus* in EP; through an integrative approach, I reveal differentiation and a high species richness in the genus that previously have not been recognized; I also include a biogeographical analysis of the genus in EP. In Appendix IV, I revise the genus *Ecnomiohyla*, with special emphasis on the species from Lower Central America and northwestern Colombia, including the description of a new species for EP. In Appendix V, I provide evidence for the taxonomic splitting of *Norops tropidogaster* and *N. gaigei*, previously recognized as a single species. In Appendix VI, I describe a new species of *Dactyloa*; also I assess the taxonomic status of *D. chocorum* and *D. purpurescens* and include comments on the other *Dactyloa* species from EP. In Appendix VII, I describe two new species of *Lepidoblepharis*, and I perform a biogeographical analysis of the origins of the genus in Panama. In the appendix VIII, I present an updated checklist of all herpetofaunal species present in EP with their respective conservation status and distributional area.

4. DISCUSSION

Recently genetic barcoding has been widely accepted as a useful tool to carry out preliminary studies, and it helps to assess the taxonomic status of problematic species that cannot be solved by traditional taxonomy alone (Chapple & Ritchie 2013). For taxonomic analyses, I used the now for herpetofaunal barcoding established barcode sequences of the 16S and COI genes. The success rate of amplification for the mtDNA-16S marker was close to the average value reached in other studies (Crawford et al., 2010, 2013); however, in mtDNA-COI, the rate was lower than what have been reported in other studies on amphibians and reptiles (77 % vs. 85 %; Nagy *et al.*, 2012; Perl *et al.*, 2014), which could have been caused either by a low amount of tissue preventing successful DNA extraction in some cases, or by contamination, which was detected in several samples. Mistakes during the application of the barcoding method could certainly lead to incorrect results (Will et al., 2005; Hickerson *et al.,* 2006; Chapple & Ritchie 2013).

A key cue for interpreting the barcoding results is establishing thresholds of genetic divergence permitting species delimitation. As these thresholds substantively vary among amphibian and reptile families (or even genera, Crawford *et al.,* 2010; Jansen *et al*., 2011; Paz & Crawford, 2012; Nagy *et al*, 2012; Perl *et al*., 2014), I used an integrative (multidisciplinary) approach, which is certainly more balanced than a simple threshold application, and should bring better understanding of which taxa likely represent biologically and evolutionary independent species.

Correct evaluation the herpetofaunal diversity on a regional scale is always risky when only a single method for identifying species is used. This is also true for barcoding, although one might assume that such a modern method is safer in this respect than so called “old fashioned” methods such as morphological analyses. The risks of misleading interpretation of results based just on barcoding is easily demonstrable with some of my data. On the one hand, I found strong molecular evidence supporting the presence of several new species in the genus *Diasporus* (Batista *et al.,* 2016 proof.), as well as a

new species of other species in different genera of amphibians and reptiles as showed in this study (*Dactyloa, Ecnomiohyla, and Bolitoglossa*). On the other hand, for other species my molecular analyses demonstrated high molecular divergence among populations, that were not supported by other lines of evidence (for example morphologically and biogeography) to be split into several species (e.g., *Pristimantis caryophyllaceus,* and *Bolitoglossa biseriata*), mainly due to the recovery of old lineages or a very high genetic variation within the species. When using only molecular evidence, I could identify or even describe at least two more new species related to *P. caryophyllaceus*, but when including an integrative approach with deep morphological, biogeographical, and ecological analyses, I found no evidence to support additional new species within *P. caryophyllaceus*. Therefore, the integrative approach enhances the delimitations of species boundaries, resulting either in the descriptions of new species or clarifying the status of cryptic species (Pante *et al.*, 2014). As part of such an approach, the application of bioacoustics in the case of anurans, and hemipenial morphology in reptiles constituted additional evidence that supported my species hypotheses in different groups (e.g. Colostethinae, *Diasporus* spp., and *Lepidoblepharis* spp.).

The taxonomic evaluation of biodiversity has direct implications on the conservation of the species. Conservation plans for species or a population can fail seriously when based on information not pertaining to the target population, which might represent an undescribed, cryptic, or rare species with a different ecology than the species from which the ecological information for the plan originates (Melville *et al.,* 2014). For example, I found a second (cryptic) species hidden under the name of *Norops tropidogaster*. The new species, *N. gaigei*, was separated based on pholidosis, hemipenial morphology, and molecular genetics. In this case, neither species is endangered, but it exemplifies the potential fallacy that could emerge from an insufficiently resolved taxonomy. Within the genus *Diasporus*, I found four new species of frogs that were treated as *Diasporus diastema* or *D. quidditus*. At least three of these

species are restricted to single mountain peaks, hence, they represent microendemics, which are of high conservation priority (IUCN, 2013). Restricting the study on morphology, the newly found endemics would be lumped together into a widespread species without the chance of receiving any specific protective measures.

This is the first regional evaluation of the herpetofaunal biodiversity in eastern Panama applying an integrative taxonomy. In EP, as anywhere else in the world, the implementation of this approach shows that the biodiversity was underestimated. Several biodiversity studies have demonstrated that a substantial number of undescribed species can be discovered if an integrative approach is applied, instead of relying solely on morphology (Padial *et al.,* 2010; Funk *et al.*, 2012; Pante *et al*, 2014). My results are in line with such studies, and emphasize that a comprehensive taxonomic evaluation through an integrative analysis of any herpetofauna should be initiated.

Before this study, 104 amphibians and 138 reptiles were reported for the region. However, including the candidate species identified in this study and those in way to be described, 119 amphibian and 156 reptile species are known to occur in EP, which means an increase in species richness of 14.4 % for amphibians and 13.0 % for reptiles diversity. The most diverse region is the lowlands, with 200 species, 93 of which are restricted to the lowlands. Among the highlands, the Serranías of Darién, de San Blas, and Pirre were the most diverse with 99, 104 and 103 species, respectively. The serranias of San Blas and Pirre harbour the major number of species restricted to each area (15 and 14 each).

The eastern Panamanian region is dominated by six principal geographical units, one in the lowland (Chucunaque-Tuira basin), and five in the highlands (the mountain ranges of San Blas, Maje, Darién, Pirre and Jingurudó-Sapo). In this region there are 251 amphibians and reptiles with a widespread distribution (196 reptiles and 55 amphibians), and 142 restrictect to each geographical unit (78 reptiles and 64 amphibians), most of them in lowlands (60 reptiles and 34 amphibians). Nevertheless,

the lowland species usually are widespread species with a broader distribution, northward and/or southward. Contrarily, few microendemic species are present in the mountain ranges, but frequently these species are macroendemics as well (e.g. *Diasporus* spp., *Pristimantis* spp., *Colostethus* spp.; see appendix VIII). On the peaks, there are isolated species and/or populations that have speciated *in situ*. One interesting example are the *Diasporus* spp. from the EP highlands (see appendix II). This allopatric speciation is closely related to the geological history of eastern Panama, and matches the rising and closure of the Panamanian isthmus (see appendix II, IV, and VII). The origin of the distributional patterns for several groups of the herpetofauna present in the region agrees well with the recent hypothesis of an earlier closure of the Panamanian isthmus (20–15 Ma), leaving the option that most of the species have colonized eastern Panama through a land bridge during the middle Miocene, and only few species have used other way of colonization before the land bridge connection (e.g., island hopping, rafting).

Substantial efforts are urgently needed to preserve the herpetofauna in eastern Panama, which is presently at alarming risk. Currently 29 species of amphibian and reptiles are in three of the following categories of the IUCN: near threatened, vulnerable or endangered. During this work I have found new and rare species with very restricted distribution, which have not been evaluated by the IUCN specialists, and are waiting for conservation assesments and/or more biological studies. The major threats that can cause disminution in the herpetofauna populations in EP are: Chytridiomycosis, fires, logging, and global warming. Chytridiomycosis is arriving and populations and/or species could decline or disappear in months or even weeks after the arrival of this disease in a population. The last published record for the fungus Bd was at Nuevo Vigia, Darién, in lowlands; this is an area with a variety of land uses, mostly crops and pasture, with very little primary forest around. There is evidence that the fungus is moving from the northwest of Panama to the south, but the way of dispersion is not clear until now. After finishing my PhD works, I was invited to participate in a monitoring project in the Serranía de Pierre, and I had the chance to visit places I had worked during my PhD field work. Unfortunately I experienced an event of amphibian mortality,

my team (Marcos Ponce, Maiden Miranda & Michele Quiroz) and I found dead animals. Later with the help of the Smithsonian institute and Roberto Ibañez, swaps from frogs from Pierre were analyzed, resulting in the bad news that the samples were positively tested for Bd. Now the Fondo Darien and the Grupo para la Educación y el Manejo Ambiental Sostenible (GEMAS) are looking for funds to support research, to evaluate the status of the amphibians in whole region.

Not enough, Darien is attractive for loggers, every summer legal and illegal loggers arrive in Darién looking for the best wood [e.g. Cocobolo (*Dalbergia retusa*), almendro (*Dipteryx oleifera*), balsamo (*Myroxylon balsamum*)]. Men equipped with chain saws, heavy duty equipment, ingress deep into the forest, sometimes farther than the reservation limits (e.g., Darien National Park). Along with the deforestation, in the same season the people use to burn their lands with the aim to “clean it” from bushes and grass. The smoke of the fires that the people make every year can be smelled in the highlands (pers. obs.) of the Serranía de Pirre. There are no studies evaluating these impacts over the amphibian fauna in the area. Although after this study I had done the evaluation for some species and groups of species (genera or species groups), an urgent monitoring program is needed in all the areas of EP.

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Appendix I

Declaration on the contributions of authors

to the publication: A new species of *Bolitoglossa* (Amphibia: Plethodontidae) from eastern Panama, with comments on other members of the *adspersa* species group from eastern Panama.
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- Abel Batista (AB). - Milan Vesely (MV), - Konrad Mebert (KM), - Gunther Köhler (GK)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 75% – field work (collecting and documenting specimens), molecular analysis
Coauthor MV: 10% – participated in the field trips.
Coauthor KM: 10% – participated in the field trips.
Coauthor GK: 5% – participated in the field trips and documenting specimens.

(3) to the creation of the data collection and figures

PhD candidate: 80% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor MV: 10% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor KM: 10% – provided photos

(4) to the analysis and interpretation of the data

PhD candidate: 70% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor GK: 10% – contributed to data analysis and interpretation
Coauthor MV: 10% – contributed to data analysis and interpretation
Coauthor KM: 10% – contributed to data analysis and interpretation

(5) to writing the manuscript

PhD candidate: 70%
Coauthor MV: 10%
Coauthor KM: 10%
Coauthor GK: 10%

Date/place: 13.04.2016 / Frankfurt am Main, Germany _

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

In 2011 and 2012, we conducted several expeditions to eastern Panama for the purpose of studying the herpetofauna of the region. We collected numerous specimens, among which were a considerable number of salamanders of the genus *Bolitoglossa*. We found all of the species expected for the area, except for *B. cuna*. After applying an integrative analysis, which included barcoding, morphology, and biogeography, we uncovered specimens that we were unable to assign to any known species. In the following study we revise the subgenus *Eladinea*, to which all of the eastern Panamanian species have been assigned, provide detailed information on these species, and describe a new species from a private reserve (Reserva Natural Privada Cerro Chucantí) in the Cordillera de Majé. © Anand Varma

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A new species of *Bolitoglossa* (Amphibia: Plethodontidae) from eastern Panama, with comments on other members of the *adspersa* species group from eastern Panama

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Abstract: We describe a new species of *Bolitoglossa* from Cerro Chucantí, Cordillera de Majé, Provincia de Darién, Panama. A phylogenetic analysis based on molecular data provides evidence for the assignment of the new taxon to the *Bolitoglossa adspersa* species group. The new species differs in color pattern and morphometrics from all other congeners found in eastern Panama. Additionally, we include comments on the other species of salamanders known to occur in the region.

Key Words: Barcoding, *Bolitoglossa biseriata, Bolitoglossa chucantiensis* sp. nov., Darién, *Eladinea,* morphology, phylogeny.

Resumen: Describimos una nueva especie de *Bolitoglossa* de Cerro Chucantí, Cordillera de Majé, Provincia de Darién, Panamá. Un análisis filogenético basado en datos moleculares proporciona evidencia de la asignación del nuevo taxón al grupo de especies *Bolitoglossa adspersa*. La nueva especie se diferencia en el patrón de color y morfometría de todos los otros congéneres que se encuentran en el este de Panamá. Además, incluimos comentarios sobre las otras especies de salamandras que son conocidas en la región.

Palabras Claves: *Bolitoglossa biseriata*, *Bolitoglossa chucantiensis* sp. nov., códigos de barras, Darién, *Eladinea,* morfología, filogenia.

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INTRODUCTION

The Neotropical salamanders (Plethodontidae) are dominated by the genus *Bolitoglossa*, globally the most diverse salamander genus (128 species) with a distribution extending from northeastern Mexico and across Central America, and into South America (AmphibiaWeb, 2014). With a surface area of only 75,416 km^2 Panama is home to 29 species of plethodontids (AmphibiaWeb, 2014); the highest diversity is in the western part of the country, where 17 species of *Bolitoglossa* are found, but only four of these species are known to occur in eastern Panama (Jaramillo et al., 2010). Two subgenera have been recognized for lower Central America and South America, *Bolitoglossa* (*sensu stricto*) and *Eladinea* (*sensu* Parra-Olea et al., 2004). *Eladinea* is comprised of the *adspersa*, *epimela*, *schizodactyla*, and *subpalmata* species groups. Of these, the distribution of only the *adspersa* species group extends far to the south, reaching central Bolivia (Frost, 2014).

The *adspersa* species group consists of 33 known species, of which four are known from eastern Panama (*B. biseriata*, *B. cuna*, *B. medemi*, and *B. taylori*); two of these are endemic to the Pirre (*B. taylori*) and San Blas (*B. cuna*) mountain ranges (Raffaëlli, 2007; Köhler, 2011; Acosta-Galvis and Gutiérrez-Lamus, 2012; Acevedo et al., 2013). Neotropical salamanders usually are difficult to identify due to their similarities in color pattern variation and morphology (Wake, 1970; Wake and Lynch, 1976; García-París et al., 2000; Wake et al., 2007; Fermin et al., 2012). The few species from eastern Panama, however, are easy to distinguish from each other; the only exceptions are *B. biseriata* Tanner, 1962 and *B. cuna* Wake et al., 1973, which are similar in overall appearance and only can be differentiated by their head width and the number of maxillary teeth (Wake et al., 1973). Wake et al. (1970) noted the occurrence of *B. phalarosoma* Wake and Brame, 1962 in the Jaqué-Imamadó divide of eastern Panama, but this record remains unsubstantiated because the authors did not indicate voucher specimens or provide other supportive data; other authors (e.g., Raffaëlli, 2007; Acosta-Galvis and Gutiérrez-Lamus, 2013) have stated that the identity of the salamanders referred to as *B. phalarosoma* and an undescribed species noted by Wake et al. (1970) needs to be confirmed. At this point, therefore, we do not consider *B. phalarosoma* as a member of the Panamanian herpetofauna. The remaining two species known to occur in eastern Panama are *B. taylori* Wake, et al., 1970 and *B. medemi* Brame and Wake, 1972.

During recent expeditions to the Darién, Jingurudó, Majé, Pirre, and San Blas mountain ranges, we collected specimens of three salamander species known to occur in eastern Panama (*B. biseriata, B. medemi,* and *B. taylori*), as well as a single adult specimen of an undescribed species of salamander from the Cordillera de Majé and a related salamander (an apparent juvenile) from the Cordillera de Jingurudó. We identified both of these specimens as members of the genus *Bolitoglossa* based on the following characteristics: absence of a sublingual fold, presence of well-developed hands and feet, presence of extensive digital webbing, and a count of 13 costal grooves between the limbs (Parra-Olea et al., 2004). Herein we describe the specimen from the Cordillera de Majé as a new species, and discuss its relationship to the juvenile specimen from the Cordillera de Jingurudó. We also provide data on molecular and morphological variation for the four species of *Bolitoglossa* found in the region.

MATERIALS AND METHODS

We conducted our fieldwork in the Darién, Jingurudó, Majé, Pirre, San Blas, and Sapo mountains of eastern Panama, (Fig. 1); see Appendix 2 for details on the collecting areas. We recorded georeferences by using a Garmin GPSmap 60CSx, in the WGS 1984 datum format and given in decimal degrees, and created the maps in ArcGIS 10 (ESRI, 2010). We euthanized the specimens collected with the euthanasia solution T61, fixed them with a preservative solution of 5ml formalin (36%) in 1L ethanol (94%), and subsequently stored them in ethanol (70%).

Morphology

We followed the methodology of Boza-Ovideo et al. (2012) for measuring the morphological characters of the holotype, and used a dial precision caliper under a dissecting microscope (Leica MZ 12) rounded to the nearest 0.1 mm. We examined the following characters: snout–vent length (standard length) from the tip of snout to the posterior end of vent (SVL), tail length from the posterior end of vent to the tip of the tail (TL), distance from the gular fold to the tip of the snout (SG), head width at the greatest width of the head (HW), head depth (height) at the posterior angle of the jaw (HD), eyelid length (EL), eyelid width (EW), distance from the anterior margin of the orbit to the tip of the

snout (ES), horizontal eye diameter (ED), intercanthal distance (IC), interorbital distance between the eyelids (IO), tip of the snout to the point where the forelimb articulates with the body (SF), internarial distance (IN), snout projection (SP), shoulder width (SW), snout to the anterior angle of the vent (SAV), axilla-groin distance (AX), hind limb length from the groin to the tip of longest digit (HLL), forelimb length from the axilla to the tip of the longest digit (FLL), hand width at the widest extent (HAW), foot width at the widest extent (FW), length of the 3rd toe (T3), and length of the 5th toe (T5); we counted premaxillary teeth (PMT), maxillary teeth (MT), and vomerine teeth (VT) by using a dissecting microscope; we provide MT and VT for left and right sides, respectively. We followed Brcko et al. (2013) for the following characters: costal folds between the adpressed limbs of the straightened specimen (limb interval, LI, as a measure of relative limb length), mental gland width (WMG), and mental gland length (LMG). We ran an exploratory analysis among the different morphological characters, since not enough useful morphological information was available (see Table 1) to conduct a statistical test among all the species; we present these diagnostics characters in graphs, showing only the range between the maximum and minimum values. We follow Köhler (2012) for the description of coloration in life and in ethanol. We used the keys to the genus *Bolitoglossa* in Savage (2002) and Köhler (2011) for a preliminary identification of the specimens collected. We obtained data for morphological characters and tooth counts for comparisons within the *adspersa* species group from the following original species descriptions and species revisions: Tanner (1962), Wake and Brame (1962), Brame and Wake (1972), Wake et al. (1973), Wake and Lynch (1976), Acosta-Galvis and Gutiérrez-Lamus (2012), and Acevedo et al. (2013). We derived osteological information on the holotype from radiographs. The capitalized colors and color codes (the latter in parentheses) are those of Köhler (2012). We followed Köhler (2012) for the terminology of markings used in the color descriptions.

Molecular Analysis

We extracted DNA from fresh liver tissue using the protocol of Ivanova et al. (2006). We amplified the mitochondrial 16S mtDNA using a Mastercycler pro S (Eppendorf, Hamburg, Germany), and performed the initial denaturation for 2 min at 94°C, which was followed by 40 cycles with denaturation for 35 s at 94°C, hybridization for 35 s at 48.5°C, and elongation for 60 s at 72°C; the final elongation proceeded for 7 min at 94°C. The reaction mix contained 1 µL DNA template, 2.5 µL Reaction Buffer ×10 (PeqGold), 4 µL 2.5 mM dNTPs, 0.4 µL (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µL H_2O, 1 µL 25 mM $MgCl_2$, and for 16S 1 µL per primer (containing 10 pmol, forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon); the COI gene was sequenced by the Southern China DNA Barcoding Center; because this project was developed along with a larger barcoding project for the amphibians and reptiles of eastern Panama, we only used the standardized genetic markers 16S and COI (Paz and Crawford, 2012), as financial resources were limited to these markers. We compared the molecular data of our specimens with the available sequences for the species of *Bolitoglossa* present in Central America and South America, which we obtained from recent publications (Boza-Oviedo et al., 2012; Hertz et al., 2013; Elmer et al., 2013; Acevedo et al., 2013). We aligned the obtained sequences with ClustalX (Thompson et al., 1997). We present a list of the specimens included in our genetic analysis, with the corresponding GenBank accession numbers, in Appendix 1. The final alignment of the 16S mtDNA comprised 32 sequences of 439 bp in length, of which 111 sites are variable and 74 are parsimony-informative (excluding outgroups). We computed Kimura 2–parameter (K2P) pairwise genetic distances for 16S and COI separately, using MEGA5 (Tamura et al. 2011). For phylogenetic inference we used 16S mtDNA (we did not include COI, because it was not available for most species of *Bolitoglossa*), and ran a Maximum Likelihood (ML) analysis with 1,000 bootstrap replicates using MEGA5, using the Kimura 2 parameter model. We used JModeltest 0.1.1 (Posada 2008) under the corrected Akaike Information Criterion (AICc) to select the substitution model for the Bayesian analysis. We determined TIM3+G as the best-fitting substitution model, and ran a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001) for 10,000,000 generations with four default chains, sampling every 100 generations and subsequently discarding 5% as burn-in. For the tree including all the species of *Bolitoglossa*, we used *Oedipina complex*, *Nototriton picadoi*, and *N. matama* as outgroups. For the tree including only the *adspersa* species group, we used *B. colonnea* and *B. schizodactyla* as outgroups.

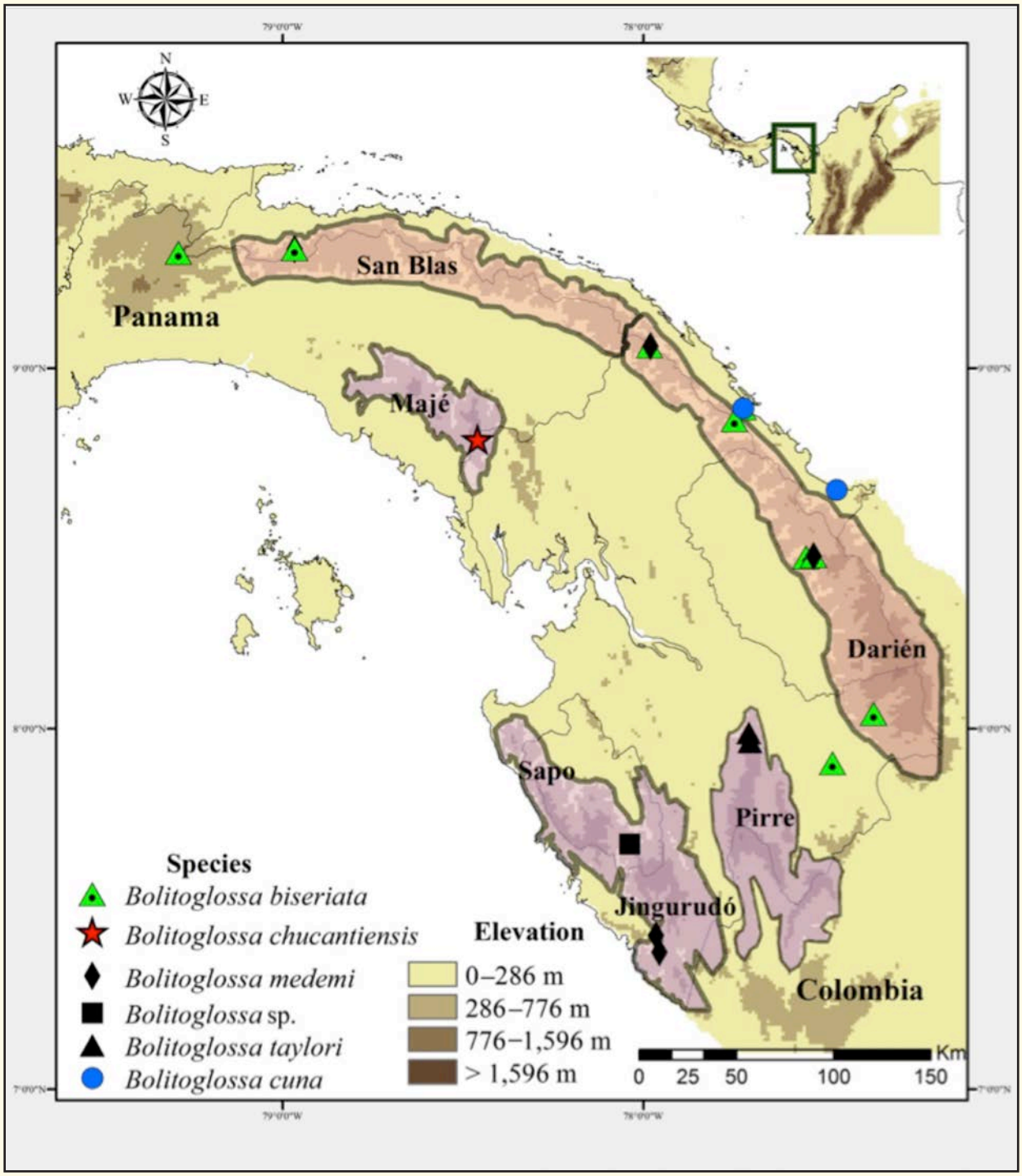

Fig. 1. Distribution of species of *Bolitoglossa* in eastern Panama. *Bolitoglossa* sp. (Black Square) pertains to specimen MHCH 2663 from the Cordillera de Jingurudó; shaded areas with borders represent the principal mountain ranges in eastern Panama, with elevations above 500 m a.s.l. (names of mountain ranges inside the shaded areas).

Table 1. Measurements and morphological proportions for the *Bolitoglossa* spp. from eastern Panama. We included data for *B. medemi, B. taylori,* and *B. cuna* taken from the species descriptions.

Characters (mm)	Species				
	***B. chucantiensis* (*n* = 1)**	***B. biseriata* (*n* = 6)**	***B. medemi* (*n* = 16)**	***B. taylori* (*n* = 7)**	***B. cuna* (*n* = 3)**
SVL	47	37–46 (40.88 ± 4.37)	33.7–58 (43.75 ± 6.38)	39.5–64.7 (48.01 ± 6.66)	46.6–55.7 (50.33 ± 4.76)
TL	55	34–44.5 (37.55 ± 4.84)	28.7–54 (39.56 ± 7.8)	40.9–73.3 (55.71 ± 8.74)	45–52.2 (48.6 ± 5.09)
SG	11.74	8.72–10.47 (9.45 ± 0.77)	10.07–12.44 (11.14 ± 1.04)	9.78–15.7 (11.53 ± 1.52)	10.4–11.6 (11.03 ± 0.6)
HW	7.63	5.44–6.89 (5.9 ± 0.68)	6–9.22 (7.46 ± 0.94)	6.6–10.1 (7.65 ± 0.91)	6.8–7.7 (7.3 ± 0.46)
HD	6.41	5.26–6.6 (5.66 ± 0.64)	6.22–8.52 (7.14 ± 0.89)	6.4–8.41 (7.45 ± 0.7)	—
AX	23.18	18.72–24.74 (20.52 ± 2.84)	16.7–27.6 (22.58 ± 3.24)	21.61–36.4 (25.64 ± 3.8)	28.4–32.5 (30.45 ± 2.9)
HLL	10	7.5–10 (8.38 ± 1.11)	8.9–13 (11.19 ± 1.38)	9–13.8 (10.91 ± 1.41)	—
FLL	11	7–10 (8.38 ± 1.25)	8–14 (11.08 ± 1.65)	9–12 (10.33 ± 0.87)	—
HAW	3.67	2.44–3.06 (2.68 ± 0.27)	2.44–3.82 (2.94 ± 0.55)	3.22–4.5 (3.8 ± 0.4)	—
FW	4.63	3.28–4.52 (3.71 ± 0.56)	3.1–5.3 (4.03 ± 0.64)	4.1–6.4 (4.92 ± 0.65)	4.4–5 (4.67 ± 0.31)
LI	13	13–13 (13 ± 0)	13–13 (13 ± 0)	13–13 (13 ± 0)	13–13 (13 ± 0)
PMT	2	1–2 (1.75 ± 0.5)	2–6 (4.17 ± 1.6)	1–5 (3 ± 1.22)	—
MT right	38	10–30 (20 ± 8.52)	20–25 (22.5 ± 2.43)	19–39 (28.33 ± 5.87)	—
MT left	37	8–27 (19.25 ± 8.18)	19–26 (21 ± 2.53)	18–39 (27.89 ± 6.13)	—
MT total	75	18–57 (39.25 ± 16.56)	28–59 (42.13 ± 7.37)	37–78 (58.94 ± 12.17)	66–77 (70.67 ± 5.69)
VT right	13	10–14 (11.5 ± 1.73)	14–23 (17 ± 3.69)	12–18 (14.78 ± 1.72)	—
VT left	12	9–22 (13.75 ± 6.18)	13–19 (15 ± 2.53)	12–20 (14.78 ± 2.28)	—
VT total	25	19–36 (25.25 ± 7.8)	22–50 (31.5 ± 7.38)	18–49 (30.72 ± 6.74)	33–38 (34.67 ± 2.89)
TL/SVL	1.17	0.79–1.01 (0.92 ± 0.09)	0.75–1.03 (0.89 ± 0.09)	1.04–1.28 (1.13 ± 0.08)	0.94–0.97 (0.95 ± 0.02)
HW/SVL	0.16	0.13–0.15 (0.14 ± 0.01)	0.16–0.19 (0.17 ± 0.01)	0.14–0.17 (0.16 ± 0.01)	0.14–0.15 (0.15 ± 0.01)
MT/SVL	1.60	0.49–1.33 (0.94 ± 0.35)	0.8–1.26 (0.97 ± 0.16)	0.8–1.59 (1.23 ± 0.22)	1.38–1.42 (1.41 ± 0.02)
VT/SVL	0.53	0.47–0.78 (0.61 ± 0.15)	0.48–1.06 (0.73 ± 0.2)	0.43–0.89 (0.64 ± 0.13)	0.59–0.82 (0.7 ± 0.11)
HAW/SVL	0.08	0.06–0.07 (0.07 ± 0.01)	0.06–0.07 (0.06 ± 0)	0.07–0.09 (0.08 ± 0.01)	—
FW/SVL	0.10	0.08–0.10 (0.09 ± 0.01)	0.08–0.11 (0.09 ± 0.01)	0.09–0.12 (0.1 ± 0.01)	0.09–0.1 (0.09 ± 0.01)
SG/SVL	0.25	0.22–0.24 (0.23 ± 0.01)	0.21–0.26 (0.24 ± 0.02)	0.20–0.27 (0.24 ± 0.02)	0.2–0.24 (0.22 ± 0.02)
VT/MT	0.33	0.35–1.06 (0.73 ± 0.29)	0.51–1.32 (0.77 ± 0.22)	0.38–0.78 (0.53 ± 0.11)	0.43–0.58 (0.49 ± 0.07)
SVL/HW	6.16	6.49–7.85 (6.95 ± 0.61)	5.24–6.44 (5.86 ± 0.34)	5.75–7.29 (6.27 ± 0.37)	6.58–7.23 (6.89 ± 0.33)

RESULTS

The salamander found on Cerro Chucantí in the Cordillera de Majé differs in color pattern and tooth counts from all its known congeners occurring in eastern Panama (Table 1) and South America. The new species showed a genetic distance to all species in the group of 7.5% (5.5–10.4%; $n = 16$) for 16S and 19.2% (5.6–28.8%; $n = 4$) for COI (only species from eastern Panama were included). In a Bayesian phylogenetic analysis based on all the taxa of *Eladinea* and *Bolitoglossa* available on GenBank (see Appendix 2), the new species clustered together with samples from the *adspersa* species group. In the Cordillera de Jingurudó, a distance of ca. 140 km from the locality of our new species, we found a very small salamander (SVL 17.9 mm) that we were unable to assign to any described species. Based on its disproportionally large head, the specimen apparently is a juvenile, and thus we excluded it from morphological comparisons with other species. According to the mtDNA results, the specimen is closely related to our new species, as it shows a K2P genetic distance of 1.4% for 16S and 5.6% for COI. Our mtDNA analysis shows that the most variable species was *B. biseriata*, with an average within-group genetic distance of 2.4% ($n = 5$) for 16S

(only one sample for COI). A specimen of *B. biseriata* from Río Púcuro (SMF 97139) was 3.5% divergent from one collected on the Cordillera de San Blas (SMF 97127) and another from the Río Tuquesa (MHCH 2659), but showed only 1.2% divergence from a second specimen from the Río Tuquesa (MHCH 2658). *Bolitoglossa biseriata* appears to be a polymorphic species or a complex with several cryptic species, possibly paralleling the high variation in dorsal color pattern (Fig. 8), in hand and foot shapes (Fig. 7 G–L), and genetic distances (Tables 2–3). Genetically, the other two species were less variable: *B. taylori* (0.2 % K2P) and *B. medemi* (1.7 % K2P).

Table 2. Mean genetic distances of 16S mtDNA among the *Bolitoglossa* samples used in the phylogenetic analysis (Fig. 2); numbers below diagonal are for K2P distances, and numbers above are standard error estimates (in percentage).

Species		K2P distance\SD (given in %)															
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
1	*B. adspersa*		1.1	1.1	1.4	1.4	1.1	1.5	1.0	1.1	1.1	1.5	1.1	1.4	1.1	1.0	1.4
2	*B. altamzonica*	4.4		1.3	1.3	1.6	1.2	1.6	1.1	1.2	1.0	1.4	0.9	1.6	1.2	1.1	1.5
3	*B. biseriata*	5.2	6.5		1.5	1.6	1.2	1.4	1.1	1.1	1.3	1.5	1.3	1.7	1.0	1.2	1.3
4	*B. chucantiensis* sp. nov.	6.8	5.8	8.2		1.6	1.3	1.5	1.3	1.5	1.5	1.7	1.2	1.6	1.5	1.5	1.5
5	*B. colonnea*	6.9	8.8	9.9	8.3		1.5	1.8	1.4	1.7	1.5	1.7	1.6	1.2	1.6	1.5	1.7
6	*B. leandrae*	4.2	4.7	5.9	5.5	7.8		1.4	0.7	1.1	1.1	1.5	1.1	1.6	1.3	1.1	1.3
7	*B. medemi*	8.3	9.0	8.7	8.5	11.5	7.7		1.5	1.6	1.5	1.7	1.5	1.7	1.5	1.6	1.5
8	*B. nicefori*	3.5	3.8	5.5	5.8	6.6	2.0	7.8		1.0	0.9	1.4	1.0	1.5	1.2	0.9	1.3
9	*B. orestes*	4.1	4.7	5.3	7.1	9.5	4.1	8.8	3.5		1.3	1.5	1.1	1.6	1.2	0.9	1.5
10	*B. palmata*	4.4	3.8	6.6	7.4	8.5	4.4	8.8	3.2	5.4		1.5	1.0	1.6	1.3	1.2	1.3
11	*B. paraensis*	8.2	7.0	8.9	10.4	10.1	7.6	10.5	6.6	7.5	7.6		1.3	1.9	1.6	1.4	1.5
12	*B. peruviana*	5.8	4.2	7.7	6.6	10.1	5.7	9.5	4.9	6.0	5.4	7.4		1.5	1.3	1.1	1.4
13	*B. schizodactyla*	7.2	8.5	10.2	8.7	5.4	9.2	10.6	7.6	9.2	8.9	11.9	9.8		1.6	1.5	1.7
14	*B. sima*	4.1	4.7	4.4	7.1	8.8	5.7	8.0	5.1	4.7	6.0	8.5	6.9	8.5		1.3	1.4
15	*B. tamaense*	3.5	4.4	5.8	7.8	7.5	4.1	9.0	2.6	2.9	4.8	6.3	5.8	7.9	5.4		1.3
16	*B. taylori*	6.4	7.0	7.0	8.2	9.4	5.9	8.2	5.4	7.0	5.7	8.3	8.0	8.9	6.3	5.7	

Table 3. Genetic distances of COI mtDNA gene among the *Bolitoglossa* samples used in the phylogenetic analysis (Fig. 2); numbers below diagonal are for K2P distances, and numbers above are standard error estimates (in percentage).

Species		K2P distance\SD (given in %)													
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	*B. chucantiensis* sp. nov. SMF 97141		1.1	2.3	2.4	2.1	2.2	2.2	2.2	2.2	2.1	2.1	2.1	2.1	1.8
2	*B.* sp. MHCH 2663	5.6		2.1	2.4	2.4	2.3	2.3	2.2	2.1	2.2	2.2	2.2	2.2	1.9
3	*B. biseriata* MHCH 2658	20.4	20.1		1.1	2.4	2.3	2.3	2.4	2.3	2.3	2.4	2.3	2.4	2.4
4	*B. biseriata* SMF 97139	21.6	23.5	6.3		2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.4
5	*B. medemi* MHCH 2660	18.5	20.9	22.1	21.5		0.8	0.8	2.2	2.2	2.2	2.2	2.2	2.2	2.4
6	*B. medemi* SMF 97131	19.0	19.8	21.3	21.8	3.6		0.0	2.3	2.2	2.3	2.3	2.3	2.3	2.5
7	*B. medemi* SMF 97130	19.0	19.8	21.3	21.8	3.6	0.0		2.3	2.2	2.3	2.3	2.3	2.3	2.5
8	*B. taylori* MHCH 2666	18.4	20.3	21.0	20.7	18.2	20.1	20.1		0.5	0.5	0.2	0.5	0.2	2.4
9	*B. taylori* MHCH 2665	17.9	18.6	19.6	20.5	17.9	19.3	19.3	1.4		0.2	0.5	0.2	0.5	2.3
10	*B. taylori* SMF 97136	17.6	18.9	19.9	20.7	17.6	19.6	19.6	1.2	0.2		0.4	0.0	0.4	2.3
11	*B. taylori* MHCH 2668	18.1	20.0	20.7	20.5	18.4	20.4	20.4	0.2	1.2	1.0		0.4	0.0	2.4
12	*B. taylori* SMF 97138	17.6	18.9	19.9	20.7	17.6	19.6	19.6	1.2	0.2	0.0	1.0		0.4	2.3
13	*B. taylori* SMF 97137	18.1	20.0	20.7	20.5	18.4	20.4	20.4	0.2	1.2	1.0	0.0	1.0		2.4
14	*B. colonnea* SMF 97128	15.9	16.7	21.5	23.0	21.8	21.8	21.8	20.1	19.0	19.2	19.8	19.2	19.8	

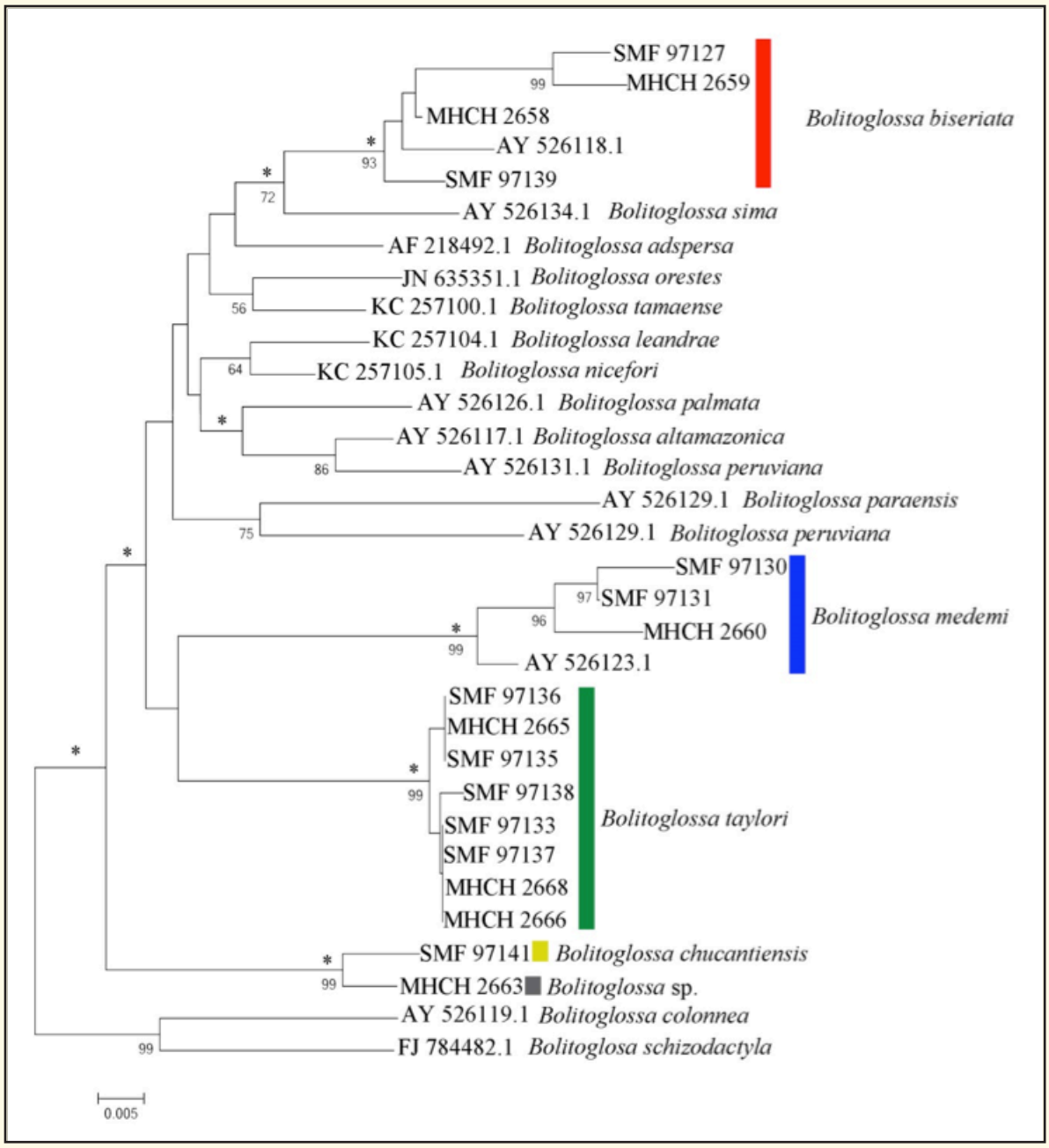

Fig. 2. Maximum likelihood consensus tree of 16S mtDNA, for the *Bolitoglossa adspersa* species group; specimen labels refer to the collection or museum number; scale bars refer to the number of substitutions per site. Maximum likelihood bootstrap values are shown below the branch, Bayesian posterior probabilities ≥ 0.95 are shown with an asterisk above the branch, ≤ probabilities are not shown. We used *B. colonnea* and *B. schizodactyla* as outgroups.

Our new species is most similar to *Bolitoglossa taylori* in body proportions, but the two species can be distinguished from one another by their color pattern and shape of their hands and feet (Fig. 7). We provide a formal description of the new species below.

Bolitoglossa chucantiensis sp. nov.

***Common names*:** Chucantí Salamander (English); Salamandra de Chucantí (Spanish). Fig. 3, Fig. 4, Fig. 7 A–B.

***Holotype*:** SMF 97141 (original field number AB 1063), an adult male from Panama, Provincia de Darién, Cordillaera de Majé, Distrito de Chepigana, Río Congo Arriba, Reserva Natural Privada Cerro Chucantí, (8.8034°N, 78.4601°W; 1,424 m elev.), collected 3 December 2013 by Abel Batista and Konrad Mebert.

***Diagnosis*:** A salamander of the genus *Bolitoglossa* and the subgenus *Eladinea* (as evidenced by the presence of a first caudal vertebra that bears unbranched transverse processes, and a prominent mental gland in males; Parra-Olea et al., 2004; Fig. 4). Our mtDNA sequence data show that this species is nested within the *adspersa* group. *Bolitoglossa chucantiensis* can be distinguished from all other eastern Panamanian and South American species of *Eladinea* by the presence of a higher number of maxillary teeth in proportion to the SVL, by the presence of completely webbed feet and hands, by its unique color pattern, and by a mtDNA genetic distance > 5.5%. *Bolitoglossa chucantiensis* is a small salamander (SVL 47 mm) with a slight indentation evident between the toe and fingertips, and in which only the longest toe and finger are pointed; the tail is relatively long (TL/SVL = 1.2); the dorsal coloration is brown, with patches of yellow speckling; and a prominent rounded hedonic mental gland and a postiliac glands are present in males. *Bolitoglossa chucantiensis* can be distinguished from other species in the *adspersa* group by the following characteristics (with contrasting features for *B. chucantiensis* in parentheses or brackets; see Table 1 for additional details): it differs from all the South American species (Acevedo et al., 2013; Brcko et al., 2013; García-Gutiérrez et al., 2013) by the presence of a higher number of maxillary teeth in males; and it differs from its closest relatives in South America by more than 5.5% of K2P pairwise genetic distance (*B. adspersa*, *B. altamazonica*, *B. leandrae*, *B. nicefori*, *B. orestes*, *B. palmata*, *B. paraensis*, *B. peruviana*, *B. sima*, *B. tamaense*, and *B. mucuyensis*). *Bolitoglossa chucantiensis* can be distinguished from the closely related *B. guaneae* by several characters. In *B. guaneae* the SVL is shorter (31.53–41.56 mm [vs. 47.3]), the 3rd finger and toe tips are pointed and protruding (vs. a slight indentation is evident between the toe and fingertips), its coloration is pale brown to gray in preservative, and in life the coloration is dark brown, sometimes mottled or streaked with white (vs. the dorsal ground color is dark brown and contains patches of yellow speckling); similarly, *B. chucantiensis* can be differentiated from *B. biseriata* because the head of this species is shorter (an HL/SVL ratio of 8.7–10.5 [vs. 11.74]), the hand and foot are narrower (vs. a broad hand and foot), the dorsal ground color is brown, cream, or red and sometimes is patterned with single small dark or yellow dots (vs. the dorsal ground color is dark brown, and contains patches of yellow speckling that are paler toward the head; Fig. 3); although males are not available for comparison, 33–38 vomerine teeth are present in female *B. cuna* (vs. 25), the head and hands of this species are narrower in relation to the body length, and fewer maxillary teeth are present (Fig. 6). Additionally, *B. cuna* is a lowland species (vs. a highland species) found near sea level (see Discussion), and the body is more slender than that of *B. chucantiensis*. *Bolitoglossa medemi* is a species with 28–59 maxillary teeth (vs. 75), generally contains fewer teeth in relation to the body length (0.8–1.3 vs. 1.6), and the head is broader. *Bolitoglossa taylori* is a species with extensive webbing (vs. completely webbed), and its dorsal coloration usually consists of small or large blotches (vs. patches of yellow speckling).

***Description of holotype*:** Male, SVL 47.3 mm; 75 MT, 2 PMT, the PMT do not pierce the lip, 23 VT; trunk length 23.18 mm between the levels of the axilla and groin; the head is moderately broad with an SVL/HW ratio of 6.2; the head is distinctly wider than the neck; the distance across shoulders is 6.0 mm; the horizontal orbit diameter is 75% of the snout length; the eyes protrude beyond the lateral margins of the head and are visible in dorsal view; the nasolabial protuberances are evident and developed; the snout is truncate in dorsal view and slightly rounded to truncate in lateral view; the canthus rostralis is indistinct; the nostrils are small and located near the tip of the snout; a mental gland is present, oval, WMG 3.1 mm, LMG 2.6 mm; the body is cylindrical, with 13 costal grooves; the hands and feet are moderately broad (HAW = 7%, HFW = 10% of SVL), the feet are completely webbed, subterminal pads are evident on digits 2–3–4 on the foot and 2–3 on the hand; the fingers, in order of decreasing length, are III–II–IV–I; the toes are III–IV–II–V–I (Figs. 3F, 7 A–B); the tail is long, 55.0 mm and exceeding the standard length 1.17 of SVL; the hind limb is 10.0 mm, the forelimb is 11.0 mm; the maxillary teeth are relatively numerous, MT/SVL 1.6 times, and extend to about the level of the end of the eye; the vomerine teeth border the anterior orbit shape in the roof of mouth; paravomerine teeth are present and numerous; the tongue is enlarged and rounded in its anterior tip, with a concavity in the center; the prevomer clearly projects from the level of the palate, bearing

vomerine teeth in long, slightly arched series, and extends laterally almost to the center of the roof of the mouth. We include other measurements and counts in Table 1.

Coloration of the holotype in life (Fig. 3)**:** The color pattern of the holotype was recorded the day after capture (at 1730 h), as follows: the upper dorsum is Crimson (62); the flanks, tail, and limbs are Maroon (39), strongly speckled with Straw Yellow (53); the iris is Light Yellow Ocher (13) with Hazel (26) reticulations, and the eyelids are Straw Yellow (53); the venter is translucent with Warm Sepia (40) pigment, with the throat Straw Yellow (53); the ventral surfaces of the limbs are speckled with Straw Yellow (53).

***Coloration of the holotype in alcohol*:** The color pattern of the holotype was recorded after the specimen spent about two years in ethanol (70%), as follows: the upper dorsum is Verona Brown (37); the head region is Vandyke Brown (282); the flanks and limbs are Grayish Olive (274), speckled with Glaucous (272); the tail is Dusky Brown (285); the eyelids are Brownish Olive (292); and the venter is Smoke Gray (267).

Osteology (Fig.4)**:** The vertebral column consists of one atlas, 14 trunk vertebrae, one sacral, two caudosacral vertebrae, and 39 caudal vertebrae, with the first caudal process directed frontally; ribs are present on all the trunk vertebrae except for the last one, and are directed forwardly; the skull is well formed, and the visible structures of the head are the following: premaxilla, maxilla, nasals, vomer bodies, orbitosphenoids, and parasphenoid; the otic capsules are well developed and attached to the squamosals, the quadrates are barely visible and connected to the squamosals; the limbs are well developed; the digits are visible on all the limbs; the phalangeal formula for the hand is 1–2–3–2, and for the foot 1–2–3–3–2; and the metacarpal IV and metatarsal V are broader than the others (Fig. 4).

***Habitat and natural history notes*:** *Bolitoglossa chucantiensis* is known only from the type locality in the eastern Panamanian montane forest (*sensu* Fund and Hogan, 2012; Fig. 1) comprised of trees attaining heights of about 15 m, with their branches densely covered with bromeliads and other epiphytes (e.g., orchids, Loranthaceae), and with palms, vines, and bromeliads dominating the understory. The holotype was found at 2200 h, active on a palm leaf about 1 m above the ground, along a trail 200 m southwest from the ridge top. A drizzling rain had fallen between 1830 and 2100 h, but the conditions had turned calm, with only a slight breeze. Other species of amphibians and reptiles observed in the area that day were: *Oedipina* aff. *complex*, *Diasporus* sp., *Colostethus* aff. *pratti, Pristimantis moro*, *P. caryophyllaceus*, *P. cruentus*, *Espadarana prosoblepon*, *Silverstoneia* sp., *Ptychoglossus festae*, *Dendrophidion percarinatum*, and *Geophis* sp.

***Etymology*:** The species name is derived from the name of the mountain (Cerro Chucantí) where the holotype was found, with the Latin suffix *-ensis* indicating a place or locality. Chucantí is the highest point in the Cordillera de Majé, with an elevation of 1,439 m, and is part of the Chucantí Private Cloudforest Reserve, a protected area owned by Guido Berguido.

DISCUSSION

Bolitoglossa chucantiensis is a member of the *adspersa* group of the subgenus *Eladinea***,** the only group of the subgenus distributed in eastern Panama and northern South America. The new species can be distinguished from other members of the group by external features (TL/SVL and MT/SVL ratios, a brown dorsal coloration containing patches of yellow speckling) and by its relatively large genetic distance from other species in the group (> 5.5% in 16S and > 16% in COI). Herein we combined morphology and molecular genetics to compare the species of *Bolitoglossa* occurring in eastern Panama. Within the *adspersa* group, several examples of distinct species exhibit a smaller sequence divergence. For example, we analyzed sequences of *B. nicefori*, *B. tamaense*, and *B. leandrae* from Colombia and found a genetic divergence of 2.6% of p and K2P for 16S between the first two species, and only 2.0% of p and K2P for 16S between *B. nicefori* and *B. leandrae*. The mean genetic divergence among these species is 3.0% of K2P (Acevedo et al., 2013). While *B. tamaense* can be distinguished from *B. nicefori* by the different amount of webbing, *B. leandrae* is morphologically similar to *B. tamaense* but shows high maxillary tooth counts (29–30 vs. 35–40) and a distinct elevational distribution (Acosta-Galvis and Gutiérrez-Lamus, 2012; Acevedo et al., 2013). Nevertheless, an even lower genetic divergence (0.5% K2P) has been found for some morphologically well-defined sister species of the genus *Bolitoglossa* (Parra-Olea et al., 2004). Finally, the minimum threshold of 3% of pairwise genetic divergence applied in barcoding analyses of 16S mtDNA of amphibians (Vieites et al., 2009;

Crawford et al., 2010; Jansen et al., 2011) is not consistent to delineate among *Bolitoglossa* spp., as the morphological differences noted above justify the use of an even lower %-divergence to recognize separate species.

Although *B. chucantiensis* can be well differentiated from other species of *Bolitoglossa*, we refrain from assigning our second specimen from the Cordillera de Jingurudó to any recognized taxon. Initially, we treated it as conspecific with *B. chucantiensis* due to their low pairwise genetic distance (1.4% K2P). The recently described species, *B. guaneae* Acosta-Galvis and Gutiérrez-Lamus, 2012, from the Cordillera Oriental of the Colombian Andes, however, appears to exhibit a similar phenotype to our Jingurudó specimen. Unfortunately, neither molecular data nor tissue samples of *B. guaneae* were available for a genetic comparison. Thus, a taxonomic assignment of our Jingurudó specimen must await a proper analysis that includes more Colombian material and/or more specimens from the Cordillera de Jingurudó.

Among the other taxa of *Bolitoglossa* we collected in eastern Panama, we detected exceptionally high variation in morphological and molecular characters in specimens of *B. biseriata*, even within geographically close metapopulations or from the same locality. As already mentioned, the sample from Río Púcuro (SMF 97139) is unusual by showing a genetic distance of 3.5% K2P to samples from Río Tuquesa and San Blas (SMF 97127, MHCH 2659), which exceed the suggested threshold of genetic distance for species level within the genus (> 3.0%). The Río Púcuro specimen also possesses fully webbed feet (Fig. 7 K–L), which is strikingly different from other specimens of *B. biseriata* sampled in the area. In the context of a sample size too low to reveal the full morphological variation of foot webbing, we provisionally consider this an anomaly. The sample was collected relatively close to the type locality of *B. biseriata* (19.7 km NE) and its genetic distance from other conspecifics (MHCH 2658, S13236) with typical *biseriata* webbing on the feet (Fig. 7 I–J) is much lower (1.7 % of K2P). These facts, together with other morphological similarities, allocate the Río Púcuro specimen to *B. biseriata*. Another case of high variation is evident among three *B. biseriata* collected within the Cordillera de San Blas: almost twice as many maxillary teeth are present in SMF 97641 and SMF 97129 that in SMF 97127, found at the same locality (57–61 vs. 36), whereas the typical counts for other specimens collected in Panama range from 18 to 46. Furthermore, molecular distances also are quite variable, as the specimen with high tooth counts, SMF 97641, exhibits 3.1% K2P distance to the syntopic SMF 97127, which has a low tooth count, but only 1.0% K2P distance to MHCH 2658 (an adult male from Río Tuquesa) whose maxillary tooth count is even lower (18 maxillary teeth) and was found at a distance of about 82 km to the northwest. Although SMF 97641 was not included in the phylogenetic analysis due to an incomplete sequence of 16S mtDNA (only 192 bp, no sequence was obtained for COI), its morphological appearance corresponds well to that of other *B. biseriata* from the region (Fig. 8). Due to these incongruences in geographic pattern of molecular and morphological data, we treat *B. biseriata* as a species complex harboring deep conspecific lineages (Vieites et al., 2009; Padial et al., 2010). Therefore, we suggest treating it as a species complex until a larger sample size allows for a more detailed comparative analysis to better understand the extent of morphological and genetic variation. In the view of these data, the validity of *B. cuna* needs to be evaluated. Although Wake et al. (1973) state that the head of *B. cuna* is narrower than that of *B. biseriata,* we did not find any differences in HW/SVL ratio between these species (Fig. 6). The only character that might be useful to differentiate between them is maxillary tooth count (see key below). Since *B. cuna* is known only for the vicinity of the type locality (Solis et al. 2004), molecular data from this locality still are needed to clarify its status in relation to the *B. biseriata* complex, whose members are similar in overall appearance.

Bolitoglossa taylori was the least genetically variable species (average genetic distance within species = 0.2% K2P), but it showed considerable variation in coloration and skin texture (Fig. 10). This variation was documented by Wake et al. (1970: 9), who stated that the dorsal surfaces of *B. taylori* can be "light grayish brown, light brown, yellowish brown, orange-brown, or rich red-brown sometimes with extensive dark brown or black dorsal markings, and often with a dark brown lateral stripe". Such variability also has been described for other members of the genus (e.g., Vial, 1966; García-París et al., 2000, 2008). We summarize the morphological variation for the species reported from eastern Panama in the key below, and emphasize the importance of conserving Panama's primary rainforests to enable the survival and long-term persistence of these beautiful and valued amphibians.

Fig. 3. *Bolitoglossa chucantiensis* holotype. A–C = head and dorsal color pattern; D = ventral coloration; E = left foot; F = right hand; and G–H = internal parts of mouth.

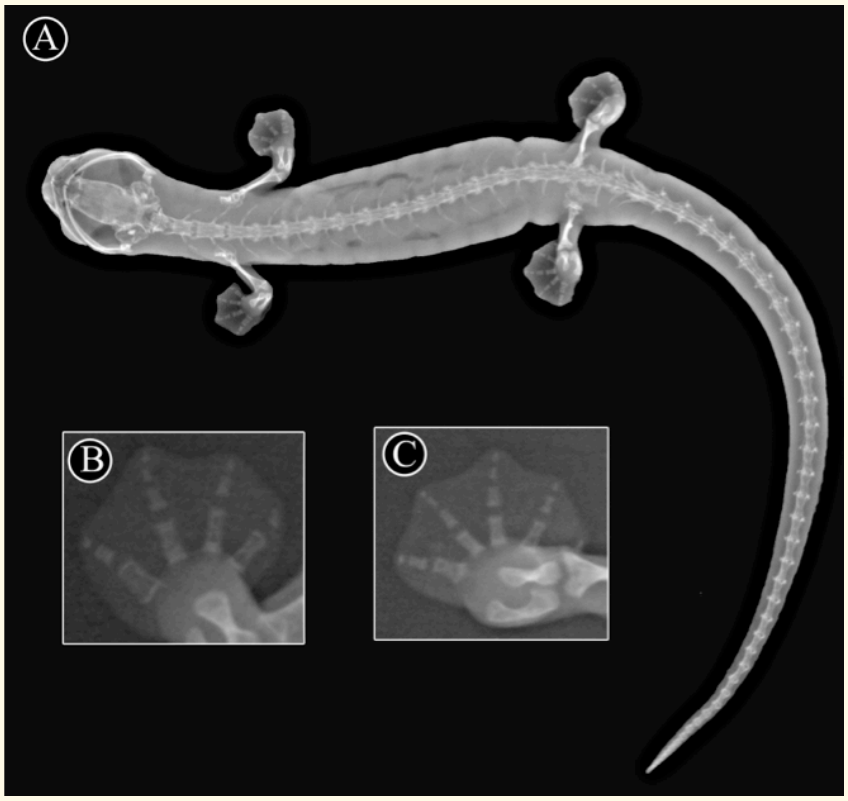

Fig. 4. X–ray images of the holotype of *B. chucantiensis*; A = entire body; B = left hand; and C = left foot.

Fig. 5. *Bolitoglossa* sp. (MHCH 2663), from the Cordillera de Jingurudó.

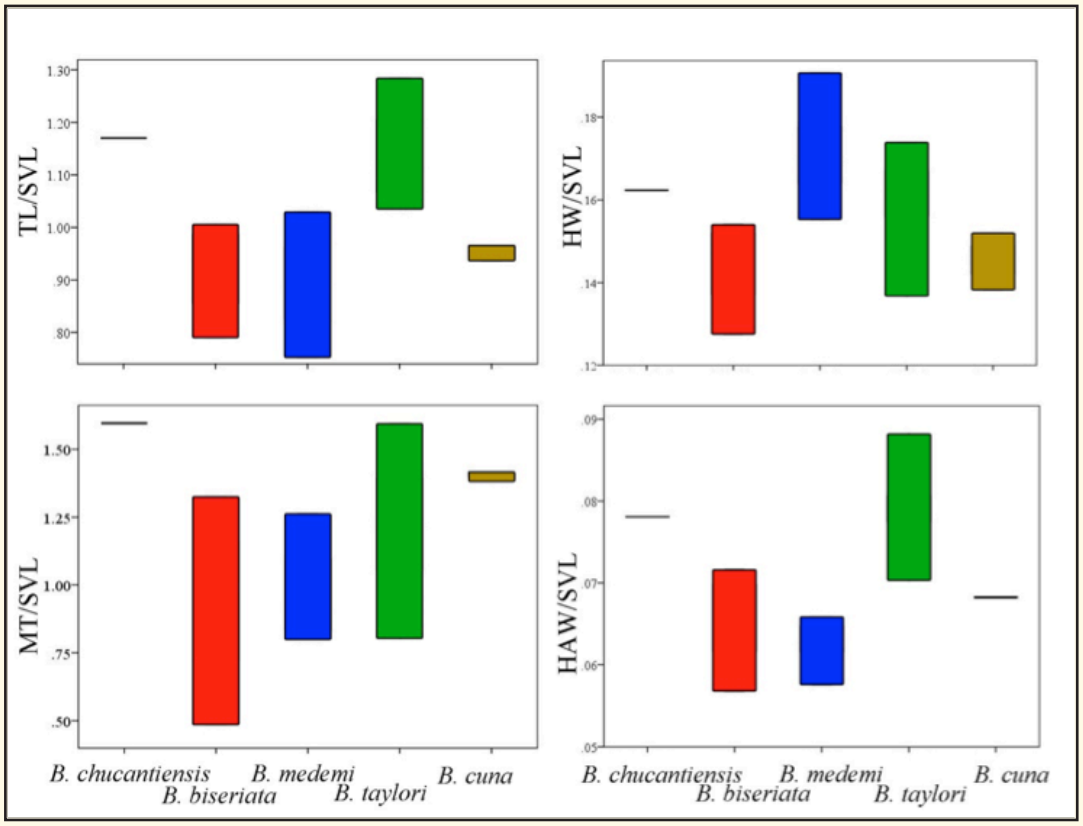

Fig. 6. Morphological diagnostic features showing differences among the species of *Bolitoglossa* from eastern Panama; data for *B. cuna* was taken from the original description (Wake et al., 1973); boxes represent the range of proportions (maximum and minimum values).

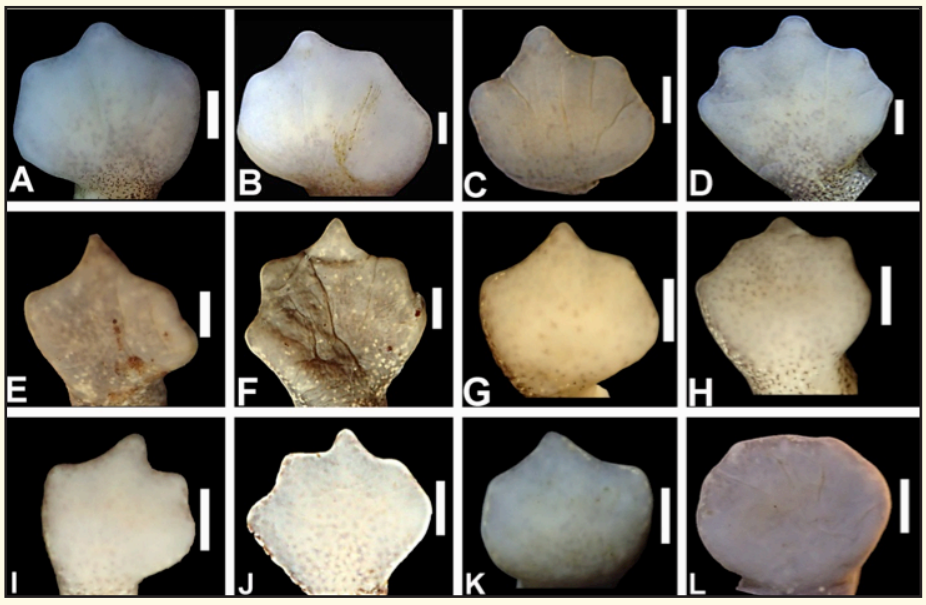

Fig. 7. Shape of the hands and feet in species of *Bolitoglossa* from eastern Panama. A–B = *B. chucantiensis* (holotype) A = left hand, B = right foot; C–D = *B. taylori* (MHCH 2667), C = right hand, D = left foot; E–F = *B. medemi* (MHCH 2662), E = right hand, F = left foot; G–H = *B. biseriata* (SMF 97129, San Blas), G = right hand, H = left foot; I–J = *B. biseriata* (MHCH 2658, Río Tuquesa), I = right hand, J = left foot; and K–L = *B. biseriata* (SMF 97139, Púcuro), K = left hand, L = right foot.

Fig. 8. Color variation in *B. biseriata*. A = Burbayar field station; B = San Blas ridge (SMF 97129); C = San Blas ridge (SMF 97127); D = San Blas ridge (SMF 97641); E = Río Pucuro (SMF 97139); F = Río Tuquesa (MHCH 2658); G = Donoso, Colón; and H = Río Tuquesa (MHCH 2659).

Fig. 9. Color variation in *B. medemi*. A–B = San Blas ridge (SMF 97130); C–D = San Blas ridge (SMF 97131); and E–F = Río Tuquesa (SMF 97132).

Fig. 10. Color variation in *B. taylori*. A = SMF 97136; B = SMF 97135; C = SMF 97133; D = SMF 97138; E = MHCH 2666; F = MHCH2669; G = left foot (SMF 97137); and H = left hand (SMF 97137).

Key to the genus *Bolitoglossa* in eastern Panama

1a. Tail length equal to SVL or distinctly longer, (TL/SVL = 1.04–1.28); tail cylindrical; dorsum mainly red or dark brown, uniform or with cream to yellow blotches or patches with speckles 2

1b. Tail shorter than SVL (TL/SVL = 0.75–1.03); tail cylindrical or laterally compressed; dorsal coloration uniform, bicolored, black, red, or brown 3

2a. Dorsum brown with large patches with yellowish speckling; feet completely webbed, with a slight indentation between tips of toes and fingers . . . . . *Bolitoglossa chucantiensis*

2b. Dorsum brown, black, cream, or red, without patches of yellow speckling; extensive webbing on feet, evident indentation between tips of toes and fingers . . . . . *Bolitoglossa taylori*

3a. Tail laterally compressed, tip of 3rd toe and 3rd finger sharply pointed, abruptly protruding the hand and foot . . . . . *Bolitoglossa medemi*

3b. Tail cylindrical; tip of 3rd toe and 3rd finger not abruptly protruding the hand or foot 4

4a. More than 66 maxillary teet . . . . . *Bolitoglossa cuna*

4b. Fewer than 61 maxillary teeth . . . . . *Bolitoglossa biseriata*

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Appendix 1. Supplementary table of specimens used in phylogenetic analyses, with their corresponding GenBank accession number of 16S and COI mtDNA.

Species	16S	COI	Country
B. adspersa MVZ 158485	AF218492 (4)		Colombia: Cundinamarca
B. alberchi MVZ 194293	AF218480 (4)		Mexico: Chiapas
B. alberchi MVZ 163959	AF218478 (4)		Mexico: Veracruz
B. alvaradoi MVZ 215735	AY526157		Costa Rica: Heredia: El Plástico
B. aureogularis UCR 19858	JQ899151		Costa Rica
B. aureogularis UCR 19859	JQ899152		Costa Rica
B. aureogularis UCR 19892	JQ899153		Costa Rica
B. aureogularis UCR 19893	JQ899154		Costa Rica
B. biseriata S13236	AY526118		Panamá: Nusagandi: Kuna Yala
B. bramei UCR 20483	JQ899159		Costa Rica
B. bramei UCR 20484	JQ899160		Costa Rica
B. bramei UCR 20851	JQ899142		Costa Rica
B. bramei MVZ 225893	—		
B. carri USNM 523276	AY526138		Honduras: Cerro Cantagallo
B. carri USNM 523277	AY526139		Honduras: Cerro Cantagallo
B. celaque SMF 78087	AY526140		Honduras: Lempira
B. celaque SMF 78088	AY526141		Honduras: Lempira
B. cerroensis MVZ 233516	AF199233		

B. cerroensis DBW5123	AF199233 (2)	Costa Rica: San José: Cuericí, 5 km E Villa Mills
B. colonnea No voucher	AY526119	Panamá: Chiriquí: Reserva Forestal Fortuna
B. colonnea SMF 94461	JX434645	Panama
B. colonnea SMF 94460	JX434644	Panama
B. colonnea CHP 6526	FJ766578	Panama
B. compacta UCR 20532	JQ899163	Costa Rica
B. conanti MVZ 225843	AY526142	Honduras: Cortés: El Cusuco
B. decora USNM 497533	AY526143	Honduras: Olancho: Monte Escondido
B. diaphora MVZ 225847	AY526144	Honduras: Cortés: El Cusuco
B. dofleini MVZ 161607	AF218497 (4)	Guatemala: Alta Verapaz
B. dunni USNM 523280	AY526145	Honduras: Cortés: San Pedro Sula
B. engelhardti MVZ 167789	AF218496 (4)	Guatemala: San Marcos
B. epimela MVZ 181260	AY526120	
B. epimela MVZ 181260	AY526120	Costa Rica: Cartago: Turrialba
B. epimela MVZ 181260	AY526120	
B. flavimembris MVZ 143698	AY526146	Guatemala: San Marcos
B. flaviventris MVZ 194288	AF218489 (4)	Mexico: Chiapas
B. franklini MVZ 185991	AY526147	Mexico: Chiapas: Volcán Tacaná
B. gomezi UCR 20399	JQ899155	Costa Rica
B. gomezi UCR 20413	—	Costa Rica
B. gomezi UCR 20414	JQ899156	Costa Rica
B. gomezi UCR 20415	JQ899157	Costa Rica
B. gomezi UCR 20417	JQ899158	Costa Rica
B. gomezi UCR 20843	JQ899140	Costa Rica
B. gomezi UCR 20844	JQ899147	Costa Rica
B. gomezi UCR 20845	JQ899148	Costa Rica
B. gomezi UCR 20846	JQ899149	Costa Rica
B. gomezi UCR 20847	—	Costa Rica
B. gomezi UCR 20848	JQ899139	Costa Rica
B. gomezi UCR 20849	JQ899141	Costa Rica
B. gomezi UCR 20850	JQ899146	Costa Rica
B. gracilis MVZ 229171	AY526122	Costa Rica
B. gracilis MVZ 229170	AY526121	Costa Rica: Cartago: Reserva Tapantí
B. gracilis MVZ 229171	AY526122	Costa Rica: Cartago: Reserva Tapantí
B. hartwegi MVZ (DBW945)	AF218494 (4)	Mexico: Chiapas
B. hermosa MVZ 163690	AF416686 (5)	Mexico: Guerrero: 11.3 mi NE Atoyac
B. jugivagans SMF 94467	KC428634	Panama
B. kamuk UCR 20852	JQ899143	Costa Rica
B. kamuk UCR 20853	JQ899144	Costa Rica
B. kamuk UCR 20854	JQ899145	Costa Rica
B. lignicolor SMF 91996	JX434643.1	Panama
B. lignicolor SMF 89803	JX434642.1	Panama
B. lignicolor SMF 91994	JX434641.1	Panama
B. lignicolor SMF 91997	JX434640.1	Panama
B. lignicolor SMF 94459	JX434639.1	Panama
B. lignicolor AH 431	JX434638.1	Panama
B. lincolni MVZ 143564	AY526148	Guatemala: San Marcos

B. longissima USNM 523285	AY526149	Honduras: Olancho: Pico La Picucha
B. macrinii GP 384	AF416689 (5)	Mexico: Oaxaca: San Gabriel Mixtepec
B. marmorea MVZ 210286	AF218493	
B. marmorea MVZ 210286	AF218493 (4)	Panamá: Chiriquí
B. medemi S13237	AY526123	Panamá: Nusagandi: Kuna Yala
B. mexicana MVZ 176838	GU725457	
B. mexicana MVZ 191635	AF177588 (4)	Belize: Toledo: Blue Creek
B. mexicana USNM 343451	AF218468 (4)	Honduras: Atlántida
B. mexicana (photo voucher Bo71)	AF218470 (4)	Mexico: Chiapas
B. minutula MVZ 225870	AY526124	
B. minutula MVZ 225870	AY526124	Costa Rica: Puntarenas: Las Tablas, Cerro Pando
B. mombachoensis SMF 78718	AY133488 (6)	Nicaragua: Granada
B. mombachoensis SMF 78725	AY133489 (6)	Nicaragua: Granada
B. morio MVZ 143677	AF218495 (4)	Guatemala: San Marcos
B. morio MVZ 232970	AY526150	Guatemala: San Marcos
B. nigrescens UCR 20539	JQ899164	Costa Rica
B. oaxacensis IBH 13374	AF416690 (5)	Mexico: Oaxaca: 40 km N San Gabriel Mixtepec
B. occidentalis MVZ 194254	AY526115	Mexico: Chiapas: Berriozabal
B. odonnelli MVZ 229068	AF218476 (4)	Honduras: Olancho
B. palmata KU 217422	AY526125	Ecuador: Napo: Cordillera de Guacamayos a 31 km de Baeza
B. palmata KU 217423	AY526126	Ecuador: Napo: Cordillera de Guacamayos a 31 km de Baeza
B. paraensis INPA 3098	AY526127	Brazil: Amazonas: Rio Juruá
B. paraensis LSUMZ H-3086	AY526128	Brazil: Amazonas: Rio Ituxi at the Madeireira Scheffer
B. paraensis LSUMZ H-13735	AY526129	Brazil: Acre: 5 km N Porto Walter
B. peruviana LSUMZ H-12838	AY526130	Ecuador: Sucumbios: Estación Científica University Católica, Cuyabeno
B. peruviana KU 217421	AY526131	Ecuador: Napo: Jatún Sacha
B. pesrubra UCR 12068	AY526132	Costa Rica
B. pesrubra MVZ 210360	EU448105	Costa Rica
B. pesrubra MVZ 190923	EU448104	Costa Rica
B. pesrubra MVZ210361	—	Costa Rica
B. platydactyla GP 108	AF218487 (4)	Mexico: Veracruz
B. platydactyla GP 587	AY133487 (6)	Mexico: Veracruz
B. porrasorum MVZ 225852	AY526151	Honduras: Atlántida: Cerro Búfalo
B. riletti MVZ 194328	AF416696 (5)	Mexico: Oaxaca: 20.9 km NE Putla
B. robinsoni UCR 20489	JQ899161	Costa Rica
B. robusta MVZ190830	EU448109	Costa Rica
B. rostrata MVZ 163683	AY526152	Guatemala: Huehuetenango
B. rostrata MVZ 163930	AY526153	Guatemala: Huehuetenango
B. rufescens MVZ 194333	AY526116	Belize: Toledo: Blue Creek National Park
B. schizodactyla No voucher	AY526133	Panamá: Coclé: Parque Nacional El Copé
B. sima MVZ 163575	AY526134	Colombia: Valle del Cauca
B. sombra CH 7478	JQ899165	Panama
B. sombra UCR 225871	AY526136	Costa Rica
B. soyoorum MVZ 190847	EU448108	Costa Rica
B. sp. 1 MVZ 167947	AY526135	Colombia: Cundinamarca: El Soche

B. sp. 2 MVZ 225871	AY526136		Costa Rica: Puntarenas
B. sp. 3 MVZ 233028	AY526154		El Salvador: Santa Ana: Metapán
B. sp. 3 MVZ 200535	AY526155		El Salvador: Santa Ana: Metapán
B. sp. 4 UCR 12066	AY526137		Costa Rica: Cartago: Macho Gaff
B. splendida UCR 19835	JQ899150		Costa Rica
B. striatula MVZ 181280	AF218488 (4)		Costa Rica: Cartago
B. subpalmata MVZ 194828	AF212091		Costa Rica
B. subpalmata MVZ 229172	AF416697 (5)		Costa Rica: Puntarenas: Monteverde Cloud Forest Preserve
B. synoria SMF 78084	AY526156		Honduras: Ocotepeque: Cerro El Pital
B. tica UCR 12065	AY526137		Costa Rica
B. tica UCR 20514	JQ899162		Costa Rica
B. tica MPG 2008	EU448106		Costa Rica
B. yucatana MVZ 197507	AF218485 (4)		Mexico: Quintana Roo
B. zapoteca IBH 13375	AF416698 (5)		Mexico: Oaxaca: Santa María Ecatepec
B. zapoteca IBH 13376	AF416699 (5)		Mexico: Oaxaca: Santa María Ecatepec
B. altamazonica KU 222111	AY526117		Perú: Loreto: 1.5 km N Teniente López
Nototriton matama UCR 20215	JQ899166		Costa Rica
Nototriton picadoi MVZ 225899	AF199144		Costa Rica
Oedipina alleni MVZ 190857	AF199207		Costa Rica
B. nicefori Clone 001	KC257105.1		Colombia
B. leandrae PAT 240	KC257104.1		Colombia
B. leandrae PAT236	KC257103.1		Colombia
B. leandrae PAT 237	KC257102.1		Colombia
B. tamaense PAT 431	KC257101.1		Colombia
B. tamaense PAT 451	KC257100.1		Colombia
B. tamaense PAT 363	KC257099.1		Colombia
B. tamaense PAT 387	KC257098.1		Colombia
B. biseriata MHCH 2658	KM527322	KM527307	Chiriquí, Panama
B. biseriata MHCH 2668	KM527334	KM527317	Darién Panama
B. chucantiensis **sp. nov.** MHCH 2665	KM527324	KM527308	Darién Panama
B. colonnea SMF 97136	KM527326	KM527310	Darién Panama
B. medemi MHCH 2660	KM527325	KM527309	Darién Panama
B. medemi SMF 97131	KM527327	KM527311	Darién Panama
B. medemi SMF 97133	KM527328	KM527312	Darién Panama
Bolitoglossa sp. SMF 97138	KM527329	KM527313	Darién Panama
B. taylori MHCH 2663	KM527331	KM527314	Darién Panama
B. taylori MHCH 2664	KM527333	KM527316	Darién Panama
B. taylori MHCH 2666	KM527340	KM527321	Darién Panama
B. taylori SMF 97128	KM527336	KM527319	Darién Panama
B. taylori SMF 97130	KM527337	KM527320	Darién Panama
B. taylori SMF 97139	KM527332	KM527315	Darién Panama
B. taylori SMF 97141	KM527335	KM527318	Darién Panama
B. biseriata SMF 97135	KM527339		Darién Panama
B. taylori SMF 97140	KM527323		Darién Panama
B. schyzodactyla SMF 97127	KM527338		Darién Panama
B. biseriata MHCH 2659	KM527330		Darién Panama

Appendix 2. Supplementary table of specimens and their respective localities, used for morphological comparisons.

Voucher	Species	Locality	Country	Coordinates		Elev. (m)
				N	W	
SMF97127	*B. biseriata*	Serranía de San Blas	Panama	9.0602	–77.9827	463
SMF97641	*B. biseriata*	Serranía de San Blas	Panama	9.0602	–77.9827	463
MHCH2663	*B.* sp.	Filo entre río Sambú and quebrada Aldo, Serranía de Jingurudó.	Panama	7.6802	–78.0387	958
SMF97141	*B. chucantiensis*	Cerro Chucantí, Serranía de Majé	Panama	8.8034	–78.4601	1,424
SMF97128	*B. colonnea*	Camino Cable Car, Reserva Forestal Fortuna, Chiriquí	Panama	8.7185	–82.2331	1,217
MHCH2658	*B. cuna*	Bajo pequeño, Río Tuquesa, camp2 Cerro Pechito parado, Serranía de Darién	Panama	8.4755	–77.5488	472
MHCH2659	*B. cuna*	Bajo pequeño, Río Tuquesa, camp2 Cerro Pechito parado, Serranía de Darién	Panama	8.4791	–77.5280	718
SMF97129	*B. cuna*	Serranía de San Blas	Panama	9.0602	–77.9827	463
MHCH2660	*B. medemi*	Bajo pequeño, Río Tuquesa, camp3 Cerro Pechito parado, Serranía de Darién	Panama	8.4800	–77.5194	859
MHCH2661	*B. medemi*	Bajo pequeño, Río Tuquesa, camp3 Cerro Pechito parado, Serranía de Darién	Panama	8.4791	–77.5280	718
MHCH2662	*B. medemi*	Bajo pequeño, Río Tuquesa, camp3 Cerro Pechito parado, Serranía de Darién	Panama	8.4791	–77.5280	718
SMF97130	*B. medemi*	Serranía de San Blas	Panama	9.0614	–77.9796	344
SMF97131	*B. medemi*	Serranía de San Blas	Panama	9.0611	–77.9797	340
SMF97132	*B. medemi*	Bajo pequeño, Río Tuquesa, camp2 Cerro Pechito parado, Serranía de Darién	Panama	8.4791	–77.5280	718
SMF97140	*B. schizodactyla*	Cerro Narices, Parque Nacional Santa Fé, Provincia de Veraguas	Panama	8.5632	–81.0524	841
MHCH2664	*B.* sp.	Cerro Chucantí, Serranía de Majé	Panama	8.8034	–78.4601	1,424
SMF97139	*B. biseriata*	Río Púcuro river, Serranía de Darien	Panama	8.0410	–77.3613	306
MHCH2665	*B. taylori*	Serranía de Pirre	Panama	7.9791	–77.7085	1,124
MHCH2666	*B. taylori*	Serranía de Pirre	Panama	7.9769	–77.7085	1,104
MHCH2667	*B. taylori*	Serranía de Pirre	Panama	7.9615	–77.7037	1,310
MHCH2668	*B. taylori*	Serranía de Pirre	Panama	7.9615	–77.7037	1,310
MHCH2669	*B. taylori*	Serranía de Pirre	Panama	7.9474	–77.7042	1,317
SMF97133	*B. taylori*	Serranía de Pirre	Panama	7.9880	–77.7076	1,135
SMF97134	*B. taylori*	Serranía de Pirre	Panama	7.9779	–77.7085	1,112
SMF97135	*B. taylori*	Serranía de Pirre	Panama	7.9786	–77.7085	1,112
SMF97136	*B. taylori*	Serranía de Pirre	Panama	7.9789	–77.7085	1,129
SMF97137	*B. taylori*	Serranía de Pirre	Panama	7.9632	–77.7043	1,267
SMF97138	*B. taylori*	Serranía de Pirre	Panama	7.9474	–77.7042	1,317
UMNH3847	*B. biseriata*	Río Paya	Panama	7.9041	–77.4755	100
KUH116520	*B. biseriata*	Campamento Sasardi, Serranía de San Blas	Panama	8.8888	–77.7229	12
KUH116521	*B. biseriata*	Campamento Summit, Serranía de San Blas	Panama	8.8558	–77.7472	358
KUH116523	*B. biseriata*	Boca de Río Concepcion	Panama	8.8320	–80.9794	10
KU116519	*B. cuna*	Campamento Sasardi, San Blas	Panama	8.8888	–77.7229	12
USNM150036	*B. cuna*	Armila, San Blas	Panama	8.6624	–77.4646	10
S13237	*B. medemi*	Nusagandi, San Blas	Panama	9.3300	–78.9667	356
KU116530	*B. medemi*	Jaque–Imamado, Cordillera de Jurado	Panama	7.3805	–77.9550	800
KU116534	*B. medemi*	Río Imamado	Panama	7.4270	–77.9647	50

KU116544	*B. taylori*	Filo sur, Cerro Cituro, Serranía de Pirre	Panama			1,100
GML6077	*B. taylori*	Filo sur, Cerro Cituro, Serranía de Pirre	Panama			1,100
KU116540	*B. taylori*	Filo sur, Cerro Cituro, Serranía de Pirre	Panama			1,100
KU116542	*B. taylori*	Filo sur, Cerro Cituro, Serranía de Pirre	Panama			1,100
KU116535	*B. taylori*	Vertiente sureste de Cerro Pirre	Panama			1,440
KU116543	*B. taylori*	Filo sur, Cerro Cituro, Serranía de Pirre	Panama			1,100
KU116545	*B. taylori*	Filo sur, Cerro Cituro, Serranía de Pirre	Panama			1100
KU116539	*B. taylori*	Filo norte, Cerro Cituro, Serranía de Pirre	Panama			900–1,000
KU116538	*B. taylori*	Filo norte, Cerro Cituro, Serranía de Pirre	Panama			960
BM 97.11.12.22	*B. phalarosoma*	Medellín, Antioquia	Panama			1,538
BM 97.11.12.21	*B. phalarosoma*	Medellín, Antioquia	Panama			1,538
KU116533	*B. medemi*	Río Jaqué, Darien	Panama	7.4167	–77.9486	50
LAMC42276	*B. medemi*	Finca Chibiguí, Río Arquía, Antioquia	Colombia	6.2499	–76.4999	300
LAMC42278	*B. medemi*	Finca Chibiguí, Río Arquía, Antioquia	Colombia			
LAMC42279	*B. medemi*	Río Opogodó at Serranía de Baudo	Colombia	6.8334	–77.2999	80
LAMC42280	*B. medemi*	Camino entre Río Opogodó y Río Napipí	Colombia	6.7167	–77.1663	60
LAMC70565	*B. medemi*	Alto de Buey, Chocó	Colombia			400
LAMC70566	*B. medemi*	Camino de Yupe, Chocó	Colombia			605
LAMC70567	*B. medemi*	Camino de Yupe, Chocó	Colombia			400–500
LAMC70568	*B. medemi*	Camino de Yupe, Chocó	Colombia			605
LAMC72067	*B. medemi*	Finca Chibiguí, Río Arquía, Antioquia	Colombia			
ICN–MHN 54440	*B. guaneae*	Río Fonce, vereda La Chapa, flanco oeste de la Cordillera Oriental	Colombia	6.1351	–73.0991	1,836

Abel Batista is a Panamanian who received a Masters degree in Biological Sciences at the Universidad de los Andes in Bogotá, Colombia, and currently is a Ph.D. student at Senckenberg Research Institute, Frankfurt am Main, Germany. Abel is specializing in the study of amphibians and reptiles from eastern Panama, and his research focuses on barcoding, biogeography, conservation, taxonomy, and bioacoustics.

Gunther Köhler received a degree in Veterinary Medicine (Staatsexamen) at the University Gießen, Germany in 1993 and a doctoral degree at Goethe University Frankfurt am Main, Germany in 1995; since that time he has been the Curator of Herpetology at the Senckenberg Research Institute, Frankfurt am Main, Germany. His research focuses on the Neotropical herpetofauna, primarily that of Central America and Mexico. To date, Gunther has authored 26 books and 165 research papers on amphibians and reptiles.

Konrad Mebert is an independent researcher and international project coordinator based in Switzerland, who focuses on reptiles. After completing a Master's degree on geographic variation and the effects of inbreeding on the Dice Snake at the University of Zürich, Switzerland, and a doctoral degree on hybrid zones in North American water snakes at Old Dominion University, Virgina, he currently is associated with the University of Basel in Switzerland. To date, Konrad has authored more than 80 professional and popular publications and two books on such topics as evolution, ecology, biodiversity, and conservation. His passion for photography and love of travel have led him to all continents except Australia, but his preference is the Neotropics where he has developed a special affection for Panama.

Milan Vesely is a biologist (Ph.D.) who is vice-president of Czech Herpetological Society. An assistant professor in the Department of Zoology at Palacky University in Olomouc, Czech Republic, for almost two decades Milan has focused his research interests on the herpetofauna of Central America. During his career, Milan has authored numerous popular and scientific papers on amphibian and reptile taxonomy, ecology, and parasites, and also is co-author (with Gunther Köhler and Eli Greenbaum) of a book entitled *The Amphibians and Reptiles of El Salvador*.

Appendix II

Declaration on the contributions of authors

to the publication: An integrative approach to reveal speciation and species richness in the genus *Diasporus* (Amphibia: Anura: Eleutherodactylidae) in eastern Panama.
status: Proof (2016).
name of journal: Zoological Journal of the Linnean Society.
Authors involved:
- Abel Batista (AB), - Milan Vesely (MV), - Konrad Mebert (KM), - Gunther Köhler (GK), - Andreas Hertz (AH)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 75% – field work (collecting and documenting specimens), molecular analysis
Coauthor MV: 10% – participated in the field trips.
Coauthor KM: 10% – participated in the field trips.
Coauthor GK: 5% – participated in the field trips and documenting specimens.

(3) to the creation of the data collection and figures

PhD candidate: 80% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor MV: 10% –provided photos, created figures
Coauthor KM: 10% – provided photos

(4) to the analysis and interpretation of the data

PhD candidate: 65% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor GK: 5% – contributed to data analysis and interpretation
Coauthor MV: 10% – contributed to data analysis and interpretation
Coauthor KM: 5% – contributed to data analysis and interpretation
Coauthor AH: 15% – contributed to data analysis and interpretation

(5) to writing the manuscript

PhD candidate: 60%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 5%
Coauthor AH: 20%

Date/place: 13.04.2016 / Frankfurt am Main, Germany

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

МЛП

Zoological Journal of the Linnean Society, 2016. With 17 figures

An integrative approach to reveal speciation and species richness in the genus *Diasporus* (Amphibia: Anura: Eleutherodactylidae) in eastern Panama

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We have applied an integrative taxonomic approach, including bioacoustics, ecology, morphology, and molecular genetics (barcoding and phylogeography), to explore species richness in the genus *Diasporus* in eastern Panama, from where only *Diasporus quidditus* (Lynch, 2001) was previously known. During fieldwork in eastern Panama in 2011 and 2012 we found six additional species, four of which we are describing here as new to science, plus two species that are new for this region. We have evaluated the presence of *Diasporus diastema* (Cope, 1875) in eastern Panama by comparing morphological, genetic, and bioacoustic characters of specimens from near the type locality in central Panama with specimens from eastern Panama. We further describe and compare male advertisement calls of most *Diasporus* species. The phylogeographic analysis suggests the allopatric speciation of *Diasporus* species in eastern Panama following the completion of the Panamanian isthmus in the middle Miocene. Subsequent geological events concur with the vicariant evolution of different lineages *in situ*, suggesting eastern Panama to be a centre of endemism for this group of frogs. We present an integrative analysis of the species from eastern Panama and include an identification key for all species of the genus.

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ADDITIONAL KEYWORDS: barcoding – bioacoustics – *Diasporus* – *Diasporus darienensis* sp. nov. – *Diasporus majeensis* sp. nov. – *Diasporus pequeno* sp. nov. – *Diasporus sapo* sp. nov. – ecology – integrative taxonomy – Panama – phylogeography.

INTRODUCTION

Cryptic species are defined as 'two or more distinct species that are erroneously classified under one species name' (Bickford *et al.*, 2007). Frogs of the genus *Diasporus* are a good example of cryptic diversity, as most species resemble each other externally, and are difficult to distinguish by morphological methods alone; however, integrative analysis (the combination of several methods and lines of evidence) reveals the true diversity in this species complex (Hertz *et al.*, 2012). In recent years, three new species of the genus *Diasporus* (formerly *Eleutherodactylus diastema* group; *sensu* Hedges, Duellman & Heinicke, 2008) have been described from Costa Rica and western Panama (Chaves *et al.*, 2009; Batista, Ponce &

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Hertz, 2012; Hertz *et al.*, 2012), and several more species presumably await discovery and description (Lynch & Duellman, 1997; Ibáñez, Rand & Jaramillo, 1999; Savage, 2002; Hertz *et al.*, 2012). There is evidence of divergence between populations in traits other than morphology, for example on geographic isolation, ecology, bioacoustics, and molecular genetics, but it is difficult to distinguish them based on morphology alone. The application of an integrative approach offers a more balanced solution (Dayrat, 2005; Vieites *et al.*, 2009; Padial *et al.*, 2010; Hertz *et al.*, 2012) that can solve the taxonomic problems within this genus.

The genus *Diasporus* comprises small frogs with short limbs and expanded disc pads, with or without lanceolate or papillate tips, that inhabit rainforests from eastern Honduras to north-western South America as far as north-western Ecuador (Hedges *et al.*, 2008). Adult males possess well-developed vocal slits and a single external subgular vocal sac. Their characteristic 'dink' call (subjective general acoustic description), which can be more like a whistle in some species, is commonly heard in wet forests throughout the distribution range of the genus. Males usually call from vegetation growing at ground level up to several metres above the ground (Savage, 2002).

To date, the genus *Diasporus* contains 11 described species (Frost, 2014). Seven species are currently known to be endemic to Central America: *Diasporus citrinobapheus* Hertz *et al.*, 2012; Serranía de Tabasará, Panama; *Diasporus diastema* (Cope, 1875), widespread between central Panama and Honduras; *Diasporus hylaeformis* (Cope, 1875), cordilleras (mountain ranges) of Costa Rica and western Panama; *Diasporus igneus* Batista *et al.*, 2012; Serranía de Tabasará, Panama; *Diasporus tigrillo* (Savage, 1997), Atlantic slopes of the Cordillera de Talamanca, Costa Rica; *Diasporus ventrimaculatus* Chaves *et al.*, 2009; Cordillera de Talamanca, Costa Rica; and *Diasporus vocator* (Taylor, 1955), western Panama and southern Costa Rica. *Diasporus quidditus* (Lynch, 2001) occurs in eastern Panama and north-western Colombia (Lynch, 2001; Köhler, 2011). The remaining three species are distributed along the Pacific side of northern South America, from Colombia to north-western Ecuador: *Diasporus anthrax* (Lynch, 2001), along the eastern foothills of the Cordillera central and the western slope of the Cordillera Oriental, Colombia; *Diasporus gularis* (Boulenger, 1898), lowlands of western Colombia and north-western Ecuador; and *Diasporus tinker* (Lynch, 2001), Pacific slopes of Colombia (IUCN, 2013; Frost, 2014).

Eastern Panama (EP) is known as an important biodiversity hot spot with high endemism, as a result of the great variety of habitats Still largely unknown forests, that are suffering from rapid habitat loss (Parker, Carrión & Samudio, 2004). The high endemism in EP is likely to be the result of the complex geohistory of the Isthmus of Panama. EP represents the northernmost part of the Chocó biogeographical region (Duque-Caro, 1990), and can be subdivided into three main geographical units: the massifs of the Dabeiba Arc in the north-east (San Blas and Darién mountain ranges) and the Baudó Arc in the south-west (Jingurudó, Majé, Pirre, and Sapo mountain ranges). Between these mountains lies the Chucunaque Basin, a sedimentary basin that forms the central part of the Choco Block, with the drainage of Chucunaque River and Tuira Basin in the lowlands (Duque-Caro, 1990; Coates & Obando, 1996). The uplift of the Choco Block is the result of the collision of the Panama Arc with South America since the middle Miocene (as early as 11 Mya; Farris *et al.*, 2011). The continuous uplift of the Choco Block shallowed the water depth in the Atrato and Chucunaque basins, as they were steadily filled with sediments (Duque-Caro, 1990; Coates *et al.*, 2004). These geohistorical dynamics periodically separated land masses (e.g. the isolation of the mountains in the Baudo and Dabeiba arcs in EP during the Middle Miocene) that promoted speciation events and an increased species diversity in this region (Batista *et al.*, 2014b).

During the last 4 years we have conducted several expeditions and collected numerous specimens and associated materials of amphibians and reptiles across large parts of EP. Besides two recent discoveries and publications on regional anuran fauna (Batista *et al.*, 2014a,b), preliminary barcoding analysis of *Diasporus* frogs from different localities in EP revealed several distinct lineages. Herein, we apply the first integrative approach on *Diasporus* taxa to evaluate the status of these lineages. We use information from bioacoustics, ecology, morphology, and biogeography to evaluate the divergence among genetic lineages and draw taxonomic conclusions. Furthermore, we discuss the distribution pattern of *Diasporus* taxa from EP in a biogeographical context by comparing analyses of regional geological events and molecular clock calibrations.

MATERIAL AND METHODS

Fieldwork was carried out in the Chucunaque and Tuira basins of the eastern Panamanian lowlands (EPLL), and in all major eastern Panamanian mountain ranges. Eastern Panama (EP) defines the eastern half of the country, corresponding to the area east of the Panama Canal. It comprises two important ecoregions of the western hemisphere, the eastern Panamanian montane forests (EPMF) in the

highlands and the Chocó-Darién moist forests (CDMF) in the lowlands (Fund, 2014). The EPMF is further split into several mountain ranges: San Blas mountain range (SBM), Darién mountain range (DM), Jingurudó-Sapo mountain range (JSM), Majé mountain range (MM), and Pirre mountain range (PM) (Figs 1 and 2). The lowlands (EPLL) are dissected by a few large rivers, the Balsas, Chucunaque, Sambú, and Tuira rivers, within the CDMF. There are a few additional, smaller (<500 m a.s.l.) mountain ranges, such as the Filo del Tallo-Canglón in middle of Darién, and the Bagre in the south-east of Darién along the Sambú River. All geographical coordinates were recorded in the WGS 1984 datum given in decimal degrees. The maps were created with ArcGIS 10 (ESRI, 2009). The voucher specimens collected were killed with agent T61 and subsequently fixed with a preservative mixture of 5 mL of formalin (40%) in 1 L of ethanol (94%), and then stored in ethanol (70%). All figures have been digitally modified for improved visibility and combined using Adobe CS3. For candidate species and their delimitation we follow the integrative concept for amphibians of Vieites *et al.* (2009).

Morphometrics

Morphological nomenclature, measurements, and diagnoses follow Duellman & Lehr (2009). All measurements were made using digital calipers and were rounded to the nearest 0.01 mm. Measurements are given as mean ± SD and range in parentheses (Table 1). Specimens were deposited in the Museo Herpetológico de Chiriquí at the Universidad Autónoma de Chiriquí, David, Panama, and at the Senckenberg Research Institute and Nature Museum, Frankfurt, Germany. The abbreviations for museum collections follow Sabaj Pérez (2013), with field numbers AB from the abbreviated name Abel Batista. Morphological data of similar *Diasporus*

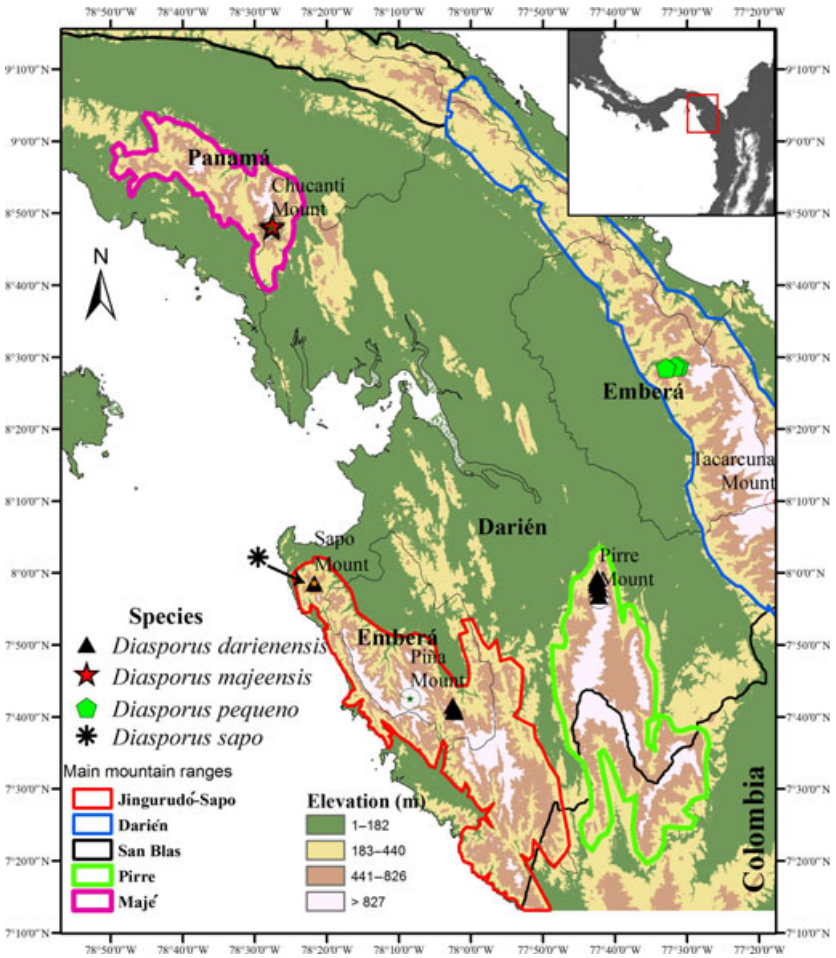

Figure 1. Map of the Darién region, eastern Panama, showing the distribution of the species of *Diasporus* described herein.

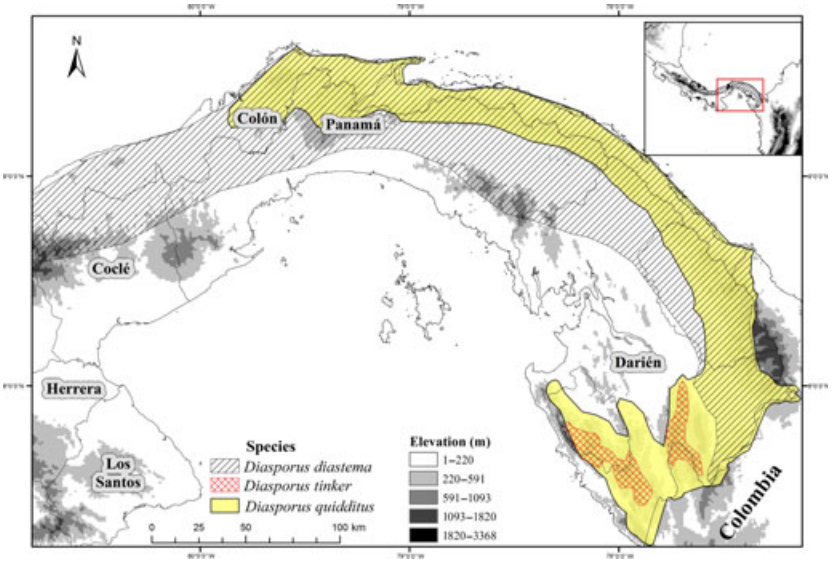

Figure 2. Map of eastern Panama (EP), showing the distribution of EP species that inhabit variable elevations, including lowlands: *Diasporus diastema* complex, *Diasporus* aff. *quidditus*, and *Diasporus tinker*.

Table 1. Genetic p–distances (in percentages) in the *16S* mtDNA between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)											
	Species	1	2	3	4	5	6	7	8	9	10	11	12
1	*D. majeensis* sp. nov.												
2	*D. darienensis* sp. nov.	5.0											
3	*D. pequeno* sp. nov.	10.3	8.5										
4	*D. sapo* sp. nov.	6.5	2.6	9.5									
5	*D. diastema* CWP	10.0	9.1	9.6	8.3								
6	*D.* aff. *diastema* EPL	11.0	9.2	9.7	9.2	4.6							
7	*D.* aff. *diastema* MM	10.8	8.0	7.1	8.0	5.0	4.2						
8	*D. citrinobapheus*	11.0	9.0	9.7	9.2	3.8	5.1	4.6					
9	*D. hylaeformis*	8.4	5.5	10.0	6.5	10.7	10.6	10.5	10.1				
10	*D.* aff. *quidditus*	10.2	6.9	9.2	7.0	7.0	6.3	5.2	7.0	8.9			
11	*D. tinker*	9.9	7.2	8.5	8.0	7.8	7.4	7.1	7.5	9.6	7.4		
12	*D. vocator*	10.5	8.9	11.1	8.3	10.7	10.8	9.8	10.1	8.2	9.2	10.6	

species for comparison were taken from holotypes (*D. anthrax*, *D. citrinobapheus*, *D. igneus*, *D. quidditus*, and *D. tinker*), as well as from original descriptions and published literature (Savage, 1997; Lynch, 2001; Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012; Jiménez *et al.*, 2013). A list of specimens examined is presented in Appendix 1.

The following morphometric measurements were taken (with abbreviations indicated, see Duellman & Lehr, 2009): snout-vent length (SVL); head length (HL), measured diagonally from angle of jaw to tip of snout; head width (HW), measured between angles of jaws; interorbital distance (IOD); eye length (EL), measured from anterior to posterior edge of externally accessible eye; hand length (HAL), measured from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), the distance from knee to distal end of the tibia; foot length (FL), measured from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW), at penultimate phalanx just anterior to disc; width of disc of third finger (3FD), at greatest width; width of third toe (3TW), at penultimate phalanx just anterior to disc; width of disc of third toe (3TD), at greatest width; width of fourth toe (4TW), at penultimate phalanx just anterior to the disc; width of disc of

fourth toe (4TD), at greatest width; tympanum diameter (TD), measured horizontally, based on an estimated circular tympanum; and body width (BW), at greatest width of body. We determined the sex of adults by the presence of vocal slits in males and the presence of eggs in females. Specimens without vocal slits or eggs, and with the correspondingly smaller SVL (relative to the standard size of confirmed adult females or males for each species), were classified as juveniles. For the description of the ungual flap we followed Lynch (2001) and Savage (2002). The ungual flap is known as the 'more or less free anterior portion of the disk cover' (see Savage, 1987), it represents the anteriormost margin of the finger/toe tip, which can be hanging in a loose flap (sheet of skin). Generalized coloration summaries were derived directly from live specimens or indirectly from photos of live specimens. For the standardized colour descriptions of selected individuals, the capitalized colours and colour codes (with the latter in parentheses) are taken from Köhler (2012).

Bioacoustics

Male advertisement calls were recorded using a Marantz Professional (PMD 620) and/or a Panasonic RR-XS410 digital recorder, with a Sennheiser ME 66 shotgun microphone capsule and a Sennheiser K6 powering module. The microphone was positioned between 0.5 and 1.5 m from the calling frog. Recordings were made at a sampling rate of 44 kHz with 16–bit resolution in uncompressed pulse-code modulation (PCM) format and saved as .wav files. The spectral and temporal parameters were analysed and the power spectra were calculated in RAVEN PRO 1.4 (Blackman DFT window; 2048 samples; 3–dB filter bandwidth of 158 Hz; grid spacing of 21.5 Hz; overlap 70.1%; Charif, Clark & Fristrup, 2004). The lowest and highest frequencies were measured at 20 dB below the peak frequency, thereby avoiding overlapping with background noise (Simões & Lima, 2011). Because our original recordings of two male *Diasporus* sp. nov. from Bajo Pequeno were lost, we extracted the calls from the background of another recording that was targeted at a *Pristimantis* sp. nov. (A. Batista, unpubl. data), using Adobe Audition 5.0. We amplified the sound (using the amplitude function) to extract details that were difficult to see in the original file. The terminology used in the description of advertisement calls follows Duellman & Trueb (1994), and follows Ursprung, Ringler & Hödl (2009) for the description of calling in bouts. The call rate was calculated as (total number of calls − 1)/(time from the beginning of the first call to the beginning of the last call) (Cocroft & Ryan, 1995). Because the dominant frequency (DF) is correlated with SVL in many frog species (Gerhardt & Huber, 2002; Bradbury & Vehrencamp, 2011), we used the non-standardized residuals between these variables for the statistical analyses. The environmental temperature and humidity were measured using an Oakton digital thermo-hygrometer. Because temporal parameters are temperature-dependent in many frog species, statistical adjustments are required (Gerhardt & Huber, 2002). In cases where we found no correlation between temperature and temporal parameters among species, we used raw data (e.g. call rate, note duration, note interval). We ran a discriminant function analysis to classify the advertisement calls of different species. The species for which SVL and/or temperature were not available were excluded from this analysis; however, all specimens were included in scatter plots of raw data that correlate DF against call rate and DF against note duration (Gerhardt & Huber, 2002; Padial *et al.*, 2008). The statistical analyses were performed using SPSS 21.0. Acoustic data for *D. anthrax* and *D. ventrimaculatus* were taken from Chaves *et al.* (2009) and Jiménez *et al.* (2013), respectively. As the terms DF, peak frequency, and high frequency were obviously confused by Jiménez *et al.* (2013), we re-estimated the real DF value from the spectrogram shown in that publication. The spectrogram figure was produced with the SEEWAVE package in R.

Molecular laboratory work and phylogenetic inference

DNA was extracted from fresh muscle or liver tissue in the Grunelius-Möllgaard Laboratory for Molecular Evolution, Senckenberg, Germany, using the protocol of Ivanova, Dewaard & Hebert (2006). The samples were amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany), performing an initial denaturation for 1 min at 94 °C, followed by 35 steps with denaturation for 9 s at 94 °C, annealing for 27 s at 45 °C, and with elongation for 1.5 min at 72 °C. Final elongation proceeded for 7 min at 94 °C. For the nuclear *recombination activating gene 1* (*RAG1*), we used: one cycle of 2 min at 96 °C; 45 cycles of 20 s at 95 °C, 25 s at 52 °C, and 2 min at 72 °C; and one cycle of 7 min at 72 °C. The reaction mixture contained 1 μL of mitochondrial DNA (mtDNA) template, 2.5 μL of reaction buffer ×10 (PeqGold), 4 μL of 2.5 mM dNTPs, 0.4 μL (containing 2.5 units) of Taq Polymerase (PeqLab), 14.1 μL of H_2O, 1 μL of 25 mM $MgCl_2$, and 1 μL per primer for *16S* (10 pmol; forward primer, L2510, 5′-CGCCTGTTTATCAAAAACAT-3′; reverse primer, H3056, 5′-CCGGTCTGAACTCAGATCACGT-3′; eurofins MWG Operon), and 3 μL per primer for *RAG1*

(forward, R182, 5′-GCCATAACTGCTGGAGCATYA T-3′; reverse, R270, 5′-AGYAGATGTTGCCTGGGT CTTC-3′; eurofins MWG Operon (Heinicke, Duellman & Hedges, 2007). Sequencing of the *16S* rRNA and *RAG1* was performed in the molecular laboratory of the Senckenberg Biodiversität und Klima Forschungszentrum (BIK–F), Germany. The mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene was sequenced in the Southern China DNA Barcoding Center. In total we extracted DNA from 48 *Diasporus* frogs from EP and received 41 sequences for *16S*, 30 sequences for *COI*, and 45 sequences for *RAG1* (Appendix 2). We compared the mtDNA data of our specimens with *Diasporus* sequences published in GenBank. The sequences were aligned with CLUSTAL W (Larkin *et al.*, 2007) and were edited by eye using GENEIOUS 4.8.5 (Drummond *et al.*, 2010). A list of specimens included in the genetic analysis with corresponding GenBank accession numbers is presented in Appendix 2. GenBank sequences of *Colostethus panamansis* (Dunn, 1933), *Craugastor opimus* (Savage and Myers, 2002), *Craugastor fitzingeri* (Schmidt, 1857), *Eleutherodactylus planirostris* (Cope, 1862), *Eleutherodactylus thorectes* Hedges, 1988, and *Pristimantis caryophyllaceus* (Barbour, 1928) were used as out-groups. The final alignment (including GenBank sequences) of the *16S* mtDNA comprised 66 sequences (excluding out-groups) and consisted of 449 positions, of which 251 sites were variable and 158 sites were parsimony-informative. The final alignment of *COI* comprised 38 sequences consisting of 552 positions, of which 250 sites were variable and 224 sites were parsimony-informative. The final alignment of *RAG1* comprised 48 sequences consisting of 571 positions, of which 172 sites were variable and 76 sites were parsimony-informative. A total of 73 samples, combining the mitochondrial genes and the nuclear *RAG1*, were obtained (excluding out-groups), consisting of 1537 positions, of which 630 sites were variable and 447 were parsimony-informative. Using MEGA 6 (Tamura *et al.*, 2011) we calculated uncorrected pairwise genetic p–distances for *COI* and *16S* both separately and combined. For each gene and for the combined gene data set (*COI*, *16S*, and *RAG1*). We selected the substitution model for the Bayesian analysis using JModeltest 0.1.1 (Posada, 2008) under the corrected Akaike information criterion (AICc; Akaike, 1974). The three-parameter model with rate heterogeneity, TPM1uf+I+G (Kimura, 1981), was implemented for the combined gene data set. We ran a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001) for 10 000 000 generations with four default chains, sampling every 1000 generations. In the final consensus tree 25% were discarded as burn-in. To test species delimitation among the *Diasporus* species, we applied the automatic barcode gap discovery (ABGD) algorithm (Puillandre *et al.*, 2011) with the following settings: 20 steps; distance calculated using the Kimura two-parameter model with a transversion/transition ratio of 2.0; and the setting for the minimum relative gap width (*X*) was set to different values between 0 and 1.5.

Phylogeography and divergence time analysis

We have used defined biogeographic areas of EP (see first paragraph in the Material and methods section) in the phylogeographic analysis. The phylogenetic relationships and divergence times were estimated for the mtDNAs *16S* and *COI* and nuclear DNA *RAG1* combined (1537 bp, 20 *Diasporus* samples included) in BEAST 1.5.4 (Drummond & Rambaut, 2007), with a relaxed clock, allowing substitution rates to vary according to an uncorrelated log-normal distribution, assuming a Yule tree prior (Drummond *et al.*, 2006). The prior distribution on substitution parameters was set to the default. To calibrate the root and one node age respectively, we used the age of approximately 57 Mya with a standard deviation of 15 Myr for the most common recent ancestor between *Pristimantis* and *Eleutherodactylus-Diasporus* (Heinicke *et al.*, 2009; Pinto-Sanchez *et al.*, 2012); and with the estimated crown ages of 32 Mya for the *Eleutherodactylus-Diasporus* (Heinicke *et al.*, 2009), and 17 Mya for *D. vocator* and *D. hylaeformis* (Pinto-Sanchez *et al.*, 2012). Parameters were estimated using 100 million generations with a burn-in of 50 million generations and trees were sampled every 10 000 generations. Results were visualized and compared using TRACER 1.5 (Rambaut & Drummond, 2009), and summary trees were generated using TreeAnnotator 1.5.4.

RESULTS

The phylogenetic analysis of the two mitochondrial genes revealed substantial molecular differences between groups with p–distances above 4.0% in *16S* mtDNA, 11% in *COI* mtDNA (Tables 1–3), and 7.0% when both genes were combined (Table 3). The divergence of these groups are further supported by substantial differences in male advertisement call and morphometric characters. The ABGD analysis generated 12 distinct phylogenetic groups for *16S* with a divergence threshold of 0.033 and a relative barcoding gap of 0.05 (*X*–value). For *COI*, it produced 12 groups assuming an a priori intraspecific divergence threshold of 0.021 and a relative gap width of 0.05 (*X*–value). The two analyses (ABGD for *16S* and *COI*) lumped all samples in one group, with a prior

intraspecific divergence of 0.050 and 0.010, respectively. For both mitochondrial genes, the groups resulting from the ABGD analysis were consistent with the species units named in this study, except for the lumping of *Diasporus* sp. nov. from Cerro Sapo and *Diasporus* sp. nov. from DM and JSM, which is also present at Cerro Sapo.

The Bayesian consensus tree of all genetic markers combined (*16S*, *COI*, and *RAG1*) as well the divergence time analyses showed two well-differentiated clades of *Diasporus* in EP, with one containing the highland species (MM, PM, and JSM) that are monophyletic and well supported (Bayesian probability >0.95). The second clade includes species primarily distributed in the lowlands, including also *D. citrinobapheus* (from western Panama, WP), various lineages of *D.* aff. *diastema* (from central Panama, CP, the eastern Panamanian lowlands, EPL, and Majé MM, below 1000 m a.s.l.) and *D.* aff. *vocator* (from WP and south-eastern Costa Rica, CR). The divergence time analyses indicated that the EP lineages split from the WP lineages around 15 Mya (95% confidence interval, 95% CI, 6–29 Myr).

Most *Diasporus* species can be differentiated morphologically by a combination of SVL, disc shape, and colour pattern (Table 4). In bioacoustics, a discriminant function analysis correctly classified all species included ($P < 0.01$; $N = 26$; Table 5). These results proved that seven species of the genus *Diasporus* occur in EP and can be diagnosed by some selected traits (e.g. SVL, ungual flap, colour pattern, dominant frequency, and distribution; for more details, see Figs 3–7 and Table 6), with a new record for the species *D. tinker* in Panama. A detailed comparison of the type series of *D. tinker* from Colombia left no doubt that the specimens collected in EP are conspecific. We took advantage of these new records and include molecular, bioacoustic, and morphological data of *D. tinker* to increase the knowledge of morphological variation in this species in Panama. Furthermore, we used this data to distinguish *D. tinker* from other *Diasporus* species in EP. Addi-

Table 2. Genetic p–distances (in percentages) in the *COI* mtDNA between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)										
	Species	1	2	3	4	5	6	7	8	9	10	11
1	*D. majeensis* sp. nov.											
2	*D. darienensis* sp. nov.	12.3										
3	*D. pequeno* sp. nov.	15.2	16.9									
4	*D. sapo* sp. nov.	13.0	10.0	14.7								
5	*D. diastema* CP	16.1	17.6	13.9	16.5							
6	*D.* aff. *diastema* EPL	16.7	16.5	14.4	17.0	10.4						
7	*D.* aff. *diastema* MM	15.2	16.0	12.3	16.2	9.6	10.0					
8	*D. hylaeformis*	18.3	19.7	18.3	18.7	18.9	19.1	17.0				
9	*D.* aff. *quidditus*	16.7	16.9	14.5	17.5	12.6	14.3	13.1	19.0			
10	*D. tinker*	16.4	16.9	14.6	17.1	14.7	14.1	14.0	20.6	16.2		
11	*D. vocator*	17.8	18.7	17.7	17.6	18.5	20.1	16.5	19.1	21.1	18.6	

Table 3. Genetic p–distances (in percentages) in the *16S* and *COI* mtDNA combined between the *Diasporus* spp. used in the phylogenetic analysis

		p–distance (in %)						
	Species	1	2	3	4	5	6	7
1	*D.* aff. *diastema* EPL							
2	*D. majeensis* sp. nov.	14						
3	*D.* aff. *quidditus*	10.4	14.3					
4	*D. darienensis* sp. nov.	13.5	9.9	12.6				
5	*D. tinker*	11.5	14.1	12.2	13.2			
6	*D. sapo* sp. nov.	13.4	10.3	12.7	7	13.6		
7	*D. pequeno* sp. nov.	12.1	14.1	12.4	14.3	12.5	12.9	

tionally, four species that clearly belong to the genus *Diasporus* on the basis of molecular (Figs 8, 9), bioacoustic, and morphological evidence cannot be assigned to any described species. Thus, we describe them as new species hereafter.

SPECIES ACCOUNTS

DIASPORUS DARIENENSIS SP. NOV.

FIGS 10, 14A, B

ELEUTHERODACTYLUS DIASTEMA – MYERS 1969: FIG. 19B

Holotype

SMF 97304 (original field number AB 1144), an adult female (Fig. 10), collected by Abel Batista & Konrad Mebert on the ridge of Pirre mountain range, ~3 km north from the peak of Cerro Pirre, Parque Nacional Darién (PND), Distrito de Pinogana, Darién, Panama, on 8 December 2012 at 20:35 h (7.97312 N, 77.70785 W; 1143 m a.s.l.).

Paratypes

MHCH 2840–41, 2844–47, 2862, SMF 97303, 97314, 97306–08, 97661–62, with same collecting data as holotype; MHCH 2850–52, SMF 97309–10, 97312, collected by Abel Batista & Milan Veselý in the Jingurudo mountain range, on a ridge between Aldo Creek and Sambú River, between the Comarca Emberá-Wounaan and the PND, Distrito de Sambú, Darién, Panama, 26–30 September 2011 (7.69271 N, 78.04200 W; 869 m a.s.l.); SMF 97313, collected by Abel Batista & Gustavo Dojirama at the top of Cerro Sapo, PND, Distrito de Garachiné, Darién, Panama, on 4 December 2011, at 22:06 h (7.97618 N, 78.36263 W; 1169 m a.s.l.).

Diagnosis

Diasporus darienensis sp. nov. is characterized by the following combination of traits (see Table 1): (1) dorsal skin texture smooth and/or with rounded or pointed scattered tubercles; (2) tympanic annulus concealed by skin, tympanic membrane absent; (3) snout acuminated in dorsal view and rounded in profile; (4) usually with a slightly enlarged and conical supraocular tubercle, cranial crests absent; (5) dentigerous processes of vomers triangular, diagonal to the eyes, near to the middle of the mouth and posteriorly separated about three-quarters of their total length from each other; (6) vocal sac moderately developed, with longitudinal gular folds evident, vocal slits present on the posterior part of the jaw, halfway under the tongue and ending at the midlevel of the eyes, no nuptial pads; (7) finger II longer than finger I, ungual flap mostly expanded, rounded, and more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid, flattened, and slightly larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles, palmar accessory tubercles small, rounded, and almost indistinguishable; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap slightly expanded to rounded, more evident on toes II–V; (12) plantar tubercle indistinguishable, between one and three non-protuberant subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle elongated; outer metatarsal tubercles conical and smaller than inner; tarsal ridge absent; (13) dorsal ground colour in life brown to reddish, some specimens with pale reticulations on a dark background colour; usually with a pair of red or pale dorsolateral lines, venter translucent or suffused with dark colour, vocal sac yellow; (14) SVL 18.1 ± 21.3 (14.9–22.9, $N = 21$), males 17.1 ± 1.11 (14.9–18.5, $N = 15$), females 20.7 ± 1.86 (18.5–22.9, $N = 6$); (15) advertisement call composed of a single, amplitude-modulated short note (49.1–51.7 ms) with harmonic structure. The dominant frequency is also the fundamental frequency, with most energy emitted at 3.34–3.81 kHz.

Description of the holotype

An adult female (SVL 17.40, Fig. 10) with slender body; dorsal skin smooth with small scattered tubercles, ventral skin smooth, discoidal fold not evident, low anal warts present; one small conical supraocular tubercle; eye twice as long as snout; tympanum of moderate size, ratio TD/EL 34%; tympanum indistinguishable, annulus tympanicus concealed by skin, tympanic membrane absent, positioned above the junction of jaws and behind the orbit; head as long as wide (HL/HW 1.03), greatest head width between angles of jaw 40% of SVL; snout subacuminate from above and rounded in profile; nares situated near tip of snout and slightly dorsolaterally directed, clearly visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes of vomer clearly visible, orbit in an oblique outline in frontal of eyes, each with five teeth; vocal slits absent; tongue long (25% of SVL) and knobbed at the end, first third attached to floor of mouth; hands moderate in size, 20% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II subequal in size to finger VI, finger II reaching the disc on finger IV when adpressed; finger III disc 1.6 times wider than distal end of adjacent phalanx; palmar tubercle low and rounded, larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles;

Table 4. Morphological proportions for *Diasporus* species

Species (*N*)	SVL	HW	HL
D. anthrax (2)*	16.5–18.7	–	–
D. tigrillo (2)*	16.8–17.5		
D. citrinobapheus (7)	19 ± 1.41 (17.3–21.8)	7 ± 0.47 (6.5–7.8)	7.6 ± 0.54 (7–8.7)
D. darienensis sp. nov. (21)	18.1 ± 2.13 (14.9–22.9)	6.5 ± 0.82 (5.2–8.4)	6.5 ± 0.64 (5.6–7.9)
D. aff. *diastema*, CP (49)	19.2 ± 2.78 (14.6–27.7)	7 ± 1.2 (5.4–10.9)	7.6 ± 0.99 (6–10.6)
D. aff. *diastema*, MM (5)	19.8 ± 3.11 (16–24.5)	7.3 ± 0.93 (6.1–8.7)	7.3 ± 1.04 (6–8.9)
D. aff. *diastema*, EPL (20)	21.3 ± 1.82 (18.3–25.2)	7.9 ± 0.83 (6.1–9.6)	7.4 ± 0.63 (6.7–8.4)
D. gularis (3)	22.1 ± 1.75 (20.4–23.9)	–	–
D. hylaeformis (28)	20.3 ± 1.51 (16.9–23.1)	7.4 ± 0.74 (5.9–8.8)	8 ± 0.71 (6.8–9.5)
D. igneus (4)	26.1 ± 0.5 (25.5–26.6)	9.9 ± 0.17 (9.7–10.1)	8.5 ± 0.31 (8.2–8.8)
D. majeensis sp. nov. (15)	21.5 ± 2.64 (15.3–25.5)	8.1 ± 0.92 (6.2–9.7)	7.8 ± 0.84 (6–9.4)
D. pequeno sp. nov. (13)	19.3 ± 2.38 (16.9–24.8)	7.2 ± 0.96 (6.2–8.9)	7.2 ± 1.04 (5.9–9.1)
D. aff. *quidditus* (51)	14.5 ± 1.44 (11.5–17.9)	5.4 ± 0.48 (4–6.5)	5.6 ± 0.48 (4.5–6.3)
D. sapo sp. nov. (11)	22.6 ± 2.86 (18.8–29.1)	8 ± 0.82 (7.1–9.7)	8.2 ± 1.05 (6.6–10.2)
D. tinker (39)	17.3 ± 1.55 (14.6–20.4)	6.4 ± 0.42 (5.6–7.3)	6.2 ± 0.58 (5.3–7.2)
D. ventrimaculatus (4)	22.8 ± 1.89 (20.2–24.7)	–	–
D. vocator (12)	14.4 ± 1.46 (12–17.2)	4.9 ± 0.41 (4–5.4)	5.4 ± 0.66 (4.4–6.3)

Species	**TL**	**HAL**	**HW/SVL**
D. anthrax	–	–	–
D. tigrillo		–	0.36 (0.34–0.37)
D. citrinobapheus	7.9 ± 0.68 (7–9.2)	–	–
D. darienensis sp. nov.	7.7 ± 0.73 (6.5–9.5)	6.8 ± 0.89 (5.4–9)	4.4 ± 0.39 (3.7–5.1)
D. aff. *diastema*, CP	8 ± 1.32 (5.5–12)	4.5 ± 0.93 (3.2–6)	0.4 ± 0.02 (0.3–0.4)
D. aff. *diastema*, MM	8.4 ± 0.96 (7.3–9.9)	4 ± 0.58 (3.5–5)	0.4 ± 0.01 (0.4–0.4)
D. aff. *diastema*, EPL	8.3 ± 0.77 (7.3–9.4)	3.9 ± 0.5 (3.4–4.8)	0.4 ± 0.02 (0.3–0.4)
D. gularis	–	–	–
D. hylaeformis	8 ± 0.75 (6.6–10)	–	–
D. igneus	11.7 ± 0.5 (11.3–12.4)	11.5 ± 0.27 (11.1–11.7)	11.9 ± 0.36 (11.5–12.2)
D. majeensis sp. nov.	9.1 ± 1.14 (6.8–10.9)	8.3 ± 1.01 (5.9–9.6)	5.1 ± 0.63 (3.5–6.1)
D. pequeño	8.4 ± 0.71 (7.5–10)	7.1 ± 0.69 (6.1–8.5)	4.4 ± 0.51 (3.7–5.5)
D. aff. *quidditus*	6.9 ± 0.56 (5.9–8)	5.5 ± 0.54 (4.3–6.3)	3.3 ± 0.32 (2.7–4)
D. sapo sp. nov.	10.4 ± 1.02 (8.3–12.1)	9.5 ± 1.07 (7.3–11.5)	5.5 ± 0.6 (4.2–6.4)
D. tinker	7.3 ± 0.66 (5.3–8.8)	6.2 ± 0.42 (5.3–7.4)	3.8 ± 0.31 (3.2–4.6)
D. ventrimaculatus	–	–	–
D. vocator	5.8 ± 0.43 (5.2–6.6)	4.9 ± 0.88 (3.9–5.5)	2.9 ± 0.38 (2.5–3.2)

Species	**HW/HL**	**HL/SVL**	**TL/SVL**
D. anthrax	–	–	–
D. aff. *tigrillo*	0.92 (0.85–0.99)	0.39 (0.38–0.40)	0.48 (0.46–0.50)
D. citrinobapheus	0.9 ± 0.04 (0.9–1)	0.4 ± 0.02 (0.4–0.4)	0.4 ± 0.01 (0.4–0.4)
D. darienensis sp. nov.	1 ± 0.06 (0.9–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. aff. *diastema*, CP	0.9 ± 0.07 (0.8–1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.04 (0.4–0.6)
D. aff. *diastema*, MM	1 ± 0.02 (1–1)	0.4 ± 0.01 (0.4–0.4)	0.4 ± 0.03 (0.4–0.5)
D. aff. *diastema*, EPL	1 ± 0.05 (0.9–1.1)	0.4 ± 0.01 (0.3–0.4)	0.4 ± 0.02 (0.4–0.4)
D. gularis	–	–	–
D. hylaeformis	0.9 ± 0.05 (0.8–1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.03 (0.3–0.5)
D. igneus	1.2 ± 0.03 (1.1–1.2)	0.3 ± 0.01 (0.3–0.3)	–
D. majeensis sp. nov.	1 ± 0.03 (1–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. pequeño	1 ± 0.05 (1–1.1)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.02 (0.4–0.5)
D. aff. *quidditus*	0.9 ± 0.07 (0.8–1.2)	0.2 ± 0.19 (0–0.4)	0.5 ± 0.03 (0.4–0.5)
D. sapo sp. nov.	1 ± 0.05 (0.9–1.1)	0.4 ± 0.02 (0.3–0.4)	0.5 ± 0.02 (0.4–0.5)
D. tinker	1 ± 0.06 (0.9–1.2)	0.4 ± 0.02 (0.3–0.4)	0.4 ± 0.03 (0.3–0.5)
D. ventrimaculatus	–	–	–
D. vocator	0.9 ± 0.07 (0.8–1.1)	0.4 ± 0.03 (0.3–0.4)	0.4 ± 0.04 (0.4–0.5)

Mean ± SD (range); see Material and methods for abbreviations. Numbers in parenthesis next to the species names represents the number of specimens analysed.
*Measurements taken from original descriptions and literature.

Table 5. Variations in advertisement call parameters in 11 species of *Diasporus*

	Traits				
Species	DF (kHz)	Low freq. (Hz)	High freq. (Hz)	Delta freq. (Hz)	Note duration (s)
*D. anthrax**	3.81	3.19 ± 0.35 (2.94–3.44)	4.45 ± 0.29 (4.25–4.65)	1.31–1.22	0.06
D. citrinobapheus (2)	2.86–3.04	2.77–2.95	2.95–3.42	0.2–0.5	0.11–0.17
D. darienensis sp. nov. (2)	3.57 ± 0.33 (3.34–3.81)	3 ± 0.29 (2.79–3.21)	4.07 ± 0.33 (3.83–4.3)	1.07 ± 0.04 (1.04–1.09)	0.05 ± 0 (0.05–0.05)
D. aff. *diastema*, EPL (7)	3.3 ± 0.12 (3.2–3.5)	2.9 ± 0.07 (2.8–3)	3.8 ± 0.11 (3.6–3.9)	0.9 ± 0.05 (0.8–0.9)	0.1 ± 0.01 (0.1–0.1)
D. aff. *diastema*, MM (4)	3.1 ± 0.2 (3–3.4)	2.7 ± 0.1 (2.7–2.9)	3.5 ± 0.15 (3.4–3.7)	0.8 ± 0.06 (0.7–0.9)	0.1 ± 0 (0.1–0.1)
D. aff. *diastema*, CP (7)	3.3 ± 0.16 (3.2–3.5)	2.97	3.82	0.86	0.09
*D. igneus** (1)	2.4	2	2.7	0.7	0.05–0.10
D. majeensis sp. nov. (1)	2.47–2.71	2.38–3.03	2.85–3.14	0.50–0.93	0.01–0.02
D. pequeño (1)	3.44–3.48	3.20–3.23	3.67–3.63	0.39–0.46	0.09–0.15
D. aff. *quidditus* (22)	4.81 ± 0.14 (4.55–5.08)	4.56 ± 0.18 (4.35–4.84)	4.97 ± 0.19 (4.77–5.29)	0.41 ± 0.02 (0.39–0.45)	0.34 ± 0.04 (0.25–0.38)
D. tinker (9)	3.5 ± 0.19 (3.14–3.71)	3.16 ± 0.16 (2.84–3.32)	3.8 ± 0.2 (3.42–4.07)	0.64 ± 0.06 (0.56–0.75)	0.17 ± 0.02 (0.14–0.19)
*D. ventrimaculatus**	2.50–2.61	2.14	2.9	0.76	0.07
D. vocator (5)	4.6 ± 0.3 (4.35–5.1)	3.83 ± 0.17 (3.71–3.94)	4.94 ± 0.25 (4.77–5.12)	1.12 ± 0.08 (1.06–1.18)	0.02 ± 0 (0.01–0.02)
Species	**Note interval (s)**	**Call rate (calls/min)**	**Notes/bouts**	**Bout duration**	**Interbout duration**
D. anthrax	0.55–5.77	23.4–44.2			
D. citrinobapheus	16.58 ± 0.47 (16.25–16.91)	3.61 ± 0.1 (3.54–3.68)	19–30	19.30–55.50	38.47–156.43
D. darienensis sp. nov.	3.08 ± 0.9 (1.65–4.84)	20.29 ± 6.17 (12.04–34.41)	–	–	–
D. aff. *diastema*, EPL	3.1 ± 0.68 (2.2–3.9)	19.7 ± 4.43 (14.8–25.8)	8.5	28.4	40.26
D. aff. *diastema*, MM	3.4 ± 1.08 (2.5–4.8)	17.9 ± 5.02 (12–22.8)			
D. aff. *diastema*, CP	1.65	34.41	10.2	17.33	30.25
D. igneus	6.40–9.67	8.18	–	–	–
D. majeensis sp. nov.	2.67–6.02	12.32	–	–	–
D. pequeño	3.51–6.85	11.61	–	–	–
D. aff. *quidditus*	4.71 ± 0.78 (3.27–5.44)	12.19 ± 2.24 (10.35–16.71)	–	–	–
D. tinker	2.96 ± 2.05 (1.35–7.55)	25.38 ± 11.84 (7.76–39.16)	11–13	15.97–27.85	19.71–30.58
*D. ventrimaculatus**	5.15	11.45			
D. vocator	1.91 ± 0.57 (1.4–2.52)	32.98 ± 9.35 (23.7–42.39)	13.23 ± 10.51 (5.8–20.67)	19.25 ± 13.31 (9.84–28.66)	311.02 ± 304.7 (95.56–526.47)

Mean ± SD (range). Number in parentheses next to the species names represents the number of individuals analysed. DF, dominant frequency.
*Information obtained from literature.

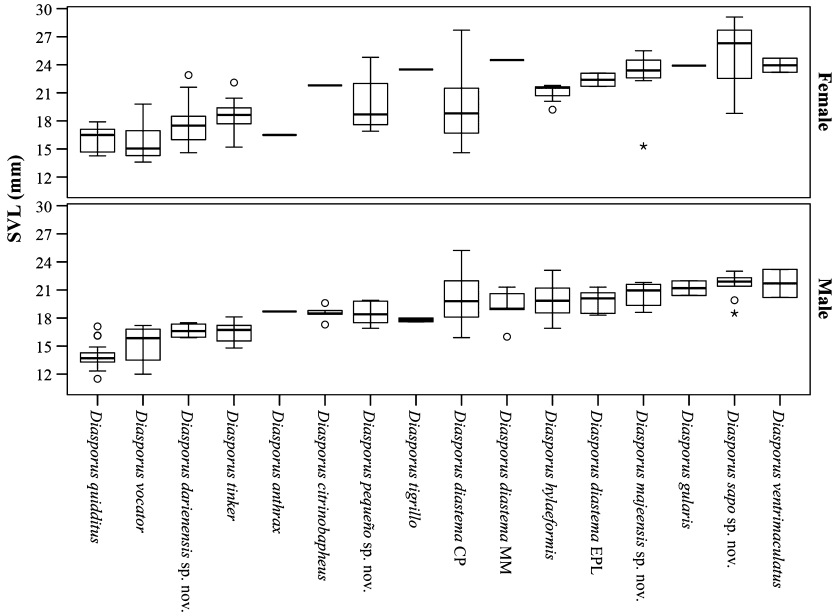

Figure 3. Differences in snout-vent length (SVL) of *Diasporus* species, separated by sex. The bottom and top of the box are the first and third percentile, and the band inside the box is the median, whiskers are the extreme values; open circles above or below the boxes represent outliers.

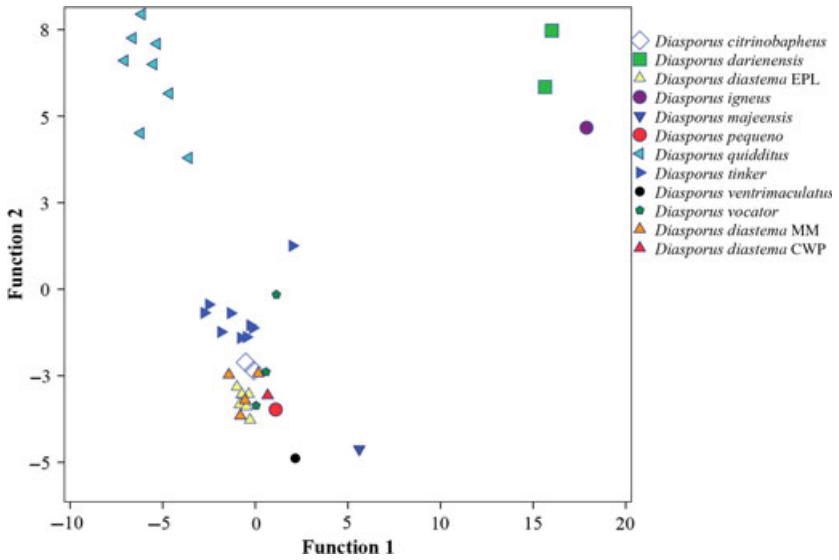

Figure 4. Discriminant function analyses of the acoustic characters of *Diasporus* species. Variables included in the analysis: note duration, note interval, dominant frequency (DF, corrected by snout-vent length), low frequency, high frequency, and call rate (temporal characters are corrected for temperature).

palmar and plantar accessory tubercles indistinguishable; no nuptial pads; no fringes on fingers; hindlimbs of moderate length, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches to tubercle of toe II; disc of toe IV slightly expanded, 1.3 times wider than distal end of adjacent phalanx; no fringes on toes; between one and three nonprotuberant subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle ovoid; outer metatarsal tubercles slightly pointed and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even, broadened; ungual flap expanded, almost

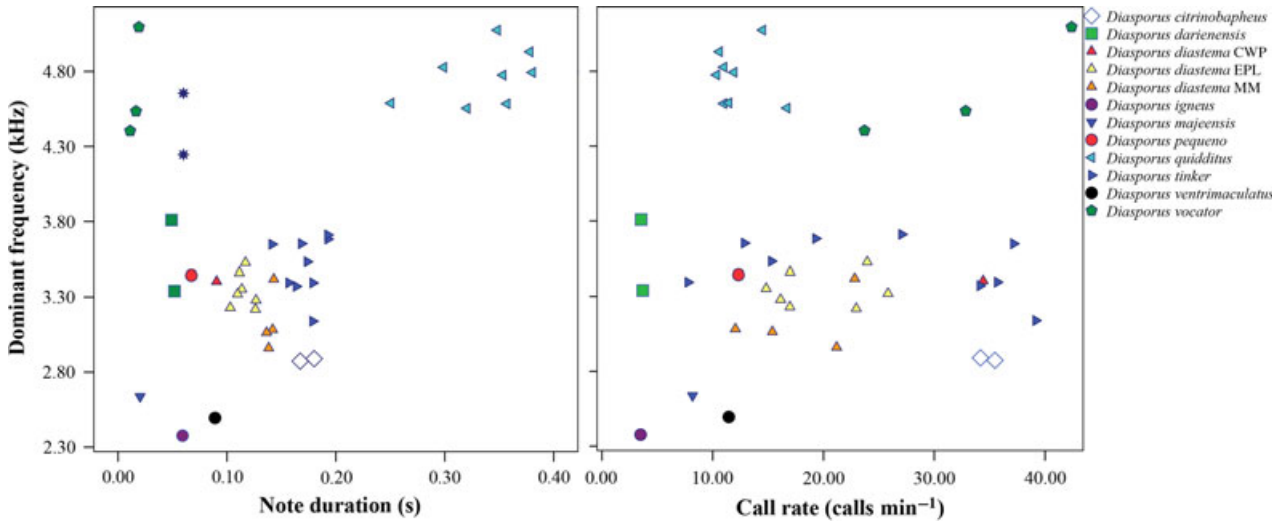

Figure 5. Scatter plot for dominant frequency/note duration (left) and dominant frequency/call rate (right) in 11 species of *Diasporus*.

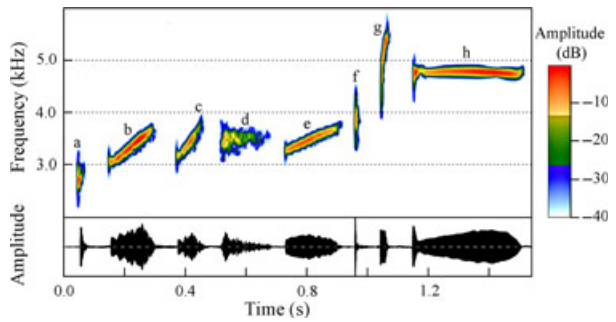

Figure 6. Spectrograms (only the harmonic containing the dominant frequency is shown) and oscillograms (below) of the advertisement calls of *Diasporus* species from eastern Panama (EP): a, *Diasporus majeensis* sp. nov. (SMF 97658); b, *Diasporus* aff. *diastema* MM (MHCH 2809); c, *Diasporus diastema* from Colón, Panama (SMF 97287, 9.26020 °N, 79.93540 °W, 36 m a.s.l.; ~9 km south-west from type locality); d, *Diasporus pequeno* sp. nov. (Bajo Pequeño, Cerro Pechito Parado, not collected); e, *Diasporus tinker* (SMF 97315); f, *Diasporus darienensis* sp. nov. (SMF 97313); g, *Diasporus vocator* (not collected; from Celmira, Bugaba, Panama, 8.55348 °N, 82.81525 °W, 242 m a.s.l.; ~60 km east from type locality); h, *Diasporus* aff. *quidditus* (SMF 97292).

rounded; pads broadened and globular in profile (Figs 7A, 10).

Coloration of holotype in life

Holotype (SMF 97304; Figs 10, 14A, B) recorded as follows: iris light Pratt's rufous (71) with a couple of lateral and irregular lines tawny (60), lumbar region tawny (60), bordered by two lines light buff (2); flanks brick red (36); groin, axilla, and ventral areas mottled with brick red (36).

Coloration in preservative

Dorsal ground colour raw amber (23), with a couple of dorsolateral lines light buff (2); groin and ventral areas buff (5), with small points sepia (279); ungual flaps dark drab (45).

Measurements of holotype (mm)

SVL 17.40; HL 6.70; HW 6.30; IOD 3.24; EL 2.72; TD 0.92; FL 6.42; TL 7.50; HAL 3.47; 3FW 0.40; 3FD 0.64; 3TW 0.36; 3TD 0.49; 4TW 0.31; 4TD 0.67; BW 5.22 (for variation of the species, see Table 1).

Vocalization

The calls produced by two specimens from Cerro Sapo (Fig. 6; Table 2), one paratype (SMF 97313, environmental temperature 21.5 °C; humidity 84%; 22:06 h) and an uncollected specimen (environmental temperature 21.7 °C; humidity 80%; 21:00 h) were analysed. The calls consist of single, short, monophasic notes that are reminiscent of a 'whistle' (Fig. 6). Note duration is 0.04–0.05 s, with an interval between calls of 16.91–16.25 s, and with a call rate of four calls per minute. The peak frequency band ranges from 2.79 to 4.30 kHz; the first harmonic contains the dominant frequency at 3.34–3.81 kHz.

Natural history

This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) along the PM and JSM (Fig. 1). The vegetation consists predominantly of trees covered with moss, bromeliads (*Werauhia* spp.

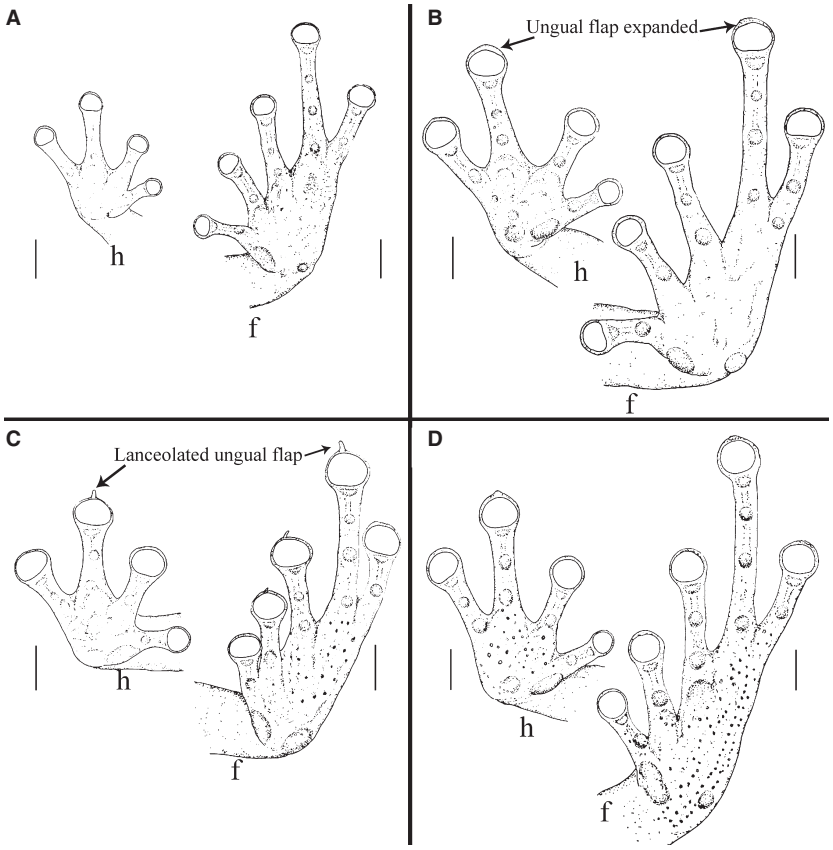

Figure 7. Drawings of ventral view of right hand and left foot of the new *Diasporus* species described here; h, hand; f, foot. Arrows indicate two examples of ungual flap shape. A, *Diasporus darienensis* sp. nov. (MHCH 2852). B, *Diasporus majeensis* sp. nov. (MHCH 2835). C, *Diasporus pequeno* sp. nov. (MHCH 2826). D, *Diasporus sapo* sp. nov. (SMF 97331). Scale bars: 1 mm.

and *Guzmania* spp.), giant ferns (*Cyathea* spp.), and orchids. *Diasporus darienensis* sp. nov. is an inhabitant of the cloud forest (869–1169 m a.s.l.), usually found 1–5 m above ground. During the day, specimens seek retreats between bromeliad leaves. At night they actively move across tree bark and bromeliads. Only two males were encountered calling, both during the end of the rainy season (December) at the top of Cerro Sapo. One male (SMF 97313) was observed calling from the underside of a leaf in a tree about 5 m above ground, the other was calling from a branch on a ridge 3 m above the ground on the same day. Diet is not known, but as with other *Diasporus* it may eat small arthropods (Batista, 2009).

Etymology

The species name is derived from the province name Darién where the holotype was found, with the Latin suffix *-ensis* donating a place or locality. The species is known to occur only in this province, and it is present in the main mountain ranges of the region.

DIASPORUS MAJEENSIS SP. NOV.

FIGS 9B, 11, 14C, D

Holotype

SMF 97293 (original field number AB 1030), an adult male (Fig. 11) collected by Abel Batista & Konrad Mebert on the top of Cerro Chucantí, at Majé mountain range, Río Congo Arriba, Distrito de Chepigana, Darién, Panama, on 2 December 2012 at 20:35 h (8.79936 N, 78.46156 W; 1380 m a.s.l.).

Paratypes

MHCH 2832–39, SMF 97655–60, with same collection data as the holotype.

Diagnosis: Diasporus majeensis sp. nov. is characterized by the following combination of characters (Figs 11, 14C, D; Table 1): (1) dorsal skin smooth with small dispersed warts, ventral skin smooth; (2) only lower part of the tympanic annulus barely visible, tympanic membrane absent; (3) snout

Table 6. Main diagnostic characters and character states to differentiate members of the *Diasporus* genus in Central and South America

Species	SVL	Ungual flap	Dorsal colour pattern	Ventral colour pattern	DF (kHz)	Distribution
*D. anthrax**	16.5–18.7	Palmate to rounded, or expanded	Dark or blackish	Black with white flecks	3.81	North-western Ecuador and Colombia
*D. tigrillo**	16.8–17.5	Spadate	Yellow to orange	White, granules on venter with base greyish	No data	Alto Lari, SE Costa Rica, 300–400 m a.s.l.
D. citrinobapheus	19 ± 1.41 (17.3–21.8)	Palmate to rounded, or spadate	Yellowish	Almost transparent	2.86–3.04	Western Panama, 680–790 m a.s.l.
D. darienensis sp. nov.	18.1 ± 2.13 (14.9–22.9)	Palmate to rounded, or spadate	Reddish, with or without reticulations	Reddish or small black speckles	3.57 ± 0.33 (3.34–3.81)	Serranía de Pirre and Jingurudó-Sapo, Darién, Panama, 869–1169 m a.s.l.
D. aff. *diastema*, CWP	19.2 ± 2.78 (14.6–27.7)	Palmate to rounded, or spadate	Yellowish	White with dark spots	3.3 ± 0.16 (3.2–3.5)	Lowlands of central and western Panama
D. aff. *diastema*, MM	19.8 ± 3.11 (16–24.5)	Palmate to rounded	Yellowish	White with dark spots	3.1 ± 0.2 (3–3.4)	Serranía de Maje, Panama
D. aff. *diastema*, EPL	21.3 ± 1.82 (18.3–25.2)	Palmate to rounded, or spadate	Yellowish	White with dark spots	3.3 ± 0.12 (3.2–3.5)	Lowlands of eastern Panama
D. gularis	22.1 ± 1.75 (20.4–23.9)	Palmate to rounded, or spadate	Pale brown with vague markings	Cream with brown stippling on throat	No data	Lowlands of western Colombia and north-western Ecuador
D. hylaeformis	20.3 ± 1.51 (16.9–23.1)	Palmate to rounded	Suffused with pink or red	Translucent, suffused with yellow or red	No data	Serranía de Talamanca Costa Rica and Panama, 1500–2500 m a.s.l.
D. igneus	26.1 ± 0.5 (25.5–26.6)	Palmate to rounded	Brownish with yellow to orange reticulations	Yellow	2.4	Western and eastern slopes of Cerro Santiago, Panama, above 1500 m a.s.l.
D. majeensis sp. nov.	21.5 ± 2.64 (15.3–25.5)	Palmate to rounded, or spadate	Reddish, with or without reticulations	Unpigmented venter	2.47–2.71	Top of Cerro Chucantí, Panama, 1400 m a.s.l.
D. pequeno sp. nov.	19.3 ± 2.38 (16.9–24.8)	Lanceolate to papillate	Brown, cream, with dark reticulations	Venter translucent, with a dark speckle and sky blue spots	3.44–3.48	Serranía de Darién, Panama, above 472 m a.s.l.
D. aff. *quidditus*	14.5 ± 1.44 (11.5–17.9)	Lanceolate to papillate	Brown	Brown	4.81 ± 0.14 (4.55–5.08)	Eastern Panama and north-western Colombia, above 100 m a.s.l.

D. sapo sp. nov.	22.6 ± 2.86 (18.8–29.1)	Palmate to rounded, or spadate	Uniform red	Translucent, suffused with red	No data	Cerro Sapo, Darién, Panama, 1169 m a.s.l.
D. tinker	17.3 ± 1.55 (14.6–20.4)	Lanceolate to papillate	Grey	Brown to orange	3.5 ± 0.19 (3.14–3.71)	Eastern Panama to north-western Colombia, in Panama 800–1350 m a.s.l., in Colombia, up to 1880 m a.s.l.
D. ventrimaculatus	22.8 ± 1.89 (20.2–24.7)	Spadate	Red to pink	White with red spots in males and white with dark spots in females	2.50–2.61	Valle del Silencio, Costa Rica, 2550 m a.s.l.
D. vocator	14.4 ± 1.46 (12–17.2)	Lanceolate to papillate	Pigmented with dark mottling and light areas	Brown	4.6 ± 0.3 (4.35–5.1)	South-western Costa Rica to central Panama, 2–1220 m a.s.l.

DF, dominant frequency.
*Information obtained from literature.

rounded in dorsal and profile view; (4) conical supraocular tubercle or cranial crests absent; (5) dentigerous processes of vomers with between one and four teeth each, straight in outline, in frontal to the orbit; (6) vocal sac small, but with visible longitudinal gular folds, vocal slits present, situated beside the tongue, from the middle side of the tongue to near the junctions of jaws, no nuptial pads; (7) finger II longer than finger I, ungual flap mostly expanded, rounded, more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid, flattened, and slightly larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular, first tubercle more evident; one or two supernumerary tubercles, palmar accessory tubercles small and rounded; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap slightly expanded to rounded, more evident on toes II–V; (12) plantar tubercle indistinguishable, subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV), first tubercle more evident; small and rounded supernumerary tubercles; inner metatarsal tubercle elongated; outer metatarsal tubercles conical and smaller than inner; tarsal ridge absent; (13) dorsal ground colour in life brown to reddish, some specimens with dark reticulations on a reddish background colour, venter translucent, vocal sac same colour as venter (Fig. 14C–D); (14) SVL 21.5 ± 2.64 (15.3–25.5, $N = 15$), males 19.9 ± 2.1 (15.3–21.8, $N = 9$), females 23.9 ± 1.22 (22.3–25.5, $N = 6$); (15) advertisement call composed of a single, amplitude-modulated short note with duration of 0.01–0.02 s, and with the DF ranging between 2.47 and 2.71 kHz (Fig. 6; Table 2).

Description of the holotype

An adult female (SVL 20.90), with slender body; dorsal skin smooth with small dispersed warts, ventral skin smooth, discoidal fold not evident; eye 1.30 times longer than snout; tympanum small, ratio TD/EL 21%; only lower part of the tympanic annulus barely visible, tympanic membrane absent, positioned 2 mm behind orbit; head slightly wider than long (HL/HW 0.85), greatest head width between angles of jaw 38% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, and also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes barely visible, in frontal of the orbit of eyes in a straight outline, each with four teeth; vocal slits absent; tongue long (20% of SVL) and broadening to the end, first third attached to floor of mouth; hands moderate in size, 23% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II smaller

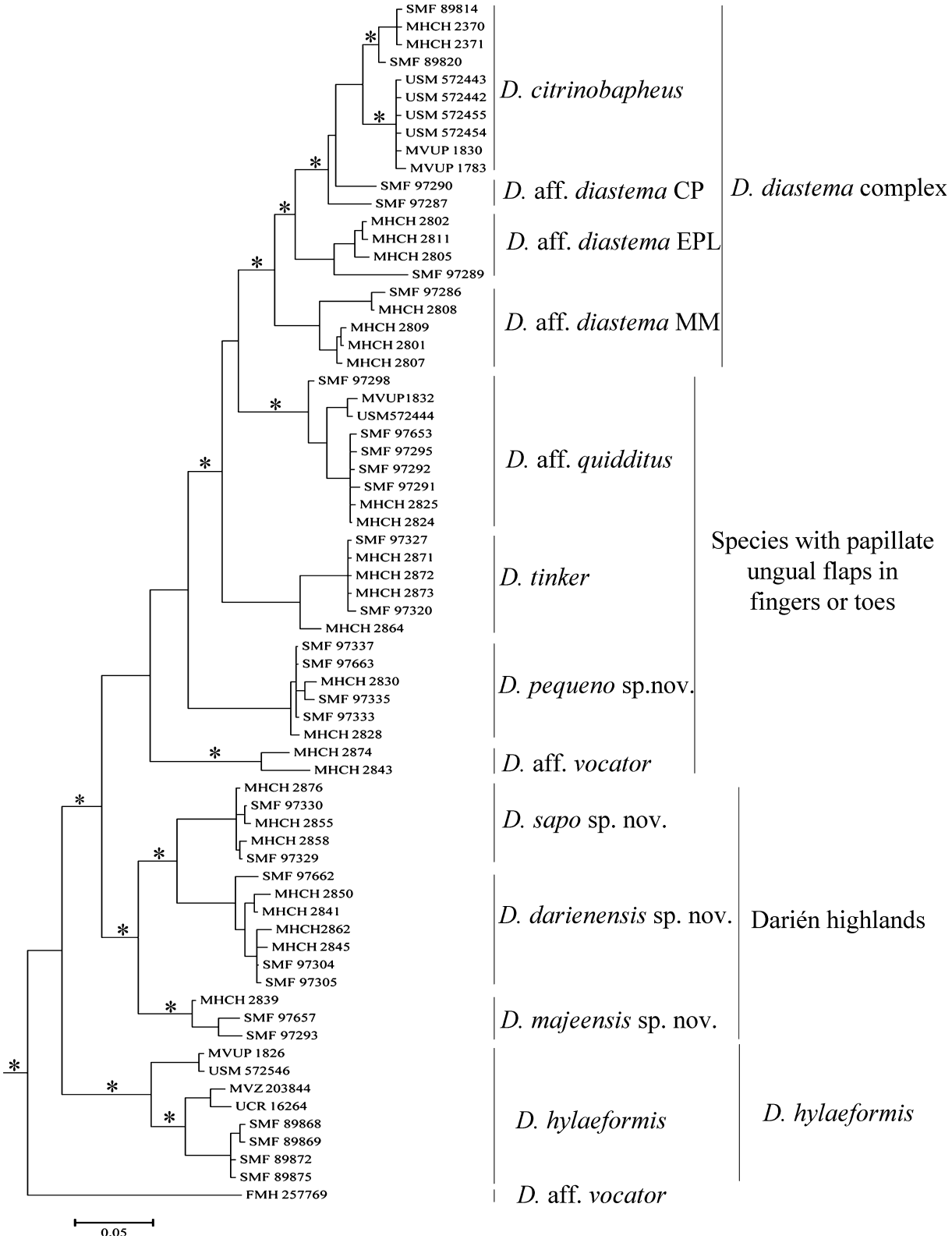

Figure 8. Bayesian consensus tree of the genus *Diasporus* based on *16S*, *COI*, and *RAG1* genes. Out-groups are not shown (*Pristimantis caryophyllaceus*, *Craugastor gollmeri*, *Craugastor fitzingeri*, *Colostethus pratti*, *Eleutherodactylus planirostris*, and *Eleutherodactylus thorectes*). Asterisks on nodes indicate estimated posterior probabilities: $P \geq 0.90$.

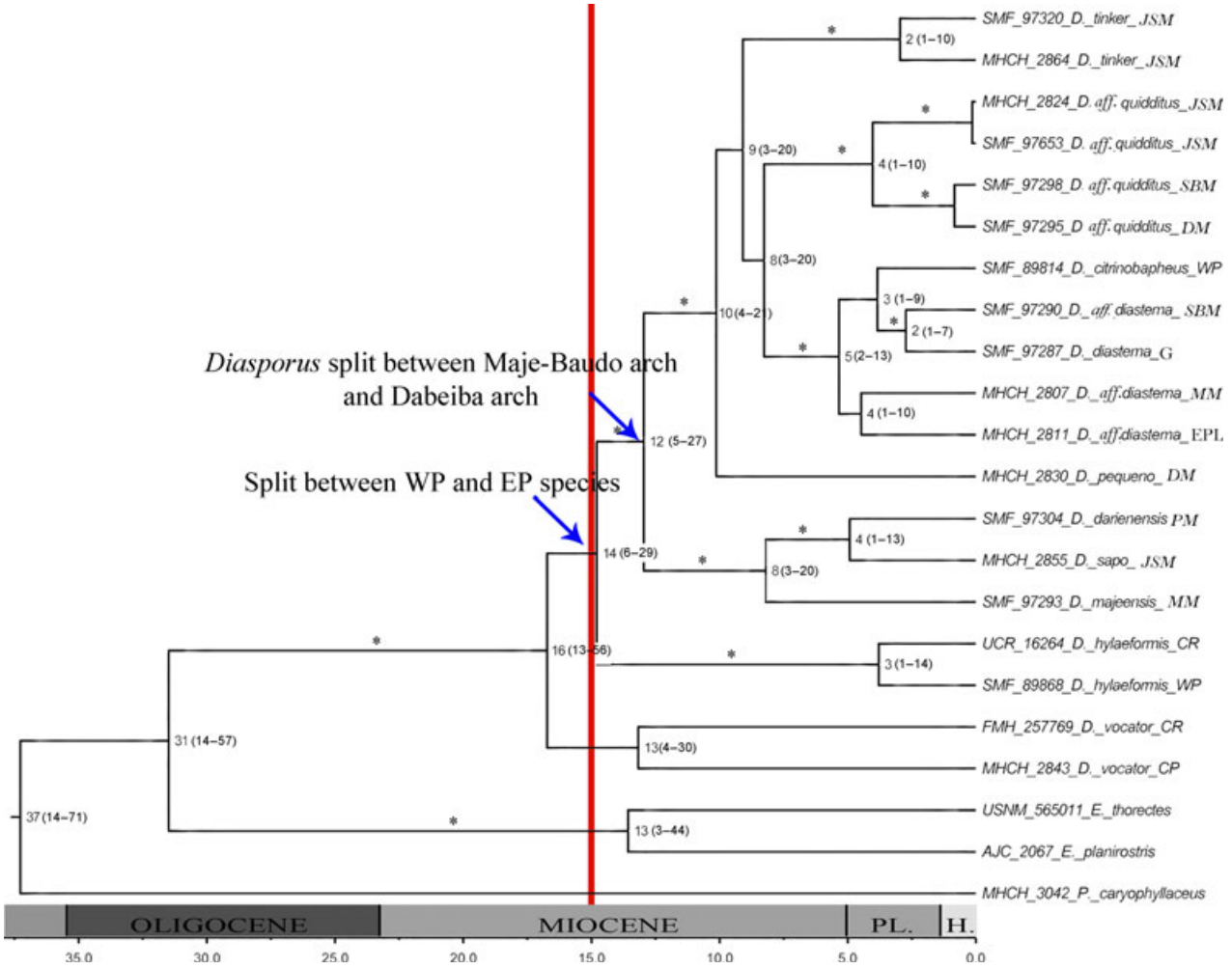

Figure 9. A chronogram of *Diasporus* species based on *16S*, *COI*, and *RAG1*, derived from a relaxed-clock Bayesian analysis, using BEAST software. The scale indicates time in Mya. The red line indicates the hypothesized completion, 15 Mya, of the Isthmus of Panama. Asterisks on nodes indicate estimated posterior probabilities: $P \geq 0.95$. Numbers at nodes represent estimated ages of diversification (SD in parenthesis). Letters at the end of species names represent biogeographic areas (for an explanation, see Material and methods); CR, Costa Rica; CP, central Panama; DM, Darién mountain range; G, Gatún lake at CP; JSM, Jingurudó-Sapo mountain range; MM, Majé mountain range; PM, Pirre mountain range; SBM, San Blas mountain range; WP, western Panama.

than finger VI, finger II reaching the base of disc on finger IV when adpressed; finger III disc 1.6 times wider than distal end of adjacent phalanx; palmar tubercle low and rounded, larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles; palmar and plantar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate lengths, TL 46% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the last third of distal phalanx of toe II; disc of toe IV slightly expanded, 1.3 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV), first subarticular tubercles more visible than the rest; inner metatarsal tubercle ovoid; outer metatarsal tubercles rounded, slightly pointed, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs slightly triangular; ungual flap expanded, even, rounded; pads globular in profile (Fig. 7B).

Coloration of holotype in life

Holotype (SMF 97293, Fig. 11) recorded as follows: iris light orange yellow (7) with middle area light Pratt's rufous (71); dorsal ground colour chestnut (30) with peach red (70) areas in the occipital, flanks,

Figure 10. Holotype of *Diasporus darienensis*> sp. nov.: A, frontal view; B, ventral view; C, left foot ventrally; D, right hand ventrally; E, flanks; F, posterior side of thighs and rear.

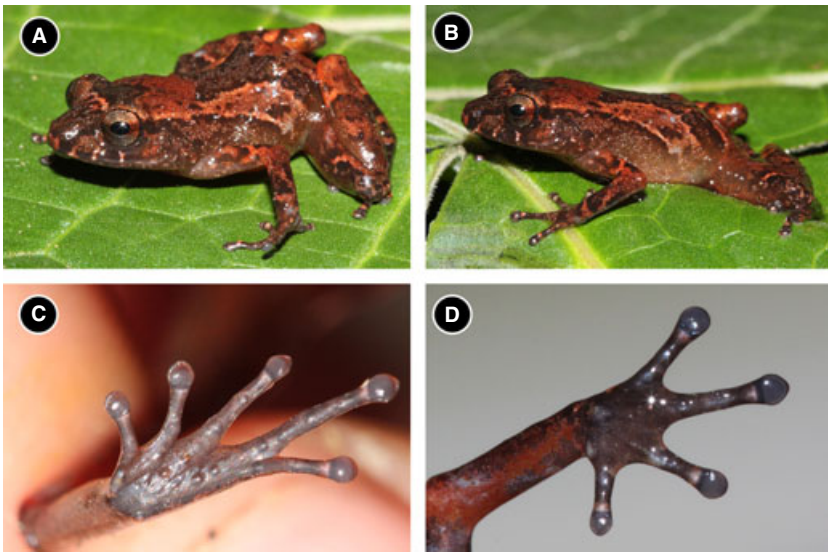

Figure 11. Holotype of *Diasporus majeensis* sp. nov.: A, B, frontal and lateral view, respectively; C, left foot ventrally; D, right hand ventrally.

and lumbar region; a spectrum red (67) interorbital band, bordered posteriorly by a sepia (286) band; axilla and groin slightly pigmented with chestnut (30); limbs same as dorsum; ventral areas translucent slightly pigmented with sepia (286); ventral part of fingers and toes dark carmine (61).

Coloration in preservative
Dorsal ground colour burnt sienna (38) with flesh ocher (57) areas in the occipital, flanks, and lumbar region; interorbital band flesh ocher (57), groin and venter light buff (2), ventral surfaces of limbs light orange yellow (7).

Measurements of holotype (mm)
SVL 20.90; HL 6.53; HW 7.68; IOD 2.21; EL 2.97; TD 0.62; FL 8.41; TL 9.51; HAL 4.79; 3FW 0.47; 3FD 0.75; 3TW 0.47; 3TD 0.65; 4TW 0.42; 4TD 0.53; BW 6.77 (for variation of the species, see Table 1).

Vocalization
The calls produced by one specimen (SMF 97658, environmental temperature 18.5 °C; 3 December 2012, 18:19 h) were analysed. The call consisted of single, short, monophasic notes that are reminiscent of a 'whistle' (Fig. 6). Note duration is 0.01–0.02 s, with an interval between calls of 2.67–6.02 s and a call rate of 12.32 calls/min; the low frequency was 2.38–3.03 kHz, the high frequency was 2.85–3.14 kHz, and the first harmonic contains the dominant frequency at 2.47–2.71 kHz.

Natural history
This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) of the Majé mountain ranges (Fig. 1). Cloud forest in this area has vegetation consisting predominantly of trees covered with moss and a large variety of understory bromeliads (*Werauhia* spp. and *Guzmania* spp.). At night, *D. majeensis* sp. nov. was found 0.5–2.0 m above ground on tree bark in bromeliad foliage. During the daytime, individuals were found hiding between bromeliad leaves. At the top of Cerro Chucantí, males were calling during the end of the rainy season (December). The recorded male was observed calling between dry bromeliad leaves 1.5 m above ground. The diet is not known, but as with other *Diasporus* it is likely to eat small crickets, cockroaches, ants, and isopods (Batista, 2009).

Etymology
The species name is derived from the name of the mountain range, Majé, where the holotype was found, with the Latin suffix *-ensis* donating a place or locality.

DIASPORUS PEQUENO SP. NOV.
FIGS 12, 14 E, F

Holotype
SMF 97663 (original field number AB 857), an adult female (Fig. 12) collected by Abel Batista, Marcial Sabugara, and Amadiel Chaquí at Cerro Pechito Parado, at the Darién mountain range, Río Tuquesa, Bajo Pequeño, Cémaco, Comarca Embera Wounaan, Darién, Panama, on 5 November 2012 at 22:35 h (8.47553 N, 77.54883 W; 472 m a.s.l.).

Paratypes
SMF 97333–34, same locality as holotype; MHCH 2828–31, SMF 97635–38, collected at Cerro Pechito Parado on 7 November 2012 at 19:00–

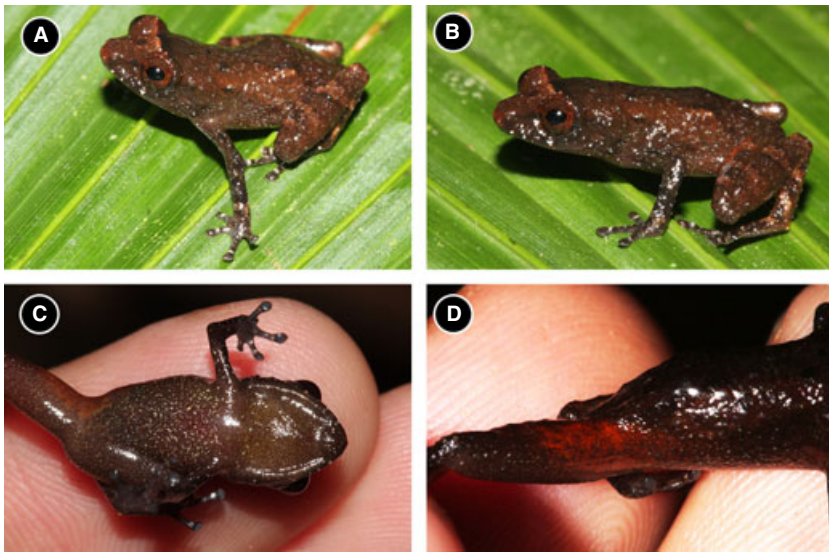

Figure 12. Holotype of *Diasporus pequeno* sp. nov.: A, B, frontal and lateral view, respectively; C, ventral view; D, flanks.

00:30 h (8.47911 N, 77.52799 W; 718 m a.s.l.), with same collectors as for holotype; MHCH 2826–27 collected at Cerro Pechito Parado, on 6 November 2012 at 19:00–01:30 h (8.47996 N, 77.51941 W; 858 m a.s.l.), with same collectors as for holotype.

Diagnosis

Diasporus pequeno sp. nov. is characterized by the following combination of characters (see Tables 4–6): (1) dorsal and ventral skin texture smooth, with small scattered tubercles, anal warts present; (2) tympanic annulus present, but with only the lower part clearly visible, tympanic membrane absent; (3) snout rounded in dorsal view and in profile; (4) rounded supraocular tubercle present, cranial crests absent; (5) dentigerous processes of vomers with between four and six teeth each, straight in outline, near to the frontal border of the orbit; (6) vocal sac well developed (Fig. 14E, F), vocal slits present, situated under the centre of the orbit level, no nuptial pads; (7) finger II longer than finger I; disc pads even broadened; ungual flap expanded, lanceolate, more evident on finger III (Fig. 7C); (8) no fringes or webbing on fingers; (9) palmar tubercle rounded, flattened, and larger than thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular, first tubercle more evident; supernumerary tubercles barely evident, palmar accessory tubercles small and rounded; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap expanded, lanceolate, more evident on toes II–V; (12) plantar tubercle indistinguishable, subarticular tubercles present (one on toes I and II, two on toes III and V, and three on toe IV), first tubercle more evident; small and rounded supernumerary tubercles; inner metatarsal tubercle elongated, outer metatarsal tubercles rounded and smaller than inner; tarsal ridge absent; (13) dorsal ground colour in life brown, cream, with dark reticulations, venter translucent (Wolffian duct visible), with a dark speckle and sky-blue spots, vocal sac bright yellow (Fig. 14E, F); (14) SVL 19.3 ± 2.38 (16.9–24.8, $N = 13$), males 18.2 ± 1.09 (16.9–19.9, $N = 10$), females 22.9 ± 1.69 (21.5–24.8, $N = 3$); (15) advertisement call composed of a single, amplitude-modulated short note (0.09–0.15 s) with harmonic structure, and with most energy emitted with the first harmonic call (3.44–3.48 kHz).

Description of the holotype

An adult female (SVL 22.68) with a slender body; dorsal skin smooth with scattered tubercles, with a small supraciliary tubercle, ventral skin smooth, discoidal fold not evident; eye 1.70 times longer than snout; tympanum of moderate size, ratio TD/EL 36%, tympanic annulus present, but only the lower part clearly visible, tympanic membrane absent; head as wide as long (HL/HW 0.95), greatest head width between angles of jaw 39% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region feebly concave; dentigerous processes in front of the orbit of eyes, perpendicular in direction to the centre of roof of mouth, in a straight outline, each with seven teeth; vocal slits absent; tongue long (18% of SVL) and broadening to the tip, first third attached to floor of mouth; hands moderate in size, 18% of SVL; relative lengths of adpressed fingers I < II < IV < III; finger II smaller than finger VI, finger II reaching the disc on finger IV when adpressed; finger III disc 2.16 times wider than distal end of adjacent phalanx; palmar tubercle rounded to ovoid, larger than thenar tubercle; thenar tubercle elongate; subarticular tubercles rounded and globular, first tubercle more evident; supernumerary tubercles rounded and small; palmar and plantar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate length, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the last third of distal phalanx of toe II; disc of toe IV expanded, 1.73 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles present (one each on toes I and II, two on toes III and V, and three on toe IV), first subarticular tubercles more evident than the rest; inner metatarsal tubercle ovoid; outer metatarsal tubercles rounded, slightly pointed, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even broadened; ungual flap expanded, fingers and toes III and IV lanceolated; pads globular in profile (Fig. 7C).

Coloration of holotype in life

Holotype (SMF 97663; Fig. 12) recorded as follows: iris geranium (66) with fine sepia (286) reticulations; dorsal ground colour walnut brown (27), with sepia (286) blotches, and small sky-blue (192) dots; a flesh ocher (57) interorbital band, bordered posteriorly by a sepia (286) band; groin Pratt's ruby (68); axilla and venter walnut brown (27) mottled with pale pinkish buff (3), throat suffused with buff (5); fingers and toes with a pale buff (1) band just before the disc cover.

Coloration in preservative

Dorsal ground colour drab (19), with a pair of dorsolateral lines light orange yellow (7); groin and

ventral areas light buff (2), with small points sepia (279); ungual flaps cinnamon–drab (50).

Measurements of holotype (mm)
SVL 22.68; HL 8.43; HW 8.89; IOD 2.52; EL 3.33; TD 1.20; FL 8.12; TL 9.75; HAL 4.49; 3FW 0.50; 3FD 1.08; 3TW 0.51; 3TD 0.91; 4TW 0.52; 4TD 0.90; BW 7.81 (see Table 4).

Vocalization
Through call amplification of a recording containing several species (4–dB amplified; for an explanation, see Material and methods), we were able to extract the calls of three species: *Pristimantis* sp. (DF 2.76 kHz), *D. diastema* (DF 2.99 kHz), and *D. pequeno* sp. nov. (environmental temperature 24 °C; 8 October 2012, 18:17 h). Eleven calls were analysed (because the similarity of the call intervals indicates a single individual), consisting of single, short, monophasic notes that are reminiscent of a 'tink' (Fig. 6). Note duration is 0.09–0.15 s, with an interval between calls of 3.51–6.85 s and a call rate of 11.61 call/min; the low frequency was 3.20–3.23 kHz, the high frequency was 3.63–3.67 kHz, and the fundamental frequency is also the dominant frequency at 3.44–3.48 kHz.

Natural history
This species is found in the eastern Panamanian montane forest (Fund & Hogan, 2012) of the Darién mountain range (Fig. 1). Most specimens were found at 0.2–1.0 m above ground, over green leaves, between branches with dry leaves or in bromeliads. At the first location (472 m a.s.l.) the understory was open. The predominant vegetation were palms, vines, and small trees; at the second location above 700 m a.s.l. bromeliads were predominant; *D.* aff. *pequeno* sp. nov. was found to be sympatric with *D. diastema* and *D. quidditus*, and all species were actively calling.

Etymology
The species name *pequeno* is derived from the name Bajo Pequeño (or Bajo Chiquito), the last village at Río Tuquesa, where this species was found.

DIASPORUS SAPO SP. NOV.
FIGS 13, 14G, H
ELEUTHERODACTYLUS SP. – MYERS 1969: FIG. 19C.

Holotype
SMF 97329 (original field number AB 429), an adult female (Fig. 13) collected by Abel Batista & Gustavo Dojirama at the top of Cerro Sapo, PND, Distrito de Garachiné, Darién, Panama, on 4 December 2011, at 20:00 h (7.97618 N, 78.36263 W; 1169 m a.s.l.).

Paratypes
MHCH 2853–58, SMF 97328, SMF 97330–32; same collecting data as for holotype.

Diagnosis
Diasporus sapo sp. nov. is characterized by the following combination of characters (see Tables 4–6): (1) dorsal skin texture slightly tuberculate, venter smooth; (2) tympanum indistinguishable, annulus tympanicus and tympanic membrane absent; (3) snout rounded in dorsal view and in profile; (4) conical supraocular tubercle and cranial crests absent; (5) dentigerous processes of vomers with between seven and 11 teeth each, straight in outline, from the centre of the orbit to the centre of the roof of mouth; (6) vocal sac and vocal slits not differentiated, only a slightly differentiated fold beside the tongue, no nuptial pads; (7) finger II longer than finger I, ungual flap expanded, spadate, more evident on fingers II–IV; (8) no fringes or webbing on fingers; (9) palmar tubercle ovoid or rounded, flattened and almost the same size as thenar tubercle; thenar tubercle elongate; subarticular tubercles rounded and globular; two or three supernumerary tubercles; (10) heel smooth; (11) no fringes or webbing on toes, ungual flap on toes expanded, spadate, more evident on toes IV and V; (12) plantar tubercle indistinguishable, subarticular tubercles rounded and globular (one on toes I and II, two on toes III and V, and three on toe IV); foot without supernumerary tubercles; inner metatarsal tubercle elongated, outer metatarsal tubercles rounded and

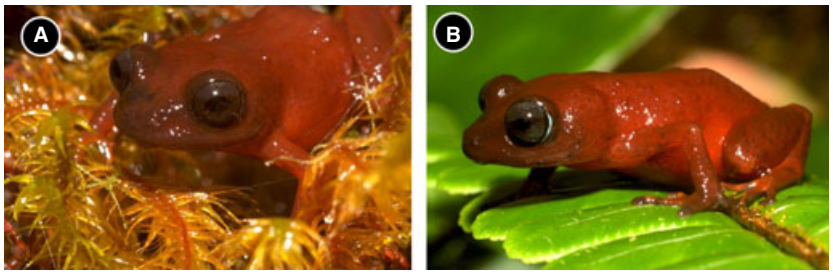

Figure 13. Holotype of *Diasporus sapo* sp. nov.: A, B, frontal and lateral views, respectively.

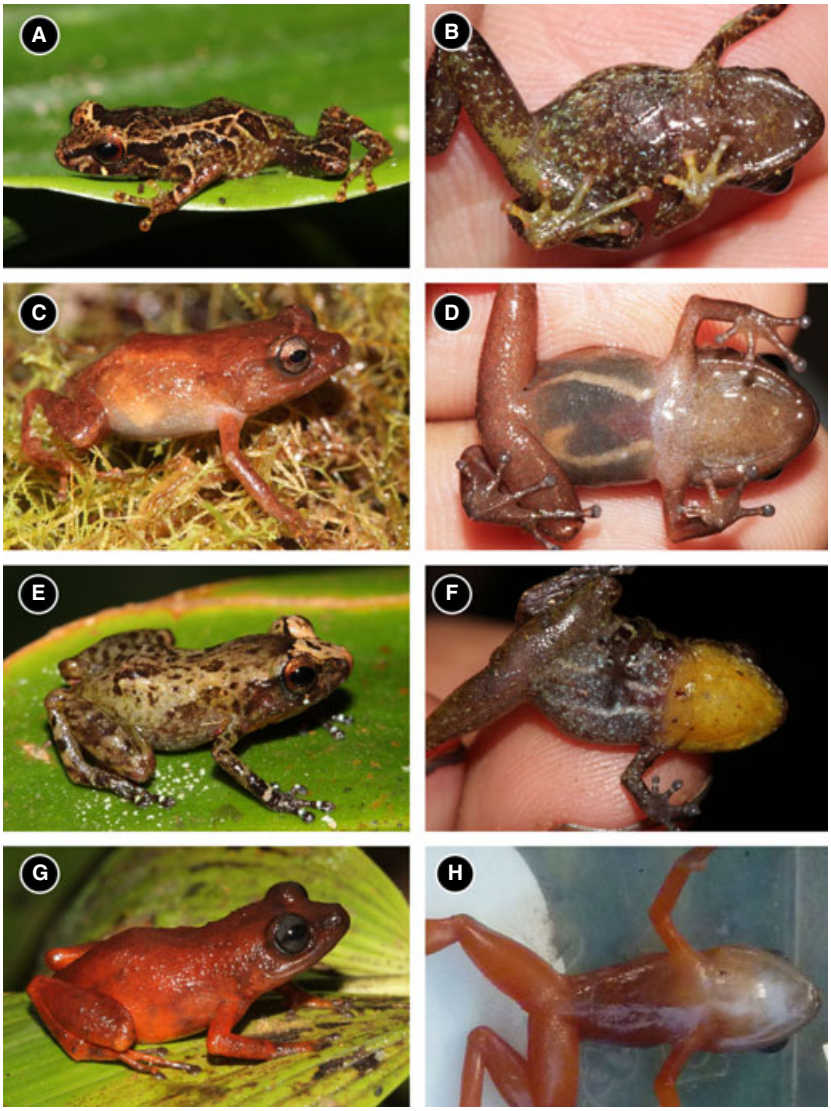

Figure 14. Colour variation of the new *Diasporus* species: A, B, *Diasporus darienensis* sp. nov. (SMF 97305); C, D, *Diasporus majeensis* sp. nov. (SMF 97658); E, F, *Diasporus pequeno* sp. nov. (MHCH 2830); G, H, *Diasporus sapo* sp. nov. (G, not collected; H, MHCH 2854).

globular, smaller than inner; tarsal ridge absent; (13) dorsal ground colour in life reddish and patternless, venter translucent, vocal sac not visible (Fig. 14H); (14) SVL 22.6 ± 2.86 (18.8–29.1, N = 11), males 22.6 ± 2.59 (19.9–29.1, N = 9), females 22.6 ± 5.3 (18.8–26.3, N = 2); (15) advertisement call unknown.

Description of the holotype

An adult female (SVL 28.91), with slender body; dorsal skin texture slightly tuberculate, venter skin smooth, discoidal fold not evident; protuberant eyes 1.78 times longer than snout; tympanum small, ratio TD/EL 23%; tympanum indistinguishable, annulus tympanicus and tympanic membrane absent, positioned 2.6 mm behind the orbit; head as wide as long (HL/HW 0.95), greatest head width between angles of jaw 35% of SVL; snout rounded from above and in profile; nares situated near tip of snout and slightly dorsolaterally directed, visible in frontal view, also visible dorsally but not ventrally; canthus rostralis rounded; loreal region concave; dentigerous processes of vomers with ten (right) and eight (left) teeth each side, straight in outline, from the centre of the orbit to the centre of the roof of mouth, and separated by a space of half of its total length; vocal slits absent; tongue long (26% of SVL) and broadening to the end, first third attached to floor of mouth; hands moderate in size, 22% of SVL; relative lengths of adpressed fingers

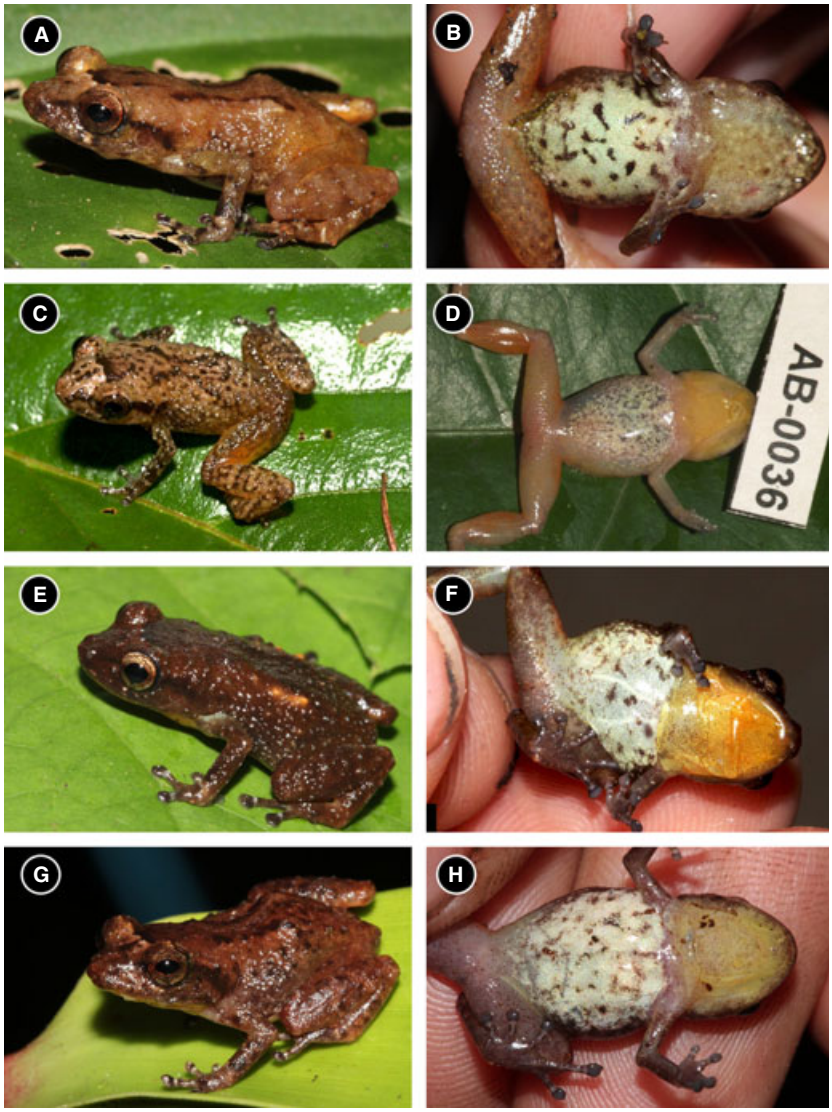

Figure 15. *Diasporus* aff. *diastema*, dorsal and ventral views: A, B, Majé mountain range (MM), near Ambroya (MHCH 2801); C, D, eastern Panamanian lowlands (EPL), Río Mono, near Bayano (MHCH 2806); E, F, Gatún, Colón, near type locality (SMF 97287); G, H, Darién mountain range (DM), Bajo Pequeño, Río Tuquesa (SMF 97289).

I < II < IV < III; finger II smaller than finger VI, finger II reaching the middle of disc on finger IV when adpressed; finger III disc 2.07 times wider than distal end of adjacent phalanx; palmar tubercle ovoid to rounded, flattened, and almost the same size as thenar tubercle; thenar tubercle low and elongate; subarticular tubercles rounded and globular; no supernumerary tubercles; two palmar accessory tubercles small and rounded; no nuptial pads; no fringes on fingers; hindlimbs of moderate lengths, TL 43% of SVL; relative lengths of adpressed toes I < II < III < V < IV; when adpressed, tip of toe I reaches the disc base of toe II; disc of toe IV expanded, 2.11 times wider than distal end of adjacent phalanx; no fringes on toes; subarticular tubercles rounded and globular (one each on toes I and II, two on toes III and V, and three on toe IV); inner metatarsal tubercle elongated; outer metatarsal tubercles rounded, globular, and smaller than inner; tarsal ridge absent; hands and feet without webbing; finger and toe discs even broadened and slightly globular in profile (Fig. 7D); ungual flap on toes expanded, spadate, more evident on toes IV and V.

Coloration of holotype in life

Coloration recorded as follows (Fig. 13): iris medium neutral gray (298) with reticulations sepia (286), iris periphery jet black (300), eye periphery sky blue (192); dorsal ground colour uniform Pratt's ruby (68), becoming darker to the front as dark carmine (61); venter and limbs chrome orange (74), throat pale buff (1).

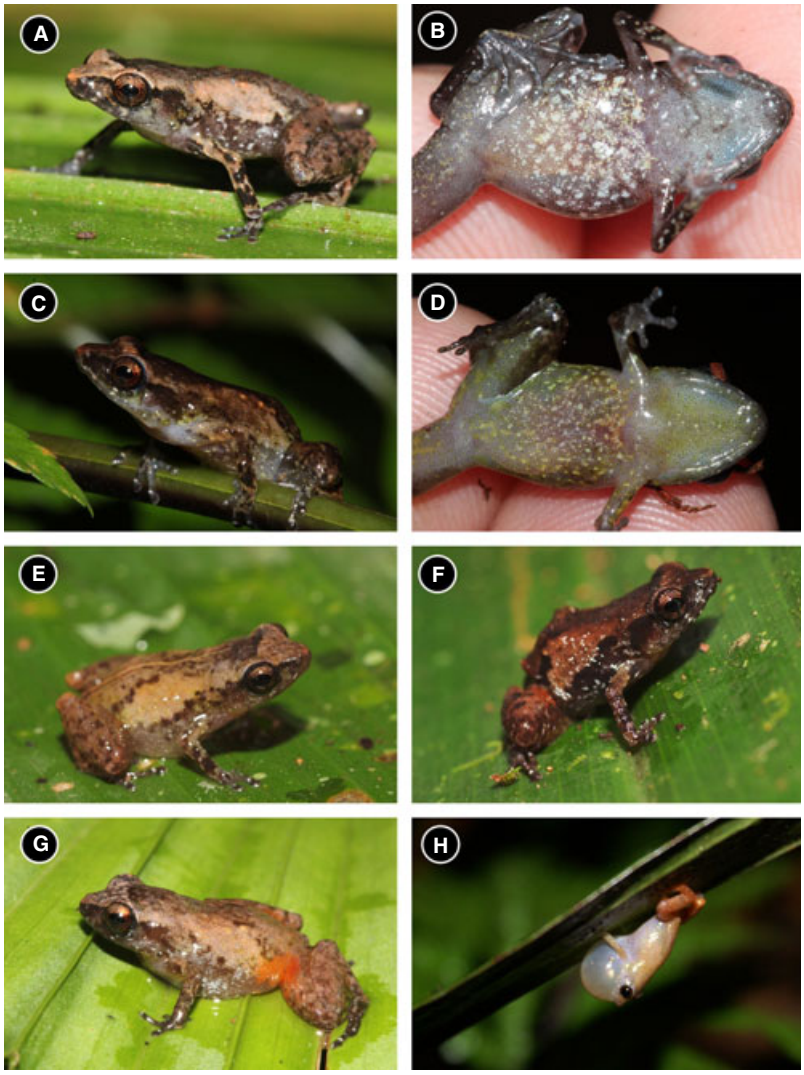

Figure 16. *Diasporus* aff. *quidditus*: A, B, Pirre mountain range (PM), Perresenico stream (MHCH 2824); C, D, Jingurudó-Sapo mountain range (JSM),, near Pavarandó (SMF 97653); E, PM, Cana Field Station (MHCH 2813); F, PM, Pirre ridge (SMF 97292); G, San Blas mountain range (SBM), Taintidu river (SMF 97298); H, calling male under leaf, 20 cm from ground, SBM, Burbayar Private Reserve.

Coloration in preservative
Dorsal ground colour cinnamon–drab (50), becoming darker to warm sepia (40) to the tip of snout; limbs and venter cream colour (12), throat buff (5), hand and foot drab (19).

Measurements of holotype (mm)
SVL 28.91; HL 9.63; HW 10.19; IOD 2.93; EL 4.29; TD 0.98; FL 11.58; TL 12.33; HAL 6.37; 3FW 0.52; 3FD 1.8; 3TW 0.61; 3TD 1.12; 4TW 0.57; 4TD 1.20; BW 9.99 (for variation in the species, see Tables 4–6).

Natural history
This species is known only from the top of Cerro Sapo, which is covered by elfin forest. The vegetation predominantly consists of small trees (roughly 10 m in height) fully covered with moss and bromeliads. *Diasporus sapo* sp. nov. was most often found at 1–2 m above ground during the night; individuals were seen walking over tree branches and tree bark.

Etymology
The species name is derived from the name of Cerro Sapo, where the species was found.

DISCUSSION

The application of an integrative approach has resulted in the description of four new species within the genus. Additionally, the historical reconstruction

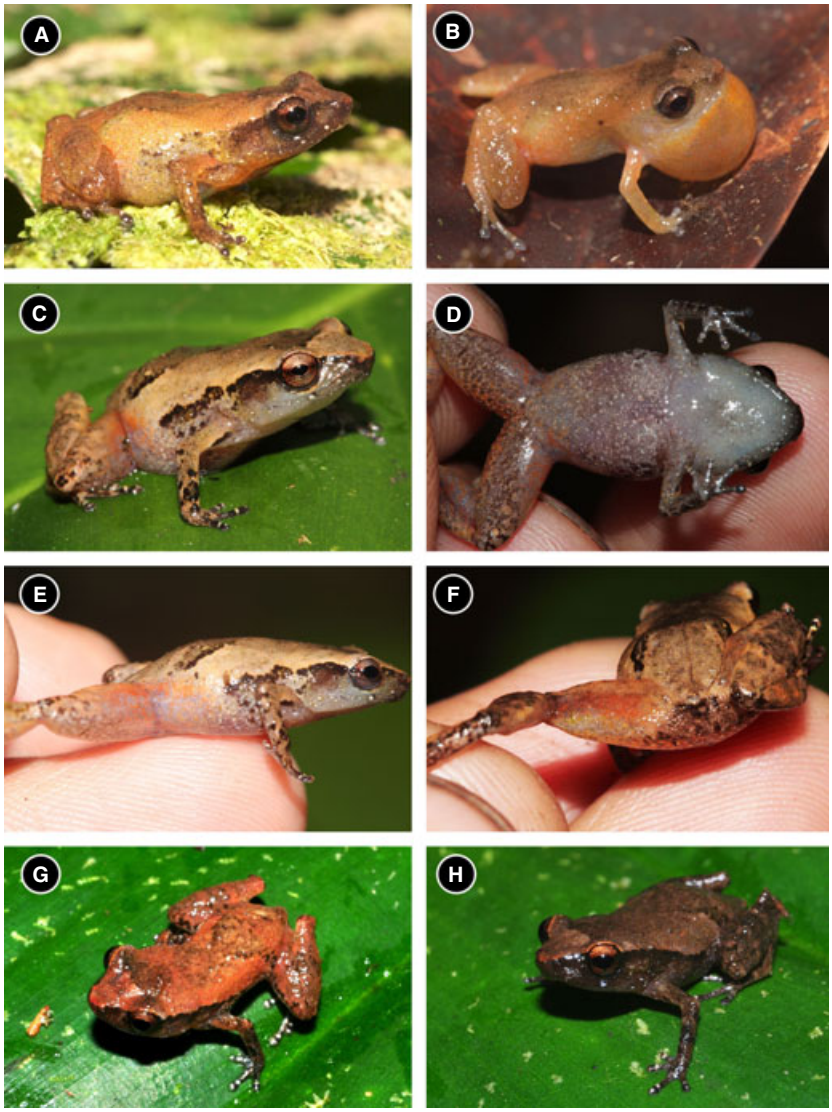

Figure 17. *Diasporus tinker*: A, B, Pirre mountain range (PM), Pirre ridge (MHCH 2864); C, F, PM, Pirre ridge (SMF 97324); G, H, Jingurudó-Sapo mountain range (JSM), showing metachrosis (same specimen); G, night coloration; H, day coloration.

of the biogeography for the *Diasporus* species confirms a Middle American origin of the genus, as revealed by previous studies (e.g. Pinto-Sánchez, Crawford & Wiens, 2014), as the older clade is represented by species distributed in western Panama (Fig. 2). Moreover, the 11 amphibian species endemic to EP (Ibáñez & Crawford, 2004; Crawford, Ryan & Jaramillo, 2010a; Crawford, Lips & Bermingham, 2010b; Batista *et al.*, 2014a; this paper) support the hypothesis of EP being a centre of endemism rather than just a pathway between two continents during the Great American Biotic Interchange (GABI; Crawford *et al.*, 2010a,b).

Morphology and ecology

Most diagnostic characters used to differentiate species within the genus *Diasporus* are morphological traits: usually SVL (body size), ungual flap (disc cover), and colour pattern (Savage, 1997; Lynch, 2001; Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012). Although SVL appears to be helpful in identifying species because of remarkable interspecific variation (Fig. 3), this character is valid only to differentiate between species with no overlap in SVL, or supported along with other morphological characters. In EP there are two groups of *Diasporus*,

separated by the ungual flap shape (see results, Fig. 3). The lanceolate or papillate ungual flap present in *D. pequeno* sp. nov., *D. quidditus*, *D.* aff. *vocator*, and *D. tinker* seems to have evolved from one common ancestor. Although those species build a monophyletic clade with members of the *D. diastema* complex, the latter do not possess lanceolate or papillate ungual flaps. This possibly means that a lanceolate/papillate ungual flap was subsequently lost in *D. diastema* and *D. citrinobapheus*. The function of the ungual flap in these frogs is not known, but interestingly all species with lanceolate or papillate ungual flaps are inhabitants of the lower understory up to 1 m above ground (including the western Panamanian *D. vocator*), and only populations of *D. quidditus* from Colombia are usually found above this height. There are other rain frogs or Terrarana (Hedges *et al.*, 2008; former genus *Eleutherodactylus*) with lanceolate or papillate ungual flaps [*Craugastor gollmeri* group, *Pristimantis chalceus* (Peters, 1873), *Pristimantis scolodiscus* (Lynch & Burrowes, 1990), *Noblella* spp.] that are also inhabitants of the forest floor or the low understory (Savage, 1987; Lynch & Duellman, 1997; Duellman & Lehr, 2009). In contrast, lanceolate or papillate ungual flaps are usually not present in other Terrarana members with more arboreal habits (e.g. *Pristimantis* spp.; Savage, 2002; Duellman & Lehr, 2009). So we suggest that the expression of this character is related to the understory habit of these frogs.

Diasporus diastema has more palmate than spadate disc covers, as stated by Savage (1997, 2002), Lynch (2001), and Köhler (2011); however, we found specimens at the mouth of Chagres River, Colón, Panama (near the type locality) and in EP with spadate rather than palmate finger disc covers. We presume that the authors mentioned above did not examine *D. diastema* specimens from central Panama, but only examined specimens from Costa Rica that are likely to represent at least one different species (Savage, 1997, 2002: plate 127; Lynch, 2001: fig. 2D KU 35149; Köhler, 2011: figs 437, 39). Moreover, the disc cover shape can be highly variable as a result of different preservation techniques (Lynch, 2001). Such high variation in disc shape has been described within and between populations of *Pristimantis caryophyllaceus* (Barbour, 1928) (Batista *et al.*, 2014b), among other species of Terrarana, leaving this trait as a controversial tool to identify species in preserved specimens.

Most species of *Diasporus* are inhabitants of the understory of tropical forests. Nevertheless, there are differences in the spatial distribution of species: whereas *D. pequeno* sp. nov., *D. quidditus* (Fig. 16), and *D. tinker* (Fig. 17) occupy the zone from near ground level up to 1 m above the forest floor, *D. diastema*, *D. majeensis* sp. nov., and *D. sapo* sp. nov. are usually found higher, 1.0–2.5 m above the ground, and *D. darienensis* sp. nov. has been found even higher, from 1 m up to 5 m above ground. There is also controversy about the smallest species, *D. quidditus*, which was originally described from Colombia, with specimens from Panama referred to this species (Lynch, 2001). Although the populations from Panama and Colombia are almost similar in appearance (size and colour), they vary substantially in ecology. Specimens from Colombia are reported to inhabit the mid-level of the forest, with males calling from the undersides of leaves at 1.5–4.0 m above ground (Lynch, 2001). After more than 3 years of field experience with this species in EP and observing more than a hundred calling males, we never encountered *D. quidditus* (Fig. 16) calling from a position higher than 0.5 m above the ground. Unfortunately, we have neither acoustic nor molecular data of topotypic specimens of *D. quidditus* to compare with our specimens. Therefore, the taxonomic relevance of these geographical behavioural variations remains unclear. Thus, we consider the populations from EP as *D.* aff. *quidditus* until more detailed molecular and bioacoustics comparison between the populations from EP and Colombia become available.

Bioacoustic

To the human ear, the calls of *Diasporus* species are very similar to each other. A typical advertisement call was described as a simple 'tink tink' or 'dink dink' (Savage, 1997, 2002; Lynch, 2001). With such a simple subjective call description it was difficult to distinguish among the calls of different species; however, in recent years detailed descriptions of advertisement calls for 12 of the 15 described species have been published (Chaves *et al.*, 2009; Batista *et al.*, 2012; Hertz *et al.*, 2012; Jiménez *et al.*, 2013; this paper). It has been shown that comparison of acoustic parameters is a powerful tool for species identification in the genus *Diasporus*. To date, only call descriptions of *D. gularis*, *D. sapo* sp. nov., and *D. tigrillo* are pending.

Spectral and temporal parameters of the calls have been used successfully to evaluate the taxonomic status in Terrarana frogs. Even with a limited sample size these parameters show differences between *Diasporus* spp. (Padial *et al.*, 2008; Figs 4 and 5). The discriminant function analysis properly sorted species when we compared dominant frequency (DF) against note duration, but not if we used DF against call rate (Figs 4 and 5). Some species, such as *D. citrinobapheus*, *D. diastema* (at least for Panamanian populations of *D.* aff. *diastema*), *D. tinker*, and

D. vocator have calls that are organized in bouts. In species with calls organized in bouts, the note interval decreases from the beginning towards the end of the call group. Thus, the high variation of DF versus call rate in those species is a result of this modulation of the note interval. We recommend that more call recordings are made in future studies (with at least ten individuals and ten calls/individual per population) to evaluate the effect of the note interval modulation on the call rate. Four species of *Diasporus* are known to call in bouts, but we did not evaluate whether this is a phylogenetic character of related species. Usually, temporal parameters can be related to evolutionary constraints or to environmental selection (Ryan, Cocroft & Wilczynski, 1990; Bosch & De la Riva, 2004); however, calling in bouts is often used to facilitate note alternation between conspecific neighbours (Schwartz, 1991), as well as to restore energetic deficits during a calling session (Leary *et al.*, 2004). It is known that orthopterans that call in bouts can affect female preferences (Hendrick, 1986), but in *Diasporus* spp. the reason for this calling pattern remains unknown.

The call of *D. diastema* from central Panama was described by Fouquette (1960) and Wilczynski & Brenowitz (1988), but their temporal and spectral measurements are highly variable, which might be an artifact of involving several species in the recording (Hertz *et al.*, 2012). We have analysed a sample of *D. diastema* from a site near the type locality (Figs 6C and 16E, F), and its DF ranged between 3.34 and 3.47 kHz, which agrees well with data from Wilczynski & Brenowitz (1988). Nevertheless, some incongruence has been detected in the note duration (Hertz *et al.*, 2012). According to our experiences, measuring temporal parameters can sometimes lead to erroneous results. This is especially true when trying to determine the end point of the call on the oscillogram, or if the recording includes considerable background noise. The *D. diastema* specimen (SMF 97287; Fig. 15E, F) that we found at the mouth of the Chagres River was the only *Diasporus* specimen found within a radius of 2 km from this site, which is approximately 9 km from the type locality near Margarita, Colón, Panama (Dunn, 1942; Taylor, 1955; Savage, 1973; Hertz *et al.*, 2012; Fig. 16E, F). Therefore, we assume that this specimen belongs to *D. diastema* as originally described by Cope (1876). Nevertheless, it is difficult to test for conspecificity by comparing the morphology of our specimen with that of the holotype of *D. diastema*, as it is in poor condition (Cochran & Goin, 1970; A. Hertz, pers. observ.); however, it should be noted that the specimen from the mouth of Chagres River and the holotype of *D. diastema* are at least of almost similar size (*D. diastema* holotype, SVL 20.0 mm; *Diasporus* sp. from Chagres River, SVL 19.0 mm). In this area the only other congeneric frog is *D.* aff. *vocator* (see also Ibáñez *et al.*, 1999), which is significantly smaller than *D. diastema*, (Fig. 3; Table 4), and calls at a higher DF.

Barcoding and phylogenetic inference

DNA barcoding is a useful tool for species identification (Hebert *et al.*, 2004; Crawford *et al.*, 2010b; Jörger *et al.*, 2012; Paz & Crawford, 2012); however, the straightforward application of this approach could yield misleading interpretations of biodiversity (see Trewick, 2008; Huang *et al.*, 2013; Shen, Chen & Murphy, 2013). DNA sequence information in the absence of other lines of evidence should never be used for species delimitations (DeSalle, 2006). Here we are using molecular barcoding along with other methods to reveal unnamed species within the genus *Diasporus* from EP. We found high genetic divergence between lineages above the suggested threshold to identify candidate species in Neotropical amphibians (>3.0% in *16S* and >10% in *COI*; Vences *et al.*, 2005; Fouquet *et al.*, 2007; Crawford *et al.*, 2013), and most of them were supported by the barcoding analysis (ABGD; Puillandre *et al.*, 2011). Differences in the barcoding genes were additionally supported by bioacoustics, ecology, morphology, and phylogeography. According to the integrative analysis, most lineages identified as species showed considerable genetic distances and are monophyletic in the reconstructed tree; however, the polyphyly in members of the *D. diastema* complex is problematic (Fig. 8). Whereas *D. citrinobapheus* is monophyletic, although consisting of two subclades, and is well differentiated from *D. diastema*, *D. diastema* itself is paraphyletic. We included two specimens from central Panama, of which SMF 97287 is most probably a 'true' *D. diastema*, whereas SMF 97290 could represents another genetic lineage. We were not able to clearly distinguish between separate lineages of *D.* aff. *diastema* from MM or EPL, nor raise any of them to species level (Tables 4–6). Despite the fact that they showed a genetic distance above the threshold used to recognize candidate species within this group (e.g. >4.0% genetic p–distance in mtDNA *16S*; Table 1), we prefer treating these populations as unconfirmed candidate species (Vieites *et al.*, 2009), and label all specimens other than SMF 97287 as *Diasporus* aff. *diastema* until more comparative data from more widespread populations become available.

Phylogeography

The results of our chronological tree indicate that species from EP are younger than those from WP

(Fig. 9). Thus, the ancestors of the genus *Diasporus* have originated somewhere in Lower Central America (see also Pinto-Sánchez *et al.*, 2014). Recent hypotheses on the formation of the land bridge between South America and North America suggest that the Isthmus of Panama was connected with the north-western landmass of South America via an island arc during the mid-Miocene around 15 Mya (Montes *et al.*, 2012a,b). *Diasporus* species from WP and EP split around this time (15 Mya), promoting the subsequent evolution into numerous *Diasporus* spp. within EP. Later on, another vicariant event took place *in situ*, probably induced by eustatic fluctuations during the middle and late Miocene (as early as 11 Mya), such as the flooding of the Atrato and Chucunaque basins (Duque-Caro, 1990; Coates *et al.*, 2004). This consequently separated populations, including the predecessors of the three closely related species *D. darienensis* sp. nov., *D. majeensis* sp. nov., and *D. sapo* sp. nov. that became isolated on separate land masses of EP during this period (5–8 Mya), and evolved allopatrically into distinct species. Around the same time (5–6 Mya), *D.* aff. *diastema* from MM split from the rest of the *D.* aff. *diastema* complex and remained isolated within the foothills of MM, evolving only minor morphological changes.

A hypothetical route of colonization and speciation for *Diasporus* frogs in EP is as follows: *Diasporus* ingresses into EP through the San Blas peninsula when it started to uplift (around 20 Ma, Montes *et al.*, 2012a). The Chucunaque and Atrato basins isolated the islands of Maje-Baudo in the south and Dabeiba (e.g. the San Blas mountain range) in the north. *Diasporus* populations colonized those islands either by over sea dispersal (debris rafting) and/or via a temporary land connection. The clade of *D. dariensis*, *D. sapo* sp. nov., and *D. majeensis* sp. nov. evolved on the southern island, whereas the remainder species (*D.* aff. *diastema*, *D. pequeno* sp. nov., *D.* aff. *quidditus*, and *D. tinker*) evolved on the northern island. *Diasporus pequeno* sp. nov. is the oldest lineage of the latter clade, whereas the other species from the southern island dispersed more recently when continuing tectonic events and sedimentation allowed occasional migrations over more shallow and narrow water bodies. Ancestors of *D. tinker* expanded east and crossed the Atrato corridor. The ancestors of *D.* aff. *quidditus* evolved during an extended period on the northern island, and began dispersing into South America when the Isthmus of Panama was nearly completed. The *D.* aff. *diastema* clade including *D. citronobapheus* expanded in both western and eastern directions.

CONCLUSION

Many species within the genus *Diasporus* in EP have been difficult to differentiate: as they were based solely on external appearance, a new approach became necessary. Based on a comprehensive analysis of *Diasporus* samples from EP, we bring new insights into bioacoustics, ecology, molecular diversification, and morphology, and reconstruct the phylogeography of the genus in this region. All new species described herein were well supported by the integration of these approaches. Although this study substantially raises the number of known species of the genus, the diversity of *Diasporus* spp. still promises to grow in the future. During the last 4 years seven species have been described (including those described here); therefore, we can expect that integrative taxonomical approaches on the genus in western Panama, Costa Rica, and Colombia may further raise the number of species.

KEY TO THE SPECIES OF THE GENUS *DIASPORUS*

1a. Ungual flap lanceolate or papillate at least on the third finger or third and fourth toe. 2
1b. Fingers and toes with rounded or spadate ungual flap. 6
2a. Very small frogs; SVL usually <17 mm. 3
2b. Small frogs; SVL usually >17 mm 4
3a. Dorsum shagreen; fingers without thick lateral fringes; toe V not partially fused with toe IV; SVL of adult males 14.0–16.0 mm, adult females 16.5–18.0 mm; calls with DF of 4.35–5.10 kHz . . . . . *Diasporus vocator*
3b. Dorsum with scattered low warts; fingers with thick lateral fringes; toe V partially fused with toe IV; SVL of adult males 10.9–14.8 mm, adult females 13.2–16.9 mm; calls with DF of 4.55–5.08 kHz . . . *Diasporus* aff. *quidditus* (populations from EP)
4a. Disk expanded with cuspidate pads, skin smooth aside from low flattened warts, no perianal warts; vocal sac pale brown or orange in males; calls with DF of 3.14–3.71 kHz . . . . . *Diasporus tinker*
4b. Disk expanded with rounded pads, skin texture smooth, with small scattered tubercles, perianal warts may or may not be present, vocal sac bright yellow in males 5
5a. Finger III with a small papillate ungual flap; dorsal pattern brownish with dark blotches; ventral areas translucent with dark speckles and small sky-blue blotches; males with bright

yellow vocal sac; calls with DF of 3.44–3.48 kHz . . . . . *Diasporus pequeno* sp. nov.

5b. Fingers without papillate ungual flap; dorsal colour yellowish tan, with brown markings; ventral surfaces white . . . . . *Diasporus gularis*

6a. Reddish colour pattern on dorsum, venter translucent or with distinct black and white blotches 7

6b. Dorsal colour pattern variable, pale, dark brown, or yellowish, venter white or cream in colour, with dark blotches or suffused (or speckled) with dark colour 10

7a. Venter translucent. 8

7b. Venter usually with distinct black and white blotches, males have white venters with red spots and females have white venters with black spots; calls with DF of 2.50–2.61 kHz. . . . . *Diasporus ventrimaculatus*

8a. Outer edge of the tibia and forearm smooth, without a series of tubercles. 9

8b. Outer edge of the tibia and forearm covered with a series of tubercles; calls with DF of 2.4 kHz . . . . . *Diasporus igneus*

9a. Dorsal colour uniform red; eye periphery sky blue; species restricted to the Sapo-Jingurudó mountain range . . . . . *Diasporus sapo* sp. nov.

9b. Dorsal colour reddish with brown or pale reticulations; eye periphery black; species restricted to the Majé mountain range; calls with DF of 2.47–2.71 kHz . . . . . *Diasporus majeensis* sp. nov.

10a. Dorsal pattern yellowish, usually suffused with pink or red; venter translucent without blotches, or with speckled pattern 11

10b. Dorsal pattern dark or pale brown, venter cream with dark spots or dark with white flecks 13

11a. Dorsum uniformly bright yellow to orange, colour of posterior surface of thigh same colour as dorsum; adults with vomerine teeth 12

11b. Posterior surface of thigh often suffused with pink or red in life; adults without vomerine teeth; calls with DF of 2.35–3.05 kHz. . . . *Diasporus hylaeformis*

12a. Dorsum smooth, uniformly bright yellow to orange, sometimes with irregularly distributed dark blotches; distal subarticular tubercle on finger I and toe I flat and rounded; SVL of adult males 17.3–19.7 mm; calls with DF of 2.86–3.04 kHz . . . . . *Diasporus citrinobapheus*

12b. Dorsum with scattered low pustules, dorsum yellow to orange with dark-brown spots confined to pustules; distal subarticular tubercle on finger I and toe I weakly bifid; SVL of adult males 16.0–17.5 mm. . . . . *Diasporus tigrillo*

13a. Dorsal pattern pale brown or reddish; venter cream with dark spots or suffused with reddish colour; axilla and groin cream in colour or same colour as dorsum. 14

13b. Dorsal pattern black with short red lines; axilla and groin scarlet; ventral surfaces black with white flecks; calls with DF of 3.81 kHz . . . . . *Diasporus anthrax*

14a. Dorsal pattern pale brown with dark spots; venter cream with dark spots; axilla and groin cream in colour; calls with DF of 2.96–3.55 kHz.. . . . . *Diasporus* aff. *diastema* (populations from CP and EP)

14b. Dorsal pattern reddish with pale lines or blotches; venter suffused with reddish colour; axilla and groin unpigmented or same colour as dorsum; calls with DF of 3.34–3.81 kHz . . . . . *Diasporus darienensis* sp. nov.

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APPENDICES

Appendix 1. Details of the museum voucher numbers (when available) and collecting locality for all *Diasporus* samples used in this study.

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 89819	*D.* aff. *citrinobapheus*	Alto de Piedra, Santa fé National Park	Panama	8.51449	81.1171	878
MHCH 2373	*D.* aff. *citrinobapheus*	Alto de Piedra, Santa fé National Park	Panama	8.51449	81.1171	878
LSt 018	*D.* aff. *citrinobapheus*	Cerro Mariposa, Santa fé National Park	Panama	8.51545	81.119	930
LSt 085	*D.* aff. *diastema*	Cerro Mariposa, Santa fé National Park	Panama	8.50128	81.11868	1215
LSt 120	*D.* aff. *diastema*	Cerro Mariposa, Santa fé National Park	Panama	8.52556	81.13168	652
ICN 41696 (holotype)	*D. anthrax*	Campamento la Miel II, near junction of quebrada Tasajos with Río la Miel, km 23 carretera la Victoria-Samaná; Caldas, Colombia	Colombia			700
ICN 41697 (paratype)	*D. anthrax*	Bosque de San Rafaél, Municipio San Rafael, Antioquia, Colombia	Colombia			1200
MHCH 2840	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94739	77.7042	1317
MHCH 2841	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98792	77.70774	1127
MHCH 2844	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98792	77.70774	1127
MHCH 2845	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98771	77.70783	1149
MHCH 2846	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97976	77.70843	1133
MHCH 2847	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98	77.70839	1139
MHCH 2850	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68289	78.03846	959
MHCH 2851	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67942	78.03868	946
MHCH 2852	*D. darienensis* sp. nov.	Bailarín mount, Jingurudó mountain range	Panama	7.69312	78.04226	865
MHCH 2862	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98724	77.70785	1140
SMF 97303	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94739	77.7042	1317
SMF 97304	*D. darienensis* sp. nov.	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9632	77.70432	1267
SMF 97305	*D. darienensis* sp. nov.	From mirador 2 to Perresenico Stream to Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field Station	Panama	7.97312	77.70785	1143
SMF 97306	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98887	77.70739	1100
SMF 97307	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97864	77.70851	1112

Appendix 1 *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
SMF 97308	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.97935	77.70872	1085
SMF 97309	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97310	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97312	*D. darienensis* sp. nov.	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68357	78.03848	948
SMF 97313	*D. darienensis* sp. nov.	Sapo Mountain	Panama	7.97632	78.36269	1152
SMF 97314	*D. darienensis* sp. nov.	Pirre mountain range	Panama	7.98741	77.70787	1137
SMF 97661	*D. darienensis* sp. nov.	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9632	77.70432	1267
SMF 97662	*D. darienensis* sp. nov.	Pirre Mountain top (1400 m a.s.l.) to camp 2; Rancho Frío Field Station, Pirre mountain range	Panama	7.94719	77.7042	1326
MHCH 2801	*D. diastema*	Amborlla, La Javillosa ridge	Panama	8.91587	78.62897	906
MHCH 2802	*D. diastema*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
MHCH 2803	*D. diastema*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2804	*D. diastema*	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2805	*D. diastema*	Río Mono, 5.7 km SE from Bayano Bridge	Panama	9.1772	78.74551	107
MHCH 2806	*D. diastema*	Río Mono, 5.7 km SE from Bayano Bridge	Panama	9.17816	78.74582	100
MHCH 2807	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89224	78.56029	943
MHCH 2808	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89182	78.56268	788
MHCH 2809	*D. diastema*	Maje mountain range, Amborlla	Panama	8.89427	78.56509	622
MHCH 2810	*D. diastema*	Maje mountain range, Amborlla	Panama	8.8972	78.56762	610
MHCH 2811	*D. diastema*	La Moneda, Meteti, Darién	Panama	8.5974	78.04934	69
SMF 97286	*D. diastema*	Maje mountain range, Amborlla	Panama	8.91681	78.61779	485
SMF 97287	*D. diastema*	400 m W from the Gatún Locks, Colón	Panama	9.26021	79.9354	36
SMF 97288	*D. diastema*	Maje mountain range, Amborlla	Panama	8.92267	78.6253	852
SMF 97289	*D. diastema*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97290	*D. diastema*	Río Terable, Chepo	Panama	9.28399	78.98383	322
MHCH 1440	*D. diastema*	Donoso, Colón	Panama			
MHCH 1469	*D. diastema*	Donoso, Colón	Panama			
SMF 80781	*D. diastema*		Panama			
SMF 81961	*D. diastema*		Panama			
SMF 79796	*D. diastema*		Panama			
SMF 79797	*D. diastema*		Panama			
SMF 83391	*D. diastema*		Panama			
SMF 85135	*D. diastema*		Panama			
SMF 78965	*D. diastema*		Panama			
SMF 82033	*D. diastema*		Panama			

SMF 82032	*D. diastema*		Panama					
SMF 82035	*D. diastema*		Panama					
SMF 81812	*D. diastema*		Panama					
SMF 78187	*D. diastema*		Panama					
SMF 78188	*D. diastema*		Panama					
SMF 78189	*D. diastema*		Panama					
SMF 78190	*D. diastema*		Panama					
SMF 78186	*D. diastema*		Panama					
SMF 78191	*D. diastema*		Panama					
LSt 018	*D. diastema*		Panama					
LSt 085	*D. diastema*		Panama					
MHCH 1360	*D. diastema*	Bocas del Toro Island, Bocas del Toro	Panama					
MHCH 1379	*D. diastema*	Donoso, Colón	Panama					
MHCH 1427	*D. diastema*	Donoso, Colón	Panama					
SMF 85938	*D. diastema*		Panama					
SMF 79794	*D. diastema*		Panama					
SMF 79800	*D. diastema*		Panama					
SMF 79799	*D. diastema*		Panama					
SMF 83390	*D. diastema*		Panama					
SMF 83389	*D. diastema*		Panama					
SMF 85068	*D. diastema*		Panama					
SMF 84997	*D. diastema*		Panama					
SMF 80977	*D. diastema*		Panama					
SMF 80978	*D. diastema*		Panama					
SMF 80979	*D. diastema*		Panama					
SMF 82034	*D. diastema*		Panama					
SMF 82031	*D. diastema*		Panama					
SMF 29859	*D. diastema*		Panama					
SMF 29874	*D. diastema*		Panama					
SMF 81811	*D. diastema*		Panama					
SMF 78561	*D. diastema*		Panama					
SMF 78185	*D. diastema*		Panama					
SMF 78184	*D. diastema*		Panama					
SMF 77231	*D. diastema*		Panama					
LSt 123	*D. diastema*		Panama					
SMF 85939	*D. diastema*		Panama					
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321		
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321		
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321		
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321		

Appendix 1 *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. diastema*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
ICN 19306	*D. gularis*	Quebrada La Miquera, Vereda Venados, Parque Natural Nacional Las Orquideas, Antioquia	Colombia	6.53802	76.30345	1060
ICN 53771	*D. gularis*		Colombia			
ICN 45169	*D. gularis*	Centro Forestal Bajo Calima, Valle del Cauca	Colombia	3.98333	76.95	50
AH 242	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1820
AH 244	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1872
AH 245	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82515	82.50204	1780
HAU 012	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82511	82.49813	1778
HAU 013	*D. hylaeformis*	Bajo Mono, Boquete	Panama	8.82595	82.49904	1800
HAU 018	*D. hylaeformis*	Cerro Guayaba, Comarca Ngöbe buglé	Panama	8.75797	82.2572	1358
AH 486	*D. hylaeformis*	Cerro Sagui, Comarca Ngöbe buglé	Panama	8.5639	81.8221	2033
AH 175	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 178	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 176	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 177	*D. hylaeformis*	Reserva Forestal Fortuna	Panama	8.67685	82.19606	1750
AH 115	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 116	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 117	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 118	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.6775	82.198	1760
AH 380	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67857	82.19329	1793
HAU 007	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67885	82.20037	1810
HAU 011	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67885	82.20037	1750

AH 381	*D. hylaeformis*	Fortuna/Westhang Pata de Macho	Panama	8.67857	82.19329	1793
SMF 89872	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91138	82.71288	2068
SMF 89867	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.91844	82.72325	2332
	D. hylaeformis	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89874	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89873	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
MHCH	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89868	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89869	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.93138	82.7137	2400
SMF 89875	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91152	82.71253	2070
SMF 89876	*D. hylaeformis*	Jurutungo, Río Sereno, Renacimientos	Panama	8.91152	82.71253	2070
AH 042	*D. hylaeformis*	La Nevera, Comarca Ngöbe buglé	Panama	8.49966	81.77238	1700
AH 343	*D. hylaeformis*	La Nevera/Cerro Santiago Westhang	Panama	8.49546	81.76718	1815
SMF 89871	*D. hylaeformis*	Las Nubes, Cerro Punta, Chiriquí	Panama	8.89418	82.6149	2117
SMF 89870	*D. hylaeformis*	Las Nubes, Cerro Punta, Chiriquí	Panama	8.89418	82.6149	2117
NH 0034	*D. hylaeformis*	Lost and Found, Reserva Forestal Fortuna	Panama	8.67445	82.2193	1283
AH 236	*D. hylaeformis*	Volcán Barú/Sendero Quezales	Panama	8.84944	82.51538	2134
MHCH 1327 (holotype)	*D. igneus*		Panama			
MHCH 1388 (paratype)	*D. igneus*		Panama			
MHCH 2072 (paratype)	*D. igneus*		Panama			
SMF 89821 (paratype)	*D. igneus*		Panama			
SMF 89821 (paratype)	*D. igneus*	La Nevera, Comarca Ngöbe buglé	Panama	8.49546	81.76718	1815
MHCH 2832	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
MHCH 2833	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
MHCH 2834	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2835	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2836	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2837	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2838	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2839	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97293	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97655	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97656	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97657	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.79936	78.46156	1380
SMF 97658	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97659	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
SMF 97660	*D. majeensis* sp. nov.	Chucantí, Maje mountain range	Panama	8.80462	78.45951	1460
MHCH 2826	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47997	77.51941	859

Appendix 1 *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
MHCH 2827	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47997	77.51941	859
MHCH 2828	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2829	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2830	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2831	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97333	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97334	*D. pequeno* sp. nov.	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
SMF 97335	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97336	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97337	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97338	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
SMF 97663	*D. pequeno* sp. nov.	Bajo pequeño, camp 3 Pechito Parao Mountain	Panama	8.47911	77.52799	718
MHCH 2813	*D. quidditus*	Rio Cana, Cana field station, Chimenea trail	Panama	7.75602	77.68565	525
MHCH 2814	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68338	78.03844	943
MHCH 2815	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67978	78.03856	947
MHCH 2816	*D. quidditus*	Sapo Mountain	Panama	7.98013	78.35546	796
MHCH 2817	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2818	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2819	*D. quidditus*	Púcuro River, Darién	Panama	8.0575	77.37022	1043
MHCH 2820	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03434	78.022	228
MHCH 2821	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
MHCH 2822	*D. quidditus*	From Taintidu River to the ridge, San Blas mountain range	Panama	9.04897	77.99753	433
MHCH 2823	*D. quidditus*	Bajo pequeño, camp 2 Pechito Parao Mountain	Panama	8.47553	77.54884	472
MHCH 2824	*D. quidditus*	Near Perresenico creek Rancho Frío Field station	Panama	7.99706	77.71084	558
SMF 97291	*D. quidditus*	Near Perresenico creek Rancho Frío Field station	Panama	7.99241	77.70941	871
SMF 97292	*D. quidditus*	Pirre mountain range	Panama	7.98728	77.70785	1135
SMF 97294	*D. quidditus*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67962	78.03859	956
SMF 97295	*D. quidditus*	Camp 2 Pícuro River	Panama	8.04887	77.37004	787
SMF 97296	*D. quidditus*	Camp 4 Pícuro River	Panama	8.0575	77.37022	1043
SMF 97297	*D. quidditus*	Camp 4 Pícuro River	Panama	8.0575	77.37022	1043
SMF 97298	*D. quidditus*	Taintidu River, Chucunaque River	Panama	9.03547	78.02637	289
SMF 97299	*D. quidditus*	San Blas mountain range	Panama	9.05929	77.98421	553
SMF 97300	*D. quidditus*	Bajo pequeño, camp 2 Pechito parao Mount	Panama	8.47553	77.54884	472
SMF 97301	*D. quidditus*	Bajo pequeño, camp 2 Pechito parao Mount	Panama	8.47997	77.51941	859

SMF 97302	*D. quidditus*	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station	Panama	7.9632	77.70432	1267
SMF 97653	*D. quidditus*	Pavarandó, from camp 1 to stream; Garra Garra Mountain	Panama	7.75898	78.09228	643
SMF 97654	*D. quidditus*	Pavarandó, from Camp 1 to stream; Garra Garra Mountain	Panama	7.75898	78.09228	643
MHCH 2825	*D. quidditus*	Pirre mountain range	Panama	7.99207	77.70947	871
ICN 38150 (paratype)	*D. quidditus*	3 km NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 38151 (paratype)	*D. quidditus*	4 km. NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 38152 (paratype)	*D. quidditus*	5 km NE de la cabecera municipal, via Cerro Macana, Bahía Solano, Chocó	Colombia			200
ICN 45173 (holotype)	*D. quidditus*	Centro Forestal Bajo Calima, Buenaventura, Valle del Cauca	Colombia	3.98333	76.94999	50
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321

Appendix 1 *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
n.a.	*D. quidditus*	Burbayar, private reservation, San Blas mountain range	Panama	9.31577	79.0058	321
MHCH 2853	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97616	78.36097	1063
MHCH 2854	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97614	78.36285	1148
MHCH 2855	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2856	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2857	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2858	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
SMF 97328	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
SMF 97329	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97606	78.36289	1158
SMF 97330	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97589	78.36254	1160
SMF 97331	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97594	78.36265	1158
SMF 97332	*D. sapo* sp. nov.	Sapo Mountain	Panama	7.97618	78.36263	1169
MHCH 2812	*D. tinker*	Pirre mountain range	Panama	7.98923	77.7074	1149
MHCH 2848	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
MHCH 2849	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
MHCH 2863	*D. tinker*	Pirre mountain range	Panama	7.96258	77.70401	1253
MHCH 2864	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
MHCH 2865	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69463	78.0426	818
MHCH 2866	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
MHCH 2867	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68423	78.03867	971
MHCH 2868	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68405	78.03865	969

MHCH 2869	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68393	78.0386	970
MHCH 2870	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68393	78.0386	970
MHCH 2871	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
MHCH 2872	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
MHCH 2873	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
SMF 97311	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68412	78.03866	962
SMF 97315	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
SMF 97316	*D. tinker*	Pirre mountain range	Panama	7.96256	77.70393	1243
SMF 97317	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69463	78.0426	818
SMF 97318	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97319	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97320	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.69271	78.042	869
SMF 97321	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.68405	78.03865	969
SMF 97322	*D. tinker*	Ridge between Aldo Creek and Sambu River, Jingurudó mountain range	Panama	7.67947	78.03861	955
SMF 97323	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
SMF 97324	*D. tinker*	Camp 2 (ridge 1300 m a.s.l.); Rancho Frío Field station, Pirre mountain range	Panama	7.9615	77.70426	1303
SMF 97325	*D. tinker*	Camp 2 (ridge 1300 m a.s.l.) to stream; Rancho Frío Field station, Pirre mountain range	Panama	7.95947	77.70365	1230
SMF 97326	*D. tinker*	Pirre top (1400) to camp 2; Rancho Frío Field station, Pirre mountain range	Panama	7.94719	77.7042	1326
SMF 97327	*D. tinker*	Bailarin mount, Jingurudó mountain range	Panama	7.66911	78.03802	1133
ICN 45174 (holotype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45175 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45176 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45177 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45178 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45179 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50
ICN 45181 (paratype)	*D. tinker*	Centro Forestal Bajo Calima; Buenaventura	Colombia	3.98333	76.95	50

Appendix 1 *Continued*

Museum no.	Species	Locality	Country	Coordinates N	Coordinates W	m a.s.l.
UCR 20491	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20492	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20493	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
UCR 20504	*D. ventrimaculatus*	Valle del Silencio at the east edge of the Cordillera de Talamanca 20 km west from the Costa Rica–Panama border	Costa Rica	9.1116	82.96172	2550
MHCH 2874	*D. vocator*	Narices Mountain, Santa fé National Park	Panama	8.56315	81.05242	841
SMF 97339	*D. vocator*	San Lucas, Donoso, colón	Panama	8.98843	80.58243	149
SMF 89949	*D. vocator* cf.	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
SMF 89950	*D. vocator* cf.	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
AH 364	*D. vocator* cf.	San San Pond Sak, Bocas del Toro	Panama	9.50555	82.52417	5
SMF 89865	*D. vocator* cf.	San San Pond Sak, Bocas del Toro	Panama	9.50814	82.52843	5
SMF 89820	*D. citrinobapheus*	Cerro Negro, Santa fé National Park	Panama	8.5691	81.09875	730
SMF 89814 (holotype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2370 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
SMF 89816 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2371 (paratype)	*D. citrinobapheus*	Paredón	Panama	8.48507	81.17273	788
MHCH 2372	*D. citrinobapheus*	Willi Mazu, Palo seco national park	Panama	8.79028	82.19893	681
SMF 89817	*D. citrinobapheus*	Willi Mazu, Palo seco national park	Panama	8.79028	82.19893	681

n.a. = voucher number not available.

Appendix 2. Voucher numbers and GenBank accession numbers for all *Diasporus* samples included in the phylogenetic analyses.

Species	Museum no.	Field number	GenBank accession no.			Country	Coordinates	
			16S	*COI*	*RAG1*		Latitude	Longitude
D. aff. *diastema*	MHCH 2801	AB 637	KT186624	KT186572	KT119471	Panama	8.91587	78.62896
D. aff. *diastema*	MHCH 2802	AB 675	KT186617	KT186563	KT119472	Panama	9.03547	78.02637
D. aff. *diastema*	MHCH 2805	AB 035		KT186555	KT119461	Panama	9.17720	78.74551
D. aff. *diastema*	MHCH 2807	AB 073		KT186580	KT119438	Panama	8.89224	78.56029
D. aff. *diastema*	MHCH 2808	AB 084	KT186627	KT186578	KT119439	Panama	8.89182	78.56268
D. aff. *diastema*	MHCH 2809	AB 086	KT186633	KT186588		Panama	8.89427	78.56509
D. aff. *diastema*	MHCH 2811	AB 218		KT186571	KT119459	Panama	8.59740	78.04934
D. aff. *quidditus*	MHCH 2824	AB 1130	KT186621	KT186569	KT119443	Panama	7.99706	77.71084
D. aff. *quidditus*	MHCH 2825	B 131		KT186560	KT119454	Panama	7.99207	77.70947
D. pequeno sp. nov.	MHCH 2828	AB 822		KT186556	KT119475	Panama	8.47553	77.54884
D. pequeno sp. nov.	MHCH 2830	AB 860		KT186559	KT119478	Panama	8.47911	77.52799
D. majeensis sp. nov.	MHCH 2839	AB 1065	KT186629		KT119442	Panama	8.80462	78.45951
D. darienensis sp. nov.	MHCH 2841	AB 1268	KT186618		KT119449	Panama	7.98771	77.70783
D. vocator	MHCH 2843	AB 1240			KT119448	Panama	8.86528	80.64383
D. darienensis sp. nov.	MHCH 2845	AB 151		KT186561	KT119456	Panama	7.98771	77.70783
D. darienensis sp. nov.	MHCH 2850	AB 329	KT186626	KT186576	KT119460	Panama	7.68289	78.03846
D. sapo sp. nov.	MHCH 2855	AB 430	KT186619	KT186568	KT119464	Panama	7.97618	78.36263
D. sapo sp. nov.	MHCH 2856	AB 431	KT186616		KT119465	Panama	7.97618	78.36263
D. sapo sp. nov.	MHCH 2858	AB 439	KT186628		KT119467	Panama	7.97618	78.36263
D. sp.	MHCH 2859	AB 032	KT186614	KT186558		Panama	8.71893	82.23161
D. darienensis sp. nov.	MHCH 2862	AB 159		KT186579	KT119458	Panama	7.98724	77.70785
D. tinker	MHCH 2871	AB 1270	KT186620		KT119451	Panama	7.66911	78.03802
D. tinker	MHCH 2872	AB 1271	KT186623		KT119452	Panama	7.66911	78.03802
D. tinker	MHCH 2873	AB 1272	KT186615		KT119453	Panama	7.66911	78.03802
D. vocator	MHCH 2874	AB 564	KT186622		KT119469	Panama	8.56315	81.05242
D. diastema	SMF 97287	AB 602		KT186566	KT119470	Panama	9.26021	79.93540
D. diastema	SMF 97289	AB 818		KT186586	KT119474	Panama	8.47553	77.54884
D. diastema	SMF 97290	AB 979		KT186577	KT119481	Panama	9.28399	78.98383
D. aff. *quidditus*	SMF 97291	AB 138	KT186613		KT119455	Panama	7.99241	77.70941
D. aff. *quidditus*	SMF 97292	AB 158	KT186634	KT186589	KT119457	Panama	7.98728	77.70785
D. majeensis sp. nov.	SMF 97293	AB 1030		KT186565	KT119440	Panama	8.79936	78.46156
D. aff. *quidditus*	SMF 97295	AB 499			KT119468	Panama	8.04887	77.37004
D. aff. *quidditus*	SMF 97298	AB 689	KT186625		KT119473	Panama	9.03547	78.02637
D. darienensis sp. nov.	SMF 97304	AB 1144		KT186581	KT119445	Panama	7.96320	77.70432
D. darienensis sp. nov.	SMF 97305	AB 1134		KT186582	KT119444	Panama	7.97312	77.70785
D. darienensis sp. nov.	SMF 97312	AB 323	KT186631	KT186585		Panama	7.68357	78.03848
D. darienensis sp. nov.	SMF 97313	AB 425			KT119462	Panama	7.97632	78.36269
D. tinker	SMF 97320	AB 308	KT186632	KT186587		Panama	7.69271	78.04200

Appendix 2 *Continued*

Species	Museum no.	Field number	GenBank accession no.			Country	Coordinates	
			16S	*COI*	*RAG1*		Latitude	Longitude
D. tinker	SMF 97326	AB 1184			KT119446	Panama	7.94718	77.70420
D. tinker	SMF 97327	AB 1269	KT186635		KT119450	Panama	7.66911	78.03802
D. sapo sp. nov.	SMF 97329	AB 429		KT186557	KT119463	Panama	7.97606	78.36289
D. sapo sp. nov.	SMF 97330	AB 435	KT186630	KT186584	KT119466	Panama	7.97589	78.36254
D. pequeno sp. nov.	SMF 97335	AB 856		KT186583	KT119476	Panama	8.47911	77.52799
D. pequeno sp. nov.	SMF 97337	AB 861		KT186570	KT119479	Panama	8.47911	77.52799
D. vocator	SMF 97339	AB 028		KT186573		Panama	8.98843	80.58243
D. aff. *quidditus*	SMF 97652	AB 931		KT186562		Panama	7.75898	78.09228
D. majeensis sp. nov.	SMF 97653	AB 1031		KT186567	KT119480	Panama	8.79936	78.46156
D. majeensis sp. nov.	SMF 97657	AB 1033			KT119441	Panama	8.79936	78.46156
D. darienensis sp. nov.	SMF 97662	AB 1185		KT186564	KT119447	Panama	7.94719	77.70420
D. pequeno sp. nov.	SMF 97663	AB 857		KT186575	KT119477	Panama	8.47911	77.52799
D. citrinobapheus	SMF 89814	AH 449	JQ927333			Panama	8.48500	81.17300
D. citrinobapheus	SMF 89820	AH 211	JQ927334			Panama	8.56900	81.09900
D. citrinobapheus	MHCH 2370	AH 450	JQ927335			Panama	8.48500	81.17300
D. citrinobapheus	MHCH 2371	AH 452	JQ927336			Panama	8.48500	81.17300
D. hylaeformis	SMF 89868	AH 267	JQ927337			Panama	8.93100	82.71400
D. hylaeformis	SMF 89869	AH 268	JQ927338			Panama	8.93100	82.71400
D. hylaeformis	SMF 89872	AH 124	JQ927339			Panama	8.91100	82.71300
D. hylaeformis	SMF 89875	AH 282	JQ927340			Panama	8.91200	82.71300
D. citrinobapheus	USNM 572442	KRL 0902	FJ784425			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572443	KRL 1181	FJ784484			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572454	KRL 0900	FJ784423			Panama	8.66700	80.59200
D. citrinobapheus	USNM 572455	KRL 0901	FJ784424			Panama	8.66700	80.59200
D. citrinobapheus	MVUP 1783	KRL 0694	FJ784338			Panama	8.66700	80.59200
D. citrinobapheus	MVUP 1830	KRL 0840	FJ784395			Panama	8.66700	80.59200
D. quidditus	USNM 572444	KRL 0647	FJ784326			Panama	8.66700	80.59200
D. quidditus	MVUP 1832	KRL 0856	FJ784405			Panama	8.66700	80.59200
D. vocator	FMNH 257769	AJC 0127	JN991419	JN991348		Costa Rica	8.79000	82.96000
D. hylaeformis	USNM 572546	KRL 0782	FJ784369	FJ766810		Panama	8.66700	80.59200
D. hylaeformis	MVUP 1826	KRL 0831	FJ784390	FJ766809		Panama	8.66700	80.59200
D. hylaeformis	MVZ 203844	1999	EU186682			Costa Rica	9.75000	83.80400
D. hylaeformis	UCR 16264	AJC 0468	JN991418	JN991347		Costa Rica	10.22000	84.54000

Appendix III

Declaration on the contributions of authors

to the publication: A new fringe-limbed frogs of the genus *Ecnomiohyla* (Anura: Hylidae) from Panama.
status: Published (2014).
name of journal: Zootaxa 3826
Authors involved:
- Abel Batista (AB), - Milan Vesely (MV), - Konrad Mebert (KM), - Gunther Köhler (GK), - Andreas Hertz (AH)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 45% – field work (collecting and documenting specimens), molecular analysis
Coauthor MV: 15% – participated in the field trips.
Coauthor KM: 5% – participated in the field trips.
Coauthor GK: 5% – participated in the field trips and documenting specimens.
Coauthor AH: 25% – participated in the field trips and documenting specimens.

(3) to the creation of the data collection and figures

PhD candidate: 60% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor MV: 5% –provided photos, created figures
Coauthor KM: 5% – provided photos
Coauthor AH: 30% – provided Figures and data for *E. veraguensis*, and *E. fimbrimembra.*

(4) to the analysis and interpretation of the data

PhD candidate: 50% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor GK: 5% – contributed to data analysis and interpretation
Coauthor MV: 15% – contributed to data analysis and interpretation
Coauthor KM: 10% – contributed to data analysis and interpretation
Coauthor AH: 20% – contributed to data analysis and interpretation

(5) to writing the manuscript

PhD candidate: 60%
Coauthor MV: 15%
Coauthor KM: 5%
Coauthor GK: 5%
Coauthor AH: 15%

Date/place: 13.04.2016 / Frankfurt am Main, Germany

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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Two new fringe-limbed frogs of the genus *Ecnomiohyla* (Anura: Hylidae) from Panama

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Abstract

Forest canopy-dwelling frogs are usually among the rarest anuran species observed in the neotropical forest, mainly because they fall outside of the scope of the standard search methods used by herpetologists. During field explorations undertaken in western and eastern Panama in recent years, we discovered two species belonging to the genus *Ecnomiohyla*, which showed significant differences in genetic distances (16S mtDNA gene) and morphological characteristics different from any known *Ecnomiohyla* species. The first specimen originates from the Serranía de Jingurudó, Darién province, southeastern Panamá, and is described herein as *E. bailarina* **sp. nov.**, and the second specimen was found at Santa Fe National Park, Veraguas province, central-western Panama, and is described as *E. veraguensis* **sp. nov.** We provide a detailed description of both new species, including comparisons of morphological and molecular characters of almost all members of the genus in lower Central America, as well as an identification key for the entire genus.

Key words: Fringe-limbed frogs, *Ecnomiohyla,* rare species, DNA barcoding, lower Central America, Panama

Introduction

After the description of a new species, subsequent sampling usually provides additional comparative specimens, which thus increases our knowledge about the biology and distribution of that species with time (Vrcibradic *et al.* 2008; Hertz *et al.* 2012a). Nonetheless, there are some apparently rare species, whose existence we know for decades based only on the type specimen(s) or material from the type locality (Pimenta *et al.* 2005; Frost 2013, Wickramasinghe *et al.* 2013). Burrowing caecilians, salamanders (*Oedipina* spp.) and forest canopy-dwelling frogs (e.g. *Pseudophilautus stellatus*) are examples of such infrequently encountered species, which are then perceived as very rare (García-París & Wake 2000; Hanken *et al.* 2005; Wilkinson *et al.* 2007; Kamei *et al*. 2009; Wickramasinghe *et al.* 2013), because the habitat of these amphibians usually falls outside of the scope of the standard search methods used by herpetologists. Thus, the perception of rarity might be only an artifact of limited or inappropriate search techniques. In this context, any information about such seldom-seen (or "rare") taxa can be relevant.

Among such rare species are most members of the fringe-limbed frogs of the genus *Ecnomiohyla* Faivovich, Haddad, Garcia, Frost, Campbell, & Wheeler 2005, which spend all their life phases in the canopy and only rarely climb down and become visible to us. Fringe-limbed frogs are large, morphologically unusual hylid frogs with a cryptic moss-like color pattern and dermal fringes on portions of the body, rendering them well camouflaged. They breed in phytotelmata (e.g. Savage 2002; Mendelson *et al.* 2008; Savage & Kubicki 2010), and most of them occur in wet lowland, premontane tropical, and cloud forests between 20–2000 m elevation (Wilson *et al*. 1985;

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Duellman 2001; Frost 2013). The genus *Ecnomiohyla* is distributed from southern Mexico through Central America and into north-western South America, containing 12 species to date (Frost 2013), which are: *Ecnomiohyla echinata* (Duellman, 1961), *E. fimbrimembra* (Taylor, 1948), *E. miliaria* (Cope, 1886), *E. minera* (Wilson, McCranie, & Williams, 1985), *E. miotympanum* (Cope, 1863), *E. phantasmagoria* (Dunn, 1943), *E. rabborum* Mendelson, Savage, Griffith, Ross, Kubicki, & Gagliardo, 2008, *E. salvaje* (Wilson, McCranie, & Williams, 1985), *E. sukia* Savage & Kubicki, 2010, *E. thysanota* (Duellman, 1966), *E. tuberculosa* (Boulenger, 1882), and *E. valancifer* (Firschein & Smith, 1956). The holotypes of *Ecnomiohyla* species were often the only specimens known for an extended period of time (Taylor 1948; Duellman 1966). However, intensified sampling during the past three decades has contributed increasingly to our knowledge about the ecology of some species, while recent genetic studies helped to understand better the species relationships inside the genus *Ecnomiohyla* and its position in the amphibian tree of life (Wilson *et al*. 1985; Faivovich *et al*. 2005; Mendelson *et al*. 2008; Crawford *et al.* 2013).

The genus *Ecnomiohyla* can be differentiated from all other genera of Hylinae by the combination of the following characters: having immense hands and feet, scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (Firschein & Smith 1956; Savage & Heyer 1969; Duellman 1970; Mendelson *et al*. 2008). The prepollices are more developed in males and usually modified with a projecting terminal spine (protruding as in *E. miliaria*), or a spade-like plate (as in *E. valancifer*). In many species, male prepollices bear also keratinized black spines (Duellman 2001), whereas in females the prepollex is slender, straight and without spines.

Some uncertainty remains in unifying all currently recognized species within *Ecnomiohyla* based on the morphological characters mentioned above (Faivovich *et al.* 2005). *Ecnomiohyla miotympanum* and *E. tuberculosa* have been catalogued as problematic species due to substantial differences in adult and larval morphology and shared behavioral ecological traits in comparison to the other members of the genus (Faivovich *et al.* 2005; Mendelson *et al*. 2008). This problem is not solved yet, partly because of the lack of fresh material for genetic approaches in many species that prevents the construction of a well-resolved phylogeny of the genus. Recent phylogenetic studies lack most species of *Ecnomiohyla* (there are no sequences available for *E. tuberculosa* yet) thus some of its species (*E. miotympanum* and *E. tuberculosa* specially) are assigned to the genus only tentatively (Faivovich *et al*. 2005; Wiens *et al.* 2010; Pyron & Wiens 2011). The issues regarding exclusion of *E. miotympanum* and *E. tuberculosa* from the genus *Ecnomiohyla* (see Savage & Kubicki 2010) are not relevant in the context of this paper, but we include *E. miotympanum* to our phylogenetic analysis to discuss its relationship with other *Ecnomiohyla* from lower Central America.

Herein, we describe two new species of *Ecnomiohyla* from Panama, based on comparative morphology of the twelve known species of *Ecnomiohyla* and a genetic analysis of the species from lower Central America (except *E. thysanota*). The new species from eastern Panama can be distinguished from its congeners by the presence of cranial and dorsal osteoderms, and two clusters of nuptial spines, one at the distal end of prepollex and one at the end of the first phalanx of the thumb in males. The new species from western Panama has scattered minute keratin tipped tubercles on the dorsal skin, and 6–8 widely spaced keratinized black spines along the outer side of the thumb.

Material and methods

Fieldwork was carried out in eastern Panama in 2011 and 2012 (Fig. 1) and in central-western Panama during two field trips in 2009. Specimens were euthanized with a euthanasia solution (T61), fixed with a mixture of 5 ml formalin (5%) in 1L ethanol (94%), and then stored in ethanol (70%). Morphological nomenclature and diagnoses usually follow the methodology of Duellman (2001), except for standards of dorsal and lateral profiles of the snout that follow Savage (2002). Coding for webbing formulae follows Savage & Kubicki (2010): considerable (C) = not extending to base of disk on one margin of any digit; substantial (S) = extending to base of disk on one margin of one digit; extensive (EX) = extending to base of disk on one margin of two to four digits; full (F) = extending to base of disk on margins of all digits.

All measurements are given in millimeters, were rounded to the nearest 0.1 mm and follow Duellman & Lehr (2009). The following measurements were taken (with abbreviations indicated): length from snout to vent (SVL); head length (HL), measured diagonally from angle of jaw to tip of snout; head width (HW) between angles of jaws;

interorbital distance (IOD); eye diameter (ED); eye length (EL) from anterior to posterior edge; eye to nostril distance (END) from anterior edge of eye to posterior corner of nostril; internarial distance (IND) between centers of nostrils; forearm length (FAL) from proximal edge of palmar tubercle to outer edge of flexed elbow; hand length (HAL) from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), distance from knee to distal end of the tibia; foot length (FL) from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW) at penultimate phalanx just anterior to disk; width of disk of third finger (3FD) at greatest width; width of third toe (3TW) at penultimate phalanx just anterior to disk; width of disk of third toe (3TD) at greatest width; width of fourth toe (4TW) at penultimate phalanx just anterior to disk; width of disk of fourth toe (4TD) at greatest width; body width (BW) at greatest width of body; tympanum diameter (TD), horizontal distance, based on an estimated circular tympanum. SVL, HL, HW, TL, and FL were measured with vernier calipers; all other variables were measured with an ocular micrometer in a Zeiss stereomicroscope.

Capitalized colors and color codes (the latter in parentheses) used in the color descriptions are those of Smithe (1975–1981), except those in the color description of the holotype of *Ecnomiohyla bailarina*, which are those of Köhler (2012). Specimens were deposited in the herpetological collection of the Senckenberg Forschungsinstitut and Naturmuseum Frankfurt (SMF) in Germany. Comparisons among similar species are based on data provided in the respective original descriptions. Geographic coordinates and altitude were taken with a Garmin GPSmap 60CSx given in decimal degrees and rounded to the fourth decimal place. Elevations are rounded up to the next tenth. All georeferences were recorded in WGS 1984 datum. The map was downloaded from the server of the Smithsonian Tropical Research Institute (http://mapserver.stri.si.edu/), and created using ArcGIS 10 (ESRI 2009). Detailed information about the specimens examined is given in Table 1.

Molecular Genetics

We took tissue samples from the two new species plus a newly collected specimen of *Ecnomiohyla fimbrimembra* (SMF89857, Hertz et al. 2012b) and a newly collected specimen of *E. sukia* (SMF94578, Köhler *et al.* 2013). Tissue for DNA was extracted by excision on finger-tips of preserved specimens, except for *Ecnomiohyla bailarina*, where the tissue was extracted from a fresh liver sample. A fragment of the mitochondrial 16S mtDNA gene was extracted following the protocol of Ivanova *et al*. (2006), and amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany) performing an initial denaturation for 60 sec at 94° C followed by 35 steps with denaturation for 15 sec at 94° C, hybridization for 45 sec at 45° C, elongation for 1.5 min at 72° C, final extension at 72° C for 7 min; reaction mix contained 1 µL DNA template, 2.5 µL Reaction Buffer x10 (PeqGold), 4 µL 2.5 mM dNTPs, 0.4 µL (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µL H2O, 1 µL 25 mM MgCl2, and 1 µL (containing 10 pmol) (forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon).

To compare the 16S mtDNA data of our specimens with published sequences, we conducted a *BLAST* search in GenBank and took the sequences with the highest scores for comparison. Additionally, we used *Bromeliohyla bromeliacia* (Schmidt, 1933), *Duellmanohyla rufioculis* (Taylor, 1952), and *D. soralia* (Wilson & McCranie, 1985) as outgroups (the phylogenetically most closely related species according to Faivovich *et al.* 2005). All sequences were aligned and manually refined using Genious (Drummond *et al*. 2010). In MEGA5 (Tamura *et al.* 2011), we computed uncorrected pairwise genetic distances prior to the maximum likelihood and Bayesian analyses. We used JModeltest 0.1.1 (Posada 2008) with likelihood settings to find the best-fitting substitution model according the Akaike Information Criterion (AICc). The Bayesian phylogenetic analysis (MrBayes 3.1.2, Huelsenbeck & Ronquist 2001) was run under the model TPM3uf+G, for 2,000,000 generations with four Metropolis-coupled Markov Chain Monte Carlo (MCMC) sampled every 100 generations. The first 5% were discarded as burn-in (burn-in= 1000). The ML analysis was assessed via 1000 bootstrap replicates, using PAUP v4.0b10 (Swofford 1998). The Automatic Barcode Gap Discovery (ABGD) algorithm (Puillandre *et al.* 2011), has been recently recommended as a reliable barcode cluster identification algorithm (Paz & Crawford 2012). Therefore, we also evaluated our sequences applying this method, using the Web interface at http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html. The following settings were chosen: steps=20, distance= Kimura 2-parameter 2.0, and the setting for the minimum relative gap width (X) was moved to different values between 0 and 1.5.

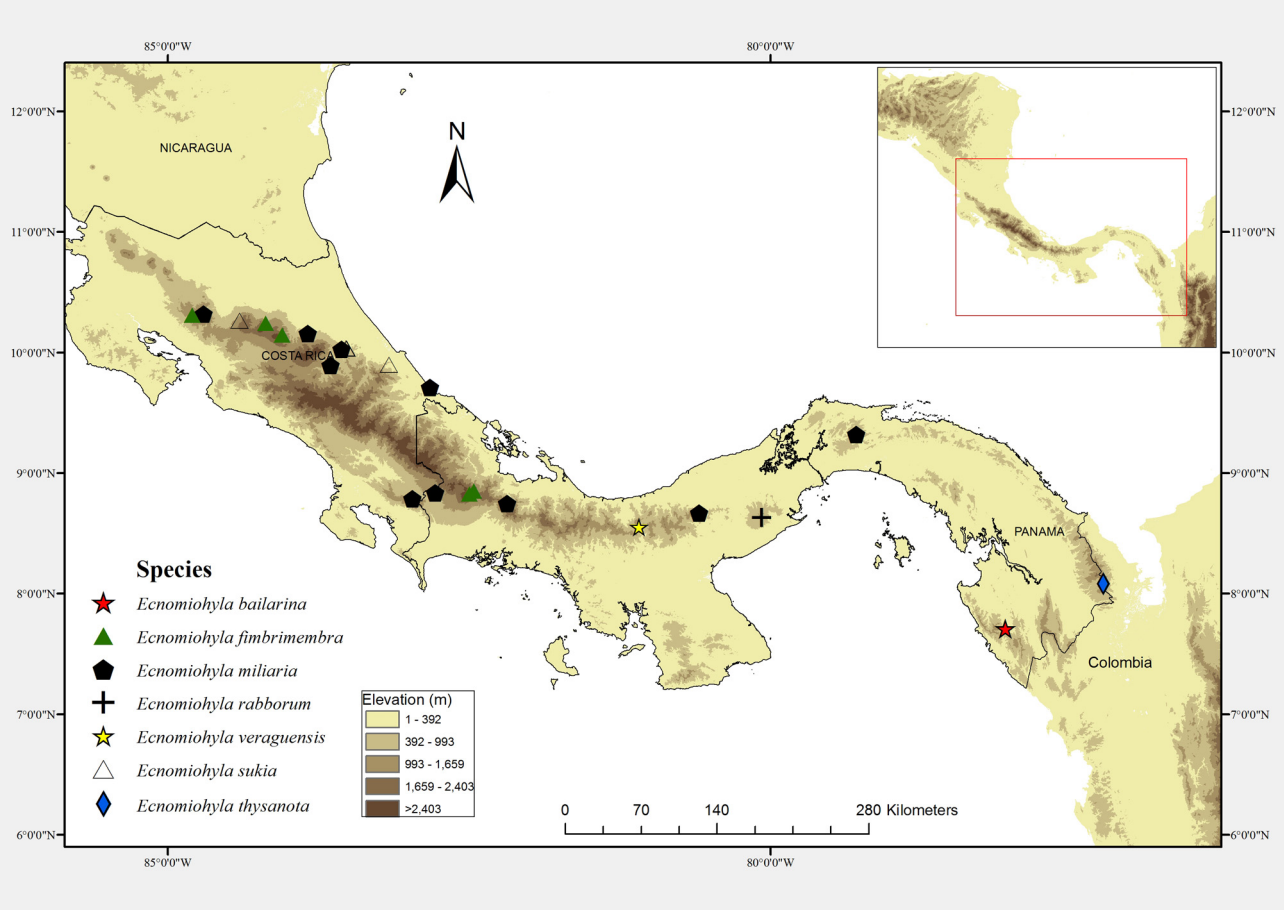

FIGURE 1. Distribution of the *Ecnomiohyla* spp. in lower Central America (main map). See Table 1 for detailed information on the localities.

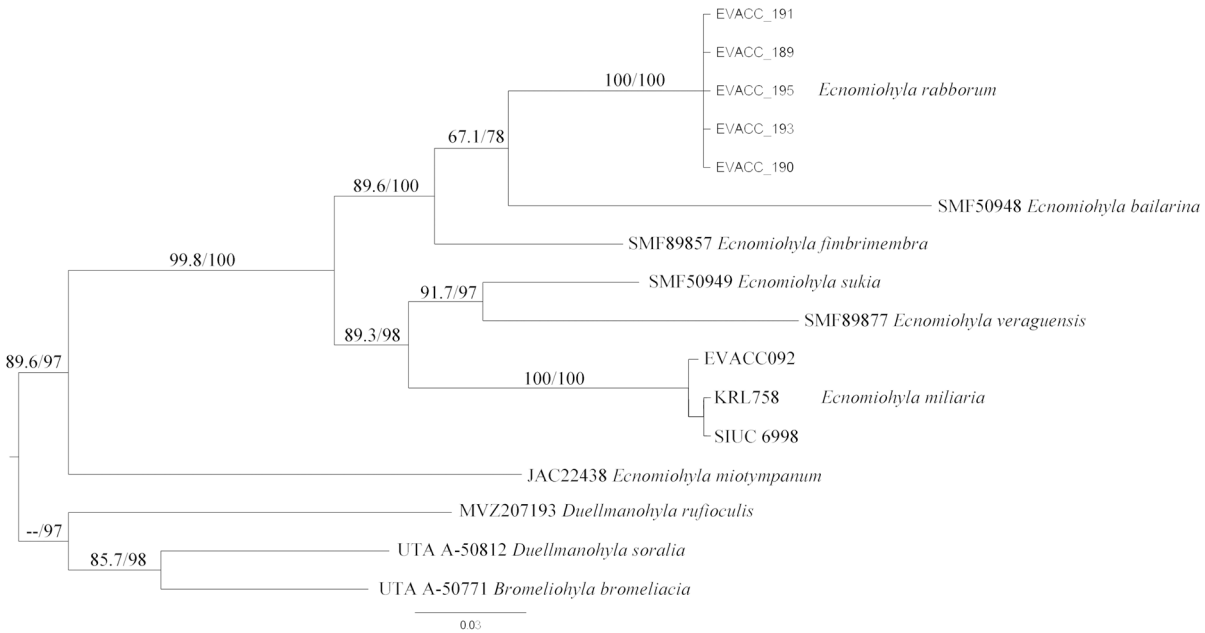

FIGURE 2. Maximum likelihood consensus tree of the 16S mtDNA gene, for the *Ecnomiohyla* spp. from lower Central America. Specimen labels refer to collection or museum number. Scale bar refers to number of substitutions per site. Maximum likelihood bootstrap values are shown in front of slash mark, Bayesian posterior probabilities (multiplied by 100) behind slash mark.

TABLE 1. Genbank accessions and detailed information on the localities of specimens mentioned in the text.

Museum number	Accession 16S	Species	Locality	Country	Coordinates N	Coordinates W	Elev.
UTA A-50771	AY843612.1	*Bromeliohyla bromeliacia*	Huehuetenango, Sierra de los Cuchumatanes, Finca Chiblac (now Aldea Buenos Aires)	Guatemala			
MVZ 207193	AY843583.1	*Duellmanohyla rufioculis*	Guanacaste, Volcan Cacao				
UTA A-50812	AY843584.1	*Duellmanohyla soralia*	Izabal, Morales, Sierra de Caral, Finca Quebradas-Cerro Pozo de Agua	Guatemala			
SMF97398	KF924240	*Ecnomiohyla bailarina*	North slope of the Jingurudó mountain range, about ca.14.6 Km S from Pavarandó village, Sambú, Comarca Emberá-Wounaam N°2	Panama	7.70903	-78.04882	750
SMF 89857	KF924242	*Ecnomiohyla fimbrimembra*	Boquete/Bajo Mono Sendero La Cascada	Panama	8.82629	-82.49907	1820
LACM 149980		*Ecnomiohyla fimbrimembra*	Pantanosa Trail, Monte Verde	Costa Rica	10.310388	-84.798056	1600
LACM 149979		*Ecnomiohyla fimbrimembra*	Northern slope of Volcan Barba, Heredia	Costa Rica	10.144033	-84.052224	1800
FMNH 191784		*Ecnomiohyla fimbrimembra*	Isla Bonita, Alajuela, Costa Rica	Costa Rica	10.241438	-84.190483	1300
CHP1036		*Ecnomiohyla fimbrimembra*	Cerro Horqueta, Boquete Panama	Panama	8.850493	-82.46196	1600
SIUC 6998	AY843777.1	*Ecnomiohyla miliaria*	EL Copé, Parque Nacional Omar Torrijos, Loop, Stream 2, Coclé.	Panama	8.667	-80.592	800
KRL758	DQ055824	*Ecnomiohyla miliaria*	EL Copé, Parque Nacional Omar Torrijos, Loop, Stream 2, Coclé.	Panama	8.667	-80.592	800
EVACC092	KC014814.1	*Ecnomiohyla miliaria*	Chagres National Park, Cerro Brewster Stream, Panamá	Panama	9.31985	-79.2889	818
KU 98451		*Ecnomiohyla miliaria*	Santa Clara, Renacimiento, Chiriquí	Panama	8.834816	-82.783559	1100
KU30404		*Ecnomiohyla miliaria*	Cartago: 2.5 km east of Turrialba	Costa Rica	9.8936	-83.6521	602
USNM 331414		*Ecnomiohyla miliaria*	Siquirres	Costa Rica	10.0269	-83.5602	62

TABLE 1. (Continued)

Museum number	Accession 16S	Species	Locality	Country	Coordinates N	Coordinates W	Elev.
UCR 4979		*Ecnomiohyla miliaria*	Estacion Biologia Las Cruces, Puntarenas	Costa Rica	8.787262	-82.972649	1200
LACM 150152		*Ecnomiohyla miliaria*	Río Peñas Blancas, Alajuela	Costa Rica	10.319491	-84.704758	800
UCR 12678		*Ecnomiohyla miliaria*	Río Blanco area, Provincia Limón.	Costa Rica	10.157077	-83.840814	450
UMMZ 149201		*Ecnomiohyla miliaria*	Comadre de Cahuita, Limón	Costa Rica	9.710069	-82.82482	20
AMNH 94887		*Ecnomiohyla miliaria*	RF fortuna Río Chiriquí	Panama	8.747156	-82.186534	1100
JAC22438	AY843645.1	*Ecnomiohyla miotympanum*	Puebla, Sierra Norte, Cuetzalan, Hotel Villas Cuetzalan	Mexico			1250
SMF94908		*Ecnomiohyla miotympanum*	Cuetzalan, Apuleo, hacienda km 7, sierra norte, Puebla	Mexico			
EVACC191	KC014813.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC189	KC014811.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC195	KC014809.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC193	KC014807.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
EVACC190	KC014812.1	*Ecnomiohyla rabborum*	El Valle, Rio Maria, Panama	Panama	8.63312	-80.0767	990
SMF94578	KF924239	*Ecnomiohyla sukia*	San Carlos, Cerro Chato, Alajuela	Costa Rica	10.2632	-84.4052	922
UCR 12787		*Ecnomiohyla sukia*	Guayacán: Alto Colorado,	Costa Rica	10.037139	-83.522889	710
UCR 10966		*Ecnomiohyla sukia*	5km from Moravia de Siquirres toward Turrialba	Costa Rica	10.033333	-83.516667	710
UCR 17024		*Ecnomiohyla sukia*	S Río Blanco (town): Fila Asunción	Costa Rica	9.9	-83.166667	400
USNM 151080		*Ecnomiohyla thysanota*	Cerro Malí, 1265 m (holotype of E. thysanota), Darién.	Panama	8.080757	-77.235448	1265
SMF82418		*Ecnomiohyla tuberculosa*					
SMF 89877	KF924241	*Ecnomiohyla veraguensis*	Cerro Negro/PN Santa Fe	Panama	8.5533	-81.09261	540

TABLE 2. Estimates of evolutionary divergence among 16S mtDNA gene sequences of the *Ecnomiohyla* spp. used in the phylogenetic analysis. Numbers below diagonal are for uncorrected p-distances and numbers above are standard error estimates.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*E. bailarina* (SMF97398)		0.02	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.02	0.02	0.01	0.02
2	*E. veraguensis* (SMF89877)	0.14		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
3	*E sukia* (SMF94578)	0.15	0.07		0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
4	*E. rabborum* (EVACC191)	0.11	0.10	0.10		0.00	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02
5	*E. rabborum* (EVACC189)	0.11	0.10	0.10	0.00		0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.02
6	*E. rabborum* (EVACC195)	0.11	0.10	0.10	0.00	0.00		0.00	0.00	0.01	0.01	0.01	0.01	0.02
7	*E. rabborum* (EVACC193)	0.11	0.10	0.10	0.00	0.00	0.00		0.00	0.01	0.01	0.01	0.01	0.02
8	*E. miliaria* (EVACC190)	0.11	0.10	0.10	0.00	0.00	0.00	0.00		0.01	0.01	0.01	0.01	0.02
9	*E. miliaria* (SIUC6998)	0.14	0.09	0.10	0.12	0.12	0.12	0.12	0.12		0.00	0.00	0.01	0.02
10	*E. miliaria* (KRL 0758)	0.14	0.09	0.10	0.12	0.12	0.12	0.12	0.12	0.00		0.00	0.01	0.02
11	*E. miliaria* (EVACCC092)	0.14	0.09	0.10	0.11	0.11	0.11	0.11	0.11	0.00	0.00		0.01	0.02
12	*E. fimbrimembra* (SMF89857)	0.12	0.10	0.10	0.08	0.08	0.08	0.08	0.08	0.11	0.11	0.11		0.02
13	*E. miotympanum* (JAC22438)	0.19	0.15	0.15	0.14	0.14	0.14	0.14	0.14	0.15	0.15	0.16	0.15	

TABLE 3. Principal differential traits of the *Ecnomiohyla* species from lower Central America and Colombia.

Trait	*E. rabborum*	*E. bailarina* sp. n.	*E. fimbrimembra*	*E. miliaria*	*E. sukia*	*E. veraguensis*	*E. phantasmagoria*	*E. thysanota*
SVL males	62.8-97.3	68	NA	86.0-110.0	56.7-63.2	57.8	95	NA
SVL females	61.3-79.9	NA	71.0 – 91.0	86.2	58.1-68.1	NA	NA	95
dorsum	granular	tuberculate	granular	tuberculate	tuberculate	finely tuberculated	tuberculate	granular
Cephalic skin co-ossified with skull	-	-	+	-	-	-	-	-
Cranial osteoderms	-	+	-	+	+	+	+	-
Dorsal osteoderms	-	+	-	+	+	+	+	-
Humeral projection in males	+	-	-	-	-	-	-	-
Prepollex (males)	blunt	rounded	blunt	recurved	obtuse	recurved	recurved	NA
Prepollical bony projection (males)	rounded	bluntly pointed. directed medially	rounded	spine	spadelike, directed laterally	spadelike, directed laterally	spine	NA
Keratinized black spines on prepollex and thumbs (males)	+	+	+	-	-	+	-	NA
Finger webbing	S	EX	C	EX	EX	EX	C	EX
Toe webbing	EX	EX	C	EX	EX	EX	EX	EX
Heel	smooth	scalloped fringe	pointed tubercles	pointed tubercles	scalloped fringe	scalloped fringe	pointed tubercles	smooth
Color in life		green with brownish flecks	brown with darker markings	brown to mottled brown and green				uniformly green

See methods for finger and toe webbing abbreviations; "-"= absent; "+"= present; NA= no specimen available for comparison.

Results

According to the barcode analysis with ABGD, we found that the comparison of all included *Ecnomiohyla* samples resulted in seven distinct species with a prior intraspecific divergence of 4.9% (Fig. 2). The overall genetic p-distance between the samples was 10.0%. Our two newly sequenced *Ecnomiohyla* specimens, one from western and one from eastern Panama, are genetically distinct and have no morphological characters that would assign them to any previously described species in the genus (Savage & Kubicki 2010; Köhler 2011). The specimen from western Panama (SMF89877) forms a sister clade to *E. sukia* from Costa Rica with an estimated evolutionary divergence of 7% (Table 2). The specimen from eastern Panama (SMF97398) is most closely related to *E. rabborum* and *E. fimbrimembra,* but is genetically distinct by 11 and 12% p-distance in the 16S gene (Table 2), respectively. SMF97398 shows the highest p-distance of 15% to *E. sukia*. *Enomiohyla miotympanum* is revealed as sister taxon to all *Ecnomiohyla* from lower Central America (Fig. 2, Table 2), separated by a p-distance of 14–19%. According to our findings of significant genetic and morphological differences in two of our newly obtained specimens (Table 3), we proceed to describe them as two species new to science.

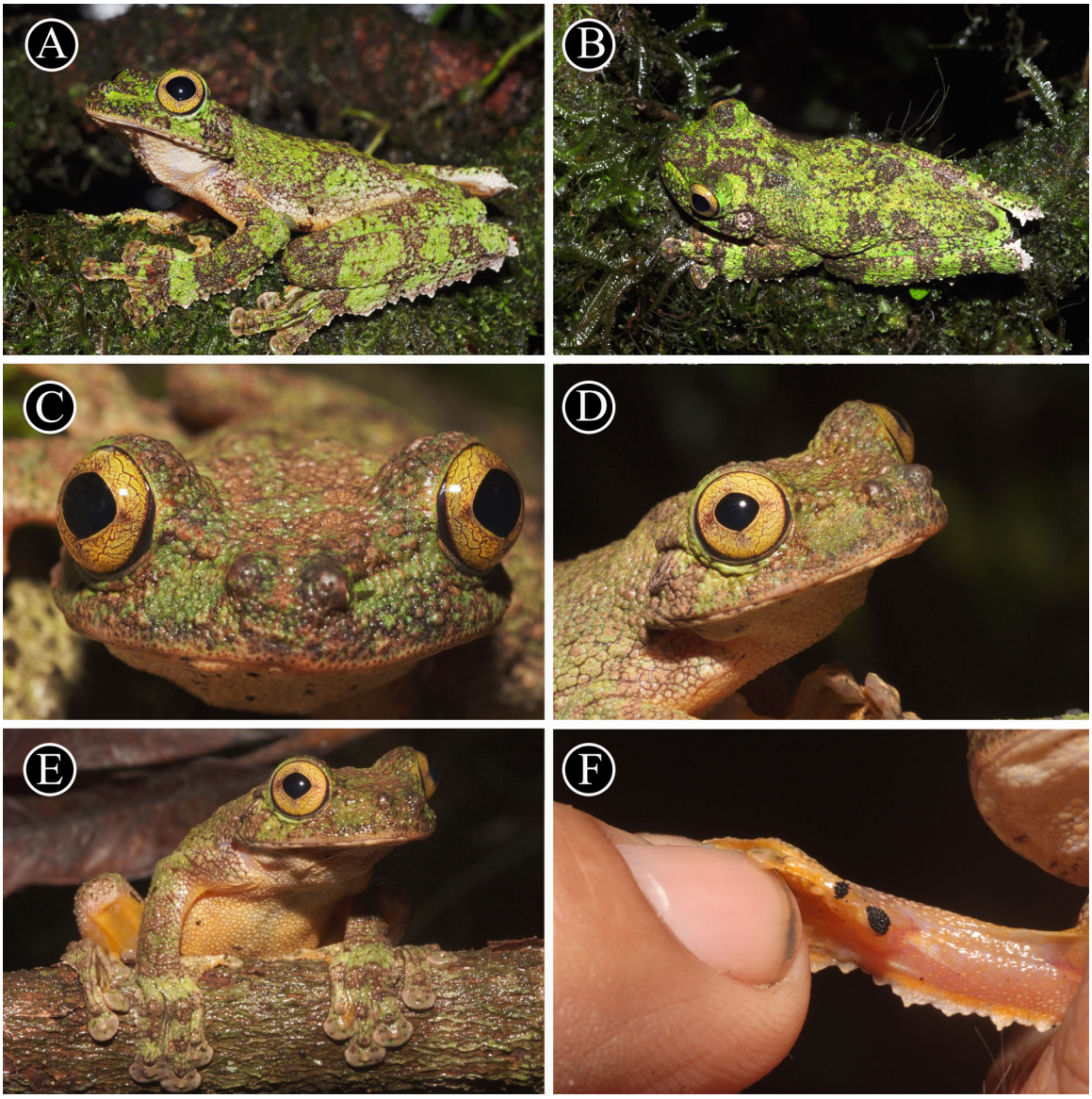

FIGURE 3. Photographs of the holotype of *Ecnomiohyla bailarina* in life. A) lateral view; B) dorsal view; C) frontal view; D) profile; E) ventral coloration; F) prepollical spines on right hand.

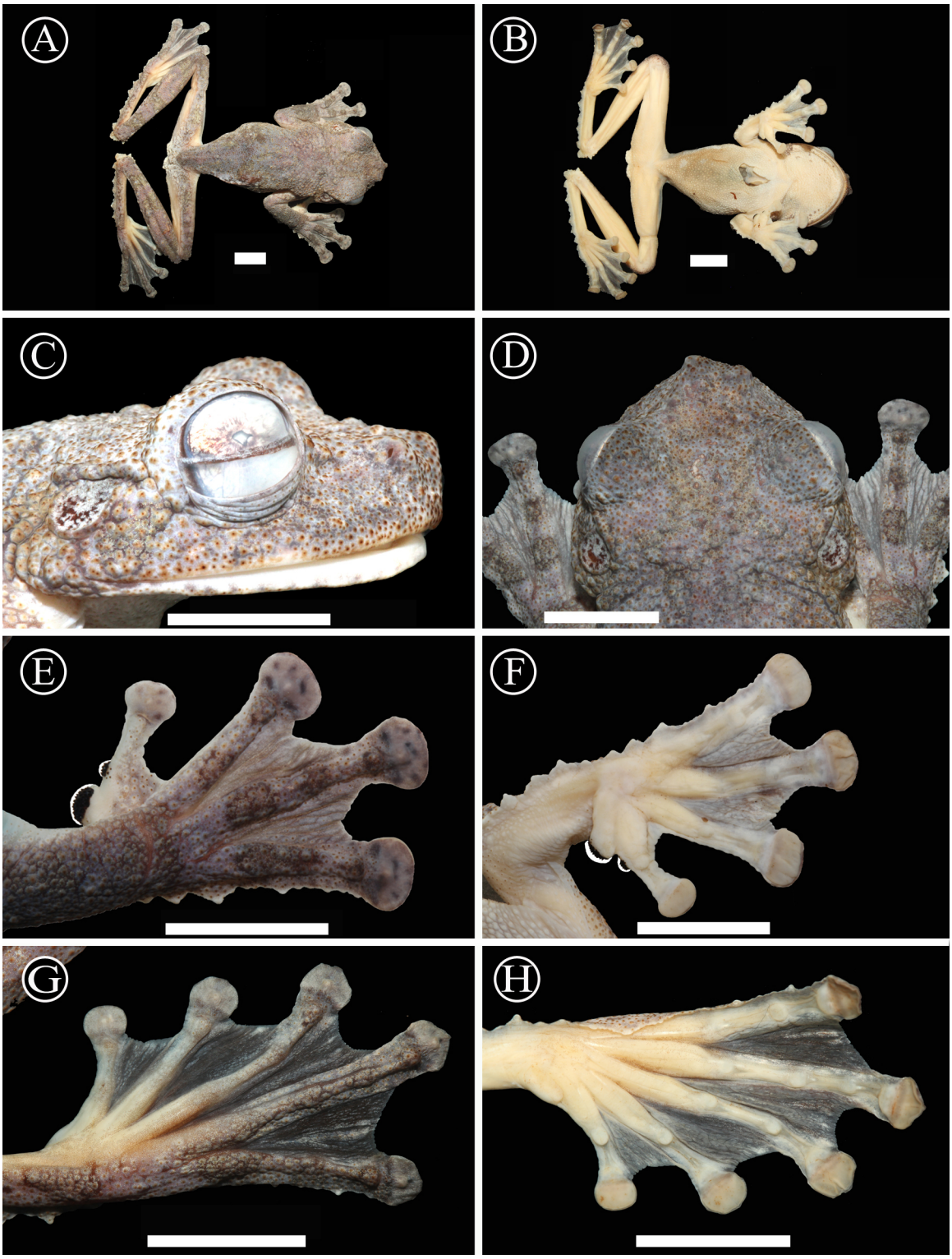

FIGURE 4. Photographs of preserved holotype of Ecnomiohyla bailarina. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars = 10 mm.

***Ecnomiohyla bailarina* sp. nov.**

Holotype. SMF97398 (original field number AB297; Fig. 3–4), an adult male from the north slope of the Jingurudó mountain range (Fig. 5), about 14.6 km S from Pavarandó village (7.70903°N, -78.04882°W, 750 m a.s.l.), Sambú, Comarca Emberá-Wounaan N°2, Darién, Panama, collected by Abel Batista and Milan Vesely on 25 September 2011 at 21:27 hrs.

Diagnosis. A medium-sized *Ecnomiohyla* (single known specimen is an adult male 68.1 mm in SVL; Figs. 3–4), differing from other known species in the genus by the following combination of characters: 1) finger webbing extensive, web reaching the finger disk on at least one side on two fingers (Fig. 4); 2) toes extensively webbed as well, web reaching the toe disk at least on one side of four toes (Fig. 4); 3) skin on dorsum strongly tuberculate; 4) cranial and dorsal osteoderms present; 5) skin on upper surface of head not co-ossified with underlying cranial elements; 6) humerus without enlarged *crista lateralis*; 7) prepollex distinct, obtuse, with bony prepollical projection rounded distally, bluntly pointed at side adjacent to thumb; 8) two clusters of nuptial spines at the distal end of prepollical tubercle and at the end of the first phalanx of the thumb; 9) a distinct scalloped fringe with pointed tubercles on a ventral surface of heel flaps, continuing almost to the disc of the 5th toe; 10) dorsal coloration in life green with scattered brownish or black flecks.

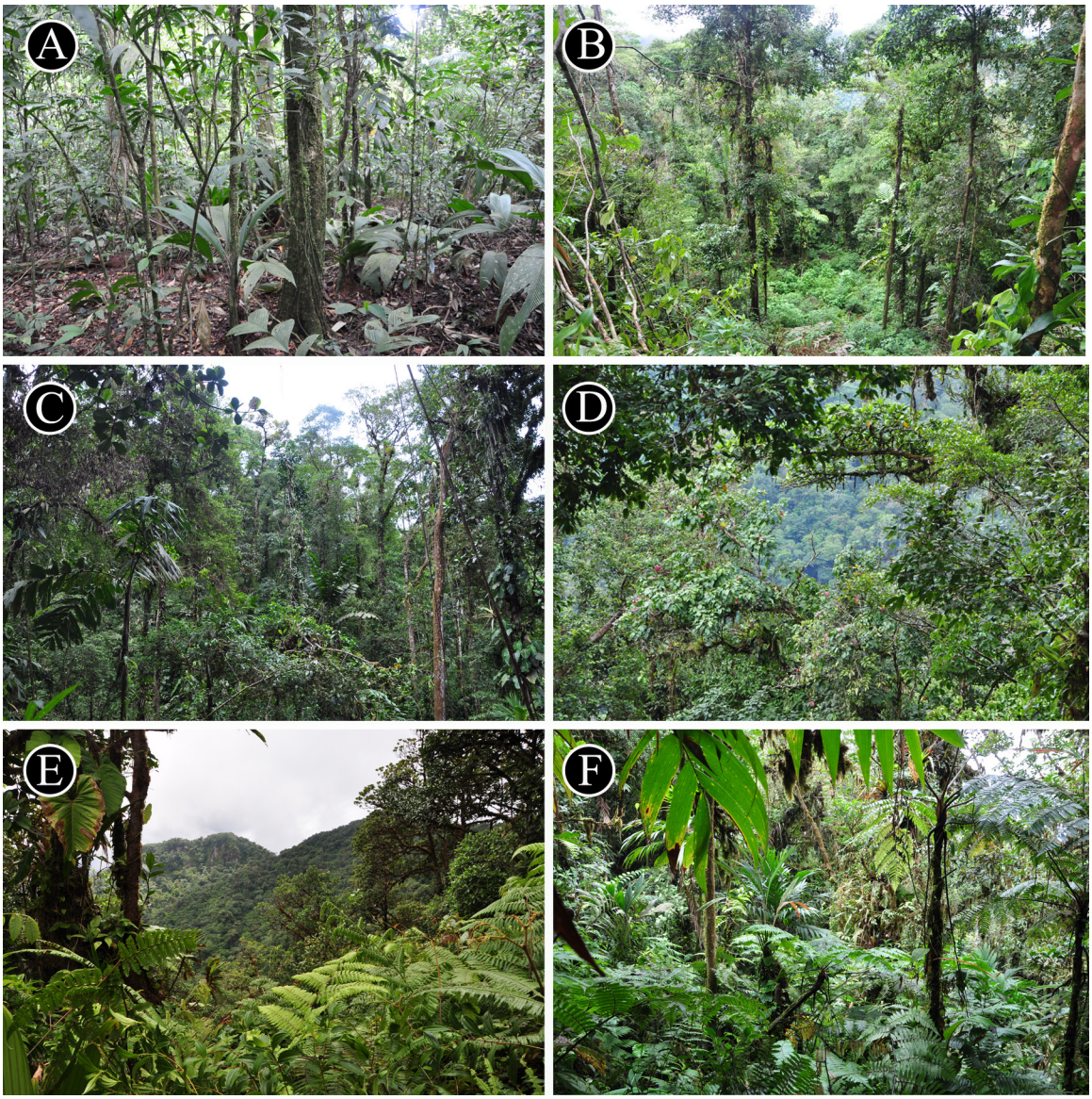

FIGURE 5. Habitat of *Ecnomiohyla bailarina* A) understory area where holotype was caught; B-C) forest structure from an open area; D) canopy forest; E) Cerro Bailarín, view from a ridge to 900 m a.s.l.; F) understory at Cerro Bailarín.

Comparison with other species of *Ecnomiohyla*. *Ecnomiohyla bailarina* can be distinguished from other species of *Ecnomiohyla* by the following characters (with contrasting features for *E. bailarina* in parentheses, see Table 3 for more details): *Ecnomiohyla minera, E. thysanota* (see Fig. 6) and *E. rabborum* are easily distinguished from the new species by having smooth heels without a scalloped fringe (triangular serrate fringe with pointed tubercles on a ventral surface of heel flaps); *E. rabborum* and *E. minera* are further distinct in having a humeral projection in males (no humeral projection); *E. rabborum* has a substantial webbing on one finger only, reaching

base of disk on one finger (webbing extensive reaching base of disk on two fingers); *E. echinata, E. fimbrimembra, E. minera, E. salvaje* and *E. valancifer* lack of cranial or dorsal osteoderms (well developed cranial and dorsal osteoderms); the type locality of the only known specimen of *E. thysanota,* a female collected at Cerro Malí, Darién (Duellman 1966), is only 100 km northeast of the type locality of *E. bailarina*, but *E. thysanota* lacks cranial and dorsal osteoderms (well developed cranial and dorsal osteoderms, see Figs. 3−4, and 6), skin on dorsum is granular (strongly tuberculate), coloration in life is reported to be uniformly green (green with scattered brown or blackish flecks); in addition, these potentially sympatric species would probably differ also in size, as the *E. thysanota* specimen is a female that is much larger (95 mm vs 68.1 mm SVL, see Table 3) than our male *E. bailarina*; males in *Ecnomiohyla* spp. tend to be bigger or at least the same size as females (Table 3, Savage & Kubicki 2010), and hence, an adult male *E. thysanota* is presumed to be considerably larger than the adult male holotype of *E. bailarina; E. fimbrimembra* (see Fig. 9), *E. miliaria*, and *E. phantasmagoria* also lack a fringe on heels (present), but have pointed heel tubercles; in addition, males of *E. miliaria* and *E. phantasmagoria* have a sharp prepollical spine directed laterally (prepollical spine vestigial, bluntly pointed and directed to the thumb); *E. fimbrimembra* and *E. salvaje* have the skin on the head co-ossified with the cranium, (Fig. 9 E–H) (skin not co-ossified with cranium); males of *E. miliaria, E, phantasmagoria, E. sukia, E. tuberculosa* and *E. valancifer* have no nuptial black spines on prepollex (numerous small black keratinized spines present on prepollex); *E. miotympanum* lacks of scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (present in *E. bailarina*); *E. tuberculosa* does not have a prepollical projection in adult males (prepollical projection present); in *E. sukia*, the prepollical spine has a similar size and direction, but is rather spade-like, not forming a sharp spine as in *E. bailarina*; *E. veraguensis* (sp. nov., see below) can be distinguished from *E. bailarina* by having only a few large, widely spaced nuptial black keratinized spines, dorsolaterally on the base of the pollex and none on the prepollex in adult males (thickly clustered smaller spines on prepollex and pollex; Fig. 10); further, it has a finely tuberculated dorsum (strongly tuberculated dorsum), and keratinized tubercles on the ventral side of the scalloped fringe on the heels are absent (present in *E. bailarina*).

TABLE 4. Measurements and morphological proportions for the holotypes of the new *Ecnomiohyla* species described herein.

Trait	Measurements (mm)		Trait	Proportions (%)	
	E. veraguensis	*E. bailarina*		*E. veraguensis*	*E. bailarina*
SVL	57.8	68.1	IND/SVL	9.7	8.2
HL	20.2	22.0	HL/SVL	34.9	32.3
HW	23.6	24.1	HW/SVL	40.8	35.4
IOD	14.7	14.1	HL/HW	85.6	91.3
ED	6.0	6.8	IOD/SVL	25.4	20.7
TD	3.6	4.2	ED/SVL	10.4	10.0
HAL	20.8	21.2	TD/SVL	6.2	6.2
FAL	11.8	16.7	HAL/SVL	36.0	31.1
IND	5.6	5.6	FAL/SVL	58.4	75.9
TL	32.8	35.8	TL/SVL	56.7	52.6
FL	28.0	27.6	FL/SVL	48.4	40.5
3FW	2.2	3.1	3FW/SVL	3.8	4.6
3FD	3.4	4.4	3FD/SVL	5.9	6.5
4TW	2.0	3.4	4TW/SVL	3.5	5.0
4TD	2.7	3.4	4TD/SVL	4.7	5.0
3TW	2.1	2.9	3TW/SVL	3.6	4.3
3TD	2.7	3.1	3TD/SVL	4.7	4.6
BW	30.5	19.7	BW/SVL	52.8	28.9

Description of the holotype. An adult male, as indicated by the presence of keratinized nuptial spines. Measurements of the holotype are shown in Table 4. Head rounded in dorsal view, slightly wider than long (HL/HW = 91.3%); snout truncate in dorsal and lateral views; nearly terminal nostrils directed laterally; top of head flat; canthus rostralis concave; loreal region concave; skin on dorsal surface of head and body tuberculate, tubercles formed by osteoderms; tubercles on upper lip, loreal and supraorbital area tipped with tiny blunt keratinous spines; lower eyelid with transparent upper part; a well-developed supratympanic fold running from midpoint of posterior margin of eye above the upper margin of tympanum, slightly curved around its upper posterior edge, tympanum prominent, opaque, smooth, 51.5% of ED, separated from eye by 3.20 mm; upper surfaces of body and limbs tuberculate, intermixed with scattered larger tubercles, cluster of tubercles above the insertion of arms; a triangular serrate-like fringe extends from the elbow along the ventrolateral margin of the forearm and continues along the outer edge of Finger IV to the base of the disk; serrate fringe largest on forearm, less evident serration along fingers; hands moderate in length (HAL/SVL = 31.1%); Finger lengths I<II<IV<III, terminal disk on Finger I 79% of diameter of disks on Fingers II–IV; which are almost the same size as tympanum (3FD/TD 1.04 times); distal subarticular tubercles on Fingers I–III large, rounded; bifid at Finger IV, larger than proximal subarticular tubercles on Fingers III–IV; indistinct supernumerary tubercles; prepollex enlarged and rounded; bony prepollical projection rounded distally, bluntly pointed at side adjacent to thumb; two clusters of nuptial spines at the distal end of prepollical tubercle and at the end of the first phalanx of the thumb; fingers extensively webbed, web extending to base of disk on at least two fingers; webbing formula: **I** $1^{3/4}$–2 **II** $^{3/4}$–$1^{1/2}$ **III** $1^{1/2}$–$1^{1/4}$ **IV**; legs relatively long and slender (TL/SVL = 52.6%), heels of adpressed limbs overlapping about 1/4 length of tibia, thigh 30.00 mm long; distinct fleshy, triangular serrate like fringe begins on heel by a striking flap and extends along ventrolateral margin of tarsus and outer margin of Toe V to base of disk; scallops deeply incised and pointed, largest on tarsus, smaller along toe; small tubercles with keratinized tips present on dorsal and ventral surface of fringe on heel; tarsal fold and outer metatarsal tubercle absent, inner metatarsal tubercle moderately large (same size as 3TD), ovoid, flat, and spadelike distally; toe lengths I<II<III=V<IV; disks on toes 75% of diameter of those on fingers, equal on Toes III–V, decreasing in size on toes II–I; subarticular tubercles rounded; supernumerary tubercles indistinct; toes extensively webbed, webs extending to base of disks on at least four toes; webbing formula: **I** $^{3/4}$–$1^{1/4}$ **II** $^{3/4}$–$1^{1/4}$ **III** $^{3/4}$–1 **IV** $1^{1/4}$–$^{3/4}$ **V**; gular area and venter strongly granulate, fine granulation on undersides of arms and proximal thighs, smooth skin on anterior surfaces of thighs and ventral parts of legs; cloacal opening directed posteriorly at mid-level of thighs, two distinct granular dermal folds under the vent; tongue slightly cordiform; vomerine ridges transverse, narrowly separated medially, placed between the posterior margins of the moderately large ovoid choanae; vomerine teeth 12–13; vocal slits not present.

Coloration of holotype in life (Fig. 3). Dorsal ground colour Light Grass Green (color 109 of Köhler 2012) with irregular Vandyke Brown (281) flecks scattered all over the head and body giving the animal a "moss cryptic" appearance; Raw Umber (22) bands present on dorsal surfaces of arms and legs, edges of scalloped fringes on arms and fleshy flaps on heels Cream Color (12); toe webbing Tawny Olive (17); tops of some dorsal granules and tubercles Orange-Rufous (56). After metachrosis (day and night coloration), ground coloration faded to Pale Emerald Green (141), brown areas to Dark Salmon color (59), pattern did not change; throat, chest, venter and ventral surfaces of arms and legs Cream Color (12) grading into Salmon (83) ventrolaterally and Orange Yellow (8) on anterior surface of thigh; a few small dark blotches on the edge of lower lip; iris Light Yellow Ocher (13), finely reticulated with Dark Brownish Olive (127); tympanum Pale Mauve (204) with scattered irregular Vinaceous Pink (245) blotches.

Coloration in preservative (Fig. 4). Dorsal surfaces Glaucous (272) with Sepia (279) mottling on upper surfaces of hind limbs; tympanum Pratt's Payne's Gray (293) with scattered irregular Maroon (39) blotches, cloacal region Pratt's Payne's Gray (293) dorsally and Cream (12) ventrally; posterior surfaces of thighs Light Yellow Ocher (13); ventral surfaces of body and limbs Cream (12); toe webbing Amber (51).

Distribution and natural history. *Ecnomiohyla bailarina* is known only from the type locality, in the eastern Panamanian montane forest (Fund & Hogan 2012; Fig. 5 A-D). The potential area of distribution of *E. bailarina* comprises the vicinities of Jingurudó and Sapo mountain ranges, between 400 to 1400 m a.s.l. (Fig. 1). Although the type locality is in a primary forest, there are some open areas with successional secondary forest. The area is on a ridge, so the trees could be affected by strong winds. In the surroundings we saw four fallen large trees probably overthrown by the wind that left clearings in the otherwise pristine forest. The largest trees in this area reached more than 20 m in height having branches in the canopy covered by bromeliads and other epiphytes (e. g., orchids

and Lorantaceae), Tree trunks were almost bare or with just a little epiphytic growth. In the understory, palms and vines were predominant. The holotype was found on a ridge in a water conserving posture (see Fig 1B in Pough *et al.* 1983) on the bark of a small tree (Fig. 5A), approximately 1.5 m above the ground. The day before the night of the capture was dry except for a drizzle that had fallen in the afternoon between 14:00–15:00 hrs. During the encounter, a slight breeze was blowing. Other amphibian species observed in the area that day were: *Colostethus* aff. *pratti* (Boulenger, 1899), *Craugastor opimus* (Savage and Myers, 2002), *Pristimantis cruentus* (Peters, 1873), *P. taeniatus* (Boulenger, 1912), *Rhinella alata* (Thominot, 1884), and *Sachatamia ilex* (Savage, 1967).

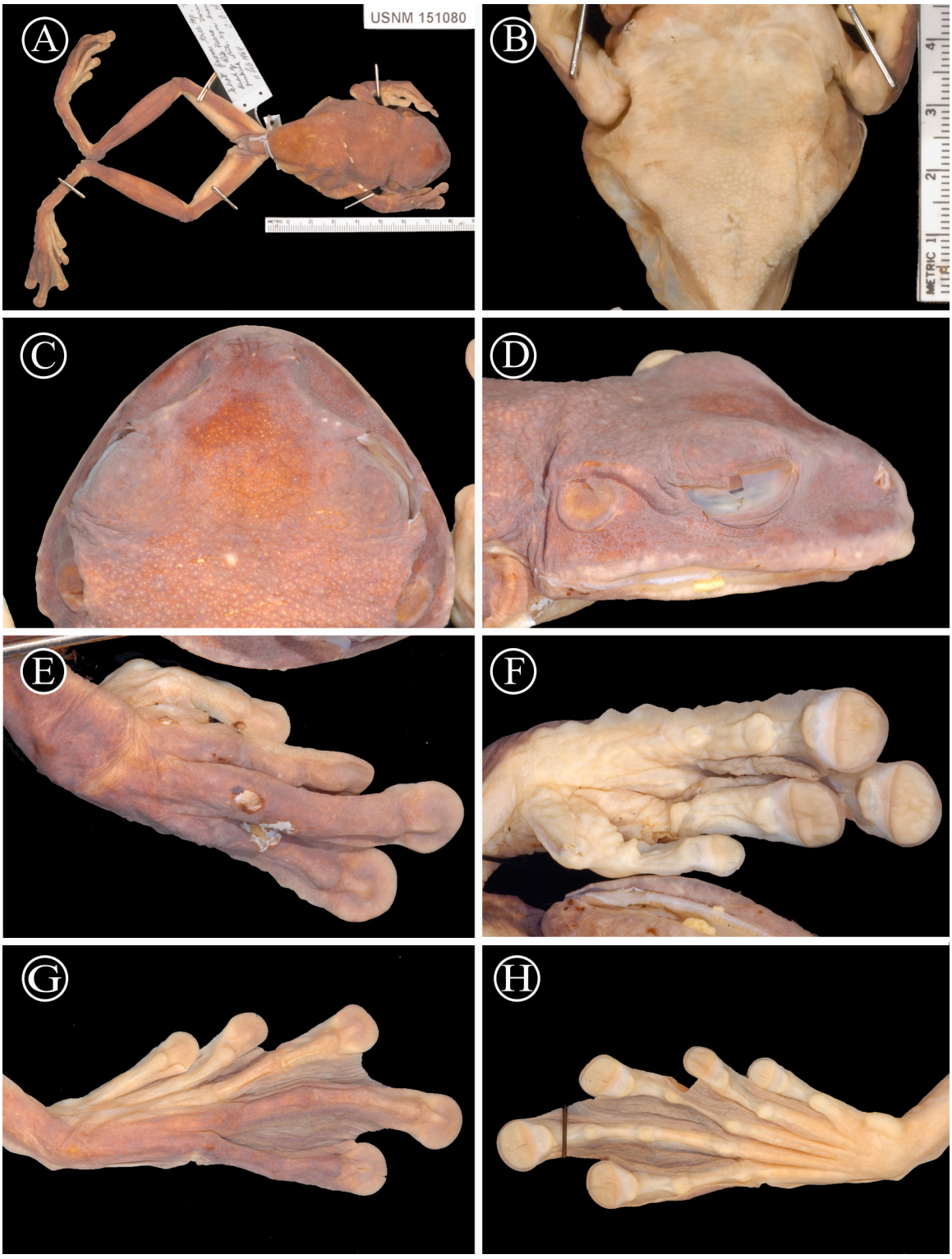

FIGURE 6. *Ecnomiohyla thysanota*, Holotype (USNM151080), preserved specimen. A) dorsal view; B) ventral view; C) head dorsally; D) head in profile; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally.

Etymology. The name *bailarina* is a noun in apposition in reference to the hill where the specimen was found. The indigenous people of the Embera call it "Cerro Bailarín", in addition, the English translation of "bailarina" is ballerina, so the name also refers to the resemblance of the fringes on arms and feet of the frog to the tutu skirt that a ballerina wears.

Conservation status. The secretive habits of *Ecnomiohyla bailarina* make the assessment of the population size difficult, as in other *Ecnomiohyla* species. Considering that the status of the *E. bailarina* population is unknown, the data deficient (DD) criterion, according the IUCN (IUCN 2013), seems appropriate for this species, until data on its population trend become available. Moreover, due to fact that *E. bailarina* and *E. thysanota* occur in a region affected by social problems and political conflicts along the border between Panama and Colombia, it is unlikely that there will be sufficient opportunity to visit the region to assess population sizes in the near future.

Ecnomiohyla veraguensis sp. nov.

Ecnomiohyla rabborum–Köhler 2011: p. 224 Fig. 537; p. 226 Fig. 541 b.

Holotype. SMF89877 (original field number AH210) an adult male (Figs. 7–8) collected near Cerro Negro (8.5533 °N, -81.09261 °W, 540 m a.s.l.), Santa Fé National Park, Veraguas, Panama, on 31 March 2009 at 12:00 hrs, collected by Smelin Abrego, Arcadio Carrizo, Andreas Hertz, and Sebastian Lotzkat.

Diagnosis. A medium-sized species of *Ecnomiohyla.* The single known specimen is an adult male, 57.8 mm in SVL) differing from other known species of the genus by following combination of characters: 1) finger webbing extensive, web touching the finger disk on at least one side on Fingers II–IV; 2) toes extensively webbed; web reaching the toe disk at least on one side on four toes; 3) skin on dorsum finely tuberculate with scattered minute keratin tipped tubercles posteriorly; 4) cranial and dorsal osteoderms present; 5) skin on upper surface of head not co-ossified with underlying cranial elements; 6) humerus without enlarged *crista lateralis*; 7) prepollex distinct, recurved, with distinct bony prepollical projection, spadelike and directed laterally; 8) 6–8 widely spaced, keratinized black spines present bordering the outer side of the thumb; 9) a distinct scalloped fringe without pointed tubercles on its ventral surface, arising at the heel and continuing on the outer side of Toe V and reaching almost to the disk of Toe V (Fig. 8); 10) dorsal coloration in life smoke gray, with upper surface of forearms bearing a suggestion of lime green (Fig. 7).

Comparison with other species of *Ecnomiohyla*. *Ecnomiohyla veraguensis* can be distinguished from other species of *Ecnomiohyla* by the following characters (with contrasting features for *E. veraguensis* in parentheses; see Table 3 for more details): *E. echinata, E. minera*, *E. rabborum, E. salvaje, E. thysanota,* and *E. valancifer* can be distinguished from the new species by the lack of cranial and dorsal osteoderms (both present); *E. rabborum* and *E. minera* are further distinct in having a humeral projection in males (no humeral projection); *E. rabborum* has substantial finger webbing, web reaching base of disk on one finger (extensive webbing, web touching the finger disk on at least one side on Fingers II–IV); *E. fimbrimembra* (Fig. 7 E-F), *E. miliaria* and *E. phantasmagoria* lack scalloped fleshy fringes on heels and have pointed heel tubercles instead (scalloped fleshy fringes present, no heel tubercles); *E. fimbrimembra* and *E. salvaje* have the skin on the head co-ossified with the cranium, (skin not co-ossified with cranium); males of *E. miliaria* and *E. phantasmagoria* have a sharp prepollical spine protruding from the prepollex (prepollex recurved, no protruding spine); *E. bailarina* has a strongly tuberculate dorsum (finely tuberculate) and two clusters of numerous, small nuptial spines at the distal end of the prepollex and the base of the pollex (only 6–8 larger, widely spaced nuptial spines along the outer side of the pollex; Fig. 10); *E. tuberculosa* lacks an enlarged prepollical bony projection or keratinized black spines on the prepollex in adult males (enlarged prepollical bony projection and 6–8 widely spaced, keratinized black spines presents); *E. miotympanum* lacks of scalloped dermal fringes on the outer margin of the forearm and foot, large digital disks, and enlarged prepollices (present in *E. veraguensis*); in terms of general appearance, *E. sukia* is most similar to the new species, but differs by a genetic distance in the 16S gene of 7% and the lack of nuptial spines in adult males (6–8 widely spaced nuptial spines on the outer side of the pollex; see Fig. 9 (B, D) and 10); further, *E. sukia* lacks keratin tipped tubercles on the dorsum (presence of keratin tipped tubercles on the dorsum).

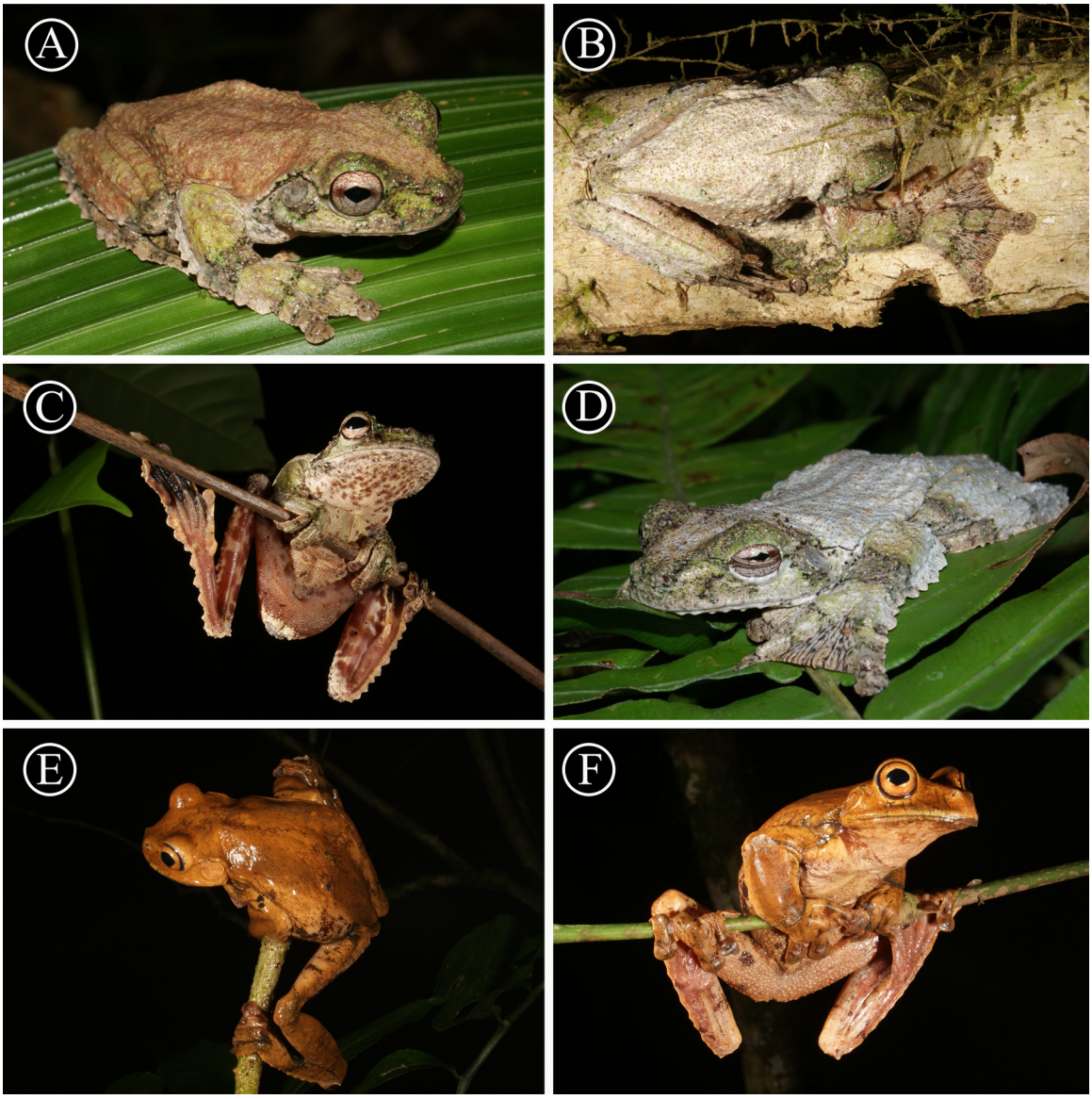

FIGURE 7. Photographs of the holotype of *Ecnomiohyla veraguensis* in life. A) lateral view (night time); B) dorsal view (day time); C) frontal view; D) at the moment of encounter; E-F) voucher specimen of *E. fimbrimembra* (SMF89857).

Description of the holotype. An adult male, as determinated by the presence of nuptial spines, and vocal slits. Measurements of the holotype are indicated in Table 4. Head rounded in dorsal view, wider than long (HL/HW= 85.6%); snout truncate in dorsal and lateral views; nostrils directed laterally; top of head flat; canthus rostralis concave; loreal region concave; a well-developed supratympanic fold running from above the upper margin of tympanum, slightly curved around its upper posterior edge; tympanum prominent, smooth, same color as dorsum, and 60.0% of ED; separated from eye by 3.04 mm; upper surface of body finely tuberculated, scattered tubercles present on dorsal surfaces of the limbs; arms robust, hypertrophied; a fleshy scalloped fringe extends from the elbow along the ventrolateral margin of forearm and continues along the outer edge of Finger IV to base of disk; scallops of fringe largest on forearm, weak scallops along finger; hands moderate in length (HAL/SVL= 36.0%); finger lengths I<II<IV<III, terminal disk on Finger I 70% of diameter of disks on Fingers II–IV, which are slightly smaller in size than tympanum (3FD/TD= 94%); distal subarticular tubercles on fingers rounded and elevated; a row of supernumerary tubercles present under the first phalanges on Fingers II–III; palmar tubercles rounded and low; prepollex enlarged and recurved, bony prepollical projection spadelike, directed laterally at side adjacent to thumb; 6–7 nuptial spines along the outer side of the pollex; fingers extensively webbed, web extending to base of disks on at least two fingers; webbing formula: **I** $1^{3/4}$–2 **II** $^{3/4}$–$1^{1/4}$ **III** $1^{1/4}$–$^{3/4}$ **IV**; legs relatively long and slender (FL/SVL= 48.4%), heels of adpressed limbs overlapping about 1/3 length of tibia, thigh 26.70 mm long; distinct fleshy,

scalloped fringe begins on heel and extends along ventrolateral margin of tarsus and outer margin of Toe V to base of disk; scallops sinuously serrated, widest on tarsus, smaller along toe; tarsal fold slightly evident; outer metatarsal tubercle barely distinct, inner metatarsal tubercle large (1.12 times 3TD), ovoid, slightly elevated, and spadelike distally; toe lengths I<II<III>V<IV; disks on toes 80–91% of diameter of those on fingers, disk on Toe IV same size as disks on Toes III and V, decreasing in size on Toes II–I; subarticular tubercles rounded and slightly elevated; a row of 5–10 supernumerary tubercles barely distinct under the proximal phalanges on toes; extensive toe webbing , webs extending to base of disks on at least four toes; webbing formula: **I** $^{3/4}$–$1^{1/2}$ **II** $^{3/4}$–$1^{1/4}$ **III** $^{3/4}$–$^{3/4}$ **IV** $^{1/4}$–$^{3/4}$ **V**; gular area and venter granulate, fine granulation on undersides of arms and proximal thighs, smooth skin on anterior surfaces of thighs and ventral parts of legs; cloacal opening directed posteriorly at mid-level of thighs, a distinct granular dermal fold under the vent. Tongue slightly cordiform, broader at the base; vomerine ridges large and transverse, well separated medially, placed between the posterior margins of choanae; vomerine teeth 10–14; paired vocal slits extending posteriorly from posterior lateral base of tongue toward angle of jaws.

Coloration of holotype in life (Fig. 7). Coloration in life was recorded at daytime: Dorsal ground color Smoke Gray (44); snout, canthus rostralis, and supraorbital regions Brownish Olive (29) suffused with Olive Green (Auxiliary 47); upper surfaces of forearms with a suggestion of Lime Green (59); dorsal surfaces of finger webbing like dorsal coloration on body, but toe webbing Vandyke Brown (121); ventral surfaces of chin and body Cream Color (54), spotted with Raw Sienna (136); ventral surfaces of hindlimbs True Cinnamon (139); ventral coloration of toe and finger webbings Vandyke Brown (121).

Coloration in preservative (Fig. 8). Dorsal surfaces Grayish Horn Color (268); snout, canthus rostralis, and supraorbital regions Medium Plumbeus (294); darker bars on upper surfaces of limbs Medium Plumbeus (294); cloacal region Pale Buff (1), suffused with Medium Plumbeus (294); groin and posterior surfaces of thighs mottled with Maroon (39) on a Pale Buff (1) ground; ventral regions Pale Buff (1); chin suffused with Maroon (39); toe and finger webbing Burnt Umber (48).

Distribution and natural history. *Ecnomiohyla veraguensis* is known only from the type locality in the Isthmian-Pacific moist forests (Fund & Hogan 2012). The holotype was found at noon on a sunny day at the end of the dry season. Relative air humidity at the moment of encounter was 68% at a temperature of 21.8 °C and it was slightly windy. The frog was sitting in a water conserving posture (Fig. 7D) on a fern leaf approximately 0.5 m above the ground, next to a water tube that is used by local people to obtain drinking water. Other amphibian species that were observed at Cerro Negro on this expedition conducted between March 31 and April 03 2009 include *Atelopus varius* (Lichtenstein & Martens, 1856), *Bolitoglossa colonnea* (Dunn, 1924), *Craugastor gollmeri* (Peters, 1863), *C. megacephalus* (Cope, 1875“1876“), *Diasporus citrinobapheus* Hertz, Hauenschild, Lotzkat & Köhler, 2012, *Lithobates warszewitschii* (Schmidt, 1857), *Pristimantis caryophyllaceus* (Barbour, 1928), *P. cerasinus* (Cope, 1875 “1876“), *P. cruentus*, *P. museosus* (Ibáñez, Jaramillo & Arosemena, 1994), *P. pardalis* (Barbour, 1928), *Rhaebo haematiticus* Cope, 1862, and *Sachatamia albomaculata* (Taylor, 1949). Since *E. veraguensis* is only known from a single specimen from a single locality, the distribution is unknown. It is expected to occur along mid-elevations of the Serranía de Tabasará.

Etymology. The species name is derived from the province name Veraguas where the holotype was found, with the Latin suffix *-ensis* donating a place or locality. The species name has been chosen to accentuate the particular role the province of Veraguas plays in terms of amphibian conservation. It is the only Panamanian province with Atlantic and Pacific coasts, thus encompassing a great variety of habitats for many amphibian species.

Conservation status. As other *Ecnomiohyla* species, *E. veraguensis* could be considered as a rare species, due to the habitats it uses, this fact makes it difficult to assess its populations. Like *E. bailarina*, the data deficient (DD) criterion, according the IUCN (IUCN 2013), seems appropriate for *E. veraguensis* too, until data on its population trend become available.

Discussion

We describe *Ecnomiohyla bailarina* based on both molecular and morphological data. This spectacular species appears to be very distinct from all other known members of the genus. The type locality of *E. bailarina* is relatively close to that of *E. thysanota* (Fig. 6), and our first assumption in the field was, that they could be conspecific. However, after comparing pictures of the *E. thysanota* holotype with our specimen we easily detected

several substantive differences in morphology, mainly demonstrated by the presence of cranial and dorsal osteoderms in *E. bailarina* and not in *E. thysanota*, the different fringe shape on the heel as well as a different skin texture, which argue for two distinct species despite of the lack of molecular genetic data from *E. thysanota*.

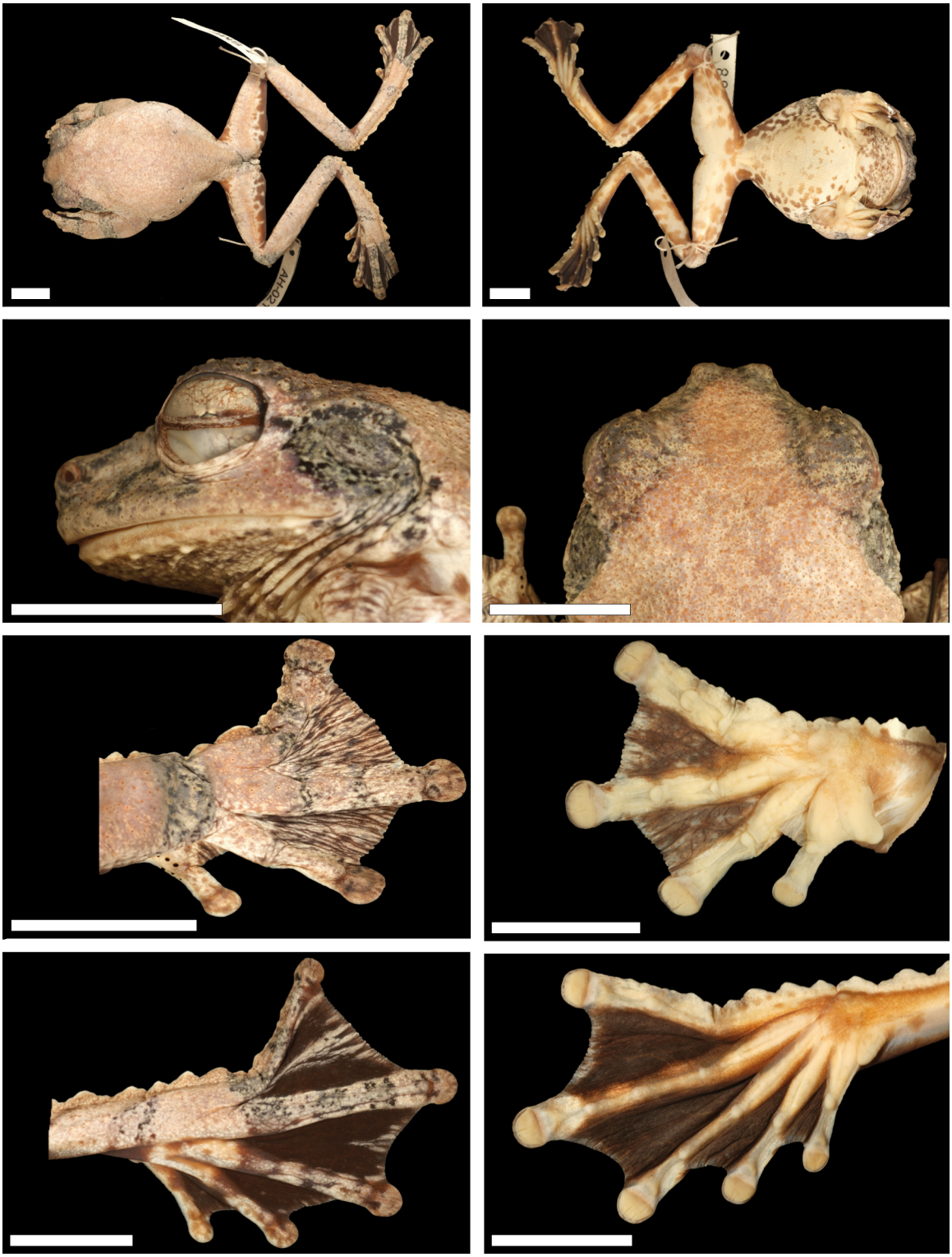

FIGURE 8. Holotype of *Ecnomiohyla veraguensis* in preservation. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars= 10 mm.

Ecnomiohyla veraguensis is similar to *E. sukia* in overall appearance and both species clusters as sister clades in the 16S tree. However, the genetic distance between them is 7% and thus far above the threshold of 3%, that is commonly used to identify potential candidate species with 16S mtDNA barcoding in the tropics (Vences *et al*.

2005; Fouquet *et al*. 2007; Jansen *et al.* 2011; Crawford *et al*. 2010, 2013). Additionally, *E. sukia* lacks nuptial spines in all examined males (Fig. 10D; Brian Kubicki pers. comm. 2011). Although the appearance of spines and tubercles could be influenced by seasonality in some other species (Mendelson *et al.* 2008), this seems not to be the case in *E. sukia*, for which two adult males have been examined, and one was kept in captivity for four years without evidence of developing any nuptial spines (Savage & Kubicki 2010). Moreover, the holotype of *E. sukia*, an adult male, was collected on 25 March 1999, thus in the same season (see ETESA 2009, and IMN 2009) as the holotype of *E. veraguensis*, but showed no nuptial spines.

So far, this is the most comprehensive phylogenetic study of the genus *Ecnomiohyla* based on molecular data. DNA sequences of few species of fringed frogs were already used in several older large-scale phylogenies (Faivovich at al. 2005, Wiens et al. 2010; Pyron & Wiens 2011) to uncover the phylogenetic relationships inside the Hylidae, but without comments to on relations inside the genus. Herein we used sequence data for six of the seven *Ecnomiohyla* species known from lower Central America and for *E. miotympanum*. Since our motivation is to identify species delimitations through genetic barcoding, we used only the widely accepted mitochondrial 16S marker (Monaghan *et al.* 2009; Vieites *et al.* 2009). Deeper analyses, including nuclear markers to support the phylogenetic relationships between the species are certainly needed.

Anyway, we found incongruence between our molecular phylogenetic analysis and the three morphological groups suggested by Savage & Kubicki (2010). In their concept *Ecnomiohyla bailarina* would clearly meet the Group 2 criteria, whereas *E. veraguensis* meets the criteria of Group 3, assuming that nuptial spines are not necessarily absent, but only largely reduced. Our phylogenetic analyses revealed two major clades in the genus. One clade contains only *E. miotympanum* and stands opposed to the rest of the species in the genus. The latter is divided into two subclades, separated by a well-supported node in both trees (bootstrap value, bs: 99.8; posterior probability, pp: 100): Sub-clade 1 contains *E. rabborum*, *E. bailarina*, and *E. fimbrimembra*; and subclade 2 contains *E. sukia*, *E. veraguensis*, and *E. miliaria*. Thus, *E. bailarina* appears to be closer related to *E. rabborum* (Group 1), than to *E. fimbrimembra* (Group 2). Our subclade 2 also contains all available species assigned to Group 3 by Savage & Kubicki (2010) (Fig. 2). As a consequence of these results, we modify the groups proposed by Savage & Kubicki (2010) into two consequent groups, characterized as follows (for the remaining species, see below): In members of the *E. fimbrimembra* species group (Group 1), male frogs may have a bony humeral projection or not, but always have conspicuous cluster of black keratinized nuptial spines on thumb and prepollex, as demonstrated in species *E. bailarina, E. echinata, E. fimbrimembra, E. minera*, *E. rabborum*, and *E. salvaje.* While in the *E. miliaria* species group (Group 2), male frogs have neither humeral projection nor black nuptial spines on the prepollex. A few nuptial spines may be present on the thumb, but if this is the case these are fewer than ten, usually light brown (not black) and widely spaced (not building a cluster); this group contains *E. miliaria*, *E. phantasmagoria*, *E. sukia*, *E. valancifer*, and *E. veraguensis* (Fig. 10).

In our phylogeny *E. miotympanum* is the only member of an own species group within *Ecnomiohyla* and appears as a sister clade to other *Ecnomiohyla* what is strongly supported (bs: 89.6; pp: 97). Thus the molecular monophyly of all members of the genus we have data for, is confirmed here. Other recent studies suggested *Ecnomiohyla* may not be monophyletic with regard to *E. miotympanum*, even though this was not strongly supported (Wiens *et al.* 2010; Pyron & Wiens 2011). While our taxon sampling of *Ecnomiohyla* species is large, the molecular dataset is restricted to a single marker, so a deeper analysis is still needed. However, as a consequence from our results we continue to treat *E. miotympanum* as a member of the genus *Ecnomiohyla* for the moment. Savage & Kubicki (2010) pointed out that *E. tuberculosa* should not be included in the genus as it lacks the enlarged prepollex and prepollical bony projection, which is present in all other species in the genus. Pro tem, we are including *E. tuberculosa* within the genus, until further data becomes available. Further, we could not assign *E. thysanota* to one of the species groups, since the male of this species is not known yet and molecular data is lacking.

In Table 4, we have summarized the morphological characteristics of the two species described here compared to the other *Ecnomiohyla* species that are present in Lower Central America (Table 3). The genus is distributed as follows: In Lower Central America, Colombia and Ecuador, the fringe-limbed frog representatives are *E. bailarina, E. fimbrimembra, E. miliaria, E. phantasmagoria, E. rabborum*, *E. sukia, E. thysanota, E. tuberculosa,* and *E. veraguensis* (Ortega-Andrade *et al.* 2010; Savage & Kubicki 2010; Köhler 2011; Ron 2012; this paper); in Nuclear Central America the fringe-limbed frogs are *E. echinata, E. miliaria, E. minera*, *E. salvaje*, and *E. valancifer*; The only known species reaching North America in eastern and central Mexico is *E. miotympanum.* The most widespread species seems to be *E. miliaria*, which is found in Nuclear and Lower Central America, distributed

from south-eastern Honduras to central Panama (Köhler 2011). However, it seems likely that *E. miliaria* represents more than one species (Solís *et al.* 2010). One evidence for this assumption is that there are two specimens from Panama assigned to *E. miliaria*, one from the Reserva Forestal Fortuna dam site (Myers & Duellman 1982) and one from El Copé (Savage & Kubicki 2010), which indeed are different to *E. miliaria* from Nuclear Central America (revised by Savage & Kubicki 2010), *E. sukia* or *E. veraguensis* (different in skin texture and fringe shape, see Fig. 13 in: Myers & Duellman 1982).

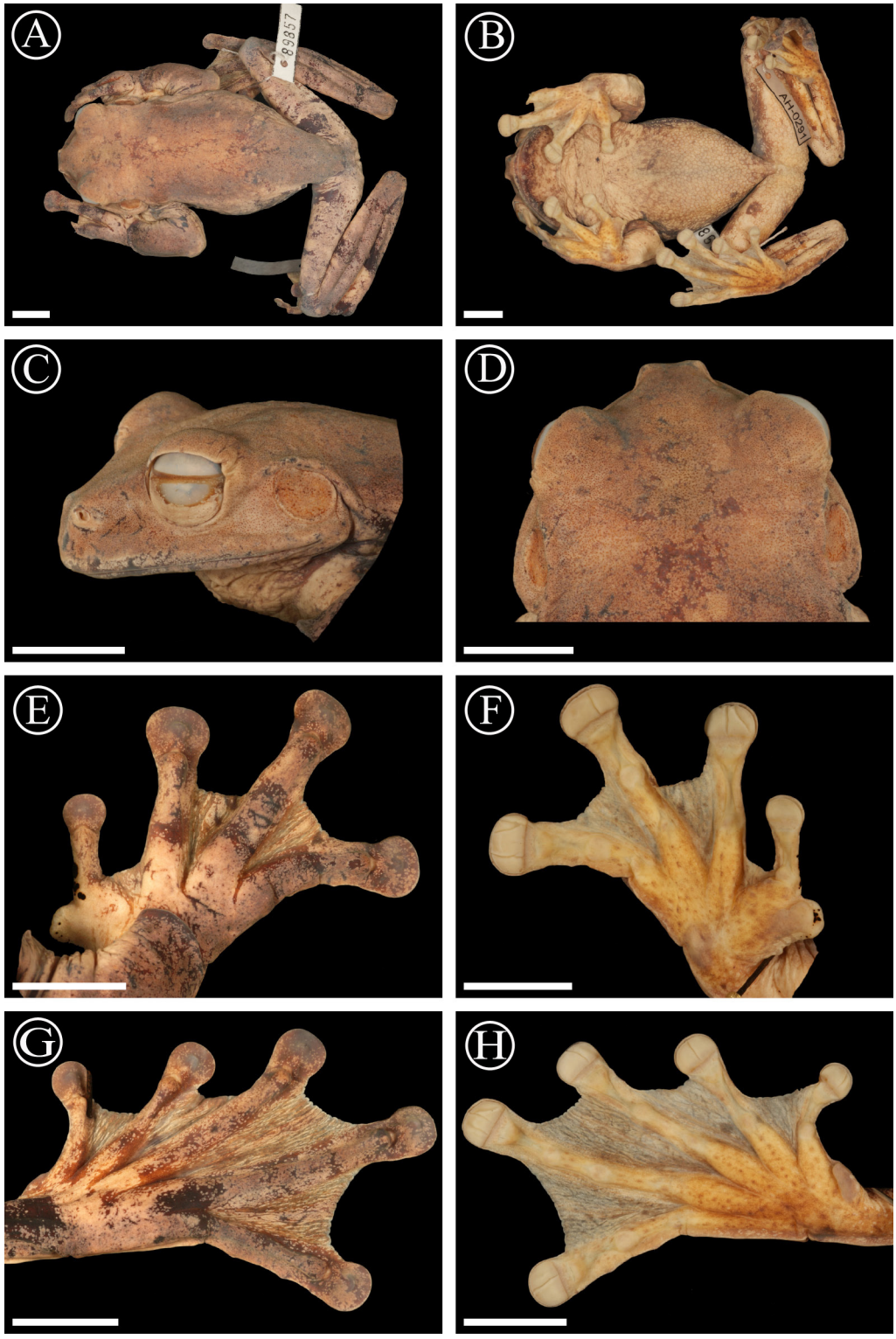

FIGURE 9. *Ecnomiohyla fimbrimembra* (SMF89857), preserved male specimen from Panama. A) dorsal view; B) ventral view; C) head in profile; D) head dorsally; E) right hand dorsally; F) right hand ventrally; G) right foot dorsally; H) right foot ventrally. Scale bars= 10 mm.

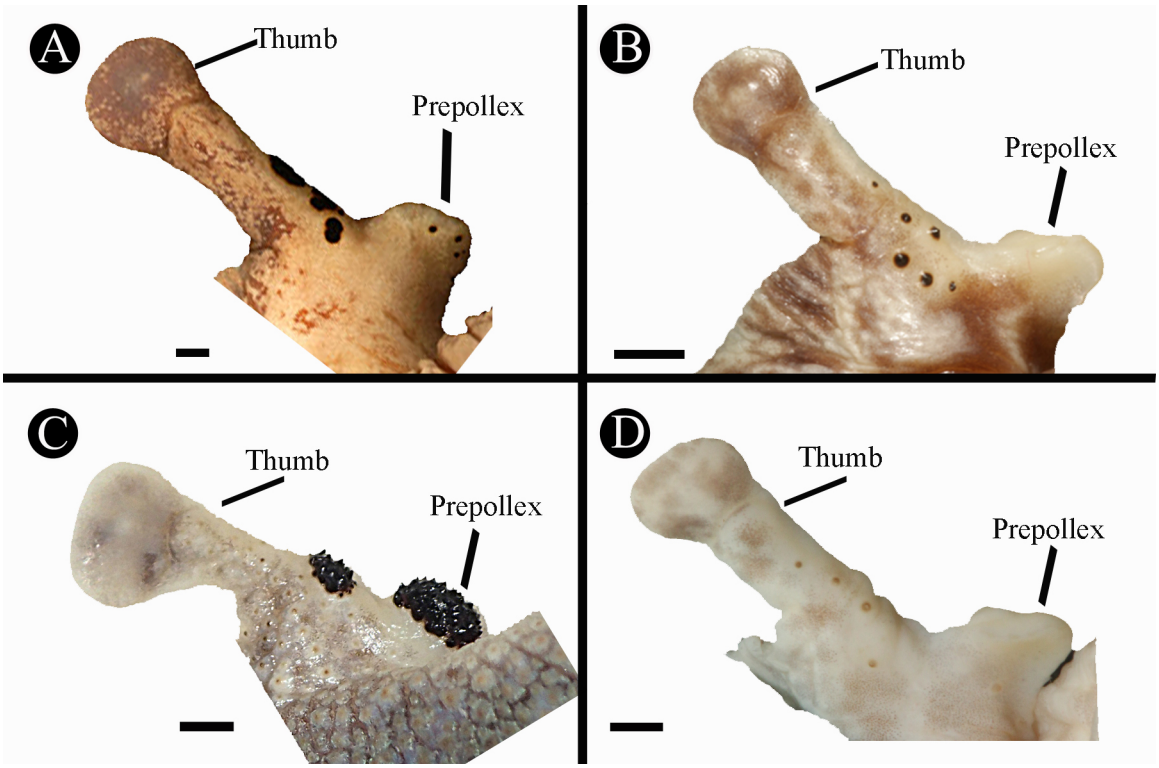

FIGURE 10. Details of the thumb and the keratinized black spines on prepollex, A) *Ecnomiohyla fimbrimembra* (SMF89857); B) *E. veraguensis* (SMF89877); C) *E. bailarina* (SMF97398); D) *E. sukia* (SMF94578). Scale bars= 1 mm

Key to the species of the genus *Ecnomiohyla*.

1a. No scalloped dermal fringes on the outer margin of the forearm and foot . . . . . *E. miotympanum*
b. Scalloped dermal fringes on the outer margin of the forearm and foot present 2
2a. Males without an enlarged prepollex and prepollical bony projection . . . . . *E. tuberculosa*
b. Males with an enlarged prepollex and prepollical bony projection 3
3a. Webbing between Finger II–IV not extending beyond penultimate subarticular tubercle on Finger III (Fig. 9F); supratympanic fold continuing posteriorly behind tympanum to terminate above axilla (Fig. 9C); dermal fringe along lateral edge of forearm and tarsus narrow and not or only weakly scalloped; dorsal skin smooth to minutely granular; skin of dorsal surface of head co-ossified with skull . . . . . *Enomiohyla frimbrimembra*
b. Webbing between Finger II–IV extending well beyond penultimate subarticular tubercle on Finger III; supratympanic fold not continuing posteriorly behind tympanum to terminate above axilla; dermal fringe along lateral edge of forearm and tarsus usually prominent and scalloped; dorsal of variable texture; skin of dorsal surface of head co-ossified with skull or not 4
4a. Dorsum uniform green in life; skin of dorsal surface of head not co-ossified with skull, and granular; heel without tubercles but with a well-defined, scalloped dermal fold (Fig. 6A) . . . . . *Ecnomiohyla thysanota*
b. Dorsum brown, reddish brown, or brown with green or darker brown markings or mottling; dorsum granular or tuberculate; skin of dorsal surface of head in adults co-ossified with skull or not; condition of heel variable 5
5a. Dorsum tuberculate; osteoderms usually present 6
b. Dorsum granular or smooth; without osteoderms 12
6a. Humeral projection present; heel without tubercles; prepollex in adult males with scattered small black spines . . . . . *Ecnomiohyla minera*
b. No humeral projection; heel with one or several tubercles or, if without tubercles then with a scalloped fringe; prepollex in adult males with or without small black spines. 7
7a. Dorsum tuberculate without cranial or dorsal osteoderms; heel with one large tubercle; males with spade-like prepollex and flattened prepollical bony projection. . . . . *Ecnomiohyla valancifer*
b. Dorsum tuberculate with cranial or dorsal osteoderms; heel with one or several pointed tubercles or, if without tubercles then with a scalloped fringe; males with variable prepollex and prepollical bony projection 8
8a. Webbing on fingers not reaching the base of disk on any digit . . . . . *Ecnomiohyla phantasmagoria*
b. Webbing on fingers reaching to base of disk on two to four but not all digits 9
9a. Heel with one or several pointed tubercles; black keratin tipped tubercles over most of flanks and venter; prepollical bony projection in males terminating in a sharp spine in adults . . . . . *Ecnomiohyla miliaria*

b. Heel without tubercles but with a scalloped fringe; without black keratin tipped tubercles over most of flanks and venter; prepollical bony projection in males, variable 10

10a. Males without keratinized black spines on prepollex; without black keratin tipped tubercles on dorsum (Fig. 10D). . . . . *Ecnomiohyla sukia*

b. Males with keratinized black spines on prepollex; black keratin tipped tubercles over most of dorsum 11

11a. Dorsum strongly tuberculated; two clusters of nuptial spines at the distal end of prepollical tubercle and the base of prepollex in males (Fig. 10C) . . . . . *Ecnomiohyla bailarina*

b. Dorsum slightly tuberculated; without nuptial spines arranged in clusters, instead 6-7 nuptial spines scattered along the pollex (Fig. 10B) . . . . . *Ecnomiohyla veraguensis*

12a. Cephalic skin co-ossifed with skull; webbing on fingers extensive, reaching to the base of disk on two to four but not all digits; toe webbing full, reaching to the base of disks on all digits . . . . . *Ecnomiohyla salvaje*

b. Cephalic skin not co-ossifed with skull; finger webbing usually not reaching to the base of disk and if, then only on one digit; toe webbing never reaching to the base of disk on all digits. 13

13a. Dorsum smooth; humeral projection absent in males; heel with few small tubercles; SVL of adult females 60.2 mm, SVL of adult males 57 mm . . . . . *Ecnomiohyla echinata*

b. Dorsum granular; humeral projection present in males; heel smooth; SVL of adult females 61.3–79.9 mm, SVL of adult males 62.8–97.3 mm . . . . . *Ecnomiohyla rabborum*

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Appendix IV

Declaration on the contributions of authors

to the publication: Morphological variation and phylogeography of frogs related to *Pristimantis caryophyllaceus* (Anura: Terrarana: Craugastoridae) in Panama.
status: Published (2014).
name of journal: Salamandra 50 (3): 155-171
Authors involved:
- Abel Batista (AB), - Milan Vesely (MV), - Konrad Mebert (KM), - Gunther Köhler (GK), - Andreas Hertz (AH)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 45% – field work (collecting and documenting specimens), molecular analysis
Coauthor MV: 15% – participated in the field trips.
Coauthor KM: 10% – participated in the field trips.
Coauthor GK: 5% – participated in the field trips and documenting specimens.
Coauthor AH: 25% – participated in the field trips and documenting specimens.

(3) to the creation of the data collection and figures

PhD candidate: 45% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor MV: 15% –provided photos, created figures
Coauthor KM: 10% – provided photos
Coauthor AH: 30% – provided tissue samples, sequences, figures and data.

(4) to the analysis and interpretation of the data

PhD candidate: 40% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor GK: 10% – contributed to data analysis and interpretation
Coauthor MV: 20% – contributed to data analysis and interpretation
Coauthor KM: 10% – contributed to data analysis and interpretation
Coauthor AH: 20% – contributed to data analysis and interpretation

(5) to writing the manuscript

PhD candidate: 60%
Coauthor MV: 15%
Coauthor KM: 5%
Coauthor GK: 5%
Coauthor AH: 15%

Date/place: 13.04.2016 / Frankfurt am Main, Germany

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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Morphological variation and phylogeography of frogs related to *Pristimantis caryophyllaceus* (Anura: Terrarana: Craugastoridae) in Panama

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Abstract. New World direct-developing frogs (Terrarana) are among the most diverse vertebrate groups in the world. Many Terrarana species are highly variable in colouration and morphology, often rendering it difficult to delineate species. Modern molecular and bioacoustic techniques are a relatively recent tool for understanding the various taxonomic entities. This affects also *Pristimantis caryophyllaceus,* a complex on which little research has previously been done. We examined the variation of morphology, genetics, and colouration in specimens affiliated to *P. caryophyllaceus* from Panama, using different Molecular Operational Taxonomic Units (MOTUs) based on molecular phylogenetic lineages. Phylogeny, ecology, and distributional information for this species shed light on the position and species delineation of *P. caryophyllaceus* and its congeners in Panama. Our results demonstrate a high level of genetic diversity in *P. caryophyllaceus*-like populations from Panama, which in fact comprise three main lineages that are geographically separated. Specimens from eastern Panama tend to be larger, with more expanded finger disks and toe pads than specimens from western Panama. However, aside from the significant morphological differences between MOTUs, the extent of variation within each MOTU is very large. Based on our extensive and integrative analysis, we suggest treating the three MOTUs of *P. caryophyllaceus* populations as a single polymorphic species with very deep conspecific lineages as a result of the dynamic geological history of the Isthmus of Panama. The validity of the recently described *P. educatoris* is not supported by our results and we therefore synonymize it with *P. caryophyllaceus*.

Key words. Amphibia, Anura, *Pristimantis caryophyllaceus*, *P. educatoris*, Panama, genetic variation, polymorphism, biogeography.

Introduction

Pristimantis caryophyllaceus (Barbour, 1928) is a widely distributed species that inhabits lowland, premontane and the lower portions of the lower montane forest domains, from Costa Rica through Panama to northwestern Colombia (Lynch 1980, Savage 2002). Although this species is distributed from sea level to 1,968 m above, it appears to be most common in the range from 300 to 1,600 m a.s.l. *Pristimantis caryophyllaceus* is characterized by a sharply projecting snout, a large and pointed heel tubercle, and a well-developed superciliary tubercle (Barbour 1928, Savage 2002). *Pristimantis caryophyllaceus* also exhibits a remarkable polychromatism and polymorphism (Hoffman & Blouin 2000, Savage 2002). Recently, one seemingly morphologically distinct lineage was described as a separate species, *P. educatoris,* by Ryan et al. (2010). These authors suggested that *P. educatoris* and *P. caryophyllaceus* are parapatric, with *P. caryophyllaceus* being distributed throughout Costa Rica and western Panama whereas *P. educatoris* occurs from west-central to eastern Panama and into Colombia. As the demarcation line separating the two taxa, these authors identified the high-altitude valley of the Río Chiriquí in the Fortuna depression. However, Ryan et al. (2010) also identified a disjunctive population of *P. educatoris* in extreme southeastern Costa Rica based on two specimens, contradicting this biogeographical concept. Moreover, the morphological analysis and description of *P. educatoris* is based only on specimens from the type locality (i.e., El Copé, Coclé, Panama; Ryan et al. 2010).

Recent genetic studies identified three deep genetic lineages with up to 14.5% divergence (Kimura 2-parameter distance) in the mitochondrial COI gene between samples of *P. caryophyllaceus* from Costa Rica, central Panama, and eastern Panama, respectively (Crawford et al. 2010, Pinto-Sánchez et al. 2012). Crawford et al. (2010) contemplated a possible co-occurrence of at least three candidate species that were concealed under the name *P. caryophyllaceus* at El Copé (Crawford et al. 2010). *Pristimantis caryophyllaceus* is indeed an old lineage, which originated in South America about 12 million years ago and subsequently evolved and expanded its range into Central America prior to the closure of the Isthmus of Panama (Pinto-Sánchez et al. 2012). As a consequence, the divergence of different lineages was triggered by the dynamic geological history in the region and the rise of the Isthmus of Panama, which ultimately resulted in various isolation processes over time.

Ecological information on *Pristimantis caryophyllaceus* (sensu lato) (Dunn 1937, Miyamoto 1984, Heinen 1992, Savage 2002, Lips et al. 2003) is mostly available on populations from western Panama and eastern Costa Rica, whereas there is only little information on the natural history of eastern Panamanian or Colombian populations (Myers 1969). Herein, we contribute new information by describing for the first time the advertisement call of *P. caryophyllaceus* from eastern Panama, and providing additional data on its ecology, biogeography and morphology in an integrative taxonomic approach to potential species delineation within the *P. caryophyllaceus* complex in Panama.

Materials and methods

Fieldwork was carried out in the mountain ranges of Darién, Jingurudó, Majé, Pirre, San Blas, and Sapo in east-

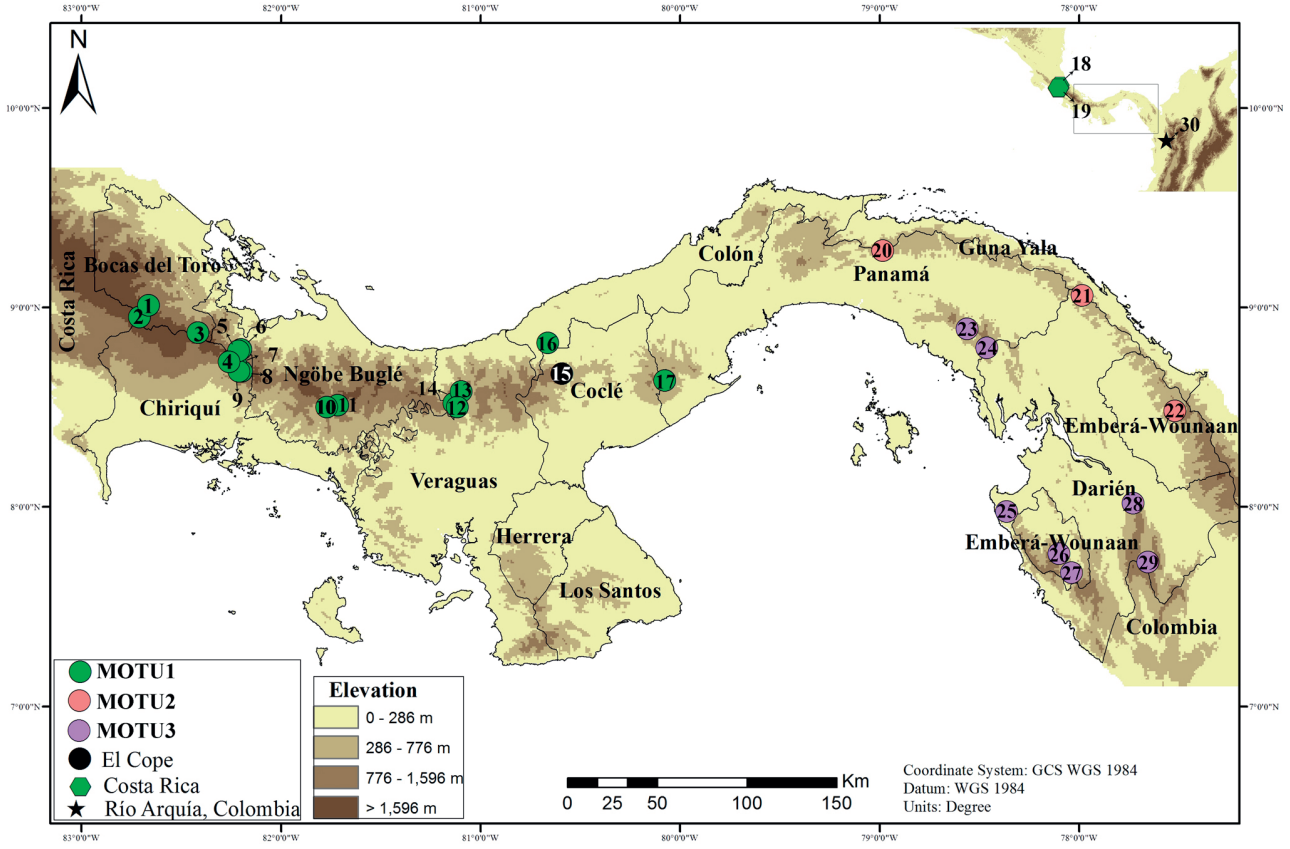

Figure 1. Localities of specimens of the *Pristimantis caryophyllaceus* complex mentioned in this study (Panama main map), lower Central America and northern Colombia (inset). Green colour represents MOTU1, pink MOTU2, and purple MOTU3. The black circle indicates El Copé, and the black star (inset map) refers to one locality in Colombia. One symbol may consolidate several localities close to each other; see text for details. (1) Río Clarito; (2) Río Changena; (3) Sendero El Pianista; (4) Fortuna village; (5) Quebrada Arena; (6) Willie Mazú; (7) Sendero los Tucanes, BP Palo Seco; (8) RF Fortuna; (9) Lost and Found; (10) western slope of Cerro Santiago, La Nevera; (11) Llano Tugrí; (12) Brazo de Mulaba; (13) Cerro Negro; (14) Cerro Mariposa; (15) El Copé; (16) Quebrada Valle Grande, Donoso; (17) Altos del María; (18) Tapanti bridge, Costa Rica; (19) Río Gacho, Costa Rica; (20) Río Terable and Burbayar; (21) Nurra; (22) Río Tuquesa; (23) Ambroya, Majé; (24) Chucantí, Majé; (25) Cerro Sapo; (26) Cerro Garra Garra, Jingurudó; (27) Cerro Bailarín, Jingurudó; (28) Pirre; (29) Cana field station; (30) Río Arquía, Colombia.

ern Panama, and the Tabasará and Talamanca mountain ranges in western Panama (Fig. 1). We evaluated the abundance of members of the *Pristimantis caryophyllaceus* complex by opportunistic search for frogs in the leaf litter and undergrowth along trails. Search transect lengths were calculated with the tracking function of a Garmin GPSmap 60CSx. All georeferences were recorded in the WGS 1984 datum format. Maps and transects were created and calculated using ArcGIS 10 (ESRI 2009). Collected specimens were euthanised with T61, fixed with 5 ml of formalin (10%) in 1 l of ethanol (94%), and subsequently stored in ethanol (70%). All figures incorporated herein have been digitally improved and combined using Adobe Photoshop CS3. For candidate species and their delimitation, we follow the integrative concept of Vieites et al. (2009).

Molecular laboratory and phylogenetic inferences

MtDNA was extracted from fresh muscle or liver tissue. The mitochondrial 16S mtDNA was amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany) by performing an initial denaturation for 1 min at 94°C followed by 35 steps with denaturation for 9 s at 94°C, annealing for 27 s at 45°C, and elongation for 1.5 min at 72°C. Final elongation proceeded for 7 min at 94°C. For the nuclear RAG1 (Recombination Activating Gene 1) we used 1 cycle: 2 min at 96°C; 45 cycles: 20 s at 95°C, 25 s at 52°C, 2 min at 72°C; 1 cycle: 7 min at 72°C. The reaction mix consisted of 1 µl mtDNA template, 2.5 µl Reaction Buffer x10 (PeqGold), 4 µl 2.5 mM dNTPs, 0.4 µl (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µl H_2O, 1 µl 25 mM $MgCl_2$, and for 16S 1 µl per primer (containing 10 pmol, forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon), and for RAG1 3 µl per primer (forward: R182, 5'- GCCATAACTGCTGGAGCATYAT -3'; reverse: R270, 5'- AGYAGATGTTGCCTGGGTCTTC -3'; eurofins MWG Operon, Heinicke et al. 2007). The COI gene was sequenced at the Southern China DNA Barcoding Center. In total we could sequence eleven samples (eight 16S, seven COI, and nine RAG1). We compared the mtDNA data of our specimens with that published for thirteen specimens in GenBank (thirteen sequences for 16S & COI and four for RAG1). The sequences were aligned with CLUSTAL W and edited visually using Geneious version 6.1 (Biomatters Inc., available online from http://www.geneious.com/). A list of specimens included in our genetic analysis with corresponding GenBank accession numbers is presented in Supplementary Table S5. *P. cerasinus* and *P. cruentus* were used as outgroups. The final alignment of the 16S mtDNA comprised 24 sequences of 473 bp in length, of which 110 sites were variable and 82 were parsimony-informative. The final alignment of the COI gene comprised 19 sequences consisting of 559 bp, of which 189 sites were variable and 158 sites were parsimony-informative. For 18 specimens, we analysed sequences of both genes, allowing us to combine COI and 16S genes consisting of 1032 bp, of which 292 were found to be variable and 233 sites parsimony-informative. Only ten samples of combined mitochondrial genes and the nuclear RAG1 gene were obtained (excluding outgroups), consisting of 1653 bp, of which 1523 sites were variable and 1461 were parsimony-informative. Using MEGA6 (Tamura et al. 2011), we computed uncorrected pairwise genetic distances for COI and 16S both separately and combined. For each gene and for the combined-gene data set, we conducted Maximum Likelihood (ML) analyses, using the Tamura-3-Parameter, with 1,000 bootstrap replicates. Prior to model-based phylogenetic inferences, JModeltest 0.1.1 (Posada 2008) was used under the corrected Akaike Information Criterion (AICc) to select the substitution model for the Bayesian analysis. We ran a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) for 20,000,000 generations with four default chains, sampling every 100th generation and subsequently discarding 5% as burn-in. To test species delimitation in the case of *P. educatoris* – *P. caryophyllaceus*, we applied two different methods. First, we conducted a statistical parsimony network analysis with gaps considered as a fifth character state (only for COI) in TCS v1.21 (Clement et al. 2000). In order to connect all haplotypes we set the connection limit to 15 steps. Second, we used the Automatic Barcode Gap Discovery (ABGD) algorithm (Puillandre et al. 2011) under the following settings: steps = 20, distance = Kimura 2-parameter model with a transversion/transition ratio of 2.0, and the setting for the minimum relative gap width (X) varied at values between 0 and 1.5. The MOTU's phylogenetic relationships and divergence times were estimated for the mtDNAs 16S and COI, and the nDNA RAG1 (10 individuals and 1,699 bp), using the program BEAST 1.5.4 (Drummond & Rambaut 2007), with a relaxed clock, allowing substitution rates to vary according to an uncorrelated log-normal distribution, assuming a Yule tree prior (Drummond et al. 2006). The prior distributions of substitution parameters were set as default, and to calibrate the root and one node age, respectively, we used an age of approximately 32 million years (Mya) with a standard deviation of seven million years for the splitting of the related species *P. cerasinus* and *P. cruentus* from *P. caryophyllaceus*, and together with the maximum and minimum estimated crown ages (10.4–14.4 Mya) for the *P. caryophyllaceus* clade obtained by Pinto-Sánchez et al. (2012). Every MOTU was treated as a monophyletic group. Parameters were estimated using 100 million generations with a burn-in of 10 million generations, and trees were sampled at every 10,000th generation. Results were visualized and compared using Tracer 1.5 (Rambaut & Drummond 2009), and summary trees were generated using TreeAnnotator 1.5.4.

Morphometrics

Morphological nomenclature, measurements and diagnosis follow Duellman & Lehr (2009) and Köhler (2011). All measurements were taken with digital callipers and rounded to the nearest 0.01 mm. Measurements are giv-

en as mean ± standard deviation and range in parenthesis (Supplementary Tab. S1). Specimens were deposited in the Museo Herpetológico de Chiriquí (MHCH) at the Universidad Autónoma de Chiriquí, Panama, and the Senckenberg Research Institute and Nature Museum (SMF), Frankfurt, Germany. Morphological data of similar *Pristimantis* species used for comparisons were taken from the respective original descriptions.

The following morphometric measurements were taken (with abbreviations): snout–vent length (SVL); head length (HL), diagonally from angle of jaw to tip of snout; head width (HW), between angles of jaws; interorbital distance (IOD); eye length (EL), from anterior to posterior edge of externally accessible eye; eye to nostril distance (END), from anterior edge of eye to posterior corner of nostril; internarial distance (IND), between centres of nostrils; forearm length (FAL), from proximal edge of palmar tubercle to outer edge of flexed elbow; hand length (HAL), from proximal edge of palmar tubercle to tip of third finger; tibia length (TL), as the distance from the knee to the distal end of the tibia; foot length (FL), from proximal edge of outer metatarsal tubercle to tip of fourth toe; width of third finger (3FW), at penultimate phalanx just anterior to disk; width of disk of third finger (3FD), at greatest width; width of fourth toe (4TW), at penultimate phalanx just anterior to the disk; width of disk of fourth toe (4TD), at greatest width; and tympanum diameter (TYMP), measured horizontally, supposing an approximately circular tympanum. We determined the sexes of adults by differences in SVL (males smaller than females), the presence of vocal slits in males, and the presence of eggs in females. Specimens with a SVL < 15 mm were classified as juveniles and excluded from the morphological analyses.

We sorted genetic lineages into Molecular Operational Taxonomic Units (MOTUs) to investigate morphological differences among these MOTUs. To test such differences between taxa currently assigned to *P. caryophyllaceus* and *P. educatoris,* we provisionally classified specimens from west of the Fortuna depression as members of the former species, whereas specimens from east of Fortuna were allocated to the latter species. We furthermore used the condition of the subarticular tubercles according to Ryan et al. (2010) as a character to distinguish between both taxa. The statistical analyses were performed using SPSS 17.0.

Bioacoustics

Advertisement calls were recorded using a Marantz Professional (PMD 620) or a Panasonic RR-XS410 digital recorder with a Sennheiser ME 66 shotgun microphone capsule with a Sennheiser K6 powering module that were set up at distances of 0.5 to 1.5 m from the calling male. Ambient temperature and humidity were measured using an Oakton digital thermo-hygrometer. Males were recorded at a sampling rate of 44 kHz and 16-bit resolution. Recordings were made in uncompressed PCM format and saved as wav-files. The spectral and/or temporal parameters were analysed and the power spectra calculated in Raven Pro 1.4 (Window: Blackman, DFT: 2048 samples, 3 dB filter bandwidth: 158 Hz; Grid spacing 21.5 Hz; overlap 70.1%; Charif et al. 2004). Lowest and highest frequencies were measured 20 db below peak frequency. Terminology used in the advertisement call description follows Duellman & Trueb (1994). To calculate call rates, we divided the average call duration by 60 (sec.) plus the average of call intervals.

Colour variation

Generalized colouration summaries were derived directly from live specimens or indirectly from photos of live specimens. Within the standardized colour descriptions of selected individuals, the capitalized colours and colour codes (the latter in parentheses) are those of Smithe (1975–1981).

Results

Based on the lineages resulting from our phylogenetic analysis (see section on molecular phylogenetics below), we assigned each specimen to one of three MOTUs. The MOTUs were largely consistent with a geographical pattern and defined as follows (Fig. 1): MOTU1 contains specimens from western Panama, MOTU2 from eastern Atlantic Panama (Daríen and San Blas mountain ranges), and MOTU3 from eastern Pacific Panama (Jingurudó, Majé, Pirre and Sapo mountain ranges). Taking into account the congruence between the phylogenetic results and the biogeographical pattern, we assigned 78 measured specimens to their respective MOTUs, with 53 to MOTU1, 18 to MOTU2, and seven to MOTU3. In the following, we present the results of our analyses of molecular genetics, morphometrics, colour variation, natural history, geographic distribution, and vocalisation of the *Pristimantis caryophyllaceus* species complex.

Molecular genetics

MOTUs based on mitochondrial data usually contained samples only from one biogeographical region, except the samples from El Copé, which are represented in all three MOTUs by at least one sample. No nuclear genetic data were available for El Copé samples to elucidate their congruence to mitochondrial data, and investigate the potential of introgression among MOTUs at El Copé. The tree topology of the combined mitochondrial genes (Fig. 2) and the COI alone (Supplementary Fig. S1) were basically congruent. The distances between and within MOTUs are shown in Table 1 (see also Supplementary Tabs S2–S4 for genetic distances between MOTUs).

In the parsimony network analysis based on the 16S gene, seven samples formed unconnected haplotype networks, and six did so in the analysis of the COI gene. The samples from El Copé in MOTU1 were connected to the

sample from Donoso (MOTU1) with eight mutational steps between them in the 16S network and four in the COI. The samples from Altos del María, Panama and Río Gacho, Costa Rica, were connected to nine unsampled haplotypes in the 16S network, but were not connected in the COI network; one sample from Tapantí, Costa Rica, was connected to the samples from Río Changena and Río Clarito, Bocas del Toro, with 12 unsampled haplotypes between them (only one of these samples was included for COI). In MOTU2, only the samples from El Copé were grouped in the same haplotype, in both 16S and COI. In MOTU3, the samples from the Majé and Jingurudó mountain ranges (16S and COI), as well as those from the Cana field station (COI) were connected. Our ABGD analysis generated five groups for 16S with a divergence threshold of 0.022 with a relative width of the barcoding gap of 0.05 in the X-value. For COI, it produced eight groups, assuming an a priori intraspecific divergence threshold of 0.068 with a relative gap width of 0.05 (X-value), whereas ten groups resulted when both genes were combined (threshold of 0.048). Our three analyses (16S, COI, and both genes combined) lumped all samples in one unit, with a priori intraspecific divergences of 0.030, 0.088, and 0.062, respectively. With ABGD, all samples from MOTU2 and almost all from MOTU1 for the COI and 16S sequences (Supplementary Figs S1–S2) were grouped in their corresponding geographic MOTU. The 16S ABGD assigned all samples of MOTU1 to one cluster, but also included samples from Majé that were placed in MOTU3 according to our phylogenetic analyses. One sample from El Copé (USNM 572338) did not nest within any cluster in the 16S analysis, but took a place within MOTU3 when using COI and both genes combined.

According to our divergence time estimates, the MOTUs started to diverge from other species of the subgenus *Hypodictyon* Cope, 1885 (sensu Hedges et al. 2008), which were present in Central America in the Oligocene 23.16 Mya (with a 95% credibility interval, CI, of 17.33–29.46 Ma). The crown age of the MOTUs dates to 13.27 Mya (CI: 11.38–14.39 Mya) during the Miocene, when MOTU1 and MOTU2 + MOTU3 split; a second break between the ancestors of MOTU2 and MOTU3 occurred 12.19 Mya, followed by several splitting processes within the respective MOTUs between 7.6 and 3.5 Mya. When all genes were used in the phylogenetic analysis, the ML analysis yielded a consensus tree that was topologically congruent with the divergence-time tree. However, in the divergence-time analyses, when each MOTU was treated as a monophyletic group, the Majé samples were nested within MOTU1, showing a divergence-time of 11.12 Mya. This is not supported (p = 0.66, see Supplementary Fig. S4), however, although when the MOTUs were not supposed to be monophyletic, the posterior probability for the divergence-time analysis was not supported either (p = 0.54, Fig. 3 and Supplementary Fig. S3).

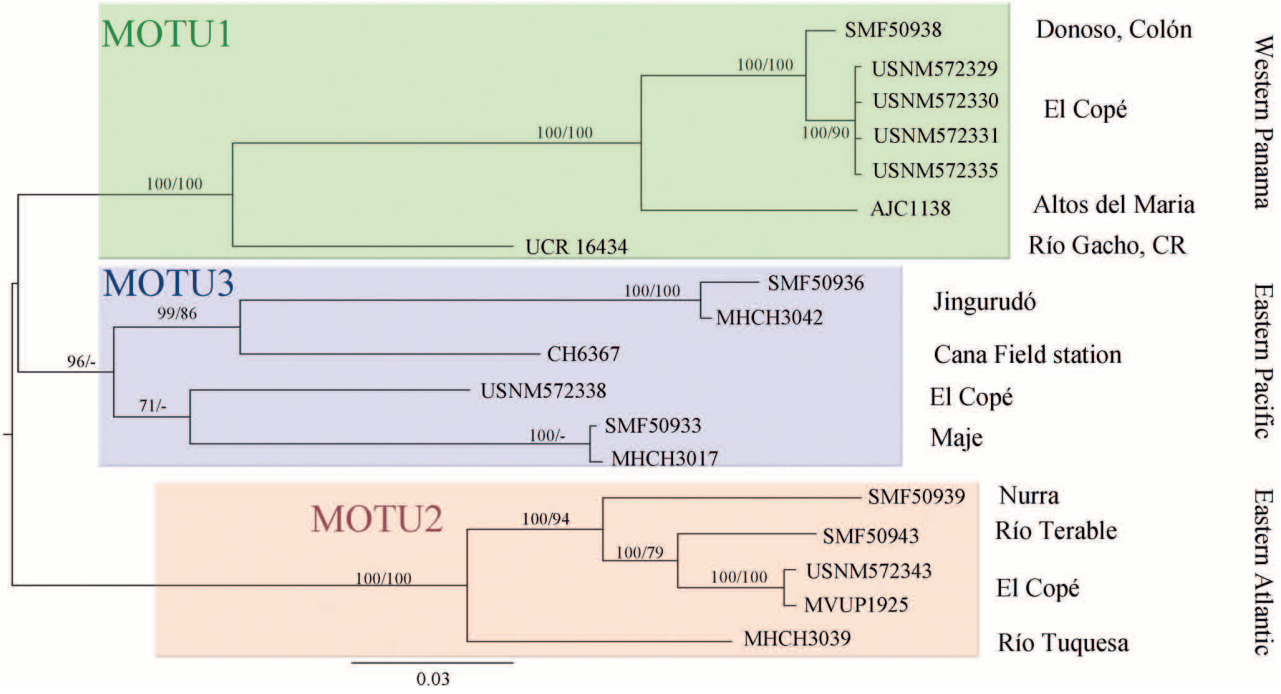

Figure 2. Maximum likelihood tree of the combined COI and 16S mtDNA sequences of the *Pristimantis caryophyllaceus* complex. Specimen labels refer to collection or museum numbers. Colour shadings of MOTUs correspond to those in Figure 1. Scale bar refers to number of substitutions per site. Maximum likelihood bootstrap values are shown above branches, and Bayesian posterior probabilities (multiplied by 100) below slash. Tree, midpoint root tree.

Table 1. Tamura 3-parameter distances among the *Pristimantis caryophyllaceus* (including "*P. educatoris*") specimens used in this study. MOTU: MOTU1 contains specimens from western Panama, MOTU2 from eastern Atlantic Panama (Daríen and San Blas mountain ranges), and MOTU3 from eastern Pacific Panama (Jingurudó, Majé, Pirre and Sapo mountain ranges).

Genes	Average genetic distance	Distance within MOTUs			Distance between MOTUs		
		MOTU1	MOTU2	MOTU3	MOTU1-MOTU2	MOTU1-MOTU3	MOTU2-MOTU3
16S	6.5	4.02	1.49	6.1	7.15	7.53	8.9
COI	14.98	6.86	7.23	9.91	19.96	18.13	15.75
16S + COI	12.43	5.4	5.53	9.12	16.05	14.8	13.76

Morphometrics

In Supplementary Table S1, we present the morphometric variables used to evaluate the differences between lineages within the *Pristimantis caryophyllaceus* complex. Our morphological analysis revealed differences between the three MOTUs (Fig. 4). A Discriminant Function Analysis (DFA) classified 83.3 % of the specimens according to our a priori groupings (94.3% MOTU1; 71.4% MOTU2; 55.6% MOTU3). The principal morphological variables contributing to the grouping were 1) TYMP/SVL, 2) 4TD/4TW, 3) IOD, 4) 3FD; the first function is: DS = 0.63 * TYMP/SVL + 0.44 × 4TD/4TW + 1.23 × IOD + -0.88 × 3FD; and the second function is DS = -0.55 × TYMP/SVL + 0.123 × 4TD/4TW + 0.17 × IOD + 0.66 × 3FD. The specimens included in MOTU3 are usually larger than those from MOTU2 and MOTU1, respectively. Likewise, MOTU2 and MOTU3 seem to be more similar to each other (MOTU2 → MOTU3: 28.6%) than either of them is to MOTU1 (3.8% and 1.9%, respectively; see Figs 4 + 5).

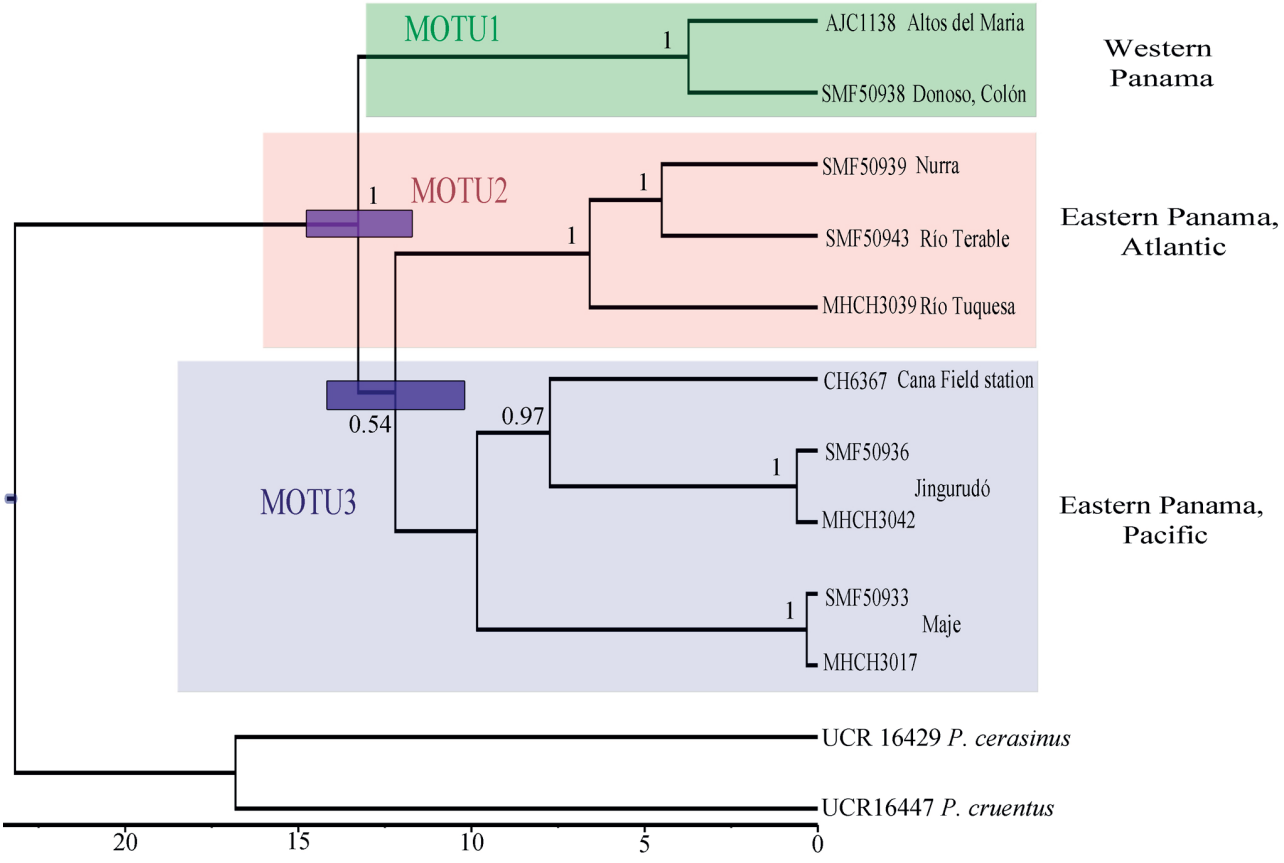

Figure 3. Timetree of *Pristimantis caryophyllaceus* based on RAG1, 16S and COI genes, with *P. cerasinus* and *P. cruentus* as outgroups. Scale along the bottom indicates time in millions of years (Mya). Colour of shading reflects MOTU designations (for species at tips), as in Figs 1 and 2. Blue horizontal bars indicate 95% credibility intervals for the divergence time of the MOTUs. Numbers on nodes indicate estimated posterior probabilities for the presence of the corresponding clade according to BEAST (see text for details).

Our analysis of differences between specimens from west of Fortuna, alias *Pristimantis caryophyllaceus*, and east of Fortuna, alias *P. educatoris* sensu Ryan et al. (2010), showed differences between each other, both according to geography (MANOVA, Pillai's trace = 0.66, $F_{12, 44}$ = 7.20, P < 0.05) and based on the condition of subarticular tubercles (MANOVA, Pillai's trace = 0.34, $F_{11, 45}$ = 2.13, P = 0.04). The DFA classified specimens on the basis of geography from western and eastern Panama correctly, with 98.4% probability, into their respective groups (DFA = 1.12 × 3FD/3FW + 0.67 × IOD/HW + 1.37 × EL + -1.68 × 3FD + 0.45 × 4TD/4TW), and when using the condition of subarticular tubercles, the DFA classified the two MOTUs with 70.5% probability into their original groups (DFA = 1.31 × EL + 0.70 × 4TD/4TW + -0.98 × SVL).

Colour variation

Pristimantis caryophyllaceus is one of the most polychromatic species in its genus. The general dorsal colouration varied from yellow to reddish with various brownish tonalities, with or without black chevron marks on the dorsum, and sometimes with sulphur-yellow spots on

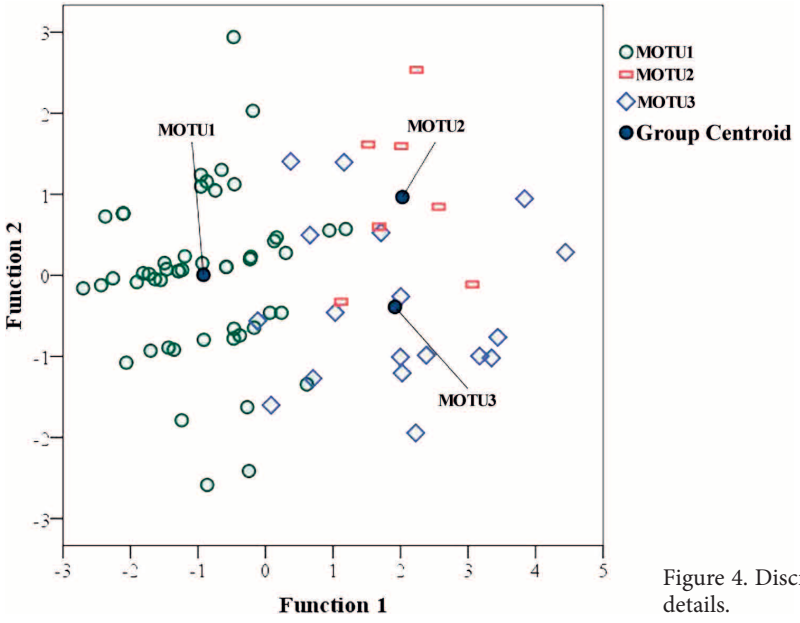

Figure 4. Discriminant function analysis of MOTUs; see text for details.

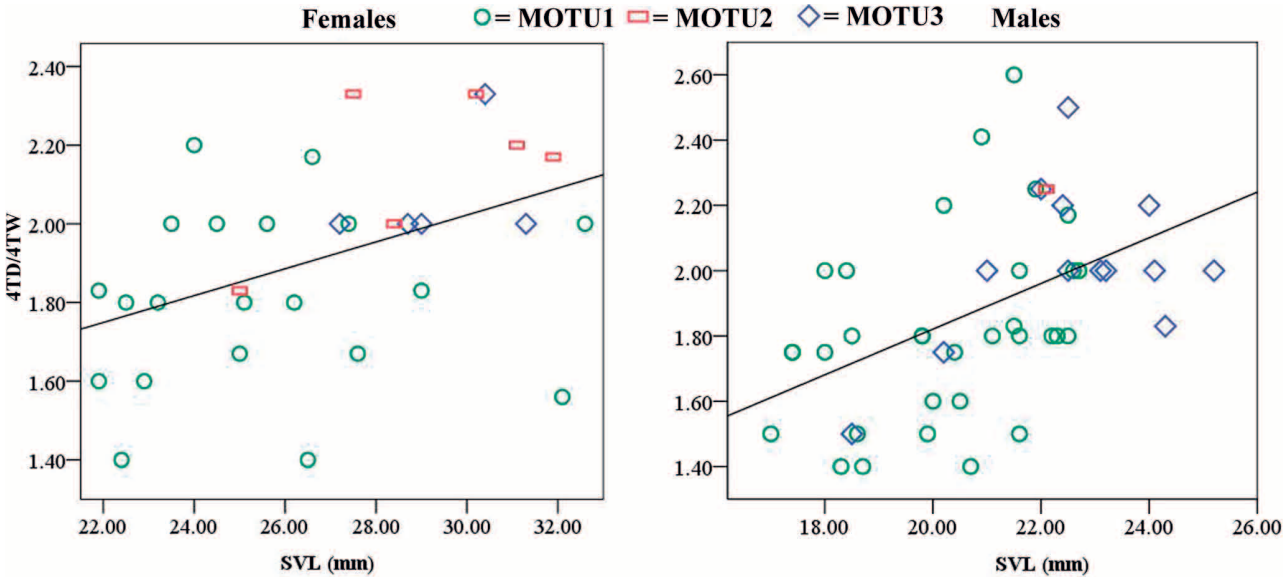

Figure 5. Relation between SVL and the expanded disk condition on the fourth toe of *Pristimantis caryophyllaceus* complex-MOTUs. Females (r^2 = 0.20; P = 0.014) and males (r^2 = 0.26; P = 0.005).

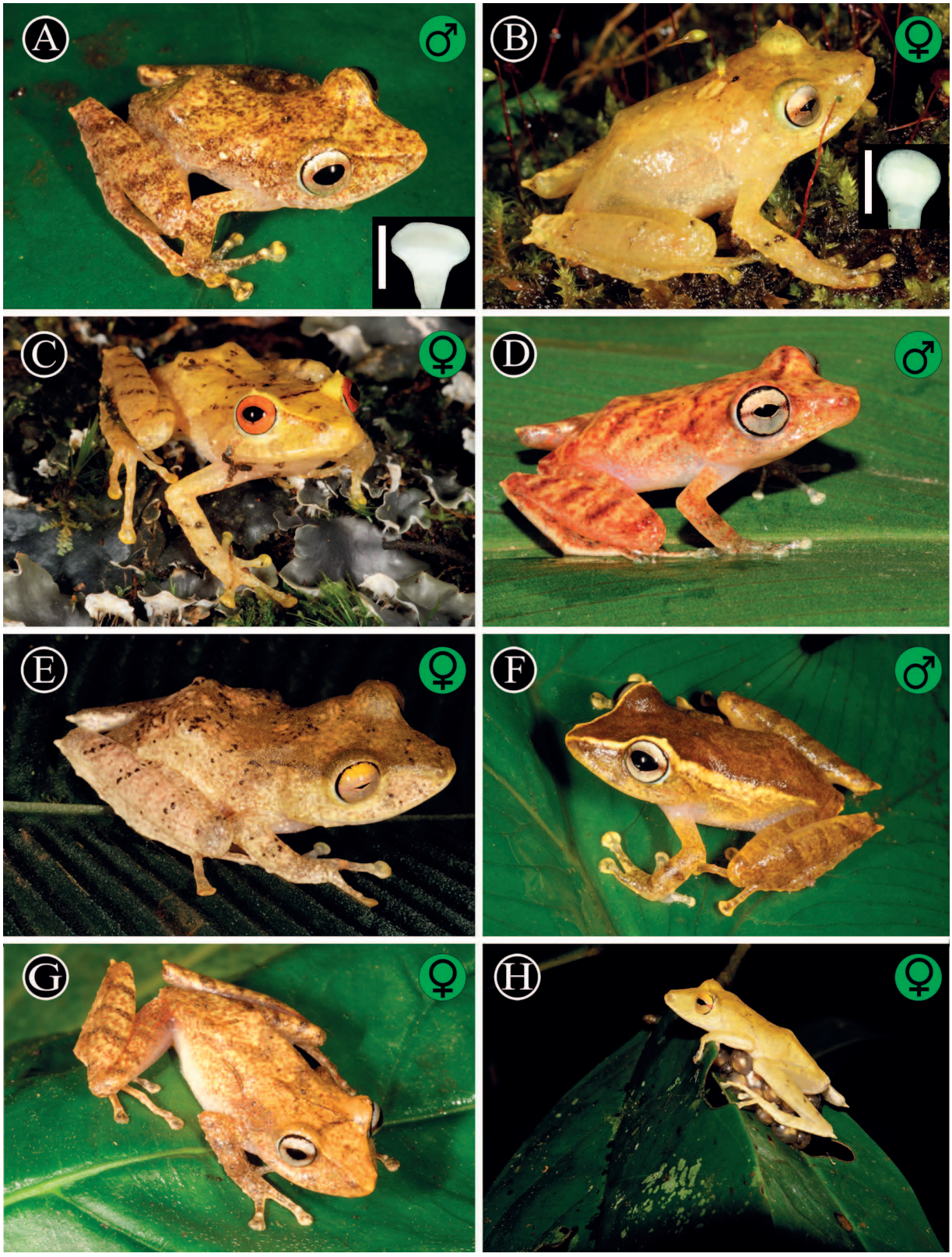

Figure 6. Colour variation in the *Pristimantis caryophyllaceus* complex. (A) Río Clarito (SMF 97037); (B) Río Changena (SMF 97035); (C) Río Changena (SMF 97034); (D) Valle grande, Donoso (SMF 50938); (E) Willy Mazú (SMF 97033); (F) La Nevera (SMF 97031); (G) Llano Tugrí (SMF 97030); (H) female during maternal care, Alto de Piedra. Colours of circles correspond to the MOTUs in Figure 1, Sex: ♀ = female, ♂ = male.

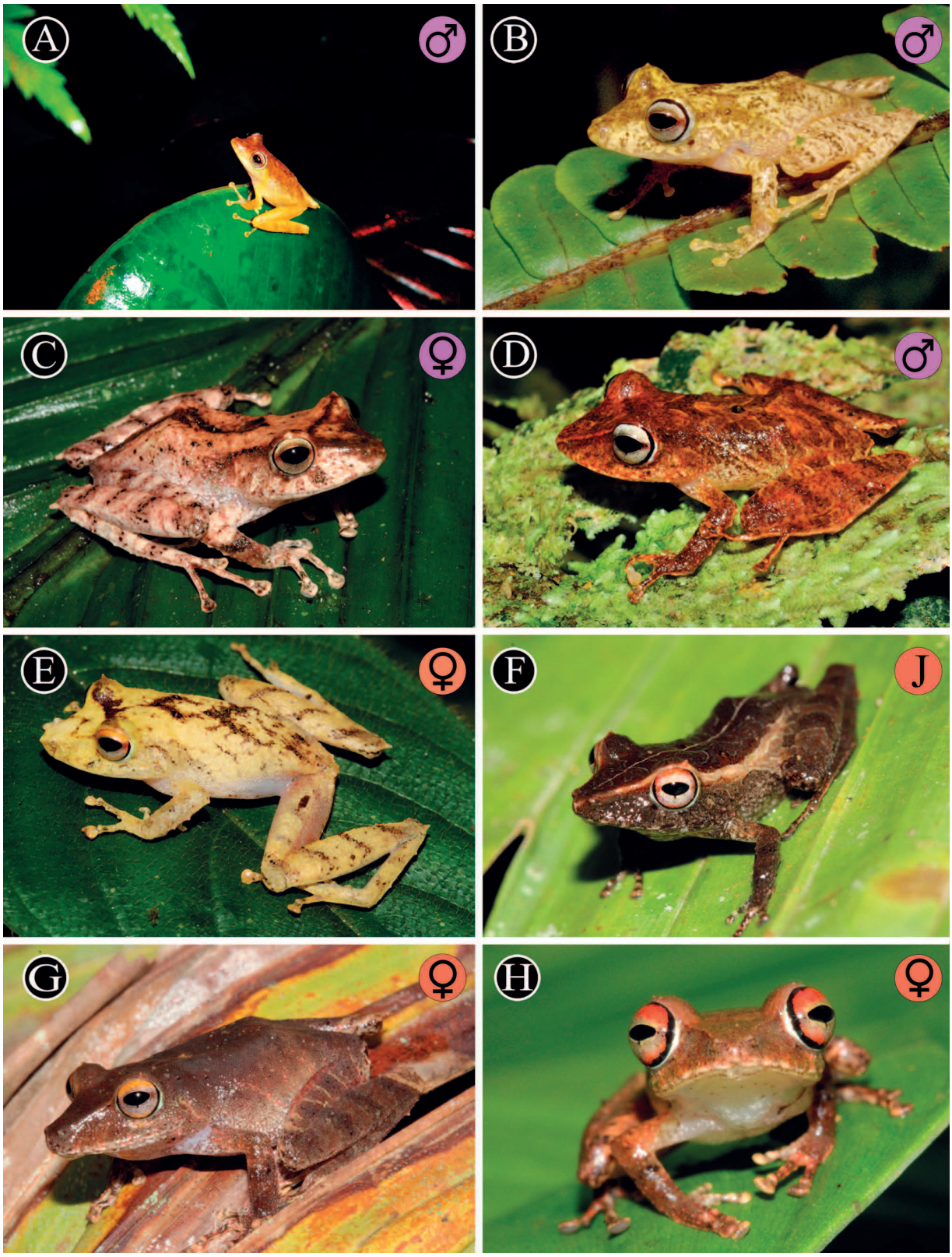

Figure 7. Colour variation in the *Pristimantis caryophyllaceus* complex. (A) Cerro Sapo (MHCH 3022, recorded); (B) Cerro Sapo (MHCH 3021); (C) Cerro Garra Garra, Jingurudó (MHCH 3042); (D) Cerro Bailarín, Jingurudó (SMF 50936); (E) Río Tuquesa (MHCH 3039); (F) Nurra (MHCH 3037); (G) Nurra (SMF 50939); (H) Nurra (SMF 50940). Colours of the right corner circles correspond to the MOTUs in Figure 1. Sex: ♀ = female, ♂ = male, J = juvenile.

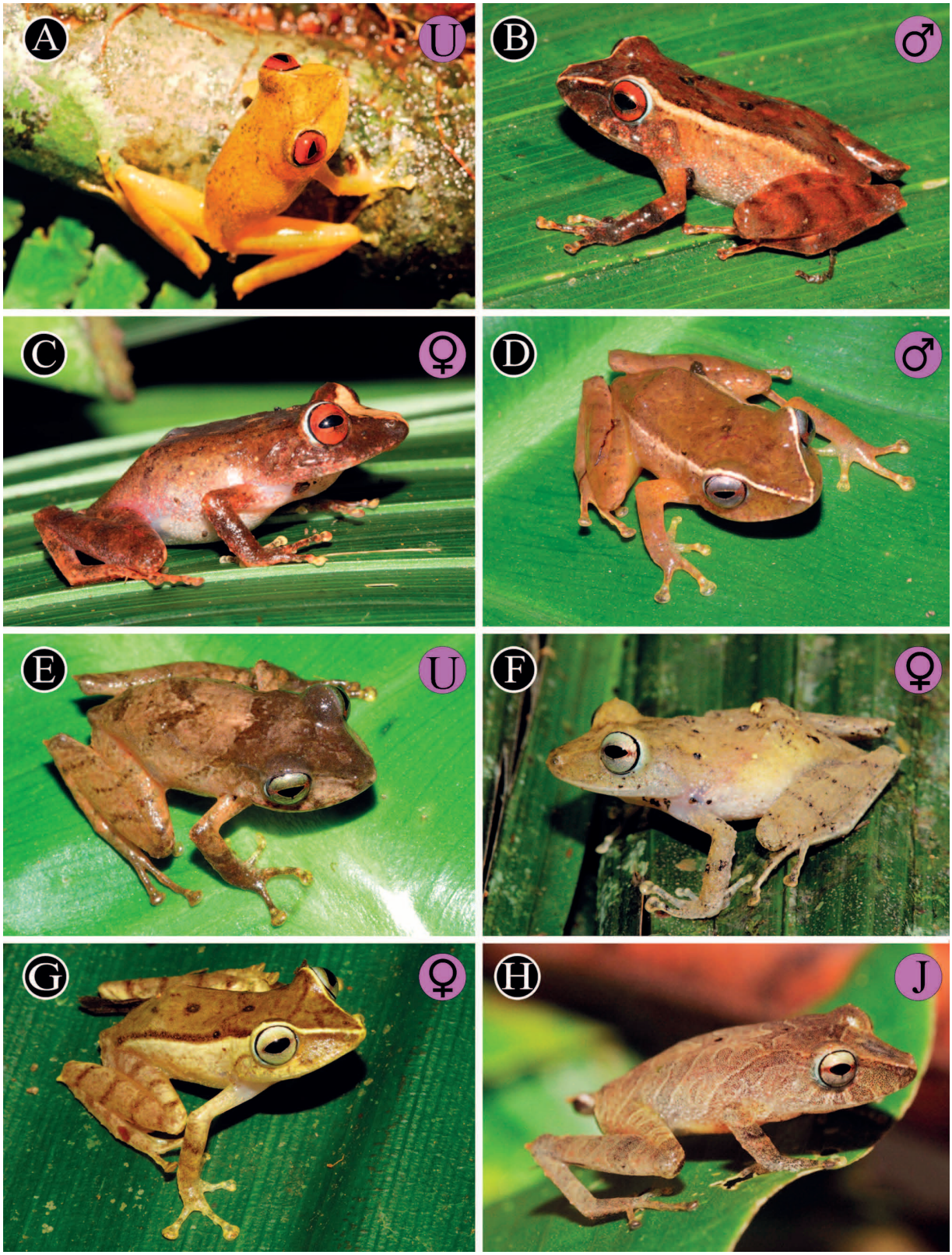

Figure 8. Colour variation in the *Pristimantis caryophyllaceus* complex. (A) Ambroya, Majé; (B) Chucanti, Majé (SMF 50945); (C) Chucanti, Majé (MHCH 3043); (D) Chucanti, Majé; (E) Chucanti, Majé; (F) Pirre (SMF 50946); (G) Pirre (MHCH 3045); (H) Cana Field station (MHCH 3019). Colours of right corner circles correspond to the MOTUs in Figure 1. Sex: ♀ = female, ♂ = male, J = juvenile, U = unidentified.

Table 2. Spectral and temporal parameters of the advertisement call of *Pristimantis caryophyllaceus*. The specimen MHCH 3022 was the only one collected, recordings 1–3 correspond to males that were not captured (see Vocalisation section for explanation). Averages for the parameters are given in the Resume column.

Variables	Males				Resume
	MHCH 3022	Recording 1	Recording 2	Recording 3	
Length recording (min)	00:20:10	00:20:10	00:11:09	00:08:26	00:59:55
Date	05-Dec-12	05-Dec-12	06-Dec-12	26-Aug-13	
Time	19:12	19:12	17:40	19:18	
SVL (mm)	22.50				
Temperature (°C)	19.4	21.6	21.6	22	21.15
Humidity (%)	83	63	63		52.25
Number of calls	1	2	3	1	7
Number of call intervals	n/a	1	2	n/a	3
Number of pulses	6	14	23	8	51
Number of pulse intervals	5	12	20	7	44
Call duration (s)	0.04	0.04	0.04	0.05	0.040±0.004 (0.035–0.046)
Call interval (s)		181.87	135.55		151±26.74 (135.45–181.87)
Call rate (call/min)	0.44		0.33		0.39±0.08 (0.33–0.44)
Pulses/call (s)	6.00	7.00	7.67	8.00	7.28±0.76 (6–8)
Pulses/duration (s)	0.003	0.003	0.004	0.003	3.34x10-3±0.7x10-3 (2.0x10-3–4.0x10-3)
Pulses/interval (s)	0.003	0.003	0.002	0.003	2.41x10-3±0.75x10-3 (1.0x10-3–4.0x10-3)
Lowest Freq (kHz)	2.48	2.30	2.24	2.41	2.32±0.11 (2.18–2.48)
Highest Freq (kHz)	3.25	3.69	3.16	3.31	3.34±0.25 (3.13–3.77)
Delta Freq (kHz)	0.76	1.38	0.92	0.90	1.03±0.26 (0.76–1.4)
Energy (dB)	85.30	82.90	88.13	107.60	89.01±9.52 (80.3–107.60)
Max. Freq (kHz)	2.76	3.01	2.56	2.80	2.75±0.23 (2.43–3.17)

the dorsum and/or limbs (Figs 6–8). Groin and posterior thigh varied from not contrasting in colour to yellow or red. *Pristimantis caryophyllaceus* also had a highly variable eye colouration, which appears not to be correlated to the dorsal colour pattern (Figs 6–8), with specimens with a red iris showing reddish (Figs 7H, 8C), yellowish (Figs 6C. 8A), uniform or striped (8B) dorsal colour patterns, as did specimens with a pale iris colouration (Figs 6–8); specimens from the same population were found to have a red, grey or cream-coloured iris (Fig. 6). A detailed colour description of specimens in life is included in Supplementary Text S6.

Vocalisation

During our trip to Cerro Sapo, we recorded four males of MOTU3, of which only one was subsequently collected (MHCH 3022, Fig. 8A). From one recording, calls of two different males could be analysed. One male was calling closer, about 0.4 m from the microphone, and the other one called from a distance of about 1.0 m. Thus, the call of the first male (MHCH 3022) appeared louder in the Raven 4.1 waveform and allowed to easily differentiate between both individuals. In the first recording (Recording1 in Tab. 2), both males were calling from a bush 1.0 m above the ground and could be observed during the recording. Simultaneously, a third male was observed, calling from a distance of approximately 2 m, but was not recorded. The next day (06/12/12, see Tab. 2), we recorded a fourth male (at 7.97692° N, 78.35969° W; 966 m a.s.l.; not captured) calling from a bush 1.5 m above the ground (Recording 2 in Tab. 2). During a second trip to Cerro Sapo, we recorded another male (at 7.97944° N, 78.35507; 834 m a.s.l.) calling from an epiphytic orchid about 0.7 m above the ground (Recording 3 in Tab. 2).

Pristimantis caryophyllaceus from MOTU3 were active at night, and males were calling sporadically throughout the night. No calls from specimens were recorded from locations other than Cerro Sapo. However, A.B. has heard and seen (but not recorded) males calling at La Nevera and Reserva Forestal La Fortuna (western Panama).

The vocalisation produced by *Pristimantis caryophyllaceus* from MOTU3 consists of a single, pulsed note that is reminiscent of a sound like "chack" and emitted at 2.75 ± 0.25 kHz (2.43–3.17; Fig. 9, Tab. 2); with a note duration of $38^{-3} \pm 3.0^{-3}$ s (35^{-3}–43^{-3}) and repeated sporadically every 151 ± 26.74 s (135.45–181.87). Every note has from six to eight pulses. The call rate is 0.39 calls/min. Although there are no recorded calls to compare the vocalisation of different MOTUs of *P. caryophyllaceus*, the call of western Panamanian specimens is also a "chack" sound that is repeated sporadically and sounded very similar to calls of specimens from eastern Panama.

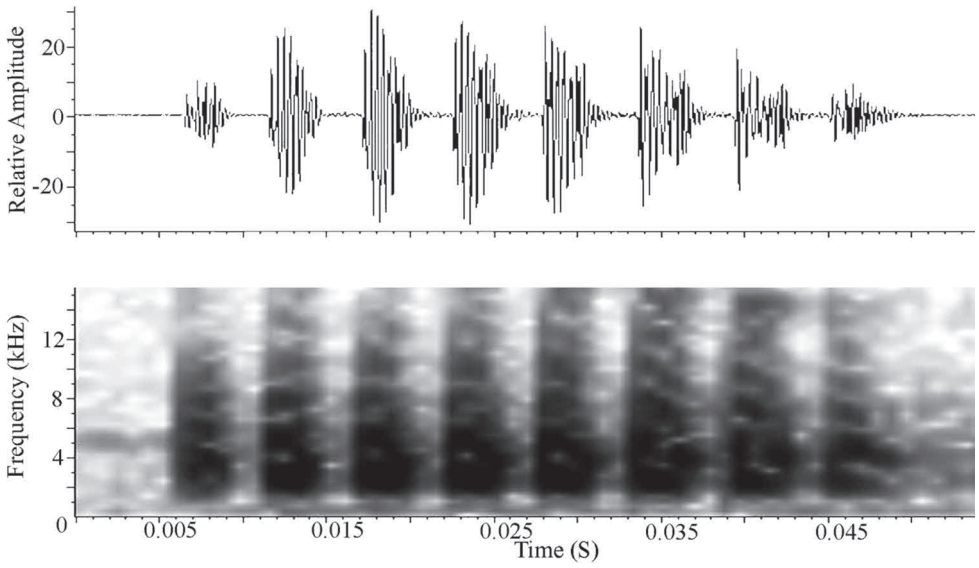

Figure 9. Oscillogram (top) and spectrogram (bottom) of the advertisement call of a *Pristimantis caryophyllaceus*-complex-male, recorded at Cerro Sapo (MHCH 3022).

Natural history notes

Frogs of the *Pristimantis caryophyllaceus* complex are primarily inhabitants of pristine forest, but are also found at the edges of forests. During seven nighttime transect searches in eastern Panama (Jingurudó, Pirre and Sapo mountain ranges), we observed 46 individuals (see Supplementary Tab. S6). The relative abundance was 2.6 indv./ 100 m trail transect; most specimens were found at heights between 0.2–2.5 m above the ground perched on bush leaves and the bark of trees. These frogs are direct-development breeders (Savage 2002), and reproduction occurs during the rainy season. Their reproductive behaviour and, especially, the maternal care have been described in detail by Myers (1969) and Ryan et al. (2010) (see also Fig. 6H).

These frogs feed on a variety of invertebrates: collembolans, cicadas, terrestrial planarians, isopods, arachnids, larvae (caterpillars, dipterans), wasps, and crickets (Batista 2009, Lieberman 1986). In one population analysed from the Burbayar Field Station in the San Blas mountain range (MOTU2; see Supplementary Tab. S5), the stomach contents of seven individuals comprised 21 prey items of 10 different taxonomic groups (see above); with 2.56 $\pm$ 3.10 mm^3 in average volume of prey. The niche breadth for this population was 6.37 (Batista 2009), whereby a value near one suggests that a species would prey exclusively on one prey category, and a value higher than one indicates that a species exploits a greater variety of prey categories (Pianka 1986, Vitt & Caldwell 1994). Thus, this species can be considered a dietary generalist. At La Nevera (MOTU1), another population of the *P. caryophyllaceus* complex was analysed (Batista 2009), but few specimens were caught, and the three stomachs analysed yielded only three prey items (one each): two crickets and one arachnid, with an average volume of 1.77 $\pm$ 1.47 mm^3.

Discussion

Our results demonstrate a high level of genetic diversity in the *Pristimantis caryophyllaceus* complex, comprising three main lineages within its currently known distribution (this study, Crawford et al. 2010, Pinto-Sánchez et al. 2012). Such a scenario has been found before, i.e., in *P. ridens* in Central America (Wang et al. 2008). The three main lineages, classified here as MOTUs, appear to be very old with an estimated crown age between 10.4–14.4 million years ago (Pinto-Sánchez et al. 2012). Due to the great genetic distances between the individual MOTUs, they could be considered three Unconfirmed Candidate Species (UCS) (Vieites et al. 2009, Crawford et al. 2010) that are statistically supported by some morphometric differences (e.g., TYMP/SVL, 4TD/4TW), which would normally support granting them specific status. However, considering the many shared traits among these MOTUs, such as the intricate results obtained from the haplotype network, the lumping of all samples in one species with low values of prior genetic distance (ABGD analyses), the high morphological variation exhibited within the MOTUs compared to that between the MOTUs, the difficulty to differentiate their phenotypes in the field, and their similarities in ecology and behaviour, these MOTUs may just as well be treated as Deep Conspecific Lineages (DCL: Vieites et al. 2009, Padial et al. 2010) of one single species with incomplete lineage sorting among them. The recognition of these MOTUs as separate species would require testing for possible reproductive, genetic, and ecological incompatibilities

in their zones of contact and/or evaluating whether these MOTUs behave/evolve as independent entities.

The ABGD has grouped successfully almost all samples of MOTU1 and MOTU2 for both mitochondrial genes. The most incongruent result obtained in the species delimitation test (ABGD) was the placement of the samples from the Majé mountain range (MOTU3) with the samples from western Panama (MOTU1) in the same group (Supplementary Fig. S2). The over-splitting detected by the ABGD analysis in MOTU3 (Supplementary Figs S1–S2) is likely due to the great genetic distances found within that group, as reflecting the inclusion of specimens from three different isolated regions (Majé mountain range, Jingurudó-Sapo mountains, and Pirre lowlands). In our analyses, the ABGD lumped all MOTUs with a low prior intraspecific divergence (3.0% for 16S, 8.8% for COI, and 6.2% both genes combined), compared to the maximum values of the a priori threshold for conspecific divergence that yielded a primary species hypothesis in the closely related *Pristimantis museosus* (10.0% for 16S, 15.0% for COI and when both genes combined; Crawford et al. 2013).

Earlier studies (Ryan et al. 2010) and our genetic analysis could support splitting the *Pristimantis caryophyllaceus* complex into *P. caryophyllaceus* and *P. educatoris*, or even into three species according to the MOTUs recovered in this study. We also detected morphological, yet non-significant, variation among MOTUs that should not be disregarded until larger samples can be tested to evaluate a potential clinal variation that forms a continuum with the variability detected within the MOTUs (Mallet 2008), corresponding to interbreeding between the MOTUs (e.g., at El Cope), or corresponds to a geographical pattern. For example, the specimens from Río Changena and Río Clarito (MOTU1) share the same haplotype, but the Río Clarito (SMF 97037) frogs have more widely expanded disks than the ones from Río Changena (SMF 97035; Figs 6A–B). Likewise, specimens from the same population in eastern Panama may or may not have expanded disk pads or disk covers. Also, the relation of Toe V and Toe I to the distal subarticular tubercle of Toe IV and Toe II, respectively, is highly variable. According to Crawford et al. (2010), one can find both or even all three lineages in sympatry at El Copé, for which reason there is a potential of substantial interbreeding. We could not detect this to be the case by analysing mitochondrial DNA (O'Donnell & Mock 2012), but this question could be resolved by investigating the nuclear DNA, which is not currently available for all MOTUs. However, the existence of these lineages in sympatry could be an indication of ongoing speciation (Mallet 2008), but this has not been tested yet.

The great colour variation among specimens of the *Pristimantis caryophyllaceus* complex has been pointed out before (Hoffman & Blouin 2000). Glaw & Vences (1997) discussed the eye colouration in *P. caryophyllaceus* on the basis of photographs in Weimer et al. (1993). They also suspected that different taxa might be involved here. The fact of the matter is, however, that the pictures in Weimer et al. (1993) show a red-eyed *P. caryophyllaceus* in Figure 10, but a *P. ridens*-like frog and not a member of the *P. caryophyllaceus* complex in Figure 11. Our comprehensive photographic data and colour descriptions confirm a wide colour variation within and between MOTUs. Specimens with different eye colours from the same populations did not exhibit other morphological differences. Therefore, eye colouration has no diagnostic value, and we are considering specimens with different eye colourations occurring within a MOTU as conspecific and/or members of the same lineage, even though we have no genetic data to corroborate this (no samples with red eyes were sequenced). Ryan et al. (2010) mentioned the presence of yellowish wart-like spots on the dorsum as being characteristic of *Pristimantis educatoris* from eastern Panama. However, we also found specimens with such yellowish marks in extreme western Panama, which otherwise agree with characters of *P. caryophyllaceus* in having rounded pads and disk covers, and low and rounded subarticular tubercles.

The most common polymorphic traits (Ford 1955, Mayr 1963) found in populations of different Terrarana species (Goin 1950, Hoffman & Blouin 2000, Savage & Emerson 1970) relate to characteristics of the dorsal skin texture, colour pattern, and iris colour (Savage 2002). In *P. caryophyllaceus*, polymorphism is most prominent in the dorsal colour pattern, iris colour and digital disk. It seems to be a balanced polymorphism (Ford 1955), inasmuch as it appears to be maintained in the different MOTUs. Colour pattern in Terrarana frogs could be inherited by a simple Mendelian genetic mechanism (Goin 1950, 1960, Summers et al. 2004, O'Neill & Beard 2010), perhaps maintained by the heterogeneity of colour compositions and shapes predominant in the habitat, selective forces exerted by visually guided predators, and fitness-related traits (Hoffman & Blouin 2000, Savage & Emerson 1970, Woolbright & Stewart 2008). At least five different colour patterns were found in a single population of *P. caryophyllaceus* (Figs 8B–E) that were irrespective of sexual affinities, so that sexual dichromatism can be disregarded (see Figs 6–8). Other potential selective agents responsible for maintaining the polymorphism in this species should be targeted in futures studies. Iris colour and the shape of the digital disks are less variable than the dorsal colour pattern, and have widely been used as diagnostic characters to identify anuran species (Glaw & Vences 1997, Lynch & Duellman 1997, Savage 2002, Köhler 2008). Even though intraspecific iris colour variation is known to occur in *Pristimantis* (Lynch & Duellman 1997, Savage 2002, Duellman & Lehr 2009, Fig. 58B), only a few members of this genus have red eyes and none shows the striking variation documented here for *P. caryophyllaceus* (Figs 6–8). Eye colour is usually species-specific and correlated to the dorsal ground colour (Amat et al. 2013), but this is not the case in *P. caryophyllaceus* (see Figs 6–8), where only one species is involved and red eye colour is apparently not linked to the dorsal colour pattern (e.g., Figs 8 A–B).

Most arboreal and semiarboreal Terrarana species have large digital disks, and most ground-dwelling species have small or no digital disks. The function of digital disks is

to facilitate improved climbing in an arboreal environment and would prove beneficial also for semiarboreal activities in Terrarana species (Savage 2002, Hedges et al. 2008). Although the exact function of the digital disks in *P. caryophyllaceus* has not been investigated yet, one possibility could be that the differences in their widths reflect different degrees of arboreality among MOTUs. However, the different degrees of arboreality appear to be not the main reason for this variation, since all specimens were found in the understorey between 0.5 and 2.5 m above the ground on leaves and branches.

The polymorphism found in *P. caryophyllaceus* (sensu lato) appears to be complex and it is not properly reflected by a two-species taxonomy sensu Ryan et al. (2010) or three species as found in this study; i.e., by splitting the species into *P. caryophyllaceus* for western Panama (MOTU1) and two *P. educatoris* taxa for eastern Panama (Caribbean-MOTU2 and Pacific-MOTU3). Based on the molecular information available as of now, all three MOTUs co-occur at El Copé. However, there is no additional evidence for implementing a three-species taxonomy, and sensu Vieites et al. (2009), we would need at least one diagnostic morphological difference, a character trait that is of low intraspecific variability and of high value, to discriminate among taxa (here: MOTUs). The morphological differences between these MOTUs are insufficient to support them as distinct species, however. Furthermore, there are currently no bioacoustic data from El Copé to test for putative interspecific differences.

Ryan et al. (2010) used the SVL to differentiate between *P. caryophyllaceus* and *P. educatoris*. However, rather than a clear difference in SVL, our data suggest a smooth clinal transition of the SVL from east to west (Fig. 5). The condition of a projecting subarticular tubercle, as suggested as a diagnostic character by Ryan et al. (2010), is another trait that is too variable to differentiate between their suggested taxa, as we found both conditions in all three regions. As stated before, the finger and toe disk widths are also variable (see Figs 6A+B). There are two major discrepancies in the species description of *P. educatoris* by Ryan et al. (2010). Firstly, they state that the distribution of *P. educatoris* stretches from Santa Fe eastwards and into Colombia, but according to their Appendix, only specimens from Santa Fe and El Copé were analysed, i.e., two sites in central-western Panama separated by less than 50 km. Hence, clear evidence of an eastern distribution for *P. educatoris* remains wanting. Secondly, they mention a disjunctive population of *P. educatoris* near the Panama border on the Caribbean versant of Costa Rica, but specimens from this site were not included in their analyses and no further information is given on this population. Moreover, this contradicts the biogeographical assumption of the authors' of one species in the west and one in the east with the Fortuna depression as a supposed barrier. Consequently, we can neither finally rule out that *P. educatoris* is a valid species nor can we promote a three-species solution yet, albeit a three-regions separation (MOTU1–3) within the *P. caryophyllaceus-educatoris* populations is evident. Due to the morphological variation within the particular MOTUs and the morphological similarities between the MOTUs, we found no additional evidence to prove the three MOTUs as respective Confirmed Candidate Species (Vieites et al. 2009). Moreover, at this point, we have no evidence for assigning *P. educatoris* to either MOTU2 or MOTU3, and our data do not support the biogeographical concept suggested by Ryan et al. (2010). For now, we suppose to see the three MOTUs within the *P. caryophyllaceus-educatoris*-complex as geographically and genetically distinct lineages in accordance with the definition of a Deep Conspecific Lineage by Vieites et al. (2009) with a small contact zone around El Copé. Consequently, we reject *P. educatoris* as a valid species and place it in the synonymy of *P. caryophyllaceus*.

The phylogenetic history of the MOTUs studied here is linked to the complex biogeography of the Isthmus of Panama and northwestern South America, more precisely of the Choco Block (Duque-Caro 1990 a–b, Coates et al. 2003, 2004), formed by the Majé-Baudó Arc (Majé, Sapo, Jingurudó and Pirre massifs) and the Dabeiba Arc (Darien and San Blas massifs). *Pristimantis caryophyllaceus* has originated in South America (Duellman 2001, Heinicke et al. 2007, Pinto-Sánchez et al. 2012), and its dispersal to Central America is consistent with the recent hypothesis of an earlier formation of the Isthmus of Panama (Farris et al. 2011, Montes et al. 2012), dating well before 3.5 Mya as was previously thought (Coates & Obando 1996, Webb & Rancy 1996, Santos et al. 2009, Weir et al. 2009). The MOTUs show a geographical pattern with almost all MOTUs (except for the El Copé populations) being distributed in different geographic areas (Figs 1–2). According to our results, the split between MOTU1 and MOTU2–MOTU3 (11–14 Mya) supports the proposed connection of the Isthmus of Panama with South America, which would have facilitated faunistic migrations across dry land between Central America and the northern Andean blocks as early as about 15 Mya (Montes et al. 2012). Consequently, the ancestral *P. caryophyllaceus* expanded into Central America during the middle Miocene. The subsequent evolution and divergence of MOTU2 and MOTU3 in eastern Panama was probably induced by eustatic fluctuations during the middle and late Miocene (as early as 11 Mya), by flooding in what are now the Atrato and Chucunaque basins; de facto separating MOTU3 on the Majé-Baudó arc (Pacific side) from MOTU2 on the Dabeiba arc (Atlantic side) (Duque-Caro 1990a, Coates & Obando 1996). Probably at least one migration has occurred from the MOTU2 and MOTU3 territory west towards the range of MOTU1, since there is evidence of the presence of all three MOTUs at El Copé in central Panama (Crawford et al. 2010, Pinto-Sánchez et al. 2012), which likely reflects a secondary contact, but for which nuclear DNA has shown neither introgression nor clear separation between the MOTUs. There might also be a remnant gene flow between MOTU1 and the MOTU3-population at Majé, since latter samples were nested within MOTU1 in the 16S tree and molecular clock (Supplementary Figs S2 + S4) with a distant divergence time of 11.12 Mya when not using the monophyly constraint for the

MOTUs in the time divergence analysis. This would mean that MOTU3 from eastern Panama had available a considerable period of time to expand into western Panama and mix with MOTU1 there.

Even though we here present a lot of new information on the distribution pattern of *Pristimantis caryophyllaceus* and its variation in morphology, genetics, colour pattern, as well as advertisement calls, it is apparently still not enough to clarify the taxonomic status of the species. Detailed molecular analyses at population level, using nuclear markers such as microsatellites to detect gene flow and past demographic bottlenecks, including populations from central Panama (especially from the Piedras-Pacora mountain range) into the analysis of morphology, and statistically supported bioacoustics data from various populations over wide areas are still needed. Further studies should include correlation analyses between geographic and genetic distances to test whether the uncovered differences express just a clinal variation among the populations, and to evaluate the existence and role of previous and current introgressions among the MOTUs, particularly at El Copé.

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Supplementary material

Additional information is available in the online version of this article at http://www.salamandra-journal.com

4 Supplementary figures and 6 Supplementary tables:

Figure S1. Maximum likelihood consensus tree of the COI mtDNA of the *Pristimantis caryophyllaceus* complex.

Figure S2. Maximum likelihood consensus tree of the 16S mtDNA of the *Pristimantis caryophyllaceus* complex.

Figure S3. Maximum likelihood consensus tree of mitochondrial (16S & COI mDNA) and nuclear (RAG1 DNA) genes combined of the *Pristimantis caryophyllaceus* complex.

Figure S4. Chronogram of the *Pristimantis caryophyllaceus* complex based on Rag1, 16S and COI genes, using *P. cerasinus* and *P. cruentus* as outgroups.

Table S1. Morphological variables taken from 78 specimens used in the analyses.

Table S2. Mean genetic distances in the 16S mtDNA gene between the *Pristimantis caryophyllaceus* samples used in the phylogenetic analysis.

Table S3. Mean genetic distances in the COI mtDNA gene between the *Pristimantis caryophyllaceus* samples used in the phylogenetic analysis.

Table S4. Mean genetic distances in the COI and 16S mtDNA genes combined between the *Pristimantis caryophyllaceus* samples used in the phylogenetic analysis.

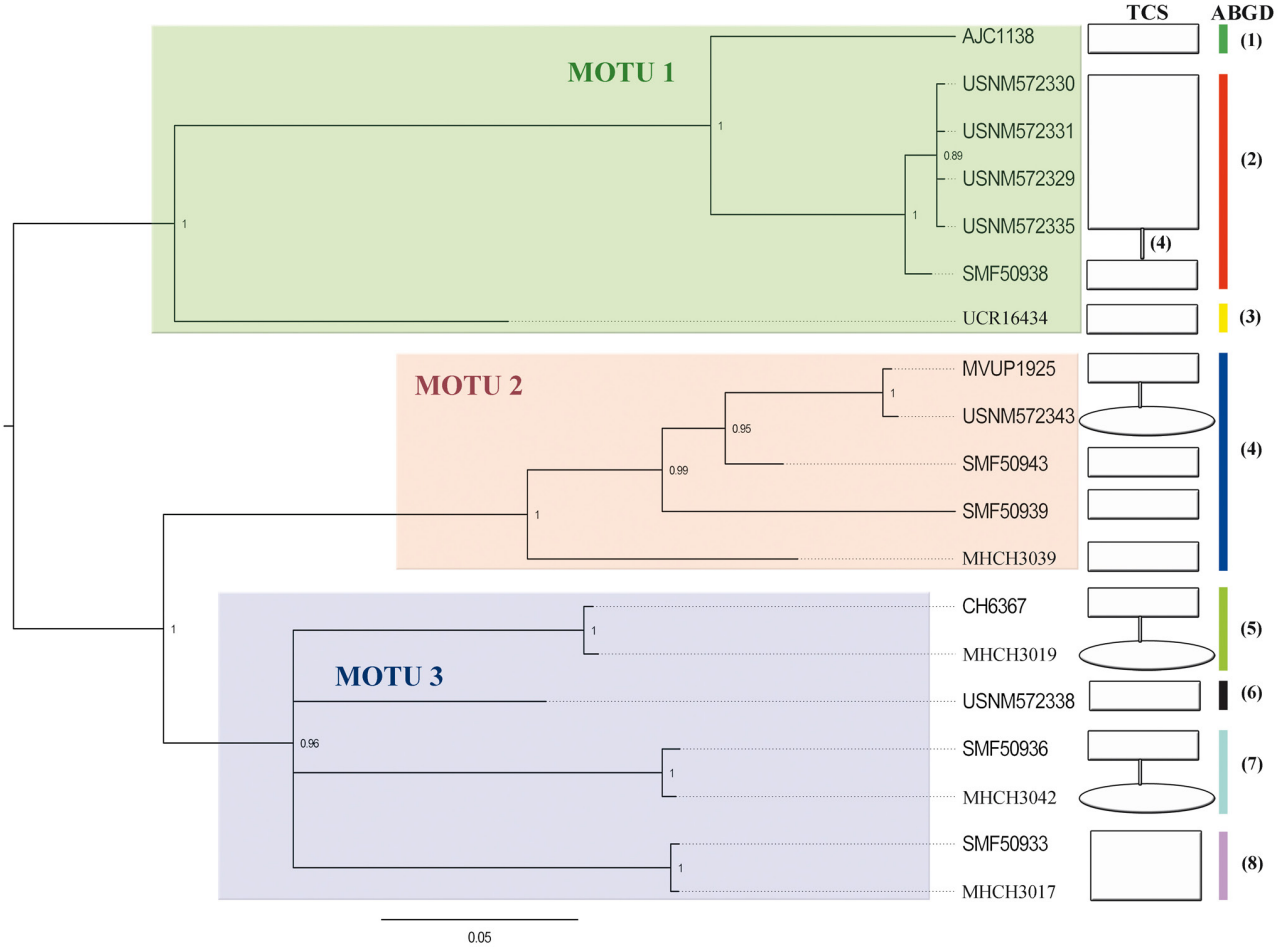
Table S5. Details of sample and museum voucher numbers.

Table S6. Transect details for *Pristimantis caryophyllaceus* (MOTU3).

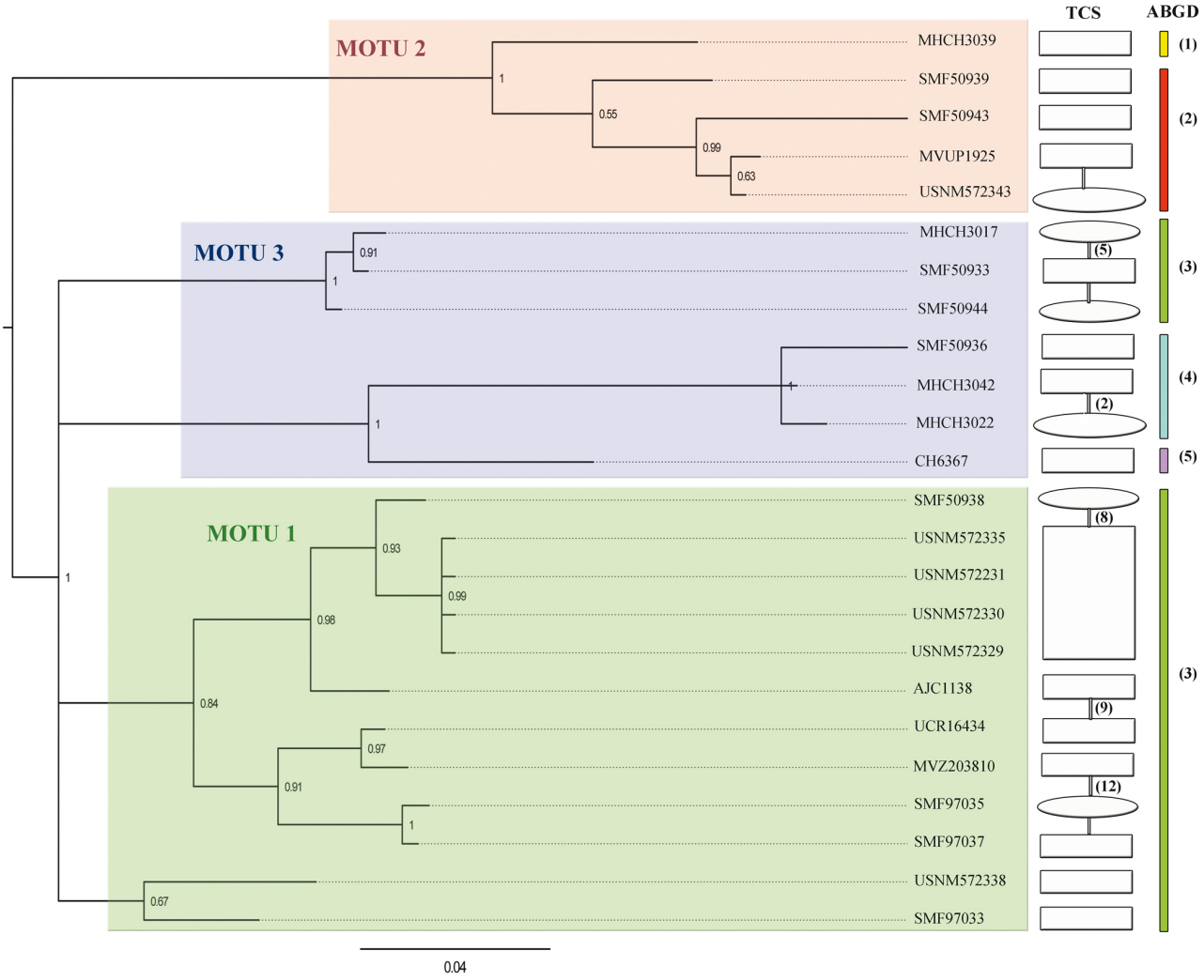
Supplementary material

Batista, A., A. Hertz, G. Köhler, K. Mebert & M. Vesely: Phylogeny, shapes, colours, and phylogeography of frogs related to *Pristimantis caryophyllaceus* (Anura: Terrarana: Craugastoridae) in Panama. – Salamandra, **50**(3): 155–171.

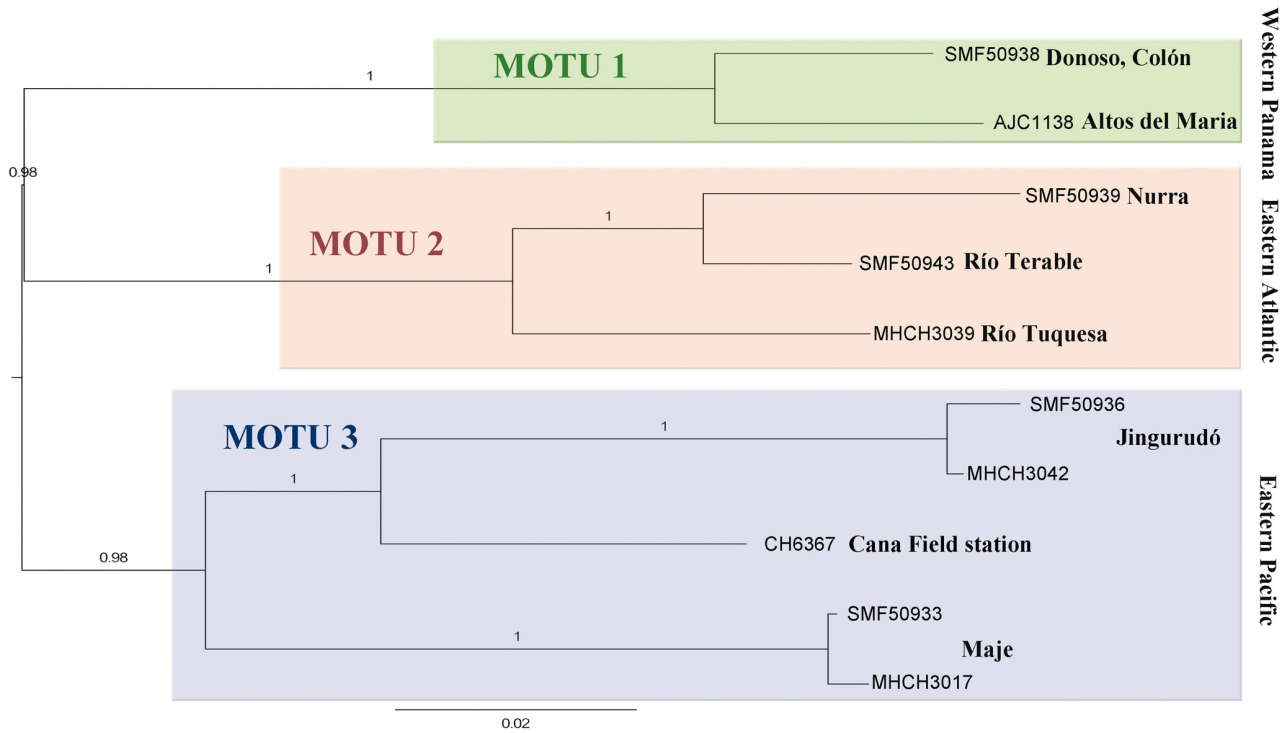
4 Supplementary Figures and 6 Supplementary Tables.



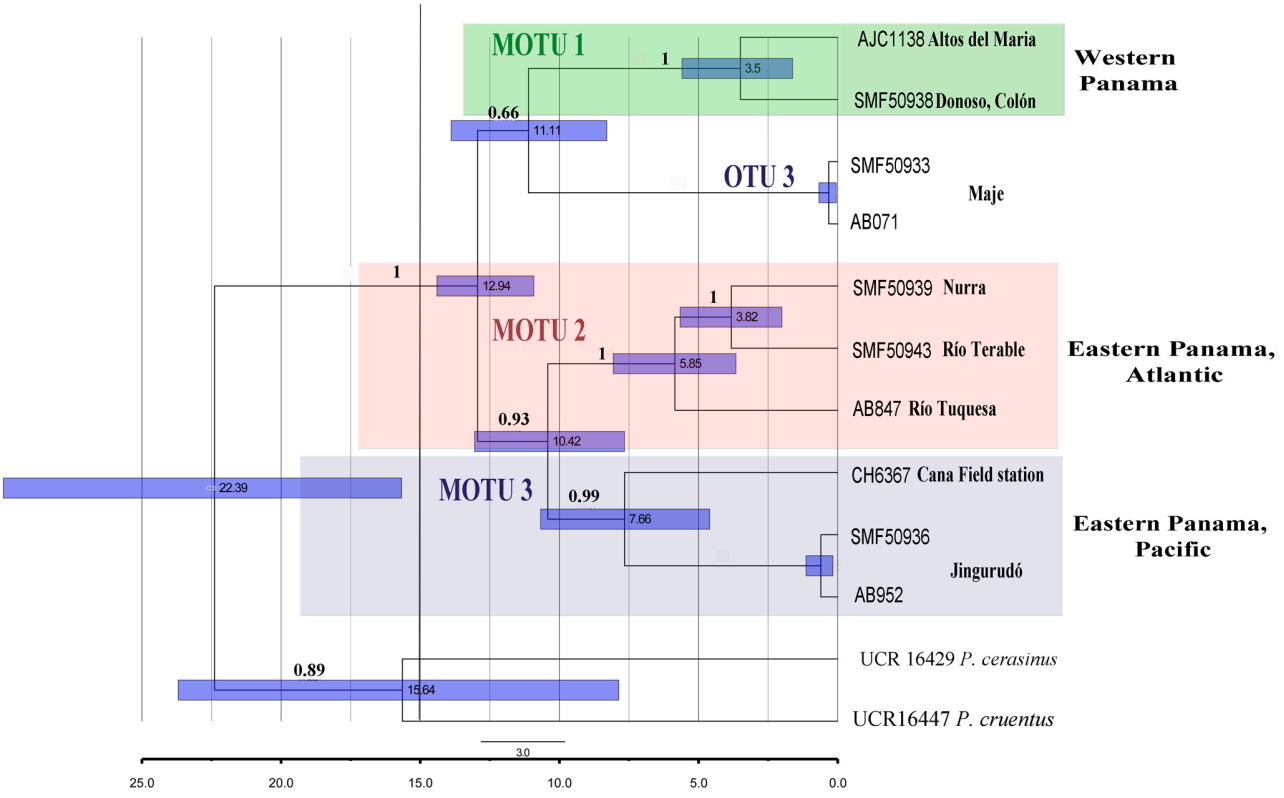
Supplementary Figure S1. Maximum likelihood consensus tree of the COI mtDNA of the *Pristimantis caryophyllaceus* complex. Specimen labels refer to collection or museum numbers. MOTU colours correspond to those in Figs. 1 and 4. Scale bar refers to the number of substitutions per site. Support of the Bayesian posterior probabilities are shown for every branch. Parsimony network with a connection limit of 90%; each node represents a unique haplotype separated from the next by one substitution step, numbers in parenthesis represents unsampled haplotypes, the rectangle the probable ancestral haplotype. ABGD colour bars represent each primary species hypothesis (number of species IDs in parenthesis), see text for details.



Supplementary Figure S2. Maximum likelihood consensus tree of the 16S mtDNA of the *Pristimantis caryophyllaceus* complex. Specimen labels refer to collection or museum numbers. MOTU colours correspond to those in Figures 1 and 4. Scale bar refers to the number of substitutions per site. Support of the Bayesian posterior probabilities are shown for every branch. Parsimony network with a connection limit of 90%; each node represents a unique haplotype separated from the next by one substitution step, numbers in parenthesis represents unsampled haplotypes, the rectangle the probable ancestral haplotype. ABGD colour bars represent each primary species hypothesis (number of species IDs in parenthesis), see text for details.



Supplementary Figure S3. Maximum likelihood consensus tree of mitochondrial (16S & COI mDNA) and nuclear (RAG1 DNA) genes combined of the *Pristimantis caryophyllaceus* complex. Specimen labels refer to collection or museum numbers. MOTUs are shadowed in the same colours as in Figures 1 and 4. Scale bar refers to number of substitutions per site. Support of the Bayesian posterior probabilities are shown for every branch.



Supplementary Figure S4. Chronogram of the *Pristimantis caryophyllaceus* complex based on Rag1, 16S and COI genes, using *P. cerasinus* and *P. cruentus* as outgroups. Scale along the bottom indicates time in Mya. Block colours reflect MOTU designations (for species at tips). The vertical line indicates the hypothesized 3.5 Mya final completion of the Isthmus of Panama. Blue horizontal bars indicate 95% credibility intervals for the divergence time of the MOTUs, numbers inside the bars indicate time in Mya. Numbers above branches indicate estimated posterior probabilities ≥ 0.95 for the presence of the corresponding clade according to BEAST (see text for details).

Supplementary Table S1. Morphological variables taken from 78 specimens used in the analyses. Number of specimens measured in parenthesis (see main text for explanation).

Trait	MOTU1		MOTU2		MOTU3	
	Females (20)	Males (33)	Females (6)	Males (1)	Females (5)	Males (13)
SVL	25.53±3.09 (21.90–32.60)	20.13±1.87 (16.10–22.70)	29.02±2.56 (25.00–31.90)	22.1	29.32±1.59 (27.20–31.30)	22.54±1.83 (18.50–25.20)
HW	9.89±1.23 (8.40–13.00)	7.80±0.80 (6.30–9.40)	11.38±1.02 (10.10–12.60)	8.1	11.58±0.31 (11.10–11.90)	8.87±0.70 (7.20–10.00)
HL	9.38± 1.16 (8.10–12.30)	7.64±0.78 (6.00–9.10)	11.27±1.02 (9.60–12.40)	8.2	11.34±0.51 (10.70–12.00)	8.82±0.91 (7.10–10.10)
IND	2.03±0.28 (1.60–2.70)	1.65±0.15 (1.40–1.90)	2.38±0.16 (2.20–2.60)	1.6	2.32±0.16 (2.10–2.50)	1.94±0.12 (1.80–2.10)
IOD	3.06±0.41 (2.50–3.90)	2.49±0.29 (2.00–3.00)	3.95±0.27 (3.80–4.50)	2.9	3.94±0.40 (3.60–4.50)	3.06±0.34 (2.60–3.50)
TYMP	0.81± 0.29 (0.50–1.70)	0.69±0.14 (0.50–1.00)	1.05±0.08 (1.00–1.20)	0.8	1.16±0.23 (0.90–1.50)	1.01±0.19 (0.70–1.40)
EL	3.33±0.32 (2.90–4.10)	2.84±0.32 (2.20–3.40)	3.72±0.22 (3.40–4.00)	3.5	3.94±0.36 (3.40–4.30)	3.28±0.38 (2.50–3.80)
END	3.27±0.50 (2.60–4.60)	2.61±0.35 (2.00–3.20)	4.28±0.44 (3.70–4.90)	3.1	4.12±0.41 (3.50–4.50)	3.07±0.36 (2.50–3.60)
TL	15.03±1.72 (13.10–19.00)	12.10±1.15 (10.00–14.10)	17.47±1.01 (15.60–18.20)	12.8	17.02±0.97 (15.70–18.00)	13.28±1.07 (10.70–14.90)
FL	11.16±1.37 (9.40–14.50)	8.85±1.08 (7.00–10.90)	12.03±0.86 (10.70–13.00)	9.9	12.14±0.46 (11.40–12.50)	9.92±1.05 (7.20–11.10)
4TW	0.56±0.09 (0.50–0.90)	0.49±0.06 (0.40–0.60)	0.58±0.04 (0.50–0.60)	0.4	0.58±0.04 (0.50–0.60)	0.48±0.07 (0.40–0.60)
4TD	1.01±0.19 (0.70–1.40)	0.89±0.19 (0.60–1.30)	1.25±0.14 (1.10–1.40)	0.9	1.20±0.14 (1.00–1.40)	0.96±0.17 (0.60–1.20)
FAL	5.38±0.60 (4.50–6.80)	4.38±0.47 (3.20–5.10)	6.08±0.58 (5.60–7.20)	4.6	6.68±0.50 (5.80–7.00)	4.95±0.52 (4.10–5.70)
HAL	6.90±1.12 (5.60–9.80)	5.51±0.70 (4.40–6.90)	7.53±0.57 (6.90–8.30)	5.5	7.98±1.12 (7.20–9.90)	6.23±0.74 (4.40–7.10)
3FW	0.57±0.07 (0.50–0.70)	0.49±0.07 (0.40–0.60)	0.62±0.08 (0.50–0.70)	0.5	0.60±0.07 (0.50–0.70)	0.48±0.07 (0.40–0.60)
3FD	1.13±0.25 (0.80–1.60)	0.93±0.20 (0.60–1.40)	1.40±0.25 (1.10–1.70)	1.1	1.26±0.18 (1.10–1.50)	1.08±0.23 (0.60–1.40)
TYMP/SVL	0.03±0.01 (0.02–0.05)	0.03±0.01 (0.02–0.05)	0.04±0.01 (0.03–0.04)	0.04	0.04±0.01 (0.03–0.05)	0.04±0.01 (0.04–0.06)
TL/SVL	0.59±0.03 (0.54–0.63)	0.60±0.03 (0.51–0.66)	0.60±0.03 (0.56–0.64)	0.58	0.58±0.03 (0.54–0.62)	0.59±0.03 (0.54–)0.66
FL/SVL	0.44±0.03 (0.39–0.48)	0.44±0.03 (0.38–0.49)	0.42±0.02 (0.38–0.44)	0.45	0.35±0.17 (0.05–0.44)	0.44±0.03 (0.39–0.49)
HW/SVL	0.390.02± (0.36–0.42)	0.39±0.02 (0.36–0.44)	0.39±0.01 (0.37–0.41)	0.37	0.40±0.02 (0.38–0.41)	0.39±0.02 (0.35–0.42)
HL/SVL	0.13±0.01 (0.11–0.14)	0.13±0.01 (0.11–0.16)	0.15±0.00 (0.14–0.15)	0.14	0.14±0.02 (0.12–0.16)	0.14±0.01 (0.12–0.15)
END/SVL	0.13±0.01 (0.11–0.14)	0.13±0.01 (0.11–0.16)	0.15±0.00 (0.14–0.15)	0.14	0.14±0.02 (0.12–0.16)	0.14±0.01 (0.12–0.15)
4TD/IVTW	1.81±0.23 (1.40–2.20)	1.81±0.29 (1.40–2.60)	2.14±0.20 (1.83–2.33)	2.25	2.07±0.15 (2.00–2.33)	2.02±0.25 (1.50–2.50)
3FD/IIIFW	1.97±0.30 (1.33–2.67)	1.91±0.35 (1.33–)	2.31±0.60 (1.83–3.40)	2.2	2.11±0.26 (1.83–2.50)	2.30±0.53 (1.40–3.50)
IOD/HW	0.31±0.03 (0.27–0.38)	0.32±0.03 (0.28–0.38)	0.35±0.02 (0.33–0.38)	0.36	0.34±0.03 (0.31–0.38)	0.35±0.04 (0.28–0.42)
HL/HW	0.95±0.04 (0.85–1.01)	0.98±0.05 (0.84–1.16)	0.99±0.04 (0.94–1.06)	1.01	0.98±0.02 (0.95–1.01)	0.99±0.06 (0.91–1.11)

Supplementary Table S2. Mean genetic distances in the 16S mtDNA gene between the *Pristimantis caryophyllaceus* samples used in the phylogenetic analysis (Tamura-3-parameter-distances).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	USNM572335 {MOTU1}																							
2	USNM572331 {MOTU1}	0.0																						
3	AJC1138 {MOTU1}	2.8	2.8																					
4	UCR16434 {MOTU1}	4.3	4.3	3.3																				
5	USNM572330 {MOTU1}	0.0	0.0	2.8	4.3																			
6	SMF97035 {MOTU1}	6.3	6.3	5.2	3.8	6.3																		
7	SMF 97037 {MOTU1}	6.3	6.3	5.2	3.8	6.3	0.0																	
8	USNM572329 {MOTU1}	0.0	0.0	2.8	4.3	0.0	6.3	6.3																
9	MVZ203810 {MOTU1}	4.8	4.8	3.8	0.5	4.8	4.3	4.3	4.8															
10	SMF50938 {MOTU1}	1.4	1.4	2.3	3.8	1.4	5.8	5.8	1.4	4.3														
11	MHCH3039 {MOTU2}	7.3	7.3	7.3	5.8	7.3	8.3	8.3	7.3	6.3	6.8													
12	SMF50939 {MOTU2}	7.3	7.3	6.2	6.8	7.3	7.8	7.8	7.3	7.3	6.8	2.3												
13	USNM572343 {MOTU2}	7.3	7.3	7.3	6.2	7.3	7.8	7.8	7.3	6.7	6.8	2.3	1.9											
14	MVUP1925 {MOTU2}	7.3	7.3	7.3	6.2	7.3	7.8	7.8	7.3	6.7	6.8	2.3	1.9	0.0										
15	SMF50943 {MOTU2}	7.8	7.8	7.8	6.7	7.8	8.3	8.3	7.8	7.3	7.3	1.9	1.4	0.5	0.5									
16	SMF50933 {MOTU3}	4.7	4.7	5.7	4.3	4.7	7.3	7.3	4.7	4.8	5.2	6.3	7.8	7.8	7.8	8.3								
17	MHCH3022 {MOTU3}	9.3	9.3	9.3	9.3	9.3	10.4	10.4	9.3	9.9	8.8	9.8	9.8	9.8	9.8	10.3	8.8							
18	MHCH3017 {MOTU3}	5.2	5.2	6.2	4.8	5.2	7.8	7.8	5.2	5.3	5.7	6.8	8.3	8.3	8.3	8.8	0.5	9.3						
19	SMF50936 {MOTU3}	9.8	9.8	9.9	8.8	9.8	9.9	9.9	9.8	9.4	10.4	11.5	11.4	11.4	11.4	12.0	8.8	2.3	9.3					
20	MHCH3042 {MOTU3}	8.8	8.8	8.8	8.8	8.8	9.9	9.9	8.8	9.4	8.3	9.3	9.3	9.3	9.3	9.8	8.3	0.5	8.8	1.9				
21	SMF50944 {MOTU3}	4.7	4.7	5.7	4.3	4.7	7.3	7.3	4.7	4.8	5.2	6.3	7.8	7.8	7.8	8.3	0.0	8.8	0.5	8.8	8.3			
22	CH6367 {MOTU3}	7.8	7.8	6.7	7.8	7.8	7.8	7.8	7.8	8.3	7.2	8.3	7.2	8.2	8.2	8.8	7.7	6.2	8.3	7.8	5.8	7.7		
23	SMF97033 {MOTU1}	3.8	3.8	4.8	4.8	3.8	7.8	7.8	3.8	5.3	4.3	6.3	6.8	6.8	6.8	7.3	3.8	9.9	4.3	10.4	9.3	3.8	8.8	
24	USNM572338 {MOTU1}	4.8	4.8	4.7	4.7	4.8	6.7	6.7	4.8	5.2	5.3	5.7	6.3	6.2	6.2	6.8	4.7	10.9	5.2	11.4	10.4	4.7	9.3	3.8

Supplementary Table S3. Mean genetic distances in the COI mtDNA gene between the *Pristimantis caryophyllaceus* samples used in the phylogenetic analysis (Tamura-3-parameter-distances).

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	USNM572331 {MOTU1}	0.0																	
2	USNM572330 {MOTU1}	0.0																	
3	USNM572329 {MOTU1}	0.0	0.0																
4	SMF50938 {MOTU1}	0.9	0.9	0.9															
5	USNM572335 {MOTU1}	0.0	0.0	0.0	0.9														
6	UCR16434 {MOTU1}	16.4	16.4	16.4	15.9	16.4													
7	AJC1138 {MOTU1}	8.4	8.4	8.4	8.2	8.4	16.9												
8	MHCH3039 {MOTU2}	20.1	20.1	20.1	20.4	20.1	16.9	21.5											
9	SMF50939 {MOTU2}	22.0	22.0	22.0	21.8	22.0	17.7	21.5	11.4										
10	SMF50943 {MOTU2}	20.1	20.1	20.1	20.1	20.1	14.6	20.9	8.8	6.9									
11	MVUP1925 {MOTU2}	19.8	19.8	19.8	19.8	19.8	16.2	21.2	9.8	8.0	4.4								
12	USNM572343 {MOTU2}	20.1	20.1	20.1	20.1	20.1	16.4	20.9	10.1	8.2	4.6	0.2							
13	SMF50933 {MOTU3}	19.7	19.7	19.7	20.5	19.7	15.5	20.5	16.6	17.3	16.8	17.0	17.3						
14	MHCH3042 {MOTU3}	19.3	19.3	19.3	19.3	19.3	15.9	20.3	14.8	17.7	14.8	15.8	16.0	12.3					
15	CH6367 {MOTU3}	17.0	17.0	17.0	17.5	17.0	14.1	19.5	13.9	16.0	13.7	14.6	14.8	11.6	11.5				
16	USNM572338 {MOTU3}	17.2	17.2	17.2	17.2	17.2	13.5	17.2	15.8	17.7	14.4	15.0	15.3	10.5	10.3	11.1			
17	MHCH3017 {MOTU3}	19.7	19.7	19.7	20.5	19.7	15.5	20.5	16.6	17.3	16.8	17.0	17.3	0.0	12.3	11.6	10.5		
18	SMF50936 {MOTU3}	18.8	18.8	18.8	18.8	18.8	15.5	19.8	15.3	17.7	14.8	15.8	16.0	12.3	0.4	12.0	10.3	12.3	
19	MHCH3019 {MOTU3}	17.2	17.2	17.2	17.7	17.2	14.3	19.7	13.7	15.7	13.4	14.3	14.6	11.8	11.8	0.2	11.3	11.8	12.2

Supplementary Table S4. Mean genetic distances in the COI and 16S mtDNA genes combined between the *Pristimantis caryophyllaceus* samples used in the phylogenetic analysis.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	USNM572329 {MOTU1}																	
2	AJC1138 {MOTU1}	6.8																
3	UCR16434 {MOTU1}	12.7	12.7															
4	USNM572335 {MOTU1}	0.0	6.8	12.7														
5	USNM572331 {MOTU1}	0.0	6.8	12.7	0.0													
6	USNM572330 {MOTU1}	0.0	6.8	12.7	0.0	0.0												
7	SMF50938 {MOTU1}	1.1	6.5	12.2	1.1	1.1	1.1											
8	CH6367 {MOTU3}	14.2	15.6	12.3	14.2	14.2	14.2	14.4										
9	MHCH3017 {MOTU3}	15.2	16.1	12.3	15.2	15.2	15.2	15.9	10.6									
10	SMF50936 {MOTU3}	16.1	16.8	13.5	16.1	16.1	16.1	16.3	10.8	11.4								
11	MHCH3042 {MOTU3}	16.1	16.9	13.8	16.1	16.1	16.1	16.0	9.8	11.2	0.8							
12	SMF50933 {MOTU3}	15.1	15.9	12.1	15.1	15.1	15.1	15.7	10.5	0.1	11.2	11.1						
13	USNM572338 {MOTU3}	13.4	13.4	10.9	13.4	13.4	13.4	13.6	10.6	8.9	10.6	10.3	8.8					
14	MVUP1925 {MOTU2}	16.0	16.9	13.2	16.0	16.0	16.0	15.8	12.7	14.4	14.5	13.8	14.2	12.4				
15	MHCH3039 {MOTU2}	16.2	17.1	13.6	16.2	16.2	16.2	16.2	12.2	13.6	14.2	13.2	13.5	12.8	7.6			
16	SMF50943 {MOTU2}	16.4	16.9	12.2	16.4	16.4	16.4	16.2	12.2	14.4	14.0	13.4	14.3	12.1	3.2	6.7		
17	USNM572343 {MOTU2}	16.2	16.7	13.4	16.2	16.2	16.2	16.0	12.9	14.6	14.6	14.0	14.4	12.6	0.1	7.8	3.4	
18	SMF50939 {MOTU2}	17.5	16.8	14.4	17.5	17.5	17.5	17.1	13.3	14.6	15.8	15.2	14.4	14.2	6.2	8.7	5.3	6.3

Supplementary Table S5. Details of sample and museum voucher numbers (where available), collecting localities, and GenBank accession numbers for all samples used in this study.

Voucher	Species	Locality	Province	Country	Genbank accession number			Coordinates		elev. (m)
					16S	COI	RAG1	N	W	
AJC1138	*P. caryophyllaceus**	Panama, corregimiento de Chame, Altos del Maria, ~7.5 km NE of El Valle de Anton, corregimiento, Chame	Panama	Panama	JN991435.1	JN991364	JQ025176	8.6330	-80.0770	
CH6367	*P. caryophyllaceus**	Panama, Darién, Distrito de Pinogana, Cana, Laguna	Darién	Panama	JN991436.1	JN991365	JQ025175	7.7220	-77.6560	
MHCH3183	*P. caryophyllaceus*	Fortuna/Westhang Pata de Macho	Chiriquí	Panama				8.6710	-82.1967	1420
MHCH3184	*P. caryophyllaceus*	Fortuna/Westhang (=western slope) Pata de Macho	Chiriquí	Panama				8.6710	-82.1967	1420
MHCH3185	*P. caryophyllaceus*	Fortuna/Westhang (=western slope) Pata de Macho	Chiriquí	Panama				8.6775	-82.1980	1760
MHCH3186	*P. caryophyllaceus*	La Nevera/Cerro Santiago Westhang (=western slope)	Comarca Ngöbe Buglé	Panama				8.5011	-81.7694	1580
MHCH3187	*P. caryophyllaceus*	La Nevera/Cerro Santiago Westhang (=western slope)	Comarca Ngöbe Buglé	Panama				8.5010	-81.7691	1600
MHCH3188	*P. caryophyllaceus*	La Nevera/Cerro Santiago Westhang (=western slope)	Comarca Ngöbe Buglé	Panama				8.5010	-81.7691	1600
MHCH3189	*P. caryophyllaceus*	Cerro Mariposa	Veraguas	Panama				8.5145	-81.1207	880
MHCH3190	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5122	-81.1214	935
MHCH3191	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5001	-81.1170	1255
MHCH3192	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5001	-81.1173	1261
MHCH3193	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5111	-81.1214	916
MHCH3194	*P. caryophyllaceus*	Fortuna	Chiriquí	Panama				8.6739	-82.2188	1292
MHCH3017	*P. caryophyllaceus*	Serrania de Majé, Ambroya, Chepo	Panamá	Panama	KJ201960	KJ201949	KJ201970	8.8921	-78.5604	911
MHCH3018	*P. caryophyllaceus*	Serrania de Majé, Ambroya, Chepo	Panamá	Panama				8.8916	-78.5617	886
MHCH3019	*P. caryophyllaceus*	Panama, Darién, Distrito de Pinogana, Cana, Laguna	Darién	Panama	KJ201961	KJ201950				
MHCH3020	*P. caryophyllaceus*	Río Sambu, Serranía de Jingurudo, Sambú.	Darién	Panama				7.6842	-78.0387	971
MHCH3021	*P. caryophyllaceus*	Cerro Sapo, Garachiné	Darién	Panama				7.9762	-78.3626	1169
MHCH3022	*P. caryophyllaceus*	Cerro Sapo, Garachiné	Darién	Panama	KJ201952	KJ201942		7.9762	-78.3628	1168
MHCH3037	*P. caryophyllaceus*	Río Taintidu, Chucunaque	Wargandi	Panama				9.0355	-78.0264	289
MHCH3038	*P. caryophyllaceus*	Río Taintidu, Chucunaque	Wargandi	Panama				9.0593	-77.9842	553
MHCH3039	*P. caryophyllaceus*	Río Tuquesa, Embera-Waounaan	Embera-Wounaan	Panama			KJ201967	8.4800	-77.5194	859
MHCH3040	*P. caryophyllaceus*	Río Tuquesa, Embera-Waounaan	Embera-Wounaan	Panama				8.4791	-77.5280	718
MHCH3041	*P. caryophyllaceus*	Río Tuquesa, Embera-Waounaan	Embera-Wounaan	Panama				8.4791	-77.5280	718
MHCH3042	*P. caryophyllaceus*	Río Sambu, Serranía de Jingurudo, Sambú.	Embera-Wounaan	Panama			KJ201969	7.7640	-78.1006	655
MHCH3043	*P. caryophyllaceus*	Chucantí ridge, río Congo, Chepigana	Darién	Panama				8.7977	-78.4623	1295
MHCH3044	*P. caryophyllaceus*	Chucantí ridge, río Congo, Chepigana	Darién	Panama				8.7965	-78.4630	1342
MHCH3045	*P. caryophyllaceus*	Rancho Frío Field station, Pinogana	Darién	Panama				7.9595	-77.7037	1230
MHCH3046	*P. caryophyllaceus*	Rancho Frío Field station, Pinogana	Darién	Panama				7.9595	-77.7037	1230
MHCH457	*P. caryophyllaceus*	El pianista, Bocas del Toro, Panamá	Bocas del Toro	Panama				8.8714	-82.4159	
MHCH523	*P. caryophyllaceus*	Qda Arena, Fortuna, Chiriquí, Panamá	Chiriquí	Panama				8.7180	-82.2284	1074
MVUP1925	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Coclé	Coclé	Panama	FJ784473.1	FJ766776		8.6670	-80.5920	800

Voucher	Species	Locality	Province	Country	Genbank accession number			Coordinates		elev. (m)
					16S	COI	RAG1	N	W	
MVZ203810	*P. caryophyllaceus**	Costa Rica: Cartago, 2.5 km S Tapanti Bridge across Rio Grande de Orosi	Cartago	Costa Rica	EU186686.1	na				
SMF 89976	*P. caryophyllaceus*	Cerro Negro/PN Santa Fe	Veraguas	Panama				8.5706	-81.1043	800
SMF 89977	*P. caryophyllaceus*	Cerro Negro/PN Santa Fe	Veraguas	Panama				8.5663	-81.0988	690
SMF 89978	*P. caryophyllaceus*	Cerro Negro/PN Santa Fe	Veraguas	Panama				8.5706	-81.1043	800
SMF 89979	*P. caryophyllaceus*	Cerro Negro/PN Santa Fe	Veraguas	Panama				8.5769	-81.0973	900
SMF 89980	*P. caryophyllaceus*	Cerro Mariposa	Veraguas	Panama				8.6757	-81.1228	1385
SMF 89981	*P. caryophyllaceus*	I Brazo Mulaba	Veraguas	Panama				8.5186	-81.1332	700
SMF 97027	*P. caryophyllaceus*	La Nevera		Panama				8.4996	-81.7710	1650
SMF 97028	*P. caryophyllaceus*	Cerro Mariposa	Veraguas	Panama				8.5145	-81.1207	880
SMF 97029	*P. caryophyllaceus*	La Nevera/Cerro Santiago Westhang	Comarca Ngöbe Buglé	Panama				8.4953	-81.7673	1800
SMF 97030	*P. caryophyllaceus*	Llano Tugri	Comarca Ngöbe Buglé	Panama				8.5082	-81.7162	1600
SMF 97031	*P. caryophyllaceus*	Willi Mazu	Comarca Ngöbe Buglé	Panama				8.7885	-82.2016	799
SMF 97032	*P. caryophyllaceus*	Willi Mazu	Comarca Ngöbe Buglé	Panama				8.7885	-82.2016	799
SMF 97033	*P. caryophyllaceus*	Willi Mazu	Comarca Ngöbe Buglé	Panama	KJ476734			8.7885	-82.2016	799
SMF 97034	*P. caryophyllaceus*	Changena Trail/Oberes Camp	Bocas del Toro	Panama				8.9505	-82.7094	1968
SMF 97035	*P. caryophyllaceus*	Changena Trail/Oberes Camp	Bocas del Toro	Panama	KJ476733			8.9505	-82.7094	1968
SMF 97036	*P. caryophyllaceus*	Rio Clarito	Bocas del Toro	Panama				9.0090	-82.6644	1258
SMF 97037	*P. caryophyllaceus*	Rio Clarito	Bocas del Toro	Panama	KJ476732			9.0090	-82.6644	1258
SMF 97039	*P. caryophyllaceus*	Cerro Guayaba	Chiriquí	Panama				8.7657	-82.2528	1565
SMF 97040	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5122	-81.1214	935
SMF 97041	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5061	-81.1196	1108
SMF 97042	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.4966	-81.1164	1356
SMF 97043	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.4997	-81.1168	1264
SMF 97044	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5000	-81.1170	1257
SMF 97045	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5000	-81.1170	1257
SMF 97046	*P. caryophyllaceus*	Cerro Mariposa, Santa Fe	Veraguas	Panama				8.5024	-81.1191	1163
SMF 97047	*P. caryophyllaceus*	Lost and Found/Fortuna	Chiriquí	Panama				8.6757	-82.2128	1364
SMF 97048	*P. caryophyllaceus*	Lost and Found/Fortuna	Chiriquí	Panama				8.6773	-82.2103	1288
SMF50931	*P. caryophyllaceus*	Serrania de Maje, Ambroya, Chepo	Panamá	Panama				8.8920	-78.5609	901
SMF50932	*P. caryophyllaceus*	Serrania de Maje, Ambroya, Chepo	Panamá	Panama				8.8920	-78.5609	901
SMF50933	*P. caryophyllaceus*	Serrania de Maje, Ambroya, Chepo	Panamá	Panama	KJ201953	KJ201943	KJ201963	8.8919	-78.5608	911
SMF50934	*P. caryophyllaceus*	Rancho Frío Field station, Pinogana	Darién	Panama				7.9891	-77.7073	1136
SMF50935	*P. caryophyllaceus*	Río Sambu, Serranía de Jingurudo, Sambú.	Darién	Panama				7.6841	-78.0387	962
SMF50936	*P. caryophyllaceus*	Río Sambu, Serranía de Jingurudo, Sambú.	Darién	Panama	KJ201954	KJ201944	KJ201964	7.6837	-78.0384	961
SMF50937	*P. caryophyllaceus*	Río Sambu, Serranía de Jingurudo, Sambú.	Darién	Panama				7.6691	-78.0380	1133
SMF50938	*P. caryophyllaceus*	Qda Valle Grande, Donoso	Colón	Panama	KJ201958	KJ201947	KJ201968	8.8216	-80.6632	211

Voucher	Species	Locality	Province	Country	Genbank accession number			Coordinates		elev. (m)
					16S	COI	RAG1	N	W	
SMF50939	*P. caryophyllaceus*	Río Taintidu, Chucunaque	Wargandi	Panama	KJ201962	KJ201951	KJ201971	9.0355	-78.0264	289
SMF50940	*P. caryophyllaceus*	Río Taintidu, Chucunaque	Wargandi	Panama				9.0355	-78.0264	289
SMF50941	*P. caryophyllaceus*	Serranía de San Blas	Guna Yala	Panama				9.0611	-77.9797	340
SMF50942	*P. caryophyllaceus*	Río Sambu, Serranía de Jingurudo, Sambú	Embera-Wounaan	Panama				7.7590	-78.0923	643
SMF50943	*P. caryophyllaceus*	Río Terable, El Llano, Chepo	Panama	Panama			KJ201965	9.2840	-78.9838	322
SMF50944	*P. caryophyllaceus*	Chucantí ridge, río Congo, Chepigana	Darién	Panama	KJ201956		KJ201966	8.7977	-78.4623	1295
SMF50945	*P. caryophyllaceus*	Chucantí ridge, río Congo, Chepigana	Darién	Panama				8.7977	-78.4623	1295
SMF50946	*P. caryophyllaceus*	Rancho Frío Field station, Pinogana	Darién	Panama				8.0168	-77.7297	133
SMF50947	*P. caryophyllaceus*	Rancho Frío Field station, Pinogana	Darién	Panama				7.9595	-77.7037	1230
SMF85008	*P. caryophyllaceus*	RF Fortuna	Chiriquí	Panama				8.7264	-82.2615	1100
SMF85010	*P. caryophyllaceus*	RF Fortuna	Chiriquí	Panama				8.7264	-82.2615	1100
SMF85011	*P. caryophyllaceus*	RF Fortuna	Chiriquí	Panama				8.7264	-82.2615	1100
SMF85012	*P. caryophyllaceus*	RF Fortuna	Chiriquí	Panama				8.7264	-82.2615	1100
SMF85014	*P. caryophyllaceus*	BP Palo seco, Los tucanes trail	Bocas del Toro	Panama				8.7817	-82.2122	1120
SMF85015	*P. caryophyllaceus*	Fortuna Town	Chiriquí	Panama				8.7313	-82.2534	1300
SMF85016	*P. caryophyllaceus*	La Nevera	Comarca Ngöbe Buglé	Panama				8.4996	-81.7710	1600
SMF85018	*P. caryophyllaceus*	La Nevera	Comarca Ngöbe Buglé	Panama				8.4996	-81.7710	1600
UCR 16429	*P. cerasinus**	Vuelta de Queque, Rio Siquirres trail, Guayacan	Limón	Costa Rica	JN991437	JN991366	JQ025177	10.0400	-83.5500	
UCR16434	*P. caryophyllaceus**	Costa Rica: San Jose, Rio Gacho, Los Juncos, Cascajal, Canton Vazquez de Coronado	San José	Costa Rica	JN991434.1	JN991363		9.9800	-83.8400	
UCR16447	*P. cruentus**	Tapantí, Cantón, Paraiso	Cartago	Costa Rica	JN991441	JN991370	JQ025179	9.6500	-83.8500	1200
USNM572329	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Cocle	Coclé	Panama	FJ784397.1	FJ766771		8.6670	-80.5920	800
USNM572330	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Cocle	Coclé	Panama	FJ784421.1	FJ766774		8.6670	-80.5920	800
USNM572331	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Cocle	Coclé	Panama	FJ784422.1	FJ766773		8.6670	-80.5920	800
USNM572335	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Cocle	Coclé	Panama	FJ784589.1	FJ766770		8.6670	-80.5920	800
USNM572338	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Cocle	Coclé	Panama	FJ784491.1	FJ766775		8.6670	-80.5920	800
USNM572343	*P. caryophyllaceus**	Parque Nacional G. D. Omar Torrijos H., El Cope, Corregimiento is Ola, Distrito La Pintada, Cocle	Coclé	Panama	FJ784375.1	FJ766772		8.6670	-80.5920	800
GK1452	*P. caryophyllaceus*	RF Fortuna	Chiriquí	Panama				8.7264	-82.2615	1100
GK1469	*P. caryophyllaceus*	BP Palo seco Los tucanes	Bocas del Toro	Panama				8.7817	-82.2122	1120
GK1595	*P. caryophyllaceus*	La nevera	Comarca Ngöbe Buglé	Panama				8.4996	-81.7710	1600

Supplementary Table S6. Transect details for *Pristimantis caryophyllaceus* (MOTU3).

Locality	Indiv.	Date	Length (m)	Duration	Climatic condition	T (°C)	Humidity (%)	Altitude **(m)**	Coordinates N	W
Serrania de Pirre	8	10/08/2011	240	03:30:00	cloudy	22.3	76	1137	7.98845	77.7076
Serrania de Pirre	10	11/08/2011	300	02:53:45	rainy	20.5	79	1110	7.9791	77.7086
Serrania de Jingurudó	7	26/09/2011	200	03:20:00	clear	20.9	84	943	7.68338	78.0384
Serrania de Jingurudó	8	27/09/2011	418	03:46:00	clear	23.5	79	953	7.68035	78.0387
Serrania de Jingurudó	3	29/09/2011	280	03:00:00	cloudy	22.6	81	865	7.69312	78.0423
Serrania de Sapo	4	05/12/2011	172	03:10:00	cloudy	21.4	72	1160	7.97589	78.3625
Serrania de Sapo	6	06/12/2011	160	03:50:00	cloudy	19.4	83	917	7.97749	78.3592

Supplementary Text S6. *Pristimantis caryophyllaceus* complex colour descriptions.

MOTU1: Alto de Piedra, Veraguas (MHCH3189, no photo): Dorsal base colour Buff (124) with dark Brownish Olive (129) v-shaped transversal stripes and dark Brownish Olive (129) mottling in between. Posterior surface of thigh Gem Ruby (110). Ventral surface Salmon Colour (106) with small dark spots in the gular region and small white spots between axilla and groin.

Reserva Forestal La Fortuna, western slope of Cerro Pata de Macho, Chiriquí (MHCH3184): Dorsal base colour Cinnamon (123A) with a suggestion of Yellow Ochre (123C), bordered by Sepia (119); dorsal tubercles Buff Yellow (53); lateral surfaces transparent lgc sparsely mottled with Buff Yellow (53) and fading dorsally into Buff Yellow (53) on transparent ground; dlc dorsal surfaces of limbs Buff Yellow (53) on transparent ground speckled with Olive-Green (Auxiliary) (47) auxiliary lines; vgc ventral ground colour dirty white fading into Smalt Blue (70) towards lateral edges; vlc ventral surfaces of hands and feet transparent with spots of Buff Yellow (53), and tubercles in Sepia (119); Ic Iris colouration Pale Pinkish Buff (121D), bordered with Sepia (119) and Robin's Egg Blue (93).

MOTU2: Río Tuquesa, Darién Mountain Range (MHCH3039, Fig. 8E): Dorsal colour Chamois (84) with Warm Sepia (40) irregular blotches and spots; Warm Sepia (40) interorbital band; upper surface of thigh with Warm Sepia (40) bars; groin and posterior surface of thigh suffused with Geranium (66); upper iris region Medium Chrome Orange (75); lower iris region Cream White (52), iris centre Dark Salmon (59); iris periphery Jet Black (300); eye periphery Smoky White (261).

Nurra, San Blas Mountain range (SMF50939, Fig 8G): Dorsal colour Russet (44) with small Warm Sepia (40) spots; upper surface of thigh with Pale Pinkish Buff (3) bars; groin and posterior surface of thigh suffused with Geranium (66); upper iris region Spectrum Orange (9); lower iris region Light Lavender (201), iris centre Dark Salmon (59); iris periphery Jet Black (300); eye periphery Pearl Gray (262). SMF50940 (Fig. 8H) same as SMF50939, but with the upper and lower iris regions Spectrum Red (67), and the eye periphery Light Sky Blue (191).

Nurra, San Blas mountain range (MHCH3037, Juvenile, Fig. 8F): Dorsal colour Sepia (279) with a mid-dorsal line in Medium Fawn (257); a series of delicate Medium Fawn (257) transverse lines on dorsum; dorsolateral line from the tip of snout to the groin Beige (254); upper and lower iris regions Spectrum Red (67); iris periphery Jet Black (300); eye periphery Pearl Gray (262).

MOTU3: Pirre Mountain range (SMF50934, 1149): Dorsal colour Buff (5), frontal region and some blotches on the rest of the dorsum Pale Buff (1); no contrasting pattern on groin or posterior surface of thigh; upper and lower iris regions Light Buff (2), iris centre Walnut Brown (27); iris periphery Jet Black (300); eye periphery Smoky White (261).

Pirre mountain range (SMF50946, Fig. 9F) Dorsal colour Buff (5), with small, scattered black spots; Pale Buff (1) spots on dorsum; no contrasting pattern on groin or posterior surface of thigh; upper and lower iris regions Light Buff (2), iris centre Walnut Brown (27); iris periphery Jet Black (300); eye periphery Smoky White (261).

Pirre Mountain range (MHCH3045, Fig. 9G): Dorsal colour Raw Umber (22) with a Light Yellow Ochre (13) dorsolateral line from the tip of the snout to the groin, separating the dorsal from the lateral colouration; face Tawny Olive (17), lateral region behind the eyes Light Yellow Ochre (13); groin and posterior surface of thigh Buff Yellow (6); upper surface of thigh with Ground Cinnamon (270) bars. Upper and lower iris region Light Buff (2); iris centre Walnut Brown (27); iris periphery Jet Black (300); eye periphery Smoky White (261).

Cerro Garra Garra, Jingurudó (MHCH3042, Fig. 8C): Dorsal colour Flesh (249) with Warm Sepia (40) irregular blotches and spots; Warm Sepia (40) interorbital band; upper surface of thigh with Warm Sepia (40) bars; groin and posterior surface of thigh suffused with Geranium (66); upper and lower iris region Olive Horn (16), iris centre Walnut Brown (27); iris periphery Jet Black (300); eye periphery Smoky White (261).

Ambroya, Maje (specimen not collected, Fig. 9A): Dorsal colour Chamois (84) with small Warm Sepia (40) spots; iris Spectrum Red (67); iris periphery Jet Black (300); eye periphery Smoky White (261). Chucanti, SMF50945 (Fig. 9B), dorsal colour Chesnut (30) with some Dusky Brown (285) blotches; dorsolateral line from the tip of snout to the groin Flesh (249); lateral colour Salmon (58); upper surface of thigh with Dusky Brown (285) bars; groin and posterior surface of thigh Flame Scarlet (73); upper and lower iris region Spectrum Red (67); iris periphery Jet Black (300); eye periphery Light Lavender (201). Chucanti, MHCH3043 (Fig. 9C), dorsal colour Deep Vinaceous (248) with the frontal region in Pale Pinkish Buff (3); groin and posterior surface of thigh Flame Scarlet (73); iris Spectrum Red (67); iris periphery Jet Black (300); eye periphery Light Lavender (201).

Chucanti (specimen not collected, Fig. 9D), dorsal colour Clay (18) with a dorsolateral line from the tip of snout to the groin in Cream (12); groin and posterior surface of thigh Flame Scarlet (73); iris Pale Neutral Gray (296); iris periphery Jet Black (300); eye periphery Light Lavender (201).

Appendix V

Declaration on the contributions of authors

to the publication: Evidence for the recognition of two species of *Anolis* formerly referred to as *A. tropidogaster* (Squamata: Dactyloidae).
status: published (2012)
name of journal: Zootaxa 3348
Authors involved:
- Abel Batista (AB), - Marcos Ponce (MP), - Milan Vesely (MV), - Gunther Köhler (GK), - Arcadio Carrizo (AC), - Sebastian Lotzkat (SL)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 20%
Coauthor AC: 20%
Coauthor GK: 60%

(2) to the implementation of the respective studies and experiments

PhD candidate: 15% – field work (collecting and documenting specimens).
Coauthor GK: 60%
Coauthor AC, MP (each coauthor): 5% – field work (collecting and documenting specimens)
Coauthor SL: 15% – field work (collecting and documenting specimens), morphological analysis, molecular analysis.

(3) to the creation of the data collection and figures

PhD candidate: 30% – contributed to create most of the morphological database
Coauthor MV: 5% – made drawings
Coauthor GK: 50% made most of figures
Coauthor MP: 5% – provided photos
Coauthor SL: 10 – provided tissue samples, photos, map, DNA sequences

(4) to the analysis and interpretation of the data

PhD candidate: 10% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor GK: 65% – contributed to data analysis and interpretation
Coauthor SL: 10% – analysis and interpretation of morphological, biogeographical, and molecular data
Coauthor MV, AC, MP (each coauthor): 5% – contributed to data analysis and interpretation

(5) to writing the manuscript

PhD candidate: 15% – Batista wrote natural history of *A. tropidogaster*, contribute to the description of the species, and made comments on the discussion.
Coauthor GK: 70%
Coauthor SL: 15%

Date/place: 13.04.2016 / Frankfurt am Main, Germany

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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Evidence for the recognition of two species of *Anolis* formerly referred to as *A. tropidogaster* (Squamata: Dactyloidae)

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Abstract

Based on differences in hemipenial morphology, male dewlap coloration, pholidosis, and 16S mtDNA, we recognize two species of anoles related to what was formerly referred to as *Anolis tropidogaster*: *Anolis tropidogaster* Hallowell 1856 and *A. gaigei* Ruthven 1916. The hemipenis in *A. tropidogaster* is large, bulbous, and bilobed whereas it is small, thin, and unilobed in *A. gaigei*; the male dewlap is almost uniform purplish red, sometimes with a paler orange central area in *A. tropidogaster* versus orange yellow with a darker orange central area in *A. gaigei*; and—aside from more subtle differences in several pholidotic characteristics—in male *A. gaigei* there is a pair of greatly enlarged postcloacal scales which is absent in *A. tropidogaster.* In the western part of its geographic range, *A. gaigei* has been confused with another anole species, *A. polylepis* Peters 1873, from which it can be readily distinguished by its strongly keeled ventral scales (smooth in *A. polylepis*).

Key words: *Anolis albi*, *Anolis cupreus*, *Anolis gaigei*, *Anolis osa*, *Anolis polylepis*, *Anolis stigmosus*, *Anolis tropidogaster*, Central America, Colombia, Dactyloidae, Panama, Reptilia, Squamata, Venezuela

Resumen

Basándonos en las diferencias morfológicas de hemipenes, coloración de la papera gular de los machos, características de escamación y 16S mtDNA reconocemos dos especies de lagartijas relacionadas con lo que hasta ahora ha sido reconocido como *Anolis tropidogaster*: *Anolis tropidogaster* Hallowell 1856 y *A. gaigei* Ruthven 1916. Las dos especies difieren en la morfología de hemipenes (hemipenes grandes, bulbosos y bilobulados en *A. tropidogaster* y pequeños, delgados y unilobulados en *A. gaigei*); en la coloración de la papera gular de los machos (casi rojo púrpura uniforme, a veces con un área central de color naranja más pálido en *A. tropidogaster*, a diferencia de un amarillo anaranjado con un área central de color naranja más oscuro en *A. gaigei*); y—además de varias diferencias más sutiles en características de escamación—machos de *A. gaigei* presentan un par de escamas postcloacales muy agrandadas las cuales están ausentes en *A. tropidogaster.* En la parte occidental de su área de distribución geográfica, *A. gaigei* ha sido confundida con otra especie de lagartija, *A. polylepis* Peters 1873, de la cual se distingue claramente mediante sus escamas ventrales fuertemente aquilladas (lisas en *A. polylepis*).

Introduction

In 1856, Hallowell described *Anolis tropidogaster* based on a single specimen (now ANSP 7618) that originated from "New Grenada," then a republic that contained the territory of today's Colombia and Panama as well as small portions of what is today Ecuador and Venezuela (Aguilera Peña 2002). However, most previous authors have interpreted the type locality of *A. tropidogaster* to be "New Grenada, Colombia" (*e.g.*, Malnate 1971) or merely

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"Colombia" (*e.g.*, Dunn 1930, Barbour 1934, Peters and Donoso-Barros 1970). GK had the privilege of examining ANSP 7618, which constitutes fragments of bone with remains of poorly preserved skin. According to Dunn (1930) and Barbour (1934), the specimen had been in this poor state at least since the early 1930s. Bocourt (1869) established his new species *Anolis stigmosus* based on two specimens (now MNHN 2427 and 2427A) from "la Colombie et ont été recueillis près de la rivière de la Magdeleine". Ruthven (1916) described *Anolis gaigei* based on a holotype (now UMMZ 48304) from "San Lorenzo, Santa Marta Mountains, Colombia, elevation of 2,700 ft." Finally, Barbour (1932) described *Anolis albi* (female holotype MCZ 32301; male paratype MCZ 32302, the latter examined by GK) from "Andagoya, Choco, western Colombia." The nominal species *A. stigmosus, A. gaigei,* and *A. albi* have been considered as synonyms of *A. tropidogaster* for a long time (Barbour 1934; Peters & Donoso-Barros 1970).

In the course of our field work in Panama, we discovered that two distinct and geographically segregated phenotypes are present among the populations currently assigned to *A. tropidogaster.* The two clusters differ most obviously in hemipenial morphology, in the coloration of the male dewlap as well as in several pholidotic characteristics. Here we report upon these results and provide evidence for the recognition of each of these morphological clusters as a distinct species. Because in parts of its geographic range one of these species has frequently been confused with another widespread lowland anole, *A. polylepis* (*e.g.*, Martínez Cortés & Rodriguez 2003, 2005, Ibáñez 2006), we also include the latter species in our comparisons.

Material and methods

In evaluating whether multiple species exist within the *Anolis tropidogaster* complex, we follow the Evolutionary Species Concept (Simpson 1961, Wiley 1978), and operationalize this concept by identifying species based on consistent differences between populations, assuming these differences are the result of different evolutionary histories (Frost & Kluge 1994). Abbreviations for museum collections follow those of Leviton *et al.* (1985) except for MHCH (Museo Herpetológico de Chiriquí, David, Chiriquí, Panama). Nomenclature of scale characters follows that of Köhler (2008). Terminology for dewlap morphology follows that of Fitch and Hillis (1984). Terminology for hemipenial morphology follows that of Myers *et al.* (1993) and Savage (1997). Scale sizes were measured using the ocular micrometer of a stereo microscope (Leica MZ 12) to the nearest 0.01 mm. All other measurements were made using precision calipers to the nearest 0.1 mm. Values are given as minimum–maximum (mean ± standard deviation). Head length was measured from the tip of the snout to the anterior margin of the ear opening. Snout length was measured from the tip of the snout to the anterior border of the orbit. Head width was determined as the distance between the oral ricti. Dorsal and ventral scales were counted at midbody along the midline. Tail height and width were measured at the point reached by the heel of the extended hind leg. Subdigital lamellae were counted on phalanges II to IV of the 4th toe. We considered the scale directly anterior to the circumnasal to be a prenasal. Relative hind leg length was examined in the field by folding the hind leg of the specimen in life towards its head and determining the point reached by the tip of the longest toe. The capitalized colors and color codes (the latter in parentheses) are those of Smithe (1975–1981). Abbreviations used are HL (head length), HW (head width), INL (infralabials), IP (interparietal plate), SO (subocular scales), SPL (supralabial scales), SS (supraorbital semicircles), and SVL (snout–vent length).

For the complementary molecular analysis, we extracted DNA following the protocol of Ivanova *et al.* (2006). To eliminate potential PCR-inhibiting contaminants, the tissue samples were incubated for 14 hours in 200 µL low PBS buffer (20 µL PBS in 180µL of water) before overnight digestion with the vertebrate lysis buffer at 56 °C. After extraction, DNA was eluted in 50 µL TE buffer. A fragment of the mitochondrial 16S rRNA gene was amplified in an Eppendorf Mastercycler® pro using the following program: initial denaturation for 2 min at 94 °C; followed by 40 cycles with denaturation for 35 s at 94 °C, hybridization for 35 s at 48.5 °C, and elongation for 60 s at 72 °C; final elongation for 10 min at 72 °C. Reaction mix for each sample contained 1 µL DNA template, 14 µL water, 2.5 µL PCR-buffer, 1 µL 25 mM $MgCl_2$, 4 µL 2.5 mM dNTPs (Invitrogen), 0.5 µL Taq Polymerase (PeqLab), and 1 µL of each primer (forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon). A total of 7 sequences (one of each Type A and *Anolis polylepis*, two of each Type B and *A. cupreus,* and one *A. kemptoni* as outgroup; see Appendix 2 for examined specimens and GenBank accession numbers) were aligned with MUSCLE (Edgar 2004) using the default settings in Geneious (Drummond *et al.* 2010). The manually refined final alignment contained 542 positions. Using

MEGA5 (Tamura *et al.* 2011), we computed uncorrected pairwise genetic distances, determined the Tamura 3-parameter model as the best-fitting substitution model, and conducted Maximum Likelihood as well as Maximum Parsimony analyses (each with 10000 bootstrap replicates). Using TCSv1.21 (Clement *et al.* 2000), we conducted a statistical parsimony network analysis, with gaps considered as a fifth character state and a connection limit of 95%.

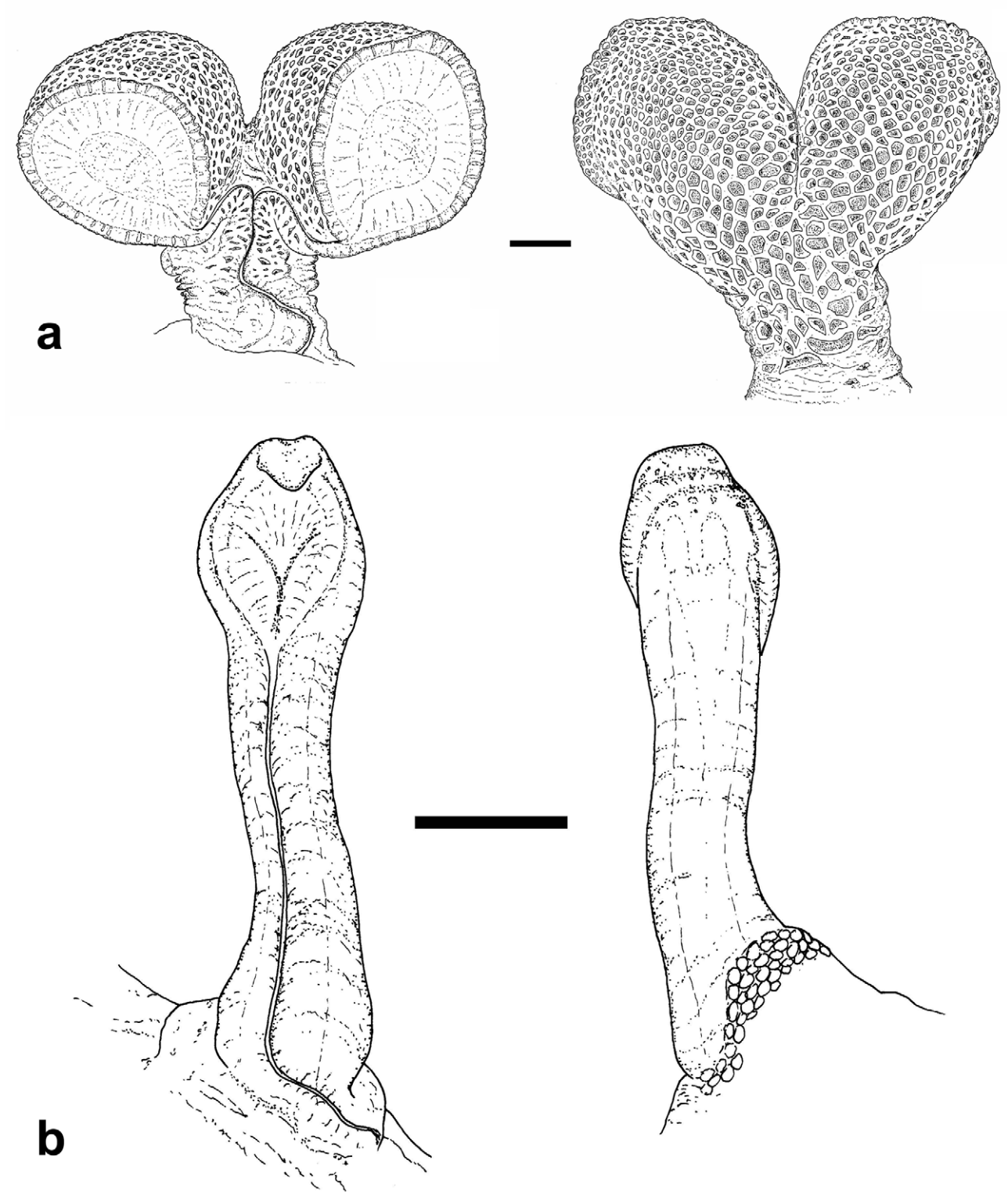

FIGURE 1. (a) Type A hemipenis (SMF 91956); (b) Type B hemipenis (SMF 91902). See text for details. Scale bar = 1.0 mm.

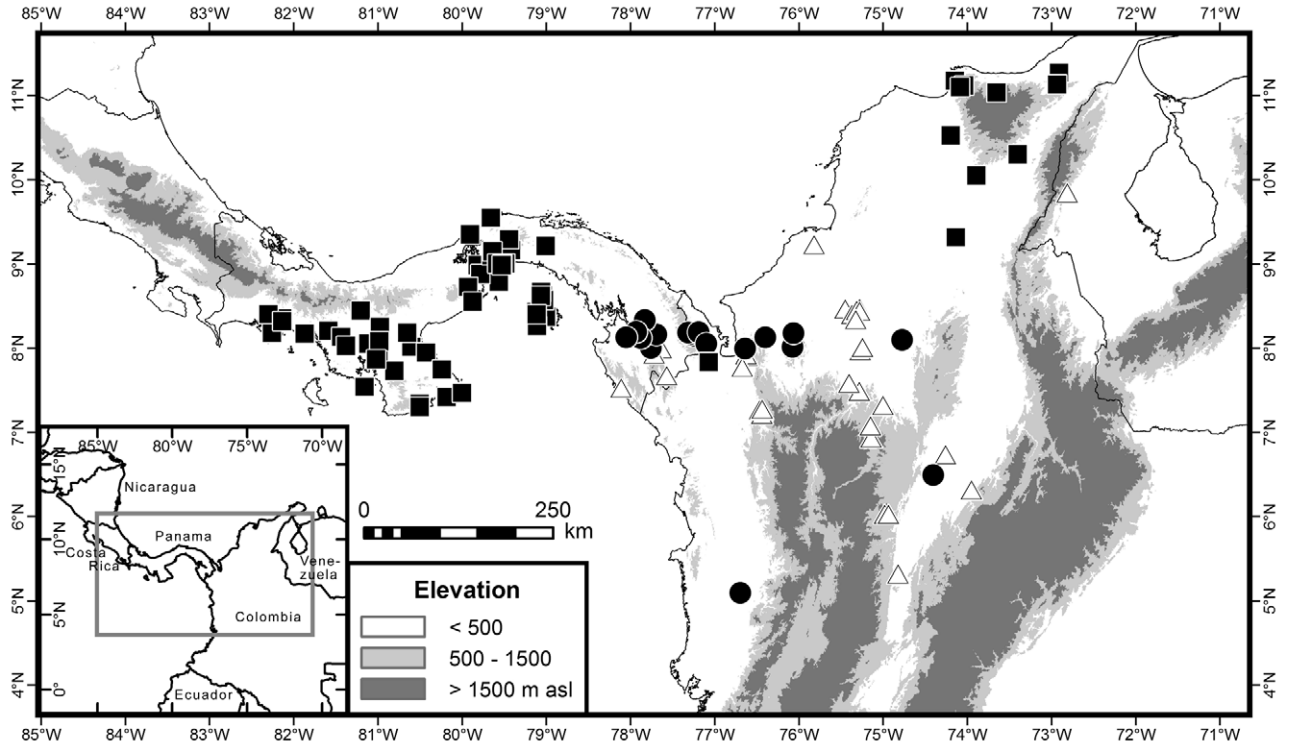

FIGURE 2. Map indicating known collecting sites mentioned in text of anoles formerly referred to as *Anolis tropidogaster*. Each symbol can represent one or more nearby localities. Circles: Type A hemipenes; squares: Type B hemipenes; triangles: localities of *Anolis tropidogaster*-like specimens not verified by authors (sources: MHUA catalogue, Donoso-Barros 1968, Carvajal-Cogollo and Urbina-Cardona 2008). See text for details.

Results

Two distinctly different hemipenial morphotypes are evident in the specimens we examined. In Type A (Fig. 1a; *N* = 5 adult males with everted hemipenes), the hemipenis is a large bilobed organ (length of lobes equal length of truncus); the sulcus spermaticus bifurcates at the base of the apex and the branches open into concave areas, one on each lobe; asulcate side of apex strongly calyculate, truncus with transverse folds. In Type B (Fig. 1b; *N* = 14 adult males with everted hemipenes), the hemipenis is unilobed and smaller and thinner relative to body size as compared to the type A hemipenis; the sulcus spermaticus opens at base of apex into a broad concave area that covers the complete sulcate side of the apex; no discernable surface structure on trunk or apex. The distribution of the two hemipenial morphs is highly correlated geographically (Fig. 2). Furthermore, the two hemipenial morphs differ readily in male dewlap coloration. The dewlap of males with a Type A hemipenis is orange yellow with a darker orange peripheral area (Figs 3a,b). The dewlap of males with a Type B hemipenis is orange yellow with a darker orange central area (Figs. 3c,d). Finally, we observed differences in several scalation characteristics (Fig. 4): (1) in males with a Type A hemipenis the postcloacal scales are usually not, or only slightly enlarged whereas males with a Type B hemipenis always have a very distinct pair of greatly enlarged postcloacal scales; (2) the dorsal head scales, especially in the parietal region: smaller and bearing minute tubercles in Type A versus larger and flat in Type B; (3) middorsal caudal scales: only slightly enlarged and somewhat irregularly arranged in Type A versus distinctly enlarged and forming a regular series in Type B; (4) postmental scales: outer postmental scales only slightly enlarged relative to medial ones in Type A versus outer scales greatly enlarged relative to medial ones in Type B.

The distinctiveness of the hemipenial morphs A and B is further corroborated by the differences in the 16S mitochondrial rRNA gene revealed by our analyses (Fig. 5). In our consensus tree (Fig. 5a), the Type A specimen (MHCH 1634) appears most closely related to the Type B specimens (SMF 91907, 91918), with *Anolis polylepis* and *A. cupreus* forming a sister clade to Type A + Type B. The mean genetic distance between Type A and Type B specimens is 4.1% (4.3 and 3.9%, respectively). This value, although being slightly lower than the genetic distance

of 6.0% observed between *A. polylepis* and both specimens of *A. cupreus*, can be interpreted to indicate a differentiation at species level. This view is supported by the fact that in the haplotype network analysis (Fig. 5b) Type A and B form unconnected subnetworks, just as *A. polylepis* and *A. cupreus* do.

Based on the combined evidence, we recognize the two hemipenial morphotypes defined above as two distinct species with Species A (= our former Type A) being distributed in Colombia and eastern Panama, and Species B (= our former Type B) being distributed from western Panama along northern Colombia, and probably into western Venezuela (Fig. 2). See Table 1 for variation in selected measurements, proportions and scale characters in the two species.

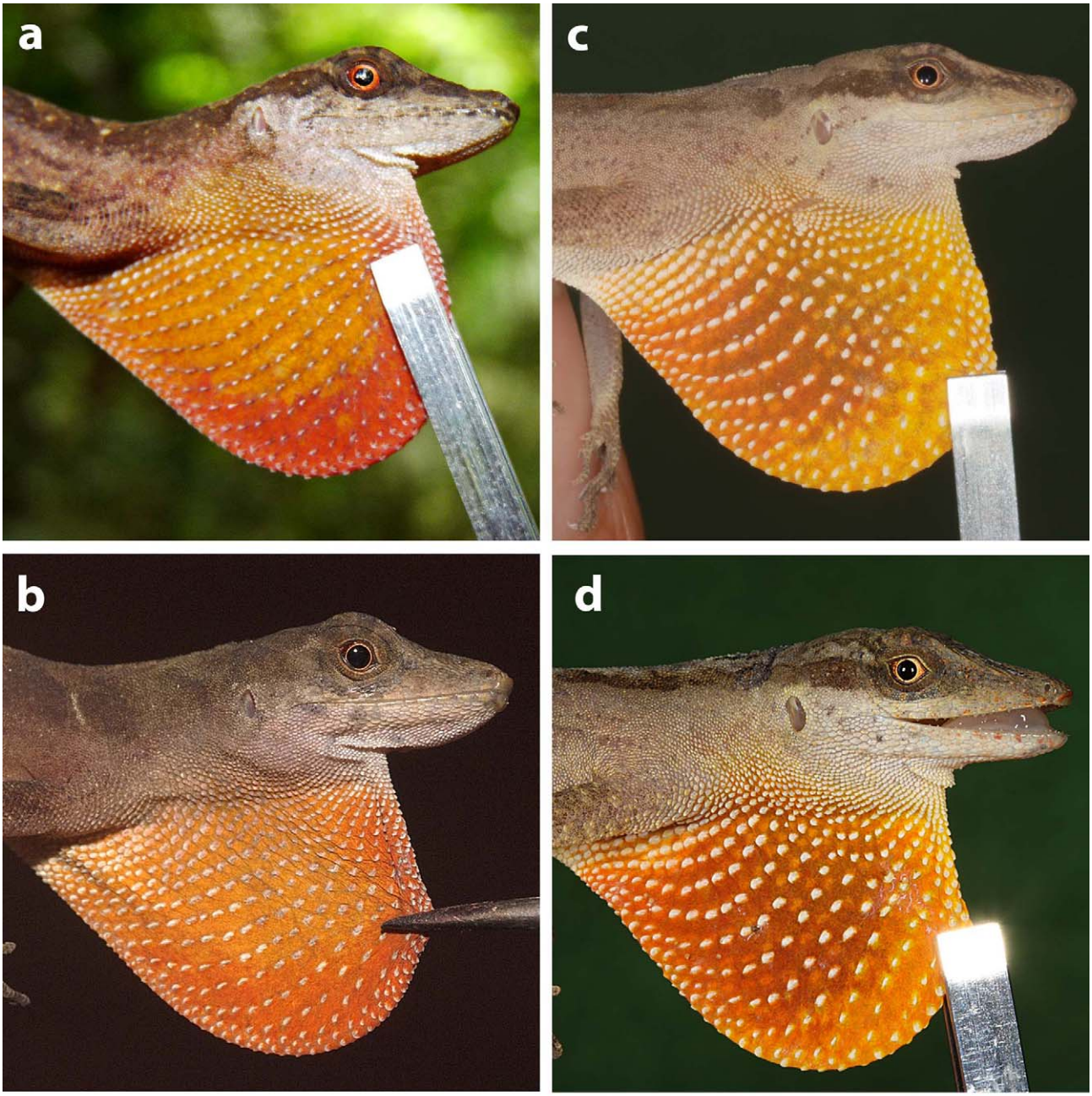

FIGURE 3. Male dewlap in life. Type A hemipenes: (a) SMF 91956; (b) SMF 93598. Type B hemipenes: (c) SMF 91902; (d) SMF 91910.

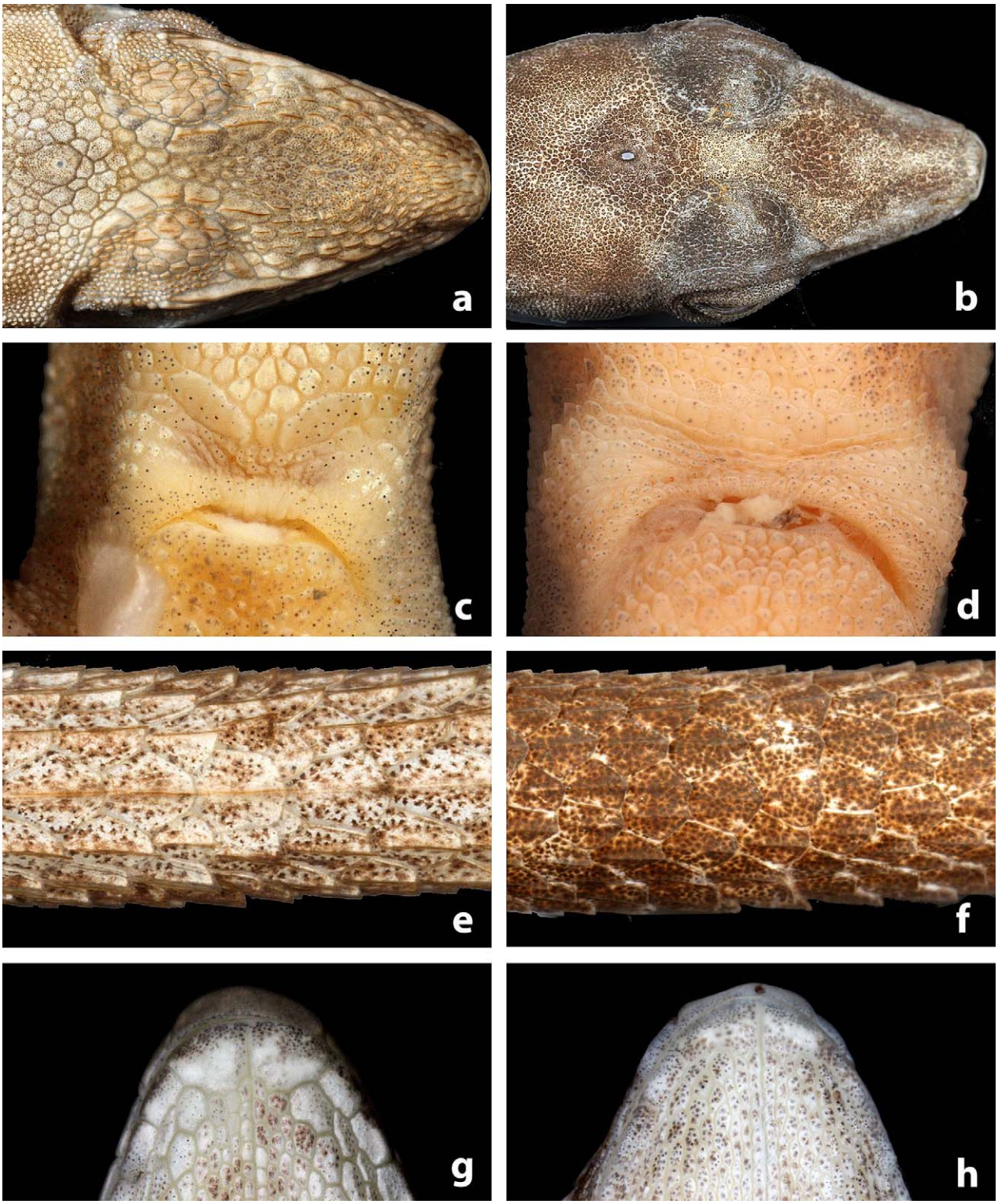

FIGURE 4. Comparison of scalation details in *Anolis gaigei* (left column) and *A. tropidogaster* (right column). Dorsal head in (a) *A. gaigei* SMF 91956 and (b) *A. tropidogaster* MHCH 1701. Cloacal region in (c) *A. gaigei* SMF 82705 and (d) *A. tropidogaster* FMNH 63793. Dorsal tail in (e) *A. gaigei* SMF 91956 and (f) *A. tropidogaster* MHCH 1636. Chin region in (g) *A. gaigei* SMF 91956 and (h) *A. tropidogaster* MHCH 1640.

As mentioned above, the holotype of *Anolis tropidogaster* (ANSP 7618; Fig. 6a) is a macerated skeleton with vague locality data (see above). Fortunately, the original description (Hallowell 1856) provides some clues as for the taxonomic identity of the male holotype (sex as indicated by the presence of "a well developed gular pouch"; Hallowell 1856:225). The information that ANSP 7618 had a "remarkably strong carination of the ventral scales"

and a "color brownish, extremities banded with brown" (Hallowell 1856:224) supports the view that this specimen belongs to the *A. tropidogaster* complex as currently understood. Somewhat odd is the remark that it had "fingers and toes without any dilation whatever" (Hallowell 1856:225), a condition that might be due to desiccation of the specimen although there is no hint for that in the original description. In the light that the original description of *A. tropidogaster* is relatively detailed (at least considering the standard at the time), the lack of mentioning a pair of distinctly enlarged postcloacal scales should be interpreted as the specimen actually lacked this characteristic. Given the distinctness of the enlarged postcloacal scales in our Species B, it seems unlikely that Hallowell simply did not mention this character in spite of being present. Thus, with reasonable confidence, ANSP 7618 can be referred to our Species A. GK has examined the type material of *A. stigmosus* Bocourt (MNHN 2427 and 2427A; Figs. 6b, c) and identified both specimens as belonging to our Species A. The examination of the paratype series of *A. gaigei* Ruthven (UMMZ 48324–30, 48332–33) demonstrated these to belong to our Species B (see also Figs. 6d, f). Furthermore, we have examined two adult males with everted hemipenes (*i.e.*, UMMZ 48322, 54815) from the Santa Marta Mountains, Colombia, and these had a small, thin unilobed organ (our type B hemipenis; Fig. 6e). Finally, GK examined the male paratype of *A. albi* (MCZ 32302; Fig. 6g, h), which can readily be identified as our Species A since it lacks enlarged postcloacal scales and also agrees well with the other diagnostic characters presented above for our Species A.

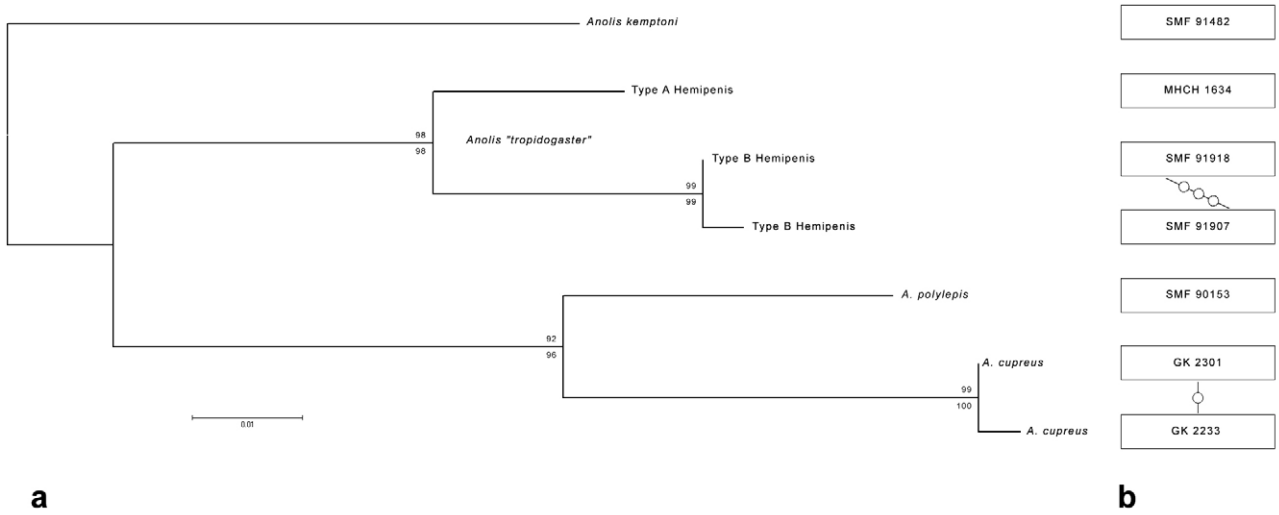

FIGURE 5. Results of 16S mtDNA analysis. a) Consensus tree from Maximum Likelihood analysis. Scale bar refers to substitutions per site. Bootstrap support values above nodes correspond to Maximum Likelihood analysis, those below the nodes to the Maximum Parsimony consensus tree of exactly the same topology. (b) Parsimony network derived from the same alignment, each node representing a unique haplotype.

In conclusion, we refer the following nominal taxa to our Species A: *Anolis tropidogaster* Hallowell, *A. stigmosus* Bocourt, and *A. albi* Barbour. Thus, our Species A has to be referred to as *A. tropidogaster* with *A. stigmosus* Bocourt, and *A. albi* Barbour remaining in its synonymy. The only available name for our Species B is *A. gaigei* Ruthven. In the following we provide standardized descriptions of these two species.

Anolis tropidogaster Hallowell, 1856

Figures 1a; 3a, b; 4b, d, f, h; 6a, b, c, g, h; 7a, b; 8

Anolis tropidogaster Hallowell 1856:224; holotype (ANSP 7618) from "New Grenada". Dunn (1930), Barbour (1934; in part.), Barbour and Loveridge (1946), Breder (1946), Evans (1947), Etheridge (1959), Donoso-Barros (1968), Peters and Donoso-Barros (1970; in part.), Williams (1976), Ayala (1986), Pefaur (1992), Auth (1994; in part.), Williams *et al.* (1995; in part.), Young *et al.* (1999; in part.), Ibáñez *et al.* (2001; in part.), Moreno-Bejarano & Álvarez-León (2003), Poe (2004; in part.), Carvajal-Cogollo and Urbina-Cardona (2008), Moreno-Arias *et al.* (2008), Medina-Rangel (2011).

Anolis albi Barbour 1932:101; holotype (MCZ 32301) from "Andagoya, Choco, western Colombia."

Anolis stigmosus Bocourt 1869:43; syntypes (MNHN 2427 and 2427A) from "la Colombie et ont été recueillis près de la rivière de la Magdeleine." Boulenger (1885).

Norops tropidogaster. Köhler (2003, 2008; in part.)

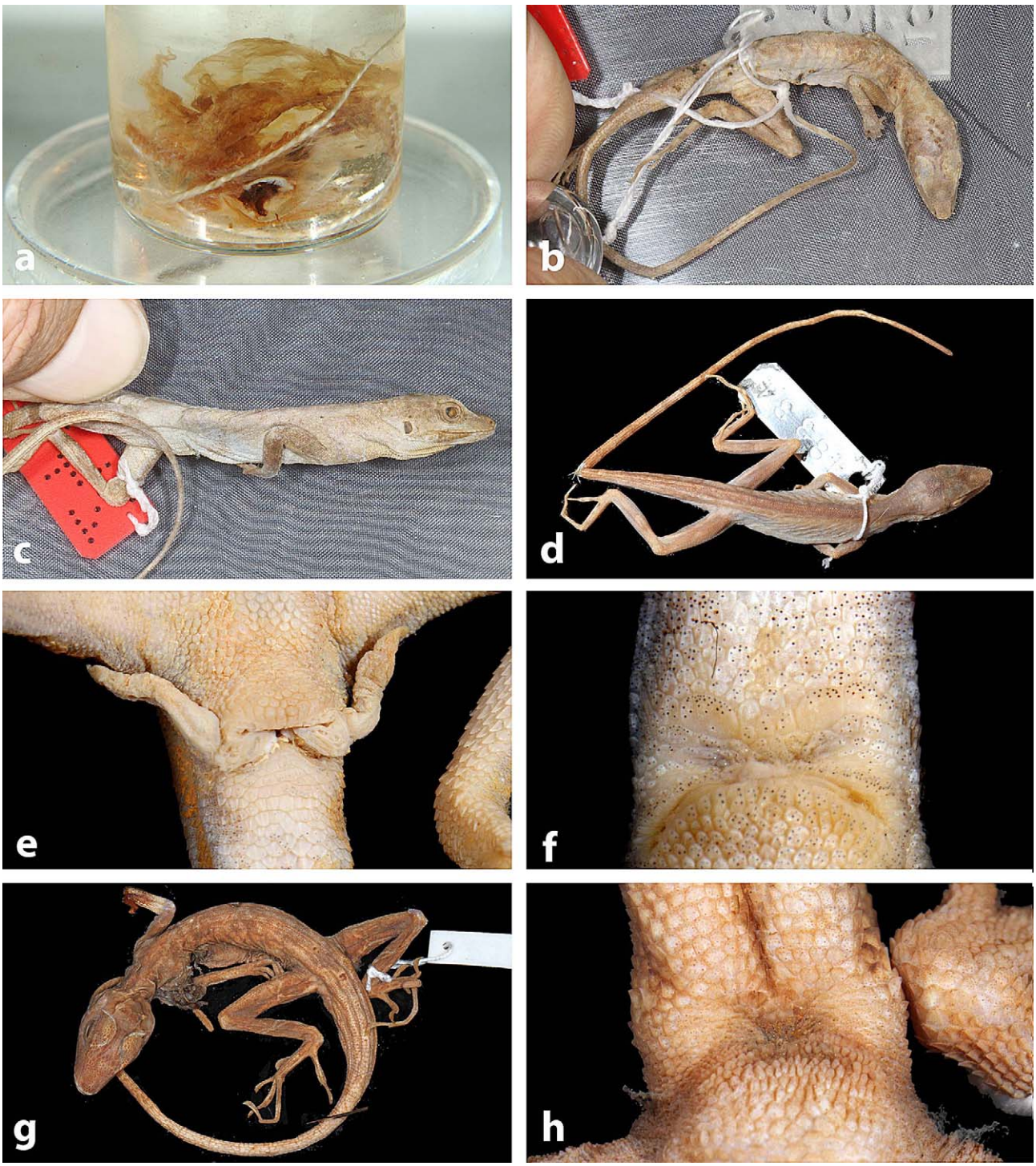

FIGURE 6. (a) Holotype of *Anolis tropidogaster* (ANSP 7618). (b) *Anolis stigmosus* syntype MNHN 2427. (c) *Anolis stigmosus* syntype MNHN 2427a. (d) *Anolis gaigei* paratype (UMMZ 48334) dorsal view. (e) *Anolis gaigei* (UMMZ 48322) hemipenis. (f) *Anolis gaigei* paratype (UMMZ 48334) cloacal region. (g) *Anolis albi* paratype (MCZ 32302) dorsal view. (h) MCZ 32302 cloacal region.

Diagnosis. A medium-sized species (SVL in largest specimen examined 55 mm) of the genus *Anolis* (sensu Poe 2004) that differs from all other Lower Central American beta anoles (sensu Etheridge 1967) in that it is long-legged (longest toe of adpressed hind leg reaches to at least center of eye, usually to a point between anterior border of eye and nostril); has strongly keeled ventral scales, a large almost uniformly purplish red (in life) colored dewlap in males; postcloacal scales not enlarged in the majority of males, some male with slightly enlarged postcloacal scales; a large bilobed hemipenis in males, and no tube-like axillary pocket. Anole species from Lower Central America that are somewhat similar in appearance to *A. tropidogaster* are *A. cupreus*, *A. gaigei*, *A. osa*, and *A. polylepis*.

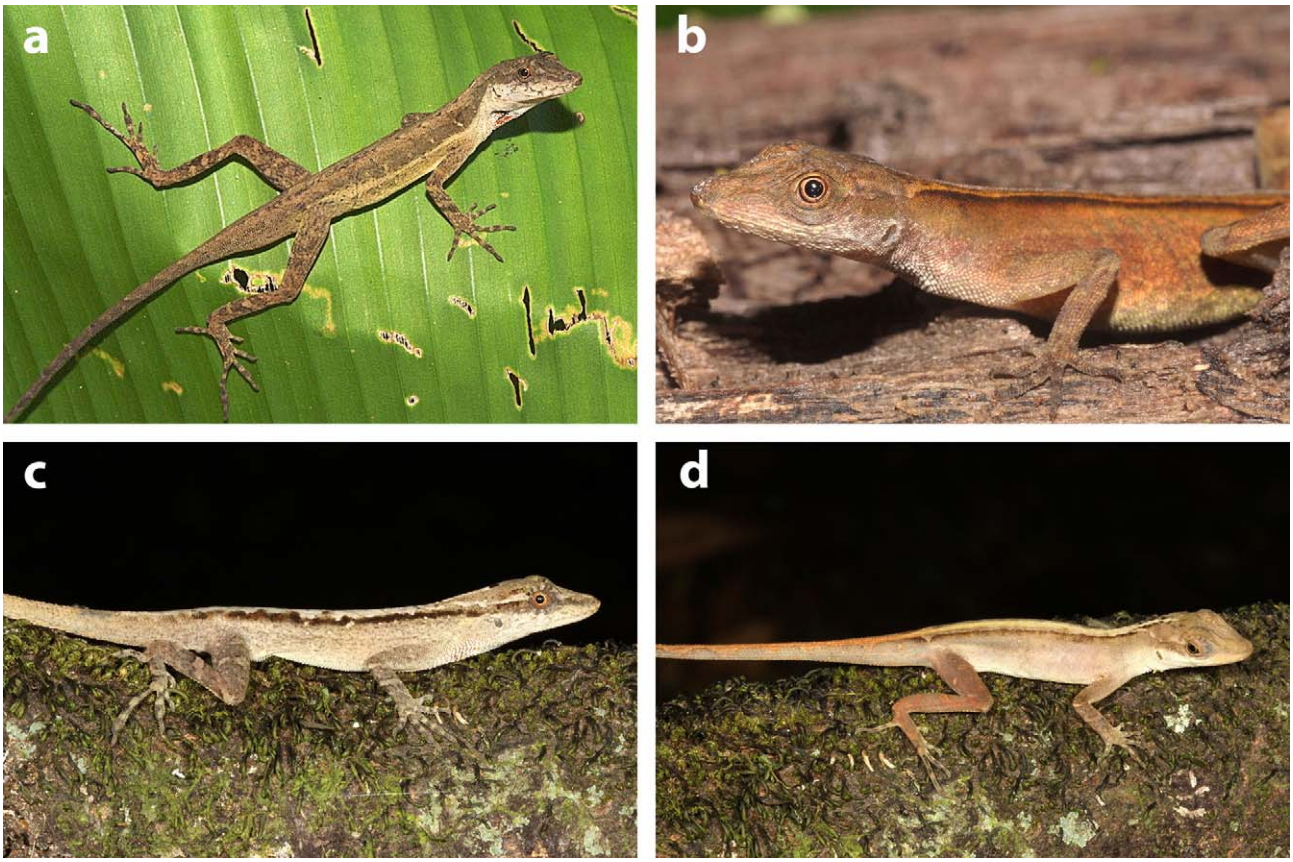

FIGURE 7. Adult individuals in life of (a) *Anolis tropidogaster* (SMF 93598), male from Laguna de Matusagaratí, Darién, Panama. (b) *Anolis tropidogaster* (SMF 93597), male from Cruce de Mono Station at Parque Nacional Darién, Darién, Panama. (c) *Anolis gaigei* (SMF 91918), male from Finca La Providencia, near Ponuga, Veraguas, Panama. (d) *Anolis gaigei* (SMF 91917), female from road from Interamericana to Horconcitos, Chiriquí, Panama.

Anolis gaigei has a small, thin, unilobed hemipenis in males (large, bulbous and bilobed in *A. tropidogaster*); a male dewlap that is orange yellow with a darker orange central area (uniform purplish red, sometimes with a paler orange central area in *A. tropidogaster*); a pair of greatly enlarged postcloacal scales in males (these scales usually not differentiated in *A. tropidogaster*); the dorsal head scales, especially in the parietal region large and flat (smaller and bearing minute tubercles in *A. tropidogaster*); middorsal caudal scales distinctly enlarged and forming a regular series (only slightly enlarged and somewhat irregularly arranged in *A. tropidogaster*); outer postmental scales greatly enlarged relative to medial ones (only slightly enlarged relative to medial ones in *A. tropidogaster*). *Anolis polylepis* and *A. osa* have smooth ventral scales at midbody and a larger, mostly uniform orange male dewlap (in some parts of its range in Costa Rica, considerable variation was observed in male dewlap coloration of *A. polylepis*, see Köhler *et al.* 2010). The male dewlap of *A. cupreus* is brown to pink with an orange margin. For variation in selected morphometric and scalation characters of *A. tropidogaster* see Table 1.

Description. *Anolis tropidogaster* is a medium-sized anole (maximum recorded SVL 54.0 mm in males, 55.0 mm in females); dorsal head scales (Fig. 8) in internasal region keeled, in prefrontal, parietal, and frontal areas rugose to tuberculate; deep frontal depression present, parietal depression absent; 5–8 (6.75 ± 0.69) postrostrals; anterior nasal usually single, occasionally divided, usually in contact with rostral and first supralabial (Fig. 9, Tab. 2); 6–11 (8.97 ± 0.94) internasals; canthal ridge sharply defined; scales comprising supraorbital semicircles weakly keeled, largest scale in semicircles about same size as largest supraocular scale; supraorbital semicircles well defined; 2–4 (3.06 ± 0.58) scales separating supraorbital semicircles at narrowest point; 2–5 (2.95 ± 0.73) scales separating supraorbital semicircles and interparietal at narrowest point; interparietal well defined, greatly enlarged relative to adjacent scales, surrounded by scales of moderate size, longer than wide, usually larger than ear opening; enlarged supraoculars not in contact with supraorbital semicircles; 2 elongate superciliaries, posterior one

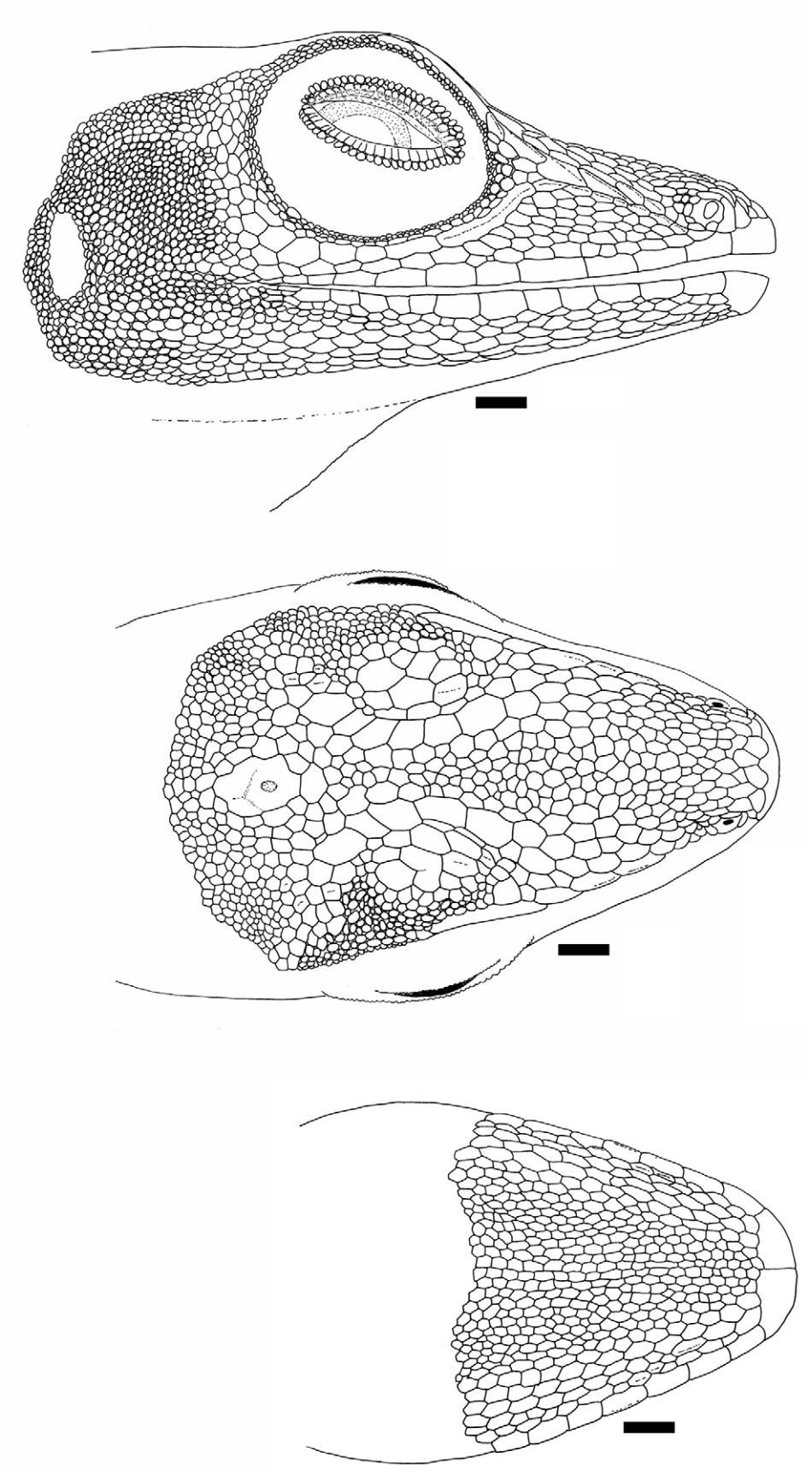

FIGURE 8. Head scalation in *Anolis tropidogaster* (MHCH 1640). Scale bars = 1.0 mm.

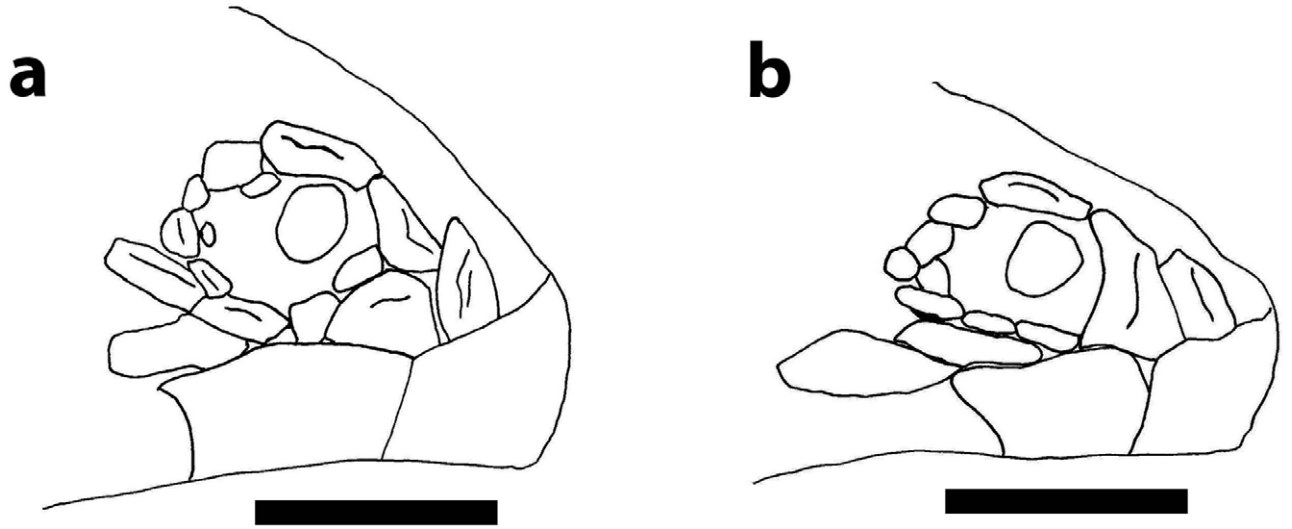

FIGURE 9. Designation of nasal scalation types. (a) Nasal type 1 (MHCH 1636); (b) Nasal type 2 (SMF 91901). Scale bars = 1.0 mm. See Text for details.

FIGURE 10. Habitat of *Anolis tropidogaster* at Cruce de Mono Station, Darién, Panama.

much shorter than anterior one; 2–3 enlarged canthals; 10–17 (13.44 ± 1.71) scales between second canthals; 12–20 (15.11 ± 1.86) scales present between posterior canthals; loreal region slightly concave, 33–64 (44.77 ± 8.80) mostly keeled (some smooth or rugose) loreal scales in a maximum of 5–8 (6.22 ± 0.83) horizontal rows; 6–9 (7.00 ± 0.93) supralabials to level below center of eye; suboculars keeled, separated from supralabials by one scale row; ear opening vertically oval; scales anterior to ear opening granular, similar in size to those posterior to ear opening; 6–10 (7.75 ± 0.97) postmentals, outer pair usually largest; keeled granular scales present on chin and throat; male

dewlap large, extending onto chest; 6–8 horizontal gorgetal-sternal rows with 26–38 scales per row, rows somewhat irregular, some of them with pairs or triplets of scales, apical portion of dewlap between marginal pairs and last gorgetal-sternal row free of scales; modal number of marginal pairs 4–5; female dewlap small or absent; no nuchal crest or dorsal ridge; 2 middorsal scale rows slightly enlarged, weakly keeled, dorsal scales lateral to middorsal series gradually larger than granular lateral scales; no enlarged scales scattered among granular laterals; 42–73 (57.0 ± 7.53) dorsal scales along vertebral midline between levels of axilla and groin in males, 53–78 (61.8 ± 10.35) in females; 29–46 (37.6 ± 4.39) dorsal scales along vertebral midline contained in one head length in males, 30–42 (35.3 ± 4.12) in females; ventral scales on midsection about the same size as largest dorsal scales; ventral body scales moderately to strongly keeled, subimbricate to imbricate; 40–65 (51.9 ± 5.50) ventral scales along midventral line between levels of axilla and groin in males, 40–64 (49.0 ± 8.60) in females; 28–46 (36.0 ± 4.52) ventral scales contained in one head length in males, 27–36 (31.6 ± 2.92) in females; 112–144 (124.7 ± 7.90) scales around midbody in males, 122–162 (133.3 ± 12.6) in females; tubelike axillary pocket absent; preanal scales not keeled; postcloacal scales usually not enlarged, or, if differentiated, then only slightly enlarged; tail laterally compressed in cross section, tail height/tail width 1.07–1.53 (1.29 ± 0.11); basal subcaudal scales smooth; lateral caudal scales keeled, homogeneous; dorsal medial caudal scale row slightly enlarged, keeled, not forming a crest; most scales on lateral surface of antebrachium weakly keeled, unicarinate; 23–29 (25.78 ± 1.46) subdigital lamellae on Phalanges II–IV of Toe IV of hind limbs; SVL 42.0–54.0 (48.9 ± 2.96) mm in males, 40.0–55.0 (47.0 ± 5.20) mm in females; HL 11.3–14.6 (12.9 ± 0.72) mm in males, 11.1–14.1 (12.5 ± 1.00) mm in females; tail length 65.0–97.0 (83.9 ± 10.70) mm in males, 69.0–87.0 (80.0 ± 6.54) mm in females; shank length 12.2–15.9 (14.0 ± 1.06) mm in males, 11.2–16.0 (13.1 ± 1.65) mm in females; tail length/SVL 1.35–1.94 (1.75 ± 0.20) in males, 1.28–1.88 (1.68 ± 0.24) in females; HL/SVL 11.3–14.6 (12.9 ± 0.72) in males, 11.1–14.1 (12.5 ± 1.00) in females; shank length/SVL 0.25–0.33 (0.29 ± 0.02) in males, 0.25–0.30 (0.28 ± 0.01) in females; shank length/HL 0.94–1.22 (1.09 ± 0.07) in males, 0.90–1.16 (1.05 ± 0.08) in females. Of 45 specimens examined, the longest toe of the adpressed hind leg reaches to mid-eye in 3 individuals (6.7%), to anterior margin of eye in 29 individuals (64.4%), and to a point between eye and snout in 13 individuals (28.9%).

Coloration in life of an adult male (MHCH 2375) was recorded as follows: Dorsal ground color Drab (27) with Olive Brown (28) vertebral band, postorbital band Dark Drab (119B) extending to level of midbody; a longitudinal level Buff (124) stripe from tympanum to insertion of hind legs; dorsal surface of head Dark Drab (119B), with a medially interrupted Dark Brownish Olive (129) interorbital bar; forelegs and hind legs Drab (27) suffused with Olive Brown (28); dorsal surface of tail Drab (27) with indistinct Olive Brown (28) bands; ventral surfaces of head, body, and limbs Straw Yellow (57); dewlap Chrome Orange (16), grading into Spectrum Orange (17) at center; gorgetals dirty white; iris Sepia (119).

Natural history notes. At the evergreen forest sites (Fig. 10) in the Darién Province, Panama, visited by AB, *Anolis tropidogaster* was an uncommon species. AB and MP encountered it within the forest on low vegetation between 0.5 and 2.0 m above the ground. Occasionally, individuals were observed on the ground. At night, these animals sleep in the usual anole-like fashion on twigs or the upper surface of leaves. One adult male was collected at a forest edge at Matusagaratí Lake, a thin forest belt between the lake and pasture areas. Another individual was captured at the base of a mangrove tree (MHCH 1636) at a riverside at Caserete, Chepigana, Darién, Panama; in the same region three individuals were seen in a cativo (*Prioria copaifera*) forest on low vegetation. Another individual (MHCH 1701) was captured during night sampling in a semideciduous forest on a bush at 1.5 m above ground at Sol Poniente, Chepigana, Darién, Panama. Some ecological observations on *A. tropidogaster* were published by Sexton *et al.* (1964).

Geographic Distribution. *Anolis tropidogaster* is distributed widely in Colombia and in eastern Panama (Figs. 2, 13). The claim that this species occurs in Ecuador seems to go back to Donoso-Barros (1968), who based this view on the holotype of *A. lemniscatus* (from "Puente del Chimbo", see Boulenger 1898), a taxon then placed in the synonymy of *A. tropidogaster.* We were unable to find additional evidence for the occurrence of *A. tropidogaster* in Ecuador and therefore remove this species from the list of known reptiles from this country. The documented vertical range of the species is from near sea level to about 1100 m.

TABLE 1. Selected measurements, proportions and scale characters of *Anolis gaigei* and *A. tropidogaster.* Range is followed by mean value and standard deviation in parentheses. For abbreviations see text.

		A. gaigei ♂ 49, ♀ 30	*A. tropidogaster* ♂ 27, ♀ 9
SVL	♂	36.0–52.5 (45.6 ± 3.40)	42.0–54.0 (48.9 ± 2.96)
	♀	37.0–52.0 (44.7 ± 4.75)	40.0–55.0 (47.0 ± 5.20)
Tail length	♂	59.0–115.0 (95.0 ± 11.63)	65.0–97.0 (83.9 ± 10.70)
	♀	82.0–100.7 (92.3 ± 8.33)	69.0–87.0 (80.0 ± 6.54)
HL	♂	10.5–13.2 (12.1 ± 0.65)	11.3–14.6 (12.9 ± 0.72)
	♀	10.6–12.1 (11.5 ± 0.48)	11.1–14.1 (12.5 ± 1.00)
HW	♂	6.5–7.9 (7.3 ± 0.37)	6.6–9.0 (7.8 ± 0.46)
	♀	6.5–7.7 (7.1 ± 0.37)	6.4–8.7 (7.5 ± 0.66)
Shank length	♂	11.2–15.6 (13.6 ± 0.85)	12.2–15.9 (14.0 ± 1.06)
	♀	10.5–14.0 (12.4 ± 0.82)	11.2–16.0 (13.1 ± 1.65)
Axilla–groin distance	♂	11.2–19.5 (17.0 ± 1.90)	16.9–24.5 (19.9 ± 1.57)
	♀	13.6–24.0 (17.9 ± 2.64)	18.3–24.9 (20.1 ± 2.12)
Tail length / SVL	♂	1.37–2.34 (2.09 ± 0.21)	1.35–1.94 (1.75 ± 0.20)
	♀	1.75–2.27 (2.10 ± 0.13)	1.28–1.88 (1.68 ± 0.24)
Tail diameter vertical / horizontal	♂	1.05–1.47 (1.23 ± 0.10)	1.07–1.53 (1.29 ± 0.12)
	♀	1.06–1.31 (1.17 ± 0.07)	1.13–1.36 (1.26 ± 0.08)
HL / SVL	♂	0.26–0.30 (0.27 ± 0.01)	0.24–0.29 (0.26 ± 0.01)
	♀	0.24–0.29 (0.26 ± 0.02)	0.24– 0.32 (0.27 ± 0.02)
HL / HW	♂	1.56–1.75 (1.66 ± 0.06)	1.49–1.89 (1.73 ± 0.09)
	♀	1.51–1.75 (1.63 ± 0.06)	1.60–1.75 (1.67 ± 0.05)
Shank length / SVL	♂	0.28–0.32 (0.30 ± 0.01)	0.25–0.33 (0.29 ± 0.02)
	♀	0.23–0.33 (0.29 ± 0.02)	0.25–0.30 (0.28 ± 0.01)
Axilla–groin distance / SVL	♂	0.24–0.43 (0.38 ± 0.04)	0.34–0.46 (0.41 ± 0.03)
	♀	0.36–0.48 (0.40 ± 0.03)	0.41–0.46 (0.44 ± 0.02)
Subdigital lamellae of 4th toe		20–28 (25.45 ± 1.65)	23–29 (25.78 ± 1.46)
Number of scales between SS		1–4 (2.10 ± 0.62)	2–4 (3.06 ± 0.58)
Number of scales between IP and SS		2–5 (3.03 ± 0.65)	2–5 (2.95 ± 0.73)
Number of scales between SO and SPL		0–1 (0.69 ± 0.47)	1–1 (1.00 ± 0.00)
Number of SPL to level below center of eye		6–9 (7.31 ± 0.65)	6–9 (7.00 ± 0.93)
Number of INL to level below center of eye		6–9 (7.33 ± 0.61)	5–9 (7.11 ± 0.85)
Total number of loreals		22–59 (40.25 ± 5.79)	33–64 (44.77 ± 8.80)
Number of horizontal loreal scale rows		5–8 (6.31 ± 0.60)	5–8 (6.22 ± 0.83)
Number of postrostrals		5–7 (6.06 ± 0.54)	5–8 (6.75 ± 0.69)
Number of postmentals		5–9 (6.35 ± 0.80)	6–10 (7.75 ± 0.97)
Number of scales between nasals		5–9 (7.03 ± 1.01)	6–11 (8.97 ± 0.94)
Number of scales between 2nd canthals		8–14 (10.93 ± 1.14)	10–17 (13.44 ± 1.71)
Number of scales between posterior canthals		10–16 (13.10 ± 1.32)	12–20 (15.11 ± 1.86)
Number of medial dorsal scales in one head length		28–56 (37.95 ± 6.09)	29–46 (37.03 ± 4.38)
Number of medial ventral scales in one head length		25–48 (33.21 ± 4.87)	27–46 (34.89 ± 4.58)
Number of scales around midbody		94–138 (117.60 ± 8.74)	112–162 (126.63 ± 9.67)
Numbers of medial dorsal scales between axilla and groin		53–83 (62.72 ± 5.92)	42–78 (58.06 ± 8.34)
Numbers of medial ventral scales between axilla and groin		40–58 (48.88 ± 4.01)	40–65 (51.26 ± 6.32)

TABLE 2. Frequency distribution of nasal region and dorsal pattern types in *Anolis gaigei* and *A. tropidogaster.*

	Anolis gaigei	*A. tropidogaster*
	35	9
Nasal region Type A	3 (8.6%)	7 (20.6%)
Nasal region Type B	32 (91.4%)	27 (79.4%)

Anolis gaigei **Ruthven, 1916**

Figures 1b; 3c, d; 4 a, c, e, g; 6 d, e, f; 7 c, d; 11

Anolis gaigei Ruthven 1916:6; holotype (UMMZ 48304) from "San Lorenzo, Santa Marta Mountains, Colombia, elevation of 2, 700 ft." Ruthven (1922).

Anolis limifrons: Martínez Cortés & Rodriguez (2005; in part.: Fig. 22B)

Anolis polylepis: Martínez Cortés and Rodriguez (2003, 2005), Rodríguez *et al.* (2004), Ibáñez (2006; in part.).

Anolis tropidogaster: Swanson (1945), Evans (1947), Donoso-Barros (1968), Myers and Rand (1969), Peters and Donoso-Barros (1970; in part.), Campbell (1971), Sexton *et al.* (1964, 1971), Kiester (1979), Kourany & Telford (1981), Rand and Myers (1990), Quintero and Cambra (1993), Auth (1994; in part.), Williams *et al.* (1995; in part.), Telford (1996), Ibáñez *et al.* (1996), Ibáñez *et al.* (1997 "1995"), Young *et al.* (1999; in part.), Ibáñez *et al.* (2001; in part.), Poe (2004, in part.), Pinto *et al.* (2008), Jaramillo *et al.* (2010; in part.).

Norops tropidogaster: Villa *et al.* (1988), Köhler (2000; in part.), Nicholson (2002), Köhler (2003; in part.), Nicholson *et. al.* (2005), Köhler (2008; in part.), Steffen (2009).

Diagnosis. A medium-sized species (SVL in largest specimen examined 52.5 mm) of the genus *Anolis* (sensu Poe 2004) that differs from all other Lower Central American beta anoles (sensu Etheridge 1967) in that it is long-legged (longest toe of adpressed hind leg reaches to at least center of eye, usually to a point between anterior border of eye and nostril), has strongly keeled mucronate imbricate ventral scales, a large almost orange red (in life) colored dewlap with a yellowish margin in males, a pair of greatly enlarged postcloacal scales in males, a small unilobed hemipenis in males, and no tube-like axillary pocket. Anole species from Lower Central America that are somewhat similar in appearance to *A. gaigei* are *A. tropidogaster, A. polylepis*, and *A. cupreus*. The males of *A. tropidogaster* have a mostly uniform purplish red dewlap, a bilobed hemipenis, and lack a pair of greatly enlarged postcloacal scales. For a more detailed comparison of *A. gaigei* and *A. tropidogaster* see the Diagnosis section for the latter species. *Anolis polylepis* and *A. osa* have smooth ventral scales at midbody and a larger, mostly uniform orange male dewlap (in some parts of its range in Costa Rica, considerable variation was observed in male dewlap coloration of *A. polylepis*, see Köhler *et al.* 2010). Also, male *A. polylepis* have a bilobate hemipenis. The males of *Anolis cupreus* lack a pair of greatly enlarged postcloacal scales, have a brown to pink dewlap with an orange margin, and a bilobate hemipenis.

Description. *Anolis gaigei* is a medium-sized anole (maximum recorded SVL 52.5 mm in males, 52.0 mm in females); dorsal head scales (Fig. 11) in internasal region keeled, in prefrontal, parietal, and frontal areas rugose to tuberculate; scales in distinct prefrontal depression slightly wrinkled, parietal depression absent; 5–7 (6.06 ± 0.54) postrostrals; anterior nasal usually single, occasionally divided, usually in contact with rostral and first supralabial (Fig. 9, Tab. 2); 5–9 (7.03 ± 1.01) internasals; canthal ridge sharply defined; scales comprising supraorbital semicircles weakly keeled, largest scale in semicircles about same size as largest supraocular scale; supraorbital semicircles well defined; 1–4 (2.10 ± 0.62) scales separating supraorbital semicircles at narrowest point; 2–5 (3.03 ± 0.65) scales separating supraorbital semicircles and interparietal at narrowest point; interparietal well defined, greatly enlarged relative to adjacent scales, surrounded by scales of moderate size, longer than wide, usually larger than ear opening; supraorbital disc composed of 6–12 distinctly enlarged keeled scales; enlarged supraoculars not in contact with supraorbital semicircles; usually a single elongated superciliary, or, if 2 elongate superciliaries, posterior one much shorter than anterior one; 2–3 enlarged canthals; 8–14 (10.93 ± 1.14) scales between second canthals; 10–16 (13.10 ± 1.32) scales present between posterior canthals; loreal region slightly concave, 22–59 (40.25 ± 5.79) mostly keeled (some smooth or rugose) loreal scales in a maximum of 5–8 (6.31 ± 0.60) horizontal rows; 6–9 (7.31 ± 0.65) supralabials to level below center of eye; suboculars keeled, suboculars separated from supralabials by 0–1 (0.69 ± 0.47) scale row; ear opening vertically oval; scales anterior to ear opening granular, similar in size to those posterior to ear opening; 5–9 (6.35 ± 0.80) postmentals, outer pair largest; keeled granular scales pres-

ent on chin and throat; male dewlap extending well onto chest, anterior insertion at level of center of eye, posterior insertion about 3.0 mm beyond level of axilla; 8–9 horizontal gorgetal-sternal rows with 11–15 scales per row, rows somewhat irregular; female dewlap small or absent; no nuchal crest or dorsal ridge; 2 middorsal scale rows slightly enlarged, weakly keeled, dorsal scales lateral to middorsal series gradually larger than granular lateral scales; no enlarged scales scattered among granular laterals; 53–75 (62.7 ± 5.57) dorsal scales along vertebral midline between levels of axilla and groin in males, 55–83 (62.7 ± 6.45) in females; 31–56 (38.5 ± 5.35) dorsal scales along vertebral midline contained in one head length in males, 28–52 (37.1 ± 7.10) in females; ventral scales on midsection about the same size as largest dorsal scales; ventral body scales strongly keeled, imbricate; 43–58 (50.0 ± 3.94) ventral scales along midventral line between levels of axilla and groin in males, 40–54 (47.6 ± 3.79) in females; 29–48 (35.0 ± 4.31) ventral scales contained in one head length in males, 25–38 (30.5 ± 4.44) in females; 94–138 (119.0 ± 9.68) scales around midbody in males, 101–128 (116.1 ± 7.5) in females; tubelike axillary pocket absent; preanal scales not keeled; males with a pair of greatly enlarged postcloacal scales; tail laterally compressed in cross section, tail height/tail width 1.05–1.47 (1.20 ± 0.09); basal subcaudal scales smooth; lateral caudal scales keeled, homogeneous; dorsal medial caudal scale row slightly enlarged, keeled, not forming a crest; most scales on lateral surface of antebrachium weakly keeled, unicarinate; 20–28 (25.45 ± 1.65) subdigital lamellae on Phalanges II–IV of Toe IV of hind limbs; SVL 36.0–52.5 (45.6 ± 3.40) mm in males, 37.0–52.0 (44.7 ± 4.75) mm in females; HL 10.5–13.2 (12.1 ± 0.65) mm in males, 10.6–12.1 (11.5 ± 0.48) mm in females; tail length 59.0–115.0 (95.0 ± 11.63) mm in males, 82.0–100.7 (92.3 ± 8.33) mm in females; shank length 11.2–15.6 (13.6 ± 0.85) mm in males, 10.5–14.0 (12.4 ± 0.82) mm in females; tail length/SVL 1.37–2.34 (2.09 ± 0.21) in males, 1.75–2.27 (2.10 ± 0.13) in females; HL/SVL 0.26–0.30 (0.27 ± 0.01) in males, 0.24–0.29 (0.26 ± 0.02) in females; shank length/SVL 0.28–0.32 (0.30 ± 0.01) in males, 0.23–0.33 (0.29 ± 0.02) in females; shank length/HL 1.04–1.24 (1.12 ± 1.04) in males, 0.94–1.18 (1.08 ± 0.05) in females. Of 20 specimens examined, the longest toe of the adpressed hind leg reached to mid-eye in 2 individuals (10%), to anterior margin of eye in 4 individuals (20%), and to a point between eye and nostril in 14 individuals (70%).

Coloration in life of an adult male (SMF 91918) was recorded as follows: Dorsal ground color Sayal Brown (223C) with a Beige (219D) vertebral band, edged by Raw Umber (223) pigment; Raw Umber (223) line continuing anteriorly through eye to tip of snout; dorsum of head Clay Color (26) with a medially interrupted Dark Brownish Olive (129) interorbital bar and a Sepia (219) nuchal spot, followed posteriorly by a Beige (219D) longitudinal line; forelegs Sayal Brown (223C); hind legs Cinnamon-Brown (33) with Raw Umber (23) crossbars; dorsal surface of tail Raw Umber (23) grading into Buff (24) distally and with indistinct Cinnamon-Rufous (40) bands; ventral surfaces of head, body, and limbs Pale Horn Color (92); ventral surface of tail suffused with Orange-Rufous (132C); dewlap Chrome Orange (16), grading into Orange Yellow (18) on anterior and distal margins; gorgetals dirty white; iris Kingfisher Rufous (240). Coloration in life of another adult male (SMF 91529) was recorded as follows: Dorsal and lateral surfaces of body and forelimbs Tawny Olive (223D); two broad Natal Brown (219A) longitudinal stripes extending from eye paravertebrally to base of tail, suffused with Walnut Brown (221B); a series of Sepia (119) blotches between occipital region and base of tail; dorsal surface of head Raw Umber (123), laterally grading into Tawny Olive (223D); ventral ground color Pale Horn Color (92), suffused with Orange-Rufous (132C) beneath tail; dorsal and lateral surfaces of tail and hind limbs Sayal Brown (223C) with the suggestion of diffuse Orange-Rufous (132C) crossbars; iris Robin Rufous (340); dewlap Burnt Orange (116), especially anterior portions suffused with Grayish Olive (43); anterior base of dewlap Warm Buff (118), posterior base Pale Horn Color (92); dewlap scales dirty white. Coloration in life of an adult female (SMF 91917) was recorded as follows: Dorsal ground color Tawny Olive (223D) with a Clay Color (123B) vertebral stripe, edged by Verona Brown (223B) pigment; dorsum of head Cinnamon Brown (33); forelegs Tawny Olive (223D); hind legs True Cinnamon (139) with Orange-Rufous (132C) spots and crossbars; dorsal surface of tail Tawny Olive (223D) with faint Orange Rufous (132C) crossbars; chin dirty white; venter Pale Pinkish Buff (121D); ventral surface of tail suffused with Orange-Rufous (132C); gular region Spectrum Orange (17) grading into Orange Yellow (18) on anterior margin; gorgetals Pale Pinkish Buff (121D); iris Robin Rufous (340). The coloration of an adult male from the Canal Zone (SMF 85304) was recorded as follows: Middorsum Army Brown (219B) bordered by a Burnt Umber (22) dorsolateral stripe; flanks Dark Drab (119B) with Drab-Gray (119D) punctuations; venter Drab-Gray (119D) suffused with Dark Drab (119B); dewlap Chrome Orange (16) with Orange Yellow (18) anterior border.

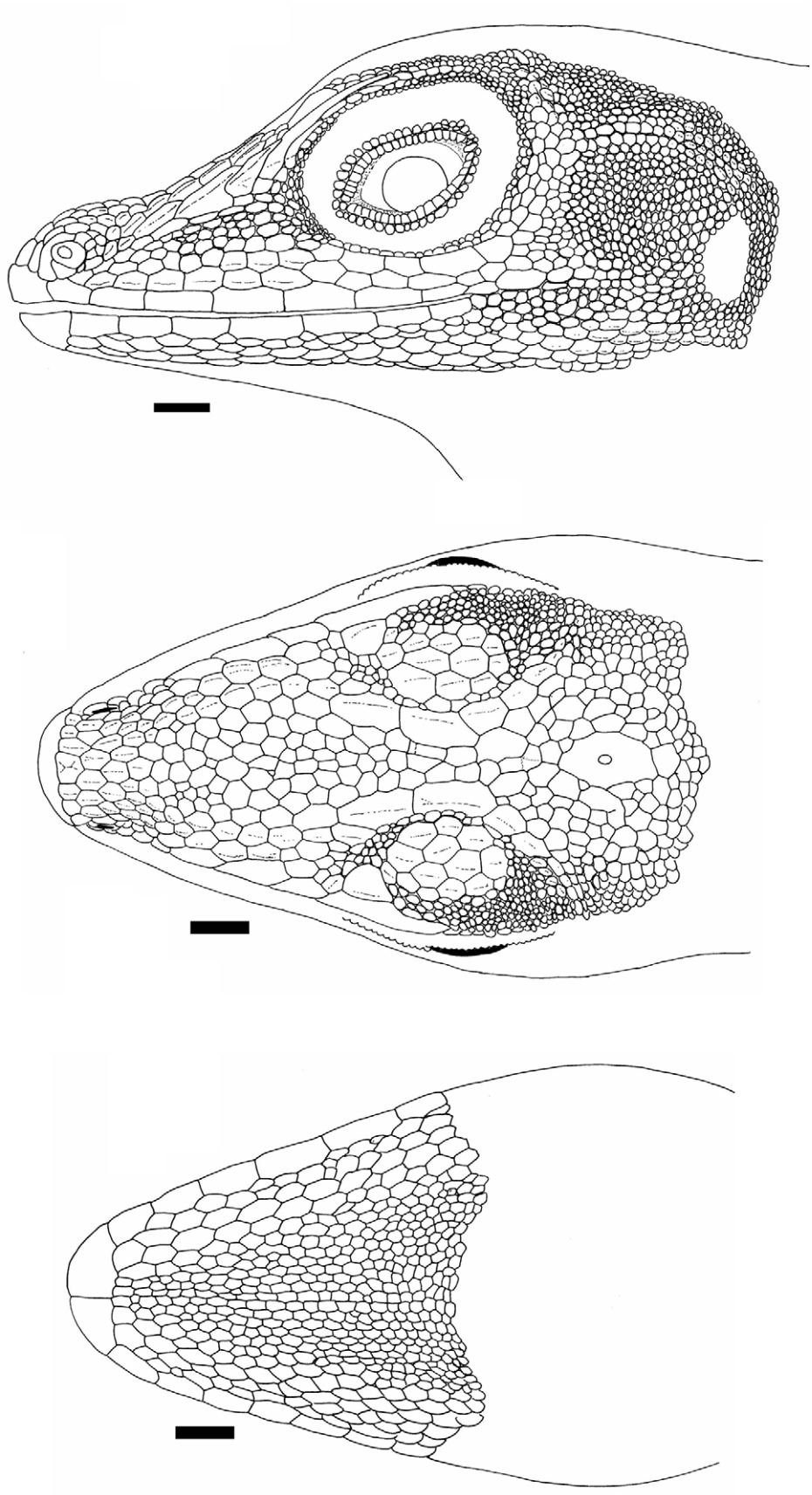

FIGURE 11. Head scalation in *Anolis gaigei* (SMF 91921). Scale bars = 1.0 mm.

FIGURE 12. Habitat of *Anolis gaigei* (a) near Santo Domingo, Los Santos, Panama, 40 masl; (b) at Finca La Providencia, near Ponuga, Veraguas, Panama, 20 masl.

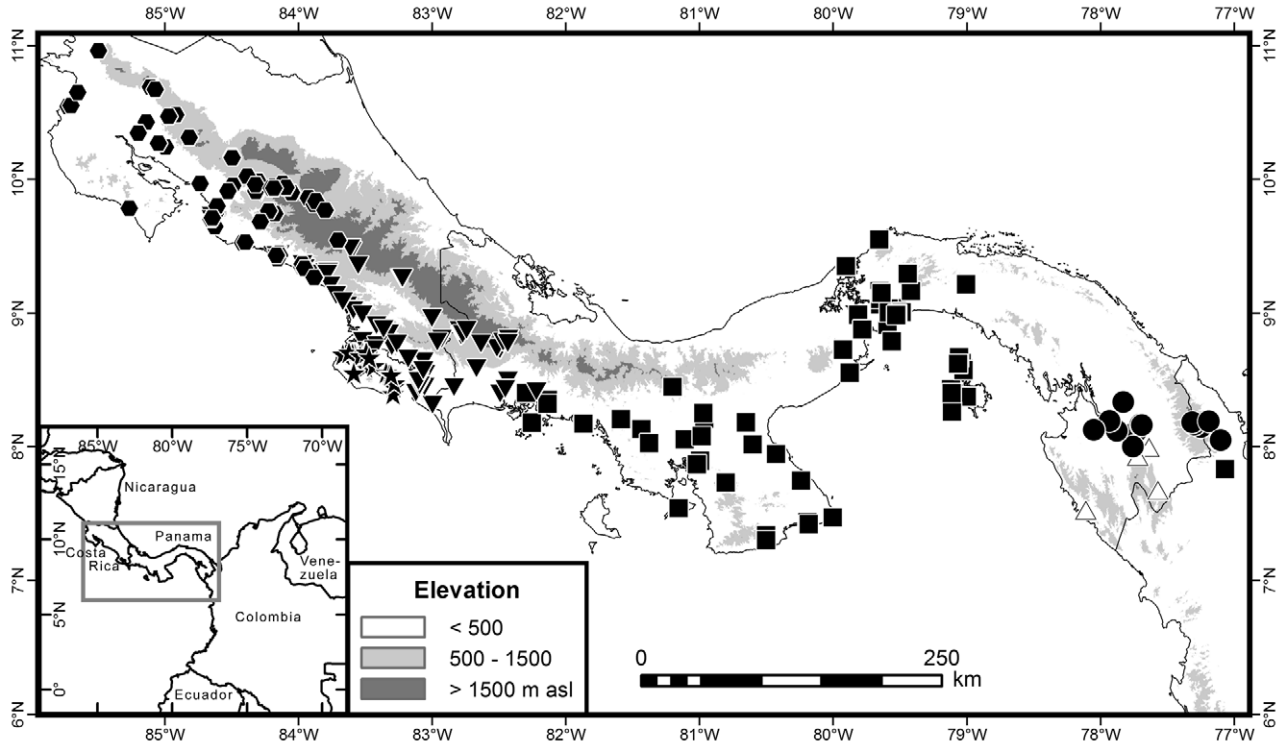

FIGURE 13. Map indicating collecting sites of selected lowland anoles inhabiting the Pacific versant of Panama and Costa Rica. Each symbol can represent one or more nearby localities. Circles: *Anolis tropidogaster;* squares: *A. gaigei;* white triangles: localities of *A. tropidogaster*-like specimens not verified by authors; black inverted triangles: *A. polylepis;* stars: *A. osa;* hexagons: *A. cupreus.* See text for details.

Natural history notes. Wherever we found it in central and western Panama, *Anolis gaigei* is an extremely common anole that reaches high population densities, being the most obvious lizard at many sites where it occurs. It is usually encountered in low vegetation between 0.5 and 2.0 m above the ground. Occasionally, individuals can be observed on the ground. At night, these animals sleep in the usual anole-like fashion on twigs or the upper surface of leaves. Although *A. gaigei* inhabits a wide range of habitats, it seems to be a typical species of the semideciduous forest and of bushy savannahs (Fig. 12). In cattle pasture they depend on the trees along the fences ("living fences," where living trees make up the actual fence posts). Aspects of the ecology of this species were studied by Sexton *et al.* (1971), Campbell (1971), and Quintero and Cambra (1993).

Geographic distribution. As currently known, *Anolis gaigei* is distributed from near the eastern city limit of Davíd, Chiriquí, along the Pacific versant of western and central Panama, including the Azuero Peninsula, to at least the Canal Zone, and widely distributed in the Santa Marta Mountains of Colombia as well as possibly northwestern Venezuela (Figs. 2, 13). The documented vertical range of the species is from near sea level to about 900 m. The elevation stated to be "8, 000 ft." (=2438 m) for a supposed locality of this species (Pueblo Viejo, Santa Marta Mountains, Colombia) by Ruthven (1916: 8) seems to be exceedingly high and therefore unlikely for this species. However, the associated specimens (UMMZ 48322–23) are clearly referable to *A. gaigei*.

Discussion

Four independent lines of evidence, *i.e.*, hemipenial morphology, pholidosis, mitochondrial DNA, and male dewlap coloration, support the recognition of two taxa of anoles formerly referred to *Anolis tropidogaster.* Although male specimens of *A. gaigei* and *A. tropidogaster* exhibit the most obvious differences (hemipenes, postcloacal scales, and dewlap coloration), the doubtless assignation of females to one of the two species is possible in most cases with the help of a little more subtle pholidotic characters. In conclusion, *A. gaigei* and *A. tropidogaster* appear well separated and readily distinguishable from each other, even in preserved material.

Notwithstanding the hiatuses between the known populations of *Anolis gaigei*, the populations which live in the Santa Marta Mountains around the type locality (including the type series) are clearly conspecific with the Panamanian populations considering the pholidotic characters that they share. Yet, the most overwhelming evidence is provided by the unique hemipenis of *A. gaigei*, which is the smallest and most delicate reproductive organ of any anole species documented so far.

Nevertheless, *Anolis gaigei* has long been regarded as a synonym of *A. tropidogaster* all over its range, and several aspects of its life history have been studied at a level of detail remarkable for anole species (Sexton *et al.* 1964, 1971; Campbell 1971; Quintero & Cambra 1993) under the latter name. Moreover, it has been confused with *A. polylepis* in the western part of its range, namely on the Azuero Peninsula and in Veraguas and eastern Chiriquí provinces of western Panama. This documents the fact that the lowland anoles along the Pacific versant of Panama have largely been neglected by herpetologists. It should be emphasized that in this case the taxa in question are not rare highland endemics restricted to a few remote localities, but very common species that abound in areas of high human population density and considerable past collection efforts. Furthermore, these species are not particularly cryptic in respect of their morphology. One only needs to examine the dewlap coloration in life, evert hemipenes, and check a few standard scalation characters to find out that several species are involved.

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APPENDIX I. Specimens Examined.

Anolis cupreus.—**Costa Rica:** Alajuela: 4 km SW San Mateo: SMF 93915–18; 6 km N San Ramón: SMF 93909; ca. 1.3 airline km NNW Angeles Norte: SMF 93910; Garita, La Garita Country Club: SMF 93895–96; Orotina: MCZ 15440; San Miguel de Turrucares: SMF 93897–99; Turrucares: KU 140620, 140622–23, 140625–27, 140629–31, 140637, USNM 37000–01; Volcán Miravalles: SMF 93920; Cartago: Cartago: KU 140665–67, 140676, 140680, 140684, 140707, 140710, 140723, 140735; Navarro: USNM 67338; Paraiso: AMNH 99677; Tapanti, 14 km E, 11.5 km S Cartago: MCZ 141383; Guanacaste: 4.2 km W Cañas, near Río Caribia: CM 117192–95; 4–5 km ENE Tilarán: KU 40758, 40762; Cañas, Tenorio, Las Flores: KU 40747, 40749, 40751; Ojatal, ca. 2 km SW El Coco: KU 66860–61; Parque Nacional Guanacaste: ZFMK 57768–70; Playa del Coco: KU 129246–47; Puerto Culebra: AMNH 62772; Río Bebedero, 2–5 km S Bebedero: KU 66862, 66864; Río Congo, ca. La Irma [Las Juntas de Abangares]: KU 140565, 140568–70, 140578–79, 140582, 140584, 140586, 140591; Río Jabillo: FMNH 167699; Taboga Camp, 20 km SE Cañas: KU 102406–07; Tilarán: ANSP 24437–45, USNM 70657–58; Puntarenas: 1 km E Sta. Elena, near Monteverde Biological Station: CM 117188–89; 2.4 km E Quepos: KU 125695; Boca de Barranca: MCZ 109931–37, 109939–42; Hacienda Barú: SMF 93876–77, 93883–84; Hacienda Barú, road to beach: SMF 93911; Hacienda El Barú, 3 km W Dominical: SMF 89310–17; Hatillo: SMF 93874–75; near Matapalo: SMF 93878–79; near Matapalo, Ecolodge Manuel Espinoso: SMF 93869–70; near Matapalo, Ecolodge Manuel Espinoso: SMF 93872; near Matapalo: SMF 93871; near Portalon: SMF 92728; near Portalon: SMF 93880–82; Neofauna near Jaco: SMF 93885–86; North of Parrita: SMF 93887–94; Parque Nacional Manuel Antonio: SMF 83092; Puente Río Tarcoles: SMF 93873; Punta Leona: SMF 92036; Punta Leona: SMF 93900–04, 93912–14, 93919; Punta Leona, Sendero Gigante: SMF 93906; Quepos: SMF 93905; Quepos: KU 125693–94, 140596–98, 140608, 140612, MCZ 129777–78; San José: 20 km N San Isidro del General: UMMZ 131790; San JoséJosé: UMMZ 70183; Zona Protedora El Rodeo: SMF 93907–08; 1 mi E Cangrejal: LSUMZ 52365–67; 1 mi W Sta. Ana: LSUMZ 52363; 2 mi E Escuadra: LSUMZ 52375; 2 mi N Santa Ana: LSUMZ 52370–71, 52378–80, 52386–87; Cangrejal: LSUMZ 52359, 52372–74, 52377; Caspirola: LSUMZ 52381–83; Finca la Baja, near San José: ZMH 4593; Finca La Pacifica: FMNH 167707, 167711; La Caja: ANSP 24453–54; San José: KU 125619, 125625, 125627, 125635, 125639, 125661, 125671, 125673–75, SMF 10993–94, USNM 74511–12, 75443, 80897–901; Santa Ana: LSUMZ 52325, 152364.

Anolis gaigei.—**Panama:** Chiriquí: 6 airline km from San Lorenzo: MHCH 1650, SMF 91915; Isla Palenque: MHCH 287–288; Playa Las Lajas: SMF 91914; Río Tabasará: MHCH 1651, SMF 91913; road btw. La Pita and Chiriqui: SMF 91916; road from Interamericana to Horconcitos: SMF 91917; Colón: Quebrada Bonita, ca Buena Vista: KU 100430–31, 113390; Cristóbal: USNM 54263; Portobelo: USNM 48528; Trinidad River: USNM 63995–99; Canal Zone, Juan Mine: AMNH 71742–43; Porto Bello: USNM 65123; Herrera: Porto Bello: USNM 65123; Los Santos: 1–1.5 mi NW Los Santos: CM 47531; Las Palmitas: USNM 148206; near Playa Venao: SMF 92195; Playita Resort: MHCH 1652–54, SMF 91904–08; Pta. Mala: AMNH 71746–49; Santo Domingo: MHCH 1655–57, SMF 91901–02; Panamá: 3 km WSW Chepo: KU 113391; Panama City: KU 117015, USNM 120552, 120564, 120584–610, 120700–01; Isleta Trapiche: USNM 102748–49; Panama City, Barrio San Miguelito: SMF 85302–03; Panama City, Metropolitan National Park: SMF 85304–06; Panamá, Parque Caminos de Cruces: MHCH 158; Pedro González Island: USNM 120695–96; 3 km W El Llano: AMNH 120006; Alto del Jobo Chorrera: UF 124419; Archipiélago de las Perlas: UMMZ 58537; Archipiélago de las Perlas, Isla Chapera: AMNH 108653–58; Archipiélago de las Perlas, Isla Contadora: AMNH 108637–51; Archipiélago de las Perlas, Isla Mogo Mogo: AMNH 108659–62; Archipiélago de las Perlas, Isla Pacheca: AMNH 108652; Archipiélago de las Perlas, Isla Saboga: AMNH 108663–67, UMMZ 51096–105; Archipiélago de las Perlas, Isla San José: AMNH 115897–99; Canal Zone, stream below Casdenas village: UF 124437; Canal Zone, Summit Garden: ANSP 24557, UF 124426; Cerro Azul above Tocumen: UF 124435–36; Chagres River: AMNH 85398; Fort Kobbe: USNM 193369–70, 193454, 532433–40; Isla Taboga: AMNH 103745–47, 107482–89, ANSP 21723–26; UMMZ 181409–10, 181424, 181427; La Chorrera, near Army Post: AMNH 71735, USNM 53821; Madden Forest Preserve: AMNH 107477–81; near Chilibre, twilight zone of Chilibre Cave: USNM 140651; near Fort Clayton Reservation: SMF 82703–07, UIMNH 41896–918; 41992; Nueva Gorgona: AMNH 89974–78; Old Panama: AMNH 71744–45, 107477–81; Panama City, El Cangrejo, Ave. E. Morales: UF 124415–16, 124427–28; Pedro González Island: USNM 120695–96; Veraguas: Finca La Providencia, near Ponuga: MHCH 2294, SMF 91529, 91918, 91956–60; Isla Cebaco: USNM 154243; Mojara: USNM 129858–62; Montuoso Ranger Station: MHCH 1658, SMF 91921; Río Coroba: USNM 148085–90; Río Santa María: SMF 91909; road from Santiago to Soná: MHCH 1659, SMF 91912; road from Soná to El María: SMF 91910–11; San Francisco: SMF 91903; Santiago: AMNH 113567; Sitio Ramsar: 91919–20. **Colombia:** Antioquia: Atrato, Sautata: FMNH 74917; Cesar: Las Pavas, Santa Marta Mountains: UMMZ 54825–30; Valencia, Santa Marta Mountains: UMMZ 54822–24; Guajira: Arroya de Arenas, Santa Marta Mountains: UMMZ 54818–20; Loma Larga, Santa Marta Mountains: UMMZ 54821; Magdalena: Agua Dulce, Santa Marta Mountains: UMMZ 48329–34; Cincinnati, Santa Marta Mountains: UMMZ 54814–15; Fundación, Santa Marta Mountains: UMMZ 48327–28; La Tigrera, Santa Marta Mountains: UMMZ 48324; Minca, Santa Marta Mountains: UMMZ 48325–26; Palomina, Santa Marta Mountains: UMMZ 48321; Pueblo Viejo, Santa Marta Mountains: UMMZ 48322–23; Quebrada, Santa Marta Mountains: UMMZ 54816; Tamocol, Santa Marta Mountains: UMMZ 54817.

Anolis osa.—**Costa Rica:** Puntarenas: ca. 6.5 km SW of Rincón de Osa, Osa Tropical Science Center: USNM 219564; ca. 2.5 km SW of Rincón de Osa, Osa Tropical Science Center: USNM 219561, 219563, 219565–66; ca. 3.5 km WNW of Rincón de Osa, Osa Tropical Science Center: USNM 219562; 5 mi SW Rincón de Osa: CM 41509; Corcovado, National Park: SMF 89260–61; dirt road to Ranger Station "Los Patos": SMF 89187–92, UCR 20731; 2 km NW Cañaza: SMF 89193–97; 4 km W Puerto Jiménez: SMF 89618–20; Puerto Jiménez: SMF 89198–202, UCR 20732, ZFMK 52335; 8, 5 km SW Puerto Jiménez, 25 m after branch to Playa la Colorada: SMF 89205–07, 89621; 11 km SW Puerto Jiménez: SMF 89208; 16.5 km S Puerto Jiménez: SMF 89209–10; 2 km W Rincón de Osa: SMF 89215–21, UCR 20733–35; 9.5 km E Agujitas, Rancho Quemado: SMF 89622–23; 8 km E Agujitas, highest point of road, 8.6945°N, 83.59161°W: SMF 89222–24; Bahía Drake, Agujitas: SMF 89624; Bahía Drake, 3–4 km W Drake: SMF 89625–26; S Rincón de Osa, 1 km after branch of road to Drake: SMF 89225–26; road 6 km SW Rincón de Osa: SMF 89227–32; road 6 km SW Rincón de Osa: SMF 80645; Puerto Escondido: SMF 89628–30; Rincón de Osa: SMF 89233–40, UCR 20740–41.

Anolis polylepis.—**Costa Rica:** Puntarenas: 1 km W Ojochal, Residential Cinco Ventanas: SMF 89607–09, UCR 20708–09, 20736; 12 mi SSW Palmar Sur, 8.7925°N, 83.51917°W: LSUMZ 52362; 2 km N Rincón de Osa, Restaurant Ventanas al Golfo: SMF 89182–86; 2 km W Venecia: SMF 89174–76, UCR 20726–27; 2 km W Villa Colón: SMF 89171–73, UCR 20739; 2.5 km N Platanillo: SMF SMF 89660–65; 2–3 km after branch of road to Rincón de Osa: SMF 89610–17, UCR 20728–29, 20737–38; 3 km E Santa Cecilia: SMF 89177–81, UCR 20730; 3 km N Pavones: SMF 89633, UCR 20743; 5 km W Conte, 8.44919°N, 83.05678°W: SMF 89632, UCR 20742; 7 mi E Golfito: LSUMZ 11866, 30259; 9 km S Zancudo: SMF 89157; Balzar: SMF 89163–65, UCR 20716–19; branch of road to Sierpe: SMF 89168–70, UCR 20720; Fairy Place at Río Cotón: SMF 89631; Golfito, Reserva natural: SMF 89251; Gromaco, 23 mi NNE Golfito, 9.5 mi ESE Potrero Grande, on Río Coto Brus: UF 16377; Las Cruces Biological Station, 6 km (by road) S of San Vito de Java: SMF 89323–24, 89333 ; Manuel Antonio: SMF 81818–20; N Rincón de Osa, suital lodge: SMF 89627; N Uvita, Reserva Oro Verde: SMF 89605; near Quepos: SMF 77658; near Trenzas: SMF 89637–38; Puerto Pilón: SMF 89634–35; Punta Mala: SMF 89639; road halfway between Pilón and Sabalo: SMF 89636; San Buenaventura: SMF 89160–62, UCR 20713–14; Uvita, La Cusinga rainforest lodge: SMF 89606, UCR 20706–07; W Los Mogos: SMF 89642–44; San José: Cedral: SMF 89305–06; 11 mi SW of San Isidro del General, on Dominical Road (Highway 22): USNM 219999–220000; **Panama:** Chiriqui: "Chiriqui": ZMB 7825–26, 7830, 58002–09; Sendero El Pianista: SMF 86384; Boquete: SMF 86383, ZSM 63/1989/1, 4, 5; Cochea, 8.72656°N, 82.49154°W: MHCH 2260–1, SMF 89747–8; El Volcán: USNM 129920; Finca C.A.S.A, 8 km NE Río Sereno, Distrito Renacimiento: SMF 85204–08, 85209–10; Hacienda Café de Eleta: MHCH 2257, SMF 89509–12; headwaters of Río Chevo: SMF 85442; Los Algarrobos: Weg zum Río Majagua: SMF 89513; Meseta de Chorcha: SMF 85211–21, 85287; near El Hato: USNM 129380; Progreso: USNM 120756; Río Chevo: SMF 85441; Santa Clara: MHCH 2262, SMF 89749; Universidad Autonoma de Chiriquí, David: SMF 85202–03; Volante: MHCH 2259, SMF 89514.

Anolis tropidogaster.—**Colombia:** Ont été recueillis près de la rivière de la Magdeleine: MNHN 2427, 2427a; Antioquia: Alto de Quimari, Sinu River side: FMNH 61676–78; Nechi, Cauca River: FMNH 55935–37; Urabá, Río Currulao: FMNH 63794–804; Chocó: Andagoya: MCZ 32302; Golfo de Urabá, Unguia: FMNH 63793; Cordobar: Murrucucu, Sinu River side: FMNH 61658–60; Tierra Alta: FMNH 61666–67, 61702–03; no Provinz: Santander: Puerto Berrio: FMNH 30791; **Panama:** Darién: 7–11 km SW El Real between Río Presencia and Río Morgentese: UMMZ 155803–04; below Río Tupisa on Río Chucunaque: AMNH 42922; Camp Creek, near Yavisa: AMNH 42920–21, 42923–25; Canclones [Canclón]: UMMZ 124957; Caserete–Chepigana: MHCH 1628, 1634, 1640, 1645; Cerro Mali, GML [Gorgas Memorial Laboratory] camp clearing: USNM 151081–83; Cerro Tacarcuna: USNM 151120; Chepigana: MHCH 1636; El Real de Santa María: MHCH 179; Pinogana, El Real: MHCH 209–210; Río Chucunaque, 3 mi W Camp Townsend: AMNH 102560–63; Sol Poniente–Chepigana: MHCH 1701; Tacarcuna Village: USNM 141814; Parque Nacional Darien, mouth of Rio Paca: MHCH 2374; Laguna de Matusagarati, Aguas Calientes, Pinogana: MHCH 2375.

APPENDIX II. Corresponding information of sequenced specimens.

species	collection number	field number	GenBank accession number	country	province	latitude	longitude
Anolis cupreus	SMF 93897	GK 2301	JQ435511	Costa Rica	Alajuela	9.94461	-84.32230
A. cupreus	SMF 93873	GK 2233	JQ435510	Costa Rica	Puntarenas	9.80211	-84.60645
A. gaigei	SMF 91907	GK 3116	JQ435508	Panama	Los Santos	7.42036	-80.18002
A. gaigei	SMF 91918	GK 3202	JQ435509	Panama	Los Santos	7.43513	-80.19132
A. kemptoni	SMF 91482	SL 680	JQ435507	Panama	Bocas del Toro	8.94736	-82.70983
A. polylepis	SMF 90153	JFB 023	JQ435506	Panama	Chiriquí	8.73761	-82.51302
A. tropidogaster	MHCH 1634	MHCH 1634	JQ435505	Panama	Darien	8.11856	-77.87800

Appendix VI

Declaration on the contributions of authors

to the publication: A new species of *Dactyloa* from eastern Panama, with comments on the other *Dactyloa* species present in the region.
status: published (2015)
name of journal: Zootaxa, 4039
Authors involved:
- Abel Batista (AB), - Milan Vesely (MV), - Konrad Mebert (KM), - Gunther Köhler (GK), - Sebastian Lotzkat (SL)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 70%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 15%

(2) to the implementation of the respective studies and experiments

PhD candidate: 75% – field work (collecting and documenting specimens), molecular analysis
Coauthor MV: 10% – field work (collecting and documenting specimens), morphological analysis
Coauthor KM: 10% – field work (collecting and documenting specimens)
Coauthor GK: 5% – morphological analysis

(3) to the creation of the data collection and figures

PhD candidate: 80% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor MV: 15% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor KM: 5% – provided photos

(4) to the analysis and interpretation of the data

PhD candidate: 70% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor MV: 5% – contributed to data analysis.
Coauthor KM: 5% – contributed to data analysis.
Coauthor GK: 5% – contributed to data analysis and interpretation
Coauthor SL: 15% – analysis and interpretation of morphological, biogeographical, and molecular data

(5) to writing the manuscript

PhD candidate: 55% – wrote most of the paper
Coauthor MV: 10% – helped to write and improve the introduction, results, discussion.
Coauthor KM: 10% – helped to write and improve the introduction, results, discussion.
Coauthor GK: 5% – helped to write and improve the introduction, results, discussion.
Coauthor SL: 20% – helped to write and improve the introduction, results, discussion.

Date/place: 13.04.2016 / Frankfurt am Main, Germany

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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A new species of *Dactyloa* from eastern Panama, with comments on other *Dactyloa* species present in the region

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Abstract

Giant anoles of the genus *Dactyloa* have been considered to be represented in eastern Panama by six species. In this contribution, we describe a seventh species that is restricted to the Majé, San Blas, Darién, and Piedras-Pacora mountain ranges. The new species resembles *D. ibanezi, D. limon,* and *D. purpurescens* in external morphology but differs from these species in dewlap coloration, dorsal color pattern, morphometrics, and scalation. The recognition of the new species is further supported by DNA barcoding (genetic distances >2.7% in 16S and >7.8% in COI between the new species and all other species of *Dactyloa*). We discuss the taxonomic identity of *D. purpurescens,* and, based on morphological evidence, we place *D. chocorum* in the synonymy of the former species. An identification key for all 11 *Dactyloa* species occurring in Panama is provided.

Key words: *Anolis,* barcoding, *Dactyloa*, Eastern Panama, Integrative taxonomy, morphology

Introduction

Ten species of *Dactyloa* Wagler occur in Lower Central America (Köhler 2008; Jaramillo *et al.* 2010; Lotzkat *et al.* 2013; Uetz & Hošek 2014): *Dactyloa casildae* (Arosemena, Ibáñez & de Sousa, 1992), *D. chloris* (Boulenger, 1898), *D. chocorum* (Williams & Duellman, 1967), *D. frenata* (Cope, 1899), *D. ginaelisae* Lotzkat, Hertz, Bienentreu & Köhler, 2013, *D. ibanezi* (Poe, Latella, Ryan & Schaad, 2009), *D. insignis* (Cope, 1871), *D. kunayalae* (Hulebak, Poe, Ibáñez and Williams, 2007), *D. latifrons* (Berthold, 1845), and *D. microtus* (Cope, 1871). The presence of an eleventh species, *D. purpurescens* (Cope, 1899), has been suggested for eastern Panama by Chun (2010), but no voucher specimens with definite locality are available to support this assumption. Recently, a detailed revision of *Dactyloa* from Lower Central America west of the Panama Canal (Lotzkat *et al.* 2013) showed that all ten confirmed species occur in Panama, where they are distributed as follows: three of them (*D. frenata, D. kunayalae, D. insignis*) have wider distributions covering eastern as well as western Panama, another four (*D. casildae, D. ginaelisae, D. ibanezi, D. microtus*) occur in western Panama only, and the last three (*D. chloris, D. chocorum, D. latifrons)* are restricted to the Chocoan biogeographical region (Williams & Duellman 1967; *www.herpnet2.org/*, accessed on August 28th 2014; Torres-Carvajal *et al.* 2014) of eastern Panama (Savage 2002; Köhler 2008; Poe *et al.* 2009; Jaramillo *et al.* 2010; Lotzkat *et al.* 2013). All mentioned species are members of the *Dactyloa latifrons* species group sensu Nicholson *et al.* (2012) and of the *latifrons* series of Castañeda & De Queiroz (2013). Only a few specimens of *Dactyloa chloris, D. kunayalae, D insignis,* and *D. latifrons,* respectively, have been reported from eastern Panama until now.

Recently, *Dactyloa limon* (Velasco & Hurtado-Gómez, 2014) was described as a new species related to *D.*

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chocorum and *D. ibanezi* from the Magdalena River Valley in Colombia. All three species are similar in appearance, having similar body size (65–95 mm SVL), smooth ventral scales, and a mostly green dorsal coloration (Velasco & Hurtado-Gómez, 2014). They also show a peculiar flank pattern, which is green with dark oblique bands or series of ocelli in males (Williams & Duellman 1967; Poe *et al*. 2009; Velasco & Hurtado-Gómez, 2014). A fourth species, *D. purpurescens,* is related to the three mentioned above (Chun 2010), although it is not included in any of the diagnoses or descriptions of *D. limon, D. chocorum,* or *D. ibanezi.* However, similarities in color pattern and several scalation characters suggest a close relationship to *D. chocorum* (Williams 1988; Chun 2010: Fig. 1–2). During recent field work in eastern Panama, we collected specimens of a potential new species related to *D. limon, D. chocorum, D. ibanezi*, and *D. purpurescens*. Here, using an integrative taxonomic approach (morphology, molecular genetics, and biogeography), we evaluate the specific status of the potential new species. We also assess the taxonomic status of *D. chocorum* and *D. purpurescens* and include comments on the other *Dactyloa* species from eastern Panama.

Material and methods

Field work was carried out in eastern Panama during July-December 2011 and May-December 2012. All georeferences were recorded in WGS 1984 datum. The maps were created using ArcGIS 10 (ESRI 2010). Collected specimens were euthanized with a pericardial injection of a euthanasia drug (T61), fixed using a solution of formalin (36%) and ethanol (94%, 1:200), and stored in 70% ethanol. All figures have been digitally improved and combined using Adobe CS3. Generalized coloration summaries were derived from photos of collected specimens in life. In the individual color descriptions in life, the capitalized colors and color codes (the latter in parentheses) are those of Köhler (2012). Abbreviations for museum collections follow Sabaj Pérez (2013). In evaluating species boundaries within the populations of alpha anoles found in eastern Panama, we follow the unified species concept (de Queiroz 2007). In an integrative approach, as lines of evidence for species delimitation we use a phenotypic criterion (external morphology: coloration, morphometrics, and pholidosis) that includes species recognition traits (dewlap size and coloration) and a criterion for reproductive isolation (genetic distinctness of the 16S and COI mtDNA). Comparisons among species are based on the examination of the available holotypes as well as photographs of type material and data from the respective original descriptions. Detailed information for the specimens utilized in this study is provided in the Appendix I.

Morphometrics. Snout-vent length and tail length measurements were taken to the nearest mm along a meter stick. Other measurements were made to the nearest 0.1 mm with precision calipers, with the aid of a dissecting microscope for diminutive characters such as scale sizes. Values are given as minimum–maximum followed by mean ± standard deviation in parentheses. Terminology for morphometrics and pholidosis follows Köhler (2014). Abbreviations of the characters used are: SVL (snout-vent length), HL (head length), HW (head width), SS (supraorbital semicircles), IP (interparietal plate), TL (tail length), AGD (axilla groin distance), SL (snout length), ShL (shank length), VDT (vertical diameter of tail), HDT (horizontal diameter of tail), ventrHL (number of ventral scales in one head length), dorsHL (number of dorsal scales in one head length), RED (number of rows of enlarged dorsal scales), ToeLam prox (lamellae under the proximal phalanges of the 4th toe, *i.e.,* from base of digit to end of dilated pad), ToeLam dist (lamellae under the distal phalanx of the 4th toe), LSR (number of loreal scale rows), LST (total number of loreal scales), SPL (number of supralabial scales to the level below the center of the eye), IFL (number of infralabial scales to the level below the center of the eye), IO (number of scales between supraorbital semicircles), IP/IO (number of scales between supraorbital semicircles and interparietal plate), 1Canths (number of scales between first canthals), 2Canths (number of scales between second canthals), PR (number of postrostral scales), IN (number of internasal scales), PM (number of postmental scales), SubL (number of sublabial scales), SAM (number of scales around midbody). The terminology for hemipenial morphology follows Myers et al. (1993) and Savage (1997). We explored the variation in measurements and meristic characters using boxplots in SPSS. Additionally, to gain another perspective on differentiation in measurements and meristic characters of the species related to *D. chocorum*, we conducted a stepwise discriminant function analysis (DFA) based on three morphometric and four pholidotic characters (i.e., SL/SVL, HL/SVL, HL/HW, LST, ToeLam dist, ToeLam prox, 1Canths dorsHL).

Genetics. DNA was extracted from fresh tail tip cuts using the protocol of Ivanova *et al.* (2006). A fragment of the mitochondrial 16S rRNA gene was amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany),

performing an initial denaturation for 1 min at 94° C followed by 35 cycles of denaturation for 0.15 min at 94° C, annealing for 0.45 min at 45° C, and elongation for 1.5 min at 72° C. Final elongation proceeded for 7 min at 94° C. Reaction mix contained 1 µL DNA template (1:10), 2.5 µL Reaction Buffer x10 (PeqGold), 4 µL 2.5 mM dNTPs, 0.4 µL (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µL H_2O, 1 µL 25 mM $MgCl_2$, and 1 µL of standard primers for 16S (containing 10 pmol, forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon). The COI fragments were sequenced in the Southern China DNA Barcoding Center. We compared the mtDNA data of our specimens with sequences published on GenBank. The resulting ClustalW alignments were reviewed and edited by eye using Geneious version 6.1 (Biomatters Inc., available online from http://www.geneious.com/), missing data was treated as N. A list of specimens included in the genetic analyses with corresponding GenBank accession numbers are presented in Appendix I. The final 16S alignment comprising 37 sequences of the genus *Dactyloa* obtained in this study and from GenBank (excluding outgroup) consisted of 458 sites of which 149 were variable, 94 parsimony-informative, and 55 singletons. The final alignment for the COI gene comprising 53 sequences obtained in this study and from GenBank consisted of 569 sites, of which 233 were variable, 219 parsimony-informative, and 14 autapomorphies. Using MEGA5 (Tamura *et al.* 2011), we calculated uncorrected p-distances for 16S and COI separately. For the combined-gene data set of 16S and COI mtDNA (69 samples and 1027 sites, Ns were filled in for taxa in which we only had one of the two genes), we used JModeltest 0.1.1 (Posada 2008) under the corrected Akaike Information Criterion (AICc) to select the substitution model for the Bayesian inference (BI) and Maximum Likelihood (ML) analyses. The best-fitting substitution model determined was TrN+G. We ran a Maximum Likelihood (ML) analysis with 1000 bootstrap replicates using PAUP v4.0b10 (Swofford 1998), and a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) for 10,000,000 generations with four default chains, sampled every 100 generations, and subsequently discarded the initial 5% of the sampled trees as burn-in. Outgroups used were *Norops capito* (SMF 97094), *N. limifrons* (SMF 97099), *N. tropidogaster* (MHCH 2646), *N. poecilopus* (SMF 97111), and *Polychrus marmoratus* (SNOMNH 36693).

Results

Based on the results of our integrative analysis we recognize seven species of *Dactyloa* in eastern Panama, one of which we describe as a new species below. Maps with the locality records of these species in eastern Panama are provided in Figures 1–2. The variation of selected morphological characters among these species is shown in Tables 1–2, and the genetic differences of the 16S and COI mtDNA are presented in Table 3.

Concerning the described species of *Dactyloa,* all of which have rather rarely been collected in eastern Panama (Savage & Talbot 1978; Hulebak *et al.* 2007; Köhler 2008; Poe *et al.* 2009; Lotzkat *et al.* 2013), our 30 recently collected specimens complement the picture of their distribution and variation. A specimen of *D. chloris* (SMF 97096; Fig. 3G–H) collected at the Cana field station represents the fourth specimen of this species collected in the country (see http://www.vertnet.org/). *Dactyloa frenata* (Fig. 3E) was one of the most common species collected, but most of the records are from Majé, San Blas, and Darien mountain ranges, whereas no specimen was collected or observed in the Pirre or Jingurudó mountain ranges. Instead, we found its closest relative, *D. latifrons* (Fig. 3F), in those areas. The specimen of *Dactyloa kunayalae* (Fig. 3C–D) collected at Cerro Pechito Parado, Río Tuquesa, is the second known specimen collected in the Darién mountain range, and represents the southern and easternmost record for the species, about 60 km southeast from the nearest known record (see Lotzkat *et al.* 2013). As in the specimens from western Panama examined by Lotzkat *et al.* (2013), our specimen also has short legs, when hindlegs are adpressed against the body, the tip of fourth toe reaches the tympanum, contrary to what is written in the original description that fourth toe reaches beyond eyes (Hulebak *et al.* 2007). *Dactyloa insignis* was previously reported for two localities in eastern Panama (Lotzkat *et al*. 2013), and here we add two more localities, one at Cerro Cituro at the Pirre Mountain Range (specimen KUH 113127, see: *http://portal.vertnet.org/*), and another at the Jingurudó Mountain Range (specimen KUH 113128; see: *http://portal.vertnet.org/*), which is the nearest record to the Colombian border, with roughly 17 km airline distance to that country.

Our data indicate conspecificity of the holotype of *Dactyloa purpurescens* with the taxonomic species *D. chocorum* of current usage, as evidenced by the extreme morphological resemblance between them that was already pointed out by Chun (2010). The holotype of *D. purpurescens* (USNM 4321) agrees with the available

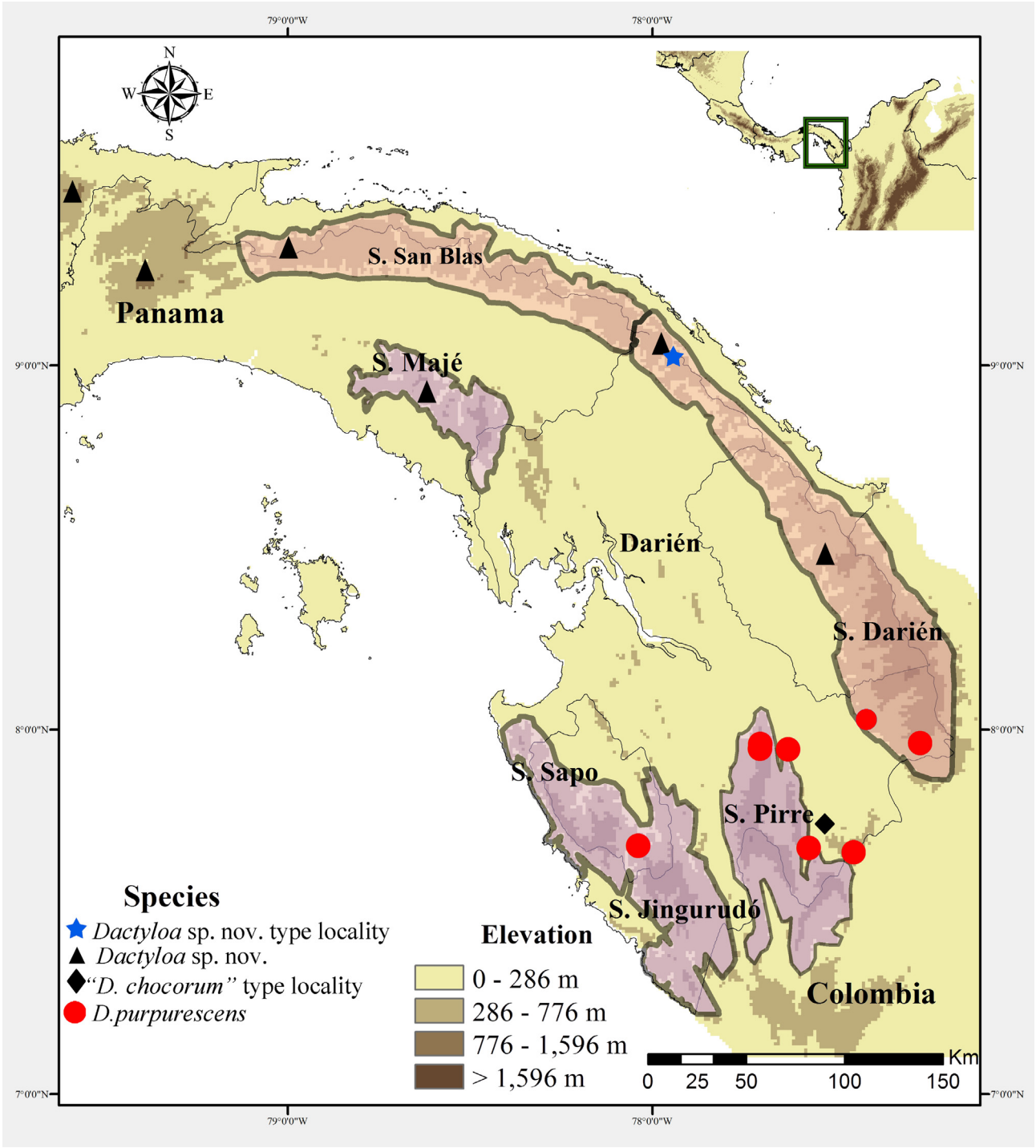

FIGURE 1. Distribution of *Dactyloa* species formerly referred to as *D. chocorum* in eastern Panama, based on specimens collected for this study, provided by A. Sosa, literature and online databases (http://www.vertnet.org/).

specimens of *D. chocorum* (from near the type locality, see Appendix I) in the following external characters [*D. chocorum* (range) vs *D. purpurescens*]: SVL (52–88 mm vs 78 mm), HW (8–13 mm vs 13 mm), HL (14–22.2 vs 16.7), ventrHL (50–74 vs 51), ToeLam prox (25–32 vs 33), ToeLam dist (11–12 vs 10). Furthermore, USNM 4321 matches specimens of *D. chocorum* in dorsal color pattern (Fig. 4–5; see Appendix II). Cope (1899) stated that *D. purpurescens* in preservative has a flank pattern that consists of "numerous small oval darker spots arranged in longitudinal lines on the back and sides, becoming rounder on the latter, and grouped into transverse agglomerations, producing the effect of bands, which are directed a little backwards as well as downwards". This color pattern perfectly agrees with the coloration of *D. chocorum* as described by Williams and Duellman (1967;

also see Appendix II). Since we are unable to find any morphological differences between the holotype of *D. purpurescens* and the available specimens of *D. chocorum*, we propose to synonymize these two nominal taxa. Therefore, the valid name for the species previously referred to as *Dactyloa* (or *Anolis*) *chocorum* in eastern Panama is *D. purpurescens*, since the latter name has priority. In Panama *D. purpurescens* (Fig. 3A–B, 4C–D) is an uncommon species, present in the Pirre and Jingurudó mountain ranges as well as in the southern portion of the Darién mountain range, around Río Pucuró. No specimens of this species were found northward or in the San Blas mountain range.

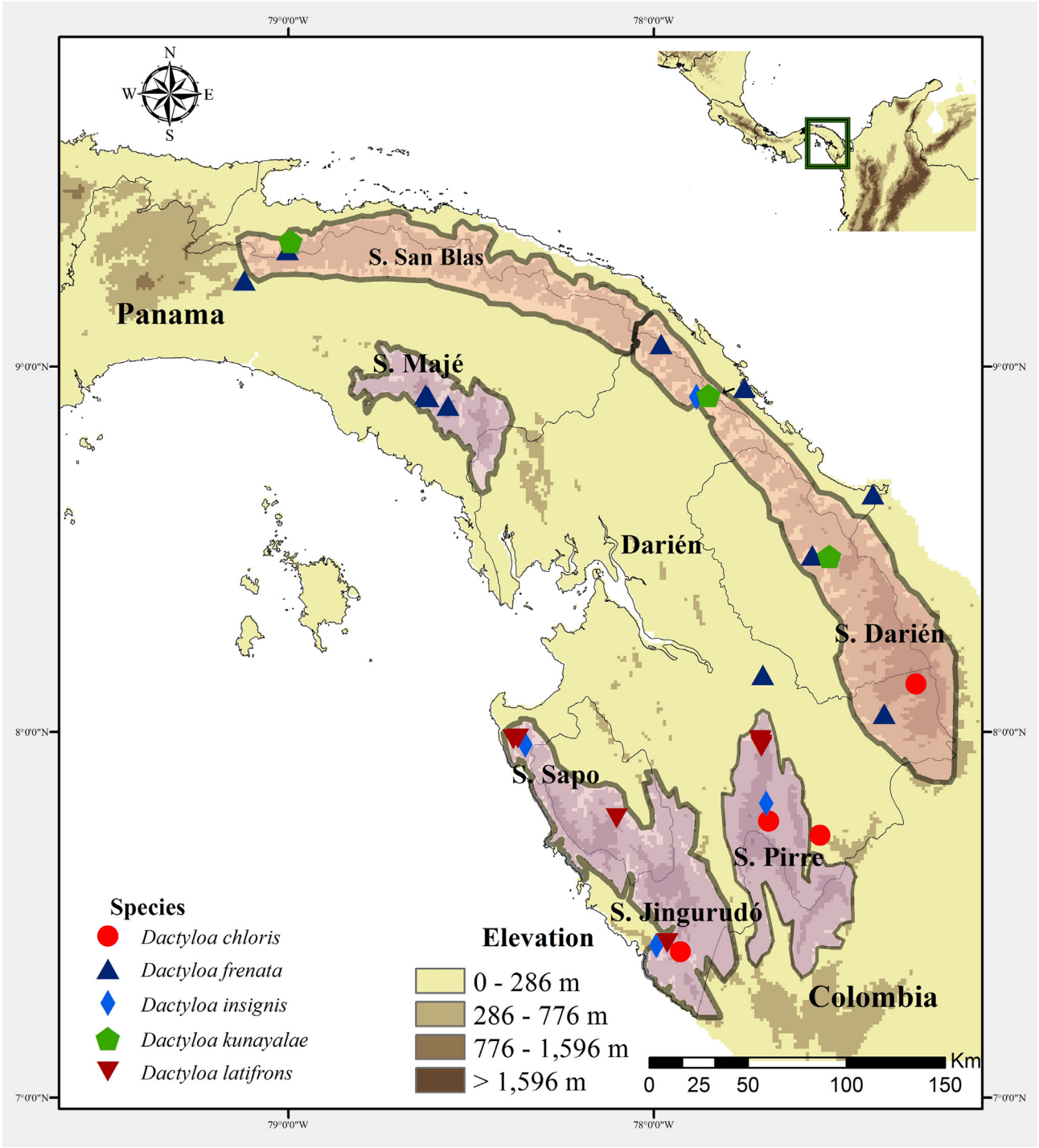

FIGURE 2. Distribution of *Dactyloa chloris, D. frenata, D. insignis, D. kunayalae,* and *D. latifrons* in eastern Panama, based on specimens collected for this study, literature and online databases (http://www.vertnet.org/).

TABLE 1. Selected measurements and proportions among our sample of *Dactyloa* from eastern Panama. Sample sizes are given in parentheses following each species name. For details and abbreviations see text.

Traits	*D. purpurescens* (13)	*D. chloris* (3)	*D. frenata* (5)	*D. ibanezi* (4)	*D. kunayalae* (1)	*D. latifrons* (4)	*D. limon* (7)	*Dactyloa* **sp. nov.** (6)
SVL	52–88 (74.58 ± 9.81)	52–54.8 (53.38 ± 1.4)	95–151 (120.2 ± 27.65)	77–79 (77.5 ± 1)	90	100–108 (103.75 ± 3.5)	70.59–81.78 (76.35 ± 3.77)	62–78 (73.67 ± 5.85)
TL	115–204 (166.25 ± 33.37)	94–115 (104.5 ± 14.85)	139–299 (208.2 ± 68.33)	172–199 (189.25 ± 11.95)	150	172–232 (205.67 ± 30.66)	176–201.92 (186.27 ± 8.92)	107–186 (146.6 ± 33.95)
AGD	21.3–36.6 (31.05 ± 4.27)	21.47–23.53 (22.74 ± 1.11)	39.66–66.03 (51.46 ± 11.96)	32.11–34.5 (33.33 ± 1.04)	37.12	41.44–44.16 (43 ± 1.36)	29.88–39.56 (33.68 ± 3.07)	27.68–35.79 (32.18 ± 3.27)
SL	7.02–11.29 (9.19 ± 1.23)	5.94–6.82 (6.45 ± 0.46)	12.43–18.42 (15.19 ± 2.71)	9.22–9.89 (9.43 ± 0.31)	11.75	12.84–14.43 (13.49 ± 0.68)	9.1–10.51 (9.84 ± 0.5)	7.94–10.76 (9.48 ± 0.92)
HW	8.03–13 (11.18 ± 1.45)	6.81–7.58 (7.3 ± 0.42)	14.51–21.66 (17.9 ± 3.25)	10.38–11.13 (10.68 ± 0.32)	14.67	15.18–17.31 (16.5 ± 0.92)	10–11.54 (10.94 ± 0.5)	9.05–11.4 (10.43 ± 0.88)
HL	13.99–22.21 (18.15 ± 2.44)	11.13–12.34 (11.74 ± 0.61)	24.6–36.95 (30.54 ± 6.03)	19.52–20.01 (19.81 ± 0.21)	24.3	25.03–30.52 (27.39 ± 2.32)	16.35–21.5 (17.77 ± 1.85)	16.55–21.26 (19.52 ± 1.6)
TL/ SVL	1.51–2.73 (2.24 ± 0.36)	0–2.16 (1.29 ± 1.14)	1.46–2.02 (1.7 ± 0.2)	2.18–2.58 (2.44 ± 0.18)	1.67	1.72–2.15 (1.99 ± 0.23)	2.39–2.49 (2.44 ± 0.05)	1.64–2.45 (1.99 ± 0.37)
HL/SVL	0.22–0.27 (0.25 ± 0.02)	0.21–0.24 (0.22 ± 0.02)	0.24–0.26 (0.25 ± 0.01)	0.25–0.26 (0.26 ± 0.01)	0.27	0.25–0.28 (0.26 ± 0.01)	0.22–0.26 (0.23 ± 0.02)	0.25–0.28 (0.27 ± 0.01)
ShL/ SVL	0.26–0.29 (0.28 ± 0.01)	0.23–0.27 (0.25 ± 0.02)	0.29–0.31 (0.3 ± 0.01)	0.27–0.3 (0.29 ± 0.01)	0.24	0.3–0.32 (0.31 ± 0.01)	0.22–0.27 (0.25 ± 0.02)	0.27–0.29 (0.28 ± 0.01)
HL/ HW	1.5–1.9 (1.69 ± 0.13)	1.48–1.81 (1.61 ± 0.17)	1.63–1.76 (1.7 ± 0.05)	1.79–1.9 (1.86 ± 0.05)	1.66	1.58–1.76 (1.66 ± 0.08)	1.48–1.91 (1.63 ± 0.18)	1.83–1.99 (1.87 ± 0.06)
VDT/HDT	1.11–1.45 (1.3 ± 0.14)	–	1.27–1.6 (1.4 ± 0.16)	1.27–1.47 (1.36 ± 0.09)	1.39	0.45–1.37 (1.12 ± 0.45)	–	1.23–1.47 (1.36 ± 0.08)
HW/ SVL	0.14–0.15 (0.15 ± 0.01)	0.13–0.14 (0.14 ± 0.01)	0.14–0.16 (0.15 ± 0.01)	0.13–0.14 (0.14 ± 0.01)	0.16	0.15–0.16 (0.16 ± 0.01)	0.13–0.16 (0.14 ± 0.01)	0.13–0.15 (0.14 ± 0.01)
AGD/ SVL	0.39–0.46 (0.42 ± 0.02)	0.39–0.45 (0.43 ± 0.03)	0.40–0.45 (0.43 ± 0.02)	0.42–0.44 (0.43 ± 0.01)	0.41	0.41–0.42 (0.41 ± 0.01)	0.4–0.48 (0.44 ± 0.03)	0.39–0.47 (0.44 ± 0.03)
ShL/ HL	0.97–1.28 (1.12 ± 0.1)	1.09–1.26 (1.16 ± 0.09)	1.16–1.2 (1.18 ± 0.02)	1.09–1.16 (1.12 ± 0.04)	0.88	1.09–1.26 (1.18 ± 0.07)	0.86–1.23 (1.07 ± 0.14)	0.98–1.1 (1.05 ± 0.04)
SL/SVL	0.1–0.14 (0.12 ± 0.01)	0.11–0.12 (0.12 ± 0.01)	0.12–0.14 (0.13 ± 0.01)	0.12–0.13 (0.12 ± 0.01)	0.13	0.13–0.13 (0.13 ± 0)	0.12–0.14 (0.13 ± 0.01)	0.12–0.14 (0.13 ± 0.01)
SL/HL	0.47–0.53 (0.5 ± 0.02)	0.48–0.59 (0.55 ± 0.06)	0.48–0.52 (0.5 ± 0.02)	0.47–0.5 (0.48 ± 0.02)	0.48	0.47–0.51 (0.49 ± 0.02)	0.49–0.61 (0.56 ± 0.05)	0.47–0.51 (0.49 ± 0.02)

TABLE 2. Selected pholidotic characters among our sample of *Dactyloa* from eastern Panama. Sample sizes are given in parentheses following each species name. For details and abbreviations see text.

Traits	*D. purpurescens* (13)	*D. chloris* (3)	*D. frenata* (5)	*D. ibanezi* (4)	*D. kunayalae* (1)	*D. latifrons* (4)	*D. limon* (7)	*Dactyloa* sp. nov. (6)
ventrHL	50–74 (57.31±6.82)	34–46 (38.67±6.43)	50–60 (55.2±4.15)	50–64 (58±5.89)	44	42–72 (59.5±13)	38–52 (44.4±5.18)	60–74 (65.33±4.84)
dorsHL	48–72 (55.23±6.41)	48–52 (50±2)	40–56 (46±6)	46–56 (52.5±4.43)	48	42–50 (45.5±3.42)	38–46 (41.6±3.29)	50–58 (53.33±3.93)
RED	2–4 (3.17±0.58)	2–4 (3±1)	3–4 (3.2±0.45)	2–4 (3.25±0.96)	0	2–4 (2.75±0.96)	2–3 (2.8±0.45)	3–4 (3.5±0.55)
ToeLam prox	25–33 (28.92±2.4)	25–28 (26.33±1.53)	36–42 (38.6±2.3)	27–28 (27.25±0.5)	18	36–38 (37±0.82)	28–30 (29±1)	28–32 (30.17±1.6)
ToeLam dist	10–12 (11±0.91)	7–8 (7.33±0.58)	14–17 (15.6±1.52)	10–12 (10.75±0.96)	11	16–19 (17±1.41)	8–12 (10±2)	10–12 (10.83±0.75)
LSR	6–8 (6.8±0.92)	–	7–8 (7.4±0.55)	6–7 (6.5±0.58)	5	7–9 (7.5±1)	–	6–7 (6.5±0.55)
LST	50–80 (62.78±9.08)	–	63–98 (74.4±13.76)	38–54 (44.5±7.19)	39	70–91 (81.75±8.85)	41–45 (43.67±2.31)	53–78 (64.67±9.89)
SPL	7–10 (8.36±0.81)	–	8–10 (9.4±0.89)	7–8 (7.75±0.5)	7	9–10 (9.25±0.5)	7–9 (8±0.71)	9–11 (9.33±0.82)
IO	1–5 (2.91±1.22)	–	3–4 (3.4±0.55)	2–2 (2±0)	4	4–5 (4.5±0.58)	1–3 (2±0.71)	2–3 (2.33±0.52)
IP/ IO	2–3 (2.64±0.5)	–	3–4 (3.8±0.45)	3–3 (3±0)	3	3–7 (5.5±1.73)	2–4 (2.8±0.84)	3–5 (3.83±0.75)
1Canths	12–14 (12.91±0.83)	–	11–15 (12.8±1.48)	10–13 (11.5±1.29)	10	11–14 (12.75±1.26)	9–11 (10±0.71)	12–18 (14.5±2.26)
2Canths	7–13 (10.18±2.09)	–	11–13 (12.2±1.1)	8–12 (10.25±1.71)	10	11–13 (12±0.82)	6–10 (8.6±1.52)	10–16 (12.33±2.25)
PR	6–10 (7.73±1.1)	–	6–8 (7±0.71)	6–8 (6.75±0.96)	4	6–9 (7.5±1.73)	6–7 (6.4±0.55)	6–8 (7.33±0.82)
IN	6–10 (8.64±1.36)	–	10–13 (11.4±1.14)	7–10 (8.5±1.29)	8	10–13 (11.25±1.26)	6–9 (7.8±1.1)	8–9 (8.83±0.41)
PM	1–6 (4.45±2.16)	–	8–9 (8.6±0.55)	6–6 (6±0)	6	8–10 (9.25±0.96)	2–7 (5.6±2.07)	6–7 (6.33±0.52)
SubL	2–87 (31.45±39.97)	3–68 (45±36.43)	0–2 (0.8±0.84)	2–3 (2.5±0.58)	3	0–0 (0±0)	2–63 (14.4±27.17)	2–4 (2.83±0.75)
SAM	152–206 (177.82±16.16)	128–136 (133.33±4.62)	128–166 (151.2±16.77)	154–170 (159.5±7.19)	98	148–164 (155±6.63)	126–156 (137.6±11.44)	154–196 (172.67±15.93)

TABLE 3. Pairwise comparison of genetic distance in 16S and COI mtDNA between the *Dactyloa* spp. used in the molecular analyses. Values represent uncorrected p-distances (in percent), with COI in lower left, and 16S in upper right (in bold); those samples without COI or 16S sequences are shown with "--".

	Dactyloa **sp. nov.**	*D. purpurescens*	*D. ibanezi*	*D. kunayalae*	*D. frenata*	*D. latifrons*	*D. microtus*	*D. insignis*	*D. ginaelisae*	*D. casildae*	*D. chloris*	*D. princeps*	*D. peraccae*	*D. agassizi*	*D. frasseri*	*D. danieli*	*D. maculigula*
Dactyloa. **sp. nov.**	--	**2.8**	**2.9**	**4.0**	**6.2**	**8.6**	**9.6**	**9.1**	**9.0**	**7.6**	**12.3**	--	--	--	--	--	--
D. purpurescens	8.7		**2.4**	**4.0**	**6.4**	**8.5**	**9.6**	**9.3**	**9.6**	**7.0**	**11.6**	--	--	--	--	--	--
D. ibanezi	7.2	8.2		**3.9**	**5.8**	**8.2**	**8.6**	**8.7**	**8.1**	**7.0**	**11.6**	--	--	--	--	--	--
D. kunayalae	--	--	--		**6.1**	**7.4**	**7.9**	**8.1**	**7.9**	**5.5**	**10.2**	--	--	--	--	--	--
D. frenata	16.0	16.8	15.8	--		**5.7**	**9.2**	**9.0**	**9.1**	**7.4**	**11.3**	--	--	--	--	--	--
D. latifrons	16.9	17.6	17.5	--	7.5		**11.8**	**12.5**	**12.2**	**10.5**	**13.7**	--	--	--	--	--	--
D. microtus	16.6	18.1	18.8	--	17.4	17.7		**4.1**	**3.7**	**10.7**	**13.5**	--	--	--	--	--	--
D. insignis	16.1	18.0	18.6	--	18.0	18.2	10.1		**3.6**	**10.5**	**11.5**	--	--	--	--	--	--
D. ginaelisae	14.0	16.7	17.1	--	16.1	16.7	9.2	9.9		**11.2**	**13.1**	--	--	--	--	--	--
D. casildae	12.6	14.0	13.4	--	15.2	14.8	16.2	16.4	15.1		**11.4**	--	--	--	--	--	--
D. chloris	19.9	20.5	20.5	--	20.2	19.7	17.5	18.2	17.9	17.8		--	--	--	--	--	--
D. princeps	16.6	17.4	16.9	--	9.1	6.9	17.6	18.4	16.9	15.6	19.8		--	--	--	--	--
D. peraccae	19.2	17.7	19.5	--	21.6	22.3	21.3	20.1	20.0	19.2	16.8	21.5		--	--	--	--
D. agassizi	16.4	18.5	17.9	--	17.5	17.2	17.4	16.1	16.2	16.3	17.7	17.3	21.3		--	--	--
D. frasseri	15.9	16.7	16.5	--	16.3	16.6	17.6	19.0	17.8	16.3	18.1	16.7	19.9	17.2		--	--
D. danieli	17.0	17.8	18.3	--	16.3	16.5	17.7	16.9	17.2	15.3	18.3	17.4	19.9	16.6	17.6		--
D. maculigula	14.9	15.4	15.5	--	16.9	16.7	17.9	16.9	16.5	13.0	20.6	17.7	21.6	18.3	18.8	17.5	

Among the specimens collected is a series of individuals that cannot be allocated to any of the described species of *Dactyloa* (Fig. 4–6), but appear to form a single, new species. In external morphology they are collectively most similar to *D. ibanezi, D. limon,* and *D. purpurescens* (as described here), but differ from these species in dewlap coloration, dorsal color pattern, and several other morphological characters (e.g., HL, HW, TL, SPL). The distinctiveness of these individuals is further supported by genetic distances between the new species and all other species of *Dactyloa* (>2.7% 16S, >7.8% COI). In a discriminant function analysis, the best predictor variables to differentiate among the species related to *D. purpurescens* were LST, SL/HL, and HL/SVL which correctly classified 85.7% of all specimens (77% of *D. purpurescens*, 100% of each *D. limon* and *D. ibanezi*, and 80% of *Dactyloa* **sp. nov.**). The first function is DF = 0.347 (LST) -1.026 (SL/HL) + 1.061 (HL/SVL), with an eigenvalue of 14.55 and a proportion of explained variance of 88.60%. The second function is DF = 1.18 (LST) + 0.58 (SL/HL) + 0.43 (HL/SVL), with an eigenvalue of 1.78 and a cumulative proportion of explained variance of 99.5%. In the phylogenetic analysis, the species related to *D. purpurescens* (molecular data for *D. limon* not available) are each monophyletic (Fig. 7). The averages of p- distance among all *Dactyloa* species were 8.4% for 16S and 16.8% for COI, respectively. The minimum average interspecific genetic distance for 16S was 2.4% between *D. ibanezi* and *D. purpurescens,* and for COI 6.9% between *D. latifrons* and *D. princeps*. The average genetic distance between closely related species (*e.g., D. ginaelisae/D. insignis/D. microtus, D. latifrons/D. princeps,* and *D. purpurescens/D. ibanezi/Dactyloa* sp. nov) was 3.2% for 16S (except *D. latifrons* and *D. princeps* since we lack 16S data for the latter) and 8.6% for COI, respectively.

We describe the new species below and provide a key to the species of *Dactyloa* known to occur in eastern Panama.

***Dactyloa maia* sp. nov.**

Figures 1, 4E–F, 5E–F, 6D, 8, 9, 10.

Holotype. SMF 97268, adult male (Figs. 4E, 5E, 6D, 9), from the ridge of the Serranía de Darién (Fig. 1) along the trail that connects the Comarca Wargandí and the Comarca Guna Yala, about 10 km northeast of the village Nurra, 9.06142° N, 77.97961° W, 344 m asl., Corregimiento de Nurra, Comarca Wargandí, Panama; collected by Abel Batista and Milan Vesely on 03 October 2012; original field number AB 760.

Paratypes. All from Panama: SMF 97269, a male, from Cerro la Javillosa Ambroya, Torti, Chepo, Panama province, collected on 28 September 2012, 19:39 hrs, 8.92267° N, 78.62530° W, 851 m asl, collected by Abel Batista and Milan Vesely; MHCH 2782, a female, same collecting data as holotype; MHCH 2781 and MHCH 2783, females, respectively, from Cerro Pechito Parado, Bajo pequeño, Lajas blancas, Cémaco, Comarca Emberá-Wounáan, on 07 November 2012, 8.47911° N, 77.52799° W, 718 m asl, collected by Abel Batista; SMF 97270, a female from la Cascada trail, Burbayar private reservation, Cartí, Narganá, Comarca Guna Yala, on 26 November 2012, 9.31577° N, 79.00580° N, 322 m asl, collected by Abel Batista and Konrad Mebert.

Diagnosis. A moderate-sized species (SVL 62–78 mm) of the genus *Dactyloa, D. latifrons* species group (*sensu* Nicholson *et al.* 2012), that is most similar in external morphology to *D. pupurescens*, *D. limon*, and *D. ibanezi*, and according to molecular evidence is most closely related to *D. pupurescens* and *D. ibanezi* (Fig. 7). These four species share a moderate adult size (SVL 62–88 mm); a large dewlap; a peculiar flank pattern in males, which is green with dark oblique bands, or blotches, or ocelli always arranged in oblique rows (Fig. 5); enlarged postcloacal scales in males; and smooth ventrals. *Dactyloa maia* can readily be distinguished from these three species by its color pattern and morphology (Figs. 3–6; Table 1–2), and from the remaining species of *Dactyloa* within the *D. latifrons* group by its moderate size (SVL < 100 mm); and the orange male dewlap with an uninterrupted white margin. *Dactyloa maia* can be differentiated from *D. limon* by its male dewlap coloration which is orange with an uninterrupted white margin (vs. yellowish near the throat and tan on distal portion, or uniformly light tan in *D. limon;* Fig. 4). *Dactyloa maia* further differs from *D. purpurescens, D. limon* and *D. ibanezi* in the color pattern of the flanks as follows: *Dactyloa maia* has oblique rows of turquoise ocelli or oblique dark green bands without sexual dimorphism (Fig. 8); in *D. limon*, males have wide dark green bands on the flanks whereas females have diffuse dark green spots distributed evenly or randomly; males of *D. purpurescens* exhibit oblique rows of ocelli or blotches whereas females have dark green spots arranged in oblique rows; in *D. ibanezi* both sexes exhibit oblique thin black lines. The hemipenis of *D. maia* is a small, unilobate organ (slightly bilobate

in *D. ibanezi;* no information available for *D. limon* and *D. purpurescens*). Also, *Dactyloa maia* differs from *D. purpurescens*, *D. limon*, and *D. ibanezi* in mean values of several morphological characters as follows (mean values for *D. purpurescens*, *D. limon*, and *D. ibanezi* presented in that order): HL/SVL 0.27 in *Dactyloa maia* vs. 0.25, 0.23 and 0.26,.LST 64.7 in *Dactyloa maia* vs. 62.8, 43.7, and 44.5; number of scales between first and second canthals 14.5/12.3 (first canthals/second canthals) in *Dactyloa maia* vs 12.9/10.2, 10/8.6, and 11.5/10.

Description of the holotype. Adult male as indicated by everted hemipenes, a pair of enlarged postcloacal scales, and presence of a large dewlap (Figs. 3E, 4E, 7D, 9); SVL 76 mm; tail length 178 mm (tail complete), tail length/SVL ratio 2.34; tail laterally compressed in cross section, tail height 2.2 mm, tail width 2.0 mm; axilla to groin distance 35.6 mm; head length 19.2 mm, HL/SVL ratio 0.25; snout length 9.3 mm; head width 9.7 mm; longest toe of adpressed hind limb reaching posterior margin of orbit; shank length 20.7 mm, shank length/SVL ratio 0.27, shank length/HL ratio 1.08; tip of longest finger of extended forelimb reaching tip of snout; longest finger of adpressed forelimb not reaching to anterior insertion of hind limbs; prefrontal ridges distinct, parietal ridges conspicuous; scales on snout mostly keeled; 6 postrostrals; 7 scales between nasals; scales in distinct prefrontal depression smooth; supraorbital semicircles differentiated, composed of smooth scales, separated by a minimum of 2 scales; supraorbital disc composed of 7 enlarged smooth scales; two elongated, smooth anterior superciliaries, followed posteriorly by a much smaller, elongate scale; about 5 rows of small keeled scales extending between enlarged supraorbitals and superciliaries; interparietal plate distinct, parietal eye visible; canthal ridge distinct, composed of 3 large (posterior) and 7 small (anterior) canthal scales; 11 scales present between second canthals; 13 scales present between posterior canthals; 78 loreal scales arranged in 7 horizontal rows; subocular scales flat, subocular row well-defined; 9 supralabials to level below center of eye; ear opening 0.73 x 1.6 mm (length x height); mental distinctly wider than long, almost completely divided medially, bordered posteriorly by 6 postmentals; 10 infralabials to level below center of eye; third and fourth sublabials posterior to mental slightly enlarged; keeled granular to elongate scales present on chin and throat; dewlap large, extending well onto body, anterior insertion is about halfway between nose and orbit, posterior insertion at a level between one-third and one half of the distance between axilla and groin, with about 4 gorgetal-sternal rows, each 2–3 scales wide, becoming less regular posteriorly; low nuchal crest present, dorsal crest barely visible; dorsum of body with keeled scales, 1–2 middorsal rows of prominently keeled, but not otherwise enlarged scales; about 50 medial dorsal scales in one HL; about 125 medial dorsal scales between levels of axilla and groin; lateral scales small, long and keeled; ventrals at midbody smooth, subimbricate; about 66 ventral scales in one HL; about 87 ventral scales between axilla and groin; about 164 scales around midbody; caudal scales strongly keeled, without whorls of enlarged scales, subcaudal scales with a single prominent keel; a pair of greatly enlarged postcloacal scales, larger one about 0.88 x 1.90 mm (length x width); tube-like axillary pocket not developed; scales on anterodorsal surface of thigh and on dorsal surface of forearm keeled; digital pads dilated, dilated pad about 3 times width of non-dilated scales under distal phalanx; distal phalanx narrower than and raised from dilated pad; 31/31 (left/right) lamellae under phalanges ii to iv of 4th toe; 11/12 scales under distal phalanx of 4th toe; 21/19 lamellae under phalanges ii to iv of 4th finger; 10/9 scales under distal phalanx of 4th finger.

Hemipenis description: The completely everted hemipenis (Fig. 10) of SMF 97269 is a small, unilobate organ; sulcus spermaticus bordered by well-developed sulcal lips, opening at base of apex into a small concave area; large asulcate processus and ridge present; a prominent fleshy fringe present on each lateral side of truncus; most of apex on asulcate side and distal portion finely calyculate, truncus with transverse folds..

Coloration in life of the holotype. (Fig. 4E) Dorsal and lateral ground color of body and limbs Light Grass Green (109); lateral surface of body with three Parrot Green (121) oblique bands directed backwards as well as downwards, each with six to seven Medium Blue (168) small oval spots; tail with Dark Green (136) transverse bands; eyelids Cream Yellow (82); iris Chrome Orange (74); ventral surfaces dirty white, suffused with Pale Cyan (157); dewlap Light Pratt's Rufous (71) with three well defined longitudinal series of Emerald Green (143) scales, free margin of dewlap dirty white, anterior insertion of dewlap suffused with Beige (254).

Coloration of the holotype after approximately two years of preservation in 70% ethanol. (Fig. 9) Dorsal and lateral ground color of body and limbs Lavender (195); lateral surfaces of body with three oblique rows of small oval Medium Water Blue (182) spots directed backwards as well as downwards; tail with Plumbeous (295) transverse bands; ventral surfaces dirty white, suffused with Medium Blue Gray (194); dewlap Pale Sulfur Yellow (92), its base suffused with Pale Neutral Gray (296).

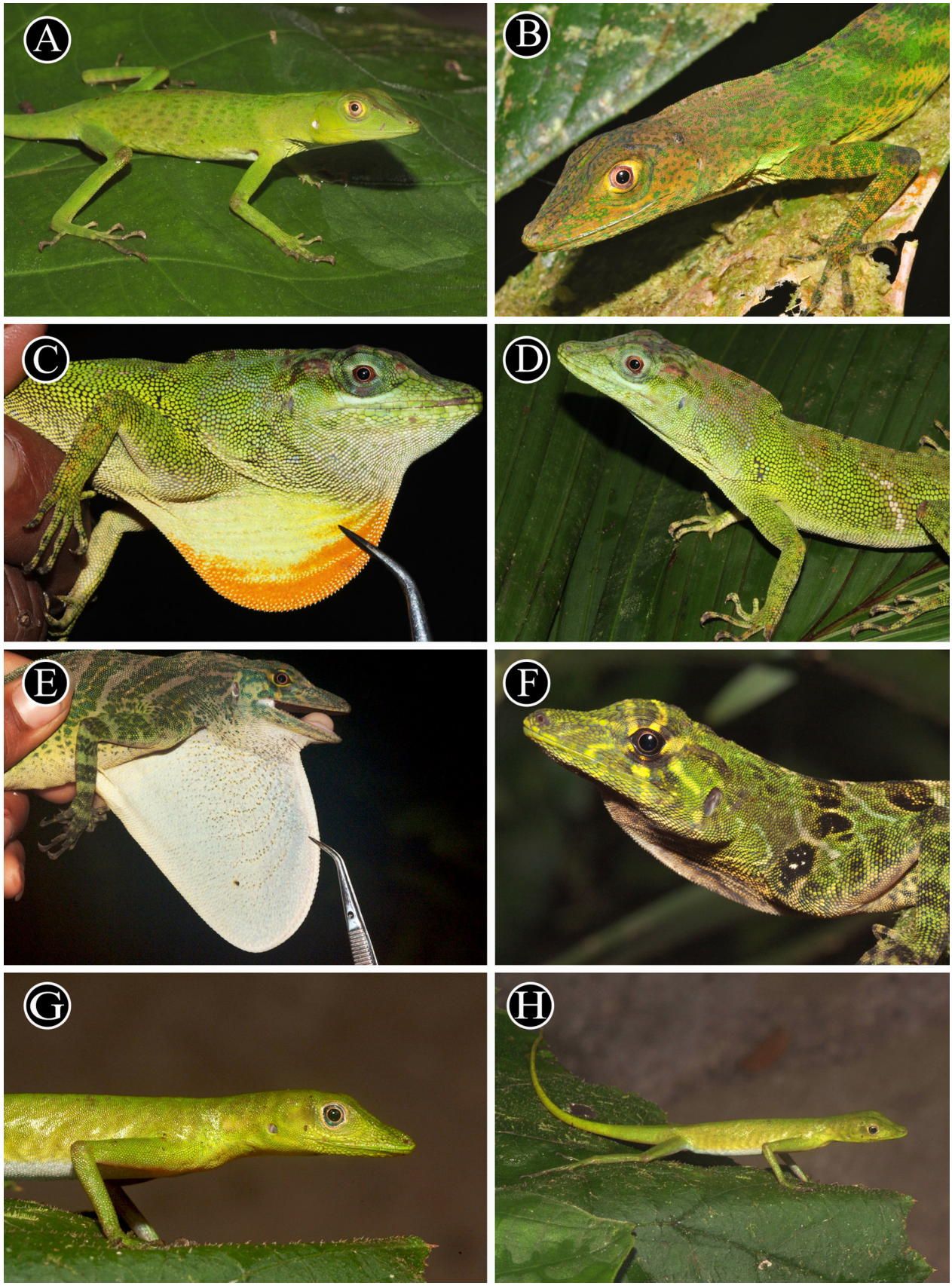

FIGURE 3. Some species of *Dactyloa* found in Eastern Panama. A–B) *D. purpurescens* (female and male, MHCH 2635–36); C–D) *D. kunayalae* (male, SMF 97266); E) *D. frenata* (male, MHCH 2785); F*) D. latifrons* (young male, SMF 96575); G–H) *D. chloris* (female, SMF 97096).

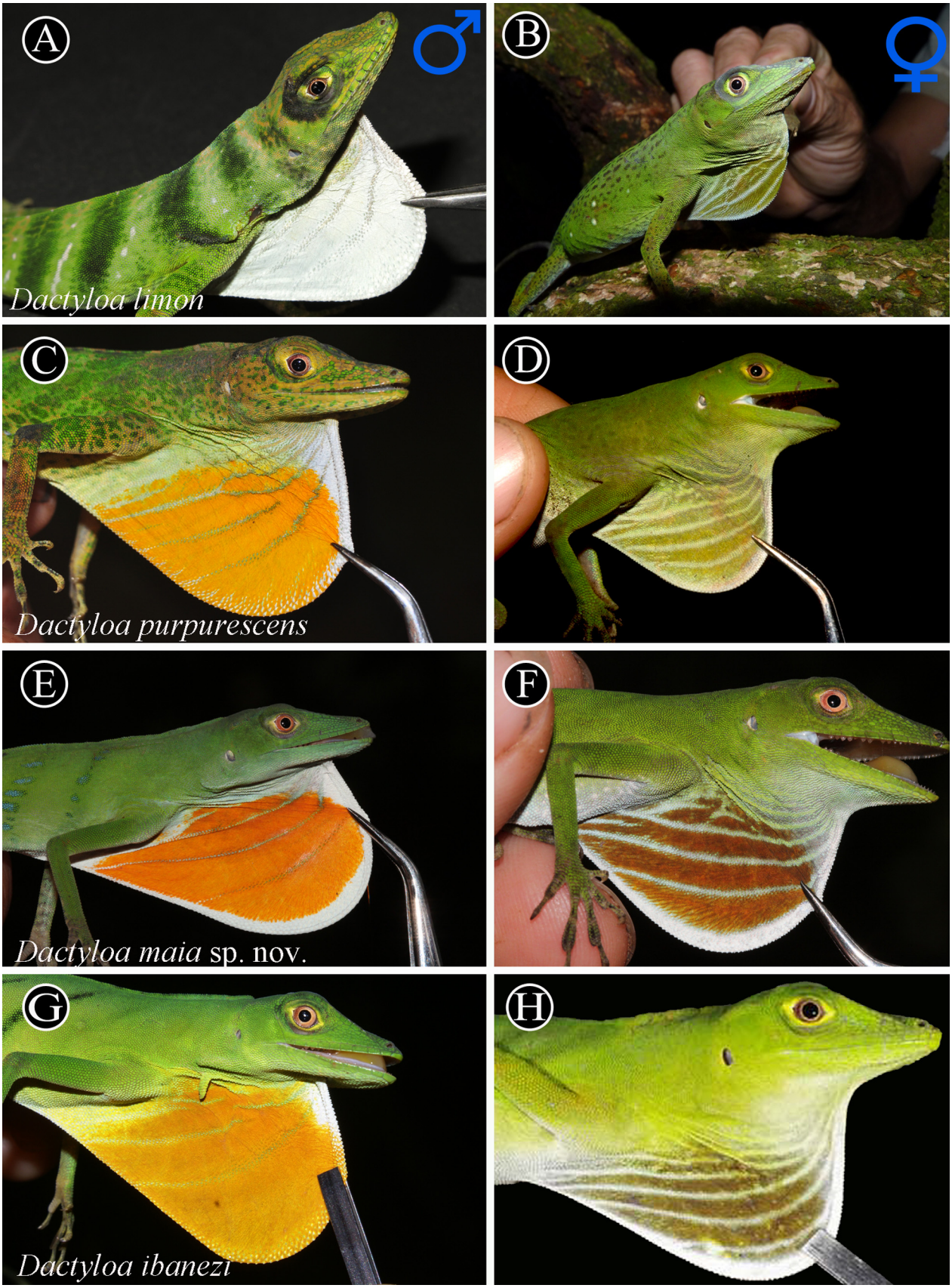

FIGURE 4. Dewlaps of the *Dactyloa* spp. related to *D. purpurescens* (left column males, right column females). A–B) *D. limon* (MHUA 11760; holotype; photo by Juan Pablo Hurtado), female not collected (from; photo by Alejandro Montoya); C–D) *D. purpurescens*, (MHCH 2636, MHCH 2635); E–F) *D. maia* **sp. nov.** (SMF 97268, MHCH 2782); G–H) *D. ibanezi* (MHCH2019, SMF 91475).

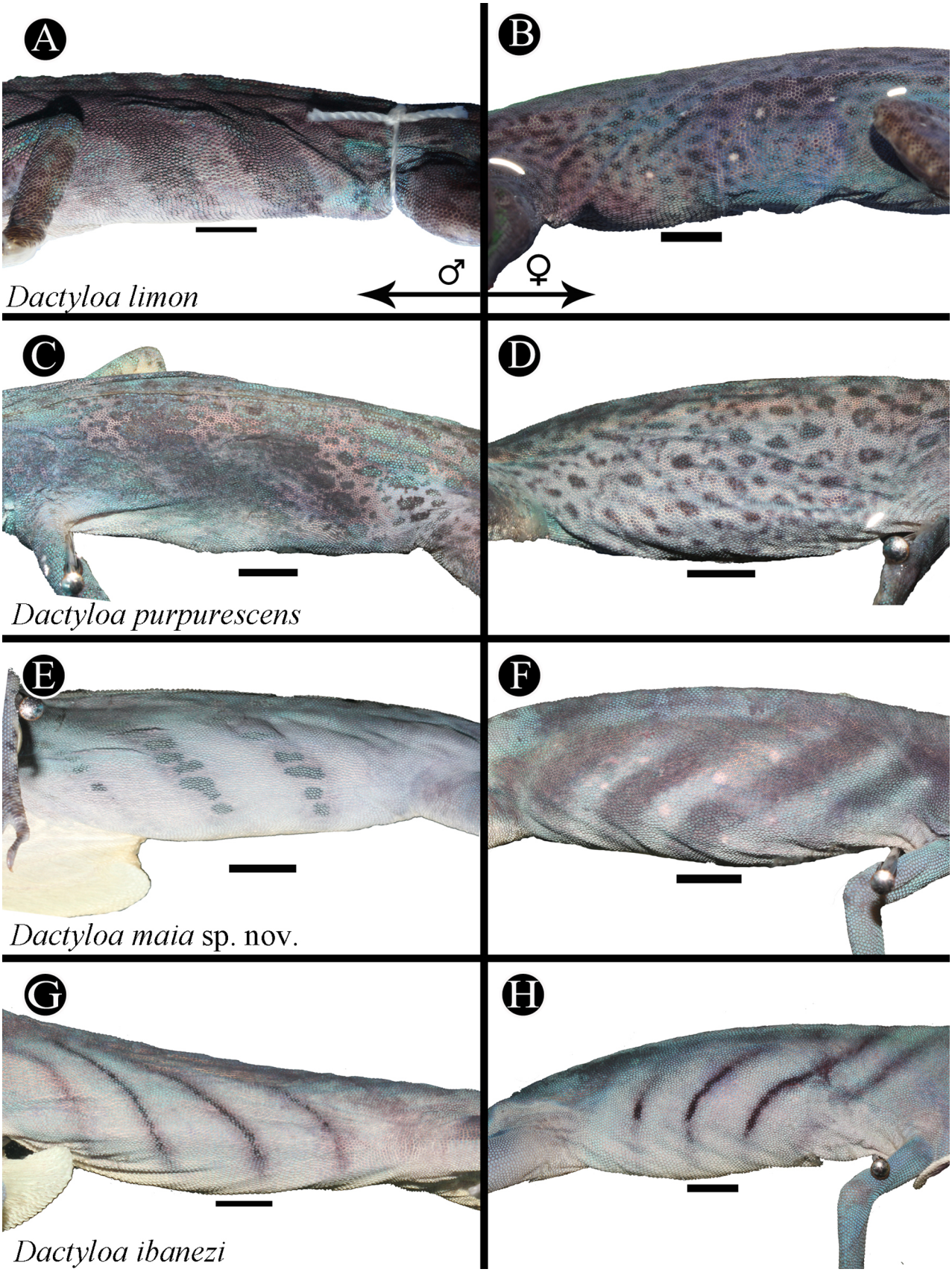

FIGURE 5. Flank pattern in species of *Dactyloa* related to *D. purpurescens*. Arrows indicate capitad direction for all males (left column) and females (right column). A) *D. limon* holotype MHUA 11760; B) D. *limon* MHUA 11248; C) *D. purpurescens* (MHCH 2636); D) *D. purpurescens* (SMF 91475); E) *D. maia* (SMF 97268); F) *D. maia* (MHCH 2782); G) *D. ibanezi*, MHCH 2184; H) *D. ibanezi*, SMF 91475. Scale bars equal 10 mm.

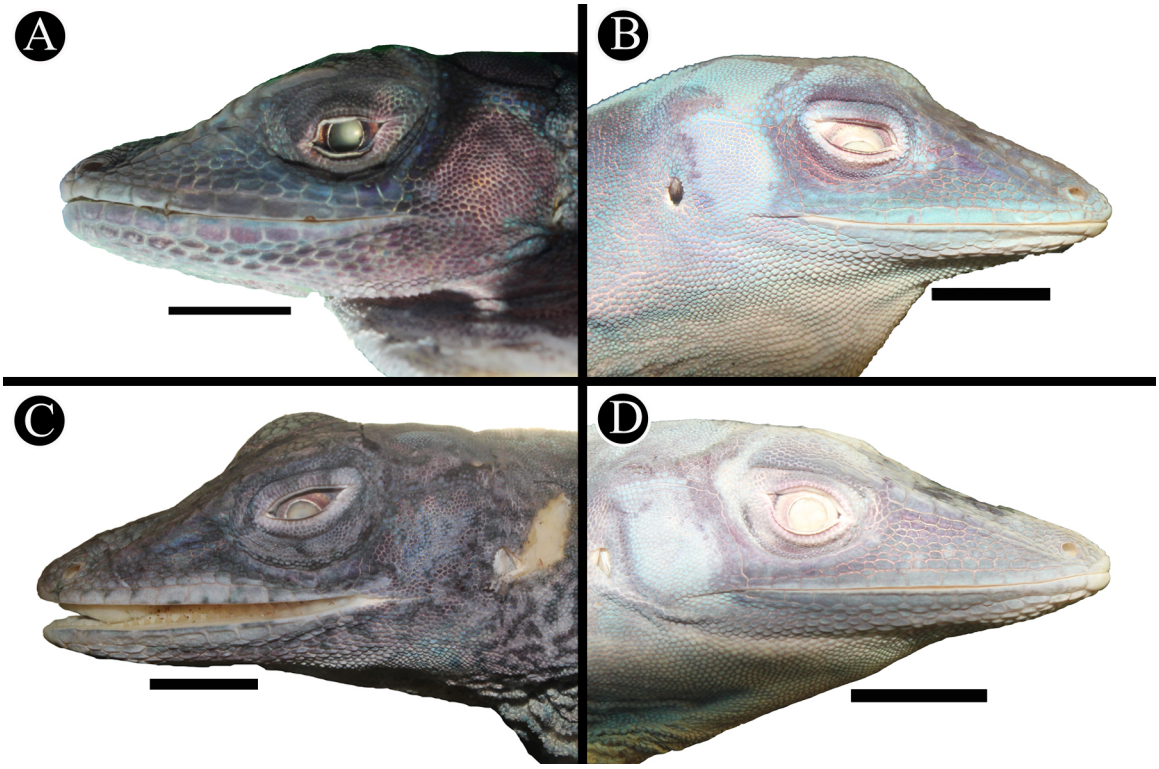

FIGURE 6. Lateral head views of the species of *Dactyloa* related to *D. purpurescens.* A) *D. limon* holotype MHUA 11760; B) *D. ibanezi* MHCH 2184; C) *D. purpurescens* SMF 97271; D) *D. maia* **sp. nov.** holotype SMF 97268. Scale bars equal 10 mm

Color variation. Another male (SMF 97269, 8E–F), at the moment of encounter, was uniform green with bluish spots on flanks arranged in oblique rows; after collection it exhibited metachrosis, and the coloration recorded was as follows: the dorsal ground color of body, head and limbs Salmon Color (58), grading into Yellow Green (103) toward the flanks; tail with Russet (44) transverse bands; four well-defined Olive Green (123) oblique bands between axilla and groin, each including four to seven irregular Cyan Black (153) blotches; ventral surfaces and dewlap as in holotype. A female (MHCH 2782, Figs. 4F, 5F, 8A–B) lacked the small oval spots, and only the three Parrot Green (121) transverse bands were present with three small Sulphur Yellow (80) spots between each band; dewlap Medium Chrome Orange (75) with three well defined longitudinal series of Emerald Green (143) scales, free margin of dewlap dirty white, anterior insertion of dewlap suffused with Light Neutral Gray (297). Another female (SMF 97270; Fig. 8G) agrees in general color pattern with female MHCH 2782. A third female (MHCH 2781; Fig. 8C–D) has the same dorsal ground color as the holotype, but with six well defined Greenish Olive (125) oblique bands in the flanks (without spots); dewlap Olive (126), with three well defined longitudinal series of Lime Green (116) scales, anterior and external border of dewlap dirty white, anterior insertion of dewlap suffused with Light Neutral Gray (297).

Distribution and Natural history. As far as we know, *Dactyloa maia* is endemic to eastern Panama, inhabiting the foothills and ridges of the Majé, San Blas, Darién (as far south and east as to Río Tuquesa, thereafter apparently replaced by *D. purpurescens*), and Piedras-Pacora (two specimens photographed by Angel Sosa-Bartuano, not plotted in Fig. 1) mountain ranges, where it occurs in the eastern Panamanian montane forest (Fund 2014) and the Isthmian-Atlantic moist forests (in the Piedras-Pacora and San Blas mountain range, Hogan & Fund 2014), at 322–852 m asl. All specimens of *Dactyloa maia* were found during night searches sleeping on branches or leaves 2 to 3 m above the ground.

Etymology. Abel Batista dedicates this beautiful new species to his recently born daughter, Maia. The name also comes from Greek mythology, where it is applied to the eldest of the Pleiades, sometimes called mountain nymphs, and are believed to live on the trees in mountains and groves as the guardians for that habitat, a role *Dactyloa maia* could also represent.

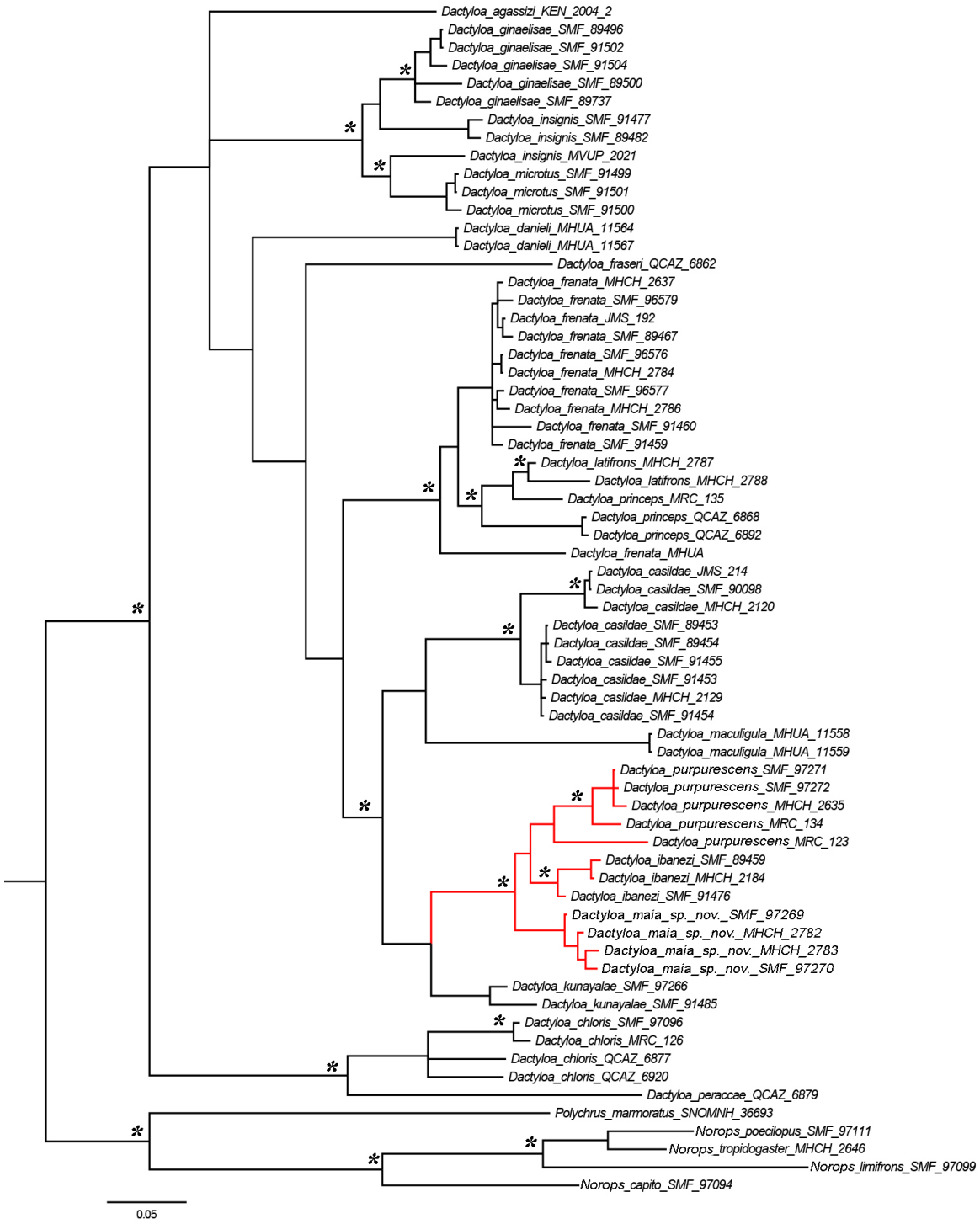

FIGURE 7. Bayesian consensus tree of the genus *Dactyloa* based on 16S and COI mtDNA. Outgroups: *Norops capito*, *Norops limifrons*, *Norops tropidogaster*, *Norops poecilopus* and *Polychrus marmoratus*. Asterisks on nodes indicate estimated posterior probabilities $P \geq 0.90$. Scale bar refers to substitutions per site.

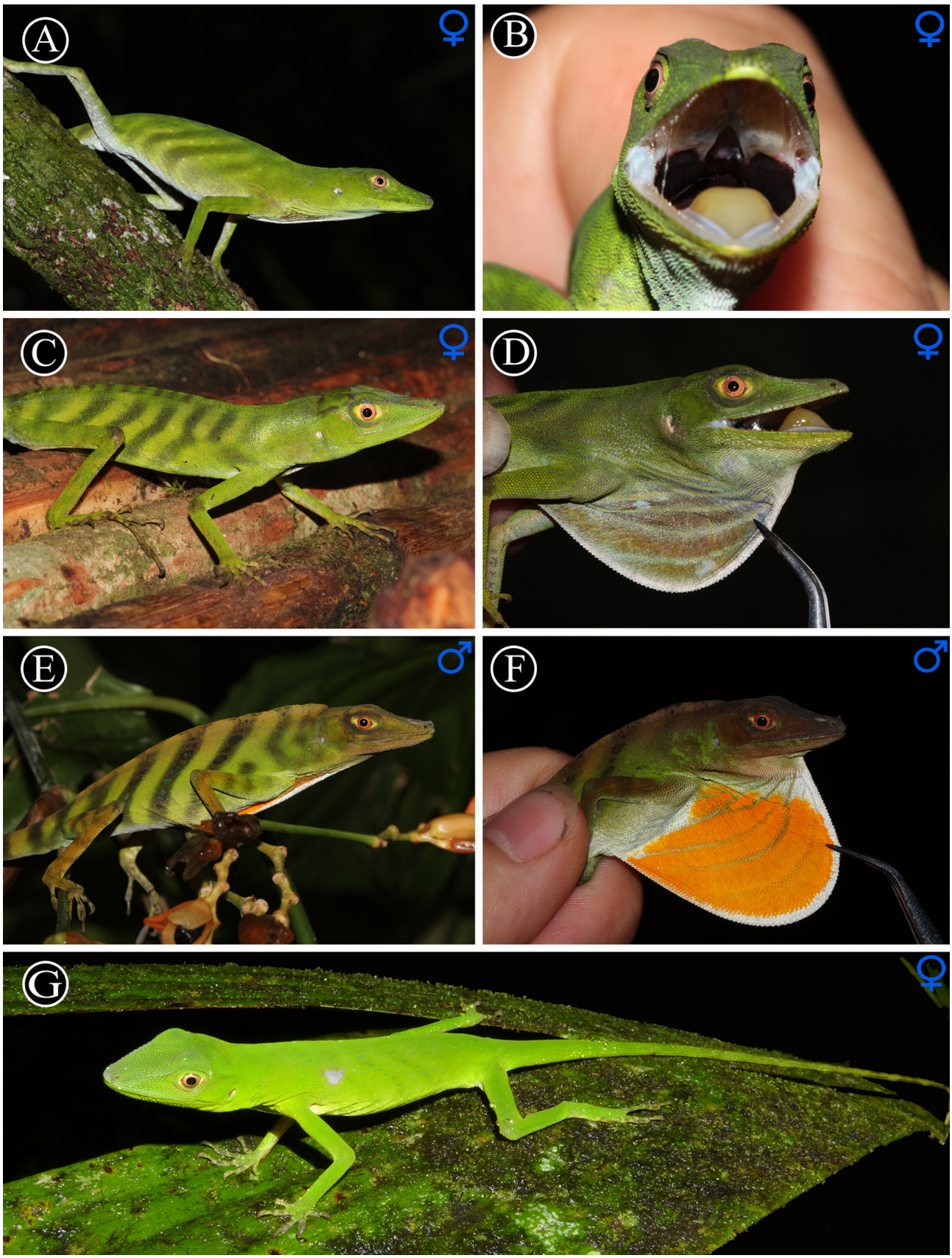

FIGURE 8. Color variation of *Dactyloa maia* **sp. nov.** in life. A–B) female MHCH 2782; C–D) female MHCH 2781; E–F) male SMF 97269; G) female SMF 97270.

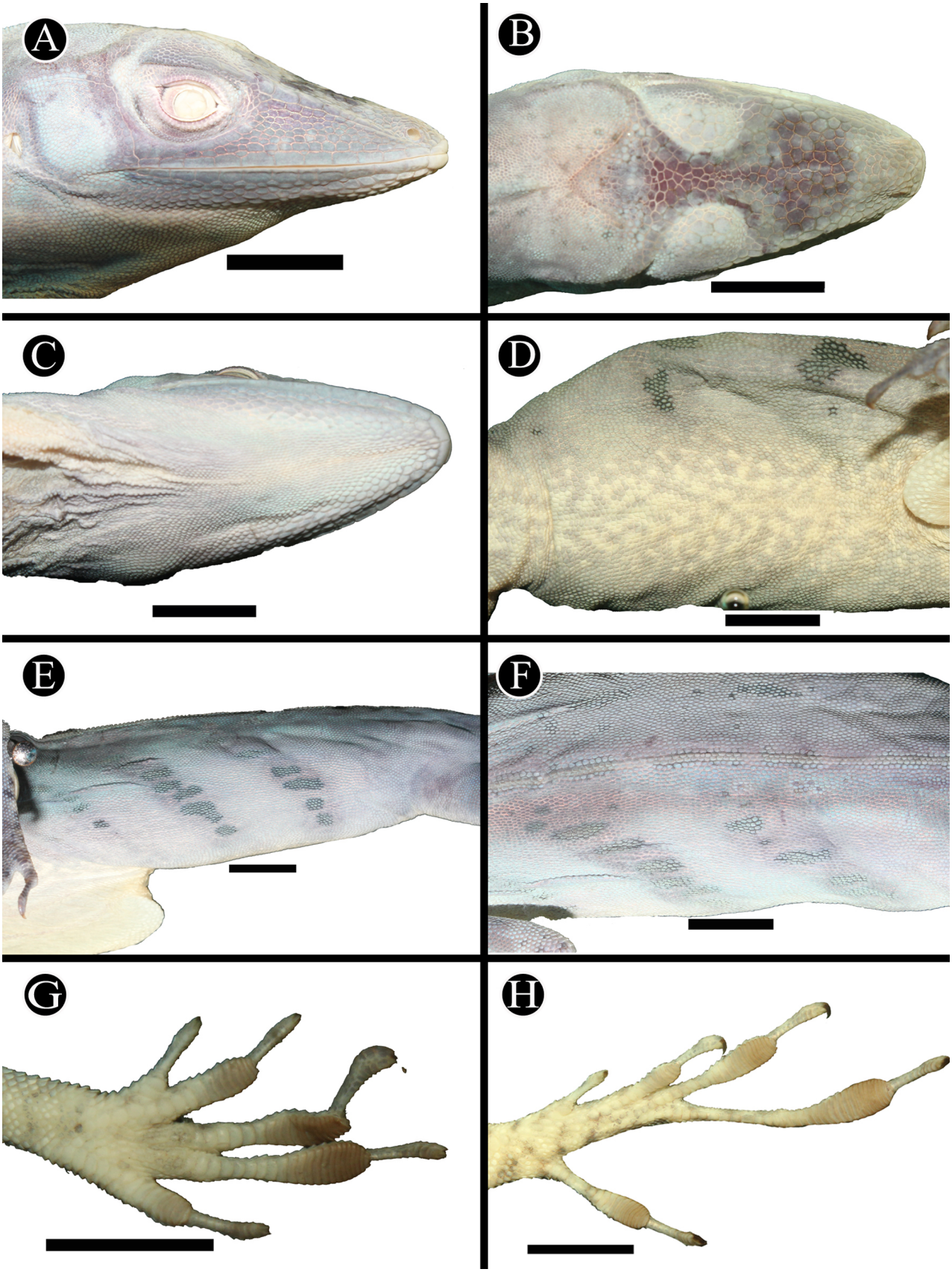

FIGURE 9. *Dactyloa maia* **sp. nov.** holotype (SMF 97268), preserved specimen. A) head, laterally; B) head, dorsally; C) head, ventrally; D) venter; E) dorsum; F) flank; G) left hand; H) left foot.

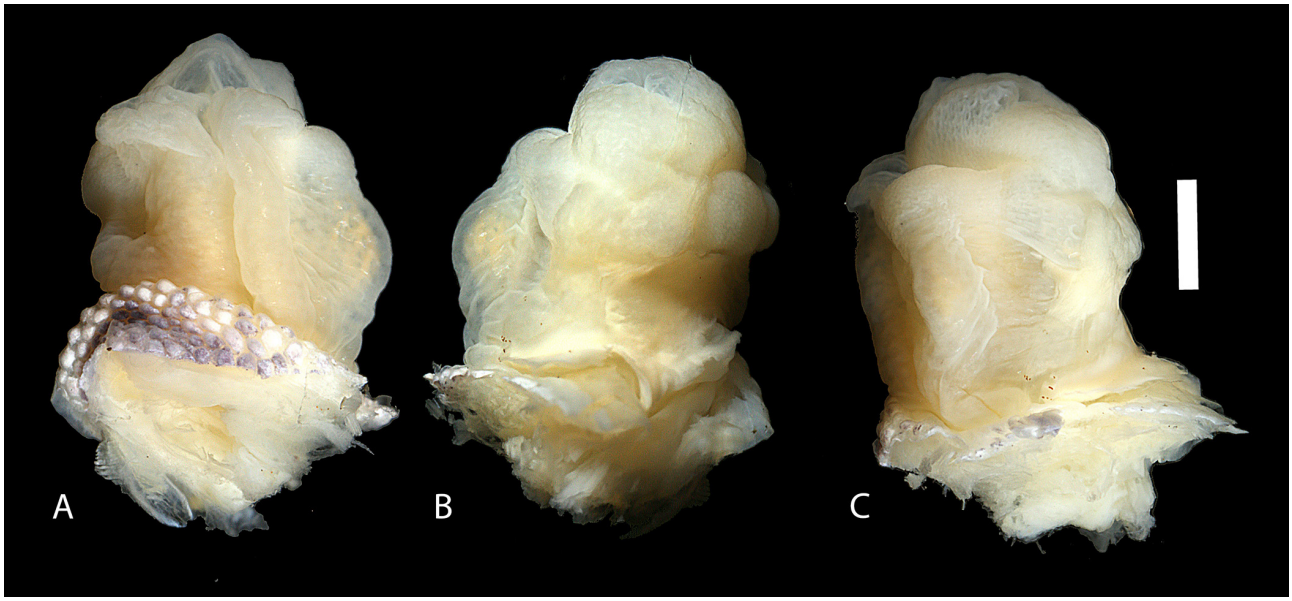

FIGURE 10. Hemipenis of the Paratype (SMF 97269) of *Dactyloa maia* **sp. nov.** A) Sulcate view; B) asulcate view; C) lateral view.

Discussion

Recently, Velasco & Hurtado-Gómez (2014) described *Dactyloa limon,* a species closely related to *D. purpurescens*. In the course of this study we collected two species of *Dactyloa* related to *D. purpurescens, D. limon*, and *D. ibanezi,* and in the beginning we thought that they were *D. ibanezi* and *"D. chocorum"*. However, clarifying the taxonomic identity of *D. chocorum* and *D. purpurescens* by realizing that the former actually is a junior synonym of the latter was the key to correctly assigning species names to the newly collected specimens. Evidence from biogeography and morphology (dewlap color, flank pattern, pholidosis) clearly assigned the name *D. purpurescens* to specimens collected in the Cerro Pirre and Jingurudó mountain ranges. The description of *D. maia* brings the number of species related to *D. purpurescens* to a total of four (*i.e., D, ibanezi, D. limon, D. maia,* and *D. purpurescens*). These four species show a parapatric distribution pattern with no known overlap among each other: *D. limon* in the Magdalena Valley, at the northeastern slopes of the Central Andes in Colombia (1000–1800 m asl), *D. purpurescens* in the biogeographical Chocoan region (20–1400 m asl) in the southern part of eastern Panama, and in the northwestern of Colombia and Ecuador; *Dactyloa maia* in the northern part of eastern Panama, along the foothills and ridges of the main mountains in that region (322–852 m asl), and finally *D. ibanezi* from central Panama to Costa Rica (400–1070 m asl; Poe *et al.* 2009; Lotzkat *et al.* 2013). We are convinced that the specimens POE 2144 (from the misspelled "Nasugundi" = Nusagandí Road) and AMNH 110568 (from "Km 12.8 on El Llano-Carti" Road), both reported as *Anolis chocorum* by Poe *et al.* (2009), are actually representatives of *D. maia* because we collected the latter species in this area (SMF 97270) and, based on the material we examined, *D. purpurescens* is absent from the San Blas mountain range (see Fig. 1). All other specimens listed as *Anolis chocorum* in the Appendix I of Poe *et al.* (2009) are from the southern portion of eastern Panama (KU 96931; MCZ 85246–7, POE 1944; as well as the specimens KU 113110–1 from Cerro Sapo and KU 96932–3 from Cerro Quía that were not plotted on their map) or Colombia (MCZ 64256, 77457, 115732, 124404; AMNH 18235) and should represent *D. purpurescens* according to the biogeographic scenario revealed herein.

Recent phylogenetic analyses have placed *Dactyloa purpurescens* and *D. ibanezi* in the *Dactyloa* clade within the *D. latifrons* series (Castañeda & de Queiroz 2013). Our molecular analyses support these assignments although no morphological synapomorphies are known that support the inclusion of the four species treated in this contribution (i.e., *D. ibanezi, D. limon, D. maia*, and *D. purpurescens*) in the *D. latifrons* series or species group (sensu Castañeda & de Queiroz 2013 or Nicholson *et al*. 2012, respectively). This work, along with the revision by Lotzkat *et al.* (2013), considerably complements the hitherto patchy distributional and specimen records of all species of *Dactyloa* found in Panama.

Key to the species of *Dactyloa* occurring in Panama (modified from Lotzkat *et al.* 2013)

1a. Large species SVL ≥ 95 mm, body robust, body pattern different: with broad bars, blotches, spots, or ocelli; if homogeneously green, then without narrow dark oblique lines on flanks 2

1b. Relatively small species SVL ≤ 88 mm, body slender, body uniform green (turning bluish in preservative) or with a parallel series of oblique narrow dark lines, broad bands, or disperse spots on flanks 8

2a. Legs short: tip of fourth toe of hind limb (adpressed along straightened specimen) reaching at most to posterior border of eye, shank length/SVL ratio < 0.24; suboculars and supralabials in contact 3

2b. Legs long: tip of fourth toe of hind limb (adpressed along straightened specimen) reaching at least to center of eye, usually to a point anterior to eye, shank length/SVL ratio 0.25 or greater; suboculars and supralabials in contact or separated by one scale row. 6

3a. At least one, often two or more, sublabials posterior to mental plate greatly enlarged; four or fewer horizontal rows of loreal scales; interparietal plate usually not distinct, usually no visible parietal eye; dorsal scales about the size of ventral scales; all scales on anterodorsal surface of thigh multicarinate; ear opening very small, less high than supralabials and infralabials together; 41–50 lamellae under fourth toe (base of digit to claw), 29–37 under fourth finger; male and female dewlap salmon, pink, or yellow. 4

3b. Sublabials not greatly enlarged, less high than adjacent infralabials; five or more horizontal rows of loreal scales; interparietal plate usually distinct, with visible parietal eye; dorsal scales smaller than ventral scales; most scales on anterodorsal surface of thigh smooth or unicarinate; ear opening moderate to large, higher than supralabials and infralabials together; numbers of lamellae under fourth toe and fourth finger higher or lower than that in 3a; dewlap coloration different 5

4a. Legs short: tip of fourth toe of hind limb (adpressed along straightened specimen) reaching to a point between anterior border of ear opening and posterior border of eye, shank length/SVL ratio 0.19–0.22; conspicuous and clear-cut coloration pattern between eye and shoulder, with a prominent light stripe extending from supralabials posteriorly above or across the ear before bending down towards shoulder, delineating a dark preaxillary blotch above and posteriorly, and paralleled above by a dark postorbital stripe with darker borders that extends at least to a level above the preaxillary blotch . . . . . *Dactyloa ginaelisae*

4b. Legs very short: tip of fourth toe of hind limb (adpressed along straightened specimen) reaching to a point between shoulder and ear, shank length/SVL ratio < 0.183; pattern of stripes between eye and shoulder more diffuse, with the light postsupralabial stripe not passing above or across the ear, and just as the dark postorbital stripe being oriented more ventrally, both losing their conspicuousness around level of ear. . . . . .*Dactyloa microtus*

5a. Subdigital lamellar pads strongly dilated, more than three times width of distal phalanx; more than 50 lamellae under fourth toe (base of digit to claw); 190 or more scales around midbody; scales on anterodorsal surface of thigh smooth, only on anterior edge unicarinate with a few bi- or tricarinate; male dewlap orange or red, female dewlap brownish, mottled . . . . . .*Dactyloa insignis*

5b. Subdigital lamellar pads barely dilated, less than two times width of distal phalanx; 35 or fewer lamellae under fourth toe (base of digit to claw); fewer than 150 scales around midbody; scales on anterodorsal surface of thigh unicarinate; male dewlap white with an orange-yellow margin; female dewlap white with a light yellow margin . . . . . *Dactyloa kunayalae*

6a. Series of dark blotches or ocelli form oblique transverse bands on dorsum, flanks, limbs, and anterior portion of tail; a pronounced light-colored interorbital bar with dark anterior and posterior borders; light and dark stripes radiating from eye in all directions; male dewlap cream white, female dewlap brown 7

6b. Coloration variable: unicolor, mottled, or with broad dark transverse bands; no pronounced interorbital bar; a dark pre- and/or postorbital stripe often present, but no light and dark stripes radiating from eye in all directions; male dewlap white with yellow and blue or green scales, female dewlap with contrasting yellow and green or blue striped or reticulate pattern . . . . . *Dactyloa casildae*

7a. Enlarged superciliaries restricted to anterior half of eye, followed by small granular scales posteriorly, not forming a stiff ridge . . . . . *Dactyloa frenata*

7b. All of upper margin of eye with enlarged superciliaries forming a stiff ridge . . . . . *Dactyloa latifrons*

8a. Relatively tiny species SVL ≤ 60 mm, body uniform green, ventrals keeled; short-legged—4th toe of adpressed hind limb does not reach to eye . . . . . *Dactyloa chloris*

8b. Somewhat larger species SVL ≥ 60 mm, body green, with a parallel series of oblique narrow dark lines, broad bands or disperse spots on flanks; ventrals smooth; long-legged—4th toe of adpressed hind limb reaches at least to anterior border of eye .9

9a. Flanks with a parallel series of oblique broad bands that are usually formed by ocelli; usually with 50–80 total loreal scales; species distributed in eastern Panama 10

9b. Flanks with a parallel series of oblique narrow dark lines that are never formed by blotches or ocelli; usually with 38–54 total loreal scales; species distributed in central and western Panama . . . . . .*Dactyloa ibanezi*

10a. Flanks in males with oblique rows of ocelli or blotches, in females with scattered dark green spots; male dewlap orange with white anterior margin; 12–14 (mean 12.9) scales between first canthals and 7–13 (mean 10.2) between second canthals; species distributed in the border between Panama and Colombia, in the mountain ranges of Pirre, and Jingurudó, and the southern corner of the Darién mountain range . . . . . *Dactyloa purpurescens*

10b. Flanks in males and females with rows of ocelli in oblique broad dark green bands; male dewlap orange with continuous white margin; 12–18 (mean 14.5) scales between first canthals and 10–16 (mean 12.3) between second canthals; species distributed in eastern Panama in the mountain ranges of Majé, Piedras-Pacora, and San Blas, and the central and northern portions of the Darién mountain range . . . . . *Dactyloa maia*

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APPENDIX I. Collecting data and GenBank accession numbers of the specimens included in the morphological and/or molecular analyses. Information is based on specimens collected for this study, literature and online databases (http://www.vertnet.org/).

Species	Collection No.	Country	Province	Locality	N	W	Elev.	16S	COI
D. agassizi	KEN 2004 2	Colombia		Colombia, Malpelo Island				JN112722	
D. casildae	JMS 214	Panama	Chiriquí	Panama, Chiriquí, near STRI Fortuna Biological Station					JN112726
D. casildae	MHCH 2120	Panama	Chiriquí	W slope Cerro Pata de Macho	8.6719	82.1997	1420	JX083229	
D. casildae	MHCH 2129	Panama	Comarca Ngöbe-Buglé	Río Flores	8.5209	81.7785	1220		
D. casildae	SMF 89453	Panama	Comarca Ngöbe-Buglé	W slope Cerro Santiago, La Nevera	8.5000	81.7722	1600	JX083230	
D. casildae	SMF 89454	Panama	Comarca Ngöbe-Buglé	W slope Cerro Santiago, La Nevera	8.5000	81.7722	1600		
D. casildae	SMF 90098	Panama	Chiriquí	Reserva Forestal Fortuna, Cerro Guayabo	8.7744	82.2398	1720	JX083228	
D. casildae	SMF 91453	Panama	Comarca Ngöbe-Buglé	Río Flores	8.5209	81.7785	1220	JX083231	
D. casildae	SMF 91454	Panama	Comarca Ngöbe-Buglé	Río Flores	8.5209	81.7785	1220		
D. casildae	SMF 91455	Panama	Comarca Ngöbe-Buglé	W slope Cerro Santiago, La Nevera	8.5000	81.7722	1600	JX083232	
D. chloris	KUH 113109	Panama	Darién	Rio Jaqué, 1.5 Km above Rio Imamado	7.4289	77.9650	150		
D. chloris	KUH 76026	Panama	Darién	Tacarcuna	8.1558	77.2892	1700		
D. chloris	KUH 96930	Panama	Darién	Río Tuira at Río Mono	7.6835	77.5525	184		
D. chloris	MRC 126	Colombia	Quibdo	Colombia, Chocó, Quibdó, Tutunendo					JN112729
D. chloris	QCAZ 6877	Ecuador		Ecuador, Pichincha, La Unión del Toachi, Centro de Interpretación Ambiental Otongachi, Otonga					JN112727
D. chloris	QCAZ 6920	Ecuador	Esmeraldas	Ecuador, Esmeraldas, San Lorenzo, grounds of Hosteria Tundaloma					JN112728

……*continued on the next page*

APPENDIX 1. (Continued)

Species	Collection No.	Country	Province	Locality	N	W	Elev.	16S	COI
D. chloris	SMF 97096	Panama	Darién	Cana field station, Setengati trai.	7.7560	77.6857	525		KP975524
D. danieli	MHUA 11564	Colombia	Antioquia	Colombia, Antioquia, Anorí, Cañadahonda					JN112732
D. danieli	MHUA 11567	Colombia	antioquia	Colombia, Antioquia, Anorí, Cañadahonda					JN112733
D. fraseri	QCAZ 6862	Ecuador		Ecuador, Pichincha, Mindo, on road to Mindo Garden at Muyu Mindala Hostal					JN112743
D. frenata	"AM, Dunn, 1937"	Panama	Darién	Three Falls Creek	8.1560	77.7020	26		
D. frenata	"US, Dunn, 1937"	Panama	San Blas	Sperdi Hills	8.6500	77.4000	482		
D. frenata	CM 170033	Panama	San Blas	Summit Camp	8.9200	77.8500	320		
D. frenata	FMNH 170033	Panama	San Blas Territory	border of Darien, Summit camp & site	8.9200	77.8500	350		
D. frenata	FMNH 170037	Panama	San Blas Territory	border of Darien, Summit camp & site	8.9200	77.8500	350		
D. frenata	JMS 192	Panama	Chiriquí	Panama, Chiriquí, near STRI Fortuna Biological Station					JN112745
D. frenata	MCZ 127717	Panama	Panamá	Cerro Campana	8.6850	79.9240	847		
D. frenata	MCZ R127717	Panama	Panama	Panama: Cerro Campana: Panama	8.6850	79.9240	800		
D. frenata	MCZ R131614	Panama	Canal Zone	Barro Colorado	9.1550	79.8480	178		
D. frenata	MHCH 2637	Panama	Colón	Petaquilla	8.9658	80.6250	59 m		KP975527
D. frenata	MHCH 2784	Panama	Emberá-Wounáan	Bajo pequeño, camp2 Pechito parao	8.4830	77.5666	211		KP975518
D. frenata	MHCH 2785	Panama	Panamá	Serrania de Maje, Ambroya	8.8926	78.5635	739		
D. frenata	MHCH 2786	Panama	Guna Yala	stream from camp2 (Yarculup)	9.0611	77.9797	340	KP975531	KP975521
D. frenata	MHUA 11519	Colombia	Antioquia	Colombia, Antioquia, San Luís, Río Claro, El Refugio Natural Reserve					JN112746
D. frenata	SMF 89467	Panama	Veraguas	PNSF: Cerro Mariposa	8.5070	81.1139	870		
D. frenata	SMF 91459	Panama	Veraguas	PNSF: Cerro Mariposa	8.5117	81.1216	900	JX083235	
D. frenata	SMF 91460	Panama	Comarca Ngöbe-Buglé	BPPS, Willie Mazú	8.7891	82.1994	700	JX083234	

……*continued on the next page*

APPENDIX 1. (Continued)

Species	Collection No.	Country	Province	Locality	N	W	Elev.	16S	COI
D. frenata	SMF 96576	Panama	Darién	camp. 3 arriba de rio pucuro	8.0496	77.3695	830		KP975515
D. frenata	SMF 96577	Panama	Panama	Amborlla, cerro la Javillosa	8.9168	78.6178	485		KP975520
D. frenata	SMF 96578	Panama	Panama	Amborlla, cerro la Javillosa filo	8.9227	78.6253	852		
D. frenata	SMF 96579	Panama	Guna Yala	Burbayar la cascada trail	9.3184	79.0027	360		KP975522
D. frenata	UF 33470	Panama	Panamá	Madrona, 8 km NNW Chepo	9.2350	79.1210	235		
D. frenata	UF 33477	Panama	Panamá	El Aguacate, 500600 m	8.9360	79.9870	550		
D. ginaelisae	SMF 89496	Panama	Comarca Ngöbe-Buglé	W slope Cerro Santiago, La Nevera	8.4997	81.7724	1700	JX083226	
D. ginaelisae	SMF 89500	Panama	Chiriquí	Reserva Forestal Fortuna, W slope Cerro Pata de Macho	8.6793	82.1930	1700		
D. ginaelisae	SMF 89737	Panama	Chiriquí	Parque Nacional Volcán Barú, Bajo Mono, Sendero La Cascada	8.8263	82.4989	1830	JX083225	
D. ginaelisae	SMF 91502	Panama	Comarca Ngöbe-Buglé	Cerro Saguí, above Quebrada Juglí	8.5636	81.8217	1960		
D. ginaelisae	SMF 91504	Panama	Comarca Ngöbe-Buglé	Cerro Saguí, above Quebrada Juglí	8.5576	81.8262	1710	JX083227	
D. ibanezi	MHCH 2019	Panama	Colón	Donoso, Colon					
D. ibanezi	MHCH 2184	Panama	Veraguas	Parque Nacional Santa Fé: Cerro Mariposa	8.5117	81.1216	900		
D. ibanezi	SMF 89459	Panama	Veraguas	Parque Nacional Santa Fé: Cerro Mariposa	8.5100	81.1166	880	JX083236	
D. ibanezi	SMF 91476	Panama	Comarca Ngöbe-Buglé	Bosque Protector Palo Seco, Willie Mazú	8.7902	82.2011	730	JX083237	
D. insignis	FM 170087	Panama	San Blas	Paradise Camp	8.9170	77.8830	200		
D. insignis	FMNH 170087	Panama	Darién	Paradise camp	8.9167	77.8833	140		
D. insignis	KUH 113127	Panama	Darien	Cerro Cituro, Pirre mountain range	7.8632	77.7053	1200		
D. insignis	KUH 113128	Panama	Darien	Ridge btw Rio Jaque & Rio Imamado	7.4300	77.9722	154		

……*continued on the next page*

APPENDIX 1. (Continued)

Species	Collection No.	Country	Province	Locality	N	W	Elev.	16S	COI
D. insignis	MCZ 16297	Panama	Darién	Mt. Sapo, eastern Panama	7.9650	78.3520	762		
D. insignis	MCZ 16297	Panama	Darien	Cerro Sapo	7.9650	78.3520	762		
D. insignis	MVUP 2021	Panama	Chiriquí	Panama, Chiriquí, Reserva Forestal Fortuna					JN112756
D. insignis	SMF 89482	Panama	Veraguas	PNSF: Cerro Mariposa	8.5100	81.1166	880	JX083223	
D. insignis	SMF 91477	Panama	Comarca Ngöbe-Buglé	BPPS, Willie Mazú	8.7885	82.2016	750	JX083224	
D. insignis	UF 33487	Panama	Panamá	El Aguacate	8.9360	79.9870	550		
D. kunayalae	FMNH 170034	Panama	San Blas Territory	border of Darien, Summit site	8.9200	77.8500	320		
D. kunayalae	SMF 91485	Panama	Comarca Ngöbe-Buglé	Río Hacha	8.5503	81.7638	970	JX083233	
D. kunayalae	SMF 97266	Panama	Emberá-Wounáan	Bajo pequeño, camp3 Pechito parao	8.4800	77.5194	859		
D. kunayalae	USNM 521924	Panama	Comarca de San Blas or Kuna Yala	Nusagandi	9.3411	78.9942	368		
D. latifrons	MCZ R17178	Panama	Darien	E.Panama,Rio Esnape,Sambu Valley	8.6000	78.1600	374		
D. latifrons	MHCH 2787	Panama	Darién	Serrania de Pirre.	7.9778	77.7086	1109 m		KP975514
D. latifrons	MHCH 2788	Panama	Emberá-Wounáan	Pavarandó, Camp 3 cerro garra garra	7.7640	78.1006	655		KP975513
D. latifrons	MHCH 2789	Panama	Darién	Camp2 (ridge 1300); Rancho Frío Field station	7.9594	77.7044	1182		
D. latifrons	Not collected	Panama	Darién	Quebrada Casa Vieja, Cerro Sapo	7.9793	78.3832	250		
D. latifrons	Not collected	Panama	Darién	Cerro Sapo	7.9819	78.3705	800		
D. latifrons	SMF 96574	Panama	Darién	Serrania de Pirre.	7.9648	77.7055	1245		
D. latifrons	SMF 96575	Panama	Darién	Serrania de Pirre.	7.9741	77.7078	1152		
D. maculigula	MHUA 11558	Colombia	Antioquia	Colombia, Antioquia, Frontino, Cuevas Peñitas, Don Luis property					JN112761

……*continued on the next page*

APPENDIX 1. (Continued)

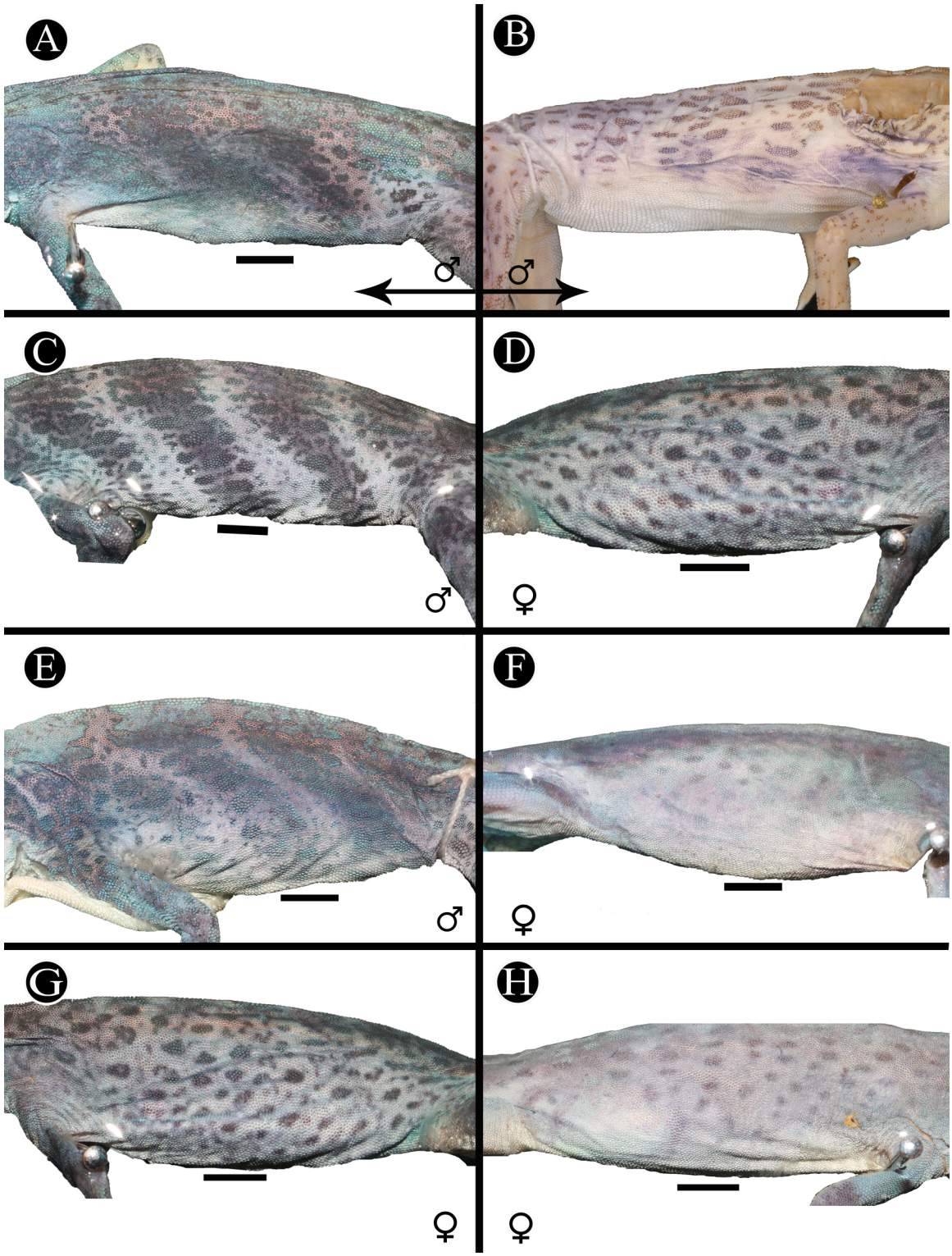
Species	Collection No.	Country	Province	Locality	N	W	Elev.	16S	COI
D. maculigula	MHUA 11559	Colombia	Antioquia	Colombia, Antioquia, Frontino, Cuevas Peñitas, Don Luis property					JN112762
Dactyloa maia	SMF 97269	Panama	Panama	Amborlla, cerro la Javillosa filo	8.9227	78.6253	852		KP975511
D. maia	MHCH 2782	Panama	Guna Yala	from top of Ridge (Yarbir) to camp 2,	9.0614	77.9796	344		KP975517
D. maia	MHCH 2783	Panama	Emberá-Wounáan	Bajo pequeño, camp3 Pechito parao quebrada muestreo entre 730850	8.4791	77.5280	718		KP975528
D. maia	SMF 97267	Panama	Darién	camp. 1 rio pucuro	8.0280	77.4130	196		
D. maia	SMF 97268	Panama	Guna Yala	from top of Ridge (Yarbir) to camp 2,	9.0614	77.9796	344		
D. maia	MHCH 2781	Panama	Emberá-Wounáan	Bajo pequeño, camp3 Pechito parao quebrada muestreo entre 730850	8.4791	77.5280	718		
D. maia	SMF 97270	Panama	Guna Yala	Burbayar la cascada trail	9.3158	79.0058	322		KP975516
D. microtus	SMF 91499	Panama	Bocas del Toro	Parque Internacional la Amistad, Río Changena	8.9785	82.6901	1640	JX083221	
D. microtus	SMF 91500	Panama	Bocas del Toro	Parque Internacional la Amistad, Río Changena	8.9785	82.6901	1640	JX083220	
D. microtus	SMF 91501	Panama	Bocas del Toro	Parque Internacional la Amistad, Río Changena	8.9785	82.6901	1640	JX083222	
D. peraccae	QCAZ 6879	Ecuador	Esmeraldas	Ecuador, Esmeraldas, Mache Chindú Reserve, Bilsa Biological Station					JN112764
D. princeps	MRC 135	Colombia	Quibdo	Colombia, Chocó, Bajo Baudó, Pilizá					JN112768
D. princeps	QCAZ 6868	Ecuador	Esmeraldas	Ecuador, Esmeraldas, Mache Chindú Reserve, Bilsa Biological Station					JN112766
D. princeps	QCAZ 6892	Ecuador	Los Ríos	Ecuador, Los Ríos, Centro Científico Río Palenque					JN112767
D. purpurescens	MCZ R85246	Panama	Darien	Panama: Rio Tuira at Rio Mono Darien	7.6750	77.5710	130		
D. purpurescens	MHCH 2635	Panama	Darién	Orilla de Rio paca.	7.9455	77.6274	140		KP975525

……*continued on the next page*

APPENDIX 1. (Continued)

Species	Collection No.	Country	Province	Locality	N	W	Elev.	16S	COI
D. purpurescens	MHCH 2636	Panama	Darién	Serrania de Jingurudo, subiendo por qda. "aldo y Río Sambu"	7.6804	78.0387	953		
D. purpurescens	MHCH 2790	Panama	Darién	Pirre top (1400) to camp2; Rancho Frío Field station	7.9472	77.7042	1326		
D. purpurescens	MRC 123	Colombia	Quibdo	Colombia, Chocó, Quibdó, Tutunendo					JN112730
D. purpurescens	MRC 134	Colombia	Quibdo	Colombia, Chocó, Bajo Baudó, Pilizá					JN112731
D. purpurescens	SMF 97271	Panama	Darién	Camp2 (ridge 1300) to stream; Rancho Frío Field station	7.9595	77.7037	1230		KP975530
D. purpurescens	SMF 97272	Panama	Darién	Camp2 (ridge 1300) to stream; Rancho Frío Field station	7.9595	77.7037	1230		KP975529
D. purpurescens	SMF 97273	Panama	Darién	Pirre top (1400) to camp2; Rancho Frío Field station	7.9472	77.7042	1326		
Norops capito	SMF 97094	Panama	Guna Yala	Ridge, Yarbir	9.0602	77.9827	463		KP975519
N. limifrons	SMF 97099	Panama	Comarca Ngöbe-Buglé	Isla Escudo de Veraguas	9.1796	81.8903	32		KP975523
N. poecilopus	SMF 97111	Panama	Darién	Rio Cana, Cana field station, Chimenea trail.	7.7560	77.6857	525		KP975512
N. tropidogaster	MHCH 2646	Panama	Darién	Laguna de Matusagarati, Aguas Calientes.	8.3628	77.9896	53 m		KP975526
Polychrus marmoratus	SNOMNH 36693	Brazil	Pará	Brazil, Pará, approx. 101 km S and 18 km E Santarem, Agropecuaria Treviso LTDA					JN112789

APPENDIX II. Flank patterns of specimens of *Dactyloa purpurescens.* Arrows indicate capitad direction. A) MHCH 2636; B) *D. purpurescens* holotype, USNM 4321; C) SMF 97271; D) SMF 91475; E) SMF 97273; F) MHCH 2635; G) SMF 97272; H) SMF 97267. Scale bars equal 10 mm.



Appendix VII

Declaration on the contributions of authors

to the publication: Revision of the genus *Lepidoblepharis* (Reptilia: Squamata: Sphaerodactylidae) in Central America, with the description of three new species
status: published (2015)
name of journal: Zootaxa 3994
Authors involved:
- Abel Batista (AB), - Marcos Ponce (MP), - Milan Vesely (MV), - Konrad Mebert (KM), - Andreas Hertz (AH)
- Gunther Köhler (GK), - Arcadio Carrizo (AC), - Sebastian Lotzkat (SL)

What has the PhD candidate contributed, and what have the coauthors contributed?

(1) to development and planning

PhD candidate: 35%
Coauthor MV: 10%
Coauthor KM: 5%
Coauthor GK: 15%
Coauthor SL: 35%

(2) to the implementation of the respective studies and experiments

PhD candidate: 35% – field work (collecting and documenting specimens), molecular analysis
Coauthor MV, AH, AC, MP, GK, KM (each coauthor): 5% – field work (collecting and documenting specimens)
Coauthor SL: 35% – field work (collecting and documenting specimens), morphological analysis

(3) to the creation of the data collection and figures

PhD candidate: 40% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor MV: 10% – created database, sequenced DNA barcodes, provided photos, created figures
Coauthor KM: 10% – provided photos
Coauthor SL: 40 – provided tissue samples, photos, map, morphological data, some DNA sequences

(4) to the analysis and interpretation of the data

PhD candidate: 30% – analysis and interpretation of molecular, morphological, and biogeographical data
Coauthor GK: 15% – contributed to data analysis and interpretation
Coauthor SL: 30% – analysis and interpretation of morphological, biogeographical, and molecular data
Coauthor MV, AH, AC, MP, KM (each coauthor): 5% – contributed to data analysis and interpretation

(5) to writing the manuscript

PhD candidate: 55%
Coauthor MV: 5%
Coauthor KM: 5%
Coauthor GK: 10%
Coauthor SL: 25%

Date/place: 13.04.2016 / Frankfurt am Main, Germany

Signature PhD candidate: _____

Affirmative confirmation of the above information:

Signature PhD advisor: _____ Date/place: _____

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Revision of the genus *Lepidoblepharis* (Reptilia: Squamata: Sphaerodactylidae) in Central America, with the description of three new species

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Table of contents

Abstract

Based on morphological and molecular data, we describe three new species of the genus *Lepidoblepharis* with granular dorsals from Panama (*Lepidoblepharis emberawoundule* **sp. nov.**, *Lepidoblepharis rufigularis* **sp. nov.**, and *Lepidoblepharis victormartinezi* **sp. nov.**). The results of our molecular analyses confirm the existence of five deeply differentiated genealogical lineages among Panamanian *Lepidoblepharis.* We present detailed descriptions of their morphology, including some new valuable scalation characters (ventral and subfemoral escutcheon) and hemipenes, as well as comparisons with the other two species of the genus known to occur in Panama (*L. sanctaemartae* and *L. xanthostigma*) and their South American congeners. Last, we provide an updated identification key for the genus *Lepidoblepharis* in Central America.

Key words: Biogeography, Central America, integrative taxonomy, *Lepidoblepharis*, Sphaerodactylidae, new species, Panama, taxonomy

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Introduction

Geckos of the genus *Lepidoblepharis* Peracca, 1897 are small, terrestrial lizards typically inhabiting the leaf-litter of forests (Savage 2002). Most of the 18 recognized species (Uetz & Hošek 2014) are distributed in northern South America, south to Brazil and Ecuador, whereas only two species have been documented to occur in Central America (Avila-Pires 2001; Köhler 2008): *L. sanctaemartae* (Ruthven 1916) that ranges from Venezuela to central Panama, and *L. xanthostigma* (Noble 1916) that has been reported to occur from Colombia to Nicaragua. These two species are readily distinguished from each other: *L. sanctaemartae* has large, flat, imbricate dorsal body scales and 6–8 lamellae under its fourth toe, which is consequently classified as short for a member of this genus. In contrast, *L. xanthostigma* has small, granular, non-imbricate dorsals and reportedly 11–14 lamellae under its fourth toe, thus being classified as a long-toed member of the genus (Ayala & Castro 1983; Lamar 1985; Köhler 2008). The only identification key available for Central American *Lepidoblepharis* (Köhler 2008) omits the lamellar counts and relies entirely on the obvious difference in dorsal scutellation. As a consequence, all Central American members of the genus that bear granular dorsals are identified as *L. xanthostigma* in the field. Recently, after a closer examination of the specimens we collected throughout Panama, we noticed that the Panamanian *Lepidoblepharis* with granular dorsals comprise four morphologically distinct lineages, *i.e.,* two long-toed and two short-toed ones. That is, besides the short-toed *L. sanctaemartae* and the long-toed *L. xanthostigma* which have long been documented to occur in Panama, there are three additional members of this genus in Panama, which we describe in the following.

Material and methods

Field work was carried out in Panama during 2008−2013; detailed information on the sample sites is provided in Appendix I and Figure 9. Geographical coordinates are in decimal degrees and the geodetic system is WGS 1984. All elevations are in meters above sea level. The map was created using ArcGIS 10 (ESRI 2010). Collected specimens were sacrificed with an euthanasia solution (T61), fixed with a solution of 5 mL formalin (36%) in 1 L ethanol (94%), and then stored in ethylic alcohol (70%). All figures have been digitally improved and combined using Adobe CS3. In the color descriptions, the capitalized colors and color codes (the latter in parentheses) are those of Köhler (2012). Abbreviations for museum collections follow Sabaj Pérez (2013). Specimens with GK field tag numbers will be deposited in the collection of the Senckenberg Forschungsinstitut Frankfurt, Germany.

Morphology. Snout-vent length and tail length measurements were taken to the nearest mm along a ruler. Other measurements were made to the nearest 0.1 mm with precision calipers, and with the aid of a dissecting microscope for diminutive characters such as scale sizes. Values are given as minimum–maximum followed by mean ± standard deviation in parentheses. Head length was measured from tip of snout to anterior margin of ear opening. Snout length was measured from tip of snout to anterior border of orbit. Head width was determined at the broadest point. Dorsal and ventral scales were counted at midbody along the midline. For the scale configuration along the median series of enlarged subcaudals, we follow the codification of Rivero-Blanco (1979; fide Avila-Pires 1995), where 1 denotes a single midventral scale, which may be bordered laterodistally by one (') or two (") scales. As an example, the codification 1'1" would denote a midventral subcaudal that is bordered laterodistally by one scale on each side and followed by a midventral subcaudal that is bordered laterodistally by two scales on each side. The escutcheon is a group of glandular scales situated on the posterior portion of the venter, and in some species (or some individuals of certain species) also on the underside of the thigh, of male sphaerodactylid lizards. In males of the genus *Lepidoblepharis,* these escutcheon scales conspicuously stand out from adjoining scales by their almost complete absence of surface pigment (Taylor 1956; Taylor & Leonard 1956). We counted the total number of scales comprising each ventral and subfemoral escutcheon patch. For subfemoral escutcheons, we further noted the number of rows in which the escutcheon scales were arranged. For ventral escutcheons, we determined the length (maximum number of escutcheon scales countable along the midline) and width (maximum number of longitudinal ventral rows countable at the escutcheon's widest portion). Abbreviations used for morphological characters are SVL (snout-vent length), TL (tail length), HL (head length), HW (head width), SPL (supralabials, counted to level of center of eye), INL (infralabials), PM (postmentals), PR (postrostrals), and SAM (scales around midbody). Scale nomenclature largely follows Köhler (2008), Savage (2002), and Avila-Pires

(1995). The species descriptions largely follow Avila-Pires (1995), the hemipenis descriptions follow the terminology of Dowling & Savage (1960). Data for the comparisons with South American species are taken from the examination of the respective holotypes or photographs of the respective holotypes and in some cases also of additional specimens, and on the original descriptions.

Genetics. DNA was extracted from fresh tail tip cuts using the protocol of Ivanova *et al.* (2006). The mitochondrial 16S rRNA gene was amplified using a Mastercycler pro S (Eppendorf, Hamburg, Germany) performing an initial denaturation for 1 min at 94° C followed by 35 steps with denaturation for 15 s at 94° C, hybridization for 45 s at 45° C, and elongation for 1.5 min at 72° C. Final elongation proceeded for 7 min at 94° C. Reaction mix contained 1 µL DNA template, 2.5 µL Reaction Buffer x10 (PeqGold), 4 µL 2.5 mM dNTPs, 0.4 µL (containing 2.5 units) Taq Polymerase (PeqLab), 14.1 µL H2O, 1 µL 25 mM MgCl2, and 1 µL of standard primers for 16S (containing 10 pmol, forward: L2510, 5'-CGCCTGTTTATCAAAAACAT-3'; reverse: H3056, 5'-CCGGTCTGAACTCAGATCACGT-3'; eurofins MWG Operon). The COI fragments were sequenced in the Southern China DNA Barcoding Center at the Kunming Institute of Zoology, China. We compared the mtDNA data of our specimens with published sequences on GenBank. The resulting ClustalW alignments were reviewed and edited by eye using Geneious version 6.1 (Biomatters Inc., available online from http://www.geneious.com/), missing data was treated as N. A list of specimens included in the genetic analyses with corresponding GenBank accession numbers is presented in Appendix II. The final 16S alignment including 21 sequences of the genus *Lepidoblepharis* and 12 outgroups (obtained in this study and from GenBank) comprised 486 sites of which (excluding outgroups) 205 were variable, 128 parsimony-informative, and 76 singletons. The final alignment for the COI gene consisted of 7 sequences of the genus *Lepidoblepharis* (all obtained in this study; no outgroups) and comprised 552 sites, of which 180 were variable, 149 parsimony-informative, and 31 singletons. Using MEGA5 (Tamura *et al.* 2011), we calculated the uncorrected genetic p-distances for 16S and COI separately. For the combined-gene data set of 16S and COI mtDNA (33 samples and 1038 sites), we used JModeltest 0.1.1 (Posada 2008) under the corrected Akaike Information Criterion (AICc) to select the substitution model for the Bayesian Inference (BI) and Maximum Likelihood (ML) analyses. TVM+G was determined as the best-fitting substitution model. We ran a ML analysis with 1000 bootstrap replicates using PAUP v4.0b10 (Swofford 1998), and a Bayesian phylogenetic analysis in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) for 20,000,000 generations with four default chains, sampled every 100 generations, and subsequently discarded the initial 5% of the sampled trees as burn-in. We estimated the divergence time for the combined 16S and COI mtDNAs using the program BEAST 1.5.4 (Drummond & Rambaut 2007), with a relaxed clock, allowing substitution rates to vary according to an uncorrelated log-normal distribution, assuming a Yule tree prior (Drummond *et al.* 2006). The prior distributions on substitution parameters were set as default. To calibrate the root and node ages, we used calibration times obtained by Gamble *et al*. (2008). Three calibration points were applied: the crown age for the Sphaerodactylidae, 70 ± 12 Ma (megaannus, *i.e.,* Million years; "ago" implied herein), the splitting between *Gonatodes* and *Lepidoblepharis* (64 ± 10 Ma), and a fossil calibrated node between *Sphaerodactylus elegans* and its sister clade to a minimum of 23 ± 6 Ma. Parameters were estimated using 50 million generations with a burn-in of 2.5 million generations and trees were sampled every 10,000 generations. Results were visualized and compared using Tracer 1.5 (Rambaut & Drummond 2009), and summary trees were generated using TreeAnnotator 1.5.4.

Results

Morphological and molecular results are summarized and compared in Figures 1–8 (also see Appendices III−VI). The results of the molecular analyses unanimously show the existence of five deeply differentiated genealogical lineages among Panamanian *Lepidoblepharis.* These findings are confirmed through morphological comparisons which revealed that these lineages differ chiefly in the respective configurations of finger and toe lamellae (Figs. 1, 3, and 4), escutcheon scales (Fig. 5), mental and postmental scales (Fig. 3), as well as in subcaudal scale pattern (Fig. 3) and hemipenial morphology (Fig. 6). Of these five lineages, the only one with flat, imbricate dorsal body scales undoubtedly is assignable to the nominal taxon *L. sanctaemartae,* which is distributed from northwestern Colombia to west-central Panama (Fig. 9). Among the remaining four lineages with granular dorsals, there are two short-toed lineages from eastern and west-central Panama, and two long-toed taxa, one of which is represented by a single specimen collected in extreme southeastern Panama, whereas the other has been collected from central and

western Panama, Costa Rica, and Nicaragua. The nominal species *L. xanthostigma* has been described from "Zent, near Puerto Limon, Costa Rica", a locality in the Caribbean lowlands of Limón province in eastern Costa Rica (Noble 1916; Fig. 9). This type locality is surrounded by our own collection sites of long-toed *Lepidoblepharis,* the nearest of which (Moin, Limón, specimens SMF 98879–80) is situated about 20 km east-southeast. The long-toed specimens from central and western Panama exhibit an overwhelming congruence in their morphological variation to the specimens from Costa Rica and Nicaragua (Fig. 1). Moreover, they comply with the descriptions of *L. xanthostigma* provided by different authors (Taylor 1956; Lamar 1985; Savage 2002; Köhler 2008), as well as with the photos of the holotype (MCZ 11658) available from the MCZ collection database (mczbase.mcz.harvard.edu). Finally, the GenBank sequences of two Costa Rican specimens of this species cluster together with our Panamanian material in the molecular analyses. Thus, the assignment of the western Panamanian long-toed specimens to this nominal taxon can confidently be reconfirmed.

Now that the two names which are available for Panamanian *Lepidoblepharis* have been assigned to two of our five inferred lineages, the three other lineages with granular dorsals that we identified in our analyses still require clarification. Comparisons with the known species of *Lepidoblepharis* (as detailed below) revealed that none is conspecific with any of our remaining three lineages. Therefore, we recognize them as undescribed species as follows: The short-toed *Lepidoblepharis* **sp. nov.** 1 is distributed in eastern Panama and probably north-western Colombia, and can be recognized by its small size, subcaudal scale pattern, and its low number of lamellae under the fourth toe and finger. The long-toed *Lepidoblepharis* **sp. nov.** 2, represented by a single male specimen that was collected in the southeastern corner of Panama ca. 15 km from the Colombian border, can be distinguished by its orange throat and its escutcheon length/width ratio. The short-toed *Lepidoblepharis* **sp. nov.** 3 is known only from central to western Panama and has a unique morphology of its subdigital lamellae, also being distinguishable by the lowest numbers of lamellae under the fourth toe and finger.

Detailed information on genetic p-distance for all samples included in the analyses can be found in Table 2 and Appendices III–V. The average of genetic p-distances between lineages was 16% for 16S and 22% for COI. The average genetic p-distance between closely related terminal clusters (equivalent to the "sibling species" of Nagy *et al.* 2012) was 13% for 16S and 18% for COI, between *Lepidoblepharis* **sp. nov.** 1 and *Lepidoblepharis* **sp. nov.** 3, and 12% in 16S between *L. xanthostigma* and *Lepidoblepharis* **sp. nov.** 3. The closest relative to *Lepidoblepharis* **sp. nov.** 2 was *L. xanthostigma* with a mean p-distance of 14% for 16S. The highest mean values of p-distances between lineages in 16S were 23% between *Lepidoblepharis* **sp. nov.** 2 and *L. sanctaemartae,* followed by 21% between *L. sanctamartae* and both *L. xanthostigma* and *Lepidoblepharis* **sp. nov.** 1. The highest genetic divergence within a terminal cluster was found within *Lepidoblepharis* **sp. nov.** 1, with individual p-distances of 4–15% (average 7%) for 16S and 4–12% (average 10%) for COI. In the phylogenetic tree (Fig. 2), the *Lepidoblepharis* were grouped in three main lineages, *Lepidoblepharis* **sp. nov.** 2 alone being the sister group to a clade comprising all other sampled species within two subgroups, one of which harbors the species which are currently only known from Lower Central America (*Lepidoblepharis* **sp. nov.** 1, *Lepidoblepharis* **sp. nov.** 3, and *L. xanthostigma*), and a second one represented by the species from Ecuador and Colombia (*Lepidoblepharis* sp. and *L. festae*) and a single species known from both South America and Panama (*L. sanctaemartae*).

The divergence time analysis yielded an estimated age of origin of around 45 (range 37–67) Ma for the genus *Lepidoblepharis* (Fig. 8). The oldest lineage was *Lepidoblepharis* **sp. nov.** 2. *Lepidoblepharis xanthostigma* originated 33 (21–45) Ma with its divergence from a clade that subsequently split into the sister species *Lepidoblepharis* **sp. nov.** 1 and *Lepidoblepharis* **sp. nov.** 3 approximately 25.2 (15–35) Ma. *Lepidoblepharis sanctaemartae* originated as a South American species around 21.4 (9–33) Ma. Three of the five species distributed in Central America originated during the uplift of the Panamanian land bridge between 15–25 Ma (Montes *et al.* 2012b). *Lepidoblepharis* **sp. nov.** 1 showed a high variation with an old lineage from Cerro Sapo (18.64, 11–27 Ma) and younger lineages at the San Blas and Darién mountain ranges (11.15, 7–16 Ma).

Integrating all evidence, it is apparent that Panama is home to five well-differentiated species of the genus *Lepidoblepharis* instead of just two as hitherto assumed. Table 1 summarizes the variation in selected morphological characters among the five species of *Lepidoblepharis* found in Panama as exhibited by our examined material. Below, we proceed to describe the three new species with granular dorsal scales, in the order of their numbering.

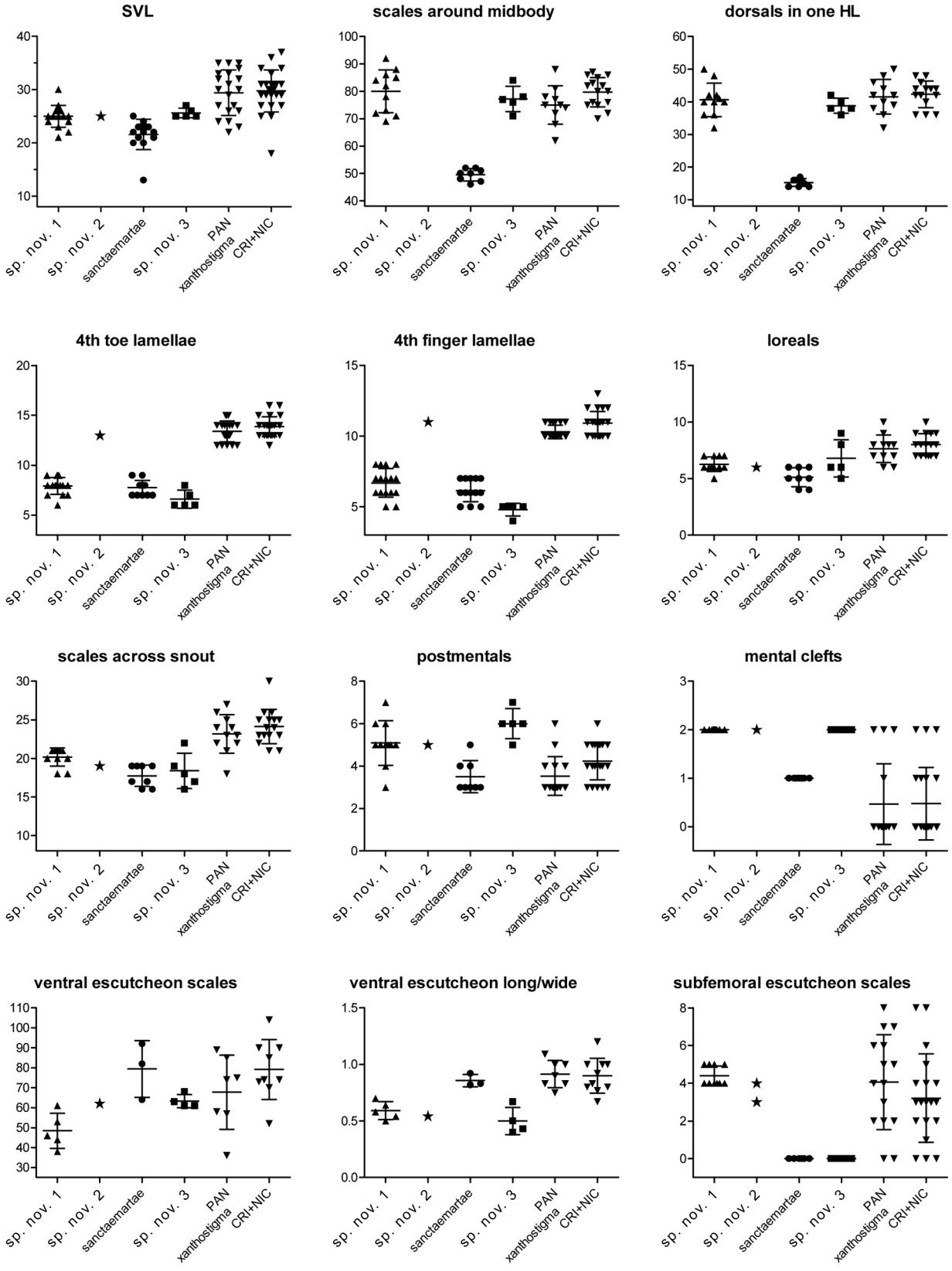

FIGURE 1. Selected morphological characteristics of Central American *Lepidoblepharis*. Horizontal bars represent mean ± standard deviation. For comparison, specimens of *L. xanthostigma* from Panama (PAN) and those from Costa Rica and Nicaragua (CRI+NIC) are shown separately.

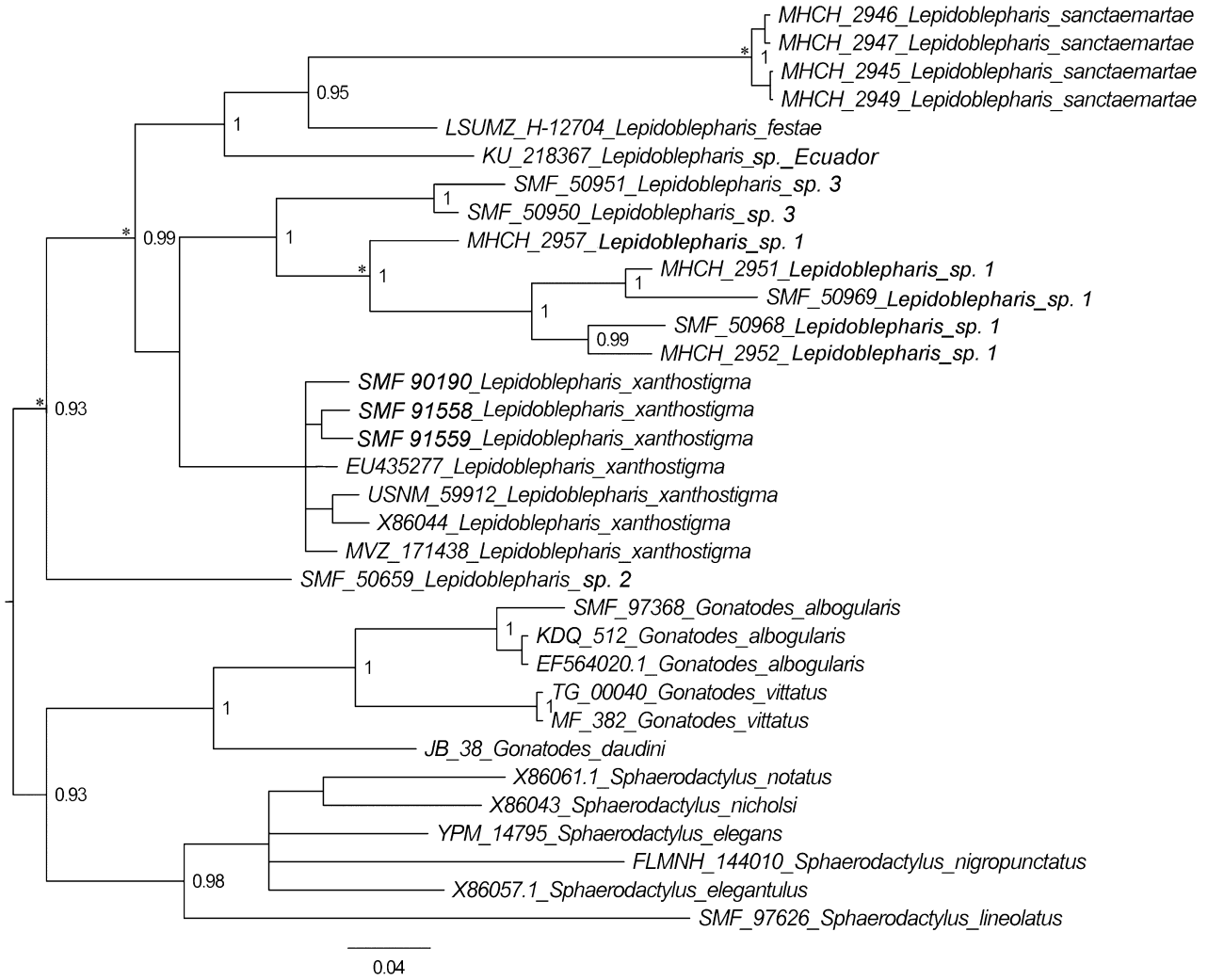

FIGURE 2. Bayesian consensus tree of the genus *Lepidoblepharis* based on 16S and COI mtDNA. Asterisks on nodes indicate bootstrap support values above 90% from the corresponding ML analysis, numbers on nodes are Bayesian posterior probabilities ($P \geq 0.90$). Scale bar refers to substitutions per site.

TABLE 1. Selected morphological characters among our examined specimens of Central American *Lepidoblepharis.*

Traits	*L. emberawoundule*	*L. rufigularis*	*L. sanctaemartae*	*L. victormartinezi*	*L. xanthostigma*
	N = 16	N=1	N = 14	N = 5	N = 44
SVL	21–30 (25.0 ± 2.07)	25	13–25 (21.6 ± 2.58)	25–27 (25.6 ± 0.89)	18–37 (29.6 ± 4.05)
TL	18–31 (24.4 ± 4.83) n = 5	33	12–21 (16.5 ± 6.36) n = 2	31 n = 1	23–49 (34.2 ± 7.18) n = 14
TL / SVL	0.82–1.24 (1.01 ± 0.15) n = 5	1.31	0.92–0.95 (0.94 ± 0.02) n = 2	1.15 n = 1	0.89–1.40 (1.17 ± 0.15) n = 14
HL / SVL	0.20–0.25 (0.22 ± 0.01) n = 11	0.22	0.20–0.22 (0.21 ± 0.01) n = 8	0.2–0.23 (0.21 ± 0.01)	0.19–0.24 (0.22 ± 0.01) n = 25
dorsals	granular	granular	flat, imbricate	granular	granular
ventrals in one HL	10–15 (12.6 ± 1.63) n = 11	13	10–14 (11.5 ± 1.31) n = 8	10–15 (12.2 ± 1.92)	11–18 (13.8 ± 2.03) n = 25

.....continued on the next page

TABLE 1. (Continued)

Traits	*L. emberawoundule*	*L. rufigularis*	*L. sanctaemartae*	*L. victormartinezi*	*L. xanthostigma*
	N = 16	N=1	N = 14	N = 5	N = 44
dorsals in one HL	32–50 (40.6 ± 5.14) n = 11	–	14–17 (15.3 ± 1.16) n = 8	36–42 (38.8 ± 2.28)	32–50 (42.0 ± 4.57) n = 25
4th toe lamellae	6–9 (7.9 ± 0.85)	13	7–9 (7.8 ± 0.73)	6–8 (6.6 ± 0.89)	12–16 (13.7 ± 1.03)
4th finger lamellae	5–8 (6.7 ± 1.01)	11	5–7 (6.2 ± 0.80)	4–5 (4.8 ± 0.45)	10–13 (10.6 ± 0.75)
SPL	3–4 (3.3 ± 0.47) n = 11	3	2–4 (3.0 ± 0.53) n = 8	3–4 (3.2 ± 0.45)	3–5 (3.6 ± 0.57) n = 27
PM	3–7 (5.1 ± 1.04) n = 11	5	3–5 (3.5 ± 0.76) n = 8	5–7 (6.0 ± 0.71)	3–6 (3.9 ± 0.95) n = 36
PR	3–4 (3.1 ± 0.30) n = 11	4	3–4 (3.6 ± 0.52) n = 8	3–5 (4.4 ± 0.89)	2–6 (3.7 ± 0.92) n = 27
clefts in posterior border of mental	2 (2 ± 0) paramedian n = 11	2 paramedian	1 (1 ± 0) median n = 8	2 (2 ± 0) paramedian	0–2 (0.47 ± 0.77) lateral, mostly short n = 36
loreals	5–7 (6.3 ± 0.65) n = 11	6	4–6 (5.1 ± 0.83) n = 8	5–9 (6.8 ± 1.64)	6–10 (7.9 ± 1.06) n = 27
scales across snout	18–21 (20.2 ± 1.17) n = 11	19	16–19 (17.8 ± 1.39) n = 8	16–22 (18.4 ± 2.30)	18–30 (23.7 ± 2.33) n = 27
SAM	69–92 (80.0 ± 7.8) n = 11	–	46–52 (49.5 ± 2.27) n = 8	71–84 (77.2 ± 4.66)	62–88 (77.8 ± 6.40) n = 25
ventrals at midbody	16–20 (18.2 ± 1.17) n = 11	17	–	15–19 (17.4 ± 1.52)	16–22 (19.1 ± 1.28) n = 27
dorsals at midbody	52–74 (61.8 ± 7.61) n = 11	–	–	53–67 (59.8 ± 5.36)	44–69 (59.0 ± 5.88) n = 25
ventral escutcheon scales	38–61 (48.4 ± 8.85) n = 5	62	64–92 (79.3 ± 14.19) n = 3	61–68 (63.3 ± 3.30) n = 4	36–104 (74.1 ± 17.07) n = 16
ventral escutcheon long	6–7 (6.8 ± 0.45) n = 5	7	9–11 (10.0 ± 1.0) n = 3	6–8 (6.8 ± 0.96) n = 4	6–12 (9.8 ± 1.59) n = 17
ventral escutcheon wide	10–13 (11.6 ± 1.14) n = 5	13	11–12 (11.7 ± 0.58) n = 3	12–15 (13.8 ± 1.26) n = 4	8–13 (10.9 ± 1.22) n = 17
ventral escutcheon long / wide	0.50–0.70 (0.59 ± 0.08) n = 5	0.54	0.82–0.92 (0.86 ± 0.05) n = 3	0.40–0.67 (0.50 ± 0.12) n = 4	0.67–1.20 (0.91 ± 0.14) n = 17
subfemoral escutcheon scales per thigh	4–5 (4.4 ± 0.52) n = 10	3–4	0 n = 6	0 n = 8	0–8 (3.6 ± 2.43) n = 34

TABLE 2. Mean values of genetic p-distances in the 16S mtDNA gene among the *Lepidoblepharis* species included in the molecular analyses.

	L. emberawoundule	*L. rufigularis*	*L. victormartinezi*	*L. sanctaemartae*	*L. xanthostigma*	*L. festae*	*Lepidoblepharis* sp. Ecuador
L. emberawoundule							
L. rufigularis	0.16						
L. victormartinezi	0.13	0.17					
L. sanctaemartae	0.21	0.23	0.18				
L. xanthostigma	0.15	0.14	0.12	0.21			
L. festae	0.17	0.16	0.14	0.16	0.14		
Lepidoblepharis sp. Ecuador	0.17	0.18	0.14	0.17	0.14	0.13	

***Lepidoblepharis emberawoundule* sp. nov.**
Figs. 3–7, 10.

Lepidoblepharis xanthostigma: all in part. (referring to certain populations in eastern Panama): Auth (1994); Young *et al.* (1999); Ibáñez *et al.* (2001); Köhler (2001: Fig. 172; 2008: Fig. 136); Jaramillo *et al.* (2010).

Holotype. Adult male SMF 50968, original field number AB 963 (Fig. 10), collected from leaf-litter at La Cascada trail, Burbayar private reserve (9.31837°N, 79.00266°W, 360 m elev.), Cartí, Narganá, Comarca Guna Yala, Panama, on 26 November 2013 at 23:40 hrs, by Abel Batista and Konrad Mebert.

Paratypes. Three adult males, two adult females, and one juvenile, all from Panama. Three males (SMF 81950–52) from Nusagandí field station and two females (SMF 81953–54) from the nearby Sendero Nusagandí, Comarca Guna Yala, collected 14–17 April 2000; one juvenile (MHCH 2952) from Río Terable, El Llano, Chepo, Panama, collected on 27 November 2012; see Appendix I for locality details.

Referred specimens. MHCH 2951, 2957; SMF 50969–70; FMNH 170029, 170042–45; see Appendix I for locality details.

Diagnosis. *Lepidoblepharis emberawoundule* (our **sp. nov.** 1) is characterized by the following combination of characters: *(1)* dorsal body scales small, granular, and juxtaposed, ventral scales large, cycloid, flat, and imbricate; *(2)* scales on head small and granular; *(3)* 3–4 (3.1 ± 0.3) postrostral scales; *(4)* a vaguely M-shaped posterior mental border with two paramedian clefts; *(5)* 3–7 (5.1 ± 1.04) postmentals, larger than the posteriorly adjacent scales on chin; *(6)* lamellae under fourth toe 6–9 (7.9 ± 0.85), lamellae under fourth finger 5–8 (6.7 ± 1.01); *(7)* median subcaudals conspicuously wider than long, but their width less than twice the width of the laterally adjacent scales or their own length, with straight or rounded posterior margins, arranged in a regular tail sequence of 1'1"; *(8)* ventral escutcheon consisting of 38–61 (48.4 ± 8.85) scales, 6–7 (6.8 ± 0.45) scales long and 10–13 (11.6 ± 1.14) wide; *(9)* subfemoral escutcheon consisting of 4–5 (4.4 ± 0.52) well-discernible scales per thigh arranged in a single row (Fig. 5 A); *(10)* 16–20 (18.2 ± 1.17) longitudinal rows of ventral scales at midbody; *(11)* 52–74 (61.8 ± 7.61) longitudinal rows of dorsal scales at midbody; *(12)* bilobate hemipenis, with a third lobule rising from the pedicel (Fig. 6 A–B); *(13)* SVL 21–30 (25 ± 2.07) mm.

Comparison with other species of the genus. *Lepidoblepharis emberawoundule* can be differentiated from many species in the genus by its small size and its low number of lamellae under the fourth toe and finger (Figs. 3–4). In the following, we provide comparisons to all other species within the genus, with the characteristics for *L. emberawoundule* in parentheses. *Lepidoblepharis emberawoundule* can be distinguished from the Panamanian species *L. xanthostigma*, *L. sanctaemartae, Lepidoblepharis* **sp. nov.** 2 (described below), and *Lepidoblepharis* **sp. nov.** 3 (described below) by uncorrected genetic p-distance (10–26% in 16S mtDNA between individuals).

Lepidoblepharis xanthostigma has greatly enlarged median subcaudal scales (slightly enlarged), and 12–16 lamellae under its fourth toe (6–9). *Lepidoblepharis sanctaemartae* has large, flat, imbricate dorsal body scales (small granular scales). *Lepidoblepharis* **sp. nov.** 2 (described below) has 13 lamellae under its fourth toe (6–9) and 11 under its fourth finger (5–8). *Lepidoblepharis* **sp. nov.** 3 (described below) has 4–5 lamellae under its fourth finger (5–8) and a unique lamellar configuration with 1–3 proximal lamellae per digit greatly enlarged, *i.e.,* about 3–4 times longer than any of the remaining lamellae, the ventral escutcheon consisting of 61–68 scales (38–61), and no discernible subfemoral escutcheon (4–5 discernible subfemoral escutcheon scales per thigh). To date, seven species of the genus *Lepidoblepharis* have been reported to possess ten or fewer lamellae under the fourth toe, *i.e.,* to be short-toed. Two of these, *L. miyatai* Lamar 1985 and *L. sanctaemartae,* possess large, flat, and imbricate dorsal scales (small, granular, and juxtaposed dorsals). Three others, *Lepidoblepharis buchwaldi* Werner 1910, *L. montecanoensis* Markezich & Taphorn 1994, and *L. williamsi* Ayala & Serna 1986, can be readily distinguished from *L. emberawoundule* because the inspection of the illustrations and photographs available for the respective holotypes showed clear differences between the species: In *L. buchwaldi*, the enlarged subcaudals are much wider than long and at least twice as wide as the laterally adjacent subcaudals (less than twice as wide as they are long or as the neighboring subcaudals are wide), the dorsal tail scales are small, *i.e.,* less than twice the size of the dorsal body scales (twice or more the size of the dorsal body scales), and the posterior border of the mental has a single median cleft (two paramedian clefts). *Lepidoblepharis montecanoensis* is a very small species with a SVL of 18–21 mm (21–30), and lacks defined occipital marks in males (two well defined occipital marks in males) as well as distinctly enlarged median subcaudals (median subcaudals distinctly enlarged). *Lepidoblepharis williamsi* also lacks enlarged median subcaudal scales (median subcaudals distinctly enlarged), and has only 25–40 ventral escutcheon scales (38–61). The holotype of *L. peraccae* Boulenger 1908 has eight lamellae under the fourth finger (5–8) and ten under the fourth toe (6–9), its plantar and palmar scales have ovoid and strongly imbricate posterior borders (those scales small, rounded, and juxtaposed; Fig. 7). The holotype of *L. microlepis* (Noble 1923) is very similar to *L. emberawoundule*, but differs in the scalation of the chin region and the ventral tail surface (Fig. 7). The posterior margin of its mental is V-shaped and lacks conspicuous clefts (posterior margin M-shaped, *i.e.,* slightly convex in the middle, with two conspicuous paramedian clefts), there are six postmentals, with one medial postmental greatly enlarged and two neighboring scales slightly enlarged (3–7 postmentals, median scales slightly larger than the others), and the posteriorly adjacent chin scales are small and conical (small and flat, some slightly pointed, and juxtaposed, Figs. 3, 7). Most decisively, each of the slightly enlarged subcaudal scales of the holotype of *L. microlepis* is bordered laterodistally by only one scale, leading to a regular tail sequence of 1'1' (the larger of the enlarged subcaudals bordered laterodistally by two scales, the smaller ones by one, forming a regular tail sequence of 1'1"; see Fig. 7 E–F). The remaining species of the genus, *i.e., L colombianus* Mechler 1968*, L conolepis* Avila-Pires 2001*, L. duolepis* Ayala & Castro 1983*, L. festae* Peracca 1897*, L. grandis* Miyata 1985*, L. heyerorum* Vanzolini 1978*, L. hoogmoedi* Avila-Pires 1995*, L. intermedius* Boulenger 1914*,* and *L. ruthveni* Parker 1926 are long-toed with eleven or more lamellae under the fourth toe (6–9 in *L. emberawoundule*).

Description of the holotype. Variation among the entire type series is given in parentheses for selected characters (see Table 1 for details and variation among all examined specimens). Adult male as judged by everted hemipenes; SVL 26 mm (21–27 mm), TL 27 mm (measured while the now broken tail was still intact) (18–27 mm), HL 5.6 mm (5.0–5.6 mm), HW 3.8 mm (3.2–4.4 mm), forelimbs 5.2 mm, hind limbs 8.6 mm, shank 3.7 mm; rostral large, clearly visible from above, with a shallow, horseshoe-shaped posterior depression and a long median cleft; postrostrals including supranasals three (3–4), the median ones smaller than supranasals, and indenting the rostral, the median postrostral about the same size as the posterior scales on snout; postnasals two (1–2), both about the same size as posteriorly adjacent loreal scales; scales on snout small and smooth, 20 (18–21) scales across snout between anterior sutures of second SPLs; loreal scales juxtaposed, elevated, and rounded, 7 (6–7) on a longitudinal line between postnasals and orbit; scales on top of head small, granular, juxtaposed, generally pointing upward, about as half as large as those on the middle area of snout; superciliary flap with two enlarged scales on anterior border, of which the first is slightly longer than the second, followed by four small scales; supralabials three (3–4), posteriormost one below center of eye; ear-opening small, oval, in oblique orientation; mental large, posterior margin slightly convex in the middle, with two small clefts bordering this convexity, resulting M-shaped; postmentals 7 (4–7), median scales slightly larger than the others, postmentals larger than the posteriorly adjacent chin scales (Figs. 3, 7); scales on chin small and juxtaposed, most of them flat but some slightly pointed, on posterior region granular, approximately vertical in position, with a slight reduction in size towards posterior

portion of chin; scales near posterior infralabials flat, subimbricate, and larger than scales in median area of chin; infralabials four, first largest, fourth below center of eye; throat with small granular scales, the posterior region with larger, granular, and pointed scales directed upward (some directed posteriorly); dorsal scales on neck and body small, granular, and juxtaposed; dorsals around midbody and on posterior portion of trunk pointed, granular, or, in frontal view, triangular, mostly directed posteriorly, some scales on flanks and in lumbar region slightly flattened; 40 (36–50) middorsal scales in one HL, 89 between levels of axilla and groin; ventrals flat, smooth, imbricate, with an ovoid posterior margin, increasing moderately in size from gular region to belly, posterior ventral scales longer than wide; 12 (11–15) midventral scales in one HL, 27 between levels of axilla and groin, 32 to border of cloaca; ventral escutcheon patch with 53 (38–46) scales, some of which have slightly pointed posterior margins, 7 (6–7) scales long and 13 (10–13) wide, escutcheon long/wide ratio 54% (50–70%); subfemoral escutcheon scales five (4–5) per thigh, arranged in a single row; transition between ventrals and scales on flanks abrupt; scales around midbody 92 (69–92), of which 18 are ventrals (16–19); scales on precloacal plate similar to ventrals, except for those on border of cloaca, which are smaller; tail dorsally and laterally with flattened, smooth, and imbricate scales, less elongated than ventrals (with a transitional zone at base of tail); underside of tail with a median row of moderately enlarged scales, mostly with a repeated series of one median scale bordered laterodistally by one scale, followed by a slightly larger median scale that is in contact laterodistally with two scales (Figs. 3, 7), constituting a regular tail sequence of 1'1"; dorsal scales on forelimbs granular; scales on hind limbs flat, smooth, imbricate on anteroventral thigh and shank surfaces, granular elsewhere; fingers, from longest to shortest, IV-III-II-V-I; toes IV-III-II-V-I, fourth and third toes about the same length; lamellae under fourth finger six (5–8), under fourth toe eight (8–9), proximal lamellae slightly larger than distal ones; claws enclosed by an ungual sheath composed of six scales, as typical for the genus.

Hemipenis morphology. The everted hemipenis of SMF 50968 (Fig. 6 A–B) is a small, bilobate organ, divided for around one third of its length, with a naked base; sulcus spermaticus bordered by well-developed, smooth sulcal lips; ornamentation of papillate calyces present on each lobe, asulcate area of the truncus covered by small spines; a third lobule-like rising from the pedicel, not connected to the sulcus spermaticus, and covered with papillate calyces.

Coloration in preservative (alcohol 70%; variation among the paratypes in parentheses). Dorsal ground color Hair Brown (277); occipital marks Beige (254) (Gray Horn Color (268) in females), posterior margin of orbit bordered with Sepia (279); dorsum of head with small Beige (254) blotches; infra- and supralabials with alternating Sepia (279) and white bars; chin and throat with Sepia (286) marks on a dirty white background; venter slightly pigmented with Vandyke Brown (281); escutcheon scales unpigmented in the center, with Olive Brown (278) borders.

Coloration in life (Fig. 10; variation among the paratypes in parentheses). Dorsal ground color Glaucous (272), with small scattered Lavender Blue (195) and Sepia (286) dots; neck region Olive Brown (278); an indistinct Sepia (286) line from tip of snout to anterior border of eye; two diffuse postorbital Sepia (286) lines, one directed towards the occipital region and the other towards the ear; infra- and supralabials with alternating Sepia (279) and white bars; a vaguely M-shaped dirty white (Smoke Gray (267) in females) occipital mark bordered with Burnt Sienna (38); top of head suffused with Fawn Color (258) and Lavender Blue (195); chin and throat with Sepia (286) reticulations on a Chamois (84) background; venter Lavender Blue (195) suffused with Cinnamon-Rufous (31); an indistinct dorsolateral pale line from behind the ear to mid tail; tail Cinnamon-Rufous (31); a Sepia (286) line from above groin to mid tail.

Distribution and habitat. *Lepidoblepharis emberawoundule* is currently known from a few sites in eastern Panamanian montane forests and Chocó-Darién moist forests (Fund 2011), from 227 to 773 m elevation in Darién and Panamá provinces as well as in the Comarcas Emberá and Guna Yala. Most probably, *L. emberawoundule* lives in the leaf-litter and feeds on small invertebrates like other *Lepidoblepharis* (Vitt *et al*. 2005).

Etymology. The name *emberawoundule* is a compound word in honor to "the forest guardians", the three indigenous peoples inhabiting eastern Panama; *embera*: Emberá Indians from the foothills of Jingurudó, Bagre, Sapo, Darién, and Pirre mountain ranges; *woun*: Wounaan Indians, mainly from the Tuira basin and Majé mountain range; *dule*: meaning people in the language of the Guna Indians from the Caribbean and Pacific versants of the San Blas and Darién mountain ranges.

***Lepidoblepharis rufigularis* sp. nov.**
Figs. 3–5, 11.

Lepidoblepharis xanthostigma: all in part. (referring to populations in extreme southeastern Panama): Auth (1994); Young *et al.* (1999); Ibáñez *et al.* (2001); Köhler (2008); Jaramillo *et al.* (2010).

Holotype. Adult male SMF 50659, original field number AB 527 (Figs. 3–5, 11), collected on a hill 1 km north of Río Púcuro (8.057501°N, 77.370217°W, 1043 m elev.), Pinogana, Darién, Panama, on 08 July 2012 at 22:40 hrs by Abel Batista.

Diagnosis. *Lepidoblepharis rufigularis* (our **sp. nov.** 2) is characterized by the following combination of characters: *(1)* dorsal scales small, granular, and juxtaposed, ventral scales large, cycloid, flat, and imbricate; *(2)* scales on head small and granular; *(3)* four postrostral scales; *(4)* two short, barely discernible paramedian clefts in the more or less U-shaped posterior mental border; *(5)* five postmentals, the two median ones larger than the posteriorly adjacent chin scales; *(6)* 13 lamellae under fourth toe, 11 lamellae under fourth finger; *(7)* median subcaudals conspicuously wider than long, almost twice as wide as the laterally adjacent scales, with straight posterior margins arranged in a regular tail sequence of 1'1"; *(8)* ventral escutcheon consisting of 62 scales, almost twice as wide (13 scales) as long (7 scales); *(9)* subfemoral escutcheon consisting of 3–4 scales per thigh; *(10)* 17 longitudinal rows of ventral scales at midbody; *(11)* bilobate hemipenis; *(12)* SVL 25 mm.

Comparison with other species of the genus. *Lepidoblepharis rufigularis* can be differentiated from all species in the genus by its small size, number of lamellae under the fourth toe and finger, the reddish throat in males (Fig. 11), and the configuration of the ventral escutcheon. In the following, we present comparisons to all other species within the genus, with the characteristics for *L. rufigularis* in parentheses. *Lepidoblepharis rufigularis* can be distinguished from the Panamanian species *L. xanthostigma*, *L. sanctaemartae, L. emberawoundule,* and *Lepidoblepharis* **sp. nov.** 3 (described below) by a genetic p-distance of 14–23% between individuals in 16S mtDNA. *Lepidoblepharis xanthostigma* is the most similar species, but has a different chin and throat coloration with dark reticulations on a pale background (orange background), and greatly enlarged median subcaudal scales which are more than two times as wide as the laterally adjacent subcaudal scales (enlarged but less than two times the width of laterally adjacent subcaudals, Fig. 3), usually 21 or more, very rarely 18, scales across snout (19), usually 18 or more, rarely 16 or 17, ventral scales a midbody (17), and an escutcheon long/wide ratio of 67–120% (54%). *Lepidoblepharis emberawoundule, L. sanctaemartae,* and *Lepidoblepharis* **sp. nov.** 3 (described below) have fewer than 10 lamellae under the fourth toe (13) and under the fourth finger (11). Additionally, *L. sanctaemartae* has large, flat, imbricate dorsal body scales (small, granular, and juxtaposed). To date, seven species of the genus *Lepidoblepharis* have been reported to possess ten or fewer lamellae under the fourth toe, *i.e.,* to be short-toed (*L. miyatai*, *L. sanctaemartae, L. buchwaldi, L. montecanoensis, L. williamsi, L. peraccae,* and *L. microlepis*), and are therefore readily differentiable from the long-toed *L. rufigularis* (13 lamellae under the fourth toe). Of the remaining members of the genus, *L. colombianus, L. conolepis, L. duolepis, L. festae, L. grandis, L. heyerorum, L. hoogmoedi, L. intermedius,* and *L. ruthveni* are relatively to very large lizards for this genus with adult SVLs between 33 and 56 mm (25 mm). Additionally, *L. conolepis* and *L. grandis* have 14–20 lamellae under the fourth toe (13). The dorsal ground color in males of *L. heyerorum* is black with yellow dorsal markings (no yellow dorsal markings). The two long-toed specimens with granular dorsals reported as *L. xanthostigma* from Colombia by Ayala & Castro (1983) are similar to *L. rufigularis* in the number of ventral scales, but they have 22–25 scales across snout (19), an escutcheon with only 25 scales (62), the gular region with blotches (gular region with longitudinal bars), and an occipital pale W-shaped mark (no occipital mark at all).

Description of the holotype. Adult male as judged by everted hemipenes; SVL 25 mm, TL 33.0 mm (measured while the now broken tail was still intact), HL 5.6 mm, HW 4.1 mm, forelimbs 5.0 mm, hind limbs 9.3, shank 3.6 mm; rostral large, clearly visible from above, with a shallow, horseshoe-shaped posterior depression and a long median cleft; postrostrals four, including supranasals, one median postrostral slightly larger than posteriorly adjacent scales on snout and indenting the rostral; postnasals two, both about the same size as posteriorly adjacent loreal scales; scales on snout rounded and pointed backward; loreal scales subimbricate, elevated toward posterior and dorsal directions, six loreal scales on a longitudinal line between postnasals and orbit; 19 scales across snout between anterior sutures of second SPLs; scales on top of head small, granular, juxtaposed, generally pointing upward, about half the size of those on the middle area of snout; superciliary flap with two enlarged scales on anterior border, of which the first is slightly longer than the second, followed by 2–5 small and globular scales;

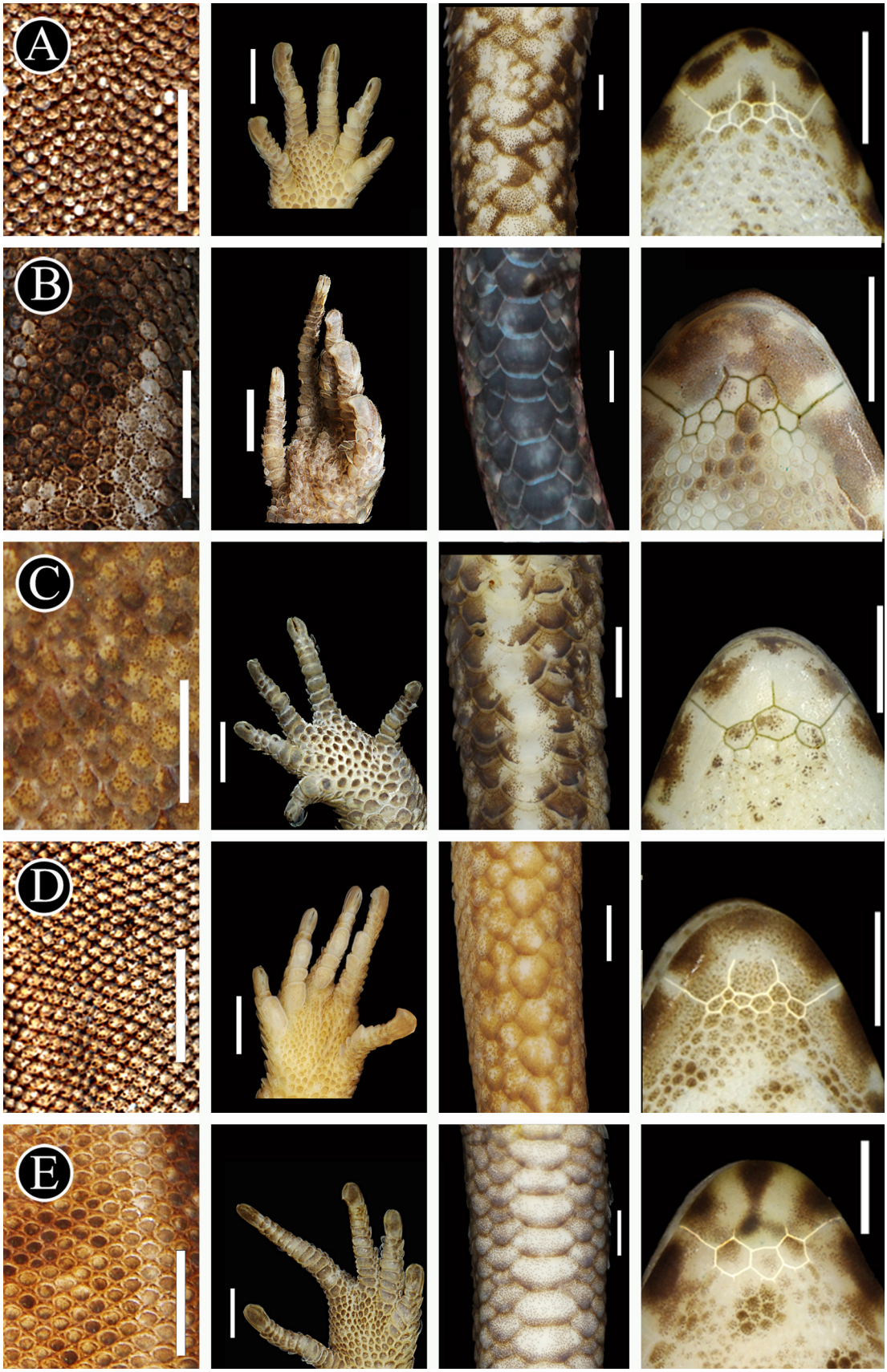

FIGURE 3. Morphological distinction among *Lepidoblepharis* from Lower Central America; images from left to right: dorsals, ventral views of foot, tail, and mental region; scale bars equal 1 mm. (A) *L. emberawoundule*; (B) *L. rufigularis*; (C) *L. sanctaemartae*; (D) *L victormartinezi*; (E) *L. xanthostigma*.

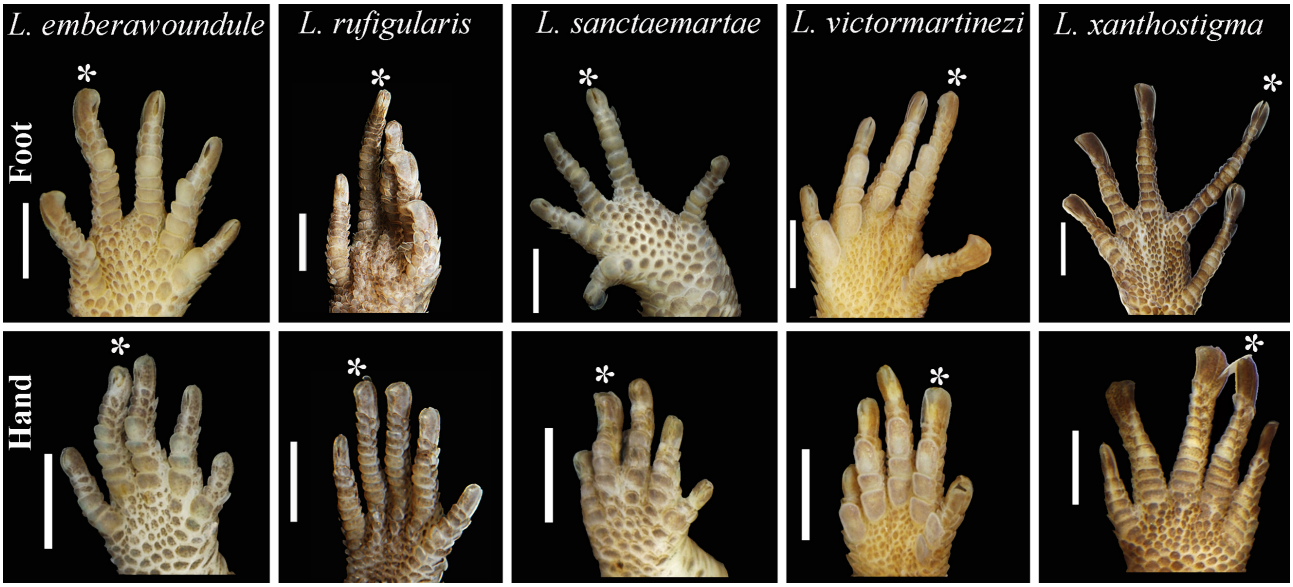

FIGURE 4. Feet and hands of Panamanian *Lepidoblepharis. Lepidoblepharis emberawoundule:* right foot of SMF 81954 and right hand of MHCH 2957; *L. rufigularis* (SMF 50659): right foot and right hand; *L. sanctaemartae:* left foot of SMF 97419 and right hand of MHCH 2948; *L. victormartinezi*: left foot and left hand of SMF 89963; *L. xanthostigma:* left foot of SMF 90190 and left hand of SMF 89576. Scale bars equal 1 mm, tip of fourth finger and toe marked with an asterisk.

supralabials three, posteriormost one below center of eye; ear-opening small, oval, in oblique orientation; mental large, posterior margin somewhat U-shaped, with two barely discernible, very short paramedian clefts; five enlarged postmentals, slightly larger than the posteriorly adjacent chin scales (Fig. 3 B); scales on chin small, flat, rounded, and juxtaposed, on posterior region flat to flat-granular, slightly inclined backward; scales near posterior infralabials flat, subimbricate, and larger than scales in median area of chin; infralabials four, first largest and almost reaching anterior level of orbit, fourth below center of eye; throat with small granular scales, pointed and directed upward; dorsal scales on neck and body small, granular, and juxtaposed, at midbody and on posterior trunk pointed, granular, or, in frontal view, triangular, mostly directed posteriorly, some scales on flanks and in lumbar region pointed; dorsal skin on body too damaged to conduct longitudinal counts of middorsal scales; ventral scales flat, smooth, imbricate, with an ovoid posterior margin, increasing moderately in size from gular region to belly, posterior ventral scales longer than wide; 13 midventral scales in one HL, 23 between levels of axilla and groin, 28 to border of cloaca; ventral escutcheon patch with 62 scales, 7 long and 13 wide, escutcheon long/wide ratio 54%; subfemoral escutcheon with 3 scales in a single row under each thigh, and a barely discernible fourth scale in a second row under the right thigh; transition between ventrals and lateral scales abrupt; 17 longitudinal rows of ventrals at midbody; scales on precloacal plate similar to ventrals, except for those on border of cloaca, which are smaller; tail dorsally and laterally with flattened, smooth, and imbricate scales that are less elongate than ventrals (with a transitional zone at base of tail); underside of tail with a median row of moderately enlarged scales (usually no more than two times wider than the laterally adjacent subcaudal scales; Fig. 3 B), mostly with a repeated series of one median scale bordered laterodistally by one scale, followed by a slightly larger median scale in contact laterodistally with two scales, constituting a regular tail sequence of 1'1"; dorsal scales on forelimbs granular to conical; scales on hind limbs flat, smooth, and imbricate on anteroventral femoral and shank surfaces, granular elsewhere; fingers, from longest to shortest, IV-III-II-V-I; toes IV-III-II-V-I, fourth and third toes about the same length; 11 lamellae under fourth finger, 13 under fourth toe; claws enclosed by an ungual sheath composed of six scales, as typical for the genus.

Hemipenis morphology. The partially everted hemipenis of SMF 50659 (Fig. 5 B) is a small organ; sulcus spermaticus bordered by well-developed and smooth sulcal lips; asulcate area of the truncus covered by papillate calyces; proximal portion of apex covered by small spinulate calyces. Due to its incomplete eversion, it is not possible to determinate whether the hemipenis is as bilobate as those of other species of the genus described and/or pictured herein (Fig. 6). Nevertheless, it is apparent that the hemipenis of *Lepidoblepharis rufigularis* does not bear a conspicuous basal third lobule, in contrast to the other two species described herein.

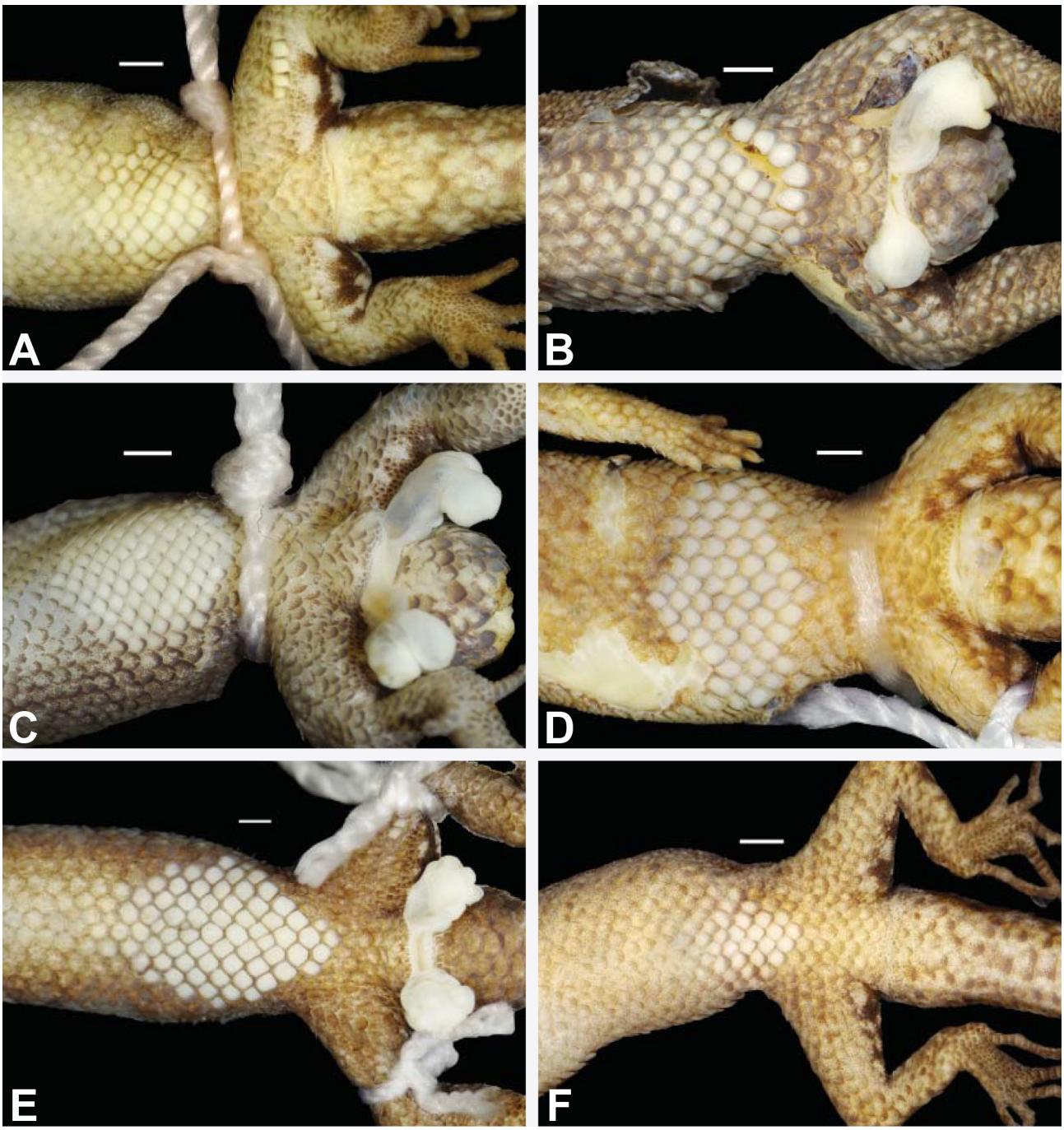

FIGURE 5. Escutcheon scales in males of Panamanian *Lepidoblepharis.* (A) *L. emberawoundule:* ventral escutcheon with 46 scales, 7 scales long x 11 wide, and subfemoral escutcheon with 4 scales per side, of SMF 81951 (SVL = 25 mm); (B) *L. rufigularis:* ventral escutcheon with 62 scales, 7 scales long x 13 wide, and subfemoral escutcheon with 3 and 4 scales per side, of SMF 50659 (SVL = 25 mm); (C) *L. sanctaemartae:* ventral escutcheon of SMF 97419 (SVL = 22 mm) with 92 scales, 10 scales long x 12 wide; (D) *L. victormartinezi:* ventral escutcheon of SMF 89963 (SVL = 27 mm) comprising 57 intact scales (an original total of 61 is assumed considering the symmetrical scale arrangement of the non-damaged portions), 7 scales long x 14 wide; (E–F) *L. xanthostigma:* (E) ventral escutcheon with 75 scales, 12 scales long x 11 wide, and subfemoral escutcheon with 2 scales per side (no additional scales hidden under strings), of SMF 90189 (SVL = 35 mm), and (F) ventral escutcheon with 36 scales, 6 scales long x 8 wide, and no subfemoral escutcheon of AB 1250 (SVL = 24 mm). All scale bars equal 1 mm.

Coloration in preservative (alcohol 70%). Dorsal ground color Hair Brown (277); posterior margin of orbit with a pale Beige (254) line; dorsum of head suffused with Beige (254); infra- and supralabials with alternating Sepia (279) and white bars; chin and throat with Sepia (286) reticulations on a dirty white background; venter pigmented with Vandyke Brown (281); escutcheon scales unpigmented in the center, with Olive Brown (278) borders.

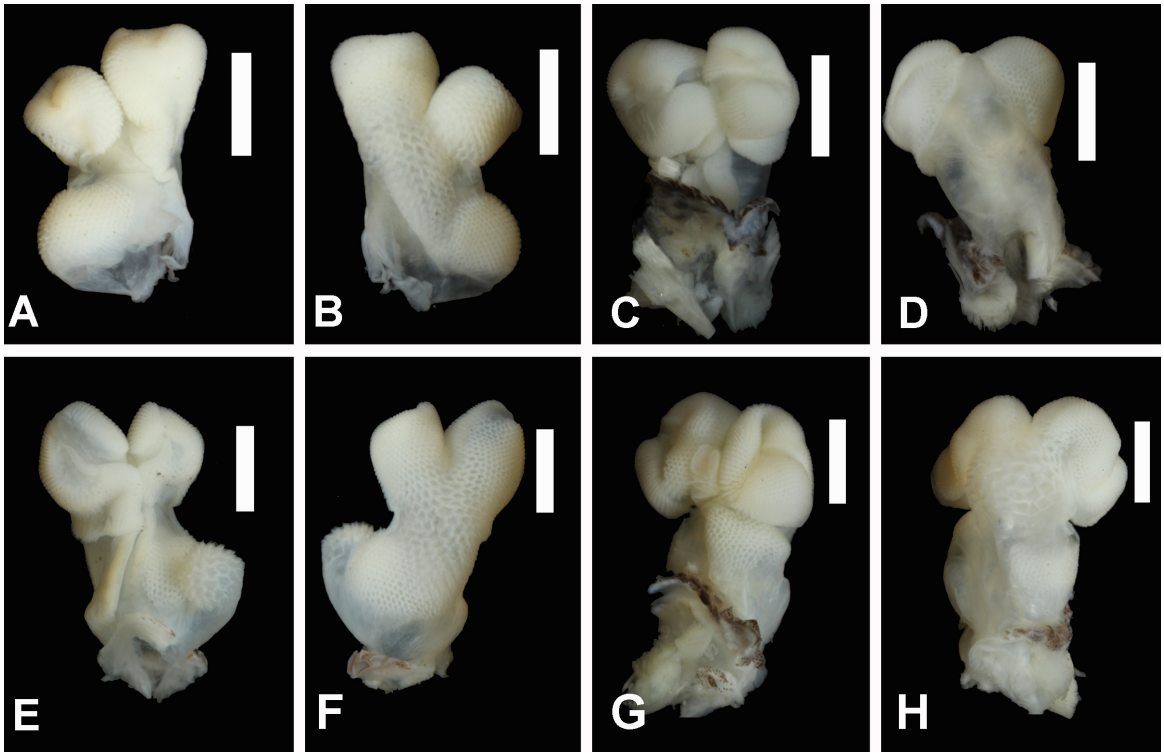

FIGURE 6. Hemipenis morphology of four *Lepidoblepharis* species from Panama. Sulcate-asulcate view, (A–B) *L. emberawoundule* SMF 50968; (C–D) *L. sanctaemartae* SMF 97419 (E–F) *L. victormartinezi* SMF 50950; (G–H) *L. xanthostigma* SMF 91558. All scale bars equal 1 mm.

Coloration in life (Fig. 11). Dorsal ground color Grayish Horn Color (268), with patches of Lavender Blue (195) and Vandyke Brown (281) scales, same pattern on neck and head; a straight Light Sky Blue (191) postorbital line, running up to level of ear; infra- and supralabials with alternating Sepia (279) and Burnt Orange (10) bars; chin and throat with Sepia (279) reticulations on a Burnt Orange (10) background; ventral parts suffused with Dusky Brown (285) and Light Sky Blue (191); escutcheon scales Smoke Gray (267), with Dusky Brown (285) scale tips; tail with a dorsolateral Salmon Color (59) line; toes and fingers suffused with Brick Red (36).

Distribution and habitat. *Lepidoblepharis rufigularis* is known only from the type locality in southeastern Darién province. Given the close proximity of the type locality to Colombia, it likely occurs there as well. The habitat at the type locality of *L. rufigularis* is part of the eastern Panamanian montane forests (Fund 2011), in the Darién mountain range, around 1000 m elev. Most probably, *L. rufigularis* lives in the leaf-litter and feeds on small invertebrates like other *Lepidoblepharis* spp. (Vitt *et al*. 2005). The specimen probably resided between the leaf-litter on a large fallen log, and was uncovered and collected after one of the local supporters in the group stepped over the log.

Etymology. The name *rufigularis* is a compound word that comes from the Latin *rufus* (red) and *gula* (throat) referring to the bright orange throat color in this species in life.

***Lepidoblepharis victormartinezi* sp. nov.**

Figs. 3–6, 12

Lepidoblepharis sp.: Martínez & Rodriguez (1994: possibly); Martínez *et al.* (1995: possibly).
Lepidoblepharis xanthostigma: all in part. (referring to certain populations in Colón and Veraguas): Auth (1994); Young *et al.* (1999); Ibáñez *et al.* (2001); Köhler (2008); Carrizo (2010: referring to SMF 89963); Jaramillo *et al.* (2010); Lotzkat *et al.* (2010: referring to SMF 89963).

Holotype. Adult female SMF 50951, original field number AB 1241 (Fig. 12) collected in leaf-litter 500 m east of the village Chicheme (8.865278°N, 80.643829°W, 100 m elev.), San José del General, Donoso district, Colón province, Panama, collected on 25 January 2013 at 11:40 hrs by Abel Batista, Lester Vásquez, and Leysi Díaz.

Paratypes. Four adult males, all from Panama: SMF 89963 from Cerro Negro, Veraguas, on 28 July 2008; SMF 50950 (collected on 20 July 2011), 50952, and MHCH 2954 from Petaquilla, Coclé del Norte, Donoso, Colón; see Appendix I for locality details.

Diagnosis. *Lepidoblepharis victormartinezi* (our **sp. nov.** 3) is characterized by the following combination of characters: *(1)* dorsal scales small, granular, and juxtaposed, ventral scales large, cycloid, flat, and imbricate; *(2)* scales on head small and granular; *(3)* 3–5 (4.4 ± 0.89) postrostrals; *(4)* two paramedian clefts demarcate the slightly convex median portion of the posterior mental border, rendering the latter vaguely M-shaped in its totality; *(5)* 5–7 (6.0 ± 0.71) postmentals; *(6)* lamellae under fourth toe 6–8 (6.6 ± 0.89), lamellae under fourth finger 4–5 (4.8 ± 0.45), the subdigital lamellae under each digit showing a peculiar morphology that is unique within the genus, with 1–3 proximal one(s) longitudinally greatly enlarged, each about 3–4 times longer than any of the remaining lamellae; *(7)* median subcaudals only slightly larger than the neighboring scales, about as long as wide, with rounded posterior margins, arranged in a regular tail sequence of 1'1" (Fig. 3 D); *(8)* ventral escutcheon consisting of 61–68 (63.3 ± 3.30) scales, 6–8 (6.8 ± 0.96) scales long and 12–15 (13.8 ± 1.26) wide; *(9)* lack of a discernible subfemoral escutcheon; *(10)* ventral scales at midbody 15–19 (17.4 ± 1.52); *(11)* dorsal scales at midbody 53–67 (59.8 ± 5.36); *(12)* bilobate hemipenis, with a third lobule rising from the pedicel; *(13)* SVL 25–27 (25.6 ± 0.89) mm.

Comparison with other species of the genus. *Lepidoblepharis victormartinezi* can be differentiated from many species in the genus by its small size and its low number of lamellae under the fourth toe and finger (Figs. 3 D; 4). Moreover, the conspicuous morphology of its subdigital lamellae is unique within the genus and immediately distinguishes it from any described congener. In the following, we provide further comparisons to all other species within the genus, with the characteristics for *L. victormartinezi* in parentheses. *Lepidoblepharis victormartinezi* can be distinguished from the Panamanian species *L. xanthostigma*, *L. sanctaemartae, L. rufigularis,* and *L. emberawoundule* by a genetic p-distance of 10–21% in 16S mtDNA between individuals. *Lepidoblepharis xanthostigma* has greatly enlarged median subcaudal scales (slightly enlarged) and 12–16 lamellae under its fourth toe (6–8). *Lepidoblepharis sanctaemartae* has large, flat, imbricate dorsal body scales (small, granular, juxtaposed). *Lepidoblepharis emberawoundule* has 5–8 lamellae under its fourth finger (4–5), a ventral escutcheon consisting of 38–61 scales (57–68), and a well-discernible subfemoral escutcheon consisting of 4–5 scales in a single row under each thigh (no discernible subfemoral escutcheon). *Lepidoblepharis rufigularis* has 13 lamellae under its fourth toe (6–8) and 11 under its fourth finger (4–5). To date, seven species of the genus *Lepidoblepharis* have been reported to possess ten or fewer lamellae under the fourth toe, *i.e.,* to be short-toed. Two of these, *L. miyatai* and *L. sanctaemartae,* possess large, flat, and imbricate dorsal scales (dorsal scales small, granular, and juxtaposed). *Lepidoblepharis buchwaldi, L. microlepis, L. montecanoensis, L. peraccae,* and *L. williamsi* can confidently be ruled out as conspecifics since the inspection of the illustrations and photographs available for the holotypes showed no subdigital lamellae to be as conspicuously enlarged as in *L. victormartinezi.* Moreover, in the holotype of *L. peraccae* the plantar and palmar scales have the posterior border ovoid and imbricated (plantar and palmar scales small rounded and juxtaposed), and in the holotype of *L. microlepis* all of the slightly enlarged median subcaudal scales are bordered laterodistally by one scale, forming a regular tail sequence of 1'1' (the slightly more enlarged median subcaudals bordered laterodistally by two scales, the slightly smaller ones by one, forming a regular tail sequence of 1'1"). The remaining species of the genus, *i.e., L colombianus, L conolepis, L. duolepis, L. festae, L. grandis, L. heyerorum, L. hogmoedi, L. intermedius,* and *L. ruthveni* are long-toed with eleven or more lamellae under the fourth toe (6–8 in *L. victormartinezi*).

Description of holotype. Variation among the entire type series is given in parentheses (see Table 1 for details). Adult female as indicated by absence of hemipenes and escutcheon scales; SVL 25 mm (25–27 mm), TL 27.2 mm (27–31), HL 5.4 mm (4.9–6.1 mm), HW 3.7 mm (3.7–4.2 mm), forelimbs 5.7 mm (6.8–5.7), hind limbs 8.3 mm (9.8–7.4), shank 3.5 mm (3.5–4.3); rostral large, clearly visible from above, with a shallow, horseshoe-shaped posterior depression and a long median cleft; postrostrals four (3–5) including supranasals, median ones smaller than supranasals, and indenting the rostral, at least one median postrostral slightly larger than posteriorly adjacent scales on snout; postnasals two (1–2), both about same size as posteriorly adjacent loreal scales; scales on snout small and smooth, 17 (16–22) scales across snout between anterior sutures of second SPLs; loreal scales subimbricate, elevated towards posterior and dorsal directions, 6 (5–9) loreal scales on a longitudinal line between postnasals and orbit; scales on top of head small, granular, and juxtaposed, generally pointing upward, about half as large as those on the central snout; superciliary flap with two enlarged scales on anterior border, of which the first

is slightly longer than the second; followed by 2–5 small and globular scales; supralabials three (3–4), posteriormost one below center of eye; ear-opening small, oval, in oblique orientation; mental large, posterior margin vaguely M-shaped and slightly convex in the middle, with two short paramedian clefts; postmentals five (5–7), flat and slightly larger than the posteriorly adjacent chin scales, the two median ones slightly larger than the others (1–2 slightly larger than the others); scales on chin small, rounded, and juxtaposed; on posterior chin region granular to flat-granular, approximately vertical in position or slightly inclined, pointing posteriorly, becoming smaller posteriorly; scales near posterior infralabials flat, subimbricate, and larger than in median area of chin; infralabials four, first largest and almost reaching anterior level of orbit, fourth below center of eye; throat with a short transition between the anterior region with small granular scales and the posterior region with larger, granular, and posteriorly pointing scales; dorsal scales on neck and body small, granular, and juxtaposed, at midbody and posterior portion of trunk pointed-granular or, in frontal view, triangular, mostly directed posteriorly, some flat-granular on flanks and in lumbar region; 38 (36–42) middorsal scales in one HL, 78 (78–96) between levels of axilla and groin; ventrals flat, smooth, imbricate, with an ovoid posterior margin, increasing moderately in size from gular region to belly, posterior ventral scales longer than wide; 13 (10–15) midventral scales in one HL, 27 (24–27) between levels of axilla and groin, 33 (29–33) to border of cloaca; transition between ventrals and scales on flanks abrupt; scales around midbody 77 (71–84), of which 19 are ventrals (15–19); scales on precloacal plate similar to ventrals, except for those on border of cloaca, which are smaller; tail dorsally and laterally with flattened, smooth, and imbricate scales, less elongated than ventrals (with a transitional zone at base of tail); underside of tail with a median row of slightly enlarged scales, mostly with a repeated series of one median scale bordered laterodistally by one scale, followed by a slightly larger median scale in contact laterodistally with two scales, constituting a regular tail sequence of 1'1"; dorsal scales on forelimbs granular to flat-granular; scales on hind limbs flat, smooth, and imbricate on anteroventral femoral and shank surfaces, granular elsewhere; fingers, from longest to shortest, IV-III-II-V-I; toes IV-III-II-V-I, fourth and third toes about the same length; lamellae under fourth finger five (4–5), with 2 (2–3) proximal ones greatly enlarged, each about 3 (3–4) times as long as the remaining distal ones; lamellae under fourth toe six (6–8), with 2 (2–3) proximal ones greatly enlarged; claws enclosed by an ungual sheath composed of six scales, as typical for the genus.

Hemipenis morphology. The everted hemipenis of SMF 50950 (Fig. 6 E–F) is a small, bilobate organ, divided for around one third of its length, with a naked base; sulcus spermaticus bordered by well-developed, smooth sulcal lips; lips opening into two broad concave areas, one on each lobe; ornamentation of papillate calyces present on each lobe; asulcate area of the truncus covered by papillate calyces and some barely visible spinulate calyces; a third lobule like rising from the pedicel, not connected to the sulcus spermaticus, and covered by spinulate calyces.

Coloration in preservative (alcohol 70%; variation among the paratypes in parentheses). Dorsal ground color Hair Brown (277); occipital marks Grayish Horn Color (268) (Drab-Gray (256) in males), posteriorly bordered with Sepia (279); dorsum of head Army Brown (46); infra- and supralabials with alternating Sepia (279) and white transverse bars; chin and throat with Sepia (286) reticulations on a dirty white background; venter strongly suffused with Vandyke Brown (281) (escutcheon of males unpigmented in the center, with Olive Brown (278) borders).

Coloration in life. Dorsal ground color Mahogany Red (34), with small scattered Lavender Blue (195) and Sepia (286) dots; neck region Glaucous (272); a Sepia (286) line from the tip of the snout to the anterior border of the eye; two postorbital Sepia (286) lines, one directed to the occipital region and the other towards the ear; infra- and supralabials with alternating Sepia (279) and dirty white transverse bars; two occipital M-shaped Smoke Gray (267) marks (Chamois (84) in males), posteriorly bordered with Sepia (279); chin and throat with Sepia (286) reticulations on a dirty white background; ventral ground color Lavender Blue (195), strongly suffused with Vandyke Brown (281); a pale dorsolateral line from above the hind limbs to mid tail, a Sepia (286) line from above groin to mid tail; toes and fingers Vandyke Brown (282).

Distribution and habitat. *Lepidoblepharis victormartinezi* is an endemic species of the Isthmian-Atlantic moist forests in west-central Panama (Fund 2011), known from around 100 m elev. in the province of Colón and 700 m elev. in Veraguas province. Most probably, *L. victormartinezi* lives in the leaf-litter and feeds on small invertebrates like other *Lepidoblepharis* (Vitt *et al*. 2005). Most specimens have been found on top of small hills, giving the impression that this species prefers drier environments on the hills rather than the more wet flat areas around the same locality. However, the specimen SMF 89963 was found in a wet flat area.

Etymology. The specific epithet *victormartinezi* is a patronym for Victor Martínez Cortés, who has pioneered Panamanian herpetology among native researchers, and was the first Panamanian herpetologist ever to publish his results in scientific journals. Since the 1980s, he has conducted herpetological inventory work at biogeographically significant localities throughout western Panama. The now unfortunately lost (V. Martínez, personal communication) specimens of "*Lepidoblepharis* sp." mentioned in his species lists of the region around Santa Fé de Veraguas (Martínez et al. 1995, Martínez & Rodriguez 1994), which includes Cerro Negro as the provenance of one of our paratypes, might have been the first specimens of this new species that were ever collected. We dedicate this species to our friend and colleague Victor Martínez in due recognition of his passionate dedication to, and great achievements for, Panamanian herpetology.

Discussion

The geckos of the genus *Lepidoblepharis* represent a typical case of a group of "small brown lizards" that are all very similar at first glance and have long been neglected by taxonomists, although they seem to be rather common in many places. Still, there is no contemporary comprehensive taxonomic revision based on an adequate number of specimens representing all known species. Ever since the only revision considering all then known species (Parker 1926), at a time when eight of the currently recognized 18 species of *Lepidoblepharis* had already been described, the subdigital lamellae have been accepted to be a character of paramount diagnostic value in *Lepidoblepharis* systematics, which also holds for the five Panamanian species reviewed herein. However, our analyses clearly show that other characters traditionally emphasized in *Lepidoblepharis* species descriptions, such as rostral shape and numbers of postrostrals, loreals, or scales across snout, are less helpful in distinguishing the four Panamanian species with granular dorsal scales, owing to the interspecific overlap in these counts (Fig. 1). Instead, we found some character sets on the ventral surface of these small lizards (*e.g.,* the escutcheon) to clearly differentiate the Panamanian species from each other as well as from the known South American species, and take the opportunity to briefly discuss them in the following.

The shape of the posterior border of the mental plate as well as the number and size of the postmentals are characters widely used in *Lepidoblepharis* systematics, and can also help to distinguish among the five Panamanian species (Figs. 3; 7 A–B). In *L. sanctaemartae,* the mental plate has a concave posterior border that may be rounded or obtusely angular with a single median cleft and usually three, sometimes up to five postmentals (Fig. 3 C). *Lepidoblepharis emberawoundule* exhibits two paramedian clefts and a vaguely M-shaped posterior mental border, and with 3–7 has a wider range of postmentals than *L. sanctaemartae* (Fig. 3 A). In *L. victormartinezi*, two paramedian clefts demarcate a convex median portion of the posterior mental border, that results vaguely M-shaped in its totality and thus similar to *L. emberawoundule,* and the number of postmentals is 5–7 in the examined specimens (Fig. 3 D). In *L. rufigularis* the posterior border is somewhat U-shaped with 5 enlarged postmentals, and two very short and barely visible clefts (Fig. 3 B). In *L. xanthostigma,* the posterior mental border may be concave, straight, or vaguely M-shaped and is bordered by 3–6 postmentals. Most specimens (21 of the 26 examined) lack clefts in the posterior border of the mental plate, but some specimens exhibit one (three of the 12 specimens from Nicaragua) or two (two of the 12 specimens from Panama) short and usually weakly developed paramedian or lateral clefts (Fig. 3 E).

In all recent species descriptions for this genus, the possible presence of enlarged median subcaudals has been noted. However, contrary to other sphaerodactylid genera (Rivero-Blanco 1979; Avila-Pires 1995), usually little attention has been paid to the exact scalation pattern along the median series of subcaudals, which we found to be of good diagnostic value. All Panamanian *Lepidoblepharis* have a single median series of enlarged subcaudal scales that originates less than ten scales posterior to the cloacal opening. Throughout this series, slightly larger and wider scales alternate with slightly smaller and narrower ones, as already noted for *L. xanthostigma* by Taylor (1956). The smaller scales are bordered laterodistally by one scale, the larger ones by two, forming a regular tail sequence of 1'1" (see Figs. 3 and 7 E–F). In *L. xanthostigma,* the enlarged subcaudals typically are at least twice as wide as the laterally adjacent subcaudals and much wider than long (twice as wide as long in most examined specimens). Moreover, their posterior margins are straight, or almost so, oriented at right angle to the longitudinal axis of the tail (Fig. 3 E). In *L. emberawoundule* and *L. rufigularis*, the median subcaudals (Figs. 3 A and B, 7 F) are also conspicuously wider than long, but neither twice as wide as the laterally adjacent scales nor twice as wide

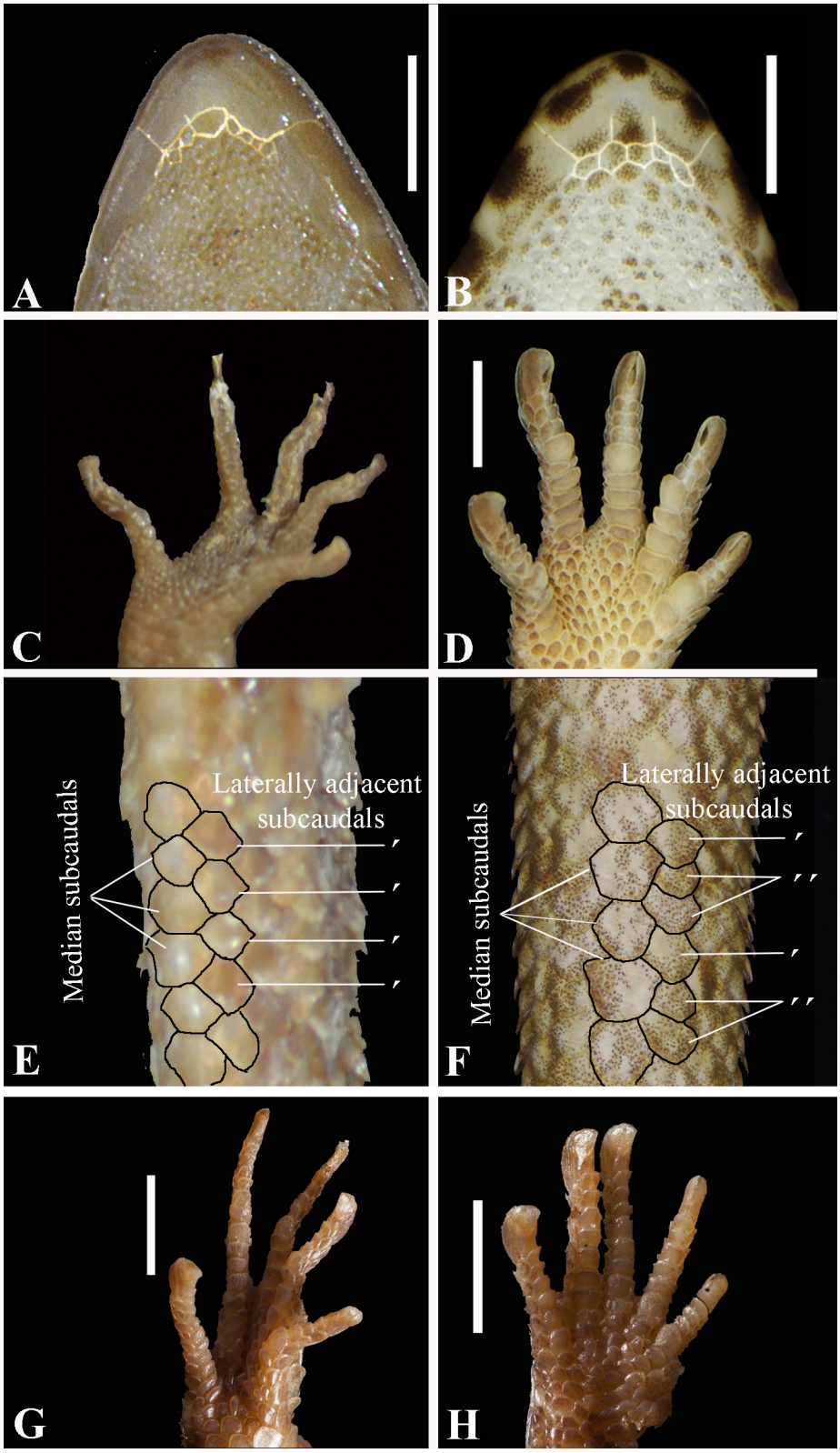

FIGURE 7. Comparison between *Lepidoblepharis emberawoundule, L. microlepis* (holotype), and *L. peraccae* (holotype). Mental plate and postmental scales in (A) *L. microlepis and* (B) *L. emerawondule*; (C) right foot of *L. microlepis;* (D) right foot of *L. emberawoundule*; (E) subcaudal scales in *L. microlepis*, each of the slightly enlarged median subcaudal scales is bordered laterodistally by only one scale, leading to a regular tail sequence of 1'1'; (F) subcaudal scales in *L. emberawoundule*, the larger of the enlarged subcaudals are bordered laterodistally by two scales, the smaller ones by one, forming a regular tail sequence of 1'1''; (G) right foot and (H) right hand of the holotype of *L. peraccae;* scale bars equal 1 mm.

as they are long. In *L. emberawoundule,* (Figs. 3 A and 7 F) their posterior margins may be straight as in *L. xanthostigma* and *L. rufigularis* (this is the case in most specimens that we have examined) or rounded. The median subcaudals of *L. sanctaemartae* have a similar morphology as those of *L. emberawoundule*, but their posterior margins are rounded in all specimens we examined (Fig. 3 C). Finally, *L. victormartinezi* has its median subcaudals only slightly larger than the neighboring scales, about as long as wide, with rounded posterior margins in all examined specimens (Fig. 3 D). It must be noted that the state of the enlarged median subcaudals can only be assessed reliably in original tails, or the original portion of a regenerated tail. Regenerated tails or tail portions generally exhibit a modified scale structure, and this also applies to the median subcaudal series in all specimens with regenerated tails that we have examined. For example, in *L. xanthostigma* the median subcaudals in regenerated tail portions are shaped very irregularly, may become much shorter and at the same time much wider, and/or apparently fuse with regular subcaudals to extend almost to or even onto the lateral surfaces, and/or be arranged in pairs or groups of three.

Another character that we found to have diagnostic value among the five species involved has to date largely been neglected in taxonomic studies of the genus *Lepidoblepharis,* although discussed in detail by Taylor (1956) and Taylor & Leonard (1956) and recognized to distinguish certain species of *Sphaerodactylus* (Harris 1982; Harris & Kluge 1984): the escutcheon scales on the venter, and in some species also on the subfemoral surfaces, of male individuals (Fig. 5). Largely or completely lacking pigmentation, these glandular scales more or less conspicuously contrast with the surrounding ventral and subfemoral scales. Apart from allowing for confidently determining the sex of all but very young individuals, the number and especially the arrangement of the escutcheon scales is quite different among the five lineages. The examined males of *L. sanctaemartae* have a ventral escutcheon consisting of 64–92 scales that spans 9–11 scales in length and 11–12 scales in width, which is relatively long and, since it spans most of the ventral body surface, also relatively wide. Along the ventral surface of the thigh, no subfemoral escutcheon scales are distinguishable (Fig. 5 C). In the males of *L. emberawoundule* that we examined, the ventral escutcheon consisting of 38–61 scales is short (6–7 scales) and relatively narrow (10–13 scales in width, leaving out some longitudinal rows of ventrals on each side). Additionally, all examined males of this lineage have a clearly visible subfemoral escutcheon consisting of 4–5 scales arranged in a single row under each thigh (Fig. 5 A). In the holotype of *L. rufigularis* the ventral escutcheon comprising 62 scales is short (7 scales) and relatively wide (13 scales in width, leaving out two longitudinal rows of ventrals on each side). Its subfemoral escutcheon consists of 3 scales arranged in a single row per thigh, plus apparently a fourth scale under the right thigh which is positioned posterior to the existing row, as if to begin a second row (Fig. 5 B). Examined males of *L. victormartinezi* have a ventral escutcheon consisting of 61–68 scales that is short (6–8 scales) but involves most if not all longitudinal rows of ventrals at its central level (12–15 scales), and can thus be considered as relatively wide. Males of this species lack a discernible subfemoral escutcheon (Fig. 5 D). In the examined males of *L. xanthostigma,* the ventral escutcheon contains 36–104 scales (in our sample; Taylor 1956 counted 28–110) and may be short or long. As noted by Taylor (1956), it is usually shorter in smaller males (6–9 scales in four of the six males with SVL < 30 mm, 10 and 12 in the other two) and longer in larger males (10–12 scales in the eight males with SVL > 30 mm). The ventral escutcheon may appear narrow to rather wide (8–12 scales), though it never covers all longitudinal rows of ventral scales at its widest level. Most examined males (16 of 18) have a distinct subfemoral escutcheon at least under one thigh, comprising 1–8 scales in one or two rows per side (Fig. 5 E–F).

In this work, we describe for the first time the hemipenis morphology of species of the genus *Lepidoblepharis* (Fig. 6). Since there are no descriptions available for other species from South America, we cannot compare them. Everting hemipenes is not always easy, especially for these diminutive lizards that have a delicate skin and a tail that breaks easily. We encourage whoever works on the taxonomy of *Lepidoblepharis* to evert hemipenes, and use the hemipenis morphology for taxonomical comparisons, since the structure of this organ has an important systematic value, is used as a standard character to differentiate species in other lizard groups (Glaw *et al*. 2006; Köhler *et al*. 2007; Maduwage & Silva 2012), and possibly plays a major role in the reproductive isolation during early stages of speciation (see examples from *Anolis* lizards by Eberhard 1985; Köhler *et al*. 2012a). At least among the four Panamanian species for which we have fully everted hemipenes available (Fig. 6), these organs are very distinct among the species and, thus, constitute valuable taxonomic tools as in other squamate groups.

DNA barcoding is a useful tool for identifying reptile species, as has been shown recently (Nagy *et al*. 2012; Lotzkat *et al*. 2013; Vences *et al*. 2014; Köhler *et al.* 2012b, 2014). For gekkonids from Madagascar, Nagy *et al.* (2012) found a threshold of 13.3% in COI p-distance for closely related and morphologically very similar species

("sister species" in their terminology), and 24.2% between "good species" (*i.e.,* species that are not too closely related and also morphologically well-distinguishable). We found average values of 18 and 23% for the same gene, respectively. Although in both places the genetic differentiation is high, both have a different geological history, which might account for the differences between genetic distances among the geckos from Madagascar and Panama (*e.g.,* via stronger isolation mechanisms having acted in the speciation among the closely related species in the latter). However, more sampling of *Lepidoblepharis* specimens is required (including more species from South America) to allow for more definitive conclusions about the genetic relationships of these species in the Neotropis. In our analysis, we have included only 6 out of the now 21 nominal species contained in the genus (not even 30% of the known species compared to about 67% of the known Malagasy gekkonids that were barcoded by Nagy *et al.* 2012). We used genetic distances in the diagnoses even though they can be very variable among and within groups (Nagy *et al.* 2012; Vences *et al*., 2014). However, we support our molecular data with robust morphological and biogeographical evidence, thus reducing the bias toward molecular data through the application of an integrative approach (Huang *et al.,* 2013; Shen *et al.,* 2013; Trewick 2008).

Looking at the phylogenetic trees and p-distances (Figs. 2 and 8; Table 2; Appendices III–VI), one is struck with the high genetic divergences inferred within the new species *Lepidoblepharis emberawoundule.* In COI, SMF 50969 from Pechito Parado (near Río Tuquesa) was most closely related to the sample from the nearest place (ca. 81 km) at Taintídu River (MHCH 2951; 3.7%), whereas the samples from Burbayar (SMF 50968) and Río Terable (MHCH 2952) showed a p-distance of 7.3% between each other, even though they have been collected only ca. 4.5 km from each other. Possible explanations for this discrepancy between geographic and genetic distances should take into consideration that one of the latter localities is on the Pacific slope and the other on the Caribbean slope, while Río Tuquesa and Río Taintídu are along the same versant. Concerning the 16S gene, SMF 50969 was the most different, with an average p-distance of 11% (ranging from 8–15%) to the other samples assigned to this species. In the phylogenetic trees (Figs. 2 and 8) it is apparent that our new species *L. emberawoundule* is composed of three deeply divergent genealogical lineages, the p-distances among which are much larger than those calculated among individuals of any other species included in our analyses, some being in the order of magnitude of the interspecific p-distances inferred herein. The first of these lineages is represented by the holotype (SMF 50968) and a paratype (MHCH 2952) from the Serranía de San Blas. The second lineage is represented by the two barcoded specimens (SMF 50969 and MHCH 2951) from the southeasterly adjacent Serranía de Darién, and the third lineage, which was inferred as the basalmost one and sister to the other two, by the single specimen collected near Cerro Sapo (MHCH 2957). Unfortunately, we lack sequences of the individual from the Serranía de Pirre (SMF 50970) and, thus, can only assume its pertainance to the third lineage based on geographical proximity and geological affinity of the respective collection sites. In view of the deep genetic divergences among these three lineages, it seems well imaginable that the second and third lineage actually represent two additional species. Yet, we refrain from describing them as such because we did not find any consistent morphological distinctions to the type series. The only suggestion of such a distinction is the ventral escutcheon of the male MHCH 2951 (lineage 2), which is composed of 61 scales while those of the type series bear merely 38–53 scales (unfortunately, all other non-type specimens that we collected are females or juveniles). To adequately address this issue and take a well-suported decision on the number of species involved, more specimens as well as more DNA sequences (preferably also of nuclear genes) from Darién province and the Comarca Emberá are required.

Furthermore, our results necessitate some remarks on the taxonomy and biogeography of the two species traditionally recognized in Panama, *Lepidoblepharis sanctaemartae* and *L. xanthostigma.* Concerning the former, Ruthven (1928) described Panamanian populations from Barro Colorado Island and Cerro Sapo as a new subspecies, *L. sanctaemartae fugax,* which he diagnosed from the nominal subspecies by their higher number of 4–5 postmentals (vs. 2–3, rarely 4). We did not find this alleged subspecific distinction reflected by our material, since five of the 8 specimens we collected in eastern Panama have only three postmentals. Regarding the westward extension of this species' distribution, our westernmost examined specimen is FMNH 60196 collected near Cerro Campana in western Panama province, probably close to the literature record from Parque Nacional Altos de Campana (Ibáñez *et al.* 1996). In our opinion, the record from Reserva Forestal La Tronosa in southern Los Santos province (Elizondo *et al.* 2007) is somewhat doubtful, possibly based on a misidentification, and thus requires verification, partially owing to the fact that these authors also listed *Sphaerodactylus homolepis,* a species with an entirely Caribbean distribution (Harris & Kluge 1984), for the same area. More decidedly, we are convinced that the record from the Humedal de Importancia Internacional San San Pond Sak in western Bocas del Toro province (ANAM 2004) must be based on a misidentification.

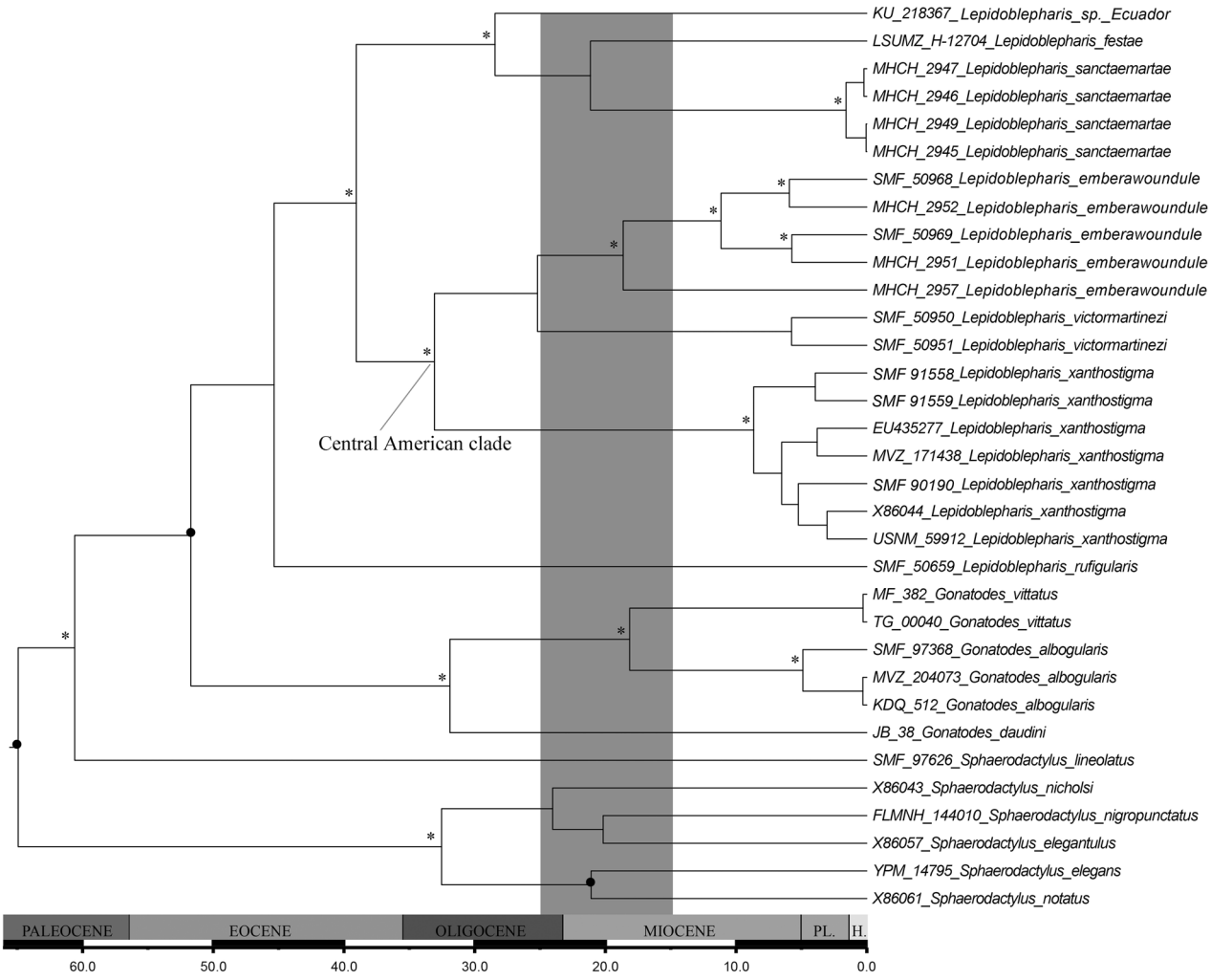

FIGURE 8. A chronogram of *Lepidoblepharis* species based on a relaxed-clock Bayesian analysis of 16S and COI mtDNA. Scale indicates time in Ma. The vertical gray shaded area indicates the ages comprising the hypothetical course of the Panamanian isthmus closure (15–25 Ma). Asterisks on nodes indicate the estimated posterior probabilities $P \geq 0.95$; black circles correspond to calibrated nodes (see methods for details).

Regarding the distribution of *Lepidoblepharis xanthostigma*, in view of our material and the absence of literature records or traceable specimens from discrete localities in eastern Panama, our results restrict the documented distribution of this species to the area between southeastern Nicaragua and central Panama just east of the canal. Our easternmost examined specimens assignable to this species (FMNH 153600 and 154549) come from Cerro La Victoria at about 79.38°W. The only literature records from more eastern sites in Panama (Cerro Guagaral or Brewster at ca. 79.25°W and Cabecera del Río Mandiga at ca. 79.2°W; Ibáñez *et al.* 1995, no voucher specimens mentioned) lie about halfway between Cerro La Victoria and the type locality of *L. emberawoundule,* and might as well be based on observations of that species in our opinion. The distribution of *L. xanthostigma* as inferred herein implies the absence of this species from Colombia, meaning that the long-toed specimens with granular dorsals reported as *L. xanthostigma* from Colombia (Ayala & Castro 1983) should represent a different species whose identity remains to be clarified, since they also differ from our new species *L. rufigularis* as detailed in its diagnosis. Concerning the variation and taxonomy of *L. xanthostigma,* we tentatively dismiss the minimum value of 11 lamellae under the fourth toe given for the species by Ayala & Castro (1983) in our diagnoses and key, since these authors do not specify the source of this value (except making clear that it was not obtained from their Colombian specimens) and the minimum value among our material is 12. Simultaneously, we raise the maximum number of lamellae under the fourth toe from 14 to 16. Our results further show that the short-toed *L. microlepis* is by no means synonymous with *L. xanthostigma* as suspected by Ruthven (1928) and discussed by Ayala & Castro

(1983), or with *L. peraccae* as suspected by Ayala & Serna (1986), and in our view confirm its status as a valid species as opposed to the views of previous authors (Lamar 1985; Markezich & Taphorn 1994; Avila-Pires 2001). Last, we are convinced that the single record of *L. peraccae* from Panama (Breder 1946, p. 426: AMNH 65296 from "Río Chagres below Gatun Dam", *i.e.,* just west of the canal) is based on a misidentification and probably referable to *L. xanthostigma* or *L. victormartinezi,* pending examination of the specimen.

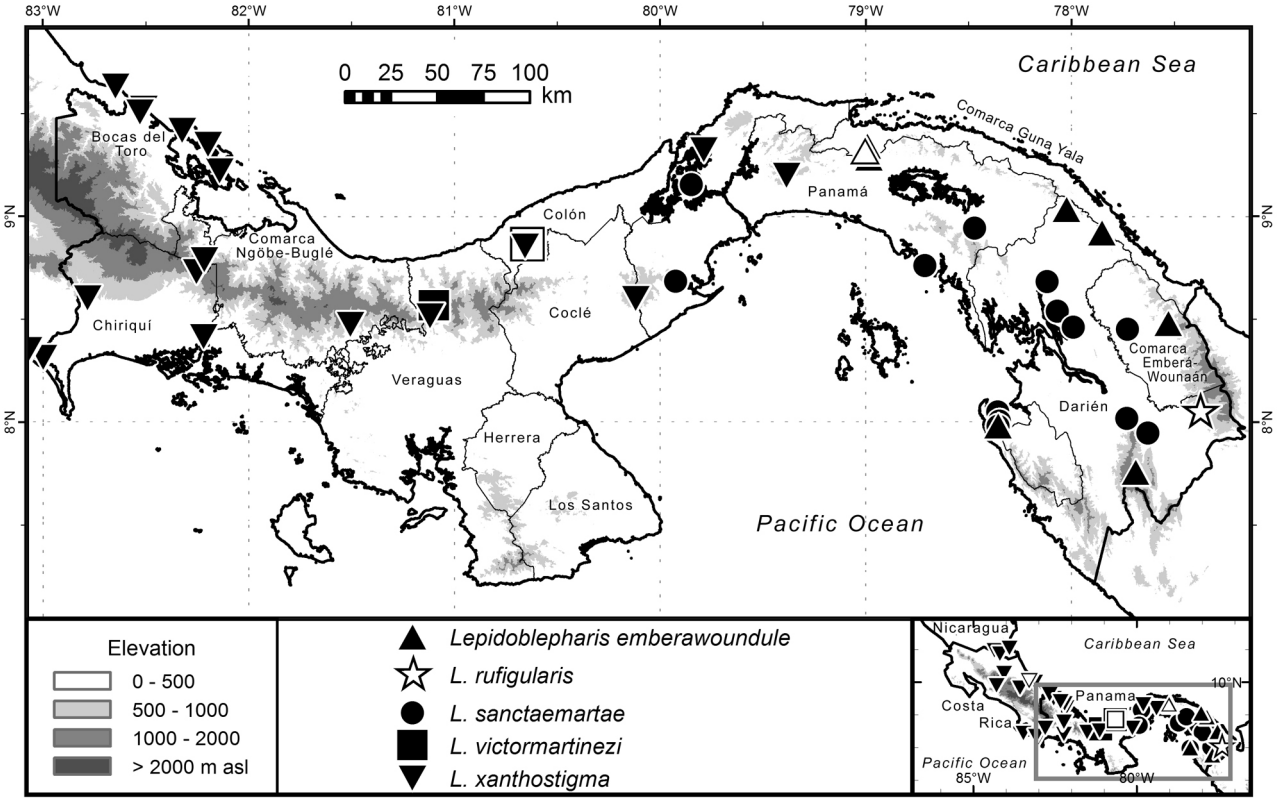

FIGURE 9. Collection localities of Central American *Lepidoblepharis* specimens examined in this study. Hollow symbols represent type localities.

FIGURE 10. Holotype of *Lepidoblepharis emberawoundule* (male SMF 50968) in life. (A) entire specimen; (B) dorsal view of the head; (C) lateral view of the head.

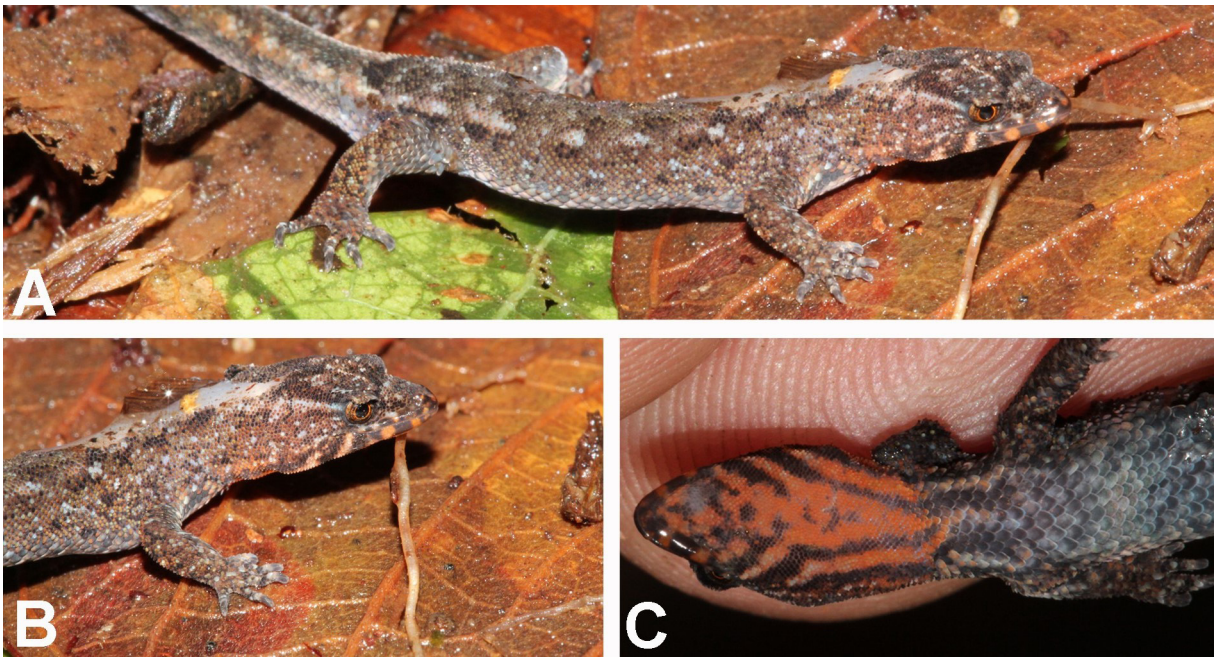

FIGURE 11. Holotype of *Lepidoblepharis rufigularis* (male SMF 50659) in life. (A–B) lateral view; (C) gular and chest region.

FIGURE 12. Holotype of *Lepidoblepharis victormartinezi* (female SMF 50951) in life. (A, C) entire specimen; (B) lateral view of the head.

In Lower Central America, the genus *Lepidoblepharis* exhibits a biogeographical pattern congruent with the phylogenetic analysis (see below), with a reduction in species numbers towards southeastern Nicaragua. In eastern Panama, there are three species present (*L. emberawoundule, L. rufigularis,* and *L. sanctaemartae*). In central Panama, there are also three species, but a different combination of species (*i.e., L. victormartinezi, L. xanthostigma,* and *L. sanctaemartae*). In western Panama, only two species occur, with *L. victormartinezi* reaching the west-central part of the country in the Tabasará mountain range. Further west, *L. xanthostigma* remains as the only *Lepidoblepharis* species ranging as far as southeastern Nicaragua. Most species prefer humid areas on the foothills of the San Blas, Sapo, Jingurudó, Pirre, Darien, and Cordillera Central mountain ranges and in the Caribbean lowlands, where annual precipitation is usually higher than 2500 mm. The only exception seems to be *L.*

sanctaemartae, which appears to tolerate drier conditions, since it is distributed also in the Pacific lowlands of Darién and west-central Panama where the annual precipitation is less than 2000 mm (http://www.hidromet.com.pa).

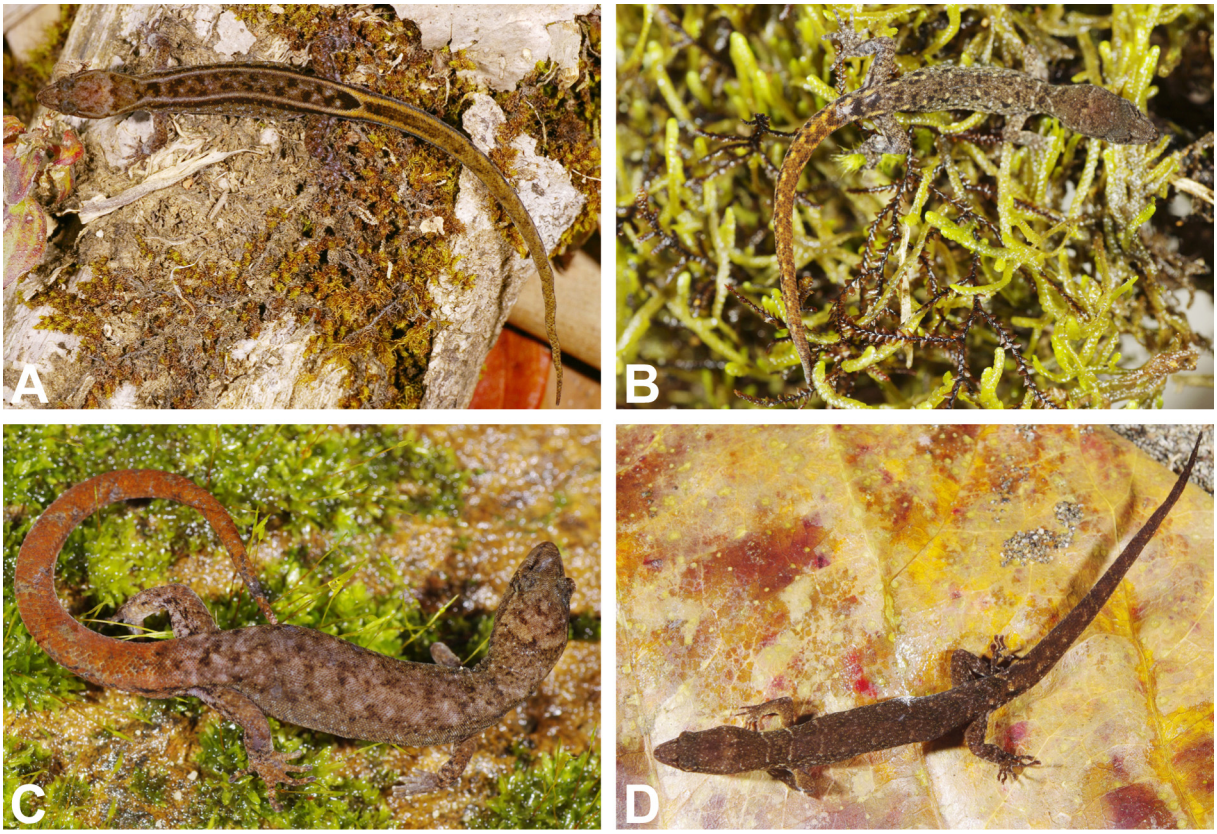

FIGURE 13. Panamanian specimens of *Lepidoblepharis xanthostigma* in life. (A) Adult male SMF 89576 and (B) juvenile SMF 91559 from Cerro Mariposa; (C) adult male SMF 91558 from Alto Tólica; (D) female MHCH 2338 from San San Pond Sak.

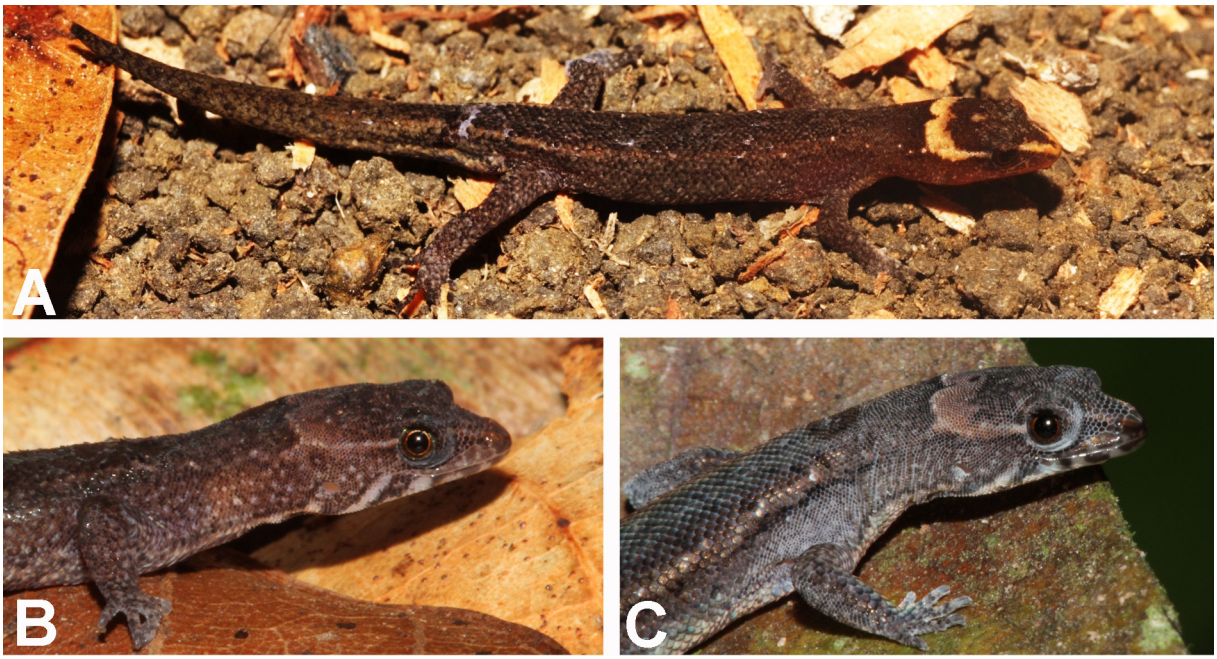

FIGURE 14. Panamanian specimens of *Lepidoblepharis sanctaemartae* from Darién province in life. (A) Adult male MHCH 2947 from Metetí; (B) male MHCH 2949 from Cerro Sapo; (C) juvenile MHCH 2946 from Serranía del Pirre.

We recognize that our divergence time analysis (Fig. 8) is very preliminary, since it leaves out several species from South America and is based on only two mtDNA markers (16S and COI, the latter having been available only for a few specimens). Nevertheless, we decided to provide a first tentative approximation to the origin of the genus. The splitting event between *Gonatodes* and *Lepidoblepharis* was dated earlier by Gamble *et al.* (2008, 64 Ma) than inferred in our analysis (52 Ma), but the crown age of the *Lepidoblepharis* spp. was very similar (42 Ma in Gamble *et al.* 2008, vs. 45 Ma in our analysis). The genus probably originated in South America, since *L. rufigularis,* which is known only from the border area with Colombia and presumably occurs further southward, but not much further northward, was inferred as the oldest lineage among the sampled members of the genus. Nevertheless, this relationship was not statistically supported (low values of ML bootstrap support and Bayesian posterior probabilities, see Figs. 2 and 8). Concerning the Central American clade (*L. xanthostigma, L. emberawoundule,* and *L. victormartinezi*), inferred to constitute the second oldest lineage, two different colonization scenarios are imaginable: Either the ancestor of the short-toed *L. emberawoundule* and *L. victormartinezi* and that of the long-toed *L. xanthostigma* have arrived in two independent dispersal events, or the common ancestor of all three species arrived in a single dispersal. The latter scenario is strongly supported by the present-day distribution of these three species, and would have occurred around the early Oligocene according to our results. This mean value of our dating precedes those inferred for other organisms in various studies, which suggest dispersals of different clades of herpetofauna and other organisms from South to Central America and vice-versa between the late Oligocene and middle Miocene (gray shading in Fig. 8), either over a temporarily emergent land bridge or by rafting or other means of island hopping along a then existing archipelago of various islands, probably aided by environmental fluctuations (*e.g.,* Flynn & Wyss 1998; Zachos *et al*. 2001; Heinicke et al. 2007; Daza *et al.* 2010; Gamble *et al*. 2011; Head *et al.* 2012; Montes *et al.* 2012a, b; Pinto-Sánchez *et al.* 2012; Elmer *et al*. 2013; Batista *et al*. 2014). The possible temporal range inferred in our analysis for the independent arrival of the South American *L. sanctaemartae* in Central America concurs quite well with the results of these works. While it may be assumed that a putative oversea dispersal of *Lepidoblepharis* and other reptiles might have been possible over longer distances, and thus perhaps occurred earlier, than that of salamanders, rainfrogs, or other amphibians (Pinto-Sánchez *et al.* 2012; Elmer *et al*. 2013), our inferred dating of a single colonization event by the ancestor of the Central American *Lepidoblepharis* clade can still be regarded as very early. Still, the lower end of our inferred temporal range does overlap the time frames suggested in the above mentioned works. At this point it must be stated that the paleogeographic evolution of the Panamanian land bridge, and with it the spatial and temporal settings of possible preliminary closures of the Panama Portal, remain unsatisfactorily resolved to date. Moreover, from the genetic viewpoint our present divergence time analysis stands on a rather weak basis that could be considerably solidified through the inclusion of additional taxa and additional genetic markers, preferably from the nuclear genome. Notwithstanding these uncertainties, the Central American short-toed species *L. emberawoundule* and *L. victormartinezi* can confidently be presumed to have originated in Central America. This, as well as the deep genealogical divergences among the populations of *L. emberawoundule* inhabiting different mountain ranges of eastern Panama, strongly advocate the importance of *in situ* speciation that probably resulted from geological activity, climatic fluctuations, and subsequent temporary isolation (Montes *et al.* 2012a, b; Pinto-Sánchez *et al.* 2012; Batista *et al*. in prep.) for the formation of the contemporary diversity.

After decades of mostly singular species descriptions from South America, our study is the first contribution to *Lepidoblepharis* taxonomy and biogeography focusing on all Central American members of the genus. Providing extensive morphological data for all species from Central America and, for the first time, DNA barcodes for almost a third of the nominal species (*i.e.,* six of the now 21), it constitutes a starting point for more in-depth studies of *Lepidoblepharis,* a group for which virtually no comprehensive studies have been carried out so far, in general as well as particularly in Lower Central America. At the same time, it is a basis for future molecular genetic and biogeographic analyses comparing material from this area with South American species.

Key to the species of *Lepidoblepharis* in Panama

1. Dorsals cycloid, flat, and distinctly imbricate . . . . . *Lepidoblepharis sanctaemartae*
   Dorsals granular, not imbricate, usually raised with a pointed or keeled appearance. 2
2. Twelve or more lamellae under fourth toe, 10 or more under fourth finger 3
   Fewer than 10 lamellae under fourth toe, fewer than 9 under fourth finger 4

3. Median subcaudal scales greatly enlarged, more than twice as wide as the laterally adjacent subcaudals or their own length; usually 21 or more, very rarely 18, scales across snout; usually 18 or more, rarely 16 or 17, longitudinal rows of ventral scales at midbody; pale throat in males, never bright orange; ventral escutcheon with a long/wide ratio of 67% or greater . . . . . *L. xanthostigma*
Median subcaudal scales enlarged, but less than two times the width of the laterally adjacent subcaudals or their own length; 19 scales across snout; 17 longitudinal rows of ventral scales at midbody; bright orange throat in males; ventral escutcheon long/wide ratio 54% . . . . . *L. rufigularis*
4. Median subcaudal scales only slightly enlarged; 6–8 lamellae under fourth toe, 4–5 under fourth finger; 1–3 proximal subdigital lamellae per digit greatly enlarged, up to 4 times as long as the distal one(s); males with a ventral escutcheon consisting of 61–68 scales (6–8 scales long and 12–15 wide), but without distinct subfemoral escutcheon scales . . . . . *L. victormartinezi*
Median subcaudal scales distinctly enlarged; 6–9 lamellae under fourth toe, 5–8 under fourth finger; 1–6 proximal subdigital lamellae per digit slightly enlarged, at most twice as long as the distal one(s); males with a ventral escutcheon consisting of 38–61 scales (6–7 scales long and 10–13 wide), and 4–5 subfemoral escutcheon scales arranged in a single row per thigh . . . . . *L. emberawoundule*

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APPENDIX I. Specimens examined morphologically for this study. Field tag numbers appear in parentheses.

Gonatodes albogularis.— PANAMA: Veraguas: Finca La Providencia: forest patch, 7.8977°N, 81.0007°W, 56 m: SMF 91553 (SL 574); Finca La Providencia: houses, 7.8973°N, 80.9865°W, 53 m: SMF 91552 (SL 573); NICARAGUA: Rio San Juan: Boca de San Carlos, 10.7905°N, 84.1938°W, 40 m: SMF 86745 (JS 525).

Lepidoblepharis buchwaldi.— ECUADOR: Los Ríos: Pichincha: Centro Cientifico Rio Palenque, 0.55°S, 79.3667°W: MCZ 151697, 151710; Pichincha: 1 km N Buena Fe, 0.88781°S, 79.48896°W: MCZ 151709.

Lepidoblepharis duolepis.— COLOMBIA: Valle: Valle Río Pance: MCZ 159596, 159597.

Lepidoblepharis emberawoundule.— PANAMA: Comarca Emberá: Distrito de Cémaco, Bajo pequeño, camp3 Pechito Parado, 8.4791°N, 77.528°W, 718 m: SMF 50969 (AB 887); Comarca Guna Yala: Distrito de Narganá, Burbayar private reserve, La Cascada trail, 9.3184°N, 79.0027°W, 360 m: SMF 50968 (AB 963); Nusagandí, Umgebung Feldstation, 9.3417°N, 78.994°W, 350 m: SMF 81950 (GK 12)–SMF 81952; Nusagandí, Sendero Nusagandi, 9.3417°N, 78.9917°W, 280 m: SMF 81954; Nusagandí, Sendero Nusagandí, 9.3417°N, 78.9917°W, 290 m: SMF 81953; Distrito de Wargandi, way back from Yarculup to Río Taintídu, 9.0345°N, 78.0221°W, 227 m: MHCH 2951 (AB 786); border of Darien, Summit camp, 8.92°N, 77.85°W, 350 m: FMNH 170042; border of Darien, Summit site, 8.92°N, 77.85°W, 320 m: FMNH 170029, 170043; Darién: Distrito de Sambú, Camino hacia Cerro Sapo, Río San Antonio, 7.9801°N, 78.3556°W, 773 m: MHCH 2957 (AB 415); border of Darien, Summit site, 8.92°N, 77.85°W, 320 m: FMNH 170044, 170045; Distrito de Pinogana, Rio Cana, Cana field station, Chimenea trail, 7.756°N, 77.6857°W, 515 m: SMF 50970 (AB 245); Panamá: Distrito de Chepo, Río Terable, 9.284°N, 78.9838°W, 322 m: MHCH 2952 (AB 989).

Lepidoblepharis festae.— COLOMBIA: Antioquia: Urrao, 2030 m: MCZ 166521.

Lepidoblepharis grandis.— ECUADOR: Pichincha: Pichincha: FMNH 177434, 177435.

Lepidoblepharis heyerorum.— BRAZIL: Pará: Urua (Parque Nacional de Amazonia, Rio Tapajos), 4.6°S, 56.2333°W: MCZ 172927, 172928.

Lepidoblepharis intermedius.— COLOMBIA: Cauca: Isla Gorgona: MCZ 159595; Isla Gorgona, beach in front of Gorganilla: MCZ 160150; Valle del Cauca: Valle Río Sabaletas, Sabaletas Piedras: MCZ 160199.

Lepidoblepharis microlepis.— COLOMBIA: Antioquia: Laguna Quesada, Puerto Limón (near Murindo) [Rio Quesado, Atrato River region according to original description (Noble 1923)], 6.95°N, 76.75°W, 34 m: AMNH R-18229.

Lepidoblepharis miyatai.— COLOMBIA: Magdalena: Ancon Guairaca (=Bahia Gairaca+Ensenada de Bayraca): MCZ 154447–49; Ancon Guairacal-Bahia Gairaca=Ensenada de Gayraca: MCZ 156973, 156974.

Lepidoblepharis montecanoensis.— VENEZUELA: Falcón: Paraguana Penninsula, 6km W Pueblo Nuevo, in the Monte Cano Biological Reserve, ca. 11.95°N, 69.975°W, 150 m: MCZ 175913.

Lepidoblepharis peraccae.— COLOMBIA: Los Mangos, S.W. Colombia, 300 m: BMNH 1908.5.29.46B.

Lepidoblepharis rufigularis.— PANAMA: Darién: Distrito de Pinogana, camp 4 arriba de Río Pucuro, 8.0575°N, 77.3702°W, 1043 m: SMF 50659 (AB 527).

Lepidoblepharis sanctaemartae.— COLOMBIA: Magdalena: Fundacion: MCZ 11304; PANAMA: Comarca Emberá: Comunidad de Villa Caleta, 8.4507°N, 77.7258°W, 30 m: SMF 85371 (MHCH 304); Darién: Distrito de Sambú, Garachine, Finca del Pana de Gustavo Dogirama, 1 hora SO del pueblo, 8.0482°N, 78.3567°W, 45 m: SMF 97419 (AB 385); Distrito de Chepigana, Río San Antonio, Cerro Sapo, Camp 2, 7.9794°N, 78.3551°W, 834 m: MHCH 2949–50 (AB 594–95); Distrito de Sambú, Rio San Antonio, Cerro Sapo, 8.0038°N, 78.3485°W, 200 m: MHCH 2945 (AB 448); Sante Fe, Cuipo site, 8.6833°N, 78.1167°W, 70 m: FMNH 170124; Distrito de Chepigana, Metetí, Arretí, 8.5382°N, 78.0674°W, 322 m: MHCH 2947 (AB 232); Distrito de Chepigana, Metetí, toma de agua, 8.4614°N, 77.989°W, 132 m: MHCH 2948 (AB 250); Distrito de Pinogana, 1 km to Rancho Frío Field station , 8.0168°N, 77.7297°W, 133 m: MHCH 2946 (AB 1216); Distrito de Pinogana, Boca de Cupe, Cruce de momo, field station, 7.9455°N, 77.6274°W, 140 m: Rio Pita: FMNH 68156; Panamá: cliffs to west of Cerro Campana, 1200', 8.685°N, 79.924°W, 366 m: FMNH 60196; Barro Colorado Island, 9.155°N, 79.848°W, 178 m: FMNH 13306, 177051, 177052; Distrito de Chepo, Wacuco, Padre Pablo Kasuboski Fram. Toma de Agua, 8.9435°N, 78.4699°W, 262 m: MHCH 2953 (AB 997).

Lepidoblepharis williamsi.— COLOMBIA: Antioquia: San Vicente (La Honda): MCZ 1170640, 170643.

Lepidoblepharis xanthostigma.— COSTA RICA: no specific locality: SMF 78437; Cartago: Rancho Naturalista, 9.83272°N, 83.56187°W, 1010 m: (GK 3554, 3557); Heredia: Schutzgebiet Rara Avis, Catarata, 10.2819°N, 84.0457°W, 675 m: SMF 81816 (CR 49); Limón: Zent, near Puerto Limón, Costa Rica, 10.033°N, 83.283°W, 13 m: MCZ 11658; Moin, 10.0036°N, 83.1044°W, 22 m: SMF 98879 (JV 310), 98880 (JV 323); Reserva Manzanillo, 9.63404°N, 82.64788°W, 30 m: (GK 2583–4); no specific locality: FMNH 176918, 176919; Puntarenas: Carate, 8.4428°N, 83.4554°W, 45 m: (GK 4944–6); Río Coco, Punta Banco, 8.3355°N, 83.0751°W, 390 m: SMF 93690 (JV 143); Cerro Incendio, 8.3522°N, 83.0713°W, 415 m: SMF 93688–89 (JV 117–18); San José: Zona Protectora El Rodeo, 9.90404°N, 84.28157°W, 790 m: (GK 2647); NICARAGUA: Rio San Juan: Bartola, 10.9728°N, 84.3392°W, 30 m: SMF 80998 (GK 303); Bartola, Orange Trail 8, 10.9728°N, 84.3392°W, 30 m: SMF 82559 (GK 367); Cerro El Bolívar, near Río Machado, 10.8672°N, 84.1695°W, 280 m: SMF 84817 (GP 095); Dos Bocas de Río Indio, 11.0486°N, 83.8801°W, 20 m: (JS 564, 585, 603, 606, 621), SMF 86741 (JS 605), 86742 (JS 602), 86743 (JS 604), 86744 (JS 569); PANAMA: Bocas del Toro: San San Pond Sak: Rio Negro: flooded forest on N bank, 9.508°N, 82.5289°W, 13 m: SMF 90190 (SL 558); San San Pond Sak: Boca San San, Centro AAMVECONA, 9.5259°N, 82.5099°W, 1 m: MHCH 2338 (SL 563); Isla Colón, near Boca del Drago, 9.41783°N, 82.32271°W, 15 m: SMF 92086–7 (GK 3456–7); Isla Bastimentos, Wizard, 9.351°N, 82.197°W, 10 m: SMF 86389 (MHCH 396); Isla Popa, 9.2206°N, 82.1411°W, 4 m: photo by Konrad Mebert; Chiriquí: Chorogo, 8.31377°N, 82.99842°W, 311 m: SMF 92085 (GK 3383); Chorogo, 8.31318°N, 82.99491°W, 304 m: SMF 92084 (GK 3382); Distrito de Bugaba, Aserríos de Gariché, Porton, proyecto hidroelectrico bajo de minas, 8.6027°N, 82.7833°W, 338 m: MHCH 1682; Reserva Forestal La Fortuna: Pfad von Finca nach Dam Site, 8.7311°N, 82.2534°W, 1300 m: SMF 85006 (GK 1494); Meseta de Chorcha, 8.4139°N, 82.2183°W, 260 m: SMF 85005 (GK 1400); Coclé: El Valle de Antón, 8.6°N, 80.1167°W, 594 m: FMNH 177522, 177523; Colón: Distrito de Donoso: Botija, Brazo, Petaquilla, Rio del Medio, 8.8423°N, 80.6564°W, 162 m: photo by Michael Castillo; Distrito de Donoso, Petaquilla, 8.8553°N, 80.6556°W, 107 m: MHCH 2955–56 (AB 1249–50); Cerro Santa Rita, ca 800', 9.324°N, 79.787°W, 244 m: FMNH 68157; Comarca Ngöbe-Buglé: Bosque Protector Palo Seco: headwaters of Río Chiriquí Malí, 8.7891°N, 82.2155°W, 1054 m: SMF 90189 (SL 489); Alto Tólica: creek near escuela, 8.4747°N, 81.5055°W, 1055 m: SMF 91558 (SL 750); Panamá: Cerro de La Victoria, Quebrada Buenos Aires, along stream, 2000', 9.2°N, 79.3833°W, 610 m: FMNH 153600; La Victoria, 2200', 9.2°N, 79.3833°W, 722 m: FMNH 154549; Veraguas: Cerro Mariposa: water supply hut near Alto de Piedra, 8.5161°N, 81.1185°W, 883 m: SMF 89576 (SL 129), 91559 (SL 760).

Lepidoblepharis victormartinezi.— PANAMA: Colón: Distrito de Donoso, Petaquilla, 8.8553°N, 80.6556°W, 107 m: SMF 50950 (AB 018), 50952 (AB 1251), MHCH 2954 (AB 1252); Distrito de Donoso, Chicheme, Mina de Cobre Panama, 8.8653°N, 80.6438°W, 100 m: SMF 50951 (AB 1241); Veraguas: Parque Nacional Santa Fé: Cerro Negro: camp, 8.569°N, 81.0989°W, 700 m: SMF 89963 (AC 0079).

APPENDIX II. Voucher numbers and GenBank accession numbers for all samples included in the phylogenetic analyses.

Species	Country	Voucher	16S	COI
Gonatodes albogularis		KDQ 512	EF564023	
Gonatodes albogularis		MVZ 204073	EF564020	
Gonatodes albogularis		SMF 97368		KP845157
Gonatodes daudinii		JB 38	EF564034	
Gonatodes vittatus		MF 382	EF564033	
Gonatodes vittatus		TG 00040	EF564032	
Lepidoblepharis emberawoundule	Panama	MHCH 2957	KP845170	
Lepidoblepharis emberawoundule	Panama	MHCH 2951	KP845171	KP845159
Lepidoblepharis emberawoundule	Panama	SMF 50969	KP845163	KP845152
Lepidoblepharis emberawoundule	Panama	SMF 50968	KP845166	KP845155
Lepidoblepharis emberawoundule	Panama	MHCH 2952	KP845162	KP845151
Lepidoblepharis festae	Colombia	LSUMZ 12704	EF564007	
Lepidoblepharis rufigularis	Panama	SMF 50659	KP845161	
Lepidoblepharis sanctaemartae	Panama	MHCH 2946	KP845160	
Lepidoblepharis sanctaemartae	Panama	MHCH 2947	KP845168	
Lepidoblepharis sanctaemartae	Panama	MHCH 2945	KP845165	KP845154
Lepidoblepharis sanctaemartae	Panama	MHCH 2949	KP845167	KP845156
Lepidoblepharis victormartinezi	Panama	SMF 50951	KP845164	KP845153
Lepidoblepharis victormartinezi	Panama	SMF 50950	KP845172	
Lepidoblepharis xanthostigma	Costa Rica	MVZ 171438	EF564009	
Lepidoblepharis xanthostigma	Costa Rica	USNM 59912	EF564010	
Lepidoblepharis xanthostigma	Panama	no voucher	X86044	
Lepidoblepharis xanthostigma	Panama	no voucher	EU435277	
Lepidoblepharis xanthostigma	Panama	SMF 90190		
Lepidoblepharis xanthostigma	Panama	SMF 91558		
Lepidoblepharis xanthostigma	Panama	SMF 91559		
Lepidoblepharis sp.	Ecuador	KU 218367	EF564008	
Sphaerodactylus elegans		YPM 14795	X86048	
Sphaerodactylus elegantulus		no voucher	X86057	
Sphaerodactylus lineolatus		SMF 97626	KP845169	KP845158
Sphaerodactylus nigropunctatus		FLMNH 144010	X86051	
Sphaerodactylus notatus		no voucher	X86061	
Sphaerodactylus nicholsi		no voucher	X86043	

APPENDIX III. Uncorrected p-distances in 16S mtDNA among the individuals of *Lepidoblepharis* included in the phylogenetic analyses.

	MHCH 2957	MHCH 2951	SMF 50968	SMF 50969	MHCH 2952	SMF 50659	SMF 50951	SMF 50950	MHCH 2946	MHCH 2949	MHCH 2945	MHCH 2947	SMF 90190	EU435277	SMF 91559	SMF 91558	MVZ 171438	X86044	USNM 59912	LSUMZ 12704	KU 219367
MHCH 2957																					
MHCH 2951	0.06																				
SMF 50968	0.05	0.05																			
SMF 50969	0.15	0.08	0.11																		
MHCH 2952	0.04	0.04	0.04	0.09																	
SMF 50659	0.17	0.16	0.16	0.17	0.16																
SMF 50951	0.10	0.10	0.11	0.20	0.11	0.17															
SMF 50950	0.10	0.10	0.11	0.21	0.11	0.18	0.01														
MHCH 2946	0.18	0.18	0.20	0.26	0.20	0.23	0.18	0.18													
MHCH 2949	0.18	0.18	0.20	0.26	0.20	0.23	0.18	0.18	0.00												
MHCH 2945	0.18	0.18	0.20	0.26	0.20	0.23	0.18	0.18	0.00	0.00											
MHCH 2947	0.18	0.18	0.20	0.26	0.20	0.23	0.18	0.18	0.00	0.00	0.00										
SMF 90190	0.13	0.13	0.14	0.22	0.14	0.15	0.11	0.12	0.20	0.20	0.20	0.20									
EU435277	0.12	0.13	0.13	0.22	0.14	0.14	0.12	0.12	0.20	0.20	0.20	0.20	0.03								
SMF 91559	0.13	0.13	0.14	0.22	0.14	0.14	0.12	0.12	0.20	0.20	0.20	0.20	0.03	0.03							
SMF 91558	0.13	0.13	0.13	0.22	0.14	0.14	0.12	0.13	0.20	0.20	0.20	0.20	0.03	0.03	0.02						
MVZ 171438	0.14	0.13	0.14	0.20	0.14	0.14	0.13	0.13	0.20	0.20	0.20	0.20	0.03	0.02	0.02	0.03					
X86044	0.15	0.14	0.14	0.18	0.15	0.14	0.12	0.12	0.22	0.22	0.22	0.22	0.03	0.03	0.03	0.03	0.03				
USNM 59912	0.13	0.13	0.14	0.20	0.14	0.14	0.11	0.12	0.21	0.21	0.21	0.21	0.03	0.03	0.04	0.04	0.03	0.02			
LSUMZ 12704	0.14	0.15	0.15	0.23	0.16	0.16	0.14	0.14	0.16	0.16	0.16	0.16	0.14	0.14	0.13	0.14	0.14	0.15	0.14		
KU 219367	0.15	0.15	0.16	0.22	0.16	0.18	0.14	0.15	0.17	0.17	0.17	0.17	0.13	0.13	0.14	0.14	0.13	0.15	0.13	0.13	

Species affiliations of the vouchers: MHCH 2957, MHCH 2951, SMF 50968, SMF 50969, MHCH 2952 = *L. emberawoundule;* SMF 50659 = *L. rufigularis;* SMF 50951, SMF 50950 = *L. victormartinezi;* MHCH 2946, MHCH 2949, MHCH 2945, MHCH 2947 = *L. sanctaemartae;* SMF 90190, EU435277, SMF 91559, SMF 91558, MVZ 171438, X86044, USNM 59912 = *L. xanthostigma;* LSUMZ 12704 = *L. festae;* KU 219367 = *Lepidoblepharis* sp. Ecuador.

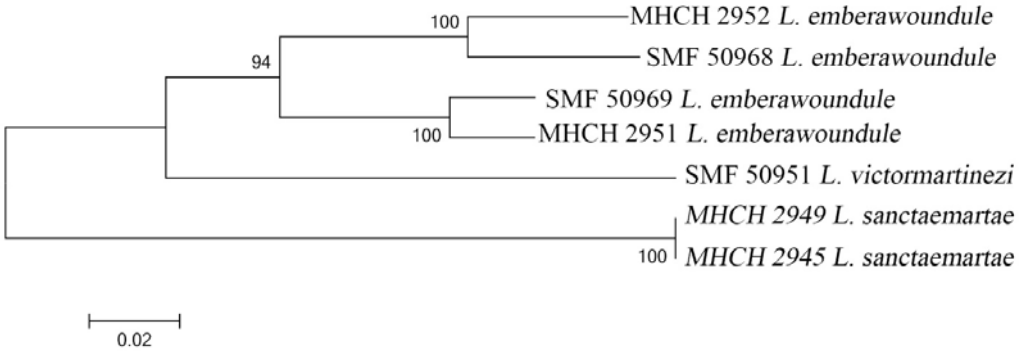
APPENDIX IV. Uncorrected p-distances in COI mtDNA among the individuals of *Lepidoblepharis* included in the phylogenetic analyses.

	SMF 50951	MHCH 2952	SMF 50968	SMF 50969	MHCH 2952	MHCH 2949	MHCH 2945
Lepidoblepharis victormartinezi SMF 50951							
Lepidoblepharis emberawoundule MHCH 2952	0.19						
Lepidoblepharis emberawoundule SMF 50968	0.18	0.07					
Lepidoblepharis emberawoundule SMF 50969	0.16	0.12	0.12				
Lepidoblepharis emberawoundule MHCH 2952	0.17	0.12	0.12	0.04			
Lepidoblepharis sanctaemartae MHCH 2949	0.24	0.23	0.24	0.22	0.22		
Lepidoblepharis sanctaemartae MHCH 2945	0.24	0.23	0.24	0.22	0.22	0.00	

APPENDIX V. Mean values of uncorrected p-distances in the COI mtDNA gene among the *Lepidoblepharis* species included in the phylogenetic analyses.

	L. victormartinezi	*L. emberawoundule*	*L. sanctaemartae*
Lepidoblepharis victormartinezi			
L. emberawoundule	0.18		
L. sanctaemartae	0.24	0.23	

APPENDIX VI. ML tree of COI mtDNA, for the *Lepidoblepharis* present in eastern Panama, midpoint rooted tree. Values on nodes represent bootstrap values, scale bar refers to substitutions per site.



Appendix VIII

Checklist of the amphibians and reptiles from eastern Panama.

Table 3.9.2. Amphibian and reptile species present in eastern Panama, its distributional regions, and conservation categories according the IUCN and EVS (see main text for explanation). *Terms: LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered; CR = Critically Endangered; DD = Data Deficient; NE = Not Evaluated; H = High; M= Medium; L = Low; * = endemic species for the country; **= endemic species for EP; Serrania de Pirre (SP), Serranía de Maje (SM), Serrania de Jingurudó-Sapo (SJS), Serranía de San Blas (SSB), Serrania del Darien (SD), TB = lowland, M = marine coast.*

Species list	Distribution	IUCN	TOTAL EVS SCORE	EVS
Class Amphibia (119)				
Order Gymnophiona (5)				
Family Caeciliidae (5)				
Caecilia isthmica	TB	DD	16	H
Caecilia leucocephala	TB	LC	15	H
Caecilia nigricans	TB	LC	15	H
Caecilia sp.	SJS	NE	NE	NE
Oscaecilia ochrocephala	TB	LC	16	H
Orden Caudata (6)				
Family Plethodontidae (6)				
Bolitoglossa biseriata	SD, TB	LC	13	M
*Bolitoglossa chucantiensis***	SM	NE	18	H
*Bolitoglossa cuna***	SSB	NE	13	M
Bolitoglossa medemi	SJS, SD	VU	15	H
*Bolitoglossa taylori***	SP	DD	17	H
Oedipina sp. aff. complex	SM	NE	17	H
Order Anura (108)				
Family Bufonidae (9)				
*Atelopus certus***	SJS, TB	EN	14	H
Atelopus glyphus	SP, TB	CR	13	M
Atelopus sp.	SSB	DD	14	H
*Atelopus limosus**	SSB	EN	14	H
Incilius coniferus	SM, SSB	LC	8	L
Rhaebo haematiticus	SJS, SP, SD, TB,SM, SSB	LC	9	L
Rhinella acrolopha	SP,SD	DD	16	H

Rhinella alata	SJS, SP, SD, TB,SM, SSB	DD	15	H
Rhinella marina	SJS, SP, SD, TB,SM, SSB	LC	3	L
Family Centrolenidae (15)				
Cochranella euknemos	SJS, SP, SD, TB,SM, SSB	LC	10	M
Cochranella granulosa	SJS, SP, SD, TB,SM, SSB	LC	15	H
Espadarana prosoblepon	SJS, SP, SD, TB,SM, SSB	LC	9	L
Hyalinobatrachium aureoguttatum	SP, TB	NT	13	M
Hyalinobatrachium chirripoi	TB	LC	12	M
Hyalinobatrachium colymbiphyllum	SJS, SP, SD, TB,SM, SSB	LC	10	M
Hyalinobatrachium fleischmanni	SJS, SP, SD, TB,SM, SSB	LC	8	L
Hyalinobatrachium talamancae	TB	LC	16	H
Hyalinobatrachium valerioi		LC	11	M
Hyalinobatrachium sp.	SSB	NE	NE	NE
Rulyrana sp.	SJS, SP, TB	NE	NE	NE
Sachatamia albomaculata	TB	LC	12	M
Sachatamia ilex	SD, TB	LC	12	M
Teratohyla pulverata	SSB, TB	LC	12	M
Teratohyla spinosa	SSB	LC	11	M
Family Aromobatidae (3)				
Allobates talamancae	SD, TB	LC	11	M
*Anomaloglossus astralogaster***	SSB	NE	18	H
*Anomaloglossus isthminus***	SSB	NE	16	H
Family Dendrobatidae (13)				
Andinobates fulguritus	SSB	LC	15	H
Andinobates minutus	SSB	LC	15	H
*Colostethus latinasus***	SP	DD	15	H
Colostethus panamansis	SJS, SP, SD, TB, SSB	LC	15	H
Colostethus pratti	SP, SD, TB	LC	15	H
Colostethus sp. 1 aff. pratti	SP	NE	NE	NE
Colostethus sp. 2 aff. pratti	SM	NE	NE	NE
Silverstoneia nubicola	SD, TB	NT	14	H
Silverstoneia sp. 1	SM	NE	NE	NE
Silverstoneia sp. 2	SJS	NE	NE	NE
Silverstoneia sp. 3	SP	NE	NE	NE
Silverstoneia sp. 4	SD, TB	NE	NE	NE

Dendrobates auratus	SJS, SP, SD, TB,SM, SSB	LC	15	H
Family Craugastoridae (23)				
Craugastor fitzingeri	SJS, SP, SD, TB, SM, SSB	LC	12	M
Craugastor gollmeri	SD	LC	16	H
Craugastor longirostris	SJS, SP, SD, TB	LC	14	H
Craugastor opimus	SJS, SP, SD, TB, SM, SSB	LC	15	H
Craugastor raniformis	SJS, SP, SD, TB, SM, SSB	LC	15	H
Craugastor sp. aff. Longirostris	SJS, SP, TB	DD		NE
*Pristimantis adnus***	SJS	NE	18	H
Pristimantis achatinus	SJS, SP, SD, TB	LC	14	H
Pristimantis caryophyllaceus	SJS, SP, SD	NT	15	H
Pristimantis cruentus	SJS, SP, SD, TB, SM, SSB	LC	14	H
Pristimantis gaigei	SJS, SP, SD, TB, SM, SSB	LC	16	H
Pristimantis moro	SP, SD	LC	16	H
Pristimantis pardalis	SD, TB	NT	17	H
*Pristimantis pirrensis***	SP	DD	18	H
Pristimantis ridens	SJS, SP, SD, TB, SM, SSB	LC	12	M
Pristimantis taeniatus	SJS, SP, SD, TB, SM, SSB	LC	16	H
Pristimantis sp. 1 aff achatinus	SP	NE	NE	NE
Pristimantis sp. 2 aff. taeniatus	SJS	NE	NE	NE
Pristimantis sp. 3 aff. latidiscus	SM, SD, SSB, SP	NE	NE	NE
Pristimantis sp. 4 aff. museosus	SP	NE	NE	NE
Pristimantis sp. 5 aff. adnus	SD	NE	NE	NE
Pristimantis sp. 6 aff. taeniatus	SP	NE	NE	NE
Strabomantis bufoniformis	SJS, SP, SD, TB, SM, SSB	LC	16	H
Family Eleutherodactylidae (7)				
Diasporus diastema	TB, SM, SSB	LC	15	H
Diasporus quidditus	SJS, SP, SD, TB	LC	16	H
Diasporus tinker	SJS, SP	LC	16	H
Diasporus sp. 1	SJS, SP	NE	NE	NE
Diasporus sp. 2	SM	NE	NE	NE

Diasporus sp. 3	SD	NE	NE	NE
Diasporus sp. 4	SJS	NE	NE	NE
Family Hemiphractidae (3)				
Gastrotheca cornuta	SP, SD, TB	EN	16	H
Gastrotheca nicefori	SJS, SP	LC	15	H
Hemiphractus fasciatus	SP, SD	NT	16	H
Family Hylidae (25)				
Agalychnis callidryas	SJS, SP, SD, TB, SM, SSB	LC	11	M
Agalychnis lemur	TB	CR	12	M
Agalychnis litodryas	TB	LC	15	H
Agalychnis spurrelli	TB	LC	14	H
Cruziohyla calcarifer	TB	LC	15	H
Dendropsophus ebraccatus	TB	LC	12	M
Dendropsophus microcephalus	TB	LC	7	L
Dendropsophus phlebodes	TB	LC	11	M
Dendropsophus subocularis	TB	LC	13	M
Ecnomiohyla bailarina	SJS	DD	18	H
*Ecnomiohyla thysanota***	TB	DD	20	H
Hyloscirtus colymba	SJS, SP, SD, TB, SM, SSB	CR	13	M
Hyloscirtus palmeri	SSB, SD	LC	13	M
Hypsiboas boans	SJS, SP, SD, TB, SM, SSB	LC	12	M
Hypsiboas crepitans	TB	LC	12	M
Hypsiboas pugnax	TB	LC	13	M
Hypsiboas rosenbergi	SJS, SP, SD, TB, SM, SSB	LC	13	M
Scinax boulengeri	SJS, SP, SD, TB, SM, SSB	LC	11	M
Scinax rostrata	TB	LC	11	M
Scinax rubra	TB	LC	11	M
Scinax sp.	TB	NE		NE
Smilisca phaeota	TB	LC	11	M
Smilisca sila	TB	LC	10	M
Trachycephalus typhonius	TB	LC	4	L
Phyllomedusa venusta	TB	LC	13	M
Family Leptodactylidae (6)	TB			
Engystomops pustulosus	TB	LC	7	L
Leptodactylus fragilis	TB	LC	5	L
Leptodactylus insularum	TB	LC	12	M
Leptodactylus melanonotus	TB	LC	6	L
Leptodactylus poecilochilus	TB	LC	12	M

Leptodactylus savagei	SJS, SP, SD, TB, SM, SSB	LC	9	L
Family Microhylidae (2)				
Ctenophryne aterrima	TB	LC	12	M
Elachistocleis panamensis	TB	LC	12	M
Family Pipidae (1)				
*Pipa myersi***	TB	EN	17	H
Family Ranidae (1)				
Lithobates vaillanti	SJS, SP, SD, TB, SSB	LC	9	L
Class Reptilia (156)				
Order Crocodylia (2)				
Family Alligatoridae (1)				
Caiman crocodilus	TB	LC	16	H
Family Crocodylidae (1)				
Crocodylus acutus	TB	VU	14	H
Order Squamata (2)				
Family Amphisbaenidae (2)				
Amphisbaena fuliginosa	TB	DD	11	M
Amphisbaena spurelli	TB	DD	12	M
Family Anguidae (2)				
Diploglossus monotropis	TB	DD	15	H
*Diploglossus montisilvestris***	SP	NT	18	H
Family Corytophanidae (3)				
Basiliscus basiliscus	TB	LC	11	M
Basiliscus galeritus	SSB	NE	13	M
Corytophanes cristatus	TB, SJS, SP, SM, SD, SSB	LC	10	M
Familly Dactyloidae (20)				
Dactyloa frenata	TB, SM, SSB, SD	LC	14	H
Dactyloa chloris	TB, SJS, SP	DD	14	H
Dactyloa insignis	TB, SP, SJS, SD	LC	14	H
Dactyloa latifrons	TB, SJS, SP	LC	13	M
Dactyloa purpurescens	TB, SJS, SP	LC	15	H
*Dactyloa kunayalae**	TB, SSB, SD	LC	***15***	H
*Dactyloa maia***	SM, SSB, TB	NE	***15***	H
Norops apletophallus	TB	LC	15	H
Norops auratus	TB	LC	13	M
Norops biporcatus	TB	LC	9	L
Norops capito	TB, SSB, SD	LC	11	M
Norops aff. fuscoauratus	TB	NE	13	M
Norops gaigei	TB	NE	14	H

Norops humilis	TB, SSB, SD	LC	14	H
Norops pentaprion	TB	LC	12	M
Norops poecilopus	TB, SJS, SP, SD, SSB	LC	14	H
Norops tropidogaster	TB	LC	13	M
*Norops triumphalis***	TB	NE	17	H
Norops vittigerus	TB	LC	14	H
Norops sp.	SP	NE		NE
Family Gekkonidae (2)				
Hemidactylus frenatus	TB	NE		NE
Lepidodactylus lugubris	TB	NE		NE
Family Gymnophthalmidae (9)				
Bachia pallidiceps	TB	DD	14	H
Cercosaura vertebralis	SP	DD	13	M
Echinosaura palmeri	TB, SJS, SP, SM, SD, SSB	DD	12	M
Gymnophthalmus speciosus	TB	LC	9	L
Leposoma rugiceps	TB, SSB	LC	15	H
Leposoma southi	TB, SM, SSB, SD	LC	14	H
Ptychoglossus festae	TB, SM	LC	14	H
Ptychoglossus myersi	SP, SJS	LC	16	H
Ptychoglossus plicatus	TB, SM, SSB	LC	11	M
Family Hoplocercidae (1)				
Enyalioides heterolepis	TB, SJS, SP, SM, SD, SSB	LC	13	M
Family Iguanidae (1)				
Iguana iguana	TB	LC	10	M
Family Mabuyidae (1)				
Marisora unimarginata	TB	LC	15	H
Family Phyllodactylidae (1)				
Thecadactylus rapicauda	TB, SJS, SP, SM, SD, SSB	LC	8	L
Family Polychrotidae (1)				
Polychrus gutturosus	TB	LC	12	M
Family Sphaerodactylidae (6)				
Gonatodes albogularis	TB	LC	9	L
Lepidoblepharis sanctaemartae	TB	LC	14	L
*Lepidoblepharis emberawoundule***	SD	NE		NE
*Lepidoblepharis rufigularis***	TB, SJS, SP, SD, SSB	NE		NE
Sphaerodactylus homolepis	TB	LC	16	H
Sphaerodactylus lineolatus	TB	LC	14	H

Family Teiidae (4)				
Ameiva praesignis	TB	LC	14	H
Holcosus festivus	TB, SJS, SP, SD, SSB	LC	10	M
Holcosus leptophrys	TB, SJS, SP, SD, SSB	LC	16	H
*Cnemidophorus duellmani***	TB	NE	16	H
Family Anomalepididae (1)				
Liotyphlops albirostris	TB	LC	9	L
Family Boidae (4)				
Boa imperator	TB	VU	8	L
Corallus annulatus	TB	DD	11	M
Corallus ruschenbergerii	TB	DD	13	M
Epicrates maurus	TB	LC	8	L
Family Charinidae (1)				
Ungaliophis panamensis	SSB	DD	12	M
Family Colubridae (27)				
Chironius flavopictus	TB	LC	15	H
Chironius exoletus	SSB, SD	LC	12	M
Chironius grandisquamis	TB, SP, SD, SSB	LC	11	M
Dendrophidion apharocybe	SP, SSB, SP	LC	16	H
Dendrophidion clarkii	SSB, SD, TB	LC	14	H
Dendrophidion percarinatum	TB	LC	11	M
Drymarchon melanurus	TB	LC	6	L
Drymobius margaritiferus	TB	LC	6	L
Drymobius rhombifer	TB	DD	14	H
Lampropeltis micropholis	TB,SSB, SD	LC	10	M
Leptophis ahaetulla	TB	LC	10	M
Leptophis depressirostris	SSB, SD	LC	14	H
Mastigodryas melanolomus	TB	LC	11	M
Mastigodryas pleei	*TB*	*DD*	14	H
Oxybelis aeneus	*TB*	LC	5	L
Oxybelis brevirostris	TB, SJS, SP, SM, SD, SSB	LC	12	M
Oxybelis fulgidus	TB	LC	7	L
Phrynonax poecilonotus	TB, SJS, SP, SM, SD, SSB	LC	7	L
Rhinobothryum bovallii	TB, SJS, SP, SM, SD, SSB	LC	16	H
Spilotes pullatus	TB, SJS, SP, SM, SD, SSB	LC	6	L
Stenorrhina degenhardtii	TB	LC	9	L
Tantilla alticola	TB, SSB	LC	11	M
Tantilla melanocephala	TB	LC	12	M
Tantilla supracincta	TB,SSB, SD	DD	16	H

Tantilla reticulata	TB,SSB, SD	NE	13	M
Tantilla ruficeps	TB,SSB, SD	LC	12	M
Tantilla sp.	SM	NE	NE	NE
Family Dipsadidae (42)				
Atractus clarki	SP	DD	14	H
*Atractus darienensis***	SP	DD	16	H
*Atractus hostilitractus***	SD	DD	***16***	H
Clelia clelia	TB,SSB, SD,SP	LC	10	M
Clelia ecuatoriana	TB,SSB, SD,SP	LC	14	H
Coniophanes fissidens	TB,SSB, SD,SP	LC	7	L
*Coniophanes joanae***	SP	DD	15	H
*Dipsas nicholsi**	SJS, SSB	DD	15	H
Dipsas temporalis	TB, SJS, SP, SD, SSB	LC	13	M
Dipsas viguieri	TB, SJS, SP, SD, SSB	LC	13	M
Enuliophis sclateri	TB, SJS, SP, SD, SSB	DD	13	M
Enulius flavitorques	TB,SSB, SD,SP	LC	4	L
Erythrolamprus bizona	TB, SJS, SP, SD, SSB	LC	12	M
Erythrolamprus epinephelus	TB, SM, SSB	LC	10	M
Erythrolamprus mimus	TB, SM, SSB	LC	15	H
*Geophis bellus**	SSB	VU	16	H
Geophis brachycephalus	SSB	LC	11	M
Geophis hoffmanni	TB, SM, SSB	DD	12	M
*Geophis tectus**	SSB	DD	13	M
Geophis sp.	SM	NE	NE	NE
Imantodes cenchoa	TB, SJS, SP, SM, SD, SSB	LC	6	L
Imantodes inornatus	TB,SSB, SD, SP	LC	12	M
*Imantodes phantasma***	SJS, SP	VU	16	H
Leptodeira maculata	TB, SJS, SP, SM, SD, SSB	LC	7	L
Leptodeira septentrionalis	TB, SJS, SP, SM, SD, SSB	LC	7	L
Ninia atrata	TB, SP	DD	13	M
Ninia maculata	SSB, SD	LC	12	M
Nothopsis rugosus	TB, SJS, SP, SM, SD, SSB	LC	10	L
Oxyrhopus petolarius	TB, SJS, SP, SM, SD, SSB	LC	12	M
Phimophis guianensis	TB	LC	13	M
Pliocercus euryzonus	TB, SJS, SP, SM, SD, SSB	LC	12	M
Rhadinaea decorata	TB, SP, SM, SD, SSB	LC	9	L
Sibon annulatus	TB, SM, SSB, SD, SJS, SP	LC	14	H

Sibon argus	SM, SSB, SD	LC	16	H
Sibon nebulatus	TB, SJS, SP, SM, SD, SSB	LC	5	L
Siphlophis cervinus	TB	DD	16	H
Siphlophis compressus	TB	DD	16	H
Tretanorhinus mocquardi	TB	VU	15	H
Urotheca decipiens	SJS	DD	10	M
Urotheca fulviceps	TB	DD	***13***	M
Urotheca guentheri	SSB	DD	12	M
Xenodon rabdocephalus	TB, SJS, SSB, SD, SP	LC	11	M
Family Elapidae (7)				
Hydrophis platurus	M	LC		NE
Micrurus ancoralis	TB	DD	15	H
Micrurus clarki	TB, SP	DD	17	H
Micrurus dumerilii	TB	DD	16	H
Micrurus mipartitus	TB, SP	LC	15	H
Micrurus multifasciatus	TB, SSB	LC	15	H
Micrurus nigrocinctus	TB	LC	10	M
Family Leptotyphlopidae (1)				
Tricheilostoma macrolepis	SJS	DD	12	M
Tropidophiidae (1)				
Trachyboa boulengeri	TB	DD	11	M
Family Viperidae (6)				
Bothriechis schlegelii	TB, SJS, SP, SM, SD, SSB	LC	11	M
Bothrops asper	TB, SJS, SP, SM, SD, SSB	LC	10	M
Bothrops punctatus	TB, SP	DD	16	H
Lachesis acrochorda	TB, SSB, SD, SP, SJS	DD	14	H
Porthidium lansbergii	TB	NT	15	H
Porthidium nasutum	TB	LC	12	M
Order Testudines (11)				
Family Geoemydidae (2)				
Rhinoclemmys annulata	TB	NT	12	M
Rhinoclemmys melanosterna	TB	DD	15	H
Family Cheloniidae (4)				
Caretta caretta	M	EN		NE
Chelonia mydas	M	EN		NE
Eretmochelys imbricata	M	CR		NE
Lepidochelys olivacea	M	EN		NE
Family Chelydridae (1)				
Chelydra acutirostris	TB	NT	11	M

Family Dermochelyidae (1)				
Dermochelys coriacea	M	CR		NE
Family Emydidae (1)				
Trachemys venusta	TB	NT	11	M
Family Kinosternidae (1)				
Kinosternon scorpioides	TB	LC	8	L
Family Testudinidae (1)				
Chelonoidis carbonarius	TB, SD	DD	17	H

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Education:

- PhD student, at the Goethe University and Senckenberg Institute, Frankfurt am Main, Germany, 2011-2016.
- Master in science at los Andes University, Bogotá, Colombia, 2008-2009.
- Bachelor degree (Zoology) at Universidad Autónoma de Chiriquí, 1997-2002

Researche experience:

- Abundancia, Distribución y Riqueza de Especies de los Anfibios del Distrito de Mironó, Comarca Ngöbe Buglé, Panamá. 2001. Convenio entre el Proyecto Agroforestal Ngöbe (PAN), Autoridad Nacional del Ambiente (ANAM), Agencia Técnica de Cooperación Alemana (GTZ), y el Instituto de Ciencias Ambientales y Desarrollo Sostenible (ICADES), Universidad Autónoma de Chiriquí (UNACHI).
- Anfibios y Reptiles del Jardín Botánico de la Universidad Autónoma de Chiriquí (2004-2005).
- Biodiversidad de la Herpetofauna del Oeste de Panamá, Unidad ejecutora: Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, Alemania (FIS), Investigador principal Dr. Gunther Köhler, Jefe del Departamento de Herpetología, FIS. 2- 26 de Enero de 2006-2012.
- Patrones de Variación en *Oophaga pumilio* (Amphibia: Dendrobatidae) del Oeste de Panamá. Marzo-Agosto 2006. Asesorado por Dr. Gunther Köhler, Jefe del Departamento de Herpetología, Forschungsinstitut und Naturmuseum Senckenberg, Frankfurt, Alemania (FIS).

- Innovaciones fisiológicas de las ranas venenosas del género *Oophaga* spp. Director del Proyecto: Adolfo Amézquita, Universidad de los Andes, Bogotá Colombia, 2008-2009.
- Comunidades acústicas, el caso de las ranas neotropicales (Terrarana). Trabajo de investigación para optar por el título de Maestría, Universidad de los Andes, Bogotá Colombia. 2009.
- Lowland *Atelopus* of western Colombia: Coexistence with *Batrachochytrium dendrobatidis* or prelude to extinction?
- Estudio bacteriológico de las partes bucales del lagarto Pollero (Teiidae: *Tupinambis* spp) En los llanos orientales de Colombia (Departamento del Casanare).
- Integrative taxonomy of the amphians and reptiles from Eastern Panama (San Blas-Darién). 2011-2015.
- Amphibian monitoring project of the amphibians and reptiles of the Darién National Park. Fondo Darién, - GEMAS, 2015.
- Herpetological expedition to Llanganates and Volcan Sumaco, Ecuador. October 2015.

Although I have done molecular and laboratory works, my research experiences are more directed to the field in the Neotropics, working in monitoring projects, conservations, ecological behavioral studies of the amphibians and reptiles.

Publications:

- Diversidad De Mamíferos en el Parque Nacional Volcán Barú, El Respingo, Chiriquí, Panamá. Julio 2002. Revista Natura, 2002 Vol 10. Pag. 99-104.
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Skills:

- **Computer:** Word, Excel, Power point, ArcGIS, Raven 4.1 (bioacoustic), Adobe Audition, Arc-GIS, Adobe Photo Shop, MapSource, GPS-Trackmaker, Google earth, ImageJ, SPSS and R (statistic, basic), EndNote.
- **Field work:** birdwatching (also mistnest capture), 4x4 car driver, trekking, camping, photography, herping for more than 15 Years.
- **Language:** Spanish, English (mid-intermediate).

References:

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