INTEGRATIVE TAXONOMY AND CONSERVATION STATUS OF AMPHIBIANS IN WESTERN PANAMA WITH AN EMPHASIS ON THE HIGHLANDS OF THE CORDILLERA CENTRAL

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"Those who have viewed at first hand the steep, dark-green, forest-covered slopes of the Cordillera de Talamanca-Chiriqui of Costa Rica and Panama, with their ever changing aspect of sun and cloud, moon and mist, bright blue sky and bright green mantle, driving rain and boiling fog, come away with a feeling of overpowering awe and mystery at the variety of nature and the magic of the human soul." J. Savage (1970)

"It seems wicked and sinful in this day and generation to describe new species of Eleutherodactylus *and its ally* Syrrhopus*." T. Barbour (1928)*

"Taxonomy can justly be called the pioneering exploration of life on a little known planet." E.O. Wilson (2004)

Inside cover: examples of new species characterized in this study: from left to right, *Diasporus citrinobapheus* (Anura), *Bolitoglossa jugivagans* (Caudata), *Oscaecilia* sp. (Gymnophiona).

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1. ABSTRACT

Amphibians have existed on the planet for over 300 million years and are today one of the most diverse vertebrate classes in the world with over 7000 known species and still many more to be discovered. However, several studies assume that approximately one third of the world´s known living amphibians are directly threatened with extinction, making it the most endangered vertebrate class.

In relation to the relatively small land mass that is occupied by the state of Panama, it supports one of the most diverse amphibian faunas. However, in many cases the ecological role of single species in a wider context and their habitat preferences are still poorly understood and subject to ongoing research. Modern taxonomic approaches in other tropical regions have shown that former assumptions of amphibian diversity were distinct underestimations of the actual species diversity; a situation that is probably also true for Panama. Concurrently, the collection of amphibian diversity data and the description of new species is a race against time. The amphibian fauna of the world and that of Panama in particular, has suffered from an unprecedented loss of diversity over the last 30 years. The reasons are manifold and include destruction, alteration, and fragmentation of their natural habitats as the main causes, but also the deadly amphibian disease chytridiomycosis caused by the fungal pathogen *Batrachochytrium dendrobatidis* (*Bd*). In Panama and Costa Rica, this Emerging Infectious Disease (EID) spread in a wave-like manner from west to east causing mass die-offs and reduced amphibian diversity even in well-preserved habitats. The disease has primarily affected stream-associated highland species. The last large-scale evaluation of the conservation status of Panama´s amphibians through the IUCN Red List of Threatened Species in 2004 concluded that approximately 30% of the known species are acutely threatened with extinction. Furthermore, around 17% of the amphibian species that have been known back then lacked adequate data to be assessed.

In view of Panama´s already overwhelming amphibian diversity, as well as the variety of habitats and the large number of sites that have not been examined with regard to amphibians before, I started this study with the conviction that the inventory of Panama´s amphibian diversity is far from being completed. Furthermore, when I started this study, it was uncertain if there would be any surviving amphibian species in areas where chytridiomycosis had emerged. The loss of whole amphibian communities in upland western Panama following *Bd* arrival led to a shift of amphibian research to lowland sites in central and eastern Panama aiming primarily on pathogen arrival and the documentation of epizootic outbreak and subsequent population decline. The situation of amphibian communities in areas post-decline was therefore largely unknown. Accordingly, the main goals of my study were to add to the taxonomic inventory of amphibians in Panama and to assess the situation of amphibian populations in habitats where chytrid-driven declines have been observed. To address these tasks I conducted fieldwork in western Panama with a focus on mountainous elevations between 1000 and 3475 m asl. Additionally, I visited different lowland sites between sea level and 1000 m asl to collect comparative material.

In the period between 2008 and 2013, I conducted five collection trips to Panama that add up to a total of approximately 13 months in the field. I have sampled nine regions in western Panama and collected 767 specimens together with student collaborators, 531 of which were collected under my personal field number. Additional data obtained from those specimens include 68 male anuran call recordings, 102 standardized color descriptions of specimens in life, and 259 tissue samples that to date yielded 185 16S mtDNA sequences. This comprises the most comprehensive data set for amphibians of Panama and the first large-scale DNA barcoding approach for western Panama to date. After a preliminary DNA barcoding and subsequent comparative examination of morphological und bioacoustic data of all specimens collected, the number of taxonomic problems that needed to be addressed was higher than I previously anticipated. For most genetic lineages deeper taxonomic analyses were required to reach conclusive results. A selection had to be made with which lineages to proceed in the analyses, in view of the substantial financial and time expenditure that would be needed for a complete taxonomic revision. Therefore, I chose to run deeper analyses on one genus from each of the three amphibian orders in Panama. The genera selection depended largely on the availability of sufficient material and the scientific relevance of the respective genus.

I selected the genus *Diasporus* from the order Anura. These small frogs are omnipresent in many habitats and thus relatively easy to find. In addition, the genus is underrepresented in taxonomic studies. This is the first taxonomic study on the genus *Diasporus* to include a molecular phylogeny and the first comparison of advertisement calls between several populations from western Panama. In total, I collected 67 *Diasporus* specimens throughout western Panama and compared them morphologically with 49 additional specimens from Central America in collections, including the primary types of *D. diasporus* and *D. hylaeformis*. Additional comparative data were taken from literature. The DNA barcoding analysis of a fragment of the 16S rRNA gene included 43 own sequences that were complemented with 15 relevant GenBank sequences. In addition, I compared the advertisement calls of 26 male individuals among each other and with call descriptions from the literature. The DNA barcoding approach revealed several unnamed genetic lineages, but in some cases also resulted in the lumping of morphologically and bioacoustically distinct specimens. Generally, the morphological examination of the collected material revealed almost no specific characters that could be used to distinguish between genetic lineages. However, it was possible to identify species using a combination of several morphological characteristics. Which ones are relevant in the individual case depends on the respective species. My extensive collection of call recordings made it possible to test for the first time the intraspecific call variation of *D. hylaeformis* in dependency of various parameters. This analysis showed that the dominant frequency depends significantly on the body size of the calling male; the smaller the calling male, the higher the frequency of the call. A similar relationship was observed between the call rate and temperature: the lower the temperature during calling, the lower the call rate. I suppose that these general patterns, which have already been observed in other anuran genera, are also true in other *Diasporus* species that could not be tested in this study. Taking into account the intraspecific variation of *Diasporus* advertisement calls, I consider comparative call analyses to be the best way to distinguish between species. This is especially true in syntopic species. Integration of the three lines of evidence (i.e., morphology, DNA barcoding, and bioacoustics) led to the identification of four new species, two of which (i.e., *D. citrinobapheus* and *D. igneus*) colleagues and I have already formally described.

I conducted an integrative taxonomic analysis of the western Panamanian representatives of the genus *Bolitoglossa* from the order Caudata, the larger of the two Panamanian salamander genera. *Bolitoglossa* is very species-rich with a centre of diversification in the high mountains of Costa Rica and western Panama. I collected 53 *Bolitoglossa* specimens and compared them to twelve specimens in collection, including the holotype and one paratype of *B. gomezi*. The dataset was complemented with information from the literature. Among the sampled specimens were two species considered to be endangered that have not been collected or observed for several decades; *B. magnifica* has not been seen for 34 years and *B. anthracina* has not been seen for 22 years. Further, I collected salamanders at several new locations. To date, my 16S mtDNA barcoding analysis represents the densest taxon sampling for Panamanian *Bolitoglossa* composed of 21 own sequences that were combined in the final alignment with 47 GenBank sequences. Even though the molecular phylogeny is based only on a single marker, the received trees largely coincide with previous studies and the nodes received high statistical support. In these trees, I retrieve all previously defined subgenera and species groups. On the basis of this molecular phylogeny, I placed *B. anthracina*, here sequenced for the first time, in the *B. subpalmata* species group. Due to the fact that *B. anthracina* is a large and dark colored species it had previously been placed by implication in the *B. schizodactyla* species group along with other large black salamanders of the *B. nigrescens* species complex. Moreover, I found deep divergent genetic lineages among geographically separated populations of *B. minutula*. However, until now there were no additional morphological characteristics detectable to distinguish between these lineages. Additionally, my colleagues and I described a new deep divergent lineage in the *B. robinsoni* species group as *B. jugivagans*, a species new to science. In contrast, I found only minor genetic differences between specimens of *B. sombra* and *B. nigrescens*. After combining morphometric data and tooth counts from literature of both species with additional data from specimens of *B. sombra* that I collected near the type locality, the distinguishing features blurred. In particular, including much larger specimens of *B. sombra*, not yet known at the time of its description, showed that the tooth count difference is dependent on the size and age of the specimen examined. Larger specimens have more maxillary and vomerine teeth. Based on this evidence I regard *B. sombra* as a junior synonym of *B. nigrescens*. Further, I revised the Panamanian distribution of the two relatively common lowland salamanders, *B. colonnea* and *B. lignicolor*. Besides filling the gaps in the fragmentary known distributions of these species, I assessed the molecular and morphological variation of both species among populations in Panama. While there was little variation in *B. lignicolor*, I found divergent genetic lineages among geographically distinct populations of *B. colonnea* that require further taxonomic examination.

Caecilians (order Gymnophiona) are among the least investigated terrestrial vertebrates. After I received a first specimen of the predominantly South American genus *Oscaecilia* (family Caeciliidae) in western Panama, I started to work more extensively on the taxonomy of Caeciliidae in Central America. The specimens from western Panama were not readily assignable to a single described species, but shared characters with *O. elongata* and *O. osae*. While *O. osae* was only known from the holotype, the type material of *O. elongata* was destroyed during World War II. On the basis of the original description, the unique feature in *O. elongata* within *Oscaecilia* is the absence of subdermal scales in the posterior part of the body. In a referred specimen of *O. elongata* mentioned in the original description from eastern Panama, this characteristic cannot be examined as it consists of head and neck only. Therefore, I used non-destructive high-resolution, synchrotron-based X-ray micro CT imaging (HRμCT) to examine cranial characters in the specimens in question and took normal radiographs to count vertebrae and to make subdermal scales visible. I found that the fragmented specimen from eastern Panama likely belongs to the well-sampled species *O. ochrocephala* and has not much in common with *O. osae* or the specimens from western Panama. Contrarily, *O. osae* and the specimens from western Panama share many morphological characters, but also show some differences. Genetic barcoding revealed that both species are close relatives, but the genetic distance could not be finally resolved, because 16S sequences obtained from blood samples of living *O. osae* were of poor quality. Thus, I compare the *Oscaecilia* from western Panama to *O. osae* in this study, but postpone a taxonomic decision until further material becomes available. Further, I designate *O. elongata* a *nomen dubium*, because the type material is lost, the type locality is not defined in more detail than “Panama”, and the original description does not allow for a definite assignment. Since previous molecular studies only considered *O. ochrocephala*, the monophyly of *Oscaecilia* was never tested before. So far, the genus *Oscaecilia* is based largely on a single cranial character, the eyes covered with bone. Here, I combined two 16S mtDNA sequences of *O. osae* from Costa Rica and two sequences from *O*. sp. from western Panama with two sequences of *O. ochrocephala* and ten sequences of four species of the genus *Caecilia*, the sister genus of *Oscaecilia*. The resulted phylogeny contains two well-supported clades, one clade containing two species of *Caecilia*, one from Panama and one from western Ecuador and all species of *Oscaecilia* tested. The other clade consists of two species of *Caecilia* from the Amazon basin. I therefore assume that the split in both clades is due to the rise of the Andes, what led to today’s cis-trans-Andean distribution of the two clades. For now, to restore monophyly, I suggest to place *Oscaecilia* within the

synonymy of *Caecilia* until more taxa have been tested.

When assessing the conservation status of the amphibian species in mountainous western Panama, I first compiled a list of known species that I potentially could have found during my fieldwork. Using the IUCN categories, I analyzed how many of the endangered species I actually found and how these are distributed over families and species groups. Surprisingly, my rediscoveries of lost species were not equally distributed among the four families that comprise most endangered amphibian species (i.e., Bufonidae, Craugastoridae, Hylidae, and Plethodontidae). While I discovered ten of eleven endangered hylids and six of nine endangered plethodontids, I found only one of four endangered bufonids and none of the nine endangered craugastorids. I assume that the secretive living plethodontids, for which no *Bd* related declines have been documented, were just overlooked in the past decades. In contrast, I propose that hylids, in which *Bd* related population decline is well documented, developed distinct evolutionary solutions permitting coexistence with the pathogen. The situation is obviously different in bufonids and craugastorids, where I found no signs of population recoveries at present. So far, the only surviving populations of species from these families exist in climatic or physiographic niches that have probably shielded them from *Bd*. My data confirm the current view that the risk for naïve amphibian populations to decline during *Bd* epizootics is predicted by ecological traits (e.g., aquatic index, vertical distribution) and not dependent on taxonomic affiliation. However, I propose that only certain amphibian families (e.g., hylids and centrolenids) have the ability to acquire immunity solutions to coexist with the pathogen during enzootic stages. This is a very new perspective on the worst infectious disease in amphibians worldwide, allowing for new research approaches to understand the host-pathogen dynamics. Moreover, I examined where the share of surviving endangered amphibian species is particularly high in mountainous western Panama. As was to be expected, most of the endangered species are found within the boundaries of protected areas. One exception is the unprotected Cerro Colorado region in the Comarca Ngöbe-Buglé that provides habitat for a wide variety of endangered and undiscovered amphibian species. Nonetheless, planned open pit mining would destroy the forests in a large part of the area. This demonstrates once again that human activities are the biggest threat to amphibians in Panama and elsewhere.

2. ZUSAMMENFASSUNG

Amphibien bevölkern die Erde seit über 300 Millionen Jahren und sind heute, mit über 7000 bekannten Arten und vielen weiteren die noch zu entdecken sind, eine der artenreichsten Wirbeltierklassen der Welt. Wie verschiedene Studien herausgefunden haben ist etwa ein Drittel der bekannten Amphibienarten unmittelbar vom Aussterben bedroht, was sie zur meist bedrohten Wirbeltierklasse macht.

ZUSAMMENFASSUNG

Panama hat im Verhältnis zu seiner relativ kleinen Landesfläche eine der artenreichsten Amphibienfaunen weltweit. Dennoch sind die Rolle im Ökosystem und die Lebensraumansprüche vieler Arten bislang nur unzureichend bekannt, was Gegenstand laufender Forschung ist. Moderne taxonomische Forschung in anderen tropischen Regionen der Welt zeigte außerdem, dass Amphibien weitaus artenreicher sind als vormals angenommen, was mutmaßlich auch auf Panama zutrifft. Gleichzeitig ist das Sammeln von Daten und die Beschreibung neuer Arten ein Wettlauf gegen die Zeit, da in den letzten 30 Jahren die Amphibienvielfalt der Welt im Allgemeinen und Panamas im Besonderen in beispielloser Weise abgenommen hat. Die Gründe dafür sind vielfältig und hauptsächlich durch Zerstörung, Veränderung und Zerstückelung natürlicher Amphibienlebensräume verschuldet, aber auch die tödliche Amphibienkrankheit Chytridiomykose, die durch den Pilz *Batrachochytrium dendrobatidis* (*Bd*) verursacht wird, hat ihren Anteil daran. In Panama und Costa Rica hat sich diese Emerging Infectious Disease (EID) (engl. für Neue Infektionskrankheit) wellenartig von Westen nach Osten ausgebreitet und selbst in geschützten Biotopen zu Massenausterbeereignissen und Biodiversitätsverlust unter den Amphibien geführt. Von der Krankheit waren in erster Linie an Bäche gebundene Hochlandarten betroffen. Die letzte großangelegte Bewertung des Erhaltungszustands der Amphibien Panamas, die von der Weltnaturschutzorganisation IUCN 2004 durchgeführt wurde ergab, dass etwa 30% der bekannten Arten vom Aussterben bedroht sind. Außerdem mangelte es bei 17% der damals bekannten Arten an genügend Daten für eine Bewertung.

In Anbetracht von Panamas ohnehin schon überwältigenden Amphibienvielfalt und der Vielfalt an Lebensräumen und Orte an denen zuvor nicht nach Amphibien gesucht worden war, startete ich diese Arbeit mit der Überzeugung, dass die Inventarisierung der Amphibienarten noch lange nicht abgeschlossen ist. Außerdem war zu Beginn unklar, ob es überlebende Amphibienarten in Gebieten gäbe in denen Chytridiomykose aufgetreten war. Der Totalzusammenbruch ganzer Amphibiengemeinschaften im Hochland Westpanamas führte zu einer örtlichen Verschiebung der Amphibienforschung hin zu Tieflandgebieten in Zentral- und Ostpanama. Dabei wurde sich verstärkt auf das Eintreffen des Pathogens, mit anschließender Dokumentation des Ausbruchs der Epizootie und dem folgenden Rückgang der Populationen konzentriert. Darum war die Situation der Amphibiengemeinschaften in Gebieten, in denen es bereits zu Populationsrückgängen gekommen war, größtenteils unbekannt. Folglich waren die Hauptziele meiner Arbeit, erstens die taxonomische Inventarisierung der Amphibienarten Panamas voran zu treiben und zweitens die Situation der Amphibiengemeinschaft in Gebieten zu bewerten, in denen Rückgänge der Populationen aufgrund von *Bd* beobachtet worden waren. Um diese Aufgaben anzugehen führte ich Feldforschung im Westen Panamas durch und legte dabei ein besonderes Augenmerk auf Höhenstufen von 1000 m NN bis auf Panamas höchsten Gipfel, den 3475 m hohen Volcán Barú. Zusätzlich besammelte ich verschiedene Orte im Tiefland zwischen 0 und 1000 m NN um Vergleichsproben zu erhalten.

Im Zeitraum zwischen 2008 und 2013 unternahm ich fünf Sammelreisen nach Panama, die

zusammengerechnet etwa 13 Monate im Feld bedeuteten. Dabei habe ich neun Regionen im Westen Panamas besucht und gemeinsam mit Studenten 767 Belegexemplare gesammelt, von denen 531 unter meiner Feldnummer gesammelt wurden. Begleitende Daten zu diesen Belegexemplaren beinhalten 68 Rufaufnahmen männlicher Anuren, 102 standardisierte Beschreibungen der Lebendfärbung und 259 Gewebeproben von denen bislang 185 16S mtDNA Fragmente sequenziert wurden. Dieser Datensatz ist der bislang größte für panamaische Amphibien und das erste großangelegte DNA Barcoding der Amphibien Westpanamas. Nachdem ich die ersten Barcodinguntersuchungen durchgeführt und die Ergebnisse mit morphologischen und bioakustischen Daten verglichen hatte, war die Anzahl der näher zu bearbeitenden taxonomischen Probleme sehr viel höher als ich zunächst annahm. Um zu endgültigen Ergebnissen zu kommen mussten die meisten genetischen Linien sehr viel genauer taxonomisch bearbeitet werden. In Anbetracht des hohen finanziellen und zeitlichen Aufwands musste also eine Auswahl getroffen werden mit welchen Arten eine komplette taxonomische Bearbeitung zu machen sei. Ich entschied mich dafür jeweils eine Gattung aus den drei Panamaischen Amphibienordnungen genauer zu analysieren. Die Auswahl dieser Gattungen wurde maßgeblich davon abhängig gemacht, ob genügend Material zur Verfügung steht und ob die Bearbeitung der jeweiligen Gattung wissenschaftliche Neuerungen versprach.

Aus der Ordnung Anura wählte ich die Gattung *Diasporus*. Diese kleinen Frösche sind in vielen Biotopen allgegenwärtig und daher relativ leicht zu finden. Außerdem sind sie nur unzureichend taxonomische bearbeitet worden. Die vorliegende Arbeit ist daher die erste die sich auf die Gattung *Diasporus* bezieht und sowohl eine molekulare Phylogenie, als auch eine vergleichende Analyse der Anzeigerufe verschiedener westpanamaischer Populationen beinhaltet. Insgesamt sammelte ich 67 *Diasporus* Belegexemplare in ganz Westpanama und verglich diese mit 49 weiteren mittelamerikanischen Belegexemplaren aus Sammlungen, einschließlich der namenstragenden Typen von *D. diasporus* und *D. hylaeformis*. Weitere Vergleichsdaten entnahm ich der Literatur. Die DNA Barcoding Analyse eines Fragments des 16S rRNA Gens beinhaltete 43 eigene Sequenzen, die mit 15 relevanten Sequenzen von GenBank verglichen wurden. Außerdem verglich ich die Anzeigerufe von 26 männlichen Individuen untereinander und mit Angaben aus der Literatur. Die DNA Barcoding Analyse ließ mehrere unbenannte genetische Linien erkennen, aber legte auch einige Individuen zusammen, die sich ansonsten morphologisch und bioakustisch gut unterscheiden ließen. Im Großen und Ganzen ergab die morphologische Untersuchung des Materials keine eindeutigen Merkmale um genetische Linien zu unterscheiden. Allerdings ist es möglich Arten zu unterscheiden, indem man eine Kombination verschiedener Merkmale heranzieht. Welche das sind muss aber von Art zu Art neu entschieden werden. Meine Umfassende Sammlung von Rufaufnahmen machte es möglich zum ersten Mal den Einfluss verschiedener Parameter auf den Ruf von *D. hylaeformis* zu testen. Die Analyse ergab, dass es eine signifikante Abhängigkeit zwischen der Dominanzfrequenz und der Körpergröße des rufenden Frosches gibt, dabei ist die Frequenz umso höher, je kleiner der Frosch ist. Ein ähnliches Verhältnis konnte zwischen Rufrate und Temperatur beobachtet werden, wobei die Rufrate mit abnehmender Temperatur ebenfalls abnimmt. Ich vermute, dass dieses allgemeingültige Muster, welches bereits für andere Anurengattungen beschrieben wurde, auch auf andere *Diasporus* Arten zutrifft. Unter Berücksichtigung dieser innerartlichen Variation betrachte ich die vergleichende Rufanalyse als die beste Methode um Arten auseinanderzuhalten. Das gilt insbesondere für syntopische Arten. Die Einbindung aller drei Beweislinien, also der Morphologie, des DNA Barcodings und der bioakustischen Analyse, führte zur Entdeckung von vier unbeschriebenen Arten von denen zwei (*D. citrinobapheus* und *D. igneus*) bereits formal von Kollegen und mir beschrieben wurden.

In der Ordnung Caudata führte ich eine integrative taxonomische Analyse der westpanamaischen Vertreter der Gattung *Bolitoglossa*, der größeren der beiden panamaischen Salamandergattungen, durch. *Bolitoglossa* ist eine sehr artenreiche Gattung mit einem Artbildungszentrum in den Höhenlagen von Costa Rica und Westpanama. Ich sammelte 53 *Bolitoglossa* Belegexemplare und verglich diese mit zwölf Belegen aus Sammlungen, einschließlich des Holotypus und eines Paratypen von *B. gomezi*. Der Datensatz wurde dann mit Daten aus der Literatur vervollständigt. Unter den gesammelten Belegen befanden sich zwei Arten, die als gefährdet eingestuft werden und die jeweils seit mehreren Jahrzehnten weder gesammelt noch gesichtet worden waren. *Bolitoglossa magnifica* wurde seit 34 Jahren nicht gesehenen und *B. anthracina* seit 22 Jahren. Außerdem fand ich Salamander an mehreren zuvor nicht besammelten Orten. Mein 16S mtDNA Barcoding, das aus 21 eigenen Sequenzen kombiniert mit 47 GenBank Sequenzen besteht, stellt das bislang umfangreichste Taxon-Sampling für panamaische *Bolitoglossa* dar. Auch wenn diese molekulare Phylogenie nur auf einem Marker basiert, so konnte ich doch weitgehende Übereinstimmungen mit vorangegangen Studien feststellen und die Knoten des Baumes sind statistisch gut unterstützt. Ich erhielt in meinen Bäumen alle zuvor definierten Untergattungen und Artengruppen. Auf Grundlage der molekularen Phylogenie ordne ich *B. anthracina*, welcher hier erstmals molekular untersucht wurde, der *B. subpalmata* Artengruppe zu. Wegen seiner Erscheinung als großer, dunkel gefärbter Salamander wurde dieser vormals ohne weitere Untersuchungen in der *B. schizodactyla* Artengruppe geführt, in der sich die großen, schwarzen Salamander aus dem *B. nigescens* Artenkomplex befinden. Außerdem fand ich mehrere weit abweichende genetische Linien zwischen geografisch getrennten Populationen von *B. minutula*. Trotzdem konnte ich bislang keine zusätzlichen morphologischen Merkmale finden, die es erlauben würden diese Linien auseinanderzuhalten. Außerdem konnten meine Kollegen und ich eine neue genetische Linie innerhalb der *B. robinsoni* Artengruppe als neue Art mit Namen *B. jugivagans* beschreiben. Im Gegensatz dazu fand ich nur geringfügige genetische Unterschiede zwischen Belegen von *B. sombra* und *B. nigrescens*. Ich kombinierte die morphometrischen Messungen und die Anzahl der Zähne, die ich an selbst gesammelten, neuen Belegen von nahe der Typuslokalität von *B. sombra* genommen hatte, mit Angaben aus der Literatur zu beiden Arten. Dabei verschwammen die Unterscheidungsmerkmale die zur

Artabgrenzung verwendet worden waren. Im Besonderen zeigte die Einbeziehung wesentlich größerer Individuen von *B. sombra* in die Analyse, die zum Zeitpunkt der Artbeschreibung nicht zur Verfügung standen, dass die Unterschiede in der Anzahl der Zähne mit dem Alter und der Größe des Individuums zusammenhängen. So haben größere Individuen mehr Zähne im Oberkiefer und Gaumendach. Auf Grund dieser Beweise sehe ich *B. sombra* als Juniorsynonym von *B. nigrescens* an. Ich habe weiterhin die panamaische Verbreitung der relativ häufigen Salamanderarten des Tieflands *B. colonnea* und *B. lignicolor* überarbeitet. Dabei untersuchte ich die molekulare und morphologische Variation beider Arten in Panama und schloss die Verbreitungslücken in den nur bruchstückhaft bekannten Verbreitungsgebieten. Während nur wenig Variation innerhalb der Art *B. lignicolor* zu beobachten war, fand ich weit auseinanderlaufende genetische Linien zwischen geographisch getrennten Populationen von *B. colonnea*, was weitere Untersuchungen nach sich ziehen wird.

Blindwühlen (Ordnung Gymnophiona) gehören zu den am wenigsten untersuchten terrestrischen Wirbeltieren. Nachdem ich ein erstes Individuum aus der vornehmlich südamerikanischen Gattung *Oscaecilia* (Familie Caeciliidae) erhalten hatte, begann ich mich intensiver mit der Taxonomie der Caeciliidae in Mittelamerika zu beschäftigen. Die Belegexemplare aus Westpanama waren nicht gleich einer beschriebenen Art zuzuordnen, sondern zeigten sowohl Merkmale von *O. elongata* als auch *O. osae*. Während *O. osae* nur anhand des Holotypus bekannt war, ist das Typusmaterial von *O. elongata* im Zweiten Weltkrieg vernichtet worden. Nach der Originalbeschreibung ist *O. elongata* von anderen Arten der Gattung durch das einzigartige Fehlen von subdermalen Schuppen im hinteren Teil des Körpers zu unterscheiden. Dieses besondere Merkmal kann in einem der Art zugeordneten Beleg aus dem Osten Panamas nicht überprüft werden, da besagtem Exemplar die hinteren zwei Drittel des Körpers fehlen. Aus diesem Grund fertigte ich hochauflösende Mikro CT Bilder mittels Synchrotron Strahlung (HRµCT) von verschiedenen Belegen an, um Schädelmerkmale vergleichen zu können. Zudem machte ich gewöhnliche Röntgenbilder um Wirbel zu zählen und subdermale Schuppen sichtbar zu machen. Dabei ergab sich, dass das bruchstückhafte Exemplar aus Ostpanama keine Gemeinsamkeiten mit *O. osae* oder den *Oscaecilia* aus dem Westen Panamas aufweist und am ehesten der relativ gut bekannten Art *O. ochrocephala* zuzuordnen ist. Im Gegensatz dazu haben die Belegexemplare aus dem Westen Panamas eine Reihe von Gemeinsamkeiten mit *O. osae* aber zeigen auch einige Unterschiede auf. Ein genetisches Barcoding ergab zwar, dass beide Arten nahe Verwandte sind, jedoch konnte die genetische Distanz nicht zufriedenstellend erhoben werden, da die 16S Sequenzen von *O. osae*, die aus Blutproben lebender Individuen gewonnen wurden, von schlechter Qualität waren. Darum vergleiche ich zwar die westpanamaischen *Oscaecilia* mit *O. osae* in dieser Arbeit, kann aber derzeit keine taxonomischen Schlüsse ziehen bis weiteres Material zur Verfügung steht. Als weiteres Ergebnis, designiere ich den Namen *O. elongata* als *nomen dubium*, da das Typusmaterial verloren ist, die Typuslokalität nur mit „Panama“ angegeben ist und die Erstbeschreibung keine eindeutige Zuordnung zulässt. Vorherige molekulare

Untersuchungen hatten bislang nur *O. ochrocephala* zur Verfügung, daher konnte die Monophylie der Gattung nie getestet werden. Bislang ist die Gattung *Oscaecilia* weitgehend an einem einzigen Schädelmerkmal, nämlich dass sich die Augen unter dem Schädelknochen befinden, festgemacht. In dieser Arbeit habe ich die 16S mtDNA Sequenzen von *O. osae* aus Costa Rica und zwei Sequenzen von *O*. sp. aus Westpanama mit zwei *O. ochrocephala* Sequenzen und zehn Sequenzen von vier Arten aus der Schwestergattung *Caecilia* kombiniert. Die daraus resultierende Phylogenie weist zwei gut unterstützte monophyletische Gruppen auf. Eine Gruppe beinhaltet neben den *Oscaecilia* Arten auch eine *Caecilia* aus Panama und eine aus dem Westen Ecuadors. Die zweite Gruppe besteht aus zwei *Caecilia* Arten aus dem Amazonas Becken. Ich vermute daher, dass die Trennung beider Gruppen auf die Hebung der Anden zurückgeht die zur heutigen cis- und trans-andinen Verbreitung beider Gruppen geführt hat. Um die Monophylie vorerst wieder herzustellen schlage ich vor *Oscaecilia* in die Synonymie von *Caecilia* zu überführen bis weitere Taxa zur Verfügung stehen.

Um den Erhaltungszustand der Amphibien Arten im Hochland Westpanamas zu bewerten, habe ich zunächst eine Liste der Arten erstellt, die ich potentiell hätte finden können. Anhand der IUCN Kategorien bestimmte ich den Anteil gefährdeter Arten, wie viele ich davon gefunden habe und welchen Familien und Artengruppen die gefundenen Arten zuzuordnen sind. Überraschenderweise, sind die wiedergefundenen Arten nicht gleichmäßig über die vier Amphibienfamilien mit den meisten gefährdeten Arten (Bufonidae, Craugastoridae, Hylidae, und Plethodontidae) verteilt. Während ich zehn der elf gefährdeten Laubfroscharten und sechs der neun Lungenlosen Salamander finden konnte, fand ich nur eine von vier gefährdeten Krötenarten und keinen der neun gefährdeten Craugastoriden. Ich glaube, dass die versteckt lebenden Salamander, bei denen auch keine Chytrid bezogenen Bestandsrückgänge beobachtet wurden, in den letzten Jahrzehnten einfach übersehen wurden. Im Gegensatz dazu vermute ich, dass die Laubfrösche, bei denen mit Chytridiomykose verbundene Bestandsrückgänge hinreichend dokumentiert wurden, einen evolutionären Weg gefunden haben mit dem Pathogen zu leben. Das steht anscheinend im Gegensatz zu Bufoniden und Craugastoriden, bei denen ich keine Hinweise auf eine Bestandserholung finden konnte. Die einzigen momentan bekannten Populationen von Arten aus diesen Familien überleben anscheinend nur in klimatischen Nischen, in denen sie vor *Bd* geschützt sind. Meine Daten bestätigen daher die derzeitige Sichtweise, dass die Wahrscheinlichkeit für Populationszusammenbrüche in einer Amphibiengemeinschaft, die zuvor keinen Kontakt mit dem Erreger hatte, für eine Art umso höher ist je höher deren Verbreitungsgebiet liegt und umso stärker die Art ans Wasser gebunden ist. Also spielt während einer Epizootie die taxonomische Zugehörigkeit eher eine untergeordnete Rolle. Hingegen haben anscheinend nur bestimmte Amphibienfamilien (z.B. Hyliden und Centroleniden) die Fähigkeit Abwehrmechanismen zu entwickeln, um während der enzootischen Phase mit dem Erreger zu koexistieren. Das ist eine sehr neue Sichtweise auf die schlimmste Amphibienkrankheit der Welt, die es erlaubt in neue Richtungen zu forschen, um die Wirt-Erreger-Beziehungen besser zu verstehen. Desweiteren untersuchte ich in welchen Gebieten im Hochland Westpanamas der Anteil an überlebenden, gefährdeten Arten besonders hoch ist. Wie zu erwarten war fanden sich die meisten gefährdeten Arten innerhalb von Schutzgebieten. Eine Ausnahme bildet die ungeschützte Cerro Colorado Region in dem Autonomiegebiet der Ngöbe-Buglé, wo ich eine Vielzahl gefährdeter und unbeschriebener Arten nachweisen konnte. Trotzdem ist hier ein Tagebau geplant, dem große Teile der Wälder zum Opfer fallen würden. Das zeigt wieder einmal, dass der Mensch die größte Gefahr für die Amphibien Panamas und der Welt darstellt.

3. LIST OF BBREVIATIONS

AB: field number of Abel Batista
AC: field number of Arcadio Carrizo
aff.: *affinis*, lat. = similar to (higher uncertainty than *cf.*)
AH: field numer of Andreas Hertz
AMP: antimicrobial peptide
asl: above sea level
AST: Audio Strip Transect
Bd: *Batrachochytrium dentrobatidis*, an amphibian pathogen
BI: Bayes Inference
BIC: Bayesian Information Criterion
BLAST: Basic Local Alignment Search Tool, algorithm for comparing DNA sequence information
BMV: body width at vent
BW1PG: body width at first PG
BWM: body width at midbody
***cf.*:** *confer*, lat. = compare (lower uncertainty than *aff.*)
CFL: number of costal folds between adpressed limbs
CG: number of costal grooves
cp.: compare
CR: IUCN Red List Category, Critically Endangered
DD: IUCN Red List Category, Data Deficient
DFT: Discrete Fourier Transform, converts time series signals into frequency components
DNA: deoxyribonucleic acid
DT: dentary tooth counts
DWF III: disk width at Finger III
DWT IV: disk width at Toe IV
e.g.: *exempli gratia*, lat = for example
ED: eye diameter
EID: Emerging Infectious Disease
EL: eyelid length
EN: IUCN Red List Category, Endangered
EVS: Environmental Vulnerability Scores

FFT: Fast Fourier Transformation, an algorithm to transform the DFT
FW: foot width
GABI: Great American Biotic Interchange
HAU: field numbers of Frank Hauenschild
HAW: hand width
HL: head length
HLL: hind limb length
HRμCT: high-resolution micro-computed tomography
HW: head width
Hz: hertz, unit of frequency
i.e.: *id est*, lat = that is
IA: index of attenuation
IND: internare distance
IOD: interorbital distance
ITCZ: Inner Tropical Convergence Zone
ITD: intertentacular distance
IUB: International Union of Biochemistry and Molecular Biology, the IUB weight matrix is the default scoring matrix in ClustalW
L: total length
LC: IUCN Red List Category, Least Concern
LF III: length of Finger III
LJL: length of lower jaw
LSt: field number of Leonhard Stadler
LT IV: length of Toe IV
Ma: megaannus, million years ago
ML: Maximum Likelihood
MPAT: maxillary-premaxilary tooth counts
MT: maxillary tooth count
mtDNA: mitochondrial DNA
NE: IUCN Red List Category, Not Evaluated
NH: field number of Nadim Hamad
NJ: Neighbor Joining
NNI: Nearest Neighbor Interchange, heuristic method
NT: IUCN Red List Category, Near Threatened
NV: number of vertebrae
PA: protected area
PCM: Puls-Code-Modulation, digital audio format
pers. comm.: personal communication
PG: primary grooves
PILA: Parque Internacional La Amistad
PMT: premaxillary tooth count
RFLF: Reserva Forestal La Fortuna
rRNA: ribosomal ribonucleic acid
SD: standard deviation
SG: secondary grooves
SG1: first secondary groove

SI: scale inception
SL: shank length
SP: snout projection
ST: splenial tooth counts
SVL: snout vent length
TD: tympanum diameter
TL: tail length
TND: tentacle to nare distance
VES: Visual Encounter Survey
VPAT: vomero-palatine tooth counts
VT: vomerine tooth count
VU: IUCN Red List Category, Vulnerable

4. INTRODUCTION

4.1. PREFACE

There is hardly one animal group that has gone through such an image change in the course of the centuries than the amphibians. In his 1758 work Systema Naturae, Carl von Linné, founder of modern taxonomy, made disparaging remarks about amphibians when he wrote „These foul and loathsome animals are abhorrent because of their cold body, pale color, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom; and so their Creator has not exerted his powers to make many of them“ (Linnaeus 1758). Nowadays, however, amphibians have turned in people‘s minds from the disgusting creatures of witches and sorcerers to a figure of sympathy for example in advertisements and as flagship species in conservation projects. It has further turned out that Linnés conclusion that there are not many amphibians on the planet was a drastic misconception. Duellman and Trueb (1986) spoke of „more than 3900 living species“; while currently there are 7273 amphibian species recognized (AmphibiaWeb 2014), thus considerably more than the 5339 (Wilson & Reeder 2005) currently recognized mammal species. Moreover, the number of recognized amphibian species is strongly increasing (Glaw & Köhler 1998). During the period 2001–2012, 420 amphibian species have been described in the taxonomic journal Zootaxa alone.

The living amphibians are organized in three, quite different orders, the biggest of which are the Anura (frogs and toads) with 6411 species, followed by Caudata (salamanders and newts) with 662 species, and Gymnophiona (caecilians) with 200 species (AmphibiaWeb 2014). Amphibians are an old vertebrate group, the first who colonized terrestrial habitats in the Mid-Devonian about 350 million years ago (Duellman & Trueb 1986) and since then evolved to a great variety of different morphologies, life modes, and ecological types, more than in any other living vertebrate group. In some respects, amphibians are transitional between the completely aquatic fishes and the terrestrial amniotes concerning in particular their reproduction. Classically, amphibians lay shell-less eggs in fresh water bodies from which a free living aquatic embryo hatches. This larva thrives as an aquatic animal before it undergoes a metamorphosis into a terrestrial animal that is morphologically completely different from the tadpole. This particularity is what the amphibians carry in their name that comes from the Greek words *amphi* (both) and *bios* (life) referring to their two different stages of life. Nevertheless, in their long evolutionary history amphibians have developed an astonishing variety of exceptions from this general rule. To date, 39 reproductive modes have been recognized in anurans (Haddad & Prado 2005), seven in salamanders (Duellman & Trueb 1986), and five in caecilians (San Mauro *et al.* 2014). In general, there is an evolutionary transition from free living larvae in large water bodies to parental care in small water bodies like phytotelmata, direct development, and even viviparity. The increasing independence of permanent water bodies in the evolutionary history of amphibians led to an almost global distribution except of the Polar Regions, some deserts like the Sahara, and some isolated volcanic archipelagos (*e.g.*, Galapagos and Hawaii).

The highest known amphibian diversity is found in the Neotropics. Lower Central America (Costa Rica and Panama), Colombia and Ecuador together are home to 1701 amphibian species, what is almost one third of the known amphibian species worldwide (AmphibiaWeb 2014).

4.2. AMPHIBIANS OF PANAMA

4.2.1. A BRIEF HISTORY OF AMPHIBIAN INVENTORY IN PANAMA

The history of herpetological studies in Panama is closely linked with the construction of the Panama Canal and the occupation of the “Canal Zone” by the United States of America from 1903–1999. Prior to the construction of the canal only sporadic work had been conducted in Panama itself. However, many of the amphibian and reptile species that occur in Panama have their type localities in neighbouring Costa Rica or in the case of widespread species even in other parts of Central America or in South America. Most of the herein provided information on great herpetologists who worked in Panama was combined from Adler (1989), Duellman (2001), Ibáñez *et al.* (2001), and Savage (2002).

Among the first who named amphibian taxa that occur in Panama are the great Swedish systematist and naturalist **Carl von Linné (1707–1778)** (*e.g.*, the caecilian genus *Caecilia*) and the Austrian naturalist and herpetologist **Joseph Nicolaus Laurenti (1735–1805)** (*e.g.*, the frog genus *Pipa*). Until well into the 19th century relatively few amphibian species that occur in Panama were described, for example not a single species of the large salamander family Plethodontidae. However, some of the larger amphibian groups like families and genera that are still extant were introduced during this period. For example, the species-rich salamander genus *Bolitoglossa* was named by the French biologists at the Muséum National d'Histoire Naturelle in Paris **Gabriel Bibron (1805–1848)**, **André Marie Constant Dumeríl (1774–1860)**, and his son **Auguste Henri André Dumeríl (1812–1870)**. The former two also named the toad genus *Atelopus* and the genus *Eleutherodactylus*. Also, the British zoologist **John Edward Gray (1800–1875)** contributed to the knowledge of the herpetofauna of Lower Central America and enhanced the herpetological collection of the British Museum of Natural History to the most important at that time. He also redefined Amphibia as a class in the meaning of amphibians today, excluding reptiles. The 19th century was also the high period of German herpetologists in tropical America headed by the Berlin zoologist **Wilhelm Carl Hartwig Peters (1815–1883)** who described around ten amphibian species that occur in Panama. Other famous amphibian taxonomists from Germany include **Oscar Schmidt (1832–1886)**, **Johann Georg Wagler (1800–1832)**, **Maximilian Prinz von Wied-Neuwied (1782–1882)**, as well as the herpetological curator at the Senckenberg Nature Museum at this time, **Oskar Boettger (1844–1910)**, who named two glass frog species from Costa Rica that also occur in Panama. However, none of these naturalists personally visited Panama, but material was sent by others, who were delegated for the purpose to collect. In this regard, the most outstanding herpetological survey in Panama in the 19th century has been conducted by the Polish **Joseph Ritter von Rawiez Warszewicz (1812–1866)** formerly gardener in the Botanical Gardens of Berlin. Although Warszewicz was particularly interested in plants, he collected most of the herpetological material examined by Schmidt and Peters. Warszewicz was the first to cross the Cordillera Central, ascending from David to Boquete, climbing the Volcán Barú and then descending the Carribean slopes to what is Chiriquí Grande today (Savage 1970). Almost all amphibians collected on this trip where new, including for example the type of *Lithobates warszewitschii* (Schmidt 1857) that was named after him (using the German spelling of his name "Warszewitsch"). With the Warszewicz expedition the time of intense exploration of Panama´s herpetofauna began. In 1875 the French ornithologist

Adolphe Boucard (1839–1905) collected amphibians in Costa Rica and Panama, at a time when France was about to develop plans for a canal that should connect the Atlantic and the Pacific Oceans. His collection has been deposited in the Museum National d´Histoire Naturelle in Paris and the amphibian material has been studied by **Paul Brocchi (1838–1898)**. In the second half of the 19th century and the early 20th century the British herpetologist at the British Museum of Natural History in London (now: Natural History Museum) **George Albert Boulenger (1858–1937)** described 16 amphibian species that occur in Panama and are valid until today. Moreover, he produced a lot of names that are considered as synonyms of other taxa today. The amphibian specimens from Latin America that Boulenger worked on were mostly obtained by **William Frederick Henry Rosenberg (1868–1957)** and came from Colombia and Ecuador. Boulenger honoured Rosenberg by naming the frog *Hypsiboas rosenbergi* (Boulenger 1898) that also occurs in Panama after him. Boulenger was only exceeded in terms of describing amphibian species that occur in Panama by the US biologist **Edward Drinker Cope (1840–1897)**, with whom amphibian taxonomy in Lower Central America reached a new era. Cope worked on the collection that **William More Gabb (1839–1878)** had brought to the United States National Museum (now: National Museum of Natural History). Gabb´s expedition was the second important expedition to the Cordillera Central of Lower Central America after that of Warszewicz. Gabb crossed the Talamancan mountains between 1873 and 1874 and collected on Cerro Utyum and the Valle de Talamanca (Savage 1970), today still an understudied area. Cope described about 50 valid amphibian species, not at least because many of his herpetological precursors focussed more on reptiles than amphibians. With Cope, the times when Europeans dominated the herpetological exploration of Panama were gone and US scientists took over. The first quarter of the 20th century was dominated by the work of **Thomas Barbour (1884–1946)** herpetologist at the Museum of Comparative Zoology at Harvard University in Cambridge, Massachusetts. During his career the United States supported the Panamanian desire for independence from Colombia what succeeded in 1903, and subsequently bought the unfinished Panama Canal construction site from the French in 1904. Barbour frequently visited Panama and became executive officer in charge for the development of Barro Colorado Island (BCI) in 1923, a hill in the jungle that had become an island in 1914 when the huge Gatun Lake was flooded. He continued in this position until 1945, while he appointed the entomologist **James Zetek (1886–1959)** as resident manager. In 1946 the Smithsonian Institution took over the administration of BCI from the National Academy of Science and since then runs a permanent research centre on the island, the Smithsonian Tropical Research Institute (STRI). Today, BCI is considered to be the best studied tropical site in the world. One of Barbour´s PhD students **Emmett Reid Dunn (1894–1956)** travelled on several trips to Costa Rica, Panama, and Colombia and soon became the leading authority on the amphibians of that area. Between 1924 and 1942 he described 29 amphibian species known to occur in Panama. He was also the first who worked intensively on salamanders and caecilians of Lower Central America. Worth mentioning is also the first women who worked on Panamanian herpetology, **Helen Beulah Thompson Gaige (1890–1970)**, curator of herpetology at the Museum of Zoology at Michigan, who visited Panama in 1923 and described the bromeliad-dwelling treefrog *Isthmohyla zeteki* (Gaige 1929) named after James Zetek. In her honor, Dunn (1931) named the frog *Pristimantis gaigei*. Between 1948 and 1959 **Edward Harrison Taylor (1889–1978)**, curator of herpetology at the University of Kansas Natural History Museum, intensively worked on the herpetofauna of Costa Rica after he has had a strong working focus in south-east Asia. His work that besides own collections also build on the collections of Dunn, produced 21 valid species, among others three of the eleven species of Gymnophiona in Panama and five of the 13 species of Centrolenidae. From the 1960s on, several herpetologists worked intensively in Lower Central America. In 1966 a expedition across the Panamanian Serranía de Talamanca has been conducted by **William Eduard Duellman (1930–)**, **Linda Trueb (1942–)**, both University of Kansas, and **Charles William Myers (1936–),** at that time at the Museum of Comparative Zoology at Harvard and from 1968 on curator of herpetology at the American Museum of Natural History (Fig. 1). More than hundred years after the Gabb expedition this was the first collection from the Caribbean slopes of the Panamanian Talamanca and produced several types and new country records as well as many call recordings of anurans, a novelty at this time. Later, in the 1980s, **John Douglas Lynch (1942–)** still described new species from this collection. Duellman worked mainly on anurans and described several new hylid frogs from Panama. Moreover, he wrote a book about the hylid frogs of Middle America that is still the most comprehensive work in this field. Recently, he contributed in the taxonomic revision of the formerly huge frog genus *Eleutherodactylus* (Hedges *et al.* 2008). The hylid frog genus *Duellmanohyla* (Campbell & Smith 1992) was named after him. Trueb contributed in the taxonomic revision of *Centrolenidae* by Guayasamin *et al.* (2009) and described several new species mostly together with Duellman. Among these is the only representative of the frog family Pipidae in Panama, *Pipa myersi* (Trueb 1984), which she named after Charles Myers. Although Myers himself focussed more on reptiles, he also described seven amphibian species

Figure 1: Participants of the 1966 expedition across the Cordillera Central at Cerro Pando: From left to right: William E. Duellman, Linda Trueb, and Charles W. Myers on a log at Río Claro, northern slope of Cerro Pando. Foto by Tomás Quintero, courtesy of William E. Duellman.

from Panama. Recently, he and colleagues described two new species of *Anomaloglossus* (Myers *et al.* 2012). Myers and Duellman were also the first to work on material from the high Serranía de Tabasará when the mining companies explored the copper deposits at Cerro Colorado. The material was collected by Grace M. Tilger for the American Museum in 1978 and 1979, and subsequently by Ronald H. Pine for the National Museum of Natural History, Smithsonian Institution (Myers & Duellman 1982). Another great name in conjunction with amphibian research in Panama is **Jay Mathers Savage (1928–)**, who first worked at the University of Southern California and later changed to the University of Miami. Savage worked primarily on the herpetofauna of Costa Rica, but with a wide influence on the knowledge of the Panamanian herpetofauna. His book about Costa Rica´s herpetofauna (Savage 2002) is still the best reference on many aspects of amphibians that occur in Lower Central America. Around 20 Panamanian amphibians were described under Savage´s contribution most with type locality in Costa Rica. Already early in his career, one of Savage´s PhD students, **David Burton Wake (1936–)** today at the University of California, Berkeley became the leading authority in the field of Central American salamanders. To date, more than half of the salamander species in Panama have been described by Wake and his coworkers. Another PhD student of Savage, **Karen Renee Lips (1966–)** now at the University of Maryland, worked on population biology of *Isthmohyla calypsa* (Lips 1996) in the Serranía de Talamanca, when the chytrid wave, coming from Costa Rica, hit Panama. Lips documented the collapse of amphibian populations in the Serranía de Talamanca and the La Fortuna area. By now, Lips papers are the most complete documentation of chytridiomycosis driven amphibian declines worldwide. The last decade of amphibian research in Panama was dominated by the work of STRI researchers, who intensively studied amphibian behavior and ecology on BCI. Especially productive in terms of amphibians was **Stanley Austin Rand (1932–2005)**, who contributed a lot to the knowledge of calling behavior in anurans. Meanwhile, conservational aspects of amphibians became more and more important and STRI researchers particularly tried to collect life specimens prior to the arrival of the chytrid wave in order to establish *ex-situ* populations. Since the wave moved from west to east the research focus shifted from west and central Panama more to eastern Panama, a region that had been only poorly studied previously. Alongside the primarily conservation aimed collecting, the deeper analysis of the collected specimens produced several new species described by researchers such as **Andrew Jackson Crawford (1968–)**, **Roberto Ibáñez (1958–)**, **César Augusto Jaramillo (1962–)**, **Joseph Ralph Mendelson III (1964–)**, and **Mason Joseph Ryan (1975–)**. Until 2006 the Cordillera Central was not in the focus of herpetological surveys. Between 2006 and 2011 the Darwin Initative of London financed a project led by the Natural History Museum of London for a large scale biological inventory of the La Amistad International Park in order to improve the sustainable management of the park. The formerly understudied 3000+ m peaks in extreme western Panama were explored what led to the discovery of several new salamander species (Boza-Oviedo *et al.* 2012). Also in 2006, the Senckenberg herpetologists **Gunther Köhler (1965–)** and his former PhD student **Javier Sunyer (1975–)**, together with the Panamanian herpetologists **Abel Batista (1978–)** and **Marcos Ponce (1979–)** visited several sites in the Serranía de Tabasará, an area that had not received much attention after Tilger and Pine collected there in the 1970s. This field trip produced a new salamander (Köhler *et al.* 2007) and several new records of amphibians from the Comarca Ngöbe-Buglé (Köhler *et al.* 2008) and further set the basis for my own work.

4.2.2. LATEST HIGHER TAXONOMY AND SYSTEMATICS OF PANAMA´S AMPHIBIANS

Amphibian supraspecific taxonomy has been relatively stable in the 20th century with globally distributed genera such as for example *Bufo* Garsault, 1764 and *Hyla* Laurenti, 1768, and huge, heterogenous families like Leptodactylidae Werner, 1896. From the 1990s on, the sequencing of DNA became easier and cheaper and the bioinformatic analysis tools developed rapidly, so that previous phylogenetic hypotheses could be tested with molecular approaches. This let to substantial changes in the amphibian tree of life, particularly in the first decade of the 21th century. Since Panama inhabits one of the most diverse amphibian faunas in Central America its amphibian assamblage was largely affected from taxonomic changes, particularly for taxa at the genus and family level. In the following, I want to give a brief overview of the taxonomic changes in the three recent amphibian orders with respect to families and genera in Panama that have been made after the comprehensive work of Savage (2002) on the herpetofauna of Costa Rica. The latest taxonomy presented here is also the higher taxonomy used in this work that is basically congruent with that of Frost (2014). An overview of the current taxonomy and nomenclature of Panamanian Amphibians is given in the table of amphibian species that occur in Panama (10.1. Appendix 1).

Anura Fischer von Waldheim, 1813: Frogs and toads, as the largest order of living amphibians, have undergone substantial taxonomic changes within recent years, a process that is still in flow. Regarding Panamanian species of the family Bufonidae Gray, 1825, Pramuk (2006) and Pramuk *et al.* (2008) placed all those Panamanian toad species that were formerly in the nearly cosmopolitan genus *Bufo* in the four genera *Chaunus* Wagler, 1828, *Incilius* Cope, 1863, *Rhaebo* Cope, 1862, and *Rhinella* Fitzinger, 1826. Especially the status of *Chaunus* with regard to *Rhinella* was subsequently discussed (Chaparro *et al.* 2007; Savage & Bolaños 2009; Köhler 2011). However, the latest molecular phylogeny by Pyron and Wiens (2011) demonstrated *Chaunus*, as used by Savage and Bolaños (2009) and Köhler (2011), to be polyphyletic, but confirmed the monophyly of the entire *Chaunus*-*Rhinella* clade. Thus, it is correct to apply the older name *Rhinella* to the entire clade until the taxonomy has been finally clarified. Van Bocxlaer *et al.* (2009) found the Central American genera *Rhinella*, *Incilius* and the North American genus *Anaxyrus* Tschudi, 1845 to be more closely related to Old World toads, with the predominantly South American toad genus *Rhaebo* as sister clade. However, this was not confirmed by Pyron and Wiens (2011) although their nodes received only low statistical support. The Central American genus *Crepidophryne* Cope, 1889 has been excluded from all those previous analysis until Mendelson *et al.* (2011) found it to be deeply nested within *Incilius*. Accordingly, four genera of Bufonidae are currently recognized in Panama (*i.e.*, *Atelopus* Duméril and Bibron, 1841, *Incilius*, *Rhaebo*, and *Rhinella*).

The exclusively Neotropical family Centrolenidae Taylor, 1951 has been revised on morphological basis (Cisneros-Heredia & McDiarmid 2007) and subsequently on the basis of a molecular phylogeny (Guayasamin *et al.* 2008; Guayasamin *et al.* 2009). In Panama, the family Centrolenidae is currently divided into the two subfamilies Centroleninae (including the genera *Cochranella* Taylor, 1951, *Espadarana* Guayasamin, Castroviejo-Fisher, Trueb, Ayarzagüena, Rada, and Vilà, 2009, *Sachatamia* Castroviejo-Fisher, Trueb, Ayarzagüena, Rada, and Vilà, 2009, and *Teratohyla* Taylor, 1951), and Hyalinobatrachinae (including only *Hyalinobatrachium* Ruiz-Carranza and Lynch, 1991 in Panama). Pyron and Wiens (2011) confirmed the monophyly of the family and the respective genera.

Hedges *et al.* (2008) made a revision of the former super-genus *Eleutherodactylus* Duméril and Bibron, 1841 that has, at that time, been the largest vertebrate genus of the world, containing more than 900 species of frogs. *Eleutherodactylus* has been a part of the large, heterogenous family Leptodactylidae until Frost *et al.* (2006) redefined the family Brachycephalidae Günther, 1858 composed of the subfamily Eleutherodactylinae Lutz, 1954 and the family Brachycephalidae. Later, Hedges *et al.* (2008), on the basis of a much larger taxon sampling created the unranked, above family-level taxon Terrarana for most direct-developing Neotropical frogs (former genus *Eleutherodactylus*), since they are doubtlessly monophyletic, but to speciose to handle as a single family. Currently, the Panamanian members of this taxon are divided into the families Craugastoridae Hedges, Duellman, and Heinicke, 2008, (containing the genus *Craugastor* Cope, 1862), Eleutherodactylidae Lutz, 1954, (containing the genus *Diasporus* Hedges, Duellman, and Heinicke, 2008), and Strabomantidae Hedges, Duellman, and Heinicke, 2008, (containing the genera *Pristimantis* Jiménez de la Espada, 1870 and *Strabomantis* Peters, 1863). Pyron and Wiens (2011) found, on the basis of a larger taxon sampling, that the former family Strabomantidae is paraphyletic and they solved this problem by placing the Strabomantidae into Craugastoridae as both were already sister taxa in the analysis of Hedges *et al.* (2008). The Panamanian former members of the family Strabomantidae were split into the two subfamilies Strabomantinae, containing *Strabomantis*; and Pristimantinae, containing *Pristimantis*. The genus *Craugastor* is placed in its own subfamily Craugastorinae.

The members of the hylid-like subfamily Hemiphractinae Gadow, 1901 that usually have direct-developing eggs, which are carried in a dermal brood pouch on the dorsum of the female until they hatch, have been removed from Hylidae Rafinesque, 1815 and placed into Leptodactylidae by Faivovich *et al.* (2005). Frost *et al.* (2006) recovered a paraphyly in the Hemiphractinae and split them into three families (*i.e.*, Amphignathodontidae Boulenger, 1882, Cryptobatrachidae Frost, Grant, Faivovich, Bain, Haas, Haddad, de Sá, Channing, Wilkinson, Donnellan, Raxworthy, Campbell, Blotto, Moler, Drewes, Nussbaum, Lynch, and Green. 2006, and Hemiphractidae Peters), 1862 until Guayasamin *et al.* (2008) found that all the families proposed by Frost et a. (2006) indeed form a monophyletic clade, which they consider as a single family, Hemiphractidae. Pyron and Wiens (2011) confirmed the monophyly of the family Hemiphractidae with their clade being placed between Terrarana and Hylidae in their phylogeny, even though this node is not well-supported.

On the basis of a molecular phylogeny, Grant *et al.* (2006) split the monophyletic poison frogs into two families Aromobatidae Grant, Frost, Caldwell, Gagliardo, Haddad, Kok, Means, Noonan, Schargel, and Wheeler, 2006 and Dentrobatidae Cope, 1865. The latter are further distinguished from Aromobatidae by the ability to excrete alkaloids. Santos *et al.* (2009) rejected this classification as unnecessary, although they recovered the same two major clades in their analysis. These clades were also confirmed by Pyron and Wiens (2011), so I see no reason to reject the classification of Grant *et al.* (2006). The genus *Colostethus* Cope, 1866 as part of the family Dentrobatidae as recognized by Grant *et al.* (2006) has been shown to be paraphyletic (Santos *et al.* 2009; Pyron & Wiens 2011), a problem that is not solved yet. However, any future solution will probably not affect Panamanian *Colostethus*, since the type species, *Colostethus latinasus* (Cope, 1863), is in one monophyletic clade with all other Panamanian *Colostethus*.

The huge family Hylidae Rafinesque, 1815 was revised on the basis of molecular data by Faivovich *et al.* (2005). According to this, two subfamilies occur in Panama Hylinae and Phyllomedusinae Günther, 1858. Previous to the work of Faivovich *et al.* (2005), many Hylinae were combined in the paraphyletic genus *Hyla* that these authors split in several genera. Faivovich *et al.* (2005) resurrected the genus *Dendropsophus* Fitzinger, 1843 from synonymy with *Hyla* for small lowland frog species that are known or suspected to have 30 chromosomes. Likewise, the two members of the *Hyla bogotensis* group of Savage (2002) in Central America were placed into the genus *Hyloscirtus* Peters, 1882. Further, Faivovich *et al.* (2005) placed the Panamanian members of the *Hyla tuberculosa* group of Savage (2002) in the newly created genus *Ecnomiohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler, 2005. The genus *Hypsiboas* Wagler, 1830 was resurrected for the larger Hylid species placed by Savage (2002) into the former *Hyla albomarginata* and *H. boans* species groups. For the members of the *Hyla lancasteri* group, the *Hyla pictipes* group, the *Hyla pseudopuma* group, and the *Hyla zeteki* groups of Savage (2002), Faivovich *et al.* (2005) proposed the new genus *Isthmohyla* Faivovich, Haddad, Garcia, Frost, Campbell, and Wheeler, 2005 containing mostly small stream-breeding frogs, but also pond and bromeliad breeders, that inhabit mid to high elevations. *Isthmohyla* is endemic to Central America with by far the highest species diversity in Lower Central America. Campbell and Smith (1992) placed *Hyla legleri* Taylor, 1958 into the genus *Ptychohyla*, an allocation not followed by Savage (2002), who remained it in *Hyla* without giving any reasons. I here follow the proposal of Campbell and Smith (1992). To date, *Ptychohyla legleri* is the only species of the genus that occurs in Panama. Molecular data for *P. legleri* is lacking, but the genus *Ptychohyl*a is paraphyletic in the tree of Pyron and Wiens (2011) with regard to *Ptychohyla spinipollex* (Schmidt, 1936). Faivovich (2005) found the genera *Phrynohyas* and *Trachycephalus* to be non-monophyletic respectively and placed *Phrynohyas* Fitzinger, 1843 in *Trachycephalus* Tschudi, 1838 to restore monophyly. The only member of this largely South American genus that enters Central America is *Trachycephalus typhonius* (Linnaeus, 1758). The name *T. venulosa* (Laurenti, 1768) that was formerly used for the Central American populations was recently assigned a junior synonym of *T. typhonius* by Lavilla *et al.* (2010). In the subfamily Phyllomedusinae, the work of Faivovich *et al.* (2005) revealed the genetical distinctiveness of *Agalychnis calcarifer* Boulenger, 1902 from other members of the subfamily. Thus, they introduced the new genus *Cruziohyla*, with *Cruziohyla carcarifer* as sole representative in Central America. They further placed the only Central American species of the *Phyllomedusa buckleyi* group, *Phyllomedusa lemur* Boulenger, 1882, in the genus *Hylomantis* Peters, 1873 „1872“. In a later work with a larger taxon sampling, Faivovich *et al.* (2010) found *Hylomantis* to be paraphyletic. Since it still appeared as sister clades to *Agalychnis* Cope, 1864 and *Pachymedusa* Duellman, 1968, they placed all members of this clade into the genus *Agalychnis* as the oldest available name.

The family Leptodactylidae has been a repository for many genera of unclear taxonomy. Frost *et al.* (2006) split the former Leptodactylidae in several families. Nascimento *et al.* (2005) removed the genus *Engystomops* Jiménez de la Espada, 1872 from the synonymy with *Physalaemus* Fitzinger, 1826, what affected only the túngara frog, *Engystomops pustulosus* (Cope, 1864), in Panama. Grant *et al.* (2006) recognized the genera *Engystomops* and

Pleurodema Tschudi, 1838, that occur in Panama among others, as own family, Leiuperidae Bonaparte, 1850. Pyron and Wiens (2011) revealed that Leptodactylidae is paraphyletic with respect to Leiuperidae, a problem that they solved by expanding Leptodactylidae, that now includes Leiuperinae as a subfamily.

The latest changes in the family Microhylidae Günther, 1858 regarding Panamanian taxa have been the result of de Sá *et al.* (2012) on the basis of molecular data, who put the controversial genus *Relictivomer* Carvalho, 1954 into synonymy with *Elachistocleis* Parker, 1927 and transferred *Chiasmocleis panamaensis* Dunn, Trapido, and Evans, 1948 from *Chiasmocleis* Méhely, 1904 to *Elachistocleis*. Additionally, de Sá *et al.* (2012) placed the genus *Nelsonophryne* Frost, 1987 in the synonymy of *Ctenophryne* Mocquard, 1904.

Regarding the Ranidae not many changes have been done, except that Frost *et al.* (2006) resurrected the genus *Lithobates* Fitzinger, 1843 for New World ranids.

Caudata Fischer von Waldheim, 1813: The taxonomy of the only two Panamanian salamander genera, *Bolitoglossa* Duméril, Bibron, and Duméril, 1854 and *Oedipina* Keferstein, 1868, which both belong to the family Plethodontidae Gray, 1850 has been relatively stable since Wake (1966) and Wake and Elias (1983). However, the family is very species rich, containing currently 441 species (AmphibiaWeb 2014), so there have always been attempts to subdivide it into tribes. First molecular studies (Chippindale *et al.* 2004; Mueller *et al.* 2004) found some conflicts with the earlier taxonomy on a suprageneric level and elevated some of Wakes (1966) tribes to subfamilies. Vieites *et al.* (2007) and Pyron and Wiens (2011) recovered only two mayor clades in the Plethodontidae that were then recognized as two subfamilies (Hemidactyliinae Hallowell, 1856 and Plethodontinae) with both Panamanian genera in the subfamily Hemidactyliinae (Wake 2012). All tropical plethodontids, and thus all that occur in Panama, are further placed in the tribe Bolitoglossini Vieites, Román, Wake, and Wake, 2011 (Wake 2012). A molecular diversification of the genus *Bolitoglossa* has been made by Parra-Olea *et al.* (2004), who defined seven subgenera. Most *Bolitoglossa* species in Panama belong to the subgenus *Eladinea* with only one species in the subgenus *Bolitoglossa*. For the genus *Oedipina*, Brame (1968) defined two species groups that were elevated to subgenera (*Oedopinola* and *Oedipina*) by Garcia-Paris and Wake (2000). Members of both subgenera are present in Panama. Later, McCranie *et al.* (2008) added the third recognized subgenus *Oeditriton*, which to date includes only Honduranian species.

Gymnophiona Müller, 1832: There is relatively little controversy in the higher taxonomy of Panamanian caecilians. Formerly, all Panamian genera were included in the heterogenous family Caeciliidae Rafinesque, 1814 that had been used for all caecilian genera that could not assigned to other families by morphology alone (Taylor 1968). Nussbaum and Wilkinson (1989) removed the aquatic and morphologically distinct genus *Typhlonectes* Peters, 1880 from the family Caeciliidae and resurrected the family Thyphlonectidae Taylor, 1968. Subsequent molecular phylogenies repeatedly revealed the paraphyly of the family Caeciliidae with respect to Typhlonectidae, as *Typhlonectes* turned out to be closely related to the genera *Caecilia* Linnaeus, 1758 and *Oscaecilia* Taylor, 1968 (Frost *et al.* 2006; Zhang & Wake 2009; Pyron & Wiens 2011). Wilkinson *et al.* (2011) proposed a nine family classification for Gymnophiona in order to solve this problem, with Caeciliidae containing only *Caecilia* and *Oscaecilia*. As a consequence the other two Panamanian genera *Gymnopis* and *Dermophis* went into the family Dermophiidae. This view was recently confirmed by San Mauro *et al.* (2014). There is still uncertainty about the monophyly of the respective genera *Caecilia* and *Oscaecilia*, because the genus *Oscaecilia* has only been represented in previous phylogenies by a single species.

4.2.3. CONSERVATION STATUS OF PANAMA´S AMPHIBIANS

The following introduction to the conservation status of Panamas amphibians has been modified and updated after Hertz *et al.* (2012c):

Amphibian populations are declining or disappearing around the globe at an alarming rate, and are now recognized as the most threatened vertebrate class on earth with more than 40% of the known species threatened with extinction (Daszak *et al.* 1999; Stuart *et al.* 2004; Gascon *et al.* 2007; Hoffmann *et al.* 2010; Hof *et al.* 2011). The causes for amphibian declines are complex, but the most obvious threats to tropical amphibians come from anthropogenic activities such as deforestation, habitat modification, and contamination, as well as indirectly from global warming through greenhouse gas emissions (Kiesecker *et al.* 2001; Young *et al.* 2004; Pounds *et al.* 2006b). In addition, from the late 1980s on, even amphibian populations in well-protected, almost undisturbed habitats have begun to decline enigmatically. These enigmatic declines have been especially severe at upland sites of Australia (Campbell 1999) and the Neotropics (Young *et al.* 2001, 2004; Lips *et al.* 2006). In particular, Lower Central America has suffered from multiple amphibian population declines that were well documented (*e.g.*, Crump *et al.* 1992; Young *et al.* 2001; Lips 1999; Lips *et al.* 2006; Ryan *et al.* 2008).

After several experts have conducted research regarding the causes, these enigmatic amphibian die-offs and population declines could be clearly associated with an Emerging

Infectious Disease (EID) (Daszak *et al.* 2000), caused by the non-hyphal zoosporic fungus *Batrachochytrium dendrobatidis* (*Bd*) Longcore, Pessier, D.K. Nichols, 1999 that became soon known as chytridiomycosis (Berger *et al.* 1998; Daszak *et al.* 1999; Ryan *et al.* 2008, Lötters *et al.* 2010). The mortality rate among infected individuals can be as high as 100% in some populations (Lips *et al.* 2003b), but not all species have been affected equally. It has been porstulated that the ecology of affected species plays a greater role in decline and possible die-out events than its taxonomic affiliation (Lips *et al.* 2003b). Due to the physiology of *Bd*, which grows best at temperatures between 17–28 °C (Piotrowski *et al.* 2004), populations in tropical mountainous habitats are more likely to be affected than those in tropical lowlands (Longcore *et al.* 1999; Andre *et al.* 2008). Montane riparian or lentic amphibian species are at higher risk to *Bd* infection and population decline than species with an exclusively terrestrial mode of life, since they come more frequently in contact with the aquatic zoospores (Lips *et al.* 2003b; Kriger & Hero 2007). Furthermore, degree of disease susceptibility of species depends on their particular immunologic defense in terms of quantity and quality of antimicrobial skin peptide mixtures (Woodhams *et al.* 2006), a research field that has not received much attention so far.

Chytridiomycosis first appeared in western Panama between 1993 and 1994 and spread in a wave-like manner south-eastward, crossing the Panama Canal in 2007 (Lips *et al.* 2008; Woodhams *et al.* 2008) and has been only recently detected in the Darién of eastern Panama (Rebollar *et al.* 2014). The disease has caused population declines and loss of amphibian diversity in most habitats where it has been detected (Crawford *et al.* 2010). Interestingly, dry lowland sites obviously serve as a refuge from *Bd* caused die-outs (Puschendorf *et al.* 2005; Zumbado-Ulate 2011; Köhler *et al.* 2012), while susceptible species in moist lowland situations may suffer from population declines (*e.g.*, *Craugastor taurus*, Solís *et al.* 2008a). Since *Bd*'s arrival, many formerly abundant species have disappeared or become rare. As an emergency measure, *ex-situ* breeding programs were established for many Panamanian species (Mendelson *et al.* 2006). However, originally meant as an emergency response (Gascon *et al.* 2007), *ex-situ* breeding programs have been criticized as too costly and with an uncertain future for the populations bred (Pounds *et al.* 2006a). Nevertheless, it is a fact that some species like the Panamanian Golden Frog (*Atelopus zeteki*) are bred with success, while they are probably extinct in the wild by now (Gratwicke *et al.* 2012).

Only recently, reports on rediscoveries or newly discovered populations of amphibian species, which were thought to be lost, have been reported from different sites of the world

(Puschendorf *et al.* 2005; Lotzkat *et al.* 2007; Rodríguez-Contreras *et al.* 2008; Kolby & McCranie 2009; Abarca *et al.* 2010; McCranie *et al.* 2010; Hertz *et al.* 2012c; Gonzales-Maya *et al.* 2013). In recent years, García-Rodríguez *et al.* (2012) try to find surviving populations of *Bd* susceptible species in predicted climatic niches in Costa Rica using species distribution models. However, there is almost nothing known about post-decline populations and how they are developing, but there is raising hope that some populations may recover after a period of decline (Newell *et al.* 2013; Scheele *et al.* 2014). Jaramillo *et al.* (2010) published the last complete evaluation of the conservation status of the amphibians of Panama. This study listed 199 species of amphibians to occur in Panama (160 Anura, 10 Gymnophyiona, and 29 Urodela) of which the IUCN Red List of Threatened Species listed 20 as Critically Endangered (CR), 25 as Endangered (EN), 13 as Vulnerable (VU), 7 as Near Threatened (NT), 105 as Least Concern, and 26 as Data Deficient (DD). Additionally, Jaramillo *et al.* (2010) calculated individual Environmental Vulnerability Scores (EVS) (Wilson & McCranie 2004) for the amphibian and reptile species of Panama and identified 60 with low (3–8), 67 with medium (9–11), and 66 with high (12–17) EVS values.

4.3. GENERAL INFORMATION ON PANAMA

Panama has the form of a sigmoid arch with an east-west axis that builds the southernmost part of Central America, as well as the narrowest part of the Mesoamerican landbridge. It is situated roughly between 7° and 10° north and 76° and 83° west. Panama borders Costa Rica to the west and Colombia to the east. Further it is bordered by the Caribbean Sea as part of the Atlantic Ocean to the north and by the Pacific Ocean to the south. At the narrowest point, at which the famous Panama Canal is located, the Isthmus of Panama measures less than 60 km. In total, Panama national territory covers about 75,500 km^2. The Republic of Panama (Spanish: República de Panamá) is a presidential representative democratic republic. The president of Panama is both head of the government and head of the state. The state of Panama is organized in ten provinces and three indigenous autonomous regions (Spanish: comarcas indígenas) (Fig. 2).

4.4. THE RISE OF THE ISTHMUS OF PANAMA AND ITS COLONIZATION BY AMPHIBIANS

The formation of Lower Central America and the closure of the Panama Portal is one of the most important geological events in Earth´s recent history. The geological history of Central America from a herpetological perspective was described in detail by Savage (1966; 1982;

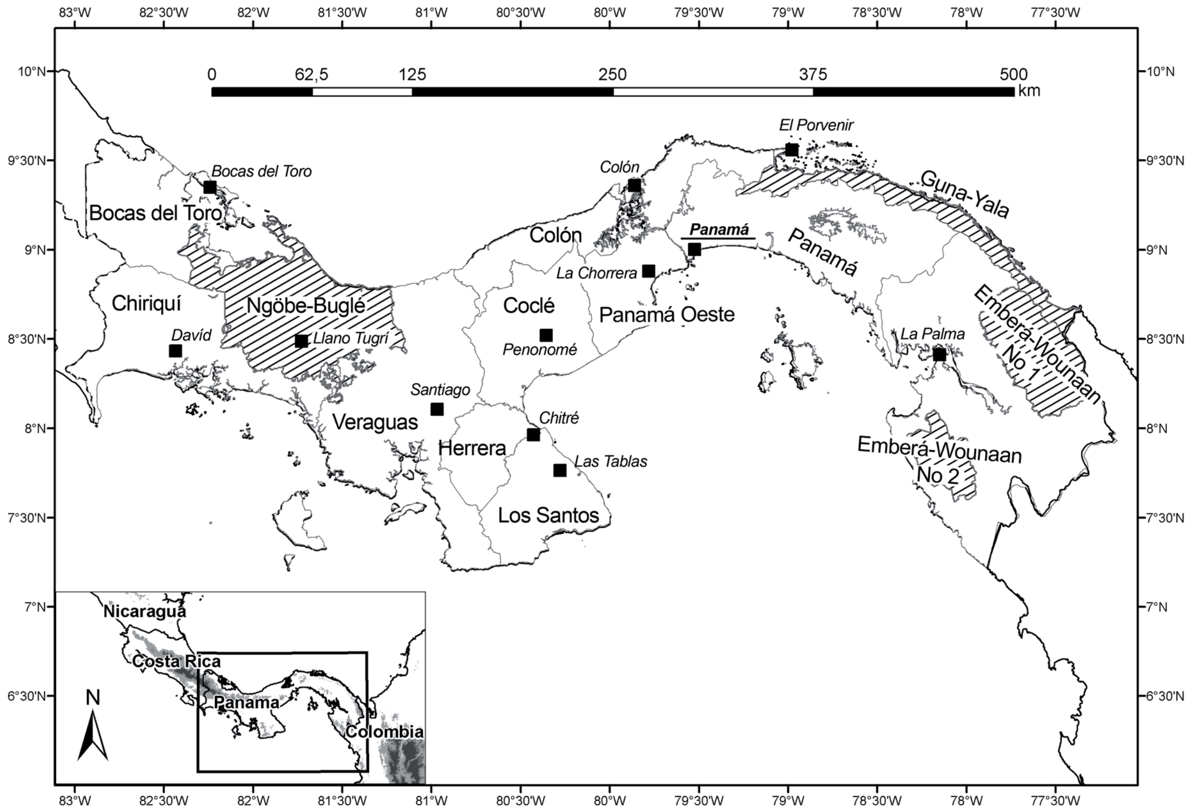

Figure 2: Political Map of the Republic of Panama. Shown are the ten provinces and the three indigenous autonomous regions (Comarcas), which are indicated by oblique parallel lines. Squares represent provincial capitals. Panama City, the capital of the Republic of Panama, is underlined. The inlet in the lower left corner shows Panama and its position in Lower Central America (Layer Source: STRI Mapserver http://mapserver.stri.si.edu/)

2002). According to that, a continuous, plane land bridge connected North and South America during the Paleocene, inhabited by a generalized amphibian fauna. It is assumed that at this time all amphibian families that are present in Panama today had already evolved. In the Eocene, flooding of the southern part of the land bridge separated the Americas again. Until the Middle Miocene, the northern part of the land bridge was a peninsula of North America followed by a series of volcanic islands (Panama Arc) with intermediate sedimentary basins and an open ocean of abyssal depth (Atrato Seaway: >2000m deep) between the Panama Canal Basin and South America (Duque-Caro 1990; Coates & Obando 1996; Kirby *et al.* 2008) (Fig. 3). From the Middle Miocene on, the South American plate compressed the Panama Arc and formed the Choco Block. As a result, the water depth in the Atrato Seaway shallowed continuously, new islands east of the Panama Canal Basin were rising, and the marine connections between these islands filled up with sediments (Fig. 4A). By the Late Miocene an archipelago surrounded by shallow waters, extended from the Chortis Block to

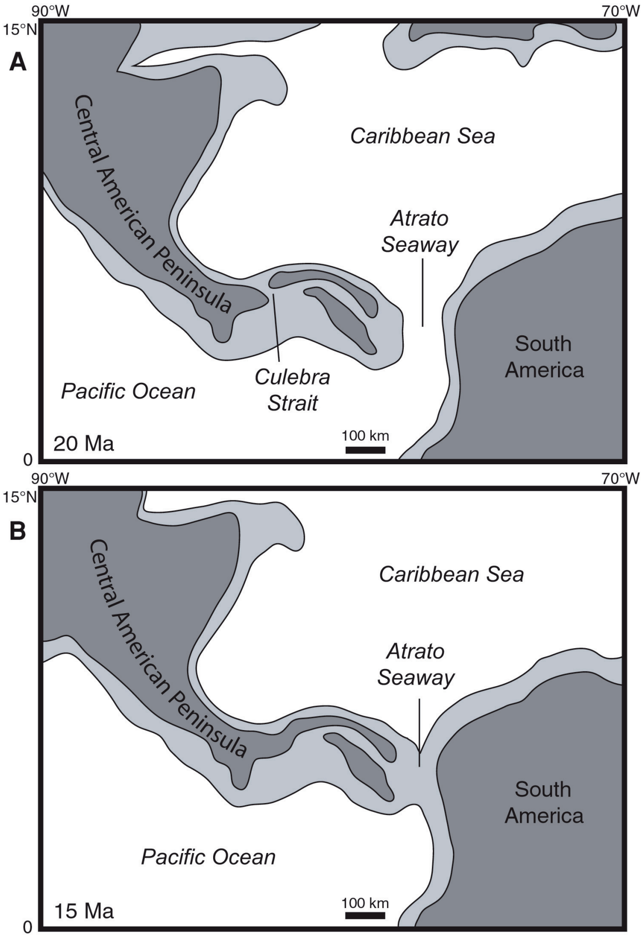

Figure 3: Position and evolution of the Central American peninsula during the Miocene (A 20 Ma; B 15 Ma). Light gray areas represent the submarine outlines of tectonic plates. Dark gray areas represent subaerial land. White areas represent deep abyssal sea. From Kirby *et al.* (2008): figure 11, page 12.

the Atrato Basin (Fig. 4B). At the beginning of the Pliocene, subduction of the Cocos Ridge eliminated all marine corridors in the portion of the Choroteca Block and uplifted the Central Cordilleras of Costa Rica and western Panama. However, the two continents remained separated by a system of shallow marine corridors east of the Choroteca Block until the complete closure of the Panama Portal due to ongoing tectonic events in the Late Pliocene (Fig. 4C). According to most recent geological studies, the complete closure of the Isthmus of Panama occurred in the Middle Pliocene about 3.5–3.1 Ma (Coates & Stallard 2013; Leigh *et al.* 2013). The zoogeographic consequences of the Central American geological evolution, known as the Great American Biotic Interchange (GABI), is certainly one of the most important examples for the clash of two faunal assemblages. The GABI model was primarily established for the faunal exchange of mammal taxa based on fossil records (*e.g.*, Simpson 1980; Marshall *et al.* 1982; Marshall 1988). However, larger mammals are usually better represented in fossil records and are believed to be better over-sea dispersers than amphibians,

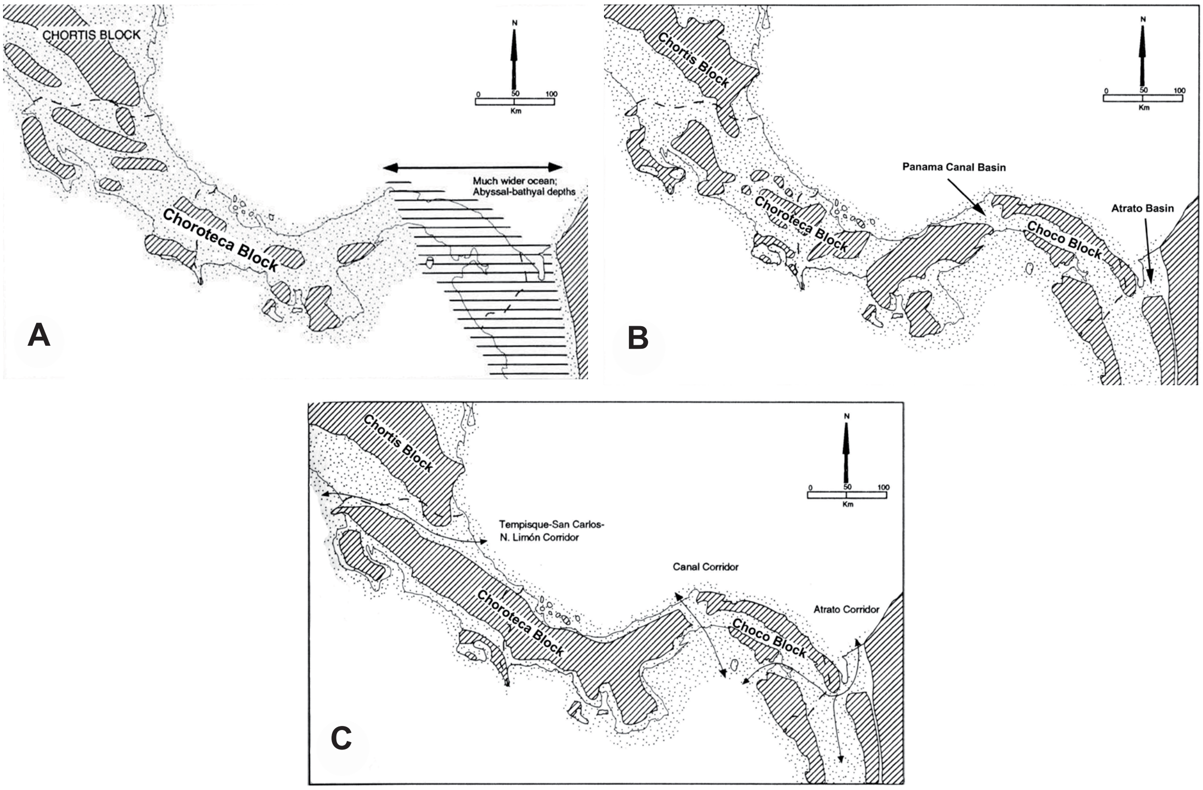

Figure 4: The geological evolution of the Central America Isthmus. **A** during the Middle Miocene (16–15 Ma), **B** during the Late Miocene (7–8 Ma), **C** during the Middle Pliocene (about 4 Ma). Subaerial land is represented by oblique parallel lines, shelf sediments by dots, and abyssal ocean by horizontal parallel lines. Modified after Coates and Obando (1996).

so that the GABI model for mammals is not necessarily the same for amphibians. On the other hand, recent molecular phylogenetic analyses suggest that anuran interchange between the Americas occurred well-before the complete closure of the Isthmus of Panama (Weigt *et al.* 2005; Pinto-Sánchez *et al.* 2011). However, the zoogeographic history of amphibians in Panama has to be considered separately by orders, families, and even genera. For example, the ancestors of the two Central American genera of the order Gymnophiona, *Gymnopis* and *Dermophis* have supposably already entered Central America when the first land connection consisted during the Paleocene, whereas the presence of the genera *Caecilia* and *Oscaecilia* in Lower Central America is likely the result of another invasion event after the closure of the Panama Portal in the Pliocene (Savage & Wake 2001). There is no doubt that plethodontid salamanders invaded South America from the north, but molecular data predate this event well-before the closure of the Panama Portal (Hanken & Wake 1982; Parra-Olea *et al.* 2004).

4.5. STUDY AREA

4.5.1. TOPOGRAPHY OF WESTERN PANAMA

Western Panama is divided into a Caribbean side to the north and a Pacific side to the south by a mountain chain that runs in east-west extension. This mountain chain, often referred to as the Cordillera Central, is the predominant element of western Panama´s topography. The Cordillera has been described in detail by Myers (1969) and Myers and Duellman (1982), although some of their altitudes are not always in accordance with more recent elevational data including my own. The Cordillera Central is frequently separated into a western part, called the Serranía (or Cordillera) de Talamanca that continues into Costa Rica and an eastern part, called the Serranía de Tabasará. After the definition of Myers and Duellman (1982), the demarcation between both Serranías is the high valley of the Río Chiriquí, often referred to as the Fortuna Depression. The Fortuna Depression, situated at an altitude of roughly 1000 m, is a break in the main crests of the Cordillera Central, which otherwise continuously lies

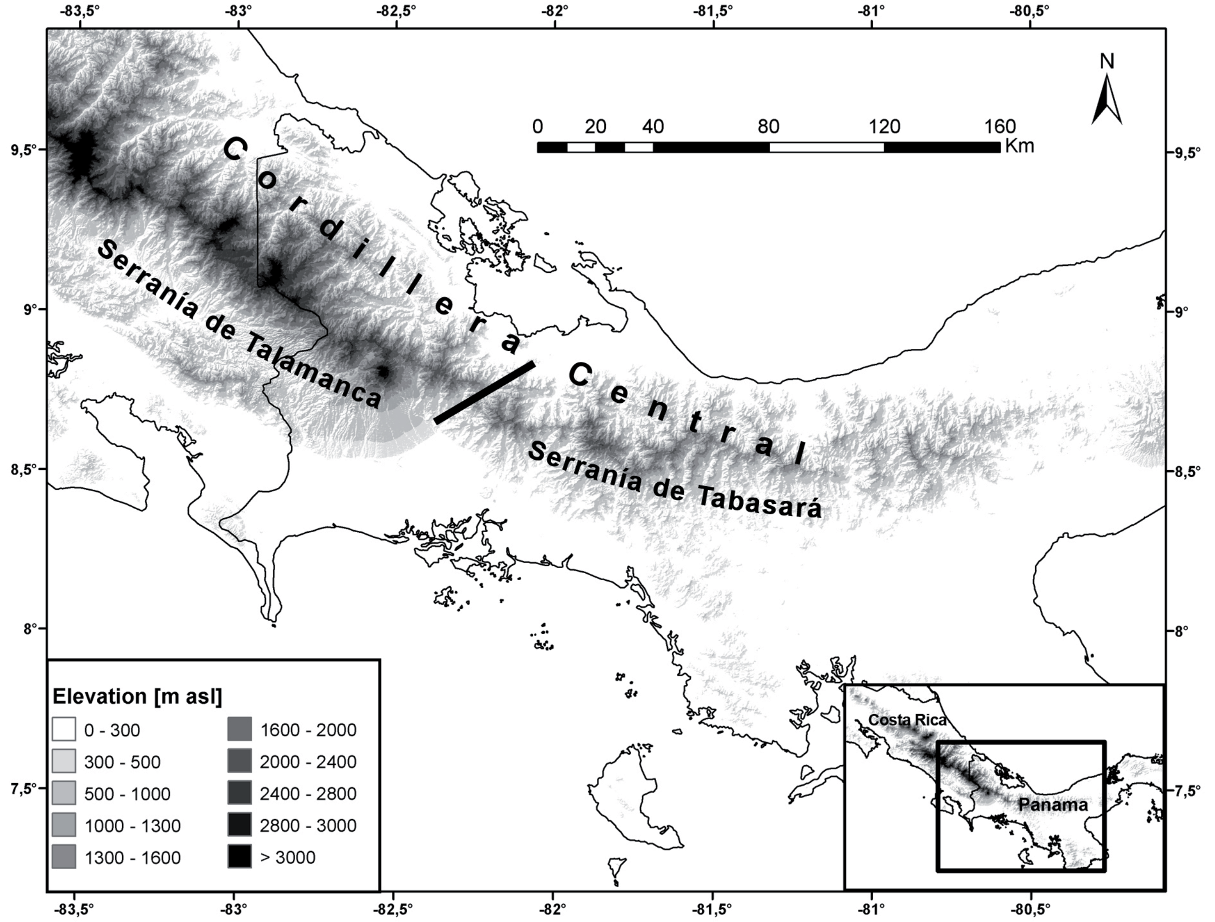

Figure 5: Position of the Cordillera Central in western Panama. The black bar indicates the position of the Fortuna Depression that separates the Serranía de Talamanca and the Serranía de Tabasará after the definition of Myers and Duellman (1982).

at 1800 m and above (Fig. 5). An overview of the prominent peaks in western Panama that are mentioned in the following text is given in Figure 6. Panama's highest peaks with 3000 m asl and more are all located in the Serranía de Talamanca. The one with the most expanded summit is the very remote Cerro Fábrega-Itamut mountain massif near the Costa Rican border. The summit region of this massif lies constantly above 3000 m and extends over approximately 10 km from north to south and 3 km from west to east. It has two prominent peaks, Cerro Fábrega (3336 m) and Cerro Itamut (3279 m) that lie close together. Only about 10 km to the south-east lies another 3000 m peak, Cerro Echandi (3162 m), situated on the Costa Rica-Panama border. From here on, the mountain crest continues in south-western direction reaching elevations between 2200 and 2800 m. Then Cerro Pando (2450 m) is located at the cross-border triangle of Costa Rica and Panama and the Panamanian provinces Chiriquí and Bocas del Toro. From here the provincial border of Chiriquí and Bocas del Toro follows the continental divide. Slightly south of the continental divide, separated by a high valley, towers Volcán Barú (3475 m) the highest mountain in the republic. While other

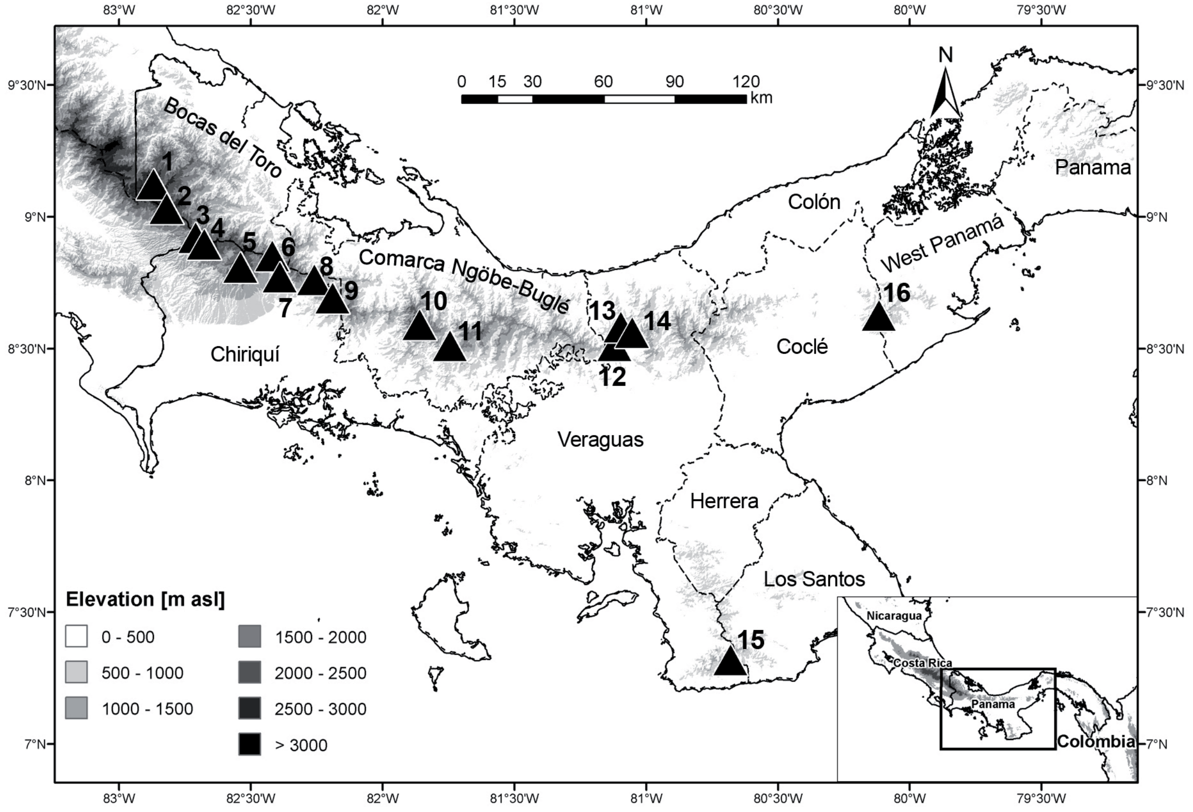

Figure 6: Position of prominent peaks mentioned in the text. From west to east: 1 Cerro Fábrega-Itamut massif; 2 Cerro Echandi; 3 Cerro Pando; 4 Cerro Totuma; 5 Volcán Barú; 6 Cerro Horqueta; 7 Cerro La Estrella; 8 Cerro Guayaba; 9 Cerro Pata de Macho; 10 Cerro Saguí; 11 Cerro Santiago; 12 Cerro Mariposa; 13 Cerro Negro; 14 Cerro Narices; 15 Cerro Hoya; 16 Cerro Gaital.

volcanoes in Panama are extinct, Volcán Barú is potentially active with its last eruption having occurred 400–500 years ago (Sherrod *et al.* 2008). The main mountain chain continues at elevations of 1800–2300 m, with Cerro Guayabo (approx 2000 m) as the last prominent peak, before it drops to 1000 m and slightly below in the Fortuna Depression. The eponymous Fortuna Lake is placed in the high valley of Río Chiriquí that is at the same time its main feeder stream, but besides it is fed by several smaller streams and creeks. Although often wrongly understood, the Fortuna Lake including all its feeder streams is part of the Pacific drainage, the continental divide lies north of the lake.

At the eastern end of the Fortuna Depression rises Cerro Pata de Macho (approx. 2100 m), the westernmost mountain of the Serranía de Tabasará. On its southern slope arises Río Hornito and on its northern slope Río Chiriquí, which both flow through the Fortuna Lake before they head towards the Pacific Ocean. The eastern slopes of Cerro Pata de Macho are already within the demarcation of the autonomous Comarca Ngöbe-Buglé. From here the watershed descends to about 1300 m before it rises again towards Cerro Saguí (approx. 2500 m), the highest and biggest mountain massif in the Serranía de Tabasará. The narrow drainage divide, formed by the southwestern extensions of Cerro Saguí, stretches towards Cerro Santiago (approx. 2100 m). The region, that includes both mountains and the land between has been frequently called the Cerro Colorado area (Myers & Duellman 1982). In its further easterly course the mountain crest stays at elevations of 1500–2000 m before it drops well below 1000 m near the town of Santa Fe at the eastern end of the Comarca Ngöbe-Buglé. The foothills of the Tabasará range east of the town of Santa Fe are not a closed barrier between Caribbean and Pacific slopes anymore. Albeit single peaks reach 1500 m and more, there are deep valleys in north-south direction that drop well below 700 m. Further east, in the province of Coclé, the Serranía de Tabasará turns into a loose chain of hills before it flattens more and more to approximately 300 m and below. After about 30 km of plain rises a relatively low volcanic elevation with Cerro Gaital (1185 m) as its highest point. This offset mountain includes the localities El Valle de Anton and Altos de Campana that have long been in the focus of herpetological collecting (Dunn 1933; Ibáñez *et al.* 1996). The Cordillera Central is certainly the "backbone" of the physiography of western Panama (Myers 1969), but there is a second, considerably smaller mountain range on the Azuero Peninsula. The Azuero highlands are isolated from the Cordillera Central by *Curatella* savannah (Myers 1969). Its highest peak is Cerro Hoya (1559 m).

4.5.2. CLIMATE AND LIFE ZONES

Panama is entirely located in the tropical latitudinal region. A day is year around twelve hours long with only little variation and solar radiation is high throughout the year, so there is not much seasonal temperature variation. However, especially western Panama has a variety of different life zones after the system of Holdridge (1967). The astonishing diversity of life zones on a relatively small area is the result of a combination of a narrow landmass surrounded by warm oceans with a high mountain range along its longitudinal axis that separates western Panama roughly into Atlantic (Caribbean) side, Pacific side, and highland life zones (Fig. 7). Examples of different habitats from various life zones are given in Figures 8 and 9. The climate of Costa Rica has been described in detail by Savage (2002), whose reasoning is here transferred to western Panama. A direct comparison of the climate of two major cities in western Panama, one on the Pacific, one on the Atlantic side is given in Figure 8. Like in other tropical countries, Panama´s climate variation over the year is rather influenced by the amount of rainfall during the different seasons, than by temperature. This occurs under the influence of the Inner Tropical Convergence Zone (ITCZ). Although the ITCZ circles the globe more than less above the geographic equator as a band

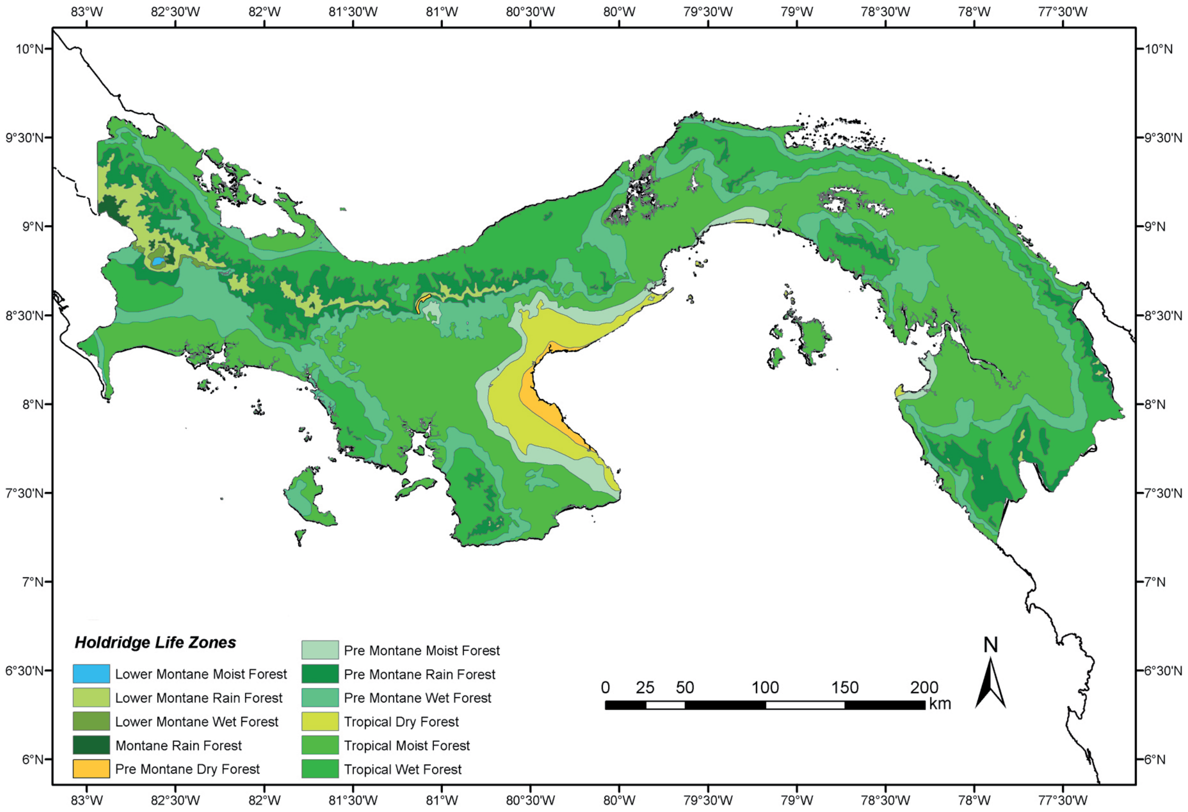

Figure 7: Position of Life Zones after Holdridge (1967) in Panama. Layer source: GIS Laboratory. Smithsonian Tropical Research Institute, Panama, 2012

of thunderstorms, it has not a steady position, but oscillates between north and south of the equator in the course of the year. This is because the ITCZ follows the thermal equator, thus the course of the sun. In the ITCZ the heated air raises what results in a few kilometers wide trough. The low pressure draws air from north and south, what generates the northeastern and

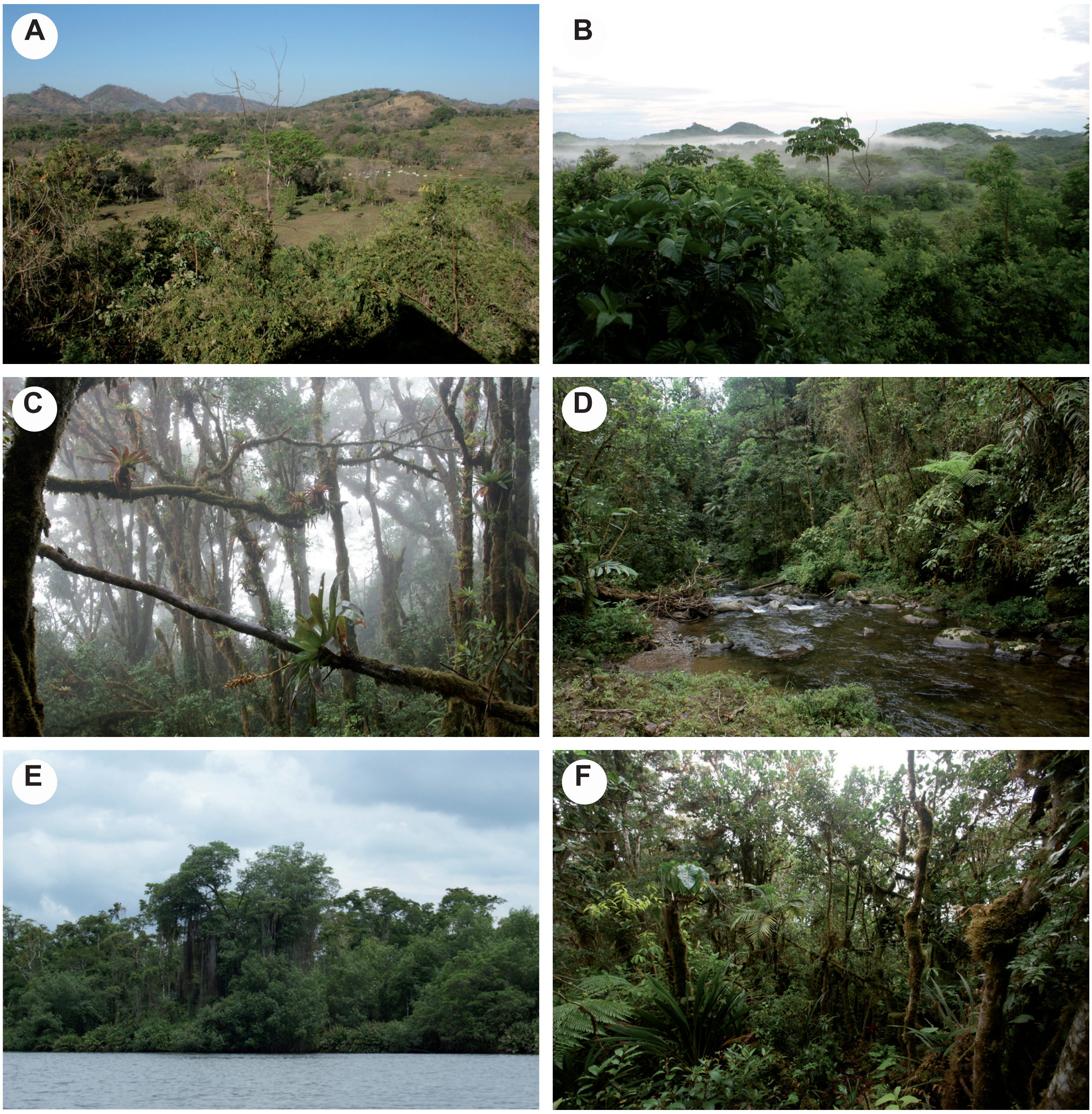

Figure 8: Examples of different habitats in western Panama. **A** Los Algarrobos, Pacific Tropical Moist Forest domain, 124 m asl, during the dry season in February. **B** The same view as in A during the rainy season in June. **C** Elfin Forest on the Continental Divide in the Costa Rica-Panama border region, Lower Montane Wet Forest domain, Cerro Pando, 2460 m asl. **D** Río Changena, Atlantic Lower Montane Rain Forest domain, 1640 m asl. **E** North bank of Río San San, river swamp forest, Atlantic Tropical Moist Forest domain, sea level. **F** Elfin Forest on a ridge on the northern slope of Cerro Santiago, La Fortuna, Lower Montane Rain Forest domain, 1740 m asl.

southeastern trade winds. The relative position of the ITCZ brings Panama a rainy season from usually May to November and a dry season from December to April. In the dry season, when the ITCZ is south of the geographic equator, rainfall patterns are dependent on Atlantic air masses. The strong northeast trade winds bring moist air masses from the Caribbean Sea, which are uprising on the steep slopes of the Atlantic versant of the Cordillera Central. The

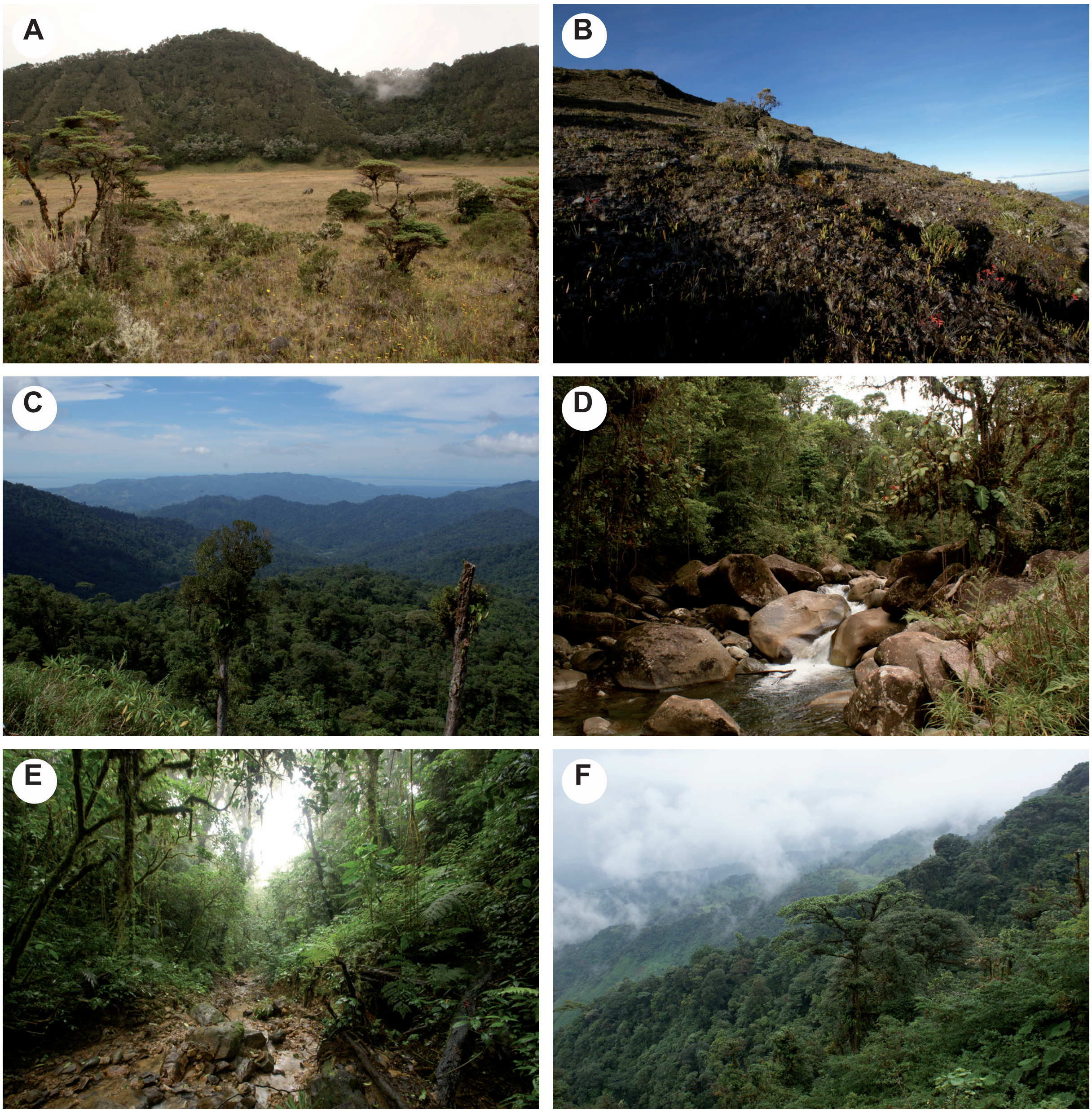

Figure 9: Examples of different habitats in western Panama. **A** Savannah in the crater of Volcán Barú, Montane Rain Forest domain, 2970 m asl. **B** Subalpine Pluvial Paramo near the summit of Volcán Barú, 3400 m asl. **C** View into the Willi Mazú valley, Atlantic Pre Montane Rain Forest domain, 700 m asl. **D** Río Bermejito, Cerro Negro, Atlantic Pre Montane Wet Forest domaine, 730 m asl. **E** Feeder stream of Río Moi, La Nevera, Atlantik Lower Montane Rain Forest domain, 1600 m asl. **F** Southern slope of Cerro Saguí, Pacific Lower Montane Rain Forest domaine, 1600 m asl.

associated adiabatic cooling of the air masses below the dew point brings abundant rainfall to the Atlantic slopes. This effect can even be increased when polar cold fronts are coming far south during the North American winter. When the air masses reach the Pacific lee side of the Cordillera Central their humidity is low and they create an inversion layer over the Pacific slopes resulting in a cloudless sky. In places where the main ridge of the Cordillera is depressed to 1000 m and below, like in the Fortuna Depression and the valleys of the eastern Serranía de Tabasará, moist Atlantic air masses reach over on the Pacific slopes bringing increased rain on the otherwise dry Pacific slopes. During the rest of the year, Panama is under the direct influence of the ITCZ. As the sun zenith shifts north, the ITCZ moves north too and reduces the influence of the northeastern trade winds. At the end of the dry season, when there are still hardly clouds on the Pacific side and the sun stands high, the Pacific side gets very much heated (Fig. 10A). Moreover, due to the east-west orientation of the Cordillera Central, the Pacific slopes are facing south and receive some more solar radiation than the north-facing Atlantic slopes. At this time of the year, in March and April, evaporation is high, the heated air rises and the water it carries condenses what brings the first heavy rains to the Pacific side in May (Fig. 10A). As the ITCZ moves north the Pacific slopes receive moist air masses from the Pacific through the southeast trade winds and bring the rainy season to the Pacific slopes. Due to the Coriolis Effect the southeast trade winds change to a southwest wind once they crossed the equator. However, at this time there is still no dry season on the Atlantic slopes, because tropical easterly waves bring thunderstorms and rainfall to the Caribbean every three to five days mainly in July and August (Fig. 10B). In addition, although Panama lies outside of the hurricane belt, offshoots of powerful hurricanes frequently bring additional heavy rains to the Caribbean coast. When the ITCZ has reached its northernmost position, in late July to

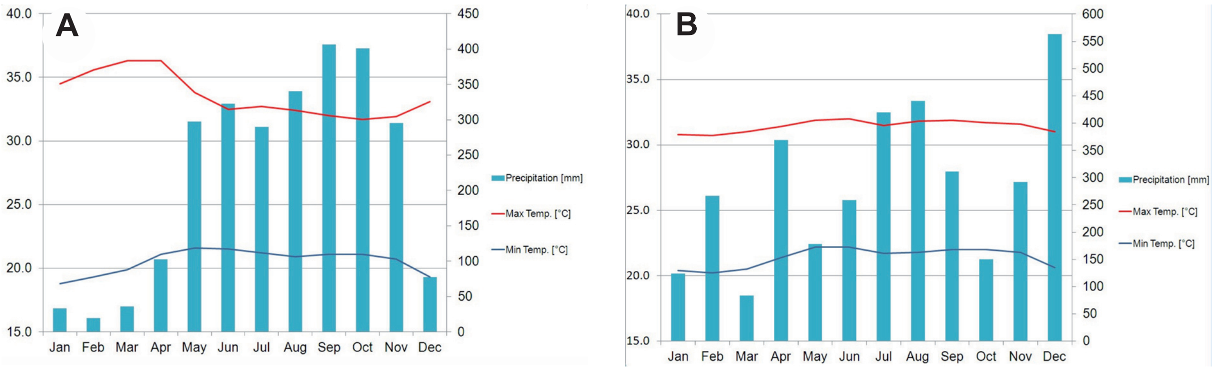

Figure 10: Climate of **A** Davíd, province of Chiriquí on the Pacific side and **B** Bocas del Toro, province of Bocas del Toro on the Caribbean side, showing differences in temperature and precipitation profiles. Data taken from WMO based on monthly averages for the 30-year period 1971-2000.

early August, there is a second short dry season on the Pacific side (Fig. 10A). This occurs for one or two weeks only and ends once the southwards returning ITCS brings back the rain. In Panama this little dry season is called in Spanish *Veranillo de San Juan*.

There are exceptions from this general climatic pattern (Figs. 11, 12). The western slopes of the Azuero highlands receive a higher amount of precipitation in the rainy season, because they lie close to the coast and are directed windward when the moist southwest winds blow. Conversely, the east coast of the Azuero Peninsula is the driest region in Panama as it lies in the rain shadow of the Azuero highlands. Another exception is the Pacific Golfo Dulce region, named after the Golfo Dulce a Pacific inlet. The Golfo Dulce region receives mean annual precipitations between 4000 and 8000 mm particularly on the Osa Peninsula (McDiarmid & Savage 2005). The dry season is very short (January through March) and the rainy season prolonged with heavy rains in October and November (Savage 2002). The Golfo Dulce rainfall regimen (Savage 2002) is mainly located in Costa Rica, but reaches the Chorogo region and the west coast of the Buríca Peninsula, albeit in weakened form. The Pacific lowlands in the surrounding of David lie in the rain shadow of the Golfo Dulce regimen, so there is slightly less rain than farther east. Despite of Panamas position on the northern hemisphere, the rainy season is called winter (span. *invierno*), the dry season summer (span.

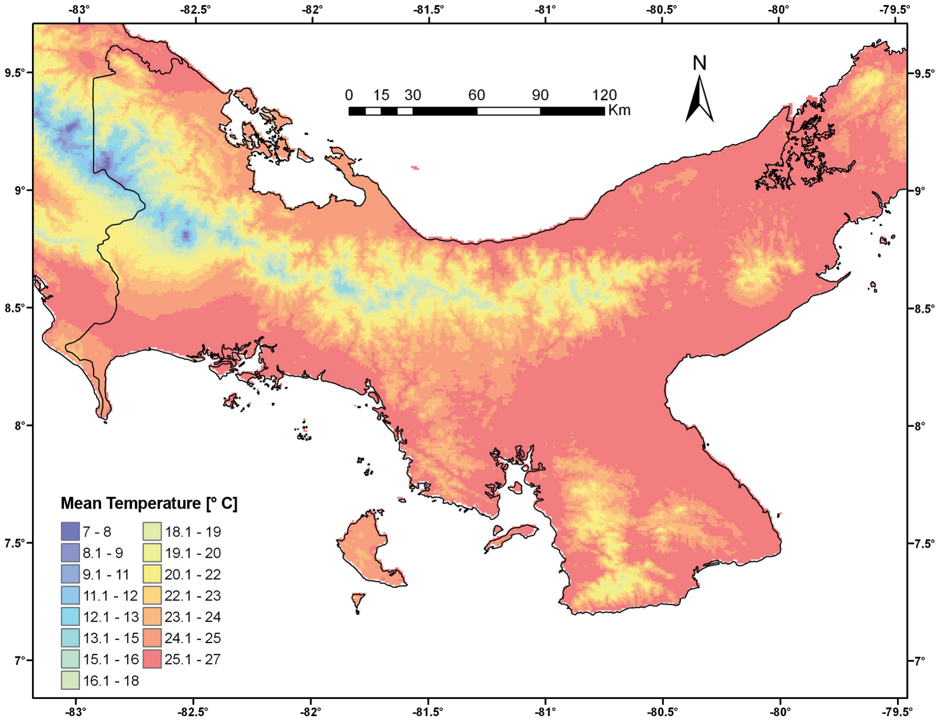

Figure 11: Mean annual temperature in western Panama. Note higher mean temperatures in the Pacific lowlands. Layer Source: WorldClim database Hijmans et al. (2005).

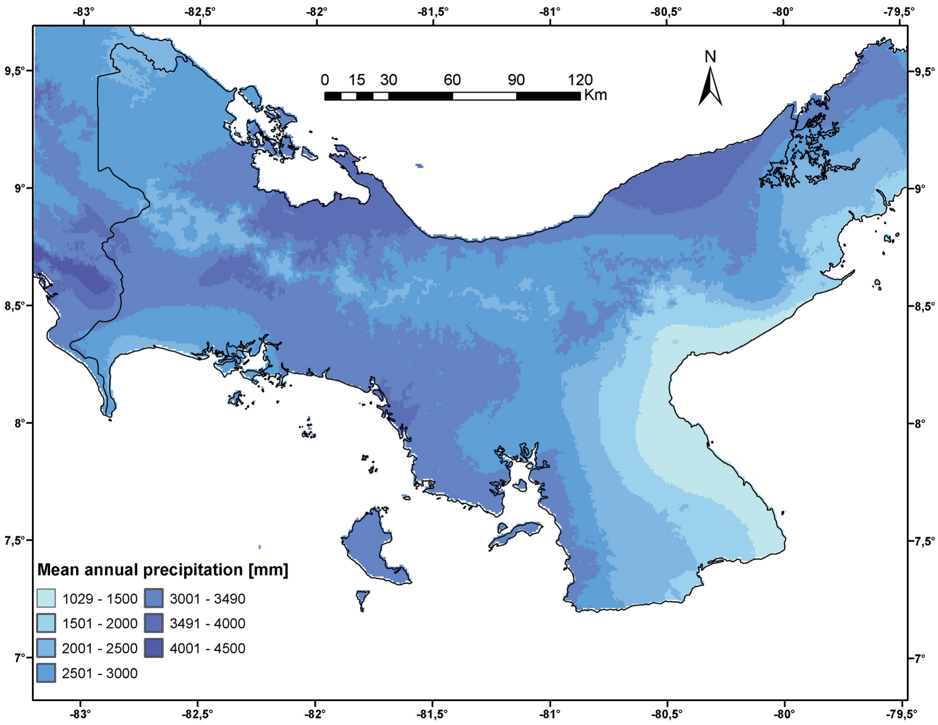

Figure 12: Mean annual precipitation in western Panama. Note increased precipitation on the Pacific versant at the Fortuna depression and at coasts with western orientation. Layer Source: WorldClim database Hijmans *et al.* (2005).

verano), and the short dry season little summer (span. *veranillo*). This is inherited from the Spanish colonial times, because in Spain and the Mediterranean region summers are hot and dry, while long intervals of rain occur in the winter.

4.6. AIMS OF THE STUDY

The objectives of this study is to assess the composition of amphibian assemblages and the conservational status of amphibian species. Some species are known to have declined due to the amphibian disease chytridiomycosis in western Panama, particularly in the Cordillera Central, in others the population status is unknown. Many mountainous amphibian species are not intensively investigated regarding cryptic diversity and geographical distribution and therefore a comprehensive study would provide a basis for a better management of species and their habitat. The aim of this study is to use integrative taxonomy to test diversity in selected Panamanian amphibian genera. Prior studies have used primarily morphological traits that could not reveal cryptic diversity and in some cases resulted in unjustified species splitting. The following hypotheses will be addressed:

1. Integrative taxonomic approaches of amphibian diversity in other tropical parts of the

world have revealed cryptic diversity, *e.g.* in Madagascar (Vieites *et al.* 2009) and Bolivia (Jansen *et al.* 2011). I assume that diversity among Panamanian amphibians is largely underestimated. By synthesizing different taxonomic approaches, I decipher diversity in selected genera, one from each recent amphibian order, as examples.

2. More than ten years after chytridiomycosis-driven declines in amphibian communities in upland western Panama have been observed for the first time, a study of how pathogen-driven extinctions are distributed among taxa is needed. Ecology and taxonomic affiliation of potential surviving populations could explain whether species habitats or phylogenetic relations define the extent of population decline and possible chance of extinction. I hypothesize that surviving populations in *Bd* positive situations are not randomly distributed over taxa. Further, regions where susceptible species appear clustered might be free from *Bd*.

3. Regions where populations of threatened species and an increased occurrence of endemics or undescribed species are located could be targeted as areas of high conservation importance. I assume that there are still such areas in western Panama that remain unidentified to date. Such areas are possibly not officially protected and thus of major conservation concern. The results of this study will help identify these regions and justify their consideration for protected areas in western Panama.

5. MATERIAL AND METHODS

5.1. DEFINITION OF THE STUDY AREA

The higher elevations of the Cordillera Central are still one of the least investigated areas in Panama. The main study area transect extends over the main ridge of the Cordillera Central from the Panama-Costa Rica border at roughly 83° west and the Santa Fe area at approximately 81° west, adding up to a total east-west extension of approximately 220 km. The investigated transect is about 60 km in width, measured 30 km from the continental divide to the north and south, respectively. The main focus was laid on elevations above 1000 m, thus the transect is broader if the elevation is not falling below 1000 m after 30 km, what is the case in extreme western Panama. Generally, it was attempted to reach as many localities within the transect, no matter in what condition relating to vegetation and conservation status of the habitat at the respective site was. However, some sites could not be reached and there remain some gaps in the transect that could not be visited. Additionally, several sites in the Pacific and Caribbean foothills and lowlands have been visited to obtain comparative material for the genera analyzed. An overview of the transect and the actual collection sites is given in Figure 13.

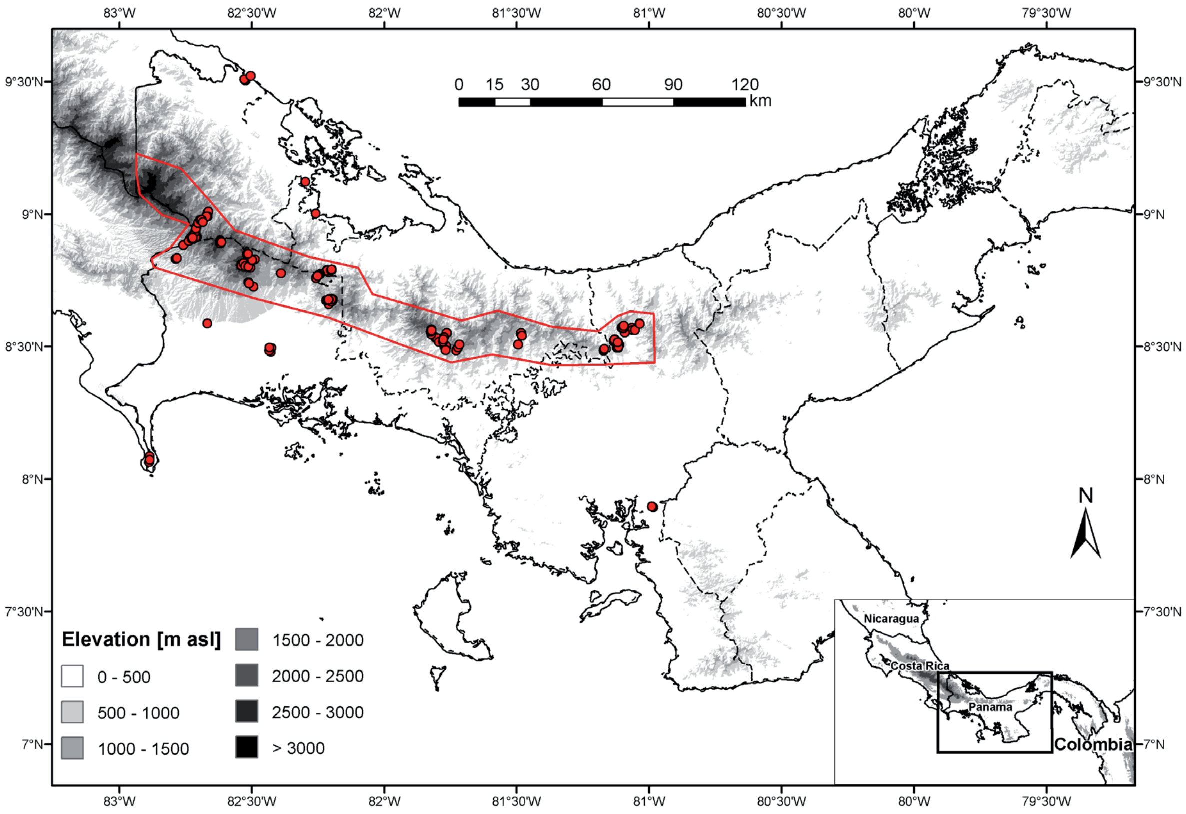

Figure 13: Map of western Panama showing the study area (red outline) and visited collection sites (red dots).

5.2. Taxon selection

In the beginning of this dissertation project I refrained from determining the amphibian taxa to work on. This was mainly because after population declines and extinction events among most Panamanian amphibian groups it was uncertain, which taxa would be the survivors. Some colleagues even doubted if there would be any surviving amphibians in Panama at all (Karen Lips pers. comm. 2007). However, field work in Panama produced many voucher specimens of around 90 different species. Preliminary analysis revealed that among the collected material where eight undescribed species and taxonomical inconsistence in many genera. Finally, to solve the entire, nearly inexorable, flood of taxonomic problems would be far beyond the scope of a dissertation. I then decided to make the dissertation a consistent taxonomic work with a strong focus on conservation aspects.

The main criteria that were used for the selection of genera for the taxonomy chapters are:

1. One genus from each order: There are various approaches for taxonomic studies for each of the three living orders of amphibians. To show the relevant spectrum of taxonomic approaches and methods one genus out of each order is addressed.

2. Scientific relevance: The selected genus has been addressed by few taxonomic works and enough questions remain open for investigation.

3. Availability of sufficient material: The species in the selected genus is abundant enough to make new scientific findings on the basis of well-supported evidence.

In the order Anura the choice fell on the genus *Diasporus*, although this large order would have had enough other interesting genera to work on. However, *Diasporus* has received little taxonomic attention in the past, especially works that include bioacoustic and molecular data are lacking. Moreover, specimens are relatively easy to obtain in large numbers; they are abundant and not endangered. Additionally, preliminary analyses at an early stage of this work had already revealed that the species diversity within the genus is largely underestimated.

In the order Caudata only two genera occur in Panama. In general, the genus *Oedipina* is certainly the poorer known genus of the Panamanian salamanders. However, the number of received specimens was very low, while among the collected specimens of *Bolitoglossa* were several rare and poorly investigated species, including a new one. Among others, the newly obtained material gave me the opportunity to face the “large black salamander problem” of Hanken *et al.* (2005).

I collected a single individual of the order Gymnophiona, thus the genus to work on was specified from the moment of encounter. Interestingly, the genus *Oscaecilia* is the least investigated of an order that is already poorly understood. The genus *Oscaecilia* has never before been recorded from western Panama and a lot of questions could be addressed.

Although this work has not a focus on historical biogeography, it is a good basis for the discussion that the three genera this work is concerned with, have very different dispersal tracks as defined by Savage (1982) and represent the three main colonization tracks of the Panamanian herpetofauna. Thus, the genus *Bolitoglossa* is a component of the Old Northern Element with long-term Laurasian affinities. By contrast, the caecilian genus *Oscaecilia* is a recent contributer of the Middle American fauna with a Gondwanan affinity that has come to Panama after the latest closure of the Panama portal in the Pliocene. Savage (2002) placed the Terrarana as *Eleutherodactylus* in the South American Element. Hedges *et al.* (2008) confirmed that Terrarana arose in South America, but the history of the several families is different and complex. The family Eleutherodactylidae colonized the Caribbean islands in the Mid-Cenozoic and through an ancient dispersal event the ancestors of the genus *Diasporus* arrived in mainland Central America and developed there to what is *Diasporus* today.

5.3. FIELD WORK METHODS

5.3.1. SURVEY METHODS

The results presented herein are based on material collected by myself and contributors during five fieldtrips between 2008 and 2013. The time in the field was distributed over the course of the year in such a way that seasonal cyclic variations could be covered. However, a focal point was set on the rainy season between April and December, because amphibian activity is increased during this time. The first fieldwork was conducted between May and August 2008, both the second and the third in 2009 from February to April and from September to December, respectively. The fourth took place from May to August 2010 and a fifth one in July 2013. To encounter adult frogs in the field, I usually used opportunistic Visual Encounter Survey transects (VES) (Heyer *et al.* 1994; Lips *et al.* 2001) along existing paths or personally cleared trails that where created at least several hours in advance. Alternatively, VES transects led along rivers and creeks. Along a VES transect objects like logs and stones that may serve as an amphibian hiding place were turned, but subsequently returned to its original position. Walking VES transects was performed at day and night, but with an emphasis on the nighttime. Searching was neither distance nor time constrained, but depended on the respective activity of the amphibian communities. Acoustic survey methods have been used for detection of anuran breeding sites and to detect concealed sitting male frogs. Provided that the species-specific male advertisement call was known, I used Audio Strip Transects (AST) (Zimmermann 1994), to quantify calling males along a transect, particularly in post-decline populations of endangered species.

5.3.2. PREPARATION AND DOCUMENTATION OF VOUCHER SPECIMENS

When a species has been found it was usually photographed on the spot. If it appeared that the animal would escape it was directly captured, or if it was a calling frog it was first recorded and then photographed. Selected specimens were captured with the hand and stored alive in 4–6 l Toppits® Freezer-Bags together with some green leaves. The bags were inflated with air and knotted. The subsequent day, specimens were redetermined by employing the keys of Savage (2002) and Köhler (2011). Afterwards, it was decided which specimens would be preserved. To these an individual field number was assigned, starting with the initials of the collector followed by a sequential number. For each field number, an individual entry in the field book was made containing the information when (date and time), where (including GPS coordinates and altitude), and what (preliminary species identification) was collected.

Each voucher specimen was photographed in detail and an individual color description using the capitalized color name and associated color codes in Smithe (1975–1981). The subsequent preparation of voucher specimens in the field follows the method described by Köhler (2001) and own modifications. Specimens were euthanized by an intrapericardial injection of 0.01–0.05 ml T 61 (Intervet, Unterschleißheim, Germany). Very small specimens (snout-vent lengths ≤ 1cm) were euthanized by the application of a small drop of T 61 on the dorsal skin. After death of the specimen, a part of the left arm was cut with sterilized scissors and stored in an Eppendorf Tube™ filled with 98% undenaturated ethanol as tissue sample. Afterwards, the specimen was laid on its ventral side with hands and feet laterally unfolded. With the end of a needle fingers and toes were unfolded until the extension of webbing was well-visible. Then, hands and feet were sprinkled with a solution of 5 ml 36% formalin in 1 l of 96% ethanol, hereafter called preparation solution. After a few minutes to let the formalin solidify the tissue of hands and feet, body cavity and thighs were injected with preparation solution through the cloacae opening. The specimen was afterwards tagged with its individual field number on the left hind limb above the knee and subsequently stored in 70% denaturated ethanol. Collected specimens will be deposited in the Senckenberg Research Institute, Frankfurt (SMF) and the Museo Herpetologico de Chiriquí (MHCH). Specimens referenced herein, which have not yet received a collection number are labeled with their original field number. A list with all collected specimens, their corresponding field numbers and, if applicable, their museum number is included in the digital appendix (10.6 SF 2). Abbreviations for museum collections for comparative voucher specimens follow those of Sabaj Pérez (2010).

5.3.3. RECORDINGS OF ANURAN VOCALIZATIONS

In most cases, specimens were only recorded after the calling individual has been definitely identified. Generally, the recorded individual was collected right after recording. If the recorded specimen escaped or could not be found after recording this was stated in the text. Recordings were made using a Sennheiser ME 66 directional microphone capsule with a Sennheiser K6 powering module at a distance of 0.50 to 1 m from the calling frog. Recordings were made with a Marantz PMD 620 digital recorder, which was connected to the microphone with a 3 m audio cable. In species that stopped calling when approached, the microphone was arranged on a tripod (Joby Gorillapod) that was erected on the ground or attached to branches or trunks. Then, withdrawed 2–3 m and waited until the frog resumed calling. Calls were recorded in uncompressed PCM format at a sampling rate of 48 kHz with 24 bit resolution and stored as wav-files on a SD Card. Relative humidity (RH) and air temperature were determined while or immediately after recording using the digital device Voltcraft HT-200 at a precision of 1 °C and 3.5% relative humidity. Measured values were rounded to the first decimal. In some cases, also the barometric pressure was measured with the build-in barometer of a handheld Garmin GPS MAP 60 CSx GPS receiver. Additionally, automated temperature and humidity data were recorded at selected places within the study area using an iButton® datalogger (Maxim Integrated™).

5.4. MOLECULAR METHODS

5.4.1. MARKER SELECTION

The purpose of using molecular markers in this study was to delimit biological species and to reconstruct their relationships. A common tool for molecular identification is the bioinformatic analysis of short standardized DNA sequences (500–800 bp), a technique commonly known as DNA barcoding. As a single, universal barcoding marker for all animals it was proposed to use a fragment of the mitochondrial Cytochrome C Oxidase subunit 1 (CO1) (Hebert *et al.* 2003). However, Vences *et al.* (2005b) found that CO1 primers had a very low success rate in sequencing this barcode locus in Madagascan frogs and proposed to use the fragment of the large mitochondrial ribosomal subunit (16S rRNA gene) in amphibians instead. In subsequent years, 16S mtDNA became more and more accepted among taxonomists as the universal barcoding marker in amphibians, although some contrary studies have been published for particular amphibian groups (Smith *et al.* 2008; Xia *et al.* 2012). However, besides in anurans, 16S became an integral part of mitogenomic studies in caecilians (Zhang & Wake 2009) and salamanders (Parra-Olea *et al.* 2004) especially from the Neotropics. This led to a good supply of comparative 16S sequences on Genbank in comparison to other markers for all three amphibian orders. Recently, Zhang *et al.* (2013) showed that 16S is especially informative in topology resolution and proposed it again as the first choice marker in anuran barcoding.

5.4.2. DNA EXTRACTION AND SEQUENCING

Small pieces of the collected tissue samples were incubated for 14 hours in 200 µl low PBS buffer (20 µl PBS in 180 µl of water) in order to eliminate potential PCR-inhibiting contaminants. Afterwards, tissues were digested with the vertebrate lysis buffer at 56 °C on a thermomixer. After vacuum extraction, DNA was eluted in 50 µl TE buffer. DNA was extracted from muscle tissue following 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 cycles with denaturation for 0.25 min at 94 °C, hybridization for 0.75 min at 45 °C, and elongation for 1.5 min at 72 °C, and a final elongation proceeded for 7 min at 72 °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 H_2O, 1 µl 25 mM $MgCl_2$, and 1 µl (containing 10 pmol) of each of the universal 16S primers 16SA-L and 16SB-H (Palumbi *et al.* 1991). The PCR products were run in a gel electrophoresis at 115 V for 20 min, with the gel containing SYBR-Green, then the DNA concentration in the PCR products was determined photometrically using an Alpha Innotech Multilmage Light Cabinet. PCR products were then diluted so that 10 µl contained 20 ng of the amplified gene fragment. The diluted solution was sent to the BiK-F Labratory Centre for sequencing.

5.4.3. TAXON AND SEQUENCE SELECTION AND ALIGNMENT

The 16S sequences that resulted from the forward and reverse reaction of every sample were combined to a consensus sequence in Geneious (Drummond *et al.* 2010). A BLAST similarity search (Altschul *et al.* 1990) of every sequence was run to detect possible contamination with DNA (*e.g.,* other amphibians, reptiles, or humans) and to identify possible sequences for comparison. The consensus 16S sequences of a taxonomic unit were opened in Geneious and combined with selected 16S mtDNA sequences from GenBank including outgroup sequences. Then, sequences were aligned with ClustalW (Larkin *et al.* 2007) using the default settings as implemented in Geneious: DNA weight matrix IUB, gap opening penalty 15, and gap extension penalty 6.66. The resulting alignment was exported from Genious as a FASTA-file.

5.4.4. MAXIMUM LIKELIHOOD ANALYSIS AND GENETIC DISTANCE

The alignment as exported from Geneious was manually refined using Mega 5.1 (Tamura *et al.* 2011) by either trimming overhanging ends or by filling up the ends of shorter sequences with question marks to indicate missing data. Furthermore, alignment gaps were manually checked and refined. With the obtained alignment a Neighbor Joining (NJ) tree was calculated to rapidly get an idea of the resulting clades before executing more exhaustive algorithms. Then, sequences were arranged according to the clades in the NJ tree and the alignment was redefined again if necessary. Once the final alignment was found, the best fitting substitution model for the Maximum Likelihood (ML) analysis in Mega 5.1 was determined by calculating the BIC (Bayesian Information Criterion) with the “find best DNA model” function in Mega 5.1. The model with the lowest BIC score was considered to describe the substitution pattern the best. A ML phylogenetic tree was calculated in Mega 5.1 choosing the following settings: Tree topology was tested with the bootstrap method using 10,000 bootstrap replications; the substitution model and rates among sites were determined for the respective alignment as described above; ambiguous sites were removed by partial deletion with a site coverage cutoff of 95%; the initial tree was found using the Nearest Neighbor Interchange (NNI) heuristic method: the branch swap filter in the ML analysis was set to “Strong”.

Moreover, to get a minimum numeric value for genetic distances within and between terminal genetic clusters, I calculated the within group and between group average p-distance in Mega 5.1. The p-distance is the proportion of nucleotide sites that are different in two compared sequences. It is calculated by dividing the number of nucleotide differences by the total number of compared nucleotide sites. The within group average distance is the arithmetic mean of individual pairwise distances within a group of sequences. I used the bootstrap variance estimation method with the following settings: 10,000 bootstrap replications, substitutions included transitions and transversions, uniform rates among sites, and the pattern among lineages homogeneous. Ambiguous sites were removed by partial deletion with a site coverage cutoff of 95%. The same settings were chosen to calculate the between groups average distance estimation.

5.4.5. BAYESIAN INFERENCE FOR TREE RECONSTRUCTION

The best fitting nucleotide substitution model for each alignment was determined using JModeltest 2.1.3. (Posada 2008), which uses the PhyML 3.0 algorithm (Guindon *et al.* 2010) to estimate the model on a maximum-likelihood principle. In the likelihood settings the number of substitution schemes was reduced to three, base frequencies +F, rate variation among sites +I and +G, and the base tree for likelihood calculations was set to maximum-likelihood optimized. After likelihoods were computed, the particular model was selected under the Akaike Information Criterion (AIC) by comparison of likelihood scores from 24 models, in compliance with the number of models that can be implemented in MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001). Bayesian Inference (BI) consisted of two independent parallel runs, each with four Markov chains (one cold, three heated). Standard analyses were run for 20,000,000 generations sampled at intervals of every 100 generations. The number of generations to discard as burn-in was estimated in MS Excel by graphical exploration of the trace plots of the log likelihood scores of both runs, discarding all samples until stationarity was reached in both runs. By default, the first 10% were discarded as burn-in, but this number was modified if necessary. In addition, convergence of runs over the length of the simulation was assessed using the compare analysis function in AWTY (Nylander *et al.* 2007) that creates bivariate plots of the split frequencies for the first and second run of the simulations for graphical comparisons. After discarding burn-in samples, the trees of the two independent runs were combined using the sumt command in MrBayes to a single majority consensus tree. The final graphic of the tree was created using FigTree 1.4 (Rambaut 2008).

5.5. BIOACOUSTICS

Call spectrum analyses were performed using Raven Pro 1.4 (Cornell Lab of Ornithology 2003–2010), computing the Discrete Fourier Transform (DFT) of the sampled call with Fast Fourier Transformation (FFT) at Hann window function (window size: 512 samples, 60% overlap). A maximum of ten calls per individual were chosen for analysis as a representative sample. If calls were emitted in call series, three entire call series were used for analysis. Temporal and spectrographic measurements were made by manually selection of calls, notes, or pulses. The following measurements were copied to the selection table in Raven Pro 1.4 and served as the basis for calculations of call parameters: Begin Time [s] = Begin of selection; End Time [s] = End of selection; Delta Time [s] = Length of selection; Peak Time [s] = Time after begin of selected component, when greatest acoustic energy is reached; Low Frequency [Hz] = Lowest frequency in selection; High frequency [Hz] = Highest frequency in selection; Peak frequency [Hz] = Dominant frequency, the frequency at which acoustic energy is highest. Frequencies were rounded to the next integer and times to the third decimal place. On the basis of the measurements made in Raven Pro 1.4, the following additional quantitative characters were calculated: Pulse length [s], Note length [s], Call length [s], Call interval [s], Pulse rate [pulses per second], Note rate [notes per second], Call rate [calls per minute], and the Rise Time [s], the time it takes from the beginning of a signal to reach the maximum power (=dominant frequency). Some additional qualitative characters are described from the visualized oscillograms and spectrograms: The presence or absence of frequency modulation in pulses or notes; the presence or absence of harmonics in pulses or notes; and the pattern of amplitude modulation in pulses, notes or entire calls. Diagnostic characters are given as range followed by mean ± standard deviation in parentheses. The call terminology follows Duellman and Trueb (1986). Images of oscillograms and audiospectrograms were created using Sound Ruler 0.9.6.0 (Gridi-Papp, 2003–2007).

5.6. MORPHOLOGICAL METHODS

5.6.1. MORPHOMETRICS

For morphological differentiation of specimens the morphological measurements were taken with Helios dial calipers under a dissecting microscope in the unit mm and rounded to the nearest 0.1 mm. Measured values served as basis for the calculation of morphometric proportions in the tables as indicated by the formula in the respective column. Morphometrics are given as range, followed by mean ± SD. Except in *Oscaecilia* where measurements of each specimen are given, because of the low number of available specimens.

For the three different genera this work is concerned with, the following standard measurements and qualitative characters have been taken (with the respective abbreviation indicated):

Anura: *Diasporus*: Snout-vent length (direct line distance from the tip of the snout to the posterior margin of the vent): SVL; length of Finger III (from distal end of Finger III including disk to the base of the second subarticular tubercle): LF III; length of Toe IV (from distal end of toe IV including disk to the base of third subarticular tubercle): LT IV; disk width at Finger III (at greatest width): DWF III; disk width at Toe IV (at greatest width): DWT IV; head length (from the commisure of the jaws to tip of the snout): HL; head width (between angles of jaw): HW; shank length (straight length of the shank): SL; eyelid length (greatest horizontal diameter of the upper eyelid): EL; interorbital distance (width of frontoparietal bone between orbits): IOD; horizontal tympanum diameter (at greatest length): TD; and horizontal eye diameter (at greatest length): ED. In addition, the following qualitative characters were taken: General dorsal coloration pattern; general ventral coloration pattern; male vocal sac coloration; groin coloration; skin texture; presence or absence of eyelid tubercles; tympanum condition: prominent, indistinct, or concealed; shape of the disk cover; presence or absence of ulna tubercles. Specimens were sexed by presence (males) or absence (females) of vocal sac and vocal slits.

Caudata: *Bolitoglossa*: Snout-vent length (called standard length by some authors, from tip of snout to posterior end of vent): SVL; tail length (from posterior end of vent to tip of tail): TL; head length (from gular fold to tip of snout): HL, head width (measured at greatest width of head): HW; hind limb length (from groin to tip of longest digit): HLL; hand width (measured at widest extent): HAW; hind foot width (measured at widest extent): FW. Further, the number of premaxillary teeth (PMT), number of maxillary teeth (MT), and number of vomerine teeth (VT); as well as the number of costal grooves (CG) and the number of costal folds between adpressed limbs (CFL) were counted under a dissecting microscope. MT and VT are provided for left and right sides, respectively. Adult specimens were sexed by the presence of cloacal folds (females) or cloacal papillae (males). Specimens that are explicitly smaller than the average of available conspecifics and that lack sexual characteristics like cloacal folds or papillae and usually also premaxillary teeth were treated as juveniles.

Gymnophiona: *Oscaecilia*: Primary grooves (total count of grooves seperating primary annuli): PG; secondary grooves (count of grooves separating primary annuli): SG; first secondary groove appears at PG number: SG1; number of vertebrae (counted on x-ray image): NV; scale inception (the first scales appear after PG number, seen on x-ray image): SI; total length: L; body width (measured at first PG): BW1PG; body width (measured at midbody): BWM; body width (measured at vent): BWV; calculated index of attenuation (L/BWM): IA; head length, (from tip of snout to first nuchal groove): HL; head width (measured at angle of mouth): HW; lower jaw length (measured from angle to tip): LJL; snout projection (protrusion of upper jaw over lower jaw): SP; tentacle to nare distance (distance between centre of the tentacle opening to centre of the nare): TND; intertentacular distance (horizontal distance from centre of one tentacle opening to the other): ITD; internare distance (horizontal distance from centre of one nare to the other): IND. Tooth counts were made from high-resolution, synchrotron-based X-ray micro CT (HRμCT) images, counted on each side and denoted as left-right tooth row count: Maxillary-premaxilary tooth counts (MPAT); vomero-palatine tooth counts (VPAT); dentary tooth counts (DT); splenial tooth counts (ST). Two preserved specimens (AB 1232; Gorgas Memorial) were not x-rayed and CT-examined, as well as the two specimens kept alive in captivity. Accordingly, skull and skeleton data are lacking for those specimens. This is also the case in the incomplete specimen (MCZ 215221). Missing data are indicated by question marks. Since sexing in caecilians is only possible by a small lateroventral incision, what is not wished by most curators and collection managers, the sex is unknown in most specimens. The holotype of *Oscaecilia osae* (LACM 138542) has been sexed before (Lahanas & Savage 1992) and the two male specimens have partly everted phallodea.

5.6.2. STATISTICAL EXPLORATION OF DATA

Series of morphological and bioacoustic measurements were statistically examined using different analysis tools in GraphPad Prism version 5.01 (GraphPad Software, La Jolla California USA, www.graphpad.com). Additional information on the respective analysis that has been conducted are contained in the appropriate parts of the text.

5.6.3. X-RAY AND HIGH-RESOLUTION MICRO-COMPUTED TOMOGRAPHY

I used x-ray images of caecilians and salamanders to count vertebrae and to make subdermal scales visible in caecilians. Contact radiographs were made at the Department of Messel Research at the Senckenberg Research Institute and Nature Museum, Frankfurt with the help of Jörg Habersetzer. The images were made with a conventional x-ray source in a full-protection device (Faxitron 43804; Hewlett Packard) on a HD storage screen (NDT 25 µm resolution) at an energy of 50 kV for either 45 s or 60 s depending on the size of the sample. The preheating time of the x-ray tube was approximately 5 min. The resulting images were digitalized using a laser scanning digitizer (HD-CR 35 NDT, Duerr-NDT, Germany) and the program CR Scan Config. The image was already checked for incorrect exposure in the preview modus by checking the gray value range (brightest pixel > 2,500; darkest pixel < 64,000). The resulting 16 bit-files were at first stored as *.raw (containing also a *.bmp-auxiliary file) and later converted into a *.tif-file for further processing in Photoshop CS3. All images were refined and trimmed using Photoshop CS3.

In order to examine cranial characters of caecilians I generated high-resolution, synchrotron-based X-ray micro CT imaging (HRµCT) at the German Electron Synchrotron (DESY) in Hamburg. HRµCT was performed at BeamlineW2 of the DORIS III accelerator ring operated by the Helmholtz Center Geesthacht. Some more background details on the method have been published by Beckmann *et al.* (2006) and Kleinteich *et al.* (2008). To prevent the specimen from drying out during the several hours lasting measurement, the whole specimen was transferred into a 50 ml Falcon Tube with the head directed in the cone-shaped bottom of the tube. The tube was then filled to the brim with 70% ethanol sealed with parafilm and capped. The tube must be completely free from any air bubbles that would interfere the measurement. The tube was then mounted in the beam line, with the head of the caecilian directed in the beam. Specimens were scanned with a 25–28 keV X-ray beam over a rotation of 180° in 0.20–0.25° steps. The resulting images have a resolution of 3.37–3.65 µm in x, y and z orientation. The further processing and reconstruction of the images has been made by Sandy Reinhard (University of Jena) using the software VG Studiomax 2.0 (Volume Graphics GmbH, Heidelberg, Germany).

5.7. SPECIES CONCEPT AND INTEGRATIVE TAXONOMY

The knowledge of the preferably exact number of species in a given area and an unequivocal system to name these is fundamental for any reproducible continuative biological study. In applied nature conservation, it is crucial to have a comprehensive overview of the number of organisms inhabiting a certain area, to know how they are distributed, and wether they need to migrate seasonally between habitats. Understanding the basic needs of species and their taxonomy is thus the basis for an efficient conservation management (*e.g.*, Hertz *et al.* 2014). Although most biologists agree that species are unitary evolving lineages, the criteria to call a lineage "species" has always been controversially discussed (*e.g.*, Frost & Hillis 1990). Accordingly, the species concept implemented and the respective characters used to delimitate species may lead to very different conclusions. De Queiroz (2007) proposed a unified species concept, as he pointed out that there is a general accordance of species being "separately evolving metapopulation lineages" in which all species concepts agree. The problem remains that there is a "Gray Zone" of intermediate stages in two divergent lineages on their way to become separate species (De Queiroz 2007; p. 882, Fig. 1). Thereby, different characters like morphology, genetics, behavior, and even reproductive isolation may evolve at different rates so that the process of speciation is not always accompanied by character change at all levels (Padial *et al.* 2010). Traditionally, amphibian species have only been described by external morphology characters, *e.g.*, morphometrics and coloration, what led in most cases to an underestimation of species diversity. However, since every line of evidence can be misleading, it is essential to integrate different, preferably independent lines of evidence to identify separately evolving lineages.

In this work I followed the unified species concept of De Queiroz (2007) and applied the species delimitation concept of Vieites *et al.* (2009). Since the three examined amphibian orders exhibit different characters, the approaches used in this study to uncover species delimitations are variable for each amphibian order (in parentheses):

1. Phenotypic criteria: external morphology, morphometrics and qualitative characters (all three orders) and osteology and toothcounts (Caudata and Gymnophiona);
2. Species recognition criterion: distinctness of male advertisement call (Anura);
3. Genetic differentiation criterion: genetic distinctness of 16S mtDNA sequence data (all

three orders).

Following the concept of Vieites *et al.* (2009), I integrate the different lines of evidence. The initial point to identify convergent lineages is the result of DNA barcoding using sequences of the 16S rRNA gene, which then entail further investigation. Integrating the different lines of evidence will then lead to the assignment of the genetic lineage to one of the following categories:

Unconfirmed Candidate Species (UCS): This is the default category for lineages with deep genetic divergence. As a benchmark I use the > 3% p-distance threshold proposed for Malagasy amphibians by Vieites *et al.* (2009), but additionally calculate between and within group mean genetic distance of terminal clusters for a better estimation of genetic divergence within and between Panamanian amphibian lineages. Specimens and populations treated as UCS usually lack enough additional material to identify divergent lines of evidence, *e.g.* specimens are juveniles, the sample size is too small, the advertisement call is unknown.

Confirmed Candidate Species (CCS): Specimens or populations treated as CCS show a considerable genetic difference of usually but not necessarily more than 3% to their closest relatives. The assumption for a separately evolving lineage is further supported by at least one additional line of evidence. Ideally, CCS occur in sympatry together with sister lineages and show no evidence of interbreeding, or in allopatry but with distinctive phenotypic differences (Padial *et al.* 2010). Moreover, there is no scientific name for the identified lineage applicable, thus formal species description is pending.

Deep Conspecific Lineages (DCL): Specimens or populations that show a considerable genetic distance to their closest relatives, usually 2–3%, but not necessarily above any threshold. In contrast to UCSs, the set of morphological and other data is good, but these data provide no additional differences between lineages.

In contrast to Lotzkat *et al.* (2013) and Jansen *et al.* (2011) I herein use the term “candidate species” for not yet described species as originally proposed by Vieites *et al.* (2009). Jansen *et al.* (2011) noted that the term candidate species is only applicable to a divergent lineage if this refers to a putative unnamed species. In order to find out if a valid name is available, a deeper taxonomic revision is required, including the examination of comparative material. However, in the three genera addressed herein, I examined most of the comparative material available, including holotypes, making the diagnoses as profound as possible with the material presently available, so I do not refrain from drawing taxonomic conclusions. Some of the species identified as new species in this study have already been described and named (see digital

Appendix 10.6. SF 1) while for others formal species description is pending. The latter ones are called candidate species herein.

6. RESULTS

6.1. TAXONOMY: ANURA – ELEUTHERODACTYLIDAE – *DIASPORUS*

Eleutherodactylidae

Sensu Hedges *et al.* (2008), the family Eleutherodactylidae comprises four genera, *Adelophryne*, *Diasporus*, *Eleutherodactylus*, and *Phyzelaphryne*, which together contain 207 species (AmphibiaWeb 2014). The first two genera are closely related and thus placed together in the subgenus Eleutherodactylinae.

Diasporus

The genus *Diasporus* is distributed from south-eastern Honduras over the Mosquitia of Nicaragua and into Costa Rica and Panama, where it is also found in the humid Pacific lowlands of the Golfo Dulce Region in the Costa Rica-Panama border region. It is then continuously distributed across Panama with exception of the dry Pacific lowlands of the Azuero Peninsula, into the Chocó of Colombia and along the Pacific coast west of the Andes

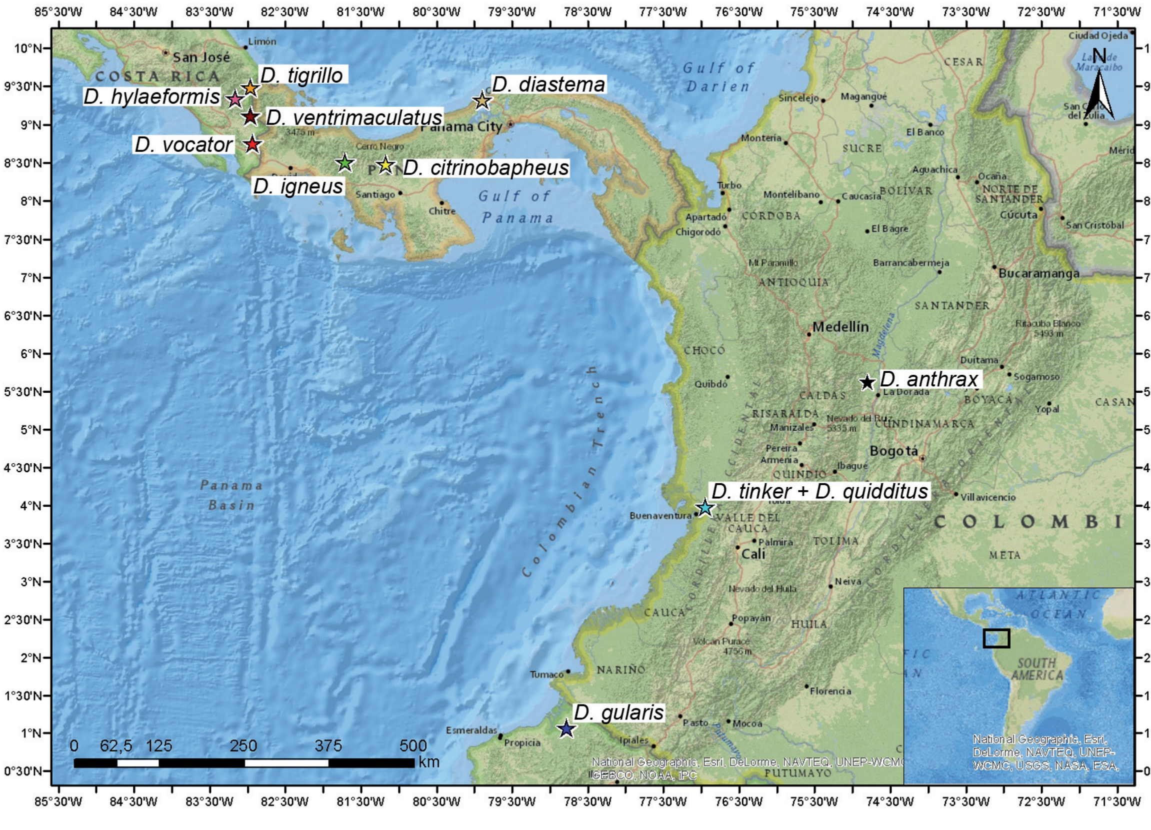

Figure 14: Map of Lower Central America and north-western South America showing the type localities of *Diasporus* species.

to northwestern Ecuador. In consideration of this distribution area, the number of eleven described species, five of which are known to occur in Panama, appears rather small. Three species in the genus have their type locality in Panama and four more in Costa Rica, but in the immediate proximity of the Panamanian border (Fig. 14). The members of this genus are found in moist forests between sea level and 2400 m asl where they are usually very abundant.

6.1.1. MOLECULAR ANALYSIS

For this study, 67 specimens of the genus *Diasporus* were collected of which 49 tissue samples that yielded 43 sequences of a fragment of the mitochondrial 16S rRNA gene have been taken. The resulting alignment was complemented by 15 sequences from GenBank. As outgroups I chose the sequence of *Eleutherodactylus johnstonei* from GenBank and a sequence of *Pristimantis ridens* from Cerro Negro from my own collection. The final alignment consisted of 515 sites of which, excluding the outgroups, 115 were variable and 87 parsimony informative.

The ML tree and the Bayes tree are basically congruent and both trees found two major clades (Fig. 15) that are well-supported (bs=94; pp=100). The p-distances within a terminal clade are given in Table 1. The upper clade comprises most of the described species from western Panama and Costa Rica. However, for this clade both trees cannot completely resolve the topology of some taxa resulting in polytomies or branches with low statistical support. Particularly, the clade that contains all of the *Diasporus hylaeformis*-like species shows several poorly supported nodes and polytomies. However, the node that forms this clade is well-supported, at least in the Bayes tree (bs=81; pp=100). A subclade containing mostly *D. hylaeformis*-like specimens from sites between Cerro Pando in extreme western Panama and La Nevera in the Serranía de Tabasará is formed with low support in the ML tree (bs=46), but with high support in the Bayes tree (pp=98). Surprisingly, it also contains a *D. vocator*-like specimen (AH 364) from the Carribbean lowlands, but the obtained sequence of this specimen is 84 positions shorter than the remaining sequences in the alignment, what might have led to a misplacement in the tree. Other specimens which were morphologically assigned to *D. hylaeformis*, that were collected at Volcán Barú (bs=89; pp=100) and La Fortuna (bs=92; pp=100) form well-supported, separate clades, respectively. However, the p-distances between these clades (Tab. 2) are comparatively low with 2.1% between the Barú and the Pando clade and 2.7% between the Barú and the Fortuna clade respectively, and only 1.4% between the Fortuna and the Pando clade. Additionally, *D. hylaeformis* appears polyphyletic in the trees. Another clade of *D. vocator*-like specimens, all from the Fortuna Depression, receives relatively low statistical support (bs=81; pp=75) and the final placement of this clade in the phylogeny is not resolved by neither one of the inferences. The p-distance

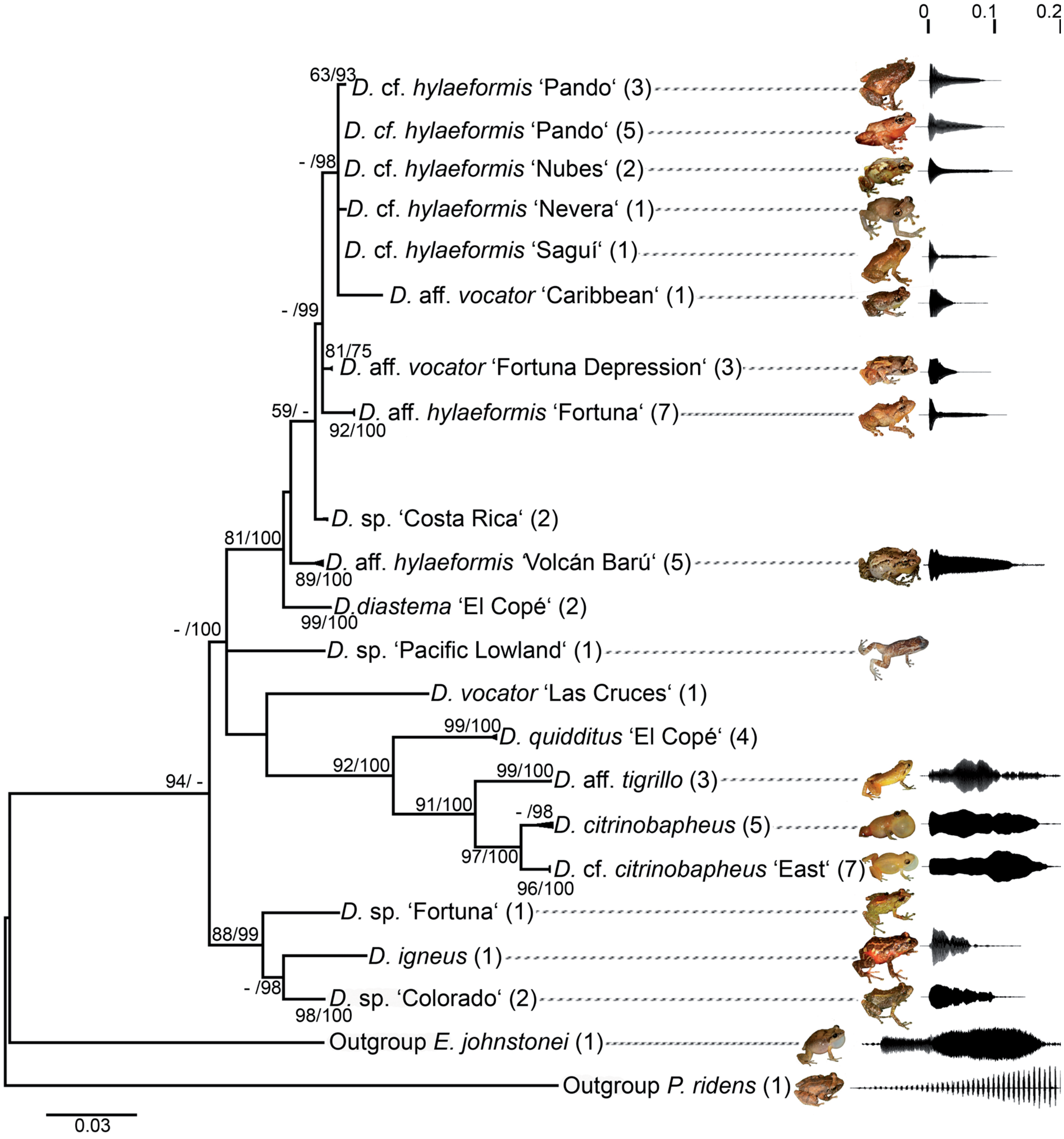

Figure 15: ML-Tree of Panamanian *Diasporus* for the 16S mtDNA marker. Numbers near nodes represent bootstrap support (before slash) and posterior propability (after slash). Branches are labeled according to morphological designation, numbers in parantheses indicate number of specimens analyzed. Depth of black triangles indicates 16S mtDNA variation within a lineage (cp. Tab. 1). Scale bar refers to number of substitutions per site. Pictures show example individuals for each clade, as well as example call oscillograms. Scale above calls refers to call length in secods.

between *D. vocator*-like specimens from the Caribbean lowlands and the Fortuna Depression is relatively low with 2.3%. However, a sequence on GenBank labeled with *D. vocator* collected very near to the type locality of *D. vocator* has a mean p-distance of 6.4% to the *D. vocator*-like specimens from the Fortuna Depression and 7.9% to the specimen from the Caribbean lowland. Another well-supported clade (bs=92; pp=100) contains besides *D. quidditus* specimens from El Copé, three entities of bright-yellow *Diasporus*. First, this is the type series and referred specimens of *D. citrinobapheus*, as well as *D.* cf. *citrinobapheus* east of the type locality, between which a p-distance of 1.6% exists. Both appear as sister clades in both trees with high statistical support (bs=97; pp=100). The third well-supported (bs=99; pp=100) distinct clade of yellow *Diasporus* is separated by p-distances of 4% to *D. citrinobapheus* and 4.1% to *D.* cf. *citrinobapheus*. The three specimens in this clade have been assigned as *D.* aff. *tigrillo* by morphological characters (see Morphology section). The placement of a single specimen from the Panamanian Pacific lowlands is not fully resolved, resulting in polytomies in both trees. The specimen has a p-distance of 4.5% to two specimens from Costa Rica from GenBank and to *D. hylaeformis*-like specimens from Volcán Barú and the highest p-distance of more than 9% to *D. citrinobapheus*. The second major clade contains exclusively specimens from the Serranía de Tabasará, none of which had been sequenced before. The only formally described species in this clade is *D. igneus* from the

Table 1: The number of base differences per site from averaging over all sequence pairs within each lineage of *Diasporus* are shown. Standard error estimates are shown in the last column. The presence of n/c in the results denotes cases in which it was not possible to estimate distances.

Lineage	p-distance	Standard error
D. citrinobapheus	0.0052	0.0023
D. cf. *citrinobapheus* 'East'	0.0006	0.0006
D. aff. *tigrillo*	0	0
D. aff. *hylaeformis* 'Fortuna'	0.0006	0.0006
D. cf. *diastema* 'El Copé'	0	0
D. sp. 'Costa Rica'	0.0025	0.0023
D. cf. *hylaeformis*	0.0009	0.0009
D. aff. *hylaeformis* 'Volcán Barú'	0.0027	0.0019
D. sp.' Fortuna'	n/c	n/c
D. igneus	n/c	n/c
D. sp. 'Colorado'	0	0
D. sp. 'Pacific Lowland'	n/c	n/c
D. aff. *vocator* 'Fortuna Depression'	0.0015	0.0015
D. aff. *vocator* 'Caribbean'	n/c	n/c
D. vocator	n/c	n/c
D. quidditus 'El Copé'	0.0015	0.0014
Outgroup *Eleutherodactylus*	n/c	n/c
Outgroup *Pristimantis*	n/c	n/c

Table 2: Estimates of p-distances over sequence pairs between lineages. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimates are shown above the diagonal.

	Lineage	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	*D. citrinobapheus*		0.005	0.009	0.012	0.012	0.013	0.013	0.012	0.012	0.013	0.012	0.013	0.012	0.015	0.013	0.01	0.015	0.017
2	*D.* cf. *citrinobapheus ’East’*	0.016		0.009	0.013	0.013	0.013	0.013	0.012	0.013	0.013	0.012	0.013	0.012	0.015	0.013	0.011	0.015	0.017
3	*D.* aff. *tigrillo*	0.04	0.041		0.011	0.012	0.012	0.012	0.011	0.013	0.012	0.012	0.012	0.012	0.014	0.013	0.011	0.014	0.016
4	*D.* aff. *hylaeformis* ’Fortuna’	0.089	0.091	0.077		0.008	0.006	0.005	0.007	0.011	0.011	0.01	0.01	0.005	0.008	0.012	0.012	0.015	0.016
5	*D.* cf. *diastema* ‘El Copé’	0.088	0.088	0.079	0.03		0.006	0.006	0.007	0.011	0.011	0.01	0.01	0.006	0.008	0.011	0.012	0.015	0.015
6	*D.* sp. ‘Costa Rica’	0.09	0.09	0.079	0.015	0.022		0.005	0.006	0.011	0.011	0.01	0.009	0.004	0.007	0.011	0.012	0.015	0.016
7	*D.* cf. *hylaeformis*	0.093	0.093	0.084	0.014	0.021	0.01		0.006	0.011	0.011	0.009	0.01	0.004	0.006	0.012	0.012	0.014	0.016
8	*D.* aff. *hylaeformis* ‘Volcán Barú‘	0.085	0.085	0.076	0.027	0.025	0.016	0.021		0.01	0.011	0.009	0.009	0.006	0.009	0.011	0.012	0.014	0.016
9	*D.* sp. ’Fortuna’	0.087	0.088	0.092	0.066	0.061	0.058	0.06	0.059		0.009	0.008	0.011	0.011	0.012	0.012	0.012	0.015	0.016
10	*D. igneus*	0.09	0.088	0.081	0.066	0.061	0.061	0.065	0.07	0.043		0.008	0.01	0.01	0.013	0.013	0.013	0.015	0.017
11	*D.* sp. ‘Colorado’	0.083	0.084	0.081	0.05	0.052	0.047	0.044	0.047	0.036	0.034		0.01	0.009	0.011	0.012	0.012	0.014	0.016
12	*D.* sp. ‘Pacific Lowland’	0.091	0.093	0.079	0.052	0.052	0.045	0.051	0.045	0.063	0.059	0.056		0.009	0.012	0.011	0.012	0.015	0.016
13	*D.* aff. *vocator* ‘Fortuna Depression’	0.086	0.086	0.081	0.012	0.023	0.007	0.008	0.02	0.059	0.059	0.044	0.045		0.008	0.011	0.012	0.014	0.016
14	*D.* aff. *vocator* ‘Caribbean’	0.104	0.106	0.094	0.028	0.028	0.018	0.015	0.033	0.067	0.072	0.053	0.064	0.023		0.014	0.015	0.017	0.018
15	*D. vocator*	0.092	0.098	0.089	0.069	0.069	0.065	0.068	0.059	0.071	0.082	0.068	0.066	0.064	0.079		0.012	0.015	0.016
16	*D. quidditus* ‘El Copé’	0.061	0.065	0.062	0.084	0.08	0.085	0.085	0.077	0.082	0.093	0.08	0.082	0.084	0.099	0.081		0.015	0.017
17	Outgroup *Eleutherodactylus*	0.131	0.131	0.12	0.124	0.122	0.118	0.119	0.117	0.122	0.124	0.115	0.117	0.116	0.136	0.128	0.121		0.016
18	Outgroup *Pristimantis*	0.156	0.16	0.153	0.144	0.138	0.141	0.144	0.142	0.151	0.153	0.144	0.151	0.137	0.153	0.151	0.166	0.142	

surroundings of Cerro Santiago. The herein sequenced specimen is the paratype of *D. igneus* (SMF 89821) from La Nevera, which shows the lowest p-distance of 3.4% to a sympatric *Diasporus* from the Cerro Colorado area. Moreover, there is a single specimen from the southwestern slope of Cerro Pata de Macho, at the western end of the Serranía de Tabasará, that has a p-distance of 4.3% to *D. igneus* and 3.6% to the other *Diasporus* from the Cerro Colorado area. The node that groups all three together is statistically well-supported (bs=88; pp=99). Even though the assumptions that *D. quidditus* is a sister taxon of the three clades of yellow *Diasporus* and that the three Tabasará clades belong together are supported by both trees, they are conflicting in the placement of the clades in the respective trees. In the ML tree, *D. igneus* and its relatives are placed as a neighboring clade to all other analyzed specimens with a high bootstrap support (bs=94), while in the Bayes tree the placement of this clade is not resolved, but the clade containing *D. quidditus* and the yellow *Diasporus* is opposed to the rest, also with high support (pp=100). In turn this node is hardly supported by the ML tree (bs=26).

6.1.2. BIOACOUSTICS

I analyzed the vocalizations of 26 male individuals of the genus *Diasporus* and grouped the calls according to the clades derived from the molecular analyses. A summary of all call parameters for the different clades are given in Table 3. Detailed descriptions of the calls, including figures of waveform and spectrogram are given in the species accounts. For a superficial comparison of call waveforms in Figure 15, I included calls of the outgroups to show similarities and differences. A specimen of *Eleutherodactylus johnstonei*, which does not correspond to the GenBank sequence, was recorded in the Palmengarten in Frankfurt am Main by myself. The call of *Pristimantis ridens*, also not corresponding to the sequence used, was taken from the supplementary audio CD of Ibáñez *et al.* (1999). Both calls are completely different from all described *Diasporus* calls. Particularly, the call of *E. johnstonei* shows also analogies that confirm the sister-taxon relationship between *Diasporus* and *Eleutherodactylus*. The call length in *E. johnstonei* is with about 0.3 s much longer than any *Diasporus* call and consists of two fused notes. The notes are differentiable, because the dominant frequency glides from slightly below 2000 Hz in the first note to about 3500 Hz in the second (Watkins *et al.* 1970). The known calls of *Pristimantis* spp. are either single, very short "ticks" repeated after a long interval, or more common, a series of short pulses (Ibáñez *et al.* 1999; Padial & de la Riva 2009; own unpublished data). In contrary, the general structure of all described *Diasporus* calls is a single, unpulsed note that is constantly emitted, usually from an elevated

Table 3: Comparison of advertisement call parameters between species and genetic lineages of *Diasporus*

Lineage	Temperature and Relative Humidity Range	Call Length (s)	Call Interval (s)	Rise Time (s)	Call rate [calls per min]	Low Frequency [Hz]	High Frequency [Hz]	Frequency Range [Hz]	Dominant Frequency [Hz]
D. cf. *hylaeformis* (n=9)	T:12.2–15.7°C RH:97–100%	0.048–0.112 (0.084±0.017)	2.103–11.974 (5.190±2.018)	0.002–0.009 (0.005±0.001)	8–23 (14±5)	1620–2900 (2280±360)	3190–4760 (3710±400)	440–2890 (1430±610)	2810–3470 (3060±220)
D. aff. *hylaeformis* 'Fortuna' (n=3)	T:13.1–19.1°C RH:94.7–100%	0.066–0.123 (0.096±0.020)	2.693–7.064 (4.365±1.353)	0.002–0.009 (0.005±0.002)	12–20 (16±4)	1580–2150 (1820±170)	2940–3500 (3190±180)	1130–1780 (1370±140)	2530–2720 (2620±63)
D. aff. *hylaeformis* 'Volcán Barú' (n=2)	T:13.5–16.0°C RH:90.7–98.0%	0.120–0.131 (0.120±0.006)	4.228–9.180 (6.000±1.153)	0.003–0.013 (0.005±0.002)	10–12 (11±1)	2420–2560 (2370±140)	3240–3300 (3270±20)	690–1100 (900±160)	2720–2910 (2830±90)
D. ventrimaculatus (Chaves et al. 2009)	T:14 °C RH:n/a	0.079 ±0.009	5.15 ±1.55	n/a	11	2140	2995	855	2495–2605 (2550±55)
D. aff. *vocator* 'Fortuna Depression' (n=1)	T:21.3°C RH:100%	0.030–0.035 (0.033±0.002)	4.769–10.767 (5.946±1.873)	0.005–0.009 (0.007±0.001)	11	3160–3390 (3300±70)	5310–5460 (5380±40)	1970–2210 (2080±90)	4590–4780 (4740±70)
D. aff. *vocator* 'Caribbean' (n=2)	T:24.3°C RH:99.9%	0.030–0.038 (0.033±0.002)	2.479–7.843 (4.713±1.739)	0.003–0.009 (0.006±0.002)	11–20 (15±6)	2900–3470 (3190±200)	5490–6000 (5750±140)	2140–3100 (2560±320)	4410–4780 (4560±170)
D. aff. *tigrillo* 'not collected' (n=1)	T: 19.6°C RH: 100%	0.151–0.165 (0.159±0.005)	2.785–4.160 (3.463±0.428)	0.009–0.053 (0.038±0.016)	18	2030–2060 (2050±8)	3050–3140 (3100±30)	990–1100 (1050±30)	2530–2630 (2620±30)
D. citrinobapheus (n=4)	T: 21.8–24.6°C RH: 93.5–100%	0.112–0.175 (0.146±0.013)	0.638–5.122 (1.805±0.984)	0.005–0.109 (0.053±0.032)	26–41 (35±7)	1910–2510 (2260±170)	3230–3660 (3460±100)	990–1550 (1190±170)	2810–3000 (2920±70)
D. cf. *citrinobapheus* 'East' (n=2)	T: 22.3 °C RH: 97.4%	0.129–0.194 (0.163±0.026)	1.230–4.459 (2.514±0.917)	0.063–0.123 (0.093±0.023)	24–26 (25±1)	2160–2320 (2240±40)	3330–3610 (3450±100)	1080–1360 (1210±90)	2910±3090 (3010±90)
D. igneus (n=1)	T: 16.9°C RH: 78.0%	0.046–0.054 (0.050±0.003)	11.085–31.909 (23.516±6.386)	0.006–0.029 (0.015±0.010)	3	1620–1730 (1660±40)	2640–2790 (2720±40)	970–1160 (1060±60)	2160–2440 (2290±110)
D. sp. 'Colorado' (n=1)	T: 15.7°C RH: 100%	0.097–0.106 (0.102±0.003)	5.142–9.941 (6.671±1.490)	0.010–0.020 (0.015±0.004)	11	2420–3190 (2710±280)	4870–5310 (5060±150)	1710–2890 (2350±410)	3750–3840 (3790±50)

position. All *Diasporus* calls used in this study are considered to be advertisement calls, because all males recorded showed no direct physical interactions with other frogs that could have provoked other call types. The general structure of the analyzed calls matches the advertisement calls that have been described for members of this genus before. The *Diasporus* cf. *hylaeformis* clade is the only one in which enough specimens from a single locality (n=9) have been recorded to statistically test the effect of temperature and body size

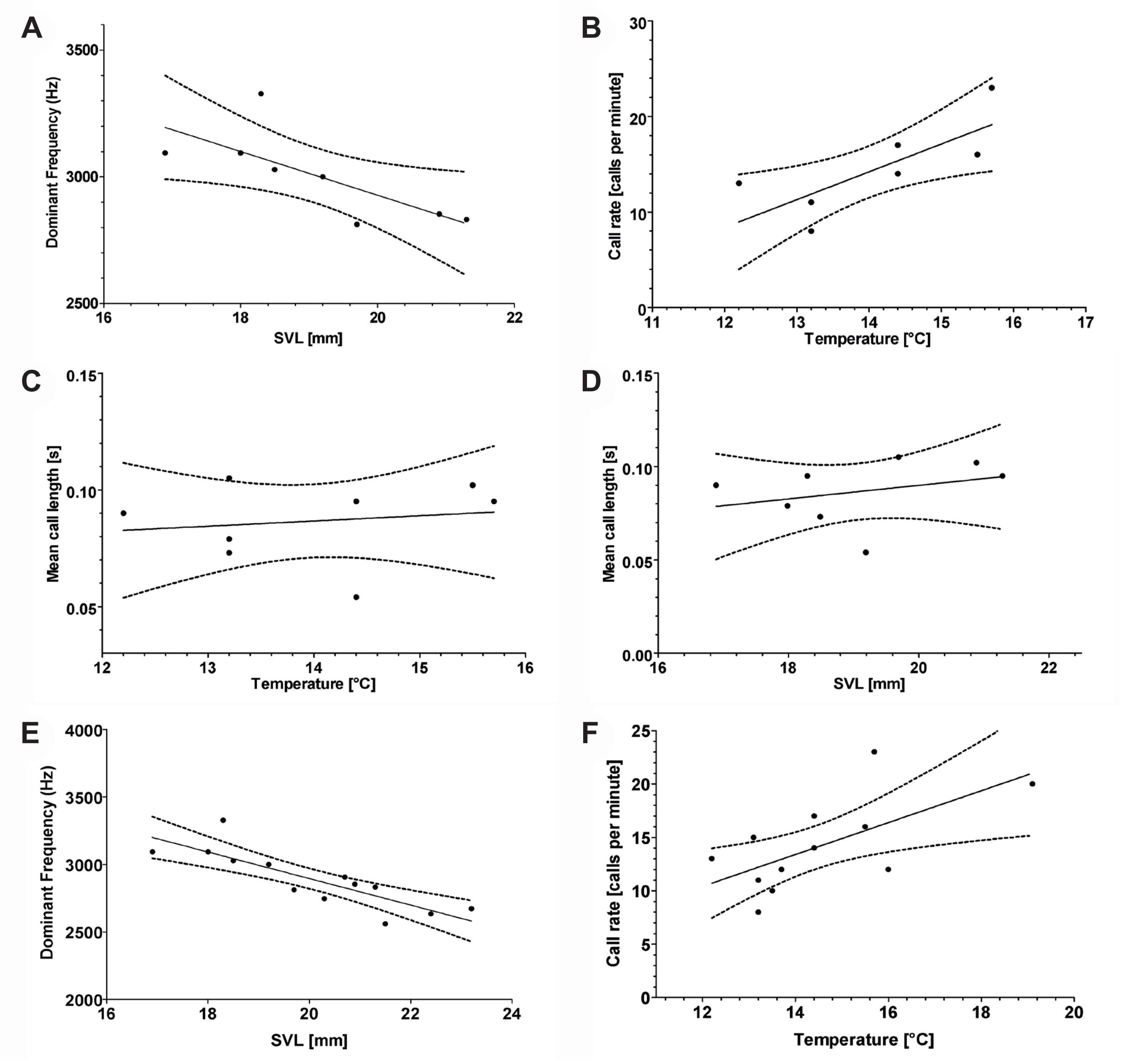

Figure 16: Linear regressions of body size and temperature versus selected call parameters in specimens of the *Diasporus* cf. *hylaeformis* clade. Dashed lines show the 95% confidence interval. **A–D** All specimens recorded at Cerro Pando and suroundings. **A** Mean dominant frequency versus snout-vent length. **B** Call rate versus temperature. **C** Mean call length versus temperature. **D** Mean call length versus snout-vent length. **E+F** All specimens of the *D.* cf. *hylaeformis* clade from all localities. **E** Mean dominant frequency versus snout-vent length. **F** Call rate versus temperature.

on the call. This has not been tested for any *Diasporus* before and I assume that the results are also true for other members of the genus. My data indicate a negative correlation between the mean dominant frequency and body size with a regression slope that is significantly different from zero (P=0.036) (Fig. 16A), albeit the effect is minor and there is only a difference of approximately 700 Hz between small (16.9 mm) and large (23.1 mm) individuals. Moreover, call rate is positively correlated with temperature with a regression slope that is significantly different from zero (P=0.023) (Fig. 16B). Both correlations (frequency/SVL, P=0.0003; call rate/temperature, P=0.0165) are also observed when testing all specimens in the *D.* cf. *hylaeformis* clade (Fig. 16E,F). However, there is neither an evident correlation between call length and temperature (P=0.701) nor between call length and body size (P=0.444) (Fig. 16C,D).

The mayor genetic clades are reflected by three different, generalized waveform types (Fig. 15). Specimens of the *Diasporus hylaeformis* species complex and the *D. vocator* species complex emit a "tink" call typical of the genus that is characterized by having the most energy at the beginning of the call, what is also expressed by a shorter mean rise time of 5–7 ms (Tab. 3). After highest energy is reached, it is rapidly descending, what gives the waveform a pointed, funnel-shaped appearance. Calls of this type are usually around 0.1 s or shorter in length. However, the calls of the members of the *D. vocator* species complex are much shorter and have a less abrupt decrease of energy in the call than members of the *D. hylaeformis* species complex what makes both complexes easy to differentiate acoustically. The calls of specimens in the *D. citrinobapheus*-*tigrillo* clade sound more like a "whistle" what is expressed in the waveform by a more consistent distribution of energy for the duration of the call and a mean rise time of 38–93 ms, which means that the dominant frequency is reached somewhere in the middle of the call what results in a sausage-shaped waveform. With 1.5 s or more, call length is clearly longer. However, the call recordings of a presumed *D*. aff. *tigrillo* from Palo Seco can only tentatively be used for a preliminary analysis since the identity of the recorded specimen is not confirmed. The specimen, a yellow *Diasporus*, escaped after the recording. The assumption that the recorded specimen was a *D*. aff. *tigrillo* is supported by the fact that *D. citrinobapheus* was not detected at this site.

Although the calls of *Diasporus igneus* and *D*. sp. 'Colorado' sound like a whistle too, their call is much shorter than in the before mentioned clade. While in *D*. sp. 'Colorado' mean call length is about 0.1 s, it is only 0.05 s in *D. igneus* (Fig. 17). The waveform is funnel-shaped, but the energy is not so rapidly descending as in the *D. hylaeformis* species complex and the *D. vocator* species complex. Besides call length and shape of the waveform, dominant frequency is apparently the best character to distinguish between the individual clades. The calls of most recorded *Diasporus* specimens have a mean dominant frequency of around 3000 Hz (Fig. 17). The clades in the *D. hylaeformis* complex vary in mean dominant frequency at a magnitude of about 440 Hz, what is less than the variation observed in specimens of the *D.* cf. *hylaeformis* clade in relation to body size of the individual frog. However, calls of *D.* aff. *vocator* specimens, both from the Caribbean and the Fortuna depression, are clearly distinct in terms of mean dominant frequency with 4560 Hz and 4740 Hz respectively. The

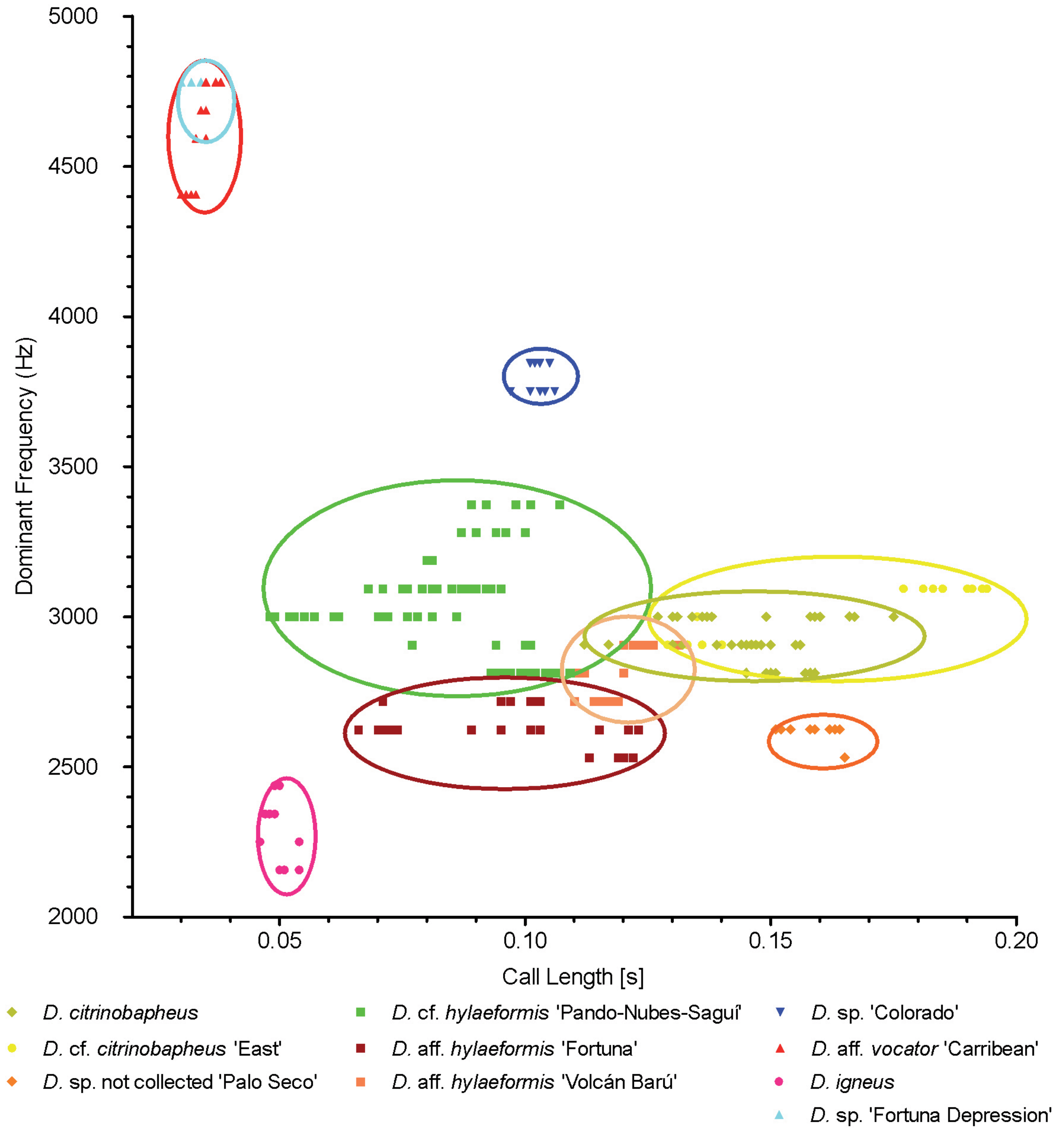

Figure 17: Comparison of dominant frequency versus call length in different *Diasporus* clades. Ovals outline the variation in the respective genetic lineages.

only difference among one another is a frequency modulation in the call of the Fortuna Depression clade specimen, while there is no observable modulation in the Caribbean clade specimens. The modulation is characterized by a rapid frequency rise within the first 0.008 s from about 4000 to 5000 Hz before falling again to slightly more than 4000 Hz. The only other clade that calls at a mean dominant frequency of clearly more than 3000 Hz is *D.* sp. 'Colorado' with a mean dominant frequency of 3790 Hz. The lowest frequencies are produced by *D. igneus* that calls at a dominant frequency of 2290 Hz. Since the calls of *Diasporus* species are very similar-sounding and simple in structure the best parameters to be used as distinctive characters are a combination of call length and dominant frequency (Fig. 17). The differences in these parameters are more pronounced in specimens that have been collected in sympatry (Fig. 18). In most sympatric species either dominant frequency, call length or both is significantly different (Tab. 4). The only exception is *D.* cf *hylaeformis* versus *D.* sp.

Table 4: The Kruskel-Wallace one-way analysis of variance showed if the medians of the analyzed parameters vary significantly ($P < 0.05$) between the analyzed groups. Shown are the results of Dunn´s post-hoc comparison test comparing the medians of the scatter plots of three selected call parameters in ten groups of the genus *Diasporus*, showing which groups differ significantly from others. The last column indicates whether both lineages occur syntopic or not.

Clades compared	Dominant frequency significantly different P<0.05?	Call length significantly different P<0.05?	Rise time significantly different P<0.05?	Known to occur syntopic
D. citrinobapheus vs. *D. citrinobapheus* 'East'	No	No	No	No
D. citrinobapheus vs. *D.* sp. not collected 'Palo Seco'	Yes	No	No	No
D. citrinobapheus vs. *D.* aff. *hylaeformis* 'Fortuna'	Yes	Yes	Yes	No
D. citrinobapheus vs. D. aff. *hylaeformis* 'Pando'	No	Yes	Yes	No
D. citrinobapheus vs. *D.* aff. *hylaeformis* 'Volcan'	No	No	Yes	No
D. citrinobapheus vs. *D.* sp. 'Colorado'	Yes	No	No	No
D. citrinobapheus vs. *D.* aff. *vocator* 'Fortuna Depression'	Yes	Yes	No	Yes
D. citrinobapheus vs. *D.* aff. *vocator* 'Caribbean'	Yes	Yes	Yes	No
D. citrinobapheus vs. *D. igneus*	Yes	Yes	No	No
D. citrinobapheus 'East' vs. *D.* sp. not collected 'Palo Seco'	Yes	No	No	No
D. citrinobapheus 'East' vs. *D.* aff. *hylaeformis* 'Fortuna'	Yes	Yes	Yes	No
D. citrinobapheus 'East' vs. *D.* aff. *hylaeformis* 'Pando'	No	Yes	Yes	No
D. citrinobapheus 'East' vs. *D.* aff. *hylaeformis* 'Volcan'	No	No	Yes	No
D. citrinobapheus 'East' vs. *D.* sp. 'Colorado'	No	No	No	No
D. citrinobapheus 'East' vs. *D.* aff. *vocator* 'Fortuna Depression'	Yes	Yes	Yes	No
D. citrinobapheus 'East' vs. *D.* aff. *vocator* 'Caribbean'	Yes	Yes	Yes	No
D. citrinobapheus 'East' vs. *D. igneus*	Yes	Yes	No	No
D. sp. not collected 'Palo Seco' vs. *D.* aff. *hylaeformis* 'Fortuna	No	Yes	Yes	No

Table 4 continued

Clades compared	Dominant frequency significantly different P<0.05?	Call length significantly different P<0.05?	Rise time significantly different P<0.05?	Known to occur syntopic
D. sp. not collected 'Palo Seco' vs. *D*. cf. *hylaeformis*	Yes	Yes	Yes	No
D. sp. not collected 'Palo Seco' vs. *D*. aff. *hylaeformis* 'Volcan'	No	No	Yes	No
D. sp. not collected 'Palo Seco' vs. *D*. sp. 'Colorado'	Yes	No	No	No
D. sp. not collected 'Palo Seco' vs. *D*. aff. *vocator* 'Fortuna Depression'	Yes	Yes	No	Yes
D. sp. not collected 'Palo Seco' vs. *D*. aff. *vocator* 'Caribbean'	Yes	Yes	Yes	No
D. sp. not collected 'Palo Seco' vs. *D*. *igneus*	No	Yes	No	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. aff. *hylaeformis* 'Pando'	Yes	No	No	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. aff. *hylaeformis* 'Volcan'	No	No	No	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. sp. 'Colorado'	Yes	No	Yes	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. aff. *vocator* 'Fortuna Depression'	Yes	Yes	No	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. aff. *vocator* 'Caribbean'	Yes	Yes	No	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. *igneus*	No	No	Yes	No
D. aff. *hylaeformis* 'Fortuna' vs. *D*. *igneus*	No	No	Yes	No
D. cf. *hylaeformis* vs. *D*. aff. *hylaeformis* 'Volcan'	Yes	Yes	No	No
D. cf. *hylaeformis* vs. *D*. sp. 'Colorado'	No	No	Yes	Yes
D. cf. *hylaeformis* vs. *D*. aff. *vocator* 'Fortuna Depression'	Yes	No	No	No
D. cf. *hylaeformis* vs. *D*. aff. *vocator* 'Caribbean'	Yes	Yes	No	No
D. cf. *hylaeformis* vs. *D*. *igneus*	Yes	No	Yes	Yes
D. aff. *hylaeformis* 'Volcan' vs. *D*. sp. 'Colorado'	Yes	No	Yes	No
D. aff. *hylaeformis* 'Volcan' vs. *D*. aff. *vocator* 'Fortuna Depression'	Yes	Yes	No	No
D. aff. *hylaeformis* 'Volcan' vs. *D*. aff. *vocator* 'Caribbean'	Yes	Yes	No	No
D. aff. *hylaeformis* 'Volcan' vs. *D*. *igneus*	No	Yes	Yes	No
D. sp. 'Colorado' vs. *D*. aff. *vocator* 'Fortuna Depression'	No	Yes	No	No
D. sp. 'Colorado' vs. *D*. aff. *vocator* 'Caribbean'	No	Yes	No	No
D. sp. 'Colorado' vs. *D*. *igneus*	Yes	No	No	Yes
D. aff. *vocator* 'Fortuna Depression' vs. *D*. aff. *vocator* 'Caribbean'	No	No	No	No
D. aff. *vocator* 'Fortuna Depression' vs. *D*. *igneus*	Yes	No	No	No
D. aff. *vocator* 'Caribbean' vs. *D*. *igneus*	Yes	No	No	No

'Colorado' when all recorded individuals are included in the analysis. However, comparing only the two individuals that where found in sympatry using a Mann Whitney test, significant differences in both dominant frequency (p=0.0001) and call length are revealed (p=0.0005) (Fig. 18).

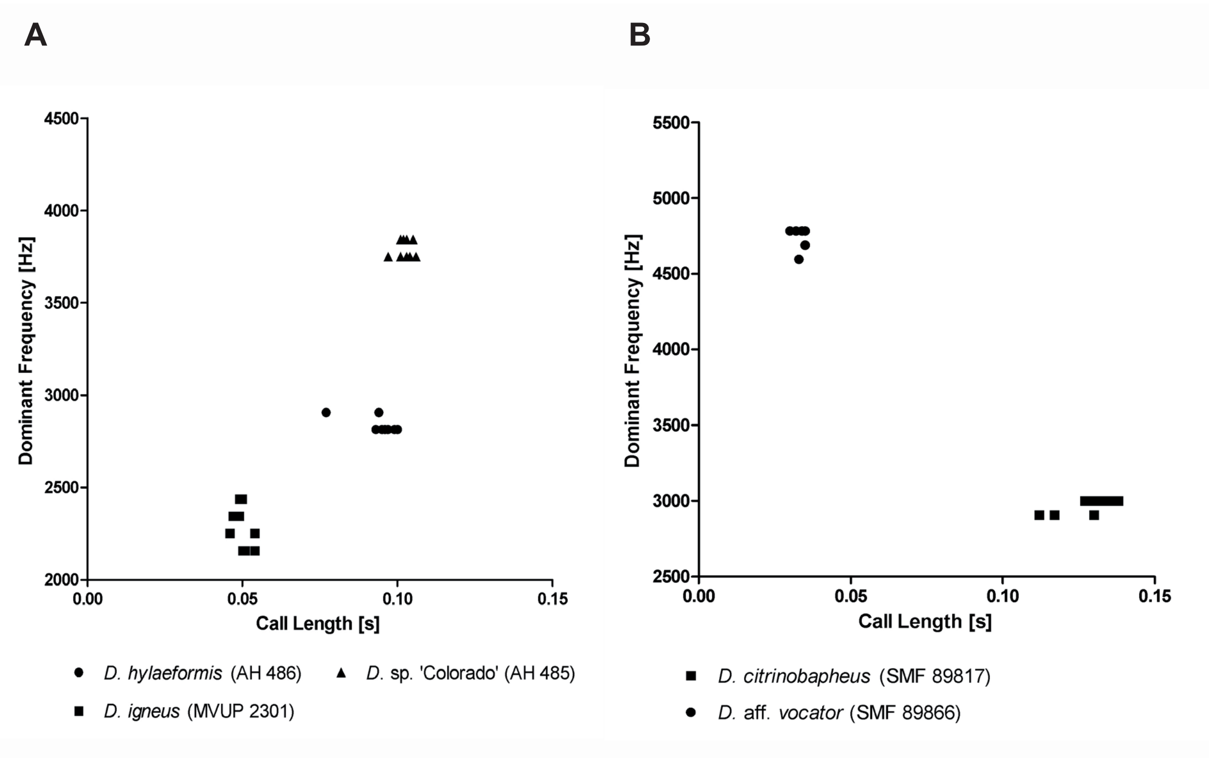

Figure 18: Scatter plots for direct comparison of dominant frequency versus call length in syntopic *Diasporus* specimens. **A** Data for three syntopic specimens of different genetic lineages collected in the Cerro Colorado area. **B** Data for two syntopic specimens of different genetic lineages collected at Willi Mazú.

6.1.3. MORPHOLOGY

The species in the genus *Diasporus* are very similar in body form and general appearance. Thus, morphometric parameters are not the best characters to distinguish between species, as they are largely overlapping (Tab. 5). Additionally, qualitative characters can be used to characterize species morphologically (Tab. 6). If a larger set of calling and thus presumed adult males are available, SVL can be used as a distinctive feature. Generally, *Diasporus* species are small, usually not exceeding 20 mm SVL and there is only a minor difference between the sexes. The largest species so far is *D. igneus* with a mean snout-vent length of 26.10±0.5 mm SVL in males, females are unknown (Batista *et al.* 2012). Other species that reach a SVL of more than 20 mm, albeit considerably smaller than *D. igneus*, are *D. ventrimaculatus* with 21.8±1.2 mm (Fig. 21A,B; Chaves *et al.* 2009) and the members of the *D. hylaeformis* complex (Tab. 5). In the latter species complex, with a mean snout-vent length of 22.7±1.2 mm the clade from La Fortuna is on average larger than specimens of the complex from the Cordillera Central and Volcán Barú. However, with 27 mm SVL the holotype of *D. hylaeformis* (Fig. 19B) is still larger than any *Diasporus* I ever saw, even larger than *D. igneus*. According to Cope (1876) the holotype of *D. hylaeformis* is a male, as indicated by the presence of a “large vocal sac” that is not visible anymore, because of the poor condition of the holotype.The smallest specimens are found in the *D.* aff. *vocator* clades, whereas specimens in the Caribbean clade with a mean snout-vent length of 14.9±2.52 mm in males and 14.67±0.79 mm in females are clearly smaller than those in the Fortuna Depression clade with 17.4±1.56 mm snout-vent length in males and 21.2 mm in a single female. The three genetic lineages of *D. hylaeformis*-like specimens show large variation in morphology

Table 5: Morphological proportions of *Diasporus* species and lineages (min–max, mean±SD). Values for *D. tigrillo* and *D. igneus* were taken from the original descriptions (Savage 1997; Batista et al. 2012). See Materials and Methods for abbreviations.

Lineage	n	sex	SVL [mm]	DWF III/ LF III	DWT IV/ LT IV	HL/SVL	HW/SVL	HW/HL
D. cf. *hylaeformis*	20	m	16.90–23.10 (19.97±1.60)	0.27–0.40 (0.32±0.04)	0.27–0.40 (0.32±0.04)	0.35–0.43 (0.39±0.02)	0.32–0.39 (0.36±0.02)	0.82–1.00 (0.92±0.05)
	8	f	19.20–21.80 (21.10±0.94)	0.27–0.36 (0.32±0.03)	0.27–0.36 (0.32±0.03)	0.37–0.42 (0.40±0.02)	0.34–0.40 (0.37±0.02)	0.85–0.96 (0.91±0.04)
D. aff. *hylaeformis* ‘Fortuna’	9	m	21.10–25.10 (22.7±1.2)	0.31–0.42 (0.36±0.04)	0.18–0.28 (0.25±0.03)	0.36–0.42 (0.38±0.02)	0.34–0.39 (0.37±0.02)	0.92–1.01 (0.97±0.03)
	1	f	24.6	0.28	0.23	0.36	0.36	1
D. aff. *hylaeformis* ‘Volcán’	4	m	20.00–21.20 (20.55±0.49)	0.29–0.33 (0.31±0.02)	0.21–0.25 (0.23±0.02)	0.33–0.37 (0.35±0.01)	0.32–0.36 (0.34±0.01)	0.95–0.97 (0.97±0.01)
	1	f	20.7	0.35	0.24	0.36	0.33	0.92
D. aff. *vocator* ‘Caribbean’	3	m	12.20–17.20 (14.90±2.52)	0.19–0.31 (0.24±0.06)	0.13–0.19 (0.17±0.03)	0.35–0.42 (0.38±0.03)	0.31–0.36 (0.33±0.03)	0.79–0.96 (0.87±0.08)
	6	f	13.60–15.70 (14.67±0.79)	0.24–0.42 (0.32±0.07)	0.14–0.19 (0.17±0.02)	0.35–0.41 (0.38±0.03)	0.31–0.36 (0.34±0.02)	0.86–0.95 (0.89±0.04)
D. aff. *vocator* ‘Fortuna Depression’	2	m	16.80–18.50 (17.40±1.56)	0.32–0.38 (0.35±0.05)	0.16–0.28 (0.22±0.08)	0.34–0.37 (0.35±0.02)	0.35–0.36 (0.35±0.00)	0.95–1.06 (1.01±0.08)
	1	f	21.2	0.32	0.24	0.38	0.38	1
D. sp. ‘Pacific Lowland’	1	m	19.5	0.43	0.26	0.37	0.35	0.93
	1	f	24.7	0.37	0.2	0.34	0.32	0.96
D. tigrillo	2	m	16.00–17.50 (16.75±1.06)	–	–	0.38–0.40 (0.39±0.01)	0.34–0.37 (0.36±0.02)	0.85–0.99 (0.92±0.10)
D. aff. *tigrillo*	2	m	17.60–18.00 (17.80±0.28)	0.27–0.29 (0.28±0.02)	0.14–0.18 (0.16±0.03)	0.40–0.43 (0.41±0.02)	0.38–0.39 (0.38±0.01)	0.91–0.94 (0.93±0.03)
	1	f	23.5	0.38	0.23	0.38	0.35	0.92
D. citrinobapheus	8	m	17.30–19.60 (18.52±0.74)	0.18–0.28 (0.24±0.04)	0.11–0.19 (0.15±0.03)	0.38–0.44 (0.40±0.02)	0.35–0.38 (0.37±0.01)	0.86–0.97 (0.92±0.05)
	1	f	21.8	0.23	0.17	0.4	0.36	0,90
D. cf. *citrinobapheus*	2	m	19.70–21.60 (20.65±1.34)	0.22	0.12–0.16 (0.14±0.03	0.37–0.40 (0.39±0.02)	0.33–0.37 (0.35±0.02)	0.90–0.91 (0.90±0.01)
D. cf. *diastema*	19	m	15.90–22.10 (18.69±2.58)	0.21–0.48 (0.36±0.07)	0.13–0.32 (0.23±0.05)	0.35–0.44 (0.39±0.02)	0.33–0.39 (0.36±0.02)	0.79–1.01 (0.91±0.07)
	22	f	14.60–23.50 (18.69±1.65)	0.21–0.44 (0.32±0.07)	0.11–0.29 (0.22±0.05)	0.36–0.44 (0.41±0.02)	0.32–0.39 (0.36±0.02)	0.78–1.04 (0.90±0.06)
D. igneus	4	m	25.50–26.60 (26.10±0.5)	–	–	0.31–0.33 (0.32±0.08)	0.37–0.38 (0.38±0.02)	–
D. sp. ‘Colorado’	2	m	18.50–18.80 (18.65±0.21)	0.28–0.31 (0.30±0.02)	0.15	0.35	0.33–0.35 (0.34±0.01)	0.95–0.98 (0.97±0.02)
D. sp. ‘Fortuna’	2	m	17.5	0.29	0.20–0.25 (0.23±0.04)	0.34	0.34	1

Table 5 continued

Lineage	n	sex	SL/SVL	EL/IOD	ED/HL	TD/ED	ED/SVL
D. cf. *hylaeformis*	20	m	0.35–0.45 (0.39±0.02)	0.84–1.25 (1.03±0.13)	0.26–0.35 (0.29±0.02)	0.30–0.55 (0.40±0.07)	0.10–0.14 (0.11±0.01)
	8	f	0.35–0.47 (0.39±0.04)	0.88–1.19 (1.06±0.09)	0.22–0.31 (0.28±0.03)	0.40–0.50 (0.45±0.03)	0.09–0.12 (0.11±0.01)
D. aff. *hylaeformis* 'Fortuna'	9	m	0.34–0.39 (0.36±0.02)	1.00–1.27 (1.15±0.14)	0.30–0.37 (0.34±0.02)	0.27–0.40 (0.34±0.04)	0.11–0.13 (0.12±0.01)
	1	f	0.35	1.18	0.31	0.36	0.11
D. aff. *hylaeformis* 'Volcán'	4	m	0.36–0.41 (0.38±0.02)	0.93–1.20 (1.06±0.14)	0.27–0.39 (0.35±0.05)	0.32–0.68 (0.44±0.17)	0.09–0.13 (0.12±0.02)
	1	f	0.39	1	0.36	0.32	0.12
D. aff. *vocator* 'Caribbean'	3	m	0.38–0.43 (0.40±0.02)	0.95–1.24 (1.10±0.14)	0.32–0.35 (0.33±0.02)	0.30–0.44 (0.35±0.08)	0.12–0.13 (0.13±0.01)
	6	f	0.36–0.42 (0.38±0.02)	1.25–1.59 (1.43±0.13)	0.33–0.37 (0.34±0.02)	0.33–0.50 (0.43±0.07	0.12–0.14 (0.13±0.01)
D. aff. *vocator* 'Fortuna Depression'	2	m	0.33–0.38 (0.36±0.04)	1.00–1.04 (1.02±0.03)	0.32–0.33 (0.33±0.01)	0.40–0.48 (0.44±0.05)	0.11–0.12 (0.12±0.01)
	1	f	0.36	1.28	0.34	0.44	0.13
D. sp. 'Pacific Lowland'	1	m	0.42	1.84	0.34	0.3	0.12
	1	f	0.38	1.37	0.34	0.37	0.11
D. tigrillo	2	m	0.46–0.50 (0.48±0.03)	–	0.28–0.35 (0.32±0.05)	0.54–0.57 (0.56±0.02)	0.11–0.13 (0.12±0.01)
D. aff. *tigrillo*	2	m	0.43–0.44 (0.44±0.00)	0.76–1.04 (0.90±0.20)	0.26–0.27 (0.27±0.00)	0.42–0.50 (0.46±0.06)	0.11 (0.11±0.01)
	1	f	0.38	1.13	0.29	0.54	0.11
D. citrinobapheus	8	m	0.40–0.42 (0.41±0.01)	0.83–1.13 (0.98±0.11)	0.28–0.36 (0.33±0.03)	0.32–0.45 (0.38±0.05)	0.11–0.15 (0.13±0.01)
	1	f	0.42	0.94	0.32	0.32	0.13
D. cf. *citrinobapheus*	2	m	0.38–0.41 (0.40±0.02)	1.12–1.15 (1.13±0.02)	0.28–0.30 (0.29±0.02)	0.42–0.45 (0.44±0.03)	0.11
D. cf. *diastema*	19	m	0.36–0.51 (0.40±0.04)	0.89–1.24 (1.04±0.10)	0.22–0.35 (0.29±0.03)	0.25–0.50 (0.36±0.07)	0.08–0.13 (0.11±0.01)
	22	f	0.36–0.56 (0.42±0.05)	0.89–1.62 (1.12±0.18)	0.21–0.37 (0.29±0.04)	0.19–0.52 (0.37±0.08)	0.09–0.15 (0.12±0.01)
D. igneus	4	m	0.43–0.44 (0.43±0.06)	–	–	–	0.13–0.14 (0.13±0.07)
D. sp. 'Colorado'	2	m	0.41–0.44 (0.43±0.02)	0.92–1.24 (1.08±0.22)	0.35–0.39 (0.37±0.03)	0.29–0.30 (0.30±0.01)	0.12–0.13 (0.13±0.01)
D. sp. 'Fortuna'	2	m	0.32–0.33 (0.33±0.01)	1.14–1.19 (1.17±0.03)	0.33–0.35 (0.34±0.01)	0.33–0.50 (0.42±0.12)	0.11–0.12 (0.12±0.00)

and coloration and are not distinguishable from each other by morphological traits (Tab. 5; Tab. 6). However, relatively stable morphological characters allow to clearly distinguish between *D.* aff. *vocator* and *D.* aff. *hylaeformis* specimens. Besides the already mentioned differences in adult SVL, morphometric values are overlapping and there is at most a tendency in some characters (Tab. 5). Anyway, qualitative morphological traits like coloration, skin texture, and presence of a supraocular tubercle in *D.* aff. *hylaeformis*, that is absent in *D.* aff. *vocator*, allow to distinguish between both species groups (Tab. 6). In addition to SVL, the two *D.* aff. *vocator* clades differ from one another by the ratio HW/HL, as well as the ratio ED/SVL (Tab. 5).

Coloration is not always the best character to differentiate taxa in anurans. However, in *Diasporus*, there are three clades of exclusively bright-yellow specimens. All three show only few morphological distinguishing features. *Diasporus citrinobapheus* and *D.* cf. *citrinobapheus* are very similar, but males of the latter clade are slightly larger (SVL: 20.65±1.34 mm vs. 18.52±0.74 mm) and have on average longer eye lids in relation to the interorbital distance (1.13±0.02 vs. 0.98±0.11). The third clade is referred to as *D.* aff. *tigrillo*, because of the dorsal skin that bears low pustules, in contrast to the smooth skin of the two *D. citrinobapheus* clades. Further, males are on average smaller (SVL: 17.80±0.28 mm), whereas the single known female is conspicuously larger (SVL: 23.50 mm) than the only known female of *D. citrinobapheus* (SVL: 21.80 mm). The head of *D.* aff. *tigrillo* is more slender in relation to snout vent length (0.38±0.01) than *D. citrinobapheus* (0.37±0.01)

Table 6: Main diagnostic characters and character states for species and genetic lineages of the genus *Diasporus*.

Species or lineage	Dorsal Color	Ventral Color	Male vocal sac coloration	Groin color	Skin texture	Eyelid tubercle	Tympanum conditions	Disk cover	Ulna tubercles
D. anthrax	Black with short red lines	Black with white flecks	Black with white flecks	Scarlet	Smooth with flat pustules	Absent	Prominent	Spadate	Absent
D. citrinobapheus	Yellow orange	Transparent	Yellow	Orange	Smooth	Absent	Concealed but visible	Spadate	Absent
D. cf. *citrinobapheus* 'East'	Yellow orange	Transparent	White	Orange	Smooth	Absent	Concealed but visible	Spadate	Absent
D. diastema	Dark brown	Pale brown	Pale brown	Yellow to Orange	Smooth with a few scattered tubercles	Absent	Indistinct, but visible ventrally	Palmate to spadate	Absent
D. gularis	Pale brown to yellow often with black spots	White	White	Yellow	Smooth	Absent	Distinct	Spadate	Absent
D. hylaeformis	Tan, gray to almost black, vertebral stipe	Yellowish to orange	Rose color	Yellow to orange	Smooth	n/a	Distinct	Spadate	Absent
D. cf. *hylaeformis*	Reddish brown to ocher, light gray, vertebral stripe common	Reddish, gray or white	Red or Yellow	Reddish, gray or white	Almost smooth to warty	Present	Concealed under pigmented skin	Expandet palmate to spadate	Present or absent
D. aff. *hylaeformis* 'Fortuna'	Reddish brown to ocher, vertebral stripe common	Reddish brown to ocher	Red or Yellow	Reddish brown to ocher	Smooth with scattered tubercles to very warty	Present, interorbital ridge in some specimens	Concealed under pigmented skin	Expanded, palmate	Present or absent
D. aff. *hylaeformis* 'Volcán'	Dark chocolate brown, beige to light gray, vertebral stripe common	Gray	Yellow	Gray to brown	Smooth with scattered tubercles to very warty	Present interorbital ridge in some specimens	Concealed under pigmented skin	Expanded, palmate	Present or absent

Table 6 continued

Species or linage	Dorsal Color	Ventral Color	Male vocal sac coloration	Groin color	Skin texture	Eyelid tubercle	Tympanum conditions	Disk cover	Ulna tubercles
D. igneus	Brownish with yellow to orange reticulations	Yellow	Yellow orange	Scarlet	Smooth with scattered tubercles	Present	Partially visible	Expanded, palmate to truncate	Present
D. quidditus	Brown with orange high-lights	Black with white flecks	Black with white flecks	Brown	warty	Absent	Prominent, upper edge concealed by supratympanic fold	Papillate	Absent
D. tigrillo	Yellow orange with each pustule dark brown	Yellow with many dark punctations	Immaculate yellow	Yellow to orange	Smooth with low pustules	Absent	Indistinct	Spadate	Absent
D. aff. *tigrillo*	Yellow orange with some pustules dark brown	Transparent	Immaculate yellow	Yellow to orange	Smooth with low pustules	Absent	Indistinct	Spadate	Absent
D. tinker	Brown	Dirty white with brown marbling	Pale brown	Brown to orange	Smooth	Absent	Prominent, upper edge concealed by supratympanic fold	Papillate	Absent
D. ventrimaculatus	Red to pink	Red to pink	Yellow, orange to bright red	Red or beige	Aerolate	Absent	Distinct	Spadate	Absent
D. vocator	Dark gray to black	Pigmented with dark mottling and light areas	Pale yellow	Brown	Finely shagreened	Absent	Concealed under pigmented skin but visible	Lanceolate	Absent
D. aff. *vocator* 'Caribbean'	Brown	Gray	Yellow	Gray	Smooth	Very low	Concealed under pigmented skin but visible	Spadate to lanceolate	Absent
D. aff. *vocator* 'Fortuna Depression'	Reddish brown	Dirty white with dark mottling	Dirty white suffused with yellow	Yellowish	Smooth	Very low	Concealed under pigmented skin, indistinct	Expandet spadate	Absent
D. sp. 'Pacific Lowland'	Brown reticulation on light ground	Light gray	n/a	Gray	Smooth	Absent	Concealed under pigmented skin but visible	Expandet, palmate	Absent

or *D.* cf. *citrinobapheus* (0.35±0.02). Additionally, *D.* aff. *tigrillo* has a longer shank in relation to the snout-vent length, than *D. citrinobapheus*-like specimens (Tab. 5) as it is the case in the types of *D. tigrillo* (Hertz *et al.* 2012b; Tab. 5). Finally, there are three genetical lineages for which no names are available. The first was collected in the Pacific lowlands of western Panama. Of *D.* sp. 'Pacific Lowland' only one male specimen was collected, but this is distinguished from other examined specimens in the genus by an extraordinary short third finger in relation to the width of the finger´s disk (DW/LF III: 0.43; compare in Tab. 5). I assume another female specimen from the SMF collection (SMF 92008), collected at the Pacific lowlands of El Chorogo, to be conspecific with the before mentioned male, because of the geographical proximity of the collection sites and morphological similarities. This specimen is relatively large (SVL: 24.2 mm) and has also a relatively short third finger in relation to the width of the finger´s disk (DW/LF III: 0.37). The other two clades revealed from molecular data contain two males, respectively. The *D.* sp. 'Colorado' specimens were collected at Cerro Santiago and Cerro Saguí, both located in the Cerro Colorado region. There are no morphometric features to differentiate this clade from other clades in my analysis, but a combination of qualitative morphological characters can be used to define this clade (Tab. 6). Another two males of an unnamed clade have been collected at Cerro Pata de Macho in the La Fortuna Forest Reserve, referred to as *D.* sp. 'Fortuna' in this study. Both specimens are comparatively small (SVL in both: 17.50 mm), but just as in the before mentioned clade there are no morphometric features to distinguish between them, but only qualitative morphological characters. The coloration of the sequenced specimen bearing greenish-yellow flanks is very distinctive, a character that was not found in any other specimen of the genus. With the exception of a bright yellow vocal sac, ventral surfaces are transparent. It further has several small eyelid tubercles, ulnar tubercles, and a tuberculated dorsal skin texture. This combination is unique among the examined *Diasporus* specimens (Tab. 6).

6.1.4. CONCLUSIONS

Appliance of the definitions for candidate species as proposed by Vieites *et al.* (2009) leads to several unnamed species. There are three genetically distinct clades of *Diasporus hylaeformis*-like specimens. The specimens from Cerro Pando and Las Nubes and their close relatives from the Cerro Colorado region (within group p-distance: 0.09%) are called *D.* cf. *hylaeformis* herein, because of the geographical proximity of Cerro Utyum in the Serranía de Talamanca of Costa Rica, the type locality of *D. hylaeformis*. However, since I have no sequences or other data of specimens from the type locality and the holotype of *D. hylaeformis* is in a poor condition (Fig. 19B) this identification is tentative. Two additional clades of *D.* aff. *hylaeformis* specimens

Figure 19: *Diasporus* types in the USNM collection (scale bar 10 mm): **A** Lectotype of *D. diastema* (USNM 25170); **B** Holotype of *D. hylaeformis* (USNM 30687).

from Cerro Pando and Volcan Barú have considerable differences in the 16S mtRNA gene, both between each other and to the *D.* cf. *hylaeformis* specimens. Although all three clades have not been found in sympatry ongoing gene flow is presumed. The Fortuna Depression is supposed to be a barrier for highland species between the Serranía de Talamanca and the Serranía de Tabasará, but since there are specimens from both mountain ranges in the *D.* cf *hylaeformis* clade, this barrier did obviously not impede migration across the depression by *D. hylaeformis*-like specimens, at least not long enough. Likewise, the high valley separating Volcán Barú from the main Cordillera Central lies continuously above 2000 m asl and thus presumably has not served long enough as an effective barrier to provoke divergent species. Problematic is the polyphyly of the *D. hylaeformis* clades in the phylogenies. However, from morphological and bioacoustic data I doubt that the *D. vocator* clades are placed correctly. Additionally, the polytomy in this branch gives enough evidence for a erroneous placement of the *D. vocator* clades. Further, two specimens on GenBank collected in Costa Rica are involved in the polytomy of *D. hylaeformis*. I prefer to call the GenBank samples *D*. sp. since I have not seen these specimens. One of these is specified as *D. diastema* (MVZ 203844) collected in the Serranía de Talamanca at 1240 m asl, the other as *D. hylaeformis* (UCR 16264) collected in the Cordillera de Tilarán at roughly 1000 m asl. From the locality and altitude data I think it is unlikely that MVZ 203844 is conspecific with *D. diastema*. I rather follow the results of my phylogenetic analyses by placing both specimens in the relationship of *D. hylaeformis*. Thus, when the *D. vocator* specimens are excluded from the clade, the polytomy of *D. hylaeformis* is resolved. Therefore, I treat all my three *D. hylaeformis* clades as Deep Conspecific Linages (Vieites *et al.* 2009) what is also supported by the lack of morphological differences (Tab. 5,6) and the high conformity in call parameters (Tab. 3, Fig. 17). There are no significant differences in the analyzed call parameters between the clades from Fortuna and Volcán Barú, whereas only dominant frequency is significantly different between *D.* cf. *hylaeformis* and the other two clades (Tab. 4). However, this is explainable by differences in male body size, since the collected males of *D.* cf. *hylaeformis* are on average smaller than the ones from the Volcán Barú and Fortuna clades (Tab. 5). There is a significant correlation observable when plotting snout-vent length of all *D.* cf. *hylaeformis* specimens against their respective mean dominant frequency (Fig. 16E). Further, call length is significantly different between the *D.* cf. *hylaeformis* and the *D.* aff. *hylaeformis* 'Volcán' clades (Tab. 4). This is inexplicable at the current state of research, since my data show that call length is neither correlated with body size nor temperature (Fig. 16C,D).

The two clades of *D.* aff. *vocator* show a partially extremely low p-distance to *D.* cf. *hylaeformis*, *e.g.*, only 1.5% between *D.* aff. *vocator* 'Caribbean' and *D.* cf. *hylaeformis,* and only 0.8% between the latter and *D.* aff. *vocator* 'Fortuna Depression'. In spite of that, morphology and bioacoustics give reason to consider *D.* aff. *vocator* as distinct from any *D. hylaeformis*-like species. Although the two clades of *D.* aff. *vocator* were placed on different branches in both trees and show a genetic p-distance of not less than 2.3%, they are very similar in general appearance. Anyway, differences in morphology are evident, *e.g.*, the head is longer than broad in the Caribbean clade, while almost as broad as long in the Fortuna Depression clade and the eye in relation to body size is much larger in the Caribbean clade than in the Fortuna clade (Tab. 5). On the contrary, the calls are almost identical with no significant differences in any analyzed parameter with exception of the frequency modulation in the specimen from the Fortuna Depression (Tabs. 3, 4; Figs. 17, 32). Further research and a larger sample size is necessary to clarify the taxonomic status of both clades. For now, I will tentatively treat them as conspecific. Batista *et al.* (2012) mentioned unpublished values for the dominant frequency of *D. vocator* from the Pacific versant of western Panama, that supposedly lie between 4600 and 4900 Hz, thus in a similar frequency range as *D.* aff. *vocator*. However, if the GenBank sequence of *D. vocator* is not based on a misidentified specimen, I am convinced that neither one nor the other clade of *D.* aff. *vocator* is conspecific with *D. vocator*. I suspect *D. vocator* to be an endemic of the Golfo Dulce region. The genetic p-distance of 6.4–7.9% to *D. vocator* is high enough to treat *D.* aff. *vocator* as, at least one, Unconfirmed Candidate Species (Vieites *et al.* 2009). However, since I cannot be absolutely sure about the identity of the *D. vocator* specimen on GenBank I will treat them all together under the name *D. vocator* for now.

Another clade with unresolved taxonomy is the one of two specimens from the Pacific lowlands, here called *D.* sp. 'Pacific Lowland'. The collection sites of the examined specimens are relatively near to the type locality of *D. vocator*, but differ morphologically from *D. vocator* by a larger adult size, different coloration, and differently shaped finger disks (Tab. 5, Tab. 6), according to these characters *D.* sp. 'Pacific Lowland' is morphologically closer to *D. diastema*. Additionally, the genetic p-distance of *D.* sp. 'Pacific Lowland' to the GenBank sequence of the presumably real *D. vocator* 'Las Cruces' is 6.4%. However, the type locality of *D. diastema* is on the Caribbean coast of central Panama from where no comparative material was available. Another aggravating factor is the poor condition of the holotype of *D. diastema* that lacks most relevant morphological characters (Fig. 19A).

Although, I recorded the advertisement call of the sequenced male specimen of *D.* sp. 'Pacific Lowland', the storage medium with the recording got stolen on my way back before any analysis could be performed. All these reasons force me to treat this clade as Unconfirmed Candidate Species (Vieites *et al.* 2009) until additional material is available.

The three clades of exclusively bright yellow *Diasporus* are distinct from other lineages in my sample in terms of coloration, male advertisement call and DNA barcoding, but are relatively similar among each other. *Diasporus* aff. *tigrillo* has a considerable p-distance to all its analyzed congeners of more than 4% and scattered low pustules that are lacking in the other two closely related clades. The presence of widely separated low pustules is a character that was used by Savage (1997) as a distinctive feature to diagnose *D. tigrillo* (Fig. 20). Moreover, the type locality of *D. tigrillo* is Río Lari on the Caribbean slopes of the Serranía de Talamanca in Costa Rica and all three specimens examined here have been found on mid-elevations on the Caribbean slopes along the Cordillera Central. Thus, a continuous distribution of *D. tigrillo* along the Caribbean slopes of the Cordillera Central is imaginable. Anyway, besides the holotype and one paratype there is no material of *D. tigrillo* available. Without fresh topotypic material and preferably recordings of the advertisement call the conspecificity of *D.* aff. *tigrillo* and *D. tigrillo* cannot be proved. *Diasporus citrinobapheus* is obviously a very close relative of *D.* aff. *tigrillo* as revealed from molecular and bioacoustic data. Especially specimens from Willi Mazú, the only collection site on the Caribbean slopes, show dark spots on yellow ground (Hertz *et al.* 2012b, Fig. 3CD), but the spots seem to

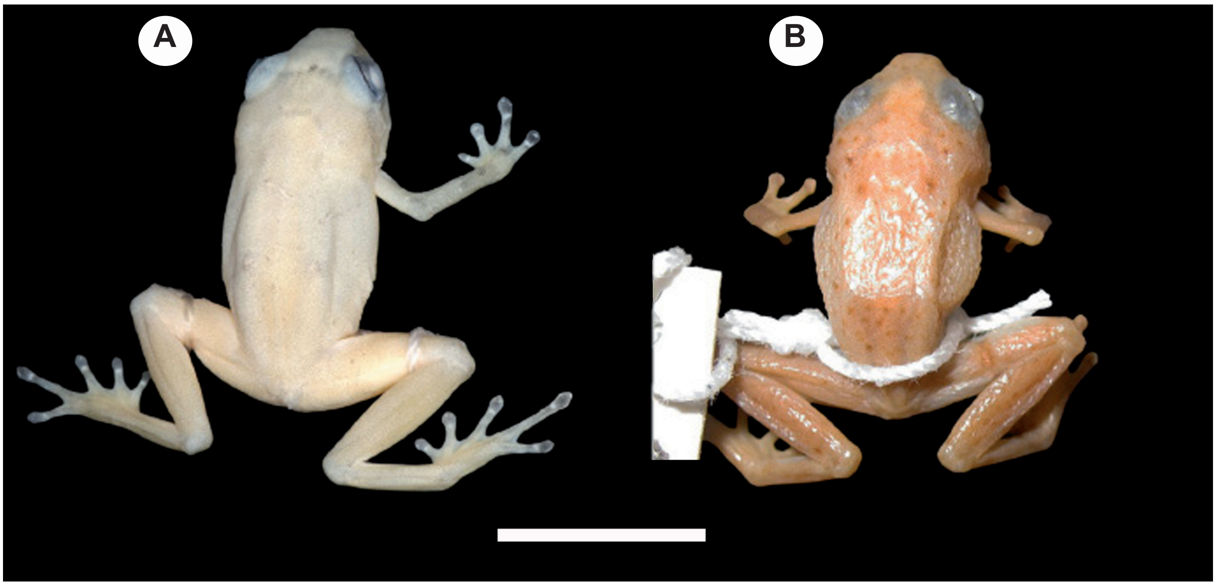

Figure 20: *Diasporus* holotypes in preservative (modified from Hertz et al. 2012b): A *D. citrinobapheus* (SMF 89814); B *D. tigrillo* (LACM 146212; photo courtesy of N. Camacho). Scale bar 10 mm.

be larger than the fine punctuations on the dorsum of the type of *D. tigrillo* (Fig. 20) and never occur in combination with low pustules. *Diasporus citrinobapheus* specimens which I collected at the type locality are immaculate (Hertz *et al.* 2012b, Fig. 3B). The recordings of the advertisement call of an uncollected, but supposed *D.* aff. *tigrillo* of unknown body size from Palo Seco differ significantly from calls of *D. citrinobapheus* in dominant frequency (Tab. 4). Moreover, *D. citrinobapheus* and *D.* aff. *tigrillo* are distinguished by a mean genetic p-distance of more than 4%. Thus, I decide to treat them as different species. The two clades, *D. citrinobapheus* and *D.* cf. *citrinobapheus* 'East', are separated by a 16S p-distance of 1.6% between them. The two male specimens of *D.* cf. *citrinobapheus* are also slightly larger and have a white instead of a yellow vocal sac (Tab. 5, Tab. 6). On the contrary, there are no significant differences in the bioacoustic parameters that would support the assumption of two distinct species. Hertz *et al.* (2012b) argued these might be two different species, mainly because of the results of a haplotype network analysis of the 16S sequences. However, herein I treat both lineages as conspecific, because the molecular, morphological and bioacoustic data do not yield enough differences to support the hypothesis of two different species.

Batista *et al.* (2012) described *D. igneus* on the basis of morphological and bioacoustic data only. The herein presented molecular data strongly support the assumption of *D. igneus* being a valid species. It is very distinct in several morphological characters as well as coloration, advertisement call, and a mean genetic distance of 5.9–9.3% to most other analyzed clades. There are only two clades that are closer relatives in terms of the 16S mtDNA sequence, but still with distances of 3.4 and 4.3% respectively. Together with these two clades, *D. igneus* forms a branch that is well-separated from its other congeners. *Diasporus* sp. 'Colorado' occurs in the same area as *D. igneus* and even syntopic near Llano Tugri on the eastern slopes of Cerro Santiago. Both species are distinct by several characters. *Diasporus* sp. 'Colorado' is much smaller and differently colored than *D. igneus*. Moreover it lacks ulnar tubercles (Tab. 5, Tab. 6). The male advertisement call of *D.* sp. 'Colorado' is measurably, but not significantly longer and dominant frequency is significantly higher than in the call of *D. igneus* (Tab. 4, Fig. 17). Additionally, *D*. sp. 'Colorado' lives syntopic with *D.* cf. *hylaeformis* from which it is well-distinct by genetic, morphology and bioacoustics. Therefore, I treat *D.* sp. 'Colorado' as an undescribed Confirmed Candidate Species (Vieites *et al.* 2009).

As a sister clade to the two previously discussed clades appears a *Diasporus* of greenish coloration from Fortuna. It shows genetic p-distances of 5.9 and 6.6% to other *Diasporus* specimens collected in the area of La Fortuna. Unfortunately, only one specimen was collected that was not calling when encountered (Frank Hauenschild pers. comm.). A second specimen, collected by Nadim Hamad (Hamad 2009), who did not record data of the specimen in life, is assigned to this species on the basis of morphometric measurements that are very close to *D*. sp. 'Fortuna' with exception of the ratio TD/ED (Tab. 5). Although crucial data like male advertisement call are missing, the large p-distances of at least 3.6% to all its congeners and its unique coloration in combination with other morphological characters

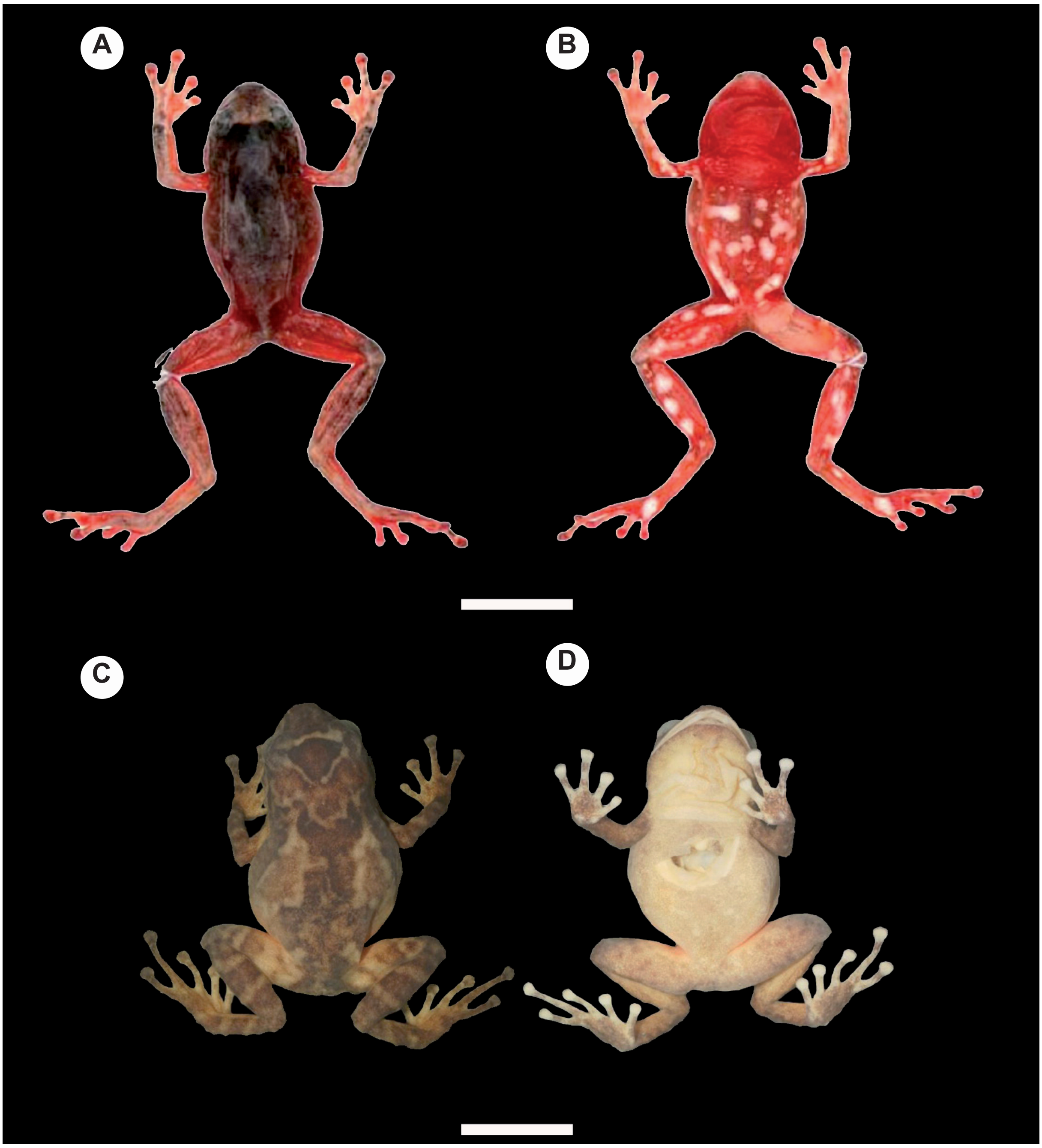

Figure 21: *Diasporus* holotypes in preservative: *D. ventrimaculatus* (UCR 20493) **A** dorsal view, **B** ventral view (modified from Chaves et al. 2009); *D. igneus* (MVUP 2301) **C** dorsal view, **D** ventral view (modified from Batista et al. 2012). Scale bar 10 mm.

leave no doubt that this is another undescribed species, for which reason I treat it as another Confirmed Candidate Species (Vietes *et al.* 2009).

6.1.5. *DIASPORUS* SPECIES ACCOUNTS

Diasporus citrinobapheus **Hertz, Hauenschild, Lotzkat, and Köhler, 2012**, ZooKeys, 196: 27.

Holotype: SMF 89814 (Fig. 20A), by original designation.

Type locality: „Quebrada Rasca (8.4851°N, 81.1727°W, 790 m elevation), near Paredón, Comarca Ngöbe-Buglé, western Panama, approximately 50 airline km NNW of the city of Santiago and 20 airline km N of Cañazas, Veraguas“.

Conservation Status: IUCN Category: Not Evaluated (NE); known from the PAs: BPPS, PNSF and PNOT, Extent of Occurrence about 2000 km^2 known from three localities. The available data are scarce. I place this species in the Near Threatened (NT) category, because the populations seem to be stable with no signs of decline. However, the species Extent of Occurrence is estimated to be less than 5000 km^2 and there is ongoing deforestation in the Serranía de Tabasará. EVS calculation: 5 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 13.

Diagnosis: From Hertz *et al.* (2012b): *Diasporus citrinobapheus* differs from all described members of its genus by the following combination of characters: coloration bright yellow to orange in life; head almost as broad as long, but comparatively broad in relation to SVL; skin of dorsum smooth; venter coarsely areolate; tympanum covered by skin but annulus clearly visible; TD 32–45% of ED; EL on average narrower than IOD; snout subacuminate in profile and rounded to subovoid in dorsal outline; disks of fingers and toes slightly expanded, disk covers of most fingers and toes spadate, but lacking papillae; disk pads of most fingers and toes triangular; subarticular tubercles of hands and feet rounded, very flat, almost not visible; vomerine odonthophores longish oval and widely separated; vomerine teeth weakly developed; upper eyelid usually smooth, very low pustules in some individuals; heel smooth.

Vocalization: From Hertz *et al.* (2012b): As in other members of the genus, the call consists of a single note, even though calls sound like a “whistle,” rather than the typical “tink” usually emitted by members of the genus *Diasporus* (Savage 2002; Chaves *et al.* 2009). Call length is around 0.150 s and thus longer than in most other *Diasporus* species (Fig. 17). The mean dominant frequency lies around 3000 Hz. An overview of the call parameters for both genetic clades of D. citrinobapheus is separately given in Tabel 3. A typical call is visualized

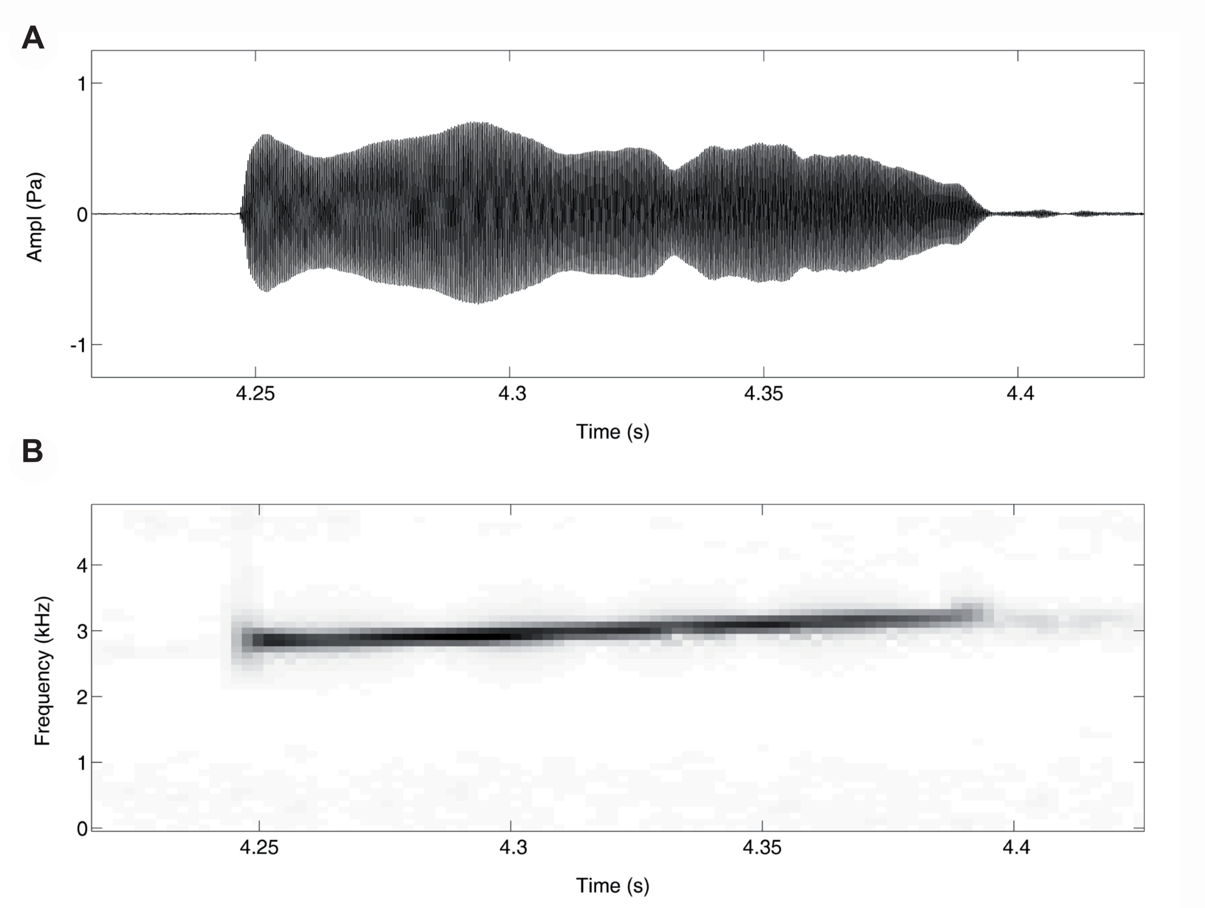

Figure 22: Example call of the male holotype of *Diasporus citrinobapheus* (SMF 89814). **A** Oscillogram, **B** Spectrogram

in Figure 22.

Coloration in life: All examined specimens show shades of bright yellow and orange dorsally (Fig. 36A,B); some have dark grayish and/or whitish-grayish spots. Ventral surfaces are almost achlorophyllaceous and transparent apart from the yellow male vocal sac. The following coloration records were made in field with life specimens (Hertz *et al.* 2012b):

MHCH 2372: Dorsal ground color Orange Yellow (18); posterior and anterior surfaces of thighs Chrome Orange (16); Raw Umber (23) interorbital and postocular stripes formed by very fine mottling; dorsum with five Dark Grayish Brown (20) blotches, forming a pattern like the five dots on a dice; scattered Dark Grayish Brown (20) blotches on dorsal surfaces of limbs; disk covers Blackish Neutral Gray (82), with white rings at the base; ventral surface of hind limbs Chrome Orange (16); ventral surface of body transparent with dirty white mottling; vocal sac white with a suggestion of Spectrum Yellow (55).

SMF 89820: In the only female, coloration in life has been recorded as follows: Dorsal surface Yellow Ocher (123 C); a Chamois (123 D) interorbital bar; anterior and posterior surfaces of thighs Chrome Orange (16); venter almost transparent; upper surfaces of disks Sepia (119) with dirty white spots and a dirty white ring around base; gular region Smoke Gray (44).

Distribution: As currently understood endemic to the Serranía de Tabasará, between the Fortuna depression and the mountainous spurs of the Cordillera Central in the province of Coclé (Fig. 23).

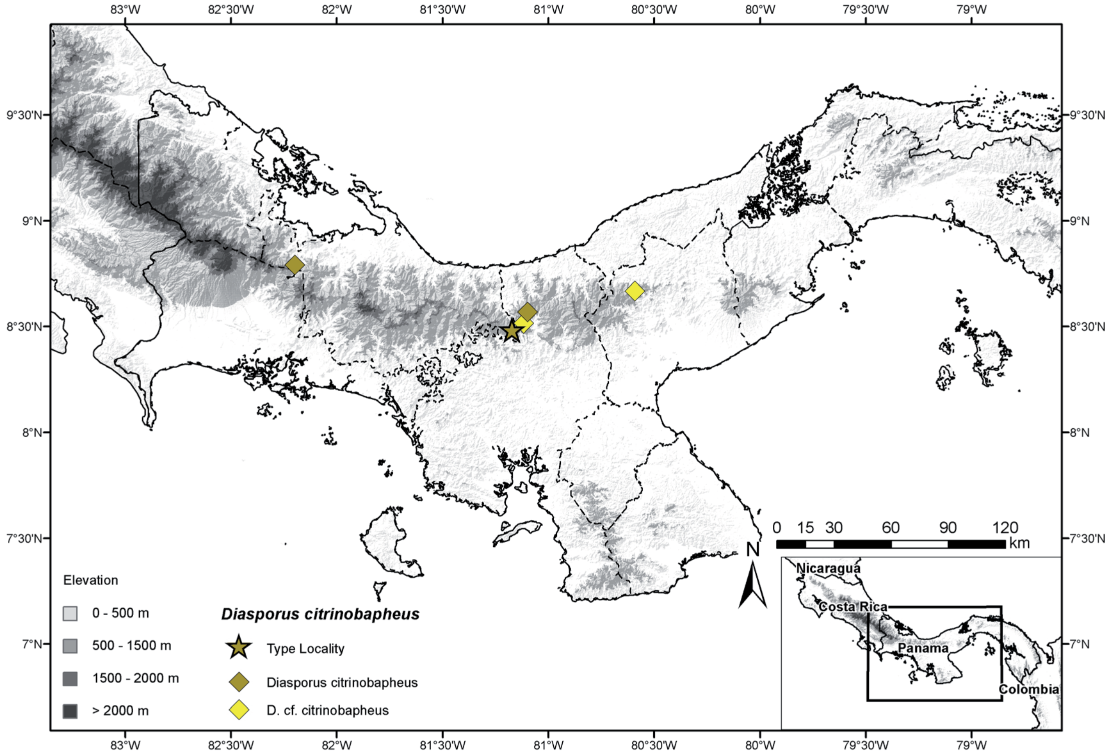

Figure 23: Collection sites of *Diasporus citrinobapheus*.

Habitat and natural history: *Diasporus citrinobapheus* inhabits premontain wet and moist forests at intermediate elevations from 680 to 790 m asl on both the Pacific and Caribbean versants. Males call from very dense vegetation and are difficult to spot. They are almost only detectable by following their characteristic vocalizations. Vocal activity is highest just after sundown, continuos during dusk, and finally stops when it is completely dark. Calling height ranges from near ground level up to three meters above ground. Calling position can be either on the upper side of a leaf or on its underside. The only female (SMF 89820) was found at daytime (15:00 h) inside an involute, young plantain leaf that apparently served as a daytime hiding place. The species does not seem to be limited to mature forest, but is also found in secondary growth and plantations. However, it appears to avoid open habitats like pasture land.

Remarks: The species description of *Diasporus citrinobapheus* is the first taxonomic work on the genus that includes a phylogeny of a molecular marker in the analysis. The subtree which contains *D. citrinobapheus*, but also *D.* aff. *tigrillo* and *D. quidditus*, is generally well-supported. Hertz *et al.* (2012b) refrained from treating the eastern populations from Alto de Piedra and El Copé (*D.* aff. *diastema*; in Crawford *et al.* 2010) as conspecific. However, from the currently available data I see no reason why they should be considered as two different species.

There has been a lot of speculation about the meaning of the readily soluble yellow coloration of this species (Fig. 24), especially among non-scientists. From my point of view, this is just a leaking of yellow pigments from the xanthophores resulting from mechanical stress when handling the frog. The xanthophores lay near to the surface of the frog´s skin, and thus are more easily damaged. *Diasporus citrinobapheus* is not the only species where this has been observed, Malkmus *et al.* (2002) report on a similar effect in the microhylid species *Chaperina fusca* from Borneo.

Figure 24: Live specimen of *Diasporus* cf. *citrinobapheus* photographed near El Copé, Coclé. The yellow coloration did rub of on the catcher´s fingers. Photo courtesy of U. Schmidt.

Diasporus diastema **(Cope, 1876 "1875"**, J. Acad. Nat. Sci Philadelphia, Ser. 2, 8**)**

Syntypes: USNM 25170-71 according to Cochran (1961); USNM 25170 designated lectotype by Dunn (1942) (Fig. 19A), who determined USNM 25171 as a specimen of *Eleutherodactylus ockendeni* (syn. *Pristimantis taeniatus*).

Type locality: „Camp Mary Caretta, Panama" according to Dunn (1942) probably Margarita, near Colón, Panama. Although Cope (1876) mentions that Camp Mary Caretta is in Panama, the title of his report "On the Batrachia and Reptilia collected by Dr. John M.

Bransford during the Nicaraguan Canal Survey of 1874" is misleading (Savage 1973).

Synonyms: *Hyla chica* Noble, 1918, Bull. Am. Mus. Nat. Hist., 38: 335–339.

Syrrhopus ineptus Barbour, 1928, Proc. New England Zool. Club, 10: 25–26.

Conservation Status: IUCN Category: Least Concern (LC); the current classification is based on the assumption that *Diasporus diastema* is continuously distributed from Honduras to central Panama. A taxonomic revision is needed before the species conservation status can be re-assessed. However, species in the genus *Diasporus* are known to show a tolerance to moderate habitat modifications and are generally common throughout their distribution area, with no sights of chytrid caused declines, and thus possibly do not face immediate threats. Moreover, there are several protected areas in the surroundings of the Panama Canal, thus the chance is high that *D. diastema* is present in at least one of these. EVS calculation: Since the geographic distribution is unclear, calculation of an EVS value is not possible at the moment.

Diagnosis: The original description by Cope (1876) does not provide much information that would allow to morphologically distinguish *Diasporus diastema* from other congeneric species. Moreover, the lectotype is not in the condition to take measurements. It is almost a skeleton and qualitative characters relating to coloration, skin texture, and the appearance of tubercles are not visible anymore. Additionally, it cannot be ruled out that publications after Cope (1876) used a set of several species, so I use morphological characters from literature other than the original description with caution. *Diasporus diastema* probably differs from *D. tigrillo*, *D. citrinobapheus*, and *D. igneus* by coloration and in the latter species also by adult size. *Diasporus vocator* is smaller and has more slender, lanceolate disk pads. *Diasporus hylaeformis* and *D. ventrimaculatus* are considerably larger, have broader heads and a truncate snout in dorsal outline.

Vocalization: There is currently no reliable data on the vocalization emitted by *Diasporus diastema*. Three papers have been published that include a description of the vocalization of *D. diastema* (Fouquette 1960; Wilczynski & Brenowitz 1988; Ibáñez *et al.*1999). All three recordings were made in the surroundings of the Panama Canal, but are incongruent in many parameters, and are possibly describing the calls of different species (Hertz *et al.* 2012b).

Coloration in life: According to the original description by Cope (1876) the type was dark brown above with a darker interorbital bar that was paler bordered anteriorly. The venter was pale brown.

Distribution: The currently accepted distribution extents from south-eastern Honduras across the Caribbean versant of Nicaragua and Costa Rica to central Panama and on the

Pacific versant from Quepos in Costa Rica into south-western Panama. It is very likely that this view includes several undescribed species.

Habitat and natural history: The habitat type in the surroundings of the Panama Canal and accordingly of the type locality is classified as Tropical Moist Forest (Holdridge 1967). Depending on the species actual distribution it might also be found in the Tropical Wet and Pre Montane Wet Forest domains.

Remarks: To clarify the identity of *Diasporus diastema,* an integrative taxonomic revision is needed that includes fresh material of tissue for molecular analysis and vocalizations from near the type locality. Since John M. Bransford collected the lectotype long before construction of the Panama Canal, the type locality might be flooded by now. However, a thorough collection of *Diasporus* specimens along the Panama Canal could reveal the true number of *Diasporus* species in that area and the one that fits the description of *D. diastema* best could then be assigned to that species. In a further step, the distribution of *D. diastema* must be reevaluated.

***Diasporus hylaeformis* (Cope, 1876 „1875“**, J. Acad. Nat. Sci. Philadelphia, Ser. 2, 8:107**)**.

Holotype: USNM 30687 according to Cochran (1961) (Fig. 19B).

Type locality: „mountain of Pico Blanco, at 7000 feet elevation“, Province of Limón, Costa Rica. Corrected to „Cerro Utyum, 7000 ft, Cantón de Talamanca, Provincia de Limón; 2134 m“, Costa Rica, by Savage (1974).

Conservation Status: IUCN Category: Least Concern (LC); as in *Diasporus diastema*, the current conservation assessment is based on an unclear taxonomy. Since I have specimens from the Serranía de Tabasará in my sample that show virtually no genetic distance to specimens from Cerro Pando, I estimate the extent of occurrence to comprise more than 10,000 km^2, thus much larger than previously thought. Thereby, the species occurs in more well-protected areas than previously thought and together with its ability to survive in disturbed habitats, there is currently no evidence to list it in a more threatened category. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 3 (forest formation) = 11.

Diagnosis: Together with *Diasporus igneus*, this is the largest species of *Diasporus*. SVL in adult males is between 20 and 27 mm, with females being slightly larger. A fine middorsal pinstripe is common in this species (Cope 1876; Taylor 1952; Savage 2002).

Vocalization: An official description of the vocalizations of *Diasporus hylaeformis* does not exist. There is a brief comment on the vocalizations of *D. hylaeformis* from different populations in Costa Rica in the discussion section of Chaves *et al.* (2009), but the authors give no exact locality data for the analyzed specimens. According to Chaves *et al.* (2009), calls of *D. hylaeformis* have a spectral bandwidth of 2355 to 3055 Hz and a call length of 0.214±0.021 s, n = 86. However, the calls I analyzed from populations in western Panama have a call length of on average 0.084–0.120 s and a dominant frequency of 2620–3060 Hz (Tab. 3). Typical calls of the respective clades are visualized in Figure 25.

Coloration in life: Cope (1876) writes in the original description: "Color above rich brown, divided on the vertebral line by a narrow red stripe. Femora light brown before and behind. Gular region with a large vocal sac, of a rose color." I recorded the coloration in life of several specimens from various localities and found them to be more variable in coloration than other species in the genus. However, predominant colors include brownish tones from very dark to lighter drab to beige colors often suffused with reddish or pink. The vocal sac is either yellow or red. A light pinstripe along the vertebra and an interorbital bar is commonly observed. The

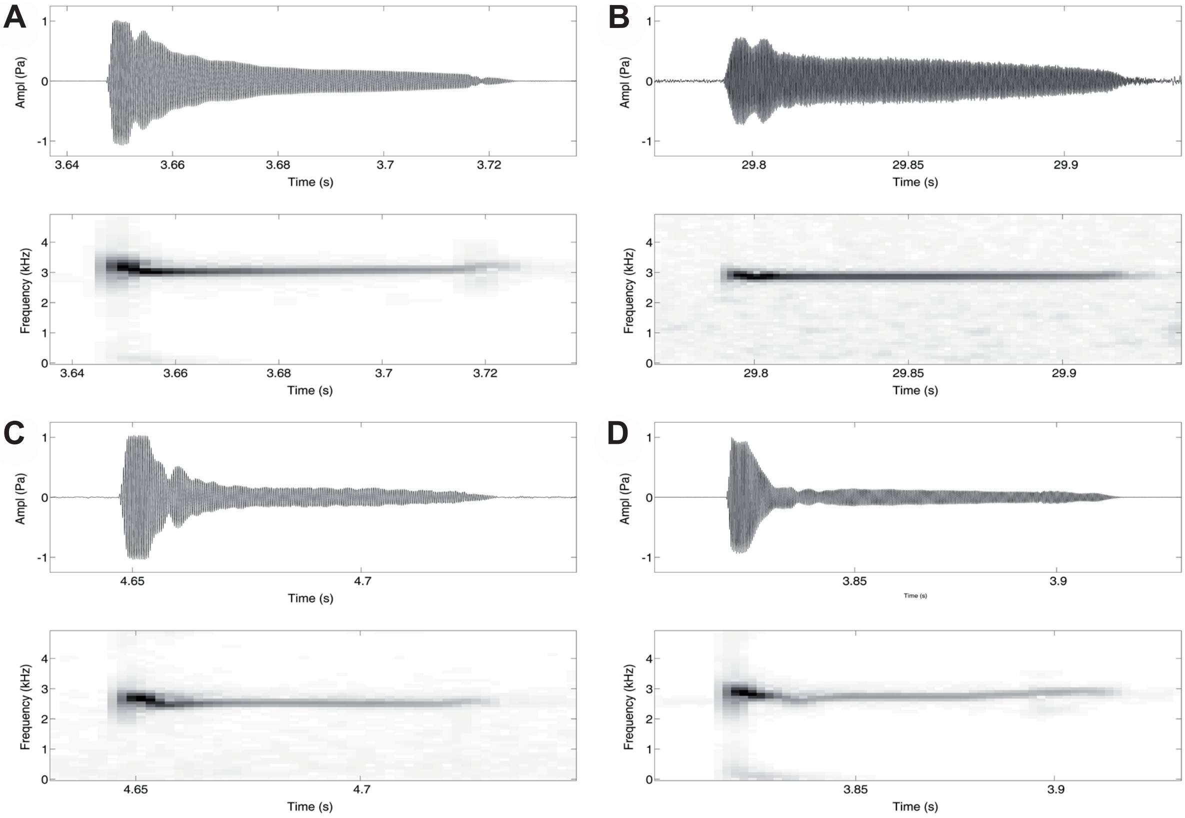

Figure 25: Oscillograms (above) and spectrograms (below) of example calls of specimens in the *Diasporus hylaeformis* species complex from different localities. **A** *D.* cf. *hylaeformis* (SMF 89874) from Cerro Pando. **B** *D.* aff. *hylaeformis* (AH 244) from Bajo Mono, Volcán Barú. **C** *D.* aff. *hylaeformis* (AH 177) from La Fortuna. **D** *D.* cf. *hylaeformis* (AH 486) from Cerro Saguí, Cerro Colorado area.

following individual colorations have been recorded in field:

La Nevera:

AH 039: Vocal sac Spectrum Yellow (17); lower surface of disk Chrome Orange (16); upper surface of disk Flame Scarlet (15); posterior surface of thigh and cloacal region suffused with Pratt´s Rufus (140); interorbital bar Chamois (123 D) edged by Raw Umber (223) posteriorly; iris with a Raw Umber (223) horizontal bar running over the pupil, Tawny Olive (223 D) above and Kingfisher Rufous (240) below this bar.

AH 042: Dorsal ground color Raw Umber (23) with Burned Umber (22) shadings; a Buff (24) interorbital bar; ventral surfaces of body and limbs Drab-Gray (119 D); lower surface of disks Crome Orange (16); vocal sac Buff (24) with a suggestion of Orange Yellow (18); iris Raw Umber (23).

AH 343: Dorsal ground color Smoke Gray (45); two parallel Cream Color (54) interorbital bars; disks on hands and feet Orange Yellow (18); ventral surfaces Pearl Gray (81) but almost translucent (heard visible through skin).

Volcán Barú:

HAU 011: Dorsal ground color Fawn Color (25) with irregular markings of Sepia (119); posterior third of dorsum and dorsal surfaces of limbs Olive Green (46) with spots of Sepia (119) and Parrot Green (60); dorsal color of toes and fingers Parrot Green (60) with spots of Buff Yellow (53); lateral coloration Cinnamon Rufous (40); ventral ground color Greenish Olive (49) interspersed with fine mottling of Parrot Green (60) and Sepia (119); iris Buff Yellow (53) bordered by Sepia (119).

HAU 012: Dorsal ground color Dark Brownish Olive (129) with patches of Pratt‘s Rufous (40) on the posterior part and on lateral surfaces; dorsal surface of head Sepia (119); tip of nose Cinnamon (39); ground color on flanks Clay Color (26); disk covers Ferruginous (41); ventral surfaces dirty white, partially transparent; ventral surface of head Cinnamon (39); posterior surfaces of thighs Ferruginous (41) with Dark Brownish Olive (129) mottling; iris Olive-Green (Basic) (46) bordered by Sepia (119).

HAU 013: Dorsal ground color Sepia (219) fading into Mars Brown (223 A) with patches of Clay Color (26) bordered by Sepia (119) towards flanks; disk covers Clay Color (26); lateral surfaces Mars Brown (223 A); ventral surfaces Mars Brown (223 A), partially transparent, with Sepia (119) mottling towards flanks; thigh Clay Color (26) with Mars Brown (223 A) transverse bars bordered by Sepia (119); iris coloration Cinnamon (39) bordered by Sepia (119); dorsal; ventral surface of head yellowish Olive-Green 50;

Jurutungo:

AH 124: Dorsal ground color Raw Umber (23); postorbital portion of head Burnt Umber (22); interorbital bar Buff Yellow (53); lateral surfaces Drab-Gray (119 D) with Pale Pinkish Buff (121 D) markings; cloacal region and upper surfaces of legs Raw Umber (23) with Pale Pinkish Buff (121 D) transversal bandings and Scarlet (14) mottling; disk covers Spektrum Orange (17); ventral surfaces of body and limbs Drab (27); body translucent, hindlimbs with Scarlet (14) mottling; lower surfaces of disks Warm Buff (118); vocal sac Orange Yellow (18); iris silvery with a suggestion of orange in the lower portion.

AH 125: Dorsal ground color of body and limbs Dark Grayish Brown (20); snout from interorbital region to tip of the snout, including upper lip Buff Yellow (53) with a suggestion of Burnt Orange (116); an irregular lateral Buff Yellow (53) circle with a suggestion of Burnt Orange (116) in the upper part, grading into Smoke Gray (44) towards the lower part; posterior dorsal surfaces of body and dorsal surfaces of hindlimbs with Smoke Gray (45) markings; upper surfaces of disks Buff-Yellow (53) with a Smoke Gray (44) band across digit tip proximal to the disk; ventral ground color Olive Gray (42) shading into Lavender (77) at posterior part of the body and lower surface of thighs; Pale Neutral Gray (86) spots present on ventral surface of body; ventral surfaces of disks Spektrum Orange (17); iris Smoke Gray (44)

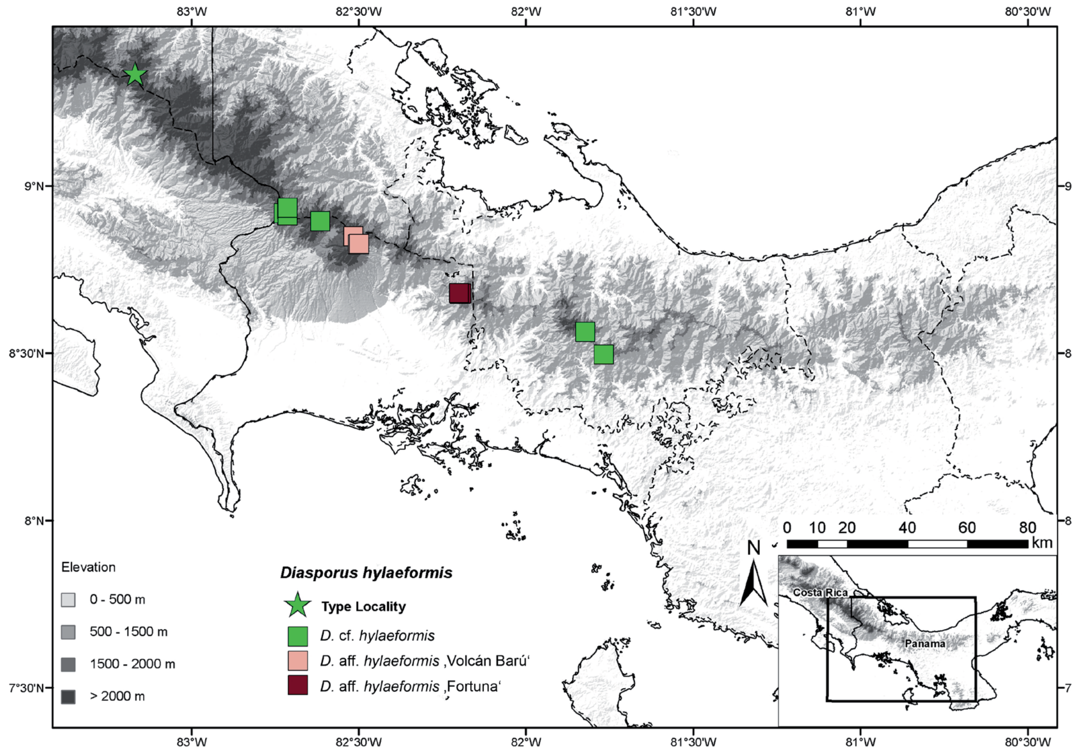

Figure 26: Collection sites of the *Diasporus hylaeformis* species complex in western Panama and southwestern Costa Rica.

metallic.

AH 263: Dorsal ground color Olive-Brown (28); lateral surfaces, interorbital bar and middorsal pinstripe Flesh Color (5); posterior thigh surfaces Geranium Pink (13); vocal sac Flame Scarlet (15) suffused with Orange Yellow (18); ventral surface of Body Salmon Color (6).

AH 265: Dorsal ground color Warm Buff (118); interorbital bar Dark Brownish Olive (129); snout Warm Buff (118); ventral surfaces dirty white; vocal sac Orange Yellow (18).

AH 268: Dorsal ground color Clay Color (26), reticulated with Hair Brown (119 A); dorsal surfaces of thighs Glaucous (80) reticulated with Hair Brown (119 A) and Pale Pinkish Buff (121 D); a Pale Pinkish Buff (121 D) triangular snout spot between orbits and tip of the snout; ventral surfaces transparent to Pale Neutral Gray (86) with Pale Pinkish Buff (121 D) mottling.

<u>Distribution:</u> As currently understood *Diasporus hylaeformis* is distributed along the ridge of the Serranías de Talamanca and Tabasará. The type locality lies in the middle of the Serranía de Talamanca (Fig. 26). There is a need for a taxonomic analysis of the species in Costa Rica to find out whether its distribution also extends into the Cordillera Central and maybe other mountain ranges in northern Costa Rica.

<u>Habitat and natural history:</u> *Diasporus hylaeformis* inhabits primarily Lower Montane Rainforest, but marginally also Montane Rainforest and Premontane Rain Forest at 1700–2400 m elevation. The species is very abundant at wooded upland sites and their typical "tink" call is a familiar background noise in the forest at night-time. Males call throughout the year, but I recognized a reduced calling activity in October and November (2009). On 19 November 2009 I found an egg clutch (Fig. 27) of 7 eggs (AH 353) in a thick moss cushion on the base of a tree trunk of approximately 20 cm diameter at Cerro Pando 2290 m elevation. Subsequent DNA barcoding identified the eggs as conspecific with *D.* cf. *hylaeformis*. Six eggs contained well-developed embryos.

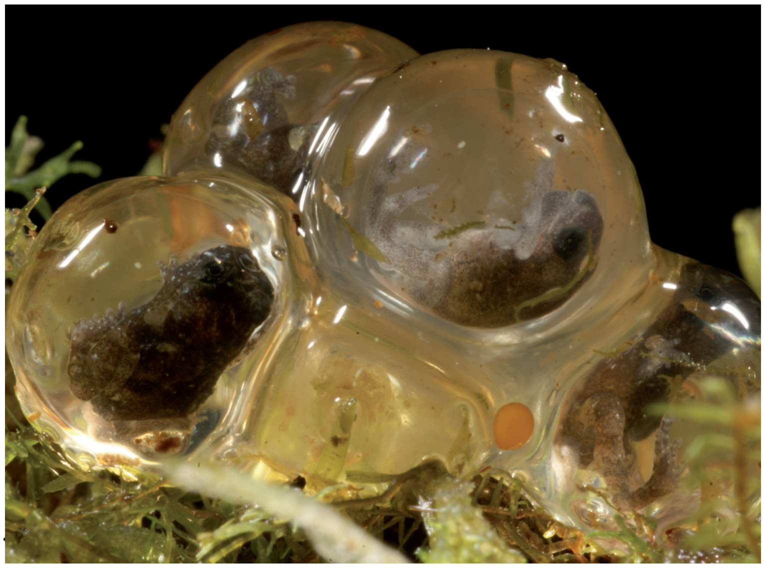

Figure 27: Egg clutch of *Diasporus* cf. *hylaeformis* with embryos

<u>Remarks:</u> My results suggest that more than one species are

currently operated under the name *Diasporus hylaeformis*. Future taxonomic studies need to include specimens from Costa Rica, preferably from near the type locality.

***Diasporus igneus* Batista, Ponce, and Hertz, 2012**, Zootaxa, 3410: 54.

Holotype: MVUP 2301 (Fig. 21C,D), by original designation.

Type locality: „southeastern slope of Cerro Santiago, Serranía de Tabasará about 4.6 km from Llano Tugrí (Buabití), capital of the Comarca Ngöbe Buglé, Müna district, Corregimiento de Peña Blanca, Panama (8.5079° N, 81.7168° W, 1710 m asl)“.

Conservation Status: IUCN Category: Not Evaluated (NE); the species has only recently been described in the course of this project. Its known extent of occurrence covers approximately 40 km^2 and it is not known to occur in degraded habitats. Further, there are no protected areas in the area and ongoing deforestation is observed. Although more data on population size is needed for an evaluation, I believe it qualifies for the Endangered (EN) category in view of its small extent of occurrence that meets criterion B1, the continuing decline of extent and quality of its habitat b (iii), what inevitably leads to a fluctuation in its extent of occurrence c (i) in the near future. EVS calculation: 6 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 14.

Diagnosis: Modified from Batista *et al.* (2012): *Diasporus igneus* can be distinguished from other species in the genus as follows: from *D. quidditus*, *D. tinker*, and *D. vocator* by its much larger body size and palmate to truncate disk covers instead of lanceolate to papillate disk covers. It differs from *D. diastema* and *D. tigrillo* by its larger adult body size, by having one to several enlarged supraocular tubercles, by having the outer edge of the tibia and forearm covered with a series of tubercles, and by having the groin and axilla colored in red. Some specimens of *D. ventrimaculatus* possess bright red colorations too, but lack supraocular and ulnar tubercles, and disk covers are spadate and not palmate to truncate. *Diasporus igneus* is further distinguished from *D. anthrax*, *D. citrinobapheus*, and *D. gularis* by its larger size, dorsal skin with scattered tubercles, and color pattern. The only described species that may grow larger than *D. igneus* is *D. hylaeformis*. *Diasporus igneus* differs by its vivid, flame-like dorsal coloration pattern and the scarlet groin coloration, while *D. hylaeformis* are mostly uniformly colored with only scattered flecking in some specimens and no scarlet groin coloration. Further, *D. igneus* has palmate to truncate disk covers, while in *D. hylaeformis* disk covers are spadate to palmate and less expanded.

Vocalization: Batista *et al.* (2012) compared vocalizations of three male *Diasporus igneus*.

Vocalizations produced by the holotype (environmental temperature = 16.9 ºC; humidity 78%; 19:30) and two other males (MHCH 1388, environmental temperature = 16.1 ºC; humidity 86%; 20:30 and MHCH 2072, environmental temperature = 15.9 ºC; humidity 93%; 02:10) were analyzed. The call consists of a single, short, monophasic note that is reminiscent of a "whistle". Note duration is 0.07±0.01 s (0.05–0.10) with an interval between calls of 13.5±3.5 s (9.0–17.5) and a call rate of approximately four calls per minute. The peak frequency band ranges from 2.0 to 2.7 kHz. The fundamental frequency is also the dominant frequency, at 2.4±0.08 kHz (2.2–2.5), followed by five main harmonic components at 4.6±0.2 kHz (4.5–5.0), 6.8±0.4 kHz (6.2–7.5), 9.3±0.4 kHz (8.7–10.1), 11.8±0.5 kHz (10.1–12.1), 13.6±0.6 kHz (12.1–14.6), with most energy emitted in the first harmonic, followed by the second (Fig. 28).

Coloration in life: The coloration of the holotype (MVUP 2301) was recorded by Batista *et al.* (2012) as follows: Iris Yellow Ocher (123) with a longitudinal Army Brown (219B) bar in the middle; dorsal ground color Verona Brown (223B), suffused with Clay Color (123D) and Burnt Orange (116); interorbital region with a Burnt Orange (116) line; groin and axilla

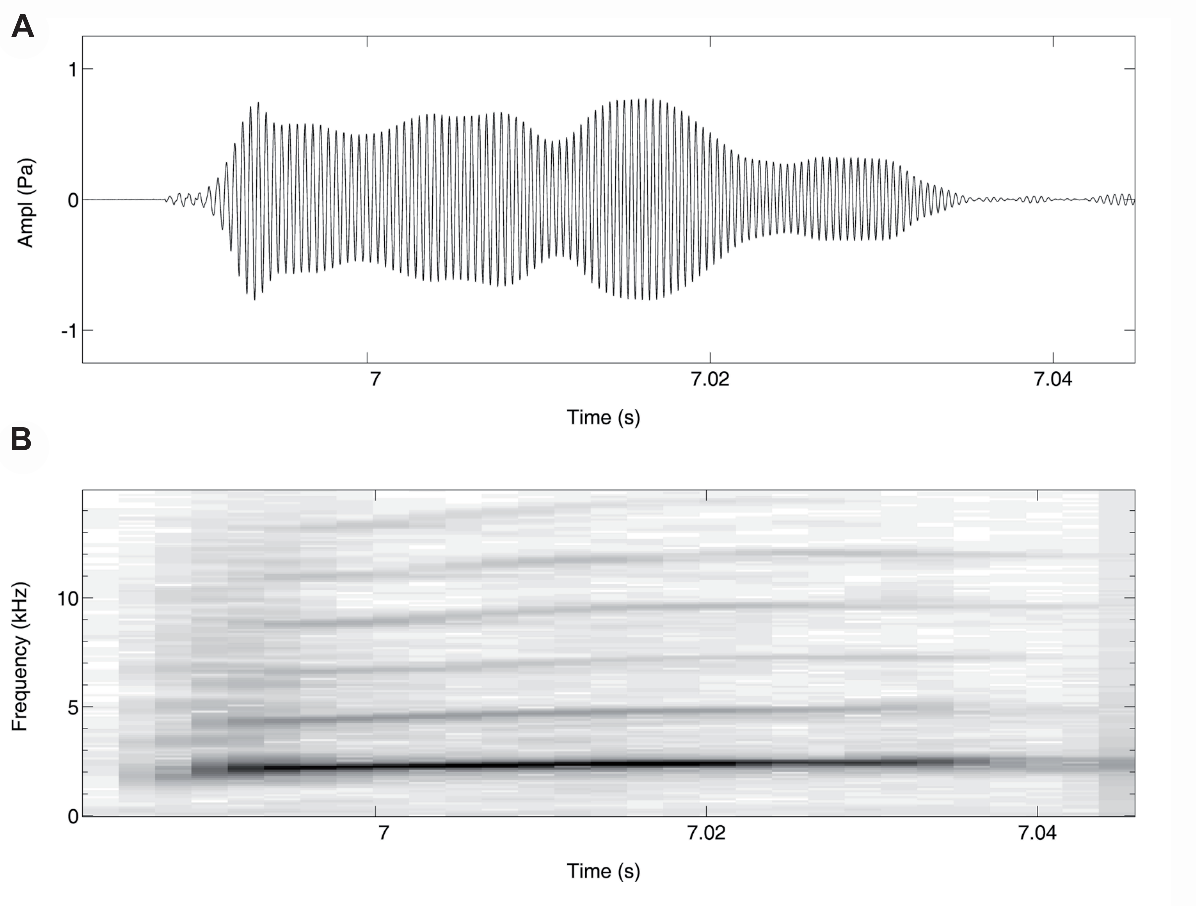

Figure 28: Example call of the male holotype of *Diasporus igneus* (MVUP 2301). **A** Oscillogram, **B** Spectrogram (note harmonics).

immaculate Scarlet (14); posterior thigh surface Scarlet (14) suffused with Raw Umber (23), and with Buff (24) bars; venter immaculate Olive Yellow (52) suffused with Scarlet (14); vocal sac immaculate Orange Yellow (18).

I further recorded the coloration of the paratype (SMF 89821) in life as follows: Dorsal ground color Sepia (219) reticulated with Cinnamon Drab (219C) suffused with Flame Scarlet (15) and Orange Yellow (18); interorbital bar between Chrome Orange (16) and Flame Scarlet (15); vocal sac Orange Yellow (18) shading into Scarlet (14) posteriorly and continous over the ventral surfaces of the posterior part of the head and the ventral surface of the body; groin Scarlet (14); iris golden.

Distribution: So far *Diasporus igneus* is only known from the slopes of Cerro Santiago above 1500 m. The type locality is Llano Tugrí on the eastern slope; the second collection site is La Nevera on the western slope. Neither A. Batista nor I have seen or heard it at sites other than these two (Fig. 29).

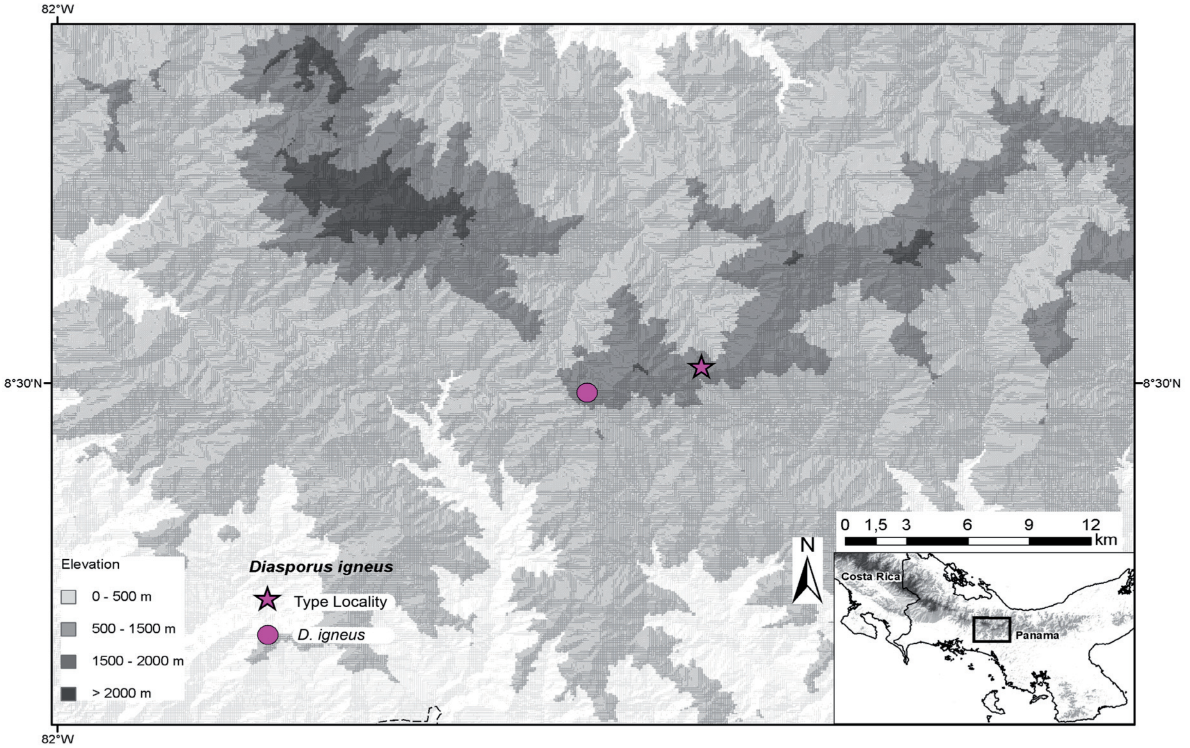

Figure 29: Collection sites of *Diasporus igneus* on the slopes of Cerro Santiago in the Cerro Colorado area, Comarca Ngöbe-Buglé.

Habitat and natural history: *Diasporus igneus* inhabits primary Lower Montane and Premontane Rain Forests. Batista *et al.* (2012) found calling males well-covered by leaves in tree holes or bromeliads between two and five and a half meters above the ground. The stomach of one specimen contained a woodlouse (Isopoda: Oniscidea) of 2.9 mm width and 8.4 mm length, the so far only known food item.

Remarks: *Diasporus igneus* is one of the largest *Diasporus* known (Hertz 2012). The SVL of the four known males is 25.5–26.6 mm. Although the male holotype of *D. hylaeformi*s is with 27 mm still larger, the average size of this species is with 19–22 mm in adult males and 20–26 mm in adult females (Savage 2002) considerably smaller. The herein presented phylogeny is the first to compare a molecular marker (16S mtDNA) of *D. igneus* with other specimens of the genus in western Panama. The results confirm the assumption of a distinct species that was based on morphological and bioacoustic data (Batista *et al.* 2012).

***Diasporus tigrillo* (Savage, 1997**, Amphibia-Reptilia, 18: 241**)**

Holotype: CRE 7177A, by original designation; now in LACM under the catalogue number 146212 (Fig. 20B).

Type locality: „Alto Lari at the confluence of the Río Lari and Río Dipari, about 21 km SW Amubri, Distrito Bratsi, Cantón de Talama[n]ca, Provincia de Limón, Costa Rica, (440 m)“.

Conservation Status: IUCN Category: Data Deficient (DD); officially this species in only known from the type material consisting of two adult males. The specimens I collected in western Panama extent the known distribution range about 160 km to the south-east and represent the first records from Panama. Including the new distribution data, its extent of occurrence is approximately 1500 km^2. Additionally, it meets criterion B1a and B1biii. The type locality is just outside the boundary of the International Park La Amistad, but it is likely that the species is present in the Park. In Panama, I found it in the Palo Seco Protected Forest. Both protected areas are suffering from ongoing deforestation (Oestreicher *et al.* 2009). The third locality in the Comarca Ngöbe-Buglé is unprotected. Although the species survives in plantations and secondary forests, the current data qualify it for the Endangered (En) category. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 12.

Diagnosis: *Diasporus tigrillo* is distinguished from most other *Diasporus* by its bright yellow coloration. It differs from the other yellow forms, *D. citrinobapheus* and the South American species *D. gularis* by the presence of low, widely spaced pustules on the dorsum. Further, *D. gularis* has papillae in the form of rounded knobs at the apex of the disk pads of Toes II–IV, which are not present in *D. tigrillo*. *Diasporus citrinobapheus* is also distinguishable from *D. tigrillo* by the ratios TD/ED and SL/SVL (Tabel 5).

Vocalization: Savage (1997) indicates that the call of *Diasporus tigrillo* is very similar to that of *D. diastema*. However, he does not give any parameters of the call, and anyway, the call of *D. diastema sensu strictu* is not known (see account of *D. diastema*).

I recorded the call of a yellow *Diasporus* at the headwaters of Río Chiriquí Malí, Palo Seco Protected Forest. Although the specimen escaped, I did not find any other yellow *Diasporus* than *D.* aff. *tigrillo* at that site. The call is most similar to that of *D. citrinobapheus* with only a significant difference in the dominant frequency (Tab. 4). Further, a lower call rate could be observed. Call length in the recorded specimen is 0.151–0.165 (0.159±0.005) s and dominant frequency 2530–2630 (2620±30) Hz. A typical call is visualized in Figure 30.

Coloration in life: *Diasporus* aff. *tigrillo* is primarily of yellowish coloration, albeit not as bright yellow as *D. citrinobapheus*. The low pustules, which are scattered over the dorsum, may be brown or not. Some specimens show also elevated dirty white spots, especially on the limbs. The coloration in life of the male specimen from Palo Seco (AH 422) was recorded

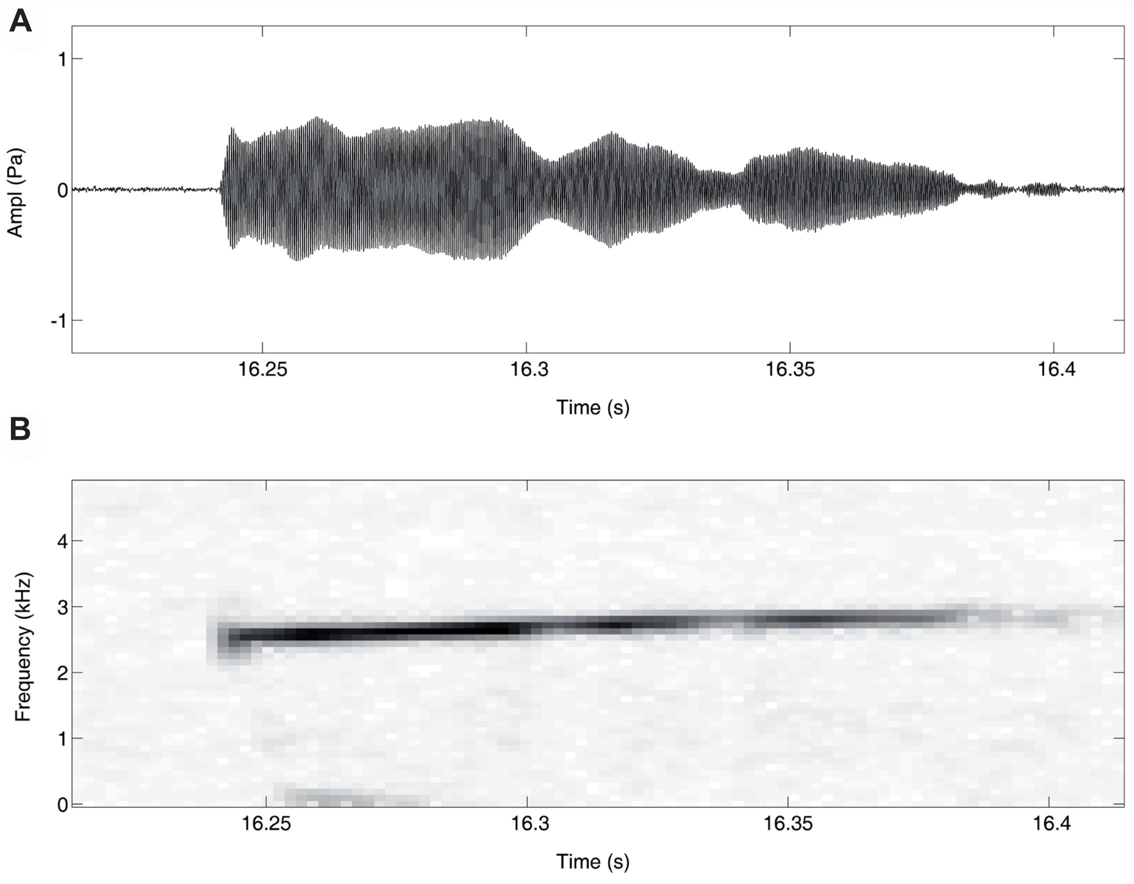

Figure 30: Example call of a presumed *Diasporus* aff. *tigrillo* recorded at Palo Seco Protected Forest that was not collected (see text for explanation). **A** Oscillogram, **B** Spectrogram.

as follows: Dorsal base color Spectrum Yellow (55) with Dark Grayish Brown (20) elevated pustules in the neck; a postocular stripe and an interorbital bar of the same color; anterior and posterior surfaces of the thighs Spectrum Orange (17); lower parts or arms mottled with elevated dirty white and Dark Grayish Brown (20) spots; finger and toe disks Dark Grayish Brown (20) with dirty white mottling and a dirty white ring around the base; gular region with vocal sac Spectrum Yellow (55), ventral surfaces of legs Spectrum Orange (17); ventral surface of body transparent.

Distribution: Assuming that *Diasporus* aff. *tigrillo* from Panama is conspecific with *D. tigrillo*, the species is distributed along the Caribbean slopes of the Serranía de Talamanca and the Serranía de Tabasará (Fig. 31).

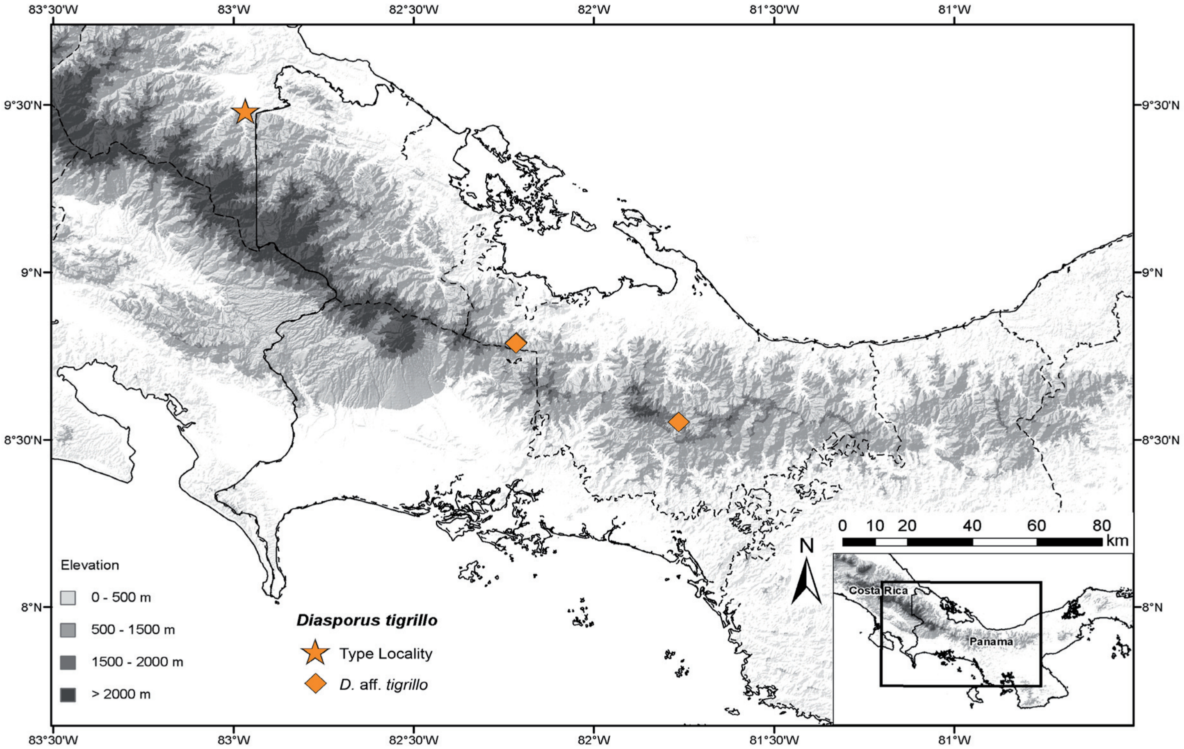

Figure 31: Collection sites of *Diasporus tigrillo* along the Caribbean slopes of the Cordilera Central.

Habitat and natural history: *Diasporus tigrillo* inhabits Tropical Wet Forest and Premontane Rain Forest along the Atlantic versant at 400–1075 m asl.

Remarks: The differences in the dominant frequency between *Diasporus* aff. *tigrillo* and *D. citrinobapheus* may be due to different body sizes. However, since the only recorded specimen of the former species escaped this could not be varified. The lower call rate could potentially result from the lower ambient temperature at the moment when the recording was made, compared to the ambient temperature during recordings of the *D. citrinobapheus* specimens (Tab. 3).

Diasporus vocator **(Taylor, 1955** Univ. Kansas Sci. Bull., 37: 522**)**

Holotype: KU 37001, by original designation.

Type locality: According to Savage (1974): „Agua Buena, Canton de Golfito, Puntarenas Province, Costa Rica, 1106 m. Near the Panamá line in the Cordillera Costeña, the region is now being heavily lumbered. Premontane Pluvial bioclimate".

Conservation Status: IUCN Category: Least Concern (LC); the current conservation evaluation assumes that *Diasporus vocator* is distributed in the humid Pacific lowlands of southern Costa Rica and adjacent Panama, as well as along the Caribbean coast of western Panama. If further studies confirm the assumption that *D. vocator* is endemic to the Golfo Dulce region of southern Costa Rica and Panama, its extent of occurrence would be less than 20,000 km^2. There is ongoing degradation of the habitat, but *D. vocator* shows the ability to tolerate moderate habitat modification. Therefore, it does not qualify for a threatened category. In view of the relatively small potential distribution area of *D. vocator*, assumed it is an endemic of the surroundings of Golfo Dulce, I would treat it as Near Threatened (NT). EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 3 (forest formation) = 11.

Diagnosis: *Diasporus vocator* is a very small species with relatively narrow, lanceolate disk pads. Specimens from the Golfo Dulce region are predominantly dark pigmented with light spots. The male vocal sac is pale yellow. A combination of these characters distinguishes *D. vocator* from other described species.

Vocalization: There is no published call description of *Diasporus vocator* from near its type locality. The only published description by Ibáñez *et al.* (1999) is based on a specimen from the surroundings of the Panama Canal. According to these authors it is "a frequently repeated soft, short and very high pitched [6000–7000 Hz] note with an insect-like quality." I analyzed calls of three specimens from the Caribbean lowlands and the Fortuna depression that are also very short (0.030–0.038 s) and high pitched (dominant frequency=4560–4740 Hz), even though not as high as in the spectrogram of Ibáñez *et al.* (1999). Batista *et al.* (2012) mention unpublished recordings of specimens from the Pacific lowlands of extreme western Panama that call at 4600–4900 Hz. Typical calls of the respective clades are visualized in Figure 32.

Coloration in life: Taylor (1955) and Savage (2002) described specimens from the Golfo Dulce region as rather dark-colored with pigmented ventral surfaces. All specimens I saw

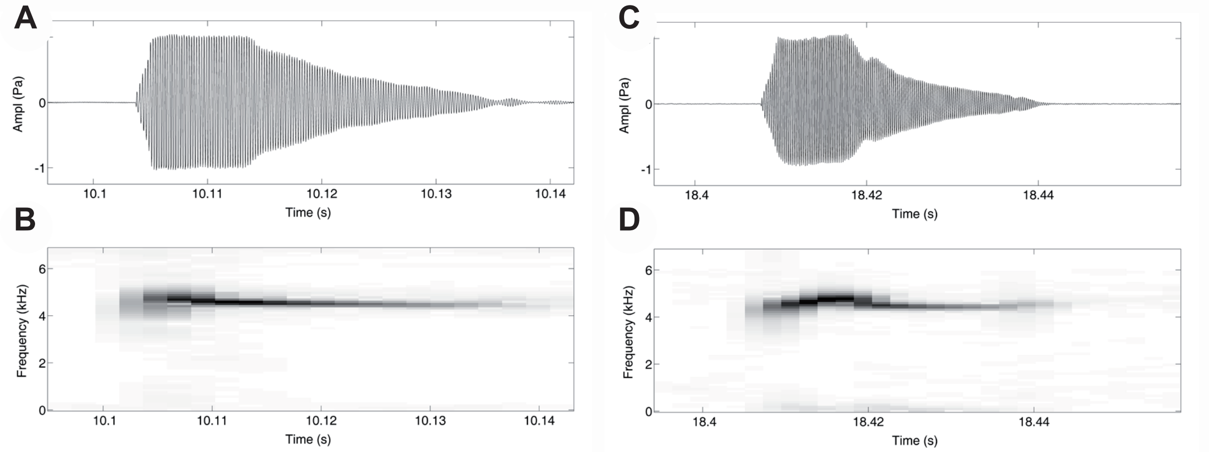

Figure 32: Example calls of *Diasporus* aff. *vocator* specimens from different localities on the Caribbean side of western Panama. Oscillogram (**A**) and spectrogram (**B**) of *D.* aff. *vocator* from San San Pond Sak, Caribbean coast. Oscillogram (**C**) and spectrogram (**D**) of *D.* aff. *vocator* from Willi Mazú, Caribbean slopes of the Fortuna Deptression, Palo Seco Protected Forest.

from the Caribbean slopes and lowlands are brownish above and have light ventral surfaces with a yellow vocal sac. The coloration in life of the specimen AH 465 from Palo Seco was recorded as follows: Dorsal ground color Drab (27); Blackish Neutral Gray (82) postocular stripes and interorbital bar, the latter bordered anteriorly by Pale Neutral Gray (86); two Cinnamon (39) paravertebral bars at the midbody; posterior part of the body and legs Tawny (38); limbs with Dark Brownish Olive (129) transversal bands; disk covers Tawny (38); ventral surfaces transparent with a suggestion of Pink (7); vocal sac dirty white, outlined with Spectrum Yellow (55).

Distribution: As currently understood, the distribution range extends from south of Quepos in Costa Rica along the humid Pacific lowlands of the Golfo Dulce region into south-western Panama, and on the Caribbean versant from Changuinola in Bocas del Toro to central Panama. Figure 33 shows collection sites of different clades examined in this study.

Habitat and natural history: *Diasporus vocator* inhabits Tropical Wet and Moist Forest and Premontane Rainforest from sea level to 1220 m (Savage 2002). In the San San Pond Sak wetlands on the Caribbean coast I found *D.* aff. *vocator* in a brackish water estuary forest with the Matomba palm (*Raphia taedigera*) and the Sangrillo (*Pterocarpus officinalis*) as the predominant tree species. Males were calling from the debris, which accumulates in leaf sheath of the palm trees or crutches of larger trees. Calling position was between 1.8 and 3 m above the ground.

Remarks: There is little doubt that several morphologically similar species are operated

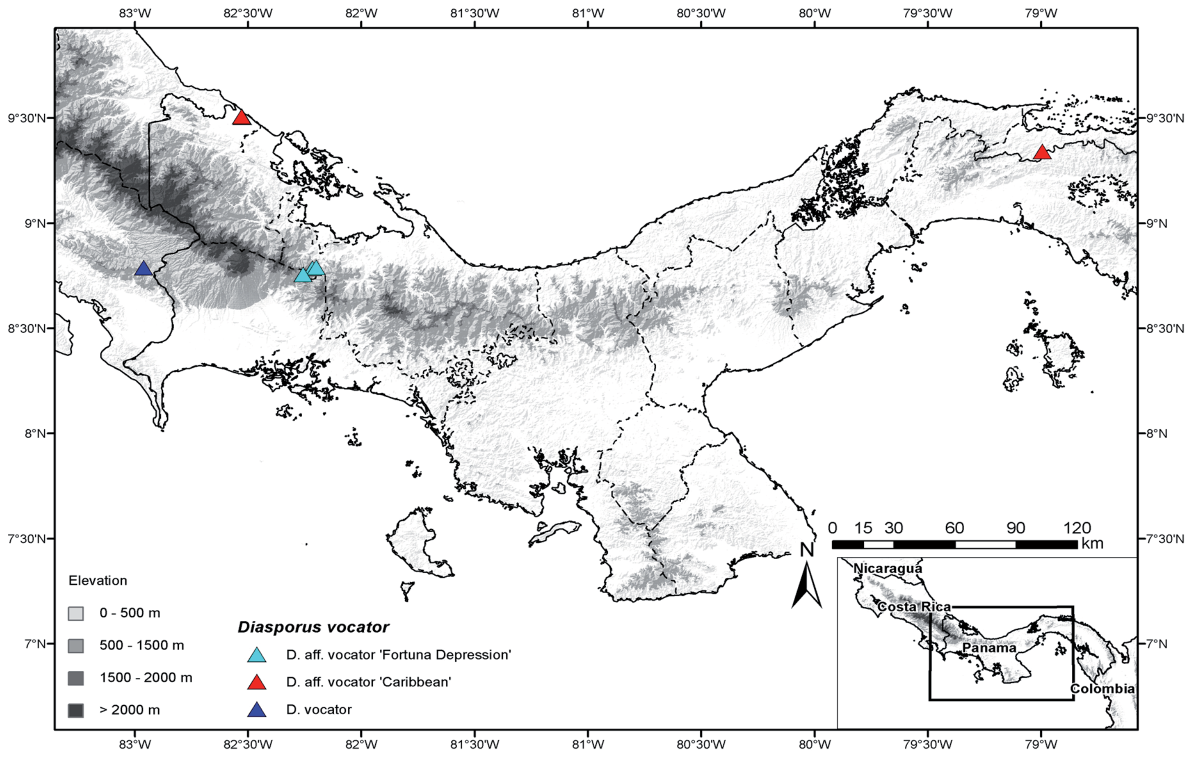

Figure 33: Collection sites of the *Diasporus vocator* species complex examined in this study from western Panama and southern Costa Rica.

under the name *Diasporus vocator* at present. However, without a larger sampling from the miscellaneous localities on the Caribbean and Pacific versants, especially from the Cordillera Costeña of Costa Rica, I see no chance to clarify the taxonomy of this complex.

Candidate species

The following candidate species are distinct by at least two lines of evidence and qualify for the Confirmed Candidate Species category sensu Vieites *et al.* (2009). Nevertheless, the designation is provisional and mostly based on very few individuals so that the formal description was postponed until additional material becomes available. Unconfirmed Candidate Species (Vieites *et al.* 2009) that I mentioned in chapter 1.1.4 are not further discussed here since the available data are not sufficient to generate species accounts.

Diasporus sp. `Colorado`

Conservation Status: The species is only known from two sites in the Cerro Colorado area, Comarca Ngöbe Buglé. There is no information on its extent of occurrence and thus an evaluation of its conservation status is not possible. Therefore, I classify this species as

Data Deficient (DD). However, the Cerro Colorado region is an unprotected area and under ongoing impact by deforestation, mining, and road construction (Hertz *et al.* 2012b). I assume it will qualify for an endangered category once sufficient data becomes available.

Diagnosis: This is a small species with adult males having a SVL of 18.50–18.80 mm. Morphometric measurements of the two available male specimens are midrange and allow not for species delimitation. It is distinguished from other species in the genus by a combination of the following characters: Eyelid tubercle present; disks expandet, disk covers palmate; ulnar tubercle absent. Based on 16S p-distance and on tree topology it is most closely related to *D. igneus* from which it is distinguished by male body size, coloration, and absence of ulnar tubercles. It is further related to *D.* sp. 'Fortuna' (characters for *D.* sp. 'Fortuna' in parentheses) from which it can be distinguished by coloration, the presence of a single eyelid tubercle (several small), absence of ulnar tubercle (low tubercle present), and palmate disk covers (spadate to lanceolate). Moreover, the shank is 0.41–0.44% of SVL (0.32–0.33%) and the eye diameter is larger in relation to the head length ED/HL=35–39% (33–35%).

Vocalization: The species calls at a dominant frequency of 3750–3840 (3790±50) Hz. The

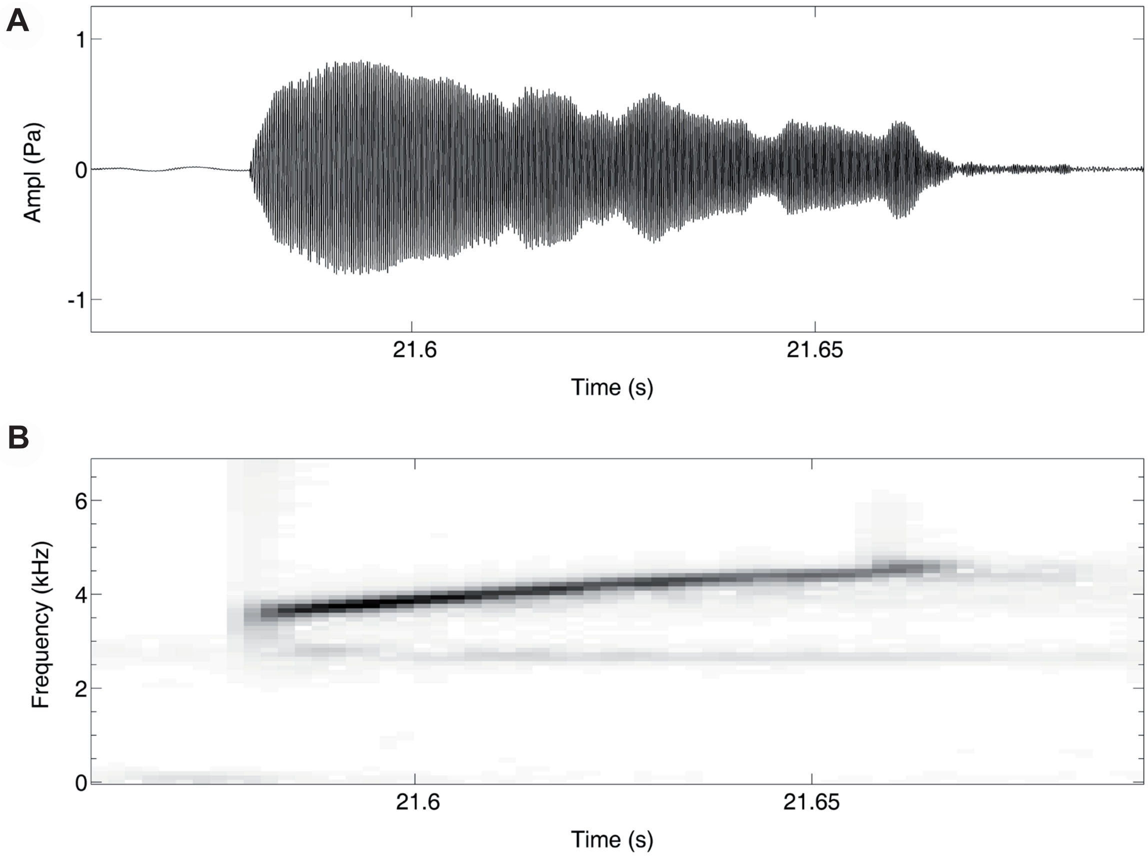

Figure 34: Example call of *Diasporus* sp. 'Colorado' (AH 485) recorded on Cerro Saguí (2033 m asl). **A** Oscillogram, **B** Spectrogram.

call is frequency modulated and rises from a low frequency of 2710±280 Hz in the beginning to a high frequency of 5060±150 Hz at the end of the call (Fig. 35). Frequency range is 2350±410 Hz. With 0.097–0.106 (0.102±0.003) s, call length relatively long. Call rate is 11 calls per minute at a temperature of 15.7 °C.

Coloration in life: Coloration in life was not recorded. Both male specimens AH 485 and 207 differ in terms of coloration. The specimen AH 485 has a darker dorsal coloration, while AH 207 shows dark spots on light ground. Both specimens have yellow vocal sacs. More specimens will be needed to reveal coloration variation.

Distribution: So far only known from two sites in the Cerro Colorado area, Comarca Ngöbe Buglé. One individual was found near Llano Tugrí, the other on Cerro Saguí (Fig. 34).

Habitat and natural history: The species inhabits the transitional zone between Premontane Rain Forest and Lower Montane Rainforest at elevations between 1600 and 2030 m. Males call from elevated positions at approximately one meter above the ground, where they sit between leaves. At Llano Tugrí it was found syntopic with *Diasporus igneus* and at Cerro Saguí with *D. hylaeformis*.

Remarks: In view of the various lines of evidence there is little doubt that this is another undescribed species. However, a larger sample size is desirable before formally describing this species.

***Diasporus* sp. `Fortuna`**

Conservation Status: At the current state of knowledge data are too scarce to evaluate the conservation status of this species; it is therefore classified as Data Deficient (DD). Both collection sites are inside the protected area RFLF.

Diagnosis: A small species (both specimens SVL=17.5 mm) that differs from most other *Diasporus* by the presence of ulnar tubercles. It shares this character with its obviously close relative *D. igneus*, but differs clearly from it in body size and coloration. It is further distinguished from *D. igneus* and its sister taxon *D.* sp. ‘Colorado’ by a conspicuously short shank in relation to the snout vent length (SL/SVL=0.32–0.33 vs. 0.41–0.44 in the other two species).

Vocalization: The vocalizations of this species is unknown.

Coloration in life: Modified after fieldnotes of F. Hauenschild: Dorsal ground color Sepia (119) with Hair Brown (119A) tubercles; a thin line of Verona Brown (223B) on dorsolateral midbody; lateral coloration Raw Umber (123) with Sepia (119) lines and Hair Brown (119A) spots; dorsal limb color Sepia (119) fading laterally into Dark Brownish Olive (129); ventral ground color Leaf Green (149) fading into Yellow Green (58) towards head; ventral surfaces of limbs Sepia (119) with Verona Brown (223B) tubercles; iris Raw Sienna (136), bordered by Sepia (119); dorsal and ventral tubercles on fingers and toes Verona Brown (223B) on Sepia (119) ground.

Distribution: Only known from the surroundings of the Lost and Found Eco Hostel on the Pacific drainage in the La Fortuna Forest Reserve.

Habitat and natural history: The only two specimens were found in the Premontane Wet and Rainforest domains between 1280 and 1810 m elevation. There is no information available on the species natural history.

Remarks: I noticed some discrepancies between the description of the coloration in life of HAU 007 by F. Hauenschild and the photos of the specimen in life made by S. Lotzkat (Fig. 36N,O). For example HAU describes the ventral color as green, while on the photos ventral surfaces seem to be transparent (Fig. 36O). Further, dorsal and lateral colors are described as rather dark brownish by Hauenschild, while especially the lateral surfaces appear light green to yellow on the photos (Fig. 36N). I have not seen the specimen in life personally.

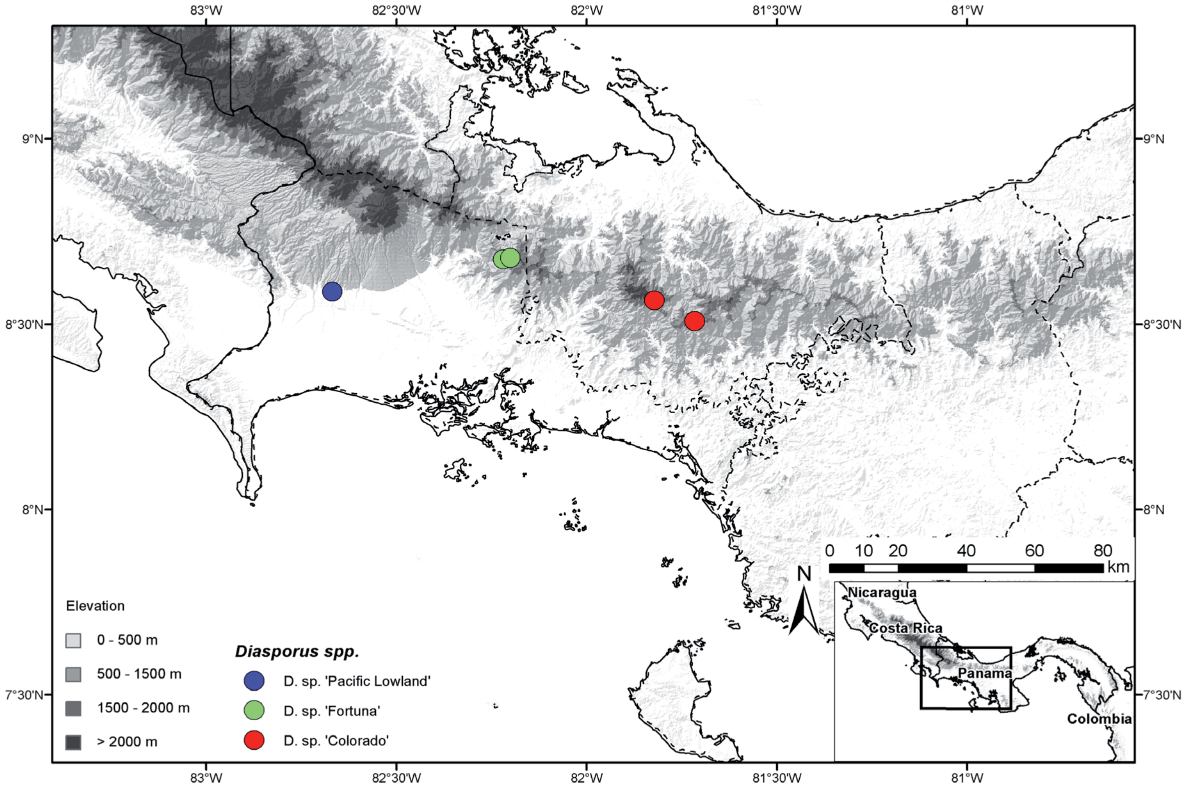

Figure 35: Collection sites of Confirmed Candidate Species in the genus *Diasporus* in western Panama.

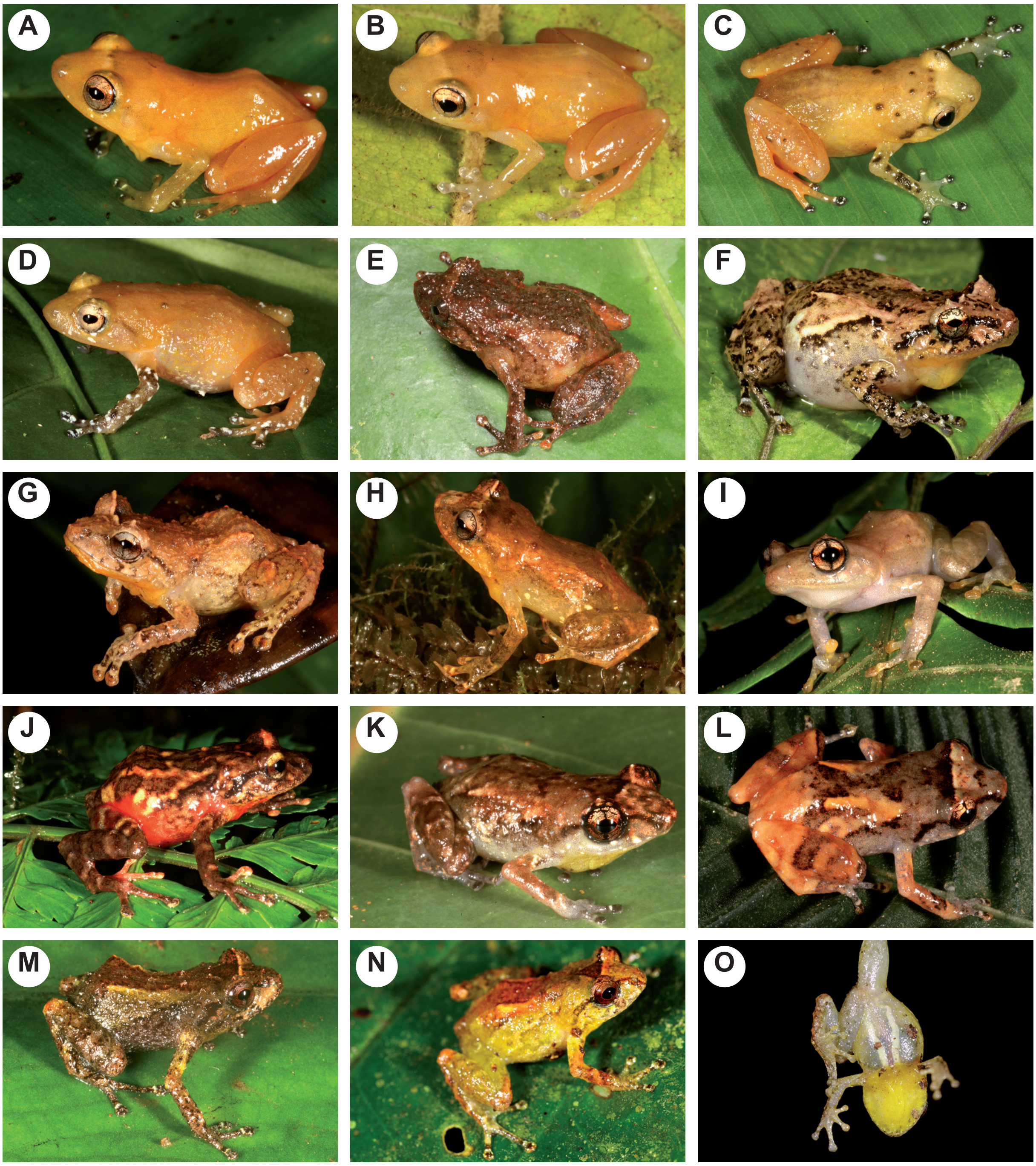

Figure 36: Examples of *Diasporus* specimens in life. **A** *D. citrinobapheus*, male holotype (SMF 89814), Paredón. **B** *D.* cf. *citrinobapheus*, male (SMF 89819), Alto de Piedra. **C** *D.* aff. *tigrillo*, male (AH 422), Río Chiriquí-Malí. **D** *D.* aff. *tigrillo*, female (SMF 89815), Río Chiriquí-Malí. **E** *D.* cf. *hylaeformis*, male (SMF 89874), Cerro Pando. **F** *D.* aff. *hylaeformis*, male (AH 244), Volcán Barú. **G** *D.* aff. *hylaeformis*, male (AH 177), La Fortuna. **H** *D.* cf. *hylaeformis*, male (AH 486), Cerro Saguí. **I** *D.* cf. *hylaeformis*, female (AH 343), La Nevera. **J** *D. igneus*, male paratype (SMF 89821), La Nevera. **K** *D.* aff. *vocator*, male (AH 364), San San Pond Sak. **L** *D.* aff. *vocator*, male (SMF 89866), Willi Mazú. **M** *D.* sp. ‚Colorado‘, male (AH 485), Cerro Saguí. **N** *D.* sp. ‚Fortuna‘, male (HAU 007), La Fortuna. **O** same specimen as N, ventral view (photo N and O courtesy of S. Lotzkat).

6.2. TAXONOMY: CAUDATA – PLETHODONTIDAE – *BOLITOGLOSSA*

Plethodontidae

The family Plethodontidae is the only salamander family that occurs in Central America. In Panama it is represented by the two genera *Bolitoglossa* and *Oedipina*, with *Bolitoglossa* being very diverse in Panama.

Bolitoglossa

The genus *Bolitoglossa* is the second most species-rich amphibian genus in Panama, after *Craugastor* (Hertz *et al.* 2012c) with currently 20 recognized species. The great majority of species belong to the subgenus *Eladinea* Miranda Ribeiro, 1937. The only exception is *B. lignicolor* that belongs to the subgenus *Bolitoglossa* Duméril, Bibron, Duméril 1854 (Parra-Olea *et al.* 2004). Most of these salamander species inhabit the high mountains of the Talamanca mountain range in the Panama-Costa Rica border region. Only four species are known to occur in the lowlands of western Panama. While lowland species seem to be more widely distributed, there is a high rate of endemism among highland species.

6.2.1. MOLECULAR ANALYSIS

In the course of this study, 61 specimens of the genus *Bolitoglossa* were collected and analyzed. Tissue samples were taken from 23 specimens and a fragment of the 16S rRNA gene could be sequenced from 21 tissue samples.Those sequences were complimented with 47 sequences from genbank. The final alignment consisted of 526 sites of which, excluding the outgroup, 145 were variable and 117 parsimony informative.

The ML tree and the Bayes tree are basically congruent. Differences are only found in clades with low posterior probability and bootstrap value. In both trees the two subgenera of *Bolitoglossa* that occur in Panama (*i.e.*, *Eladinea* and *Bolitoglossa*) appear as separate clades (bootstrap score [bs]=60; posterior probability [pp]=1) (Fig. 37C). Within the subgenus *Eladinea*, four species groups can be identified as separate clades (Fig 37B). These are three of the four species groups as defined by Parra-Olea *et al.* (2004), plus the recently discovered *B. robinsoni* species group (Boza-Oviedo *et al.* 2012; Hertz *et al.* 2013a). Only the *B. adspersa* group, that should solely be represented by *B. compacta* in Panama, did not appear in the tree topology. However, a sequence of *B. compacta* from Valle del Silencio in Costa Rica is found in the *B. schizodactyla* species group in my trees. The sequences of two

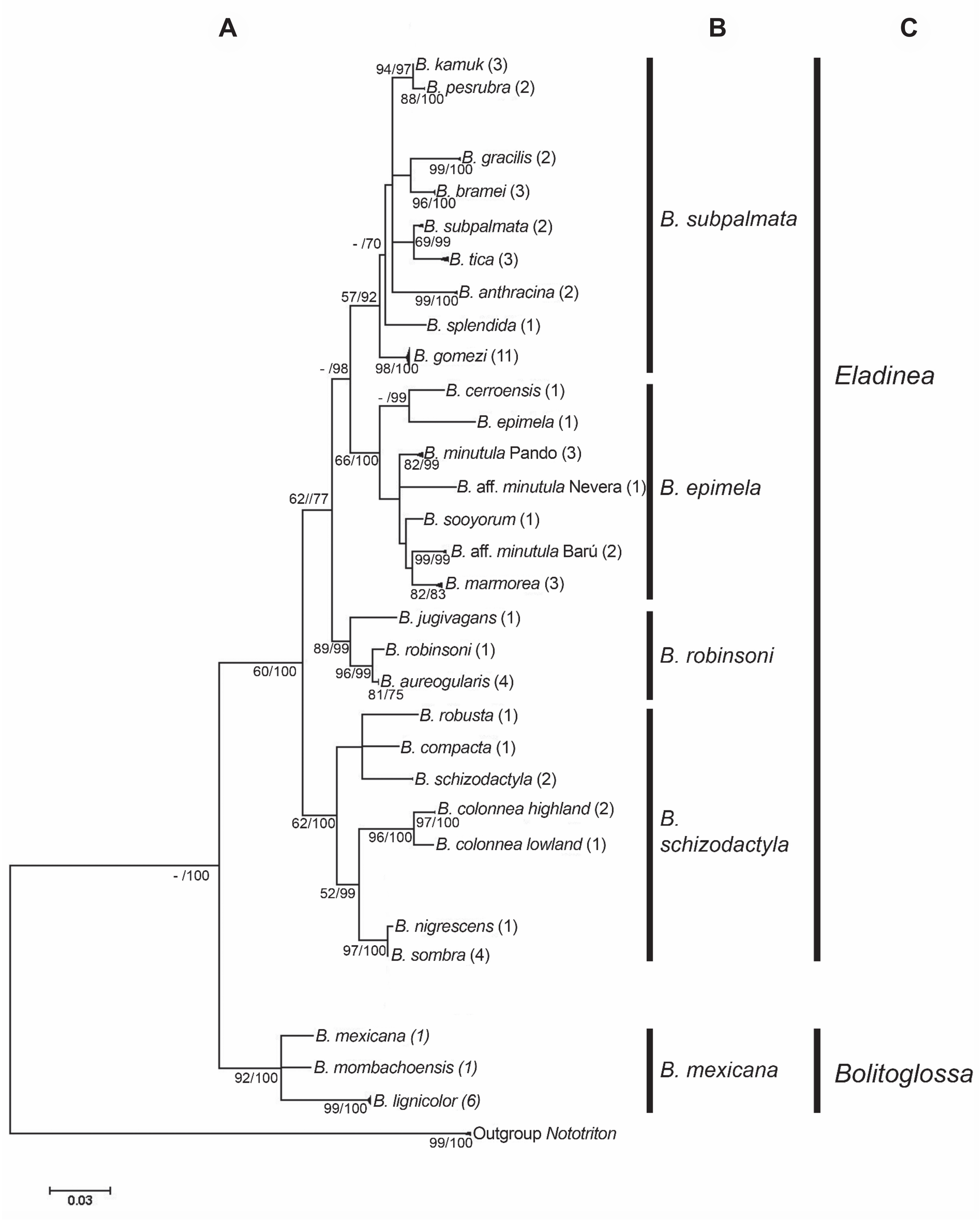

Figure 37: A ML-Tree of the 16S mtDNA marker for Panamanian *Bolitoglossa* and selected congeners. Numbers near nodes represent bootstrap support (before slash) and Bayes posterior propabilities multiplied by 100 (after slash). Branches are labeled according to morphological designation, numbers in parantheses indicate number of specimens analyzed. Depth of black triangles indicates 16S mtDNA variation within a lineage. Scale bar refers to number of substitutions per site. **B** Taxonomic subdivision of clades into species groups. **C** Taxonomic subdivision of clades into subgenera.

specimens of *B. anthracina*, both collected in the La Fortuna Forest Reserve, are nested in the *B. subpalmata* clade, but there is a noticeable genetic within-group distance between these two specimens of 0.3% (Tab. 7). In the *B. epimela* species group, *B. minutula* falls into three geographical definable, polyphyletic clades. However, the polyphyly is not finally confirmed as the nodes receive not sufficient statistical support and the subbranch of the *B. epimela* group containing *B. minutula* and its relatives is polytomous. *Bolitoglossa minutula* from near the type locality at Cerro Pando show a mean p-distance of 2.4% to specimens of *B*. cf. *minutula* from Volcán Barú and 3.2% to a single specimen of *B*. cf. *minutla* from La Nevera. The p-distance of the latter specimen to the Volcán Barú specimens is even 3.9%. The

Table 7: The number of base differences per site from averaging over all sequence pairs within each lineage of *Bolitoglossa* are shown. Standard error estimates are shown in the last column. The presence of n/c in the results denotes cases in which it was not possible to estimate distances.

Lineage	p-distance	Standard error
B. pesrubra	0	0
B. kamuk	0	0
B. anthracina	0.002985075	0.002849017
B. splendida	n/c	n/c
B. subpalmata A	n/c	n/c
B. subpalmata B	n/c	n/c
B. tica	0.00703606	0.003268036
B. gracilis	0.002645503	0.002511599
B. bramei	0.001759015	0.001694318
B. gomezi	0.000479731	0.00047265
B. sooyorum	n/c	n/c
B. aff. *minutula* Nevera	n/c	n/c
B. aff. *minutula* Baru	0.002638522	0.002597164
B. minutula Pando	0.003695129	0.002472033
B. marmorea	0.00351803	0.002353848
B. cerroensis	n/c	n/c
B. epimela	n/c	n/c
B. jugivagans	n/c	n/c
B. robinsoni	n/c	n/c
B. aureogularis	0	0
B. robusta	n/c	n/c
B. compacta	n/c	n/c
B. colonnea Highland	0	0
B. colonnea Lowland	n/c	n/c
B. schizodactyla	0	0
B. nigrescens	n/c	n/c
B. sombra	0	0
B. lignicolor	0.000881834	0.000870372
B. mexicana	n/c	n/c
B. mombachoensis	n/c	n/c
N. picadoi	n/c	n/c
N. matama	n/c	n/c

newly defined *B. robinsoni* species group is moderately well supported in both trees (bs=62; pp=0.77). The recently described species *B. jugivagans* is well-differentiated by a genetic p-distance of 3.4 and 3.2% to its nearest congeners *B. robinsoni* and *B. aureogularis* (Hertz *et al.* 2013a). The nodes are well-supported in both trees (bs=89; pp=0.99). On the other hand, the genetic distance between *B. robinsoni* and *B. aureogularis* is comparatively low with only 0.8%. In the *B. schizodactyl*a species group there is a notable distance of 1.9% between specimens of *B. colonnea* which I collected on the north slope of Cerro Pando and a specimen originated from the Caribbean lowlands that is on GenBank (Hertz *et al.* 2013b). Further, specimens of *B. sombra* collected near the type locality show almost no genetic distance to a specimen of *B. nigrescens* from the northern margin of the Serranía de Talamanca in central

Table 8: Estimates of p-distances over sequence pairs between lineages of *Bolitoglossa*. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimates are shown above the diagonal. Outgroup not shown.

	Lineage	1	2	3	4	5	6	7	8	9	10
1	*B. pesrubra*		0.004	0.010	0.009	0.008	0.008	0.009	0.010	0.008	0.009
2	*B. kamuk*	0.005		0.009	0.008	0.007	0.007	0.008	0.009	0.007	0.008
3	*B. anthracina*	0.038	0.032		0.010	0.010	0.010	0.010	0.011	0.010	0.010
4	*B. splendida*	0.032	0.026	0.045		0.008	0.008	0.010	0.010	0.010	0.009
5	*B. subpalmata* A	0.024	0.018	0.041	0.029		0.004	0.006	0.009	0.009	0.009
6	*B. subpalmata* B	0.024	0.018	0.042	0.029	0.005		0.006	0.008	0.008	0.009
7	*B. tica*	0.039	0.033	0.047	0.044	0.020	0.017		0.009	0.009	0.009
8	*B. gracilis*	0.044	0.038	0.052	0.049	0.033	0.028	0.039		0.008	0.011
9	*B. bramei*	0.025	0.019	0.040	0.040	0.033	0.027	0.039	0.031		0.009
10	*B. gomezi*	0.034	0.029	0.041	0.034	0.031	0.031	0.041	0.046	0.036	
11	*B. sooyorum*	0.045	0.040	0.056	0.055	0.042	0.042	0.049	0.062	0.054	0.042
12	*B.* aff. *minutula* ‚Nevera‘	0.056	0.050	0.066	0.069	0.058	0.058	0.068	0.078	0.059	0.058
13	*B.* aff. *minutula* ‚Baru‘	0.054	0.049	0.067	0.065	0.054	0.057	0.066	0.074	0.060	0.062
14	*B. minutula*	0.043	0.037	0.063	0.052	0.044	0.044	0.060	0.064	0.055	0.051
15	*B. marmorea*	0.046	0.040	0.064	0.055	0.044	0.044	0.054	0.058	0.055	0.048
16	*B. cerroensis*	0.061	0.055	0.076	0.055	0.061	0.061	0.070	0.073	0.064	0.058
17	*B. epimela*	0.058	0.053	0.059	0.048	0.053	0.053	0.063	0.065	0.057	0.053
18	*B. jugivagans*	0.053	0.048	0.066	0.053	0.050	0.056	0.059	0.070	0.057	0.058
19	*B. robinsoni*	0.055	0.050	0.065	0.055	0.053	0.047	0.056	0.067	0.059	0.055
20	*B. aureogularis*	0.053	0.047	0.062	0.053	0.050	0.045	0.054	0.065	0.056	0.053
21	*B. robusta*	0.066	0.066	0.074	0.071	0.069	0.069	0.077	0.073	0.067	0.061
22	*B. compacta*	0.066	0.066	0.087	0.082	0.069	0.063	0.074	0.073	0.069	0.074
23	*B. colonnea* ‚Highland‘	0.069	0.064	0.089	0.080	0.077	0.077	0.086	0.081	0.073	0.071
24	*B. colonnea* ‚Lowland‘	0.077	0.071	0.093	0.079	0.079	0.079	0.088	0.084	0.080	0.079
25	*B. schizodactyla*	0.063	0.058	0.077	0.063	0.061	0.061	0.069	0.067	0.067	0.063
26	*B. nigrescens*	0.047	0.047	0.072	0.063	0.061	0.061	0.069	0.065	0.056	0.056
27	*B. sombra*	0.045	0.045	0.071	0.061	0.059	0.059	0.067	0.063	0.054	0.058
28	*B. lignicolor*	0.094	0.088	0.099	0.102	0.099	0.099	0.113	0.105	0.100	0.099
29	*B. mexicana*	0.090	0.085	0.099	0.090	0.093	0.093	0.104	0.099	0.099	0.087
30	*B. mombachoensis*	0.090	0.085	0.096	0.095	0.098	0.098	0.110	0.102	0.099	0.093

Table 8 continued

	Lineage	11	12	13	14	15	16	17	18	19	20
1	*B. pesrubra*	0.010	0.011	0.010	0.010	0.010	0.011	0.012	0.011	0.011	0.011
2	*B. kamuk*	0.009	0.011	0.010	0.009	0.009	0.011	0.011	0.011	0.011	0.010
3	*B. anthracina*	0.011	0.012	0.012	0.012	0.012	0.013	0.012	0.013	0.012	0.012
4	*B. splendida*	0.011	0.012	0.012	0.011	0.011	0.011	0.011	0.011	0.011	0.011
5	*B. subpalmata* A	0.010	0.011	0.010	0.010	0.010	0.012	0.011	0.011	0.011	0.011
6	*B. subpalmata* B	0.010	0.011	0.011	0.010	0.010	0.012	0.011	0.012	0.010	0.010
7	*B. tica*	0.010	0.012	0.011	0.011	0.010	0.012	0.012	0.012	0.011	0.011
8	*B. gracilis*	0.012	0.013	0.012	0.012	0.011	0.012	0.012	0.013	0.012	0.012
9	*B. bramei*	0.011	0.011	0.011	0.011	0.011	0.012	0.011	0.011	0.011	0.011
10	*B. gomezi*	0.010	0.011	0.012	0.011	0.010	0.011	0.011	0.012	0.011	0.011
11	*B. sooyorum*		0.009	0.007	0.007	0.007	0.010	0.010	0.011	0.011	0.010
12	*B.* aff. *minutula* ‚Nevera‘	0.034		0.009	0.009	0.009	0.011	0.012	0.012	0.012	0.012
13	*B.* aff. *minutula* ‚Baru‘	0.025	0.038		0.008	0.008	0.010	0.010	0.011	0.012	0.011
14	*B. minutula*	0.020	0.033	0.028		0.008	0.009	0.010	0.012	0.012	0.011
15	*B. marmorea*	0.023	0.035	0.027	0.025		0.009	0.010	0.012	0.011	0.011
16	*B. cerroensis*	0.042	0.053	0.044	0.035	0.035		0.010	0.011	0.012	0.011
17	*B. epimela*	0.045	0.064	0.046	0.046	0.044	0.040		0.011	0.012	0.011
18	*B. jugivagans*	0.056	0.069	0.052	0.059	0.060	0.058	0.053		0.009	0.009
19	*B. robinsoni*	0.053	0.072	0.062	0.058	0.060	0.058	0.058	0.034		0.004
20	*B. aureogularis*	0.050	0.064	0.059	0.055	0.057	0.055	0.056	0.032	0.008	
21	*B. robusta*	0.071	0.093	0.086	0.078	0.077	0.084	0.074	0.074	0.066	0.063
22	*B. compacta*	0.074	0.093	0.088	0.080	0.076	0.084	0.085	0.072	0.053	0.055
23	*B. colonnea* ‚Highland‘	0.085	0.099	0.089	0.087	0.083	0.085	0.088	0.074	0.066	0.069
24	*B. colonnea* ‚Lowland‘	0.087	0.101	0.091	0.084	0.080	0.082	0.085	0.082	0.069	0.071
25	*B. schizodactyla*	0.069	0.082	0.075	0.074	0.062	0.082	0.082	0.061	0.058	0.061
26	*B. nigrescens*	0.058	0.077	0.067	0.064	0.064	0.066	0.069	0.066	0.058	0.061
27	*B. sombra*	0.061	0.075	0.065	0.062	0.062	0.064	0.067	0.064	0.056	0.059
28	*B. lignicolor*	0.110	0.113	0.108	0.105	0.105	0.123	0.115	0.102	0.110	0.107
29	*B. mexicana*	0.106	0.104	0.103	0.097	0.091	0.108	0.109	0.093	0.090	0.093
30	*B. mombachoensis*	0.101	0.104	0.103	0.103	0.102	0.114	0.109	0.088	0.090	0.093
31	*N._picadoi*	0.151	0.173	0.157	0.159	0.154	0.167	0.157	0.154	0.156	0.153
32	*N._matama*	0.153	0.176	0.160	0.162	0.157	0.169	0.160	0.157	0.153	0.151

Table 8 continued

	Lineage	21	22	23	24	25	26	27	28	29	30
1	*B. pesrubra*	0.012	0.012	0.013	0.013	0.012	0.010	0.010	0.014	0.014	0.014
2	*B. kamuk*	0.012	0.012	0.012	0.013	0.012	0.010	0.010	0.014	0.014	0.014
3	*B. anthracina*	0.013	0.014	0.014	0.014	0.014	0.012	0.012	0.015	0.015	0.015
4	*B. splendida*	0.013	0.014	0.014	0.013	0.012	0.012	0.012	0.015	0.014	0.014
5	*B. subpalmata* A	0.012	0.013	0.013	0.013	0.012	0.012	0.011	0.015	0.014	0.015
6	*B. subpalmata* B	0.012	0.012	0.013	0.013	0.012	0.012	0.011	0.015	0.014	0.015
7	*B. tica*	0.013	0.013	0.014	0.014	0.013	0.012	0.012	0.015	0.015	0.015
8	*B. gracilis*	0.013	0.013	0.014	0.014	0.013	0.012	0.012	0.015	0.014	0.015
9	*B. bramei*	0.012	0.012	0.013	0.013	0.012	0.011	0.011	0.014	0.015	0.015
10	*B. gomezi*	0.012	0.013	0.013	0.014	0.013	0.011	0.012	0.015	0.014	0.014
11	*B. sooyorum*	0.012	0.013	0.013	0.013	0.012	0.011	0.011	0.015	0.015	0.015
12	*B.* aff. *minutula* ‚Nevera‘	0.014	0.014	0.014	0.014	0.013	0.013	0.013	0.015	0.015	0.015
13	*B.* aff. *minutula* ‚Baru‘	0.013	0.014	0.013	0.014	0.013	0.012	0.012	0.015	0.015	0.015
14	*B. minutula*	0.013	0.014	0.014	0.014	0.013	0.012	0.012	0.015	0.015	0.015
15	*B. marmorea*	0.013	0.013	0.013	0.013	0.012	0.012	0.011	0.015	0.014	0.015
16	*B. cerroensis*	0.014	0.014	0.014	0.014	0.014	0.012	0.012	0.016	0.016	0.016
17	*B. epimela*	0.013	0.014	0.014	0.013	0.014	0.012	0.012	0.016	0.016	0.016
18	*B. jugivagans*	0.013	0.013	0.013	0.014	0.012	0.012	0.012	0.015	0.015	0.014
19	*B. robinsoni*	0.012	0.011	0.013	0.013	0.012	0.012	0.011	0.015	0.014	0.014
20	*B. aureogularis*	0.012	0.012	0.013	0.013	0.012	0.012	0.012	0.015	0.014	0.014
21	*B. robusta*		0.009	0.012	0.012	0.010	0.010	0.011	0.014	0.014	0.014
22	*B. compacta*	0.040		0.012	0.011	0.009	0.010	0.010	0.015	0.015	0.014
23	*B. colonnea* ‚Highland‘	0.064	0.058		0.007	0.012	0.010	0.009	0.015	0.014	0.013
24	*B. colonnea* ‚Lowland‘	0.061	0.056	0.019		0.012	0.010	0.009	0.015	0.014	0.014
25	*B. schizodactyla*	0.045	0.037	0.058	0.056		0.011	0.011	0.015	0.014	0.014
26	*B. nigrescens*	0.047	0.042	0.040	0.042	0.055		0.003	0.014	0.014	0.014
27	*B. sombra*	0.051	0.040	0.037	0.040	0.053	0.003		0.014	0.014	0.013
28	*B. lignicolor*	0.094	0.110	0.105	0.113	0.102	0.096	0.095		0.011	0.010
29	*B. mexicana*	0.090	0.095	0.085	0.088	0.085	0.087	0.085	0.046		0.008
30	*B. mombachoensis*	0.090	0.095	0.080	0.093	0.090	0.082	0.080	0.042	0.027	

Costa Rica.

6.2.2. MORPHOLOGICAL ANALYSIS

Morphological characters that allow for a reliable identification on the species level during field work are few in the genus *Bolitoglossa*. An overview of several morphological standard characters is given in Table 9. The best characters to be used to distinguish between species with the unaided eye under field conditions are a combination of extent of webbing, coloration, and conditionally also size. However, the most reliable morphological characters to distinguish between species are tooth counts of maxillary teeth (MT) and vomerine teeth (VT) what is only applicable to conserved material under a stereo microscope. Furthermore, one has to keep in mind that the number of teeth increases with increasing size and age of the

Table 9: Morphological proportions and toothcounts in *Bolitoglossa* lineages from western Panama

Lineage	n	Sex	SVL	TL/SVL	HL/SVL	HW/SVL	HLL/SVL	HAW/SVL
B. jugivagans	1	f	31.2	1.24	0.21	0.14	0.2	0.07
B. anthracina	1	f	43.8	1.26	0.25	0.15	0.23	0.09
	1	m	55.4	1.35	0.23	0.15	0.24	0.1
B. colonnea	7	f	33.0–49.0 (43.91±5.51	0.71–1.15 (0.96±0.15)	0.23–0.26 (0.24±0.01)	0.16–0.18 (0.16±0.01)	0.19–0.23 (0.21±0.02)	0.08–0.09 (0.08±0.01)
	2	m	40.0–40.3 (40.15±0.21)	1.04–1.09 (1.06±0.04)	0.24–0.26 (0.25±0.01)	0.16–0.14 (0.15±0.01)	0.23–0.25 (0.24±0.01)	0.09
B. compacta	1	f	39.1	0.69	0.24	0.16	0.23	0.09
B. gomezi	3	f	38.2–54.5 (44.67±8.66	1.14–1.19 (1.17±0.02	0.22–0.23 (0.22±0.01	0.14–0.15 (0.15±0.01	0.22–0.23 (0.22±0.01)	0.08–0.09 (0.08±0.01)
B. lignicolor	8	f	36.1–69.2 (53.83±11.47)	0.70–0.89 (0.78±0.06)	0.20–0.24 (0.23±0.02)	0.15–0.17 (0.15±0.01)	0.10–0.22 (0.18±0.04)	0.07–0.08 (0.07±0.00)
	6	m	45.4–67.0 (53.72±7.82)	0.93–1.07 (0.97±0.05)	0.22–0.25 (0.24±0.01)	0.16–0.17 (0.16±0.00)	0.16–0.21 (0.19±0.02)	0.07–0.08 (0.08±0.00)
	4	juv	30.0–32.4 (30.65±1.17)	0.65–0.77 (0.71±0.06)	0.25–0.28 (0.26±0.01)	0.17–0.19 (0.18±0.01)	0.17–0.23 (0.19±0.03)	0.06–0.07 (0.07±0.00)
B. magnifica	4	f	44.2–61.2 (50.38±7.68)	0.52–0.73 (0.66±0.10)	0.23–0.27 (0.25±0.02)	0.16–0.19 (0.17±0.01)	0.19–0.23 (0.21±0.02)	0.08–0.09 (0.08±0.01)
	3	m	33.7–43.5 (36.97±5.66)	0.77–0.89 (0.84±0.06)	0.24–0.26 (0.24±0.01)	0.16–0.18 (0.17±0.01)	0.21–0.23 (0.22±0.01)	0.08–0.11 (0.09±0.01)
	2	juv	29.7–30.3 (30.00±0.42)	0.55–0.78 (0.66±0.16)	0.24	0.17	0.20–0.23 (0.21±0.03)	0.08–0.09 (0.08±0.00)
B. minutula ‚Pando‘	6	f	25.20–35.40 (31.47±4.58)	0.84–0.93 (0.88±0.09)	0.20–0.23 (0.22±0.01)	0.13–0.18 (0.15±0.02)	0.17–0.24 (0.20±0.03)	0.07–0.08 (0.07±0.01)
	3	m	29.40–38.70 (33.50–4.75)	0.50–1.22 (0.81±0.37)	0.19–0.27 (0.24±0.05)	0.11–0.17 (0.15±0.03)	0.13–0.23 (0.19±0.05)	0.06–0.09 (0.08±0.02)
	2	juv	22.0–23.3 (22.65±0.92)	0.58–0.86 (0.72±0.20)	0.24–0.26) 0.25±0.01	0.16–0.17 (0.17±0.00)	0.18–0.20 (0.19±0.01)	0.05–0.06 (0.06±0.00)
B. aff. *minutula* ‚Barú'	2	f	31.8–33.1 (32.45±0.92)	1.01–1.05 (1.03±0.03)	0.21–0.22 (0.21±0.00)	0.14–0.15 (0.15±0.00)	0.17–0.19 (0.18±0.01)	0.05–0.07 (0.06±0.01)
	2	m	29.7–34.6 (32.15±3.46)	1.05–1.22	0.24	0.14	0.16–0.19	0.06–0.07
	1	juv	21.1	0.57	0.31	0.18	0.19	0.08
B. aff. *minutula* ‚Nevera'	1	juv	25.7	0.37	0.21	0.13	0.18	0.05
B. nigrescens	5	f	35.80–84.50 (50.24±19.61)	0.69–0.93 (0.84±0.09)	0.21–0.29 (0.26±0.03)	0.16–0.21 (0.18±0.02)	0.20–0.26 (0.23±0.02)	0.09–0.10 (0.09±0.01)
	2	m	57.90–70.00 (63.95±8.56)	0.88–1.03 (0.96±0.10)	0.25–0.26 (0.25±0.01)	0.16–0.18 (0.17±0.01)	0.22–0.23 (0.23–0.01)	0.1

Table 9 continued

Lineage	n	Sex	HFW/SVL	PMT	MT (left)	MT (right)	MT (total)	MT/SVL
B. jugivagans	1	f	0.09	4	23	28	51	1.63
B. anthracina	1	f	0.11	6	30	24	54	1.23
	1	m	0.12	4	29	32	61	1.1
B. colonnea	7	f	0.08–0.12 (0.10±0.01)	0–3 (2.14±1.07)	0–7 (4.43±2.23)	0–7 (3.29±2.21)	0–11 (7.71±3.64)	0.00–0.24 (0.17±0.08)
	2	m	0.11– 0.12 (0.11±0.01)	5–6 (5.5±0.71)	0	0–2 (1.00±1.41)	0–2 (1.00±1.41)	0.00–0.05 (0.03±0.04)
B. compacta	1	f	0.1	0	9	7	16	0.41
B. gomezi	3	f	0.10–0.11 (0.10±0.01	4.00–5.00 (4.67±0.58)	25.00–27.00 (26.00±1.00)	23.00–27.00 (25.67±2.31)	48.00–54.00 (51.67±3.21)	0.97–1.41 (1.18±0.22)
B. lignicolor	8	f	0.08–0.10 (0.09±0.01)	0–4 (1.25±1.75)	7–22 (15.63±4.98)	11–21 (16.38±3.25)	18–40 (32.00±8.00)	0.50–0.69 (0.59±0.06)
	6	m	0.09–0.10 (0.10±0.01)	3–5 (3.83±0.75)	13–26 (18.83±5.27)	9–25 (18.50±5.58)	22–51 (37.33±10.60)	0.48–0.82 (0.68±0.11)
	4	juv	0.08–0.09 (0.09±0.00)	0	6–10 (7.50±1.73)	5–10 (7.00±2.16)	12–20 (14.50±3.70)	0.40–0.62 (0.47±0.10)
B. magnifica	4	f	0.09–0.12 (0.10±0.01)	1–3 (2.00±1.15)	10–14 (12.00±1.83)	10–16 (12.50±2.65)	20–29 (24.50–4.20)	0.44–0.58 (0.49±0.06)
	3	m	0.10–0.12 (0.11±0.01)	1–4 (2.00±1.73)	2–15 (8.00±6.56)	4–18 (9.33±7.57)	6–33 (17.33±14.01)	0.18–0.76 (0.44±0.29)
	2	juv	0.10–0.11 (0.10±0.00)	2	3–6 (4.50±2.12)	6	9–12 (10.50±2.12)	0.30–0.40 (0.35±0.07)
B. minutula ‚Pando‘	7	f	0.08–0.10 (0.09±0.01)	2–5 (3.50±1.22)	7–22 (16.67±5.32)	5–24 (16.83±6.77)	12–46 (31.50±12.05)	0.48–1.36 (1.04±0.30)
	3	m	0.07–0.11 (0.10±0.03)	2–3 (2.33±0.58)	16–23 (19.67±3.51)	17–24 (20.67±3.51)	33–47 (40.33±7.02)	0.85–1.60 (1.24±0.37)
	2	juv	0.07	0–1 (0.50±0.71)	3–9 (6.00±4.24)	2–6 (4.00±2.83)	5–15 (10.00±7.07)	0.21–0.68 (0.45±0.33)
B. aff. *minutula* ‚Barú'	2	f	0.06–0.08 (0.07±0.01)	4	21–22 (21.50±0.71)	17–20 (18.50±2.12)	38–42 (40.00±2.83)	1.19–1.27 (1.23±0.05)
	2	m	0.07–0.08	3	16–20	14–20	30–40	1.01–1.16
	1	juv	0.09	0	1	0	1	0.05
B. aff. *minutula* ‚Nevera'	1	juv	0.06	2	1	1	2	0.08
B. nigrescens	5	f	0.10–0.13 (0.11±0.01)	1–3 (2.00±1.00)	8–34 (16.20±10.35)	7–30 (15.40±8.85)	15–64 (31.60±18.84)	0.42–0.76 (0.60±0.13)
	2	m	0.12	3–5 (4.00±1.41)	22–25 (23.50±2.12)	21–25 (23.00±2.83)	43–50 (46.50±4.95)	0.71–0.74 (0.73±0.02)

Table 9 continued

Lineage	n	Sex	VT (left)	VT (right)	VT(total)	VT/SVL	MT/VT	CG	CFL
B. jugivagans	1	f	12	15	27	0.87	1.89	13	4
B. anthracina	1	f	11	16	27	0.62	2	12	4
	1	m	12	11	23	0.42	2.65	12	3
B. colonnea	7	f	5–16 (8.29±4.23)	5–18 (9.43±4.61)	10–34 (17.71±8.71)	0.23–0.74 (0.40±0.17)	0.00–0.91 (0.47±0.29)	12	2.5–4.0 (3.21±0.49)
	2	m	8–20 (14.00±8.49)	9–28 (18.50±13.44)	17–48 (32.50±21.92)	0.43–1.19 (0.81±0.54)	0.00–0.12 (0.06±0.08)	12	4
B. compacta	1	f	8	12	20	0.51	0.8	12	3
B. gomezi	3	f	9.00–14.00 (11.00±2.65)	8.00–15.00 (11.33±3.51)	17.00–29.00 (22.33±6.11)	0.41–0.55 (0.50±0.08)	1.83–2.82 (2.41±0.52)	12	3
B. lignicolor	8	f	6–16 (11.13±3.18)	4–19 (11.13±4.55)	10–35 (22.25±7.50)	0.27–0.70 (0.42±0.15)	0.94–2.29 (1.55±0.53)	12	3.5–4.0 (3.93±0.19)
	6	m	8–18 (12.00±3.52)	8–18 (12.17±4.36)	16–35 (24.17±7.55)	0.33–0.68 (0.46±0.17)	0.71–2.47 (1.69±0.68)	12	4–4.5 (4.08±0.20)
	4	juv	5–9 (7.00±1.83)	5–8 (6.75±1.26)	10–17 (13.75±2.99)	0.33–0.56 (0.45±0.10)	0.71–1.54 (1.10±0.38)	12	3.5–4.0 (3.88±0.25)
B. magnifica	4	f	8–16 (11.75±3.86)	9–16 (12.75±3.77)	18–32 (24.50±7.55)	0.39–0.60 (0.48±0.10)	0.84–1.22 (1.04±0.17)	12	2.5–3.0 (2.88±0.25)
	3	m	5–10 (8.00±2.65)	5–10 (7.33±2.52)	10–20 (15.33±5.03)	0.30–0.47 (0.41±0.10)	0.38–1.65 (1.11±0.66)	12	2–2.5 (2.00±0.00)
	2	juv	6	5–7 (6.00±1.41)	11–13 (12.00±1.41)	0.37–0.43 (0.40±0.04)	0.82–0.92 (0.87±0.07)	12	2
B. minutula ‚Pando‘	7	f	6–11 (7.83±1.94)	5–10 (7.33±2.07)	11–20 (15.17±3.60)	0.42–0.59 (0.48±0.06)	1.09–2.64 (2.17±0.55)	12	2–5 (3.80±1.10)
	3	m	9–10 (9.33±0.58)	10–12 (10.67±1.15)	19–22 (20.00±1.73)	0.49–0.75 (0.61±0.13)	1.74–2.16 (2.01±0.24)	12	2.0–2.5 (2.25±0.35)
	2	juv	5–7 (6.00±1.41)	6	11–13 (12.00±1.41)	0.50–0.56 (0.53±0.04)	0.38–1.36 (0.87±0.69)	12	4
B. aff. *minutula* ‚Barú'	2	f	6–8 (7.00±1.41)	7–9 (8.00±1.41)	15	0.45–0.47 (0.46±0.01)	2.53–2.80 (2.67±0.19)	12	5
	2	m	7–8	7	14–15	0.43–0.47	2.14–2.67	12	4–5 (4.50±0.71)
	1	juv	4	7	11	0.52	0.09	12	2.5
B. aff. *minutula* ‚Nevera'	1	juv	7	7	14	0.54	0.14	12	5
B. nigrescens	5	f	7–19 (13.00±4.42)	7–20 (12.20±4.97)	14–39 (25.20±9.26)	0.39–0.64 (0.51±0.09)	1.04–1.64 (1.20±0.26)	12	2.0–3.5 (2.67±0.76)
	2	m	17–19 (18.00±1.41)	17	34–36 (35.00±1.41)	0.51–0.59 (0.55±0.05)	1.26–1.39 (1.33±0.09	12	2.0–2.5 (2.25±0.35)

specimen, for which reason tooth counts are usually given in relation to the snout-vent length.

Since I obtained additional material of the large, black *Bolitoglossa* sensu Hanken *et al.* (2005), I lend particular attention to the exploration of morphological data of this group. Two specimens of large, black salamanders, the male AH 284 and the female AH 385, can easily be assigned to *Bolitoglossa anthracina*, especially by the high maxillary tooth counts (Fig.

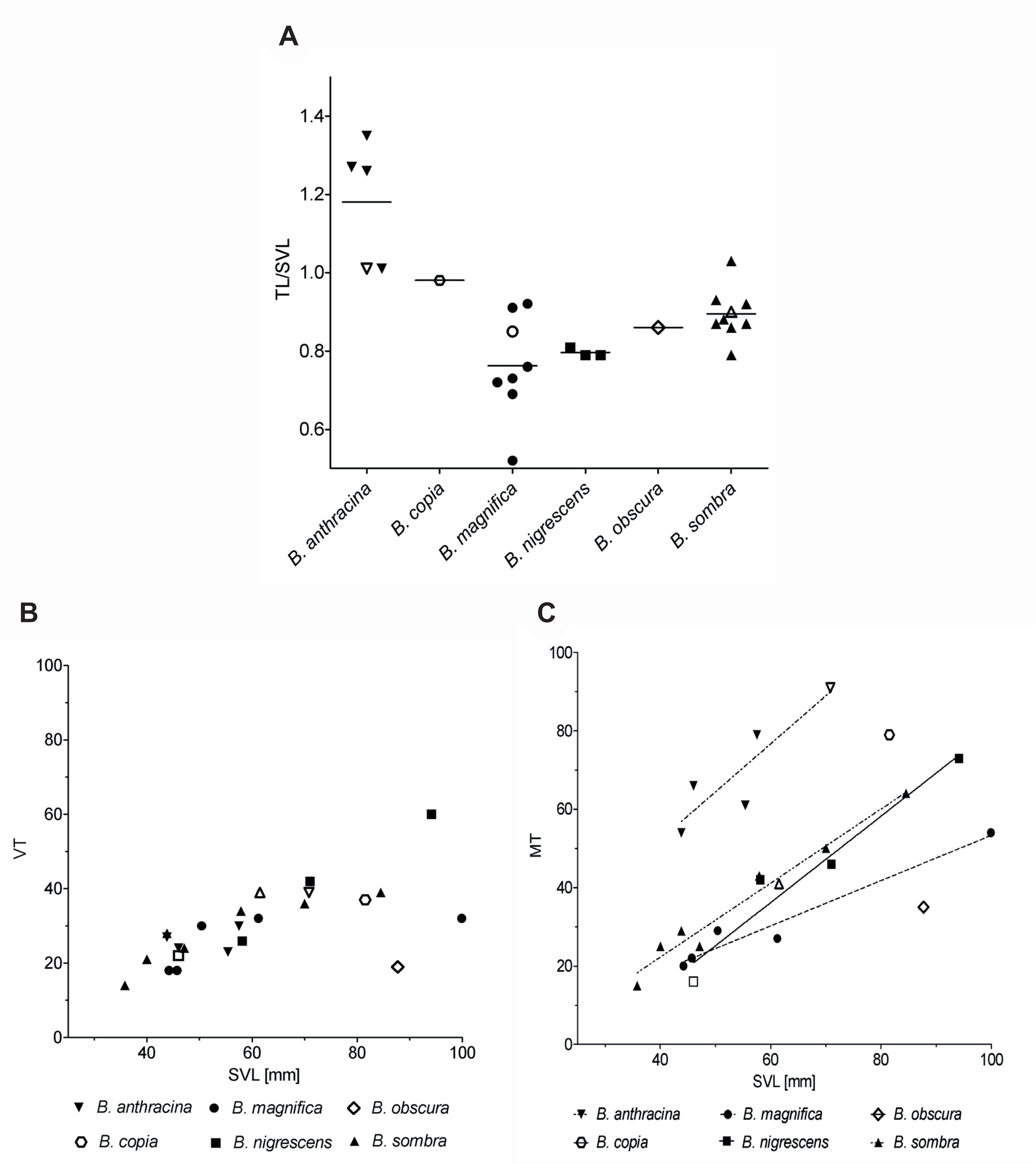

Figure 38: Comparison of morphological proportions in large, black *Bolitoglossa*. Hollow symbols represent holotypes. **A** Tail length in relation to snout-vent length. **B** Total vomerine tooth count versus snout-vent length. **C** Total maxillary tooth count versus snout-vent length.

38C) and the long prehensile tail (Fig. 38A). Both traits, especially in combination, are very different in other species of large black salamanders, which they superficially may resemble. With the exception of size and minor sex differences both specimens share all morphologic characters.

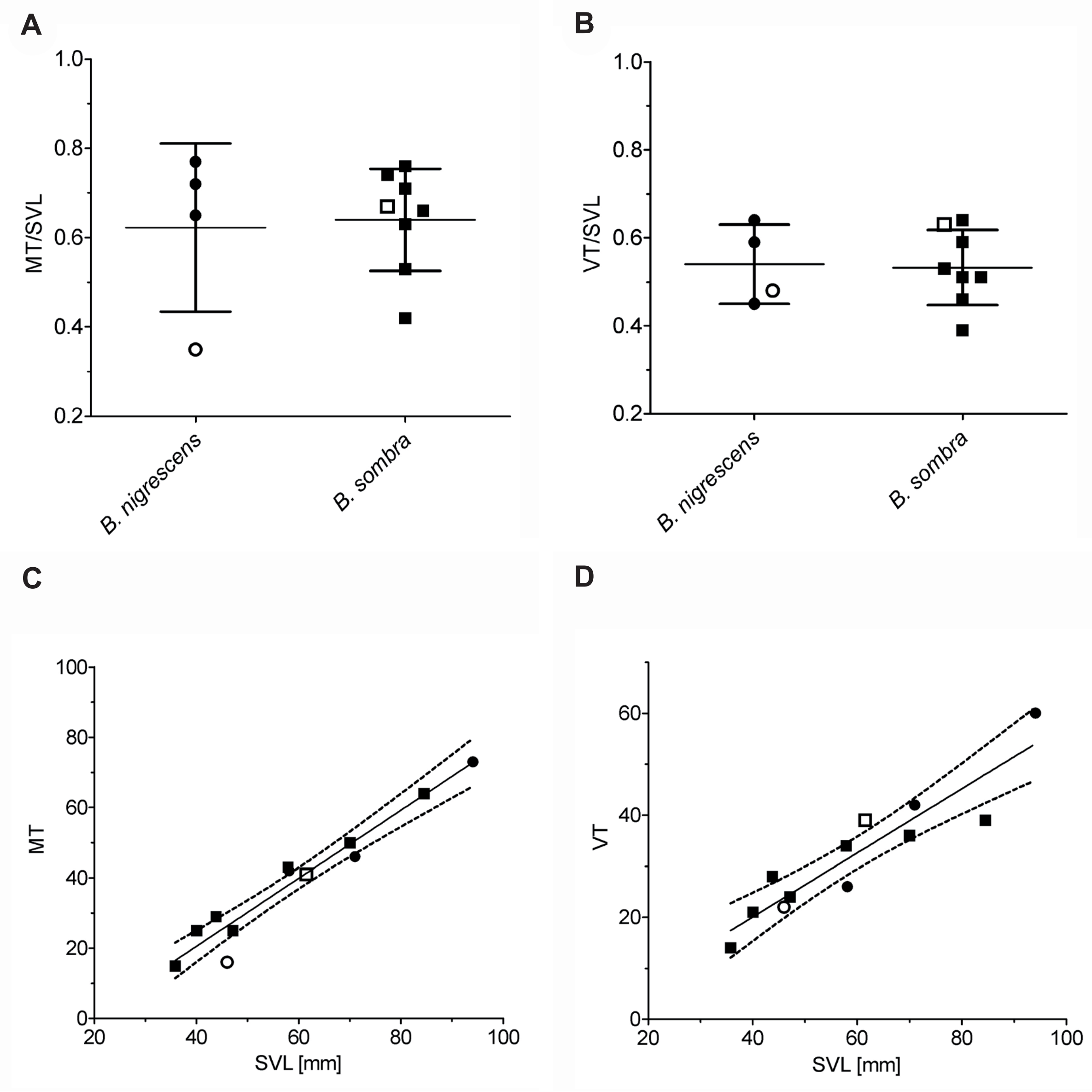

Figure 39: Tooth counts in relation to body size for specimens of *Bolitoglossa nigrescens* (circles) and *B. sombra* (squares). Hollow symbols represent holotypes. **A** Scatter plot of total number of maxillary teeth divided by snout-vent length. Lines represent mean with standard deviation. **B** Scatter plot of total number of vomerine teeth divided by snout-vent length. Lines represent mean with standard deviation. **C** Total maxillary teeth of both taxa plotted against snout-vent length. Solid line shows linary regression through all points, dashed lines represent the 95% confidence level. **D** Total vomerine teeth of both taxa plotted against snout-vent length. Solid line shows linary regression through all points, dashed lines represent the 95% confidence level.

Since I have not personally examined voucher specimens of *Bolitoglossa nigrescens* I used morphological data from Hanken *et al.* (2005) of *B. nigrescens* and compared these with their data of *B. sombra*, combined with my own data. The Mann-Whitney test showed no significant difference ($P > 0.05$) between the medians of both species in neither vomerine nor maxillary tooth counts in relation to body size (Fig 39A,B). However, a high correlation between tooth counts and size, with a regression slope that is significantly different from

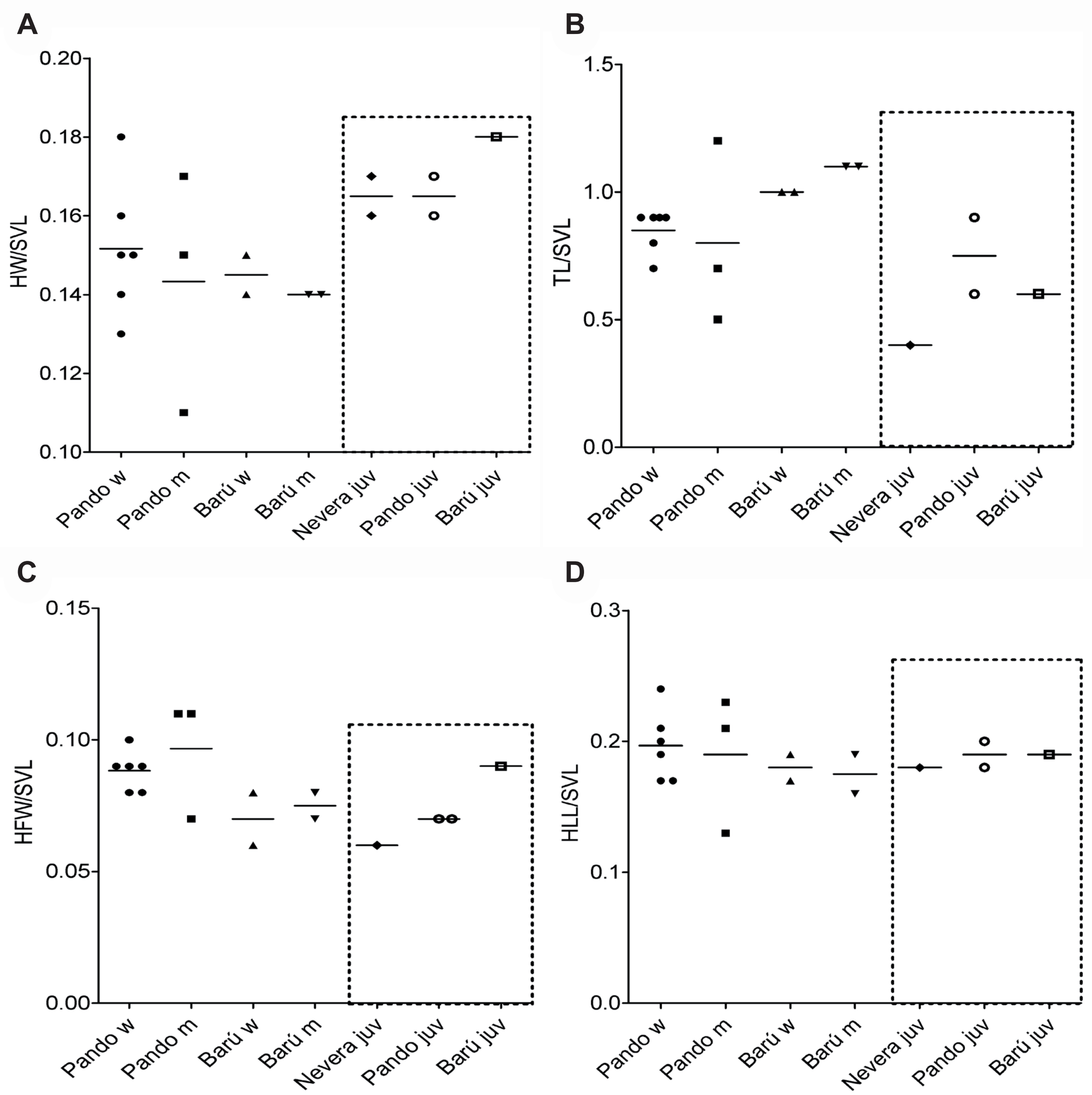

Figure 40: Comparison of morphometric proportions in specimens assigned to *Bolitoglossa minutula*. Scatter plots for specimens by gender from different localities are shown. Data for juveniles are outlined by dashed squares. Horizontal lines represent means. **A** Head width divided by snout-vent length. **B** Tail length divided by snout-vent length. **C** Hind foot width divided by snout-vent length. **D** Hind limb length divided by snout-vent length.

zero (P < 0.0001), is observable when combining available tooth counts of both species for vomerine and maxillary teeth in relation to size (Fig 39C,D). Only few values lie outside the 95% confidence level.

All specimens morphologically assigned to *Bolitoglossa minutula* share the same standard characters described for this species in relation to their body size. There is a large morphological overlap in all these characters, especially between specimens from Cerro Pando and Volcán Barú. In many cases the variation between sexes is higher than between clades (Fig. 40, Fig. 41). The single specimen from La Nevera is obviously a juvenile as characterized by its small size, comparatively short tail (Fig. 40B), and very low maxillary tooth count (Fig. 41A). When comparing only juveniles of the three genetic clades (Fig. 40, ig. 41: dashed squares), there are differences observable, especially in tail length (Fig. 40B) and foot width (Fig. 40C). However, there is no significant difference among the means of any of the proportions tested. For a more exhaustive analysis, a larger sample size is necessary.

Although there is only a single specimen of *Bolitoglossa jugivagans* known, it is well differentiated from other species in the genus by morphological characters. Especially the high tooth counts in relation to its size, in combination with a slender body, a long prehensile tail, coloration, and the less pronounced webbing, it is unlikely to be mistaken for other described salamander species in the Cordillera Central of Panama and Costa Rica (Tab. 9).

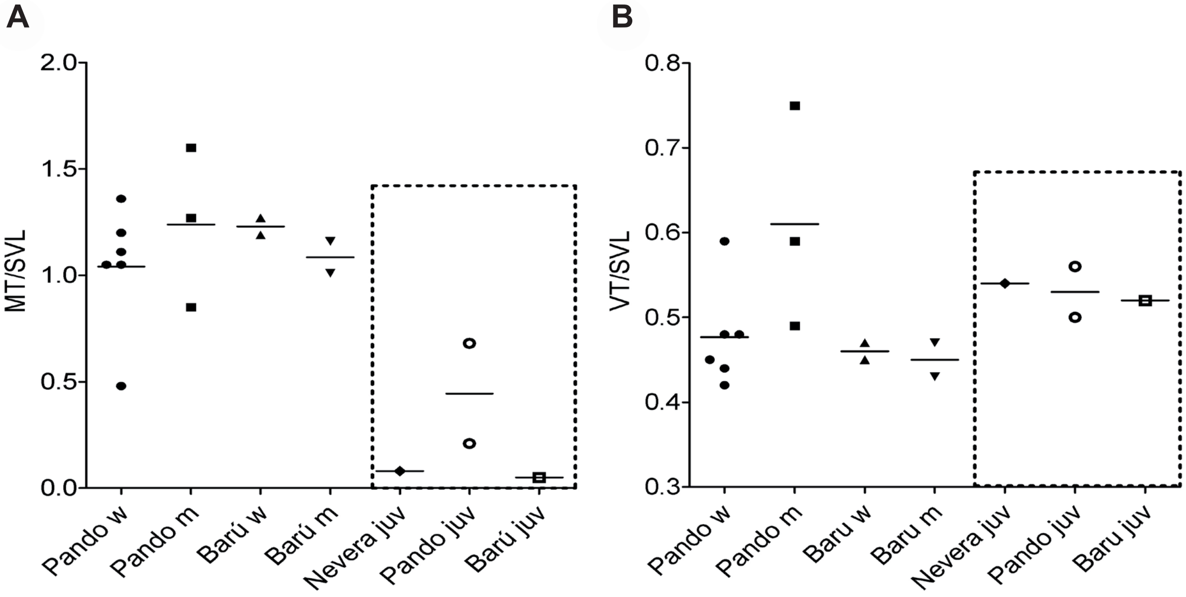

Figure 41: Comparison of tooth counts in relation to body size in specimens assigned to *Bolitoglossa minutula*. Scatter plots for specimens by gender from different localities are shown. Data for juveniles are outlined by dashed squares. Horizontal lines represent means. **A** Total maxillary tooth count divided by snout-vent length. **B** Total vomerine tooth count divided by snout-vent length.

6.2.3. CONCLUSIONS

Combination of molecular and morphological data leads to several new perceptions concerning Panamanian species of *Bolitoglossa*. There are obviously some taxonomic problems to solve within all five species groups that have been included in the analysis as apparent from the polytomies in the respective subbranches. Most of the sequences of the *B. subpalmata* species group have been taken from GenBank, so I do not have own morphological data to compare. Surprisingly, *B. anthracina* is found nested in the *B. subpalmata* species group, despite of its appearance as a relatively large and dark colored species, a character that was used to propose close relationships with *B. nigrescens,* and thus consequently place it in the *B. schizodactyla* species group (Brame *et al.* 2001; Hanken *et al.* 2005). However, younger individuals, *e.g.*, AH 385, are not coal black, but rather grayish and maxillary tooth counts are much higher, the body is more slender, and the tail longer than in other species of large black salamanders. The black coloration in adult specimens is most likely the result of convergence. There is a low (0.3%), but notable genetic p-distance between both specimens of *B. anthracina*, what is most likely due to the fact that sequencing in one specimen (AH 385) did not work well, resulting in a short and ambigious 16S mtDNA sequence of that specimen. The fact that both specimens are still grouped together with high support in both trees (bs = 99; pp = 100) in combination with their morphological similarities, left no doubt that both specimens are conspecific. Many species of the subgenus *Eladinea* known from Panama and South America have not been included in the study of Parra-Olea *et al.* (2004). Thus, the composition of most groups remained doubtful, in particular as all species groups defined by Parra-Olea *et al.* (2004) on the basis of molecular data are morphologically heterogeneous. Although Parra-Olea *et al.* (2004) did not include *B. compacta* in their study, they surprisingly placed it in the *B. adspersa* species group without further explanations, despite of its moderately large size and its predominantly black coloration. Besides one sequence from Genbank, no additional sequences of *B. compacta* have been included in my molecular analysis. However, I favor the view of *B. compacta* being a member of the *B. schizodactyla* species group as revealed from my phylogeny and as already proposed by Boza-Oviedo *et al.* (2012) until further data become available.

To date, the taxonomic status of specimens assigned to *B. minutula* cannot finally be clarified. Specimens morphologically assigned to *B. minutula* appear polyphyletic with respect to *B. sooyorum* and *B. marmorea* in my phylogenetic trees. Albeit the genetic p-distances are not especially high, they cannot be ignored mainly because other morphologically well-defined species show comparable distances. *Bolitoglossa marmorea* for instance, is morphologically separated from *B. minutula* by many characters, but the p-distance to the three clades of *B. minutula* is with 2.3–3.7% relatively low. However, I did not find any morphological characters which might be used to separate the three populations. A deeper analysis of this species complex would require more adult specimens, especially from the Serranía de Tabasará. Well-supported is the distinctness of *B. jugivagans* that is clearly separable by both molecular and morphological data from any other salamander species (Hertz *et al.* 2013a). Generally, the entire *B. robinsoni* clade is well-supported and well-resolved. However, since the identity of the specimen referred to as *B. robinsoni* on GenBank is doubtful, the clade will need a name change if it appeares that this specimen is not conspecific with *B. robinsoni* (see *B. robinsoni* species account for further comments). Undoubtful seems the synonymy of *B. sombra* with *B. nigrescens* as already proposed by Boza-Oviedo *et al.* (2012) and confirmed by my own denser molecular and morphological data set. *Bolitoglossa sombra* has been described exclusively on the basis of morphological differences that are only minor and largely overlapping compared to *B. nigrescens*. The differences in tooth counts observed by Hanken *et al.* (2005) are probably a consequence of the small sample size in combination with the increase of tooth counts with increasing size in *Bolitoglossa* species (Fig. 38B,C, Fig. 39C,D). Now, that I collected additional larger specimens from Panama (Hertz *et al.* 2011) the supposed differences blur. Nevertheless, the sample size of specimens assigned to *B. nigrescens* is still very low and there is no topotypic material available besides the holotype. An additional biogeographic evidence for the synonymy of *B sombra* and *B. nigrescens* is that the specimen UCR 20539, which I used in the phylogeny, was collected at Tarbaca, Province of San José, Costa Rica, a locality at the north-western rim of the Serranía de Talamanca and therefore north of the type locality. This makes a continuous distribution of *B. nigrescens* along the higher portions of the Pacific slopes of the Serranía de Talamanca likely. Based on this evidence I treat *B. sombra* as a junior synonym of *B. nigrescens*.

6.2.4. *BOLITOGLOSSA* SPECIES ACCOUNTS

***Bolitoglossa anthracina* Brame, Savage, Wake, and Hanken 2001**, Copeia 2001: 700.

Holotype: KU 116671, by original designation.

Type locality: „north slope of Cerro Pando, Prov. Bocas del Toro, Panamá, elevation 1450 m. Estimated coordinates: 8.966° N, 82.7° W."

Conservation Status: IUCN Category: Data Deficient (DD); known from two PAs: PILA and RFLF, Extent of Occurrence about 660 km^2 known from four localities. The available data qualify *Bolitoglossa anthracina* for the IUCN category Endangered (EN), because its extent of occurrence is less than 5,000 km^2, all individuals are in fewer than five locations, and there is continuing decline in the extent and quality of its habitat in PILA. EVS calculation: 5 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 13.

Diagnosis: This species is a large, almost completely black species. SVL ranges from 46–55.4 mm in males, and from 43.8–70.8 in females. It differs from other large black salamanders by the following combination of characters: Tail longer than SVL, TL/SVL 1.27–1.4, in males 1.01–1.3; webbing of hands and feet reduced; mental gland present in males; high number of MT, 54–91 in three females, and 61 and 66 in two males.

Coloration in life: The coloration of the adult male (SMF 94465) was recorded as uniformly Sepia (119). In contrast, the subadult female´s (SMF 94466) coloration is much lighter. C. Myers recorded a difference between night and day coloration; with night coloration being by far lighter (Brame *et al.* 2001). The coloration of the female by night and day as derived from photographs (Fig. 57 B,C) is as followes: Night: dorsal surfaces Straw Yellow (56), suffused with Dark Grayish Brown (20) on the head; lateral and ventral surfaces dirty white. Day: Dorsal surfaces Dark Grayish Brown (20) with a weak and inconspicuous Buff (24) reticulation; larger Buff (24) flecks on the middle part of the tail; lateral and ventral surfaces Medium Neutral Gray (84).

Distribution: *Bolitoglossa anthracina* is known to occur in the Cordillera de Talamanca of western Panama. Two collection sites are located on the northern slope of Cerro Pando and three, including my own, on both slopes in the La Fortuna Forest Reserve (Fig. 42). Occurrence in adjacent Costa Rica is possible.

Habitat and natural history: The species has previously been recorded in Premontane Wet Forest between 1100 and 1450 m asl from only a few localities in the Cordillera de Talamanca of western Panama. The holotype and one paratype were collected at night, while climbing in vegetation one to two meters above the ground (Brame *et al.* 2001). I found two specimens on a mountain crest between 1740 and 1760 m asl in the La Fortuna Forest Reserve, both at night. These collection sites are situated only 22 m apart from each other. The male was found inside a bromeliad about four meters above the ground. The female was also taken from a bromeliad, about one meter above ground. I agree with Wake *et al.* (2007) in considering this species to be highly arboreal. This is also supported by the very long tail that is about 30–40%

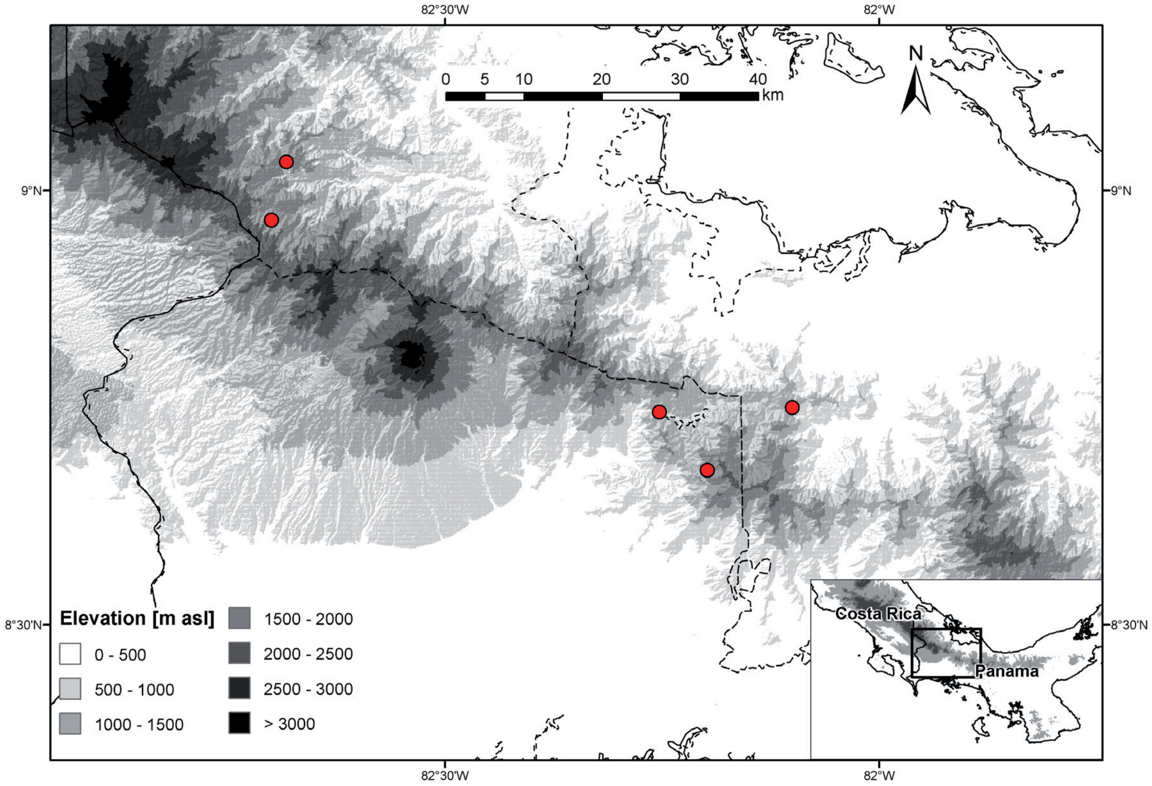

Figure 42: Collection sites of *Bolitoglossa anthracina* (red dots).

longer than the SVL and prehensile. All specimens known have been found active while climbing in vegetation between one and 18 meters above ground (Brame *et al.* 2001; Wake *et al.* 2007; own observation). The habitat where I have found this species may be characterized as ridge top or elfin forest covered with low-growing, hard-wooded trees that support a variety of epiphytes (Fig. 8F). The annual precipitation is approximately 2500 mm; mean annual temperature is around 17 °C. This indicates the habitat as Lower Montane Wet Forest, extending the known climatic preferences of this species to a second life zone.

Remarks: Formerly, this species was known mainly from the type series. The female holotype was collected on the north slope of Cerro Pando in 1966. One of the paratypes was collected in 1961 at Río Changena, which runs down the northern slope of Cerro Pando, the other in 1988 in the La Fortuna Forest Reserve (Brame *et al.* 2001). An unidentified male *Bolitoglossa* collected by C. Myers at the La Fortuna dam site, prior to road and dam construction in 1976 (Savage & Myers 2002), has been subsequently identified as *B. anthracina* by Wake *et al.* (2007). However, there have been no records for 22 years until I obtained these two additional specimens from La Fortuna Forest Reserve. Consequently, there have been no molecular data available in previous studies. Based on my molecular analysis, *B.*

anthracina is, despite of its appearance as a so called "Large Black Salamander" (Brame *et al.* 2001; Hanken *et al.* 2005), a member of the *B. subpalmata* species group and not as proposed by Parra-Olea *et al.* (2004) of the *B. schizodactyla* species group, where all the other "Large Black Salamanders" belong to.

Bolitoglossa bramei **Wake, Savage, and Hanken, 2007**, Copeia, 2007: 561.

Holotype: MVZ 225893, by original designation.

Type locality: „Costa Rica, Provincia Puntarenas, trail to Cerro Pando, above Las Tablas, 8° 56′ N, 82° 46′ [8.933°, -82.766], 2200 m".

Conservation Status: IUCN Category: Data Deficient (DD); known from two Panamanian PAs: PILA and PNVB. The most recent findings have been made in Costa Rica in 2007 and 2008 (Boza-Oviedo *et al.* 2012). It was not collected or observed in Panama since 1982 (Wake *et al.* 2007). So far, there is too little information on the species population status and possible threats. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 3 (forest formation) = 11

Diagnosis: Data derived from Wake *et al.* (2007): A small and slender species (SVL 37.6–41.0), with a high number of MT in adults (49–66), and long limbs (folds between adpressed

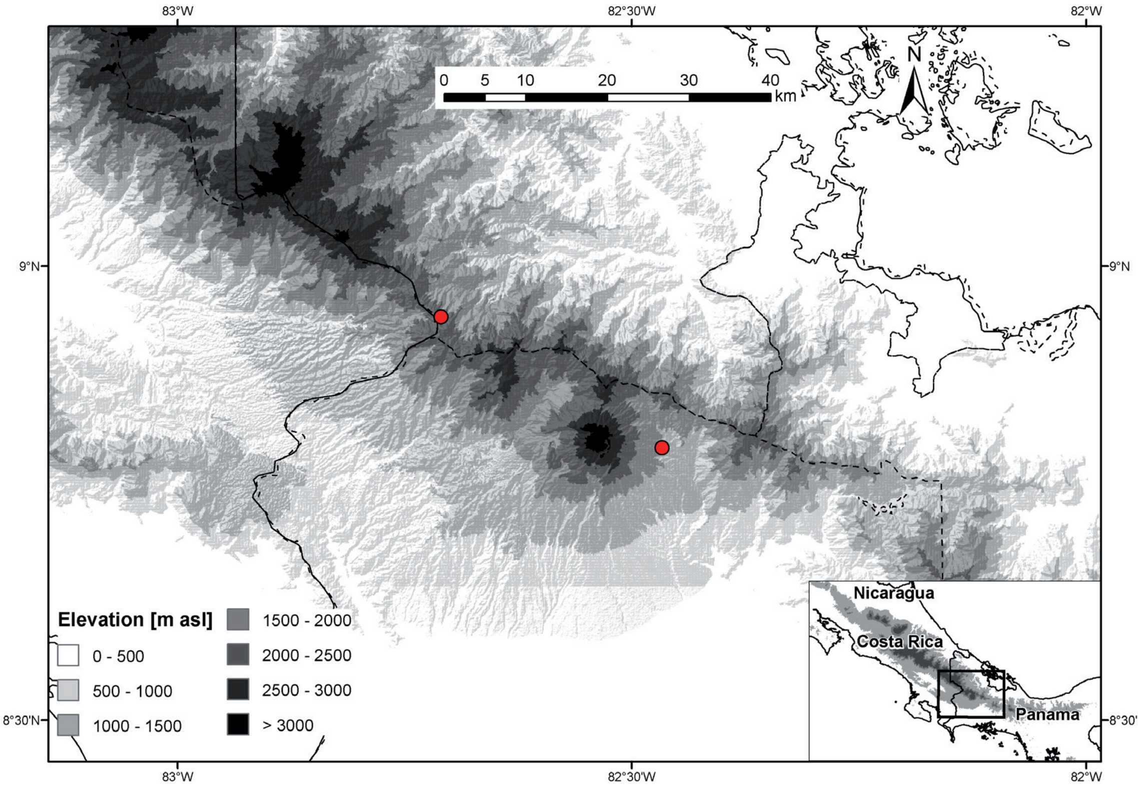

Figure 43: Collection sites of *Bolitoglossa bramei* (red dots).

limbs: 0–1.0). Hands and feet are moderately webbed, fewer than two phalanges free of web on the longest digits.

Coloration in life: Based on field notes of D. Wake and C. Myers in Wake *et al.* (2007) the usual coloration pattern of *Bolitoglossa bramei* can be briefly summarized as follows: Dorsal ground color dark brown with brick-red streaks. In some specimens the tail shows a vivid orange coloration towards the distal end of the tail. Ventral ground coloration is dark gray or blackish, but lighter than the dorsal ground color.

Distribution: *Bolitoglossa bramei* is known from both Atlantic and Pacific slopes of the Cordillera de Talamanca of Costa Rica and Panama (Fig. 43). One paratype has been collected at Bajo Mono near Boquete on the eastern slope of Volcán Barú.

Habitat and natural history: This species has been found at altitudes between 1900 and 3056 m asl. The habitat ranges from Lower Mountain and Mountain Wet Forest to sub-paramo on the highest peaks. It has been encountered on the ground, under moss, as well as climbing on vegetation (Wake *et al.* 2007; Boza-Oviedo *et al.* 2012). Wake *et al.* (2007) noted that it has a prehensile tail and is therefore suspected to be a good climber.

Remarks: Even though I visited both Panamanian sites where *Bolitoglossa bramei* is known to occur, I did not find any specimen that meets the characters described for this species.

Bolitoglossa colonnea (**Dunn 1924**, Field Mus. Nat. Hist. Publ., Zool. Ser., 12: 96.)

Holotype: MCZ 9406, by original designation.

Type locality: “La Loma, on trail from Chiriquícito to Boquete [Atlantic side], altitude about 2000 feet [610 m], Province of Bocas del Toro [today: Comarca Ngöbe-Buglé], Panama [estimated coordinates: 8.833°, -82.217°]”.

Conservation Status: IUCN Category: Least Concern (LC); known from the Panamanian PAs: BPPS, PNIB, PILA, PNSF and RFLF, including the new distribution records (Hertz *et al.* 2013b) the extent of occurrence is about 22,000 km^2. There is currently no reason for a change of the IUCN category. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 2 (forest formation) = 10.

Diagnosis: *Bolitoglossa colonnea* is a moderate-sized species of salamander with fully webbed digits and only 0–11 MT, but up to 48 VT in adults. It is readily identified by the unique character of a fleshy transverse ridge between the eyes.

Coloration in life: Coloration of *Bolitoglossa colonnea* includes mostly shades of drab brown and beige, often with indistinct stripes and stippling. Coloration in life of two specimens was recorded in the field as follows:

SMF 94460: Dorsal ground color Cinnamon (39); two Vandyke Brown (121) lines start at the interorbital transverse ridge and meet posterior to head, thence continuing as single vertebral stripe to base of tail; laterally, Prout´s Brown (121 A) demarcated above by a fine Pale Pinkish Buff (121 D) line; ventral surfaces mostly Pale Pinkish Buff (121 D) with fine Mikado Brown (121 C) longitudinal lines, except Vandyke Brown (221) gular region.

SMF 94463: Dorsal ground color Chamois (123 D), irregularly mottled with Sepia (119); distal 20% of tail grading into Pale Horn Color (92); ventral surfaces Pale Horn Color (92) with fine mottling of Pratt´s Paynes Gray (88) and Sepia (119) spots and blotches, the larger ones grading into Dark Neutral Gray (83).

Distribution: Collection sites on the Caribbean side of the central mountain range extend over the humid lowlands and premontane elevations from northern Costa Rica to western Panama, including the islands of the Bocas del Toro archipelago and Isla Escudo de Veraguas, Bocas del Toro (Fig. 44). On the Pacific side it is known from several Costa Rican localities, but so far no Panamanian sites in the humid lowlands around Golfo Dulce. Records from the Pacific drainage of western Panama have been made at three sites in the Fortuna Depression

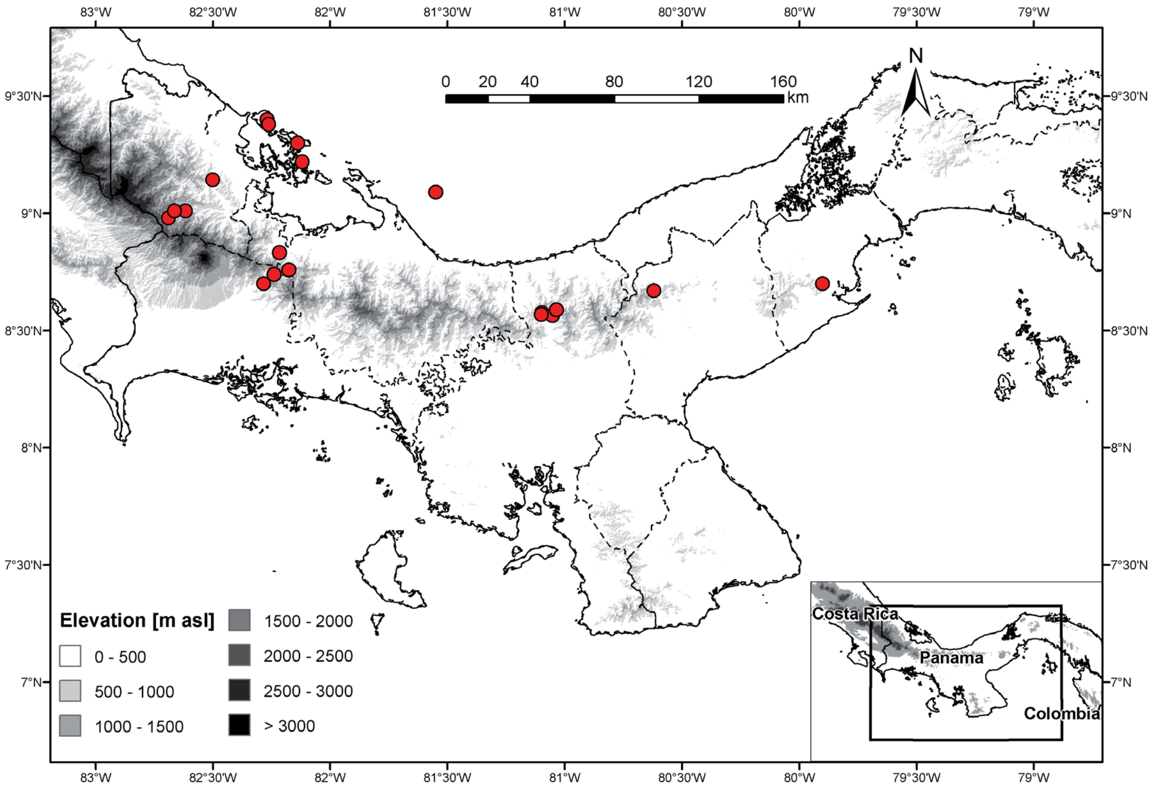

Figure 44: Collection sites of *Bolitoglossa colonnea* in Panama (red dots).

as well as in the eastern Serranía de Tabasará and on the southern slopes of Cerro La Campana in west-central Panama, the last of which is also the easternmost collection site for *B. colonnea*.

Habitat and natural history: The species is commonly found in the Lowland Atlantic, Lowland Pacific, and Premontane Wet Forest formations. Hertz *et al.* (2013b) recorded a specimen from Río Changena, Province of Bocas del Toro, at 1650 m asl, what extends the known vertical distribution of *Bolitoglossa colonnea* from 1250 m (Köhler 2011). This record also expands the known habitat of *B. colonnea* from the Lowland and Premontane Life Zones to the Lower Montane Wet Forest.

Remarks: Modified from Hertz *et al.* (2013b): There is no other salamander species in Lower Central America that is so easily recognized, owing to its conspicuous and unique fleshy interorbital ridge. Nevertheless, there is variation in morphology and genetics (Hertz *et al.* 2013b). I found the highest counts of VT in two specimens from Río Chilagre, Veraguas. This difference is considerable between the two males, from widely separated localities, one (SMF 94460) from Rio Changena, Bocas del Toro, in extreme western Panama on the Caribbean slopes of the Serraná de Talamanca, the other (MHCH 2600) from Rio Chilagre, Veraguas on the eastern end of the central mountain range. Although both specimens are about the same size, the male from Rio Chilagre (MHCH 2600) has 48 VT, 31 more than the male from Rio Changena (SMF 94460). Savage (2002) gave a maximum of 36 VT for adults of *B. colonnea*. In females, the specimen (SMF 94461) from Río Clarito, Bocas del Toro, the largest in our sample, has 11 VT fewer than the considerably smaller female (SMF 94463) from Río Chilagre, Veraguas, which has 34 VT. Another subadult female (SMF 85066) from Isla Colón, Bocas del Toro, the smallest in my sample, has one VT fewer than the next largest female (SMF 94464) from Cerro Negro, Veraguas. My data suggest that specimens from the eastern portion of the range tend to have more VT that those from the west, even considering that number of teeth increases with size and age.

I also observed genetic differences in 16S rRNA gene sequences. The distance between the specimens (AY526119, no voucher) from Hornito, La Fortuna Forest Reserve, Chiriquí and the others (SMF 94460–1; CH 6526) from Bocas del Toro is large enough to suggest that two species may be involved. Unfortunately, there is no voucher specimen corresponding to the sequence of the specimen from Hornito, La Fortuna Forest Reserve (Parra-Olea *et al.* 2004). During my field work in the La Fortuna Forest Reserve I did not obtain specimens of *B. colonnea* or any other species that may resemble *B. colonnea*. This is a subject for future studies.

***Bolitoglossa compacta* Wake, Brame, and Duellman 1973**, Contrib. Sci. Nat. Hist. Los Angeles Co., 248: 12.

Holotype: KU 116662, by original designation.

Type locality: „north slope of Cerro Pando, 1920–1970 m elevation [1815 m according to digital elevation model], Provincia de Bocas del Toro, western Panamá".

Conservation Status: IUCN category: Endangered (EN); known from the Panamanian PAs: PILA and RFLF, the extent of occurrence is about 200 km^2, it has been recorded from five Panamanian sites. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 12.

Diagnosis: This species is distinguished from other salamanders in the Cordillera de Talamanca by its coloration of bright red to yellow blotches or stripes on dark ground. Adult SVL ranges from 44.9–53.4 mm in males and 68.5–84.7 mm in females (Wake *et al.* 1973; Hanken 1979). The feet are moderately webbed. Adult tooth counts are 11–20 MT in males and 36–50 in females, plus 19–22 VT in males and19–33 females (Savage 2002).

Coloration in life: The coloration of the only specimen collected (SMF 89849) has been recorded as follows: Ground color of head, body, limbs, and tail Jet Black (89); dorsum with large Flame Scarlet (15) paravertebral blotches.

Distribution: *Bolitoglossa compacta* occurs in the Cordillera de Talamanca of western Panama and adjacent Costa Rica (Fig. 45), and on the slopes of Volcán Barú (Lips 1993).

Habitat and natural history: The species has been found in Premontane and Montane Wet Forest at elevations between 2000 and 3000 m. So far, the type locality is the only site on the Atlantic slope as well as the lowest at about 1800 m. Annual precipitation at collection sites is between 2400 and 2650 mm. Average temperature at these sites range from 10 °C in 3000 m on Volcán Barú to 16 °C at the type locality. It is considered to be rare by nature (Solís *et al.* 2004c). Hanken (1979) reported on a captive specimen which laid 39 eggs, only two of which hatched after a developing time of more than eight month.

Remarks: I found *Bolitoglossa compacta* only once on the south-eastern slopes of Volcán Barú at the roadside of Volcancito road, the road that leads from Boquete to the summit of the volcano. The specimen, a female, was hiding under a rotten trunk at daytime. I assign it to *B. compacta*, because it shows the typical coloration of bright red on dark ground, has moderately webbed feet, a stout body form, narrow feet (HAW 3.4 mm; HFW 4.0 mm) as

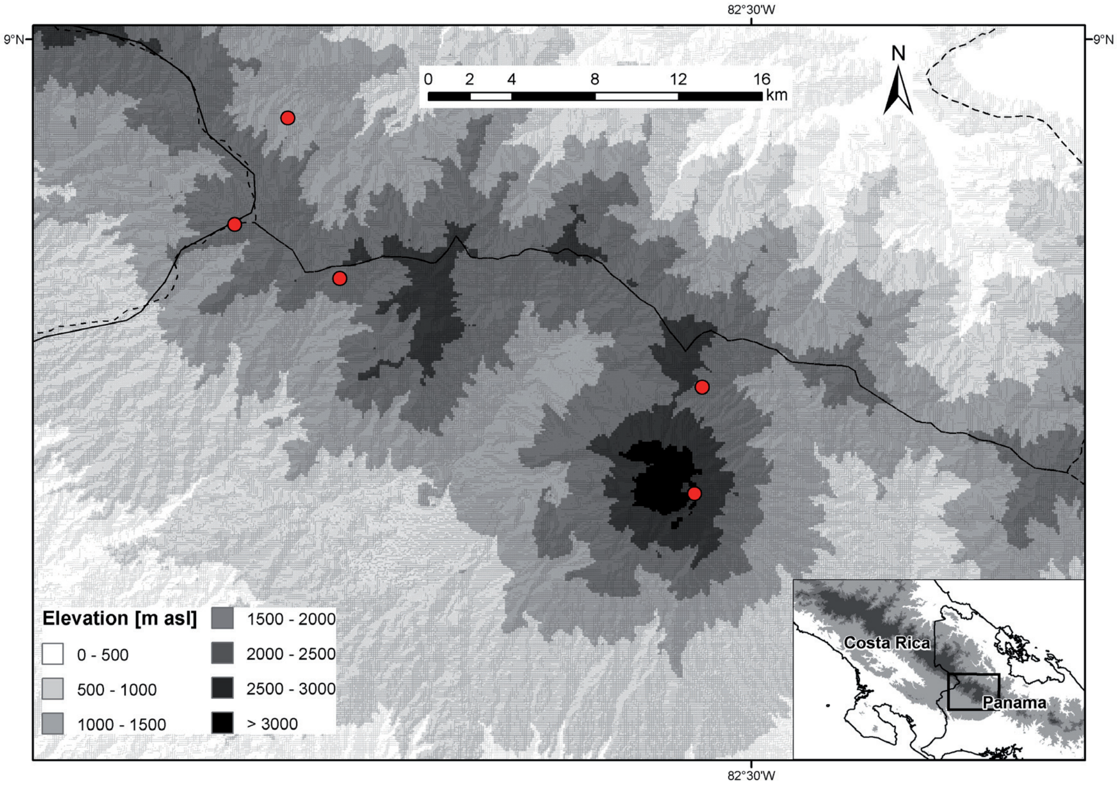

Figure 45: Collection sites of *Bolitoglossa compacta* (red dots).

well as by dentitional features (16 MT; 20 VT). I consider it to be subadult, because of its size (SVL 39.1 mm) and relatively low tooth counts. The collection site is located only 5 km south of a previous collection site at Cerro Respingo (Hanken 1979). By examination of a photo (provided by Jeffrey Dietrich pers. comm. 2012, Fig. 57G) I assign an uncollected specimen from Mount Totumas Cloud Forest also to *B. compacta*. This specimen looks more slender than the specimen I collected. I therefore suspect it to be a male.

***Bolitoglossa gomezi* Wake, Savage, and Hanken, 2007**, Copeia, 2007: 557.

Holotype: USNM 219116, by original designation.

Type locality: „Costa Rica, Provincia Puntarenas, Las Cruces Biological Station, 8° 47′ 35″ N, 82° 57′ 30″ W, 1250 m“.

Conservation Status: IUCN category: Data Deficient (DD); known to occur in the Panamanian PA PILA. There is very little information on population status and possible threats. In view of its small extent of occurrence it may qualify for a threatened category. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 12.

Diagnosis: A medium sized salamander (SVL: 29.3–49.9 mm in males; 42.3–54.5 in females) that shares several characters with *Bolitoglossa bramei*. *Bolitoglossa gomezi* differs from *B. bramei* in the following characters (character for *B. bramei* in parentheses): shorter legs, costal folds between adpressed limbs 1.5–3 (0–1); fewer MT in relation to SVL: MT/SVL=0.97–1.22 (1.30–1.60); less extensive webbing, usually two or more phalanges on longest digits free of web (less than two phalanges free of web).

Coloration in life: Coloration in life of the only specimen was recorded in the field as follows: Dorsal ground color Buff (24); dorsal surfaces of head, neck, and forelimbs with Cinnamon Rufous (40) blotches, separated by Raw Umber (23) lines; Burned Umber (23) around nostrils; lateral head from nostrils to gular fold Buff Yellow (53); a Buff-Yellow (53) dorsal stripe and interrupted Cream Color (54) lines spread over lateral parts of dorsum; dorsal surface of tail Buff (24) with only a few spots and Cream Color (54) lines; ventral surface of head from snout to gular fold Buff-Yellow (53); ventral surfaces of body and tail Indigo (73) grading into Dark Grayish Brown (20) with Cream Color (54) spots; iris Yellow Ocher (123 C), pupil horizontal.

Distribution: *Bolitoglossa gomezi* inhabits the Pacific slopes of the southern Cordillera de Talamanca of Costa Rica and into extreme western Panama. The type locality lies in the Fila Costeña, a southern branch of the Talamanca that is separated from the main Cordillera by the Valle de Coto Brus. It has recently been collected above Tres Colinas on the Pacific slope of

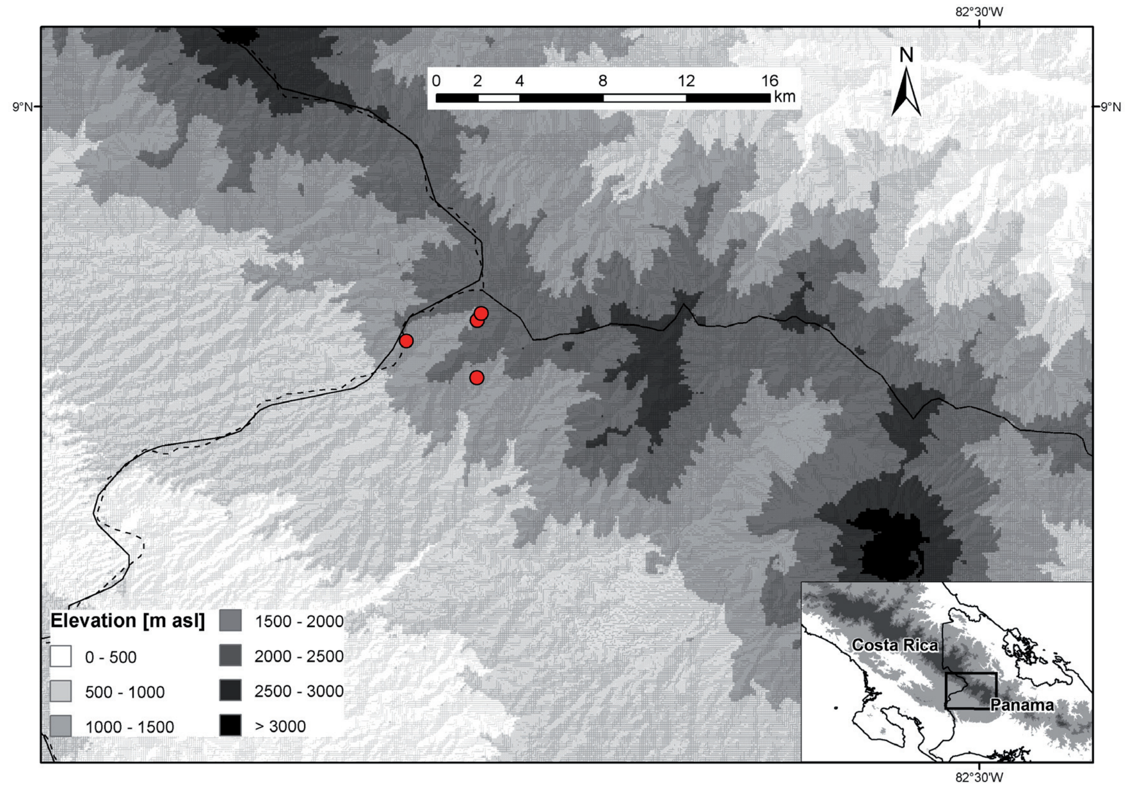

Figure 46: Collection sites of *Bolitoglossa gomezi* in Panama (red dots).

Cerro Kasir (Boza-Oviedo *et al.* 2012) that is the most north-western site so far. In Panama it has only been collected on the Pacific drainage of Cerro Pando in the valleys of Río Candela and Río Cotito.

Habitat and natural history: There is still very little information on the species habitat preferences and natural history. In the Fila Costeña of Costa Rica it inhabits elevations from 1170 to about 1250 m (Wake *et al.* 2007) and at Cerro Kasir it has been found between 2100

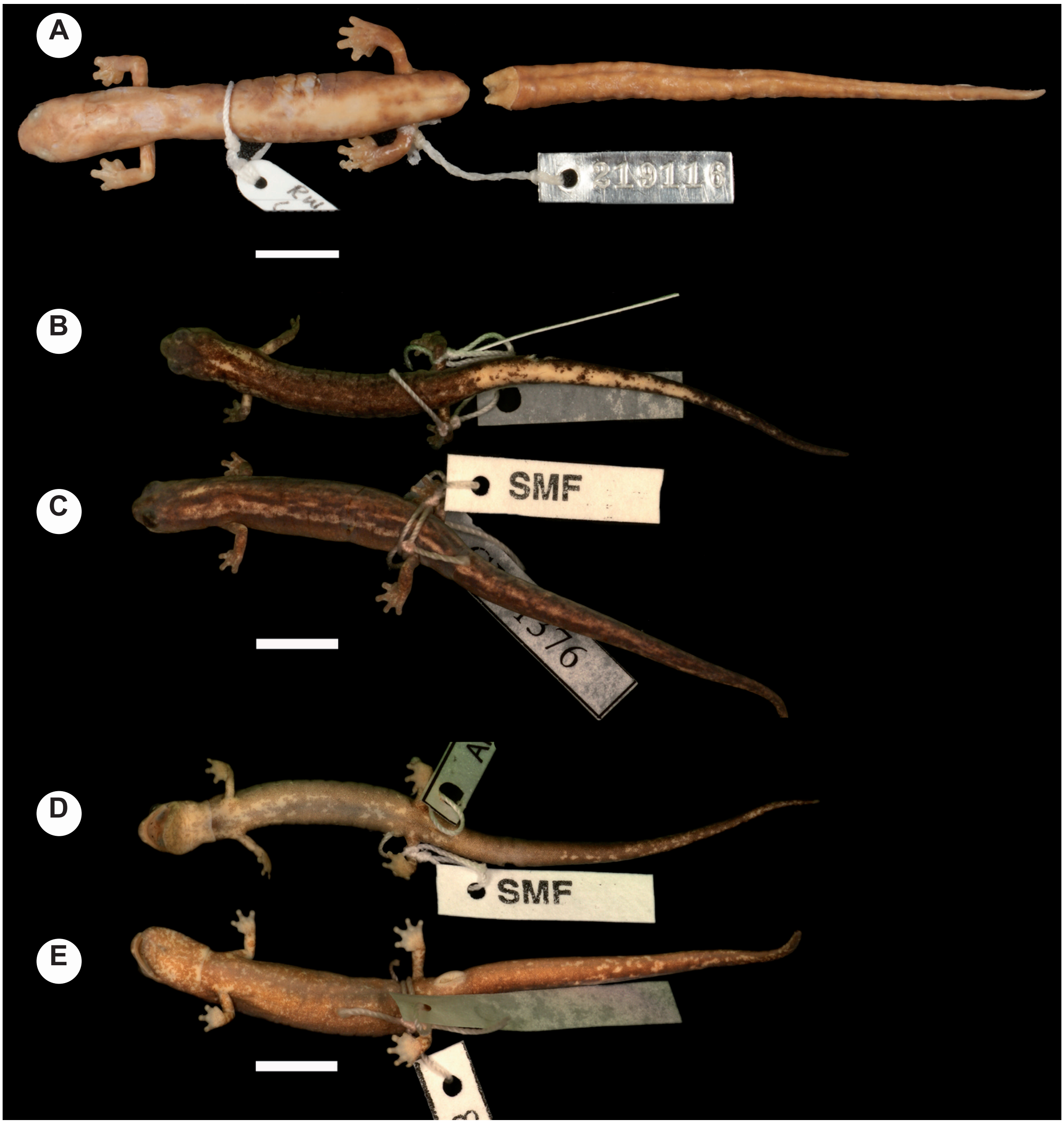

Figure 47: Specimens of *Bolitoglossa gomezi* in preservative. Scale bars 10 mm. **A** Dorsal view of the female holotype USNM 219116. **B** Dorsal view of female specimen SMF 94462. **C** Dorsal view of the female paratype SMF 85062. **D** Ventral view of female specimen SMF 94462. **E** Ventral view of the female paratype SMF 85062.

and 2150 m (Boza-Oviedo *et al.* 2012). In Panama, it was collected between 1700 and 2120 m on the slopes of Cerro Pando (Wake *et al.* 2007). The inhabited life zones are Premontane and Lower Montane Wet Forest. The Panamanian sites receive an annual precipitation of 2400–2530 mm at a mean temperature of 14–17 °C. At the type locality both annual mean temperature 20–22 °C and annual precipitation (over 3500 mm) are significantly higher. Most specimens have been taken at heights between one and three meters above ground from bromeliads, leaves or branches (Wake *et al.* 2007). I found a single female specimen (SMF 94462) that was active at night, climbing about one meter high on a rotten tree butt at the side of a small path in the high valley of Jurutungo on the southern slope of Cerro Pando.

Remarks: Although the surroundings of Cerro Pando are one of the salamander-richest areas which I visited in Panama, a single specimen remained all I could find of *Bolitoglossa gomezi*. Unfortunately, I did not take tissue samples and long time I was unclear about the identity of this specimen. For comparison, I morphologically examined the holotype (USNM 219116) and one paratype (SMF 85062) that was also collected in Jurutungo. The holotype is a large animal that at first sight seems to be very different from the paratype and the newly collected specimen (Fig. 47). However, the morphometric proportions of all three specimens vary only slightly and at this point I am not seeing any evidence to refer it to any other described or undescribed species but *B. gomezi*. The only notable difference is the slightly higher tooth counts of SMF 94462 in relation to its body size. Tissue samples for molecular approaches and additional specimens from Cerro Pando are needed.

***Bolitoglossa lignicolor* (Peters 1873,** Monatsber. Preuss. Akad. Wiss. Berlin, 1873: 617.)

Syntypes: ZMB 7736 (2 specimens); ZMB 7736A designated as lectotype by Bauer *et al.* 1993.

Type locality: “Chiriquí”, Panama; corrected by Bauer *et al.* (1993) to “Camarón, Provinz Chiriquí”, Panama; specified by Hertz *et al.* (2013b) to “Camarón Arriba, District of Bugaba, Provinz Chiriquí [estimated coordinates: 8.65°, -82.6666°]” (see remarks).

Synonyms: *Spelerpes punctatum* Brocchi, 1883, Miss. Scient. Mex. Amer. Centr., Rech Zool., 3(2, livr. 3): 115.

Oedipus ahli Unterstein, 1930, Zool. Anz., 87: 270–272.

Bolitoglossa palustris Taylor, 1949, Univ. Kansas Sci. Bull., 33: 283.

Conservation Status: IUCN Category: according to Solís et al (2004a), Endangered (EN). This needs to be reevaluated since Hertz *et al.* (2013b) found *Bolitoglossa lignicolor* to be more widely and less fragmentary distributed than formerly known. Thus, its extent of occurrence is about 25,000 km^2. It has been recorded from the Panamanian PAs: PNVB, RFEM, PNCH and RFLT, albeit most collection sites are outside of protected areas. *Bolitoglossa lignicolor* has been found in modified and even suburban habitats (Batista & Ponce 2011; Lotzkat & Hertz 2011), so I consider it to be less susceptible to habitat degradation than previously thought (Solís *et al.* 2004a). Taking the new data in account, it is not justified to list *B. lignicolor* as Endangered (EN), as it neither meet criterion B1, nor any other criterion A to E. However, I propose to list it as Near Threatened (NT), because most of the known populations exist outside of PAs, where its habitat is continuously declining in both extent and quality. Therefore it is likely to qualify for a threatened category in the near future. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 3 (forest formation) = 11.

Diagnosis: *Bolitoglossa lignicolor* is a relatively large and robust species with fully webbed hands and feet. Maximum SVL of males is 67.7 mm, of females 81.2 mm. MT count in adult males is 22–48 and 24–48 in adult females. VT count is 18–40 in males and 10–38 in females. The ratio VT/SVL is slightly higher in males.

Coloration in life: The coloration is usually a light brown dorsum, while the venter is dark brown. The usual coloration pattern is a broad, light-colored dorsal band, which may be light tan, beige or reddish. The ground coloration is darker, mostly chocolate brown on the day and silvery grey at night. However, the light dorsal coloration may be broken up into blotches and even smaller spots. In contrast, some individuals are predominantly light colored with darker mottling. The coloration in life of three specimens has been recorded in the field as follows: SMF 91996 (not pictured): Dorsum Tawny (38) with Raw Umber (223) streaks; iris Verona Brown (223 B). SMF 91994: Dorsal ground color Sepia (119) with dirty white stipples grading into Tawny Olive (223 D) on tail; ventral surfaces the same as dorsal surfaces, but with finer and less dense stippling; iris Drab Gray (119 D), peripherally Mars Brown (223 A). SMF 94458: Dorsal ground color Clay Color (26) with longitudinal, broken lines of Sepia (119) and Cream Color (54); ventral ground color Dark Brownish Olive (129) powdered with dirty white spots; limbs Dark Brownish Olive (129), dorsally suffused with Clay Color (26).

Distribution: *Bolitoglossa lignicolor* has been collected at various sites in southwestern Costa Rica, from Manuel Antonio National Park along the Pacific versant to the Osa Peninsula and the Golfo Dulce region, into western Panama (Fig. 48) as far as the mountains of the western Azuero Peninsula (Brame & Wake 1963; Hertz *et al.* 2013b). Hertz *et al.*

(2013b) pointed out that the two sites “Cerro Mangillo” and “Tiger Ridge Camp” are not in Los Santos in the central Azuero as denoted by Brame and Wake (1963). The collection data on the ANSP specimens read “Veraguas prov., Cerro Mangillo, 2800 ft. [853 m]” and “Veraguas prov.?, Tiger Ridge Camp 2600 ft. [792 m]”. However, none of these place names is traceable on recent maps. There is only one mountain at the stated elevation named Cerro Manguillo (or Cerro Manglillo) on Azuero Peninsula, located near the present day province triangle Veraguas, Herrera, and Los Santos in the Sierra Central de Azuero. It is assumed that E.R. Dunn, who collected the specimens, started his expedition to Cerro Manguillo from the Veraguas side and was not sure if he was still in Veraguas when he reached “Tiger Ridge Camp”, which he expressed by the question mark. It is suspected that “Tiger Ridge Camp” has been on the crest above Quebrada El Tigre, about 4 km north of Cerro Manguillo in Herrera province, as it is the only place that is name-wise linked to “Tiger Ridge”, as well as at the stated elevation. This view is supported by the fact that ANSP specimens with interjacent numbers were collected at “Macaraquito Camp” which is situated between these two places. It seems obvious that Brame and Wake (1963) confused Cerro Manguillo, also written Cerro Manglillo, with Manglillo village in Los Santos, which lies at only 380 m asl. The documented vertical distribution of *B. lignicolor* ranges from sea level to approximately 1200 m asl (Köhler 2011; own data).

Habitat and natural history: *Bolitoglossa lignicolor* is a strictly Pacific species that inhabits the slopes of the Cordillera Central, the wet Pacific lowlands around Golfo Dulce and the mountainous parts of the western Azuero Peninsula that receive more rain than the rest of the rather dry Azuero Peninsula (cp. Fig. 12). Localities in the Caribbean drainage of northern Costa Rica, as mentioned by Brame and Wake (1963) refer to misidentified specimens of *B. alvaradoi*. In Panama it is found in a variety of habitats that receive between 2300 to 3450 mm annual precipitations and have a mean temperature of 16–26 °C. Sites at the upper temperature limit are also at the upper precipitation limit and vice versa. Only Puerto Armuelles, Chiriquí seems to be an outlier as it lies in the rain shadow of the Golfo Dulce regimen with an average annual rainfall of only 2300 mm at a mean temperature of 25 °C. Dunn (1940) wrote that he collected material at “the holdings of the Chiriquí Land Company at Puerto Armuelles in Chiriquí”, which included acreage from Puerto Armuelles west to the Costa Rican border and beyond (Royo 2004). Hence, it is not clear where exactly he collected. Since only 10–20 km west of Puerto Armuelles annual precipitation is 2800 to more than 3000 mm, I suspect the material has been more likely collected further west. Life zones

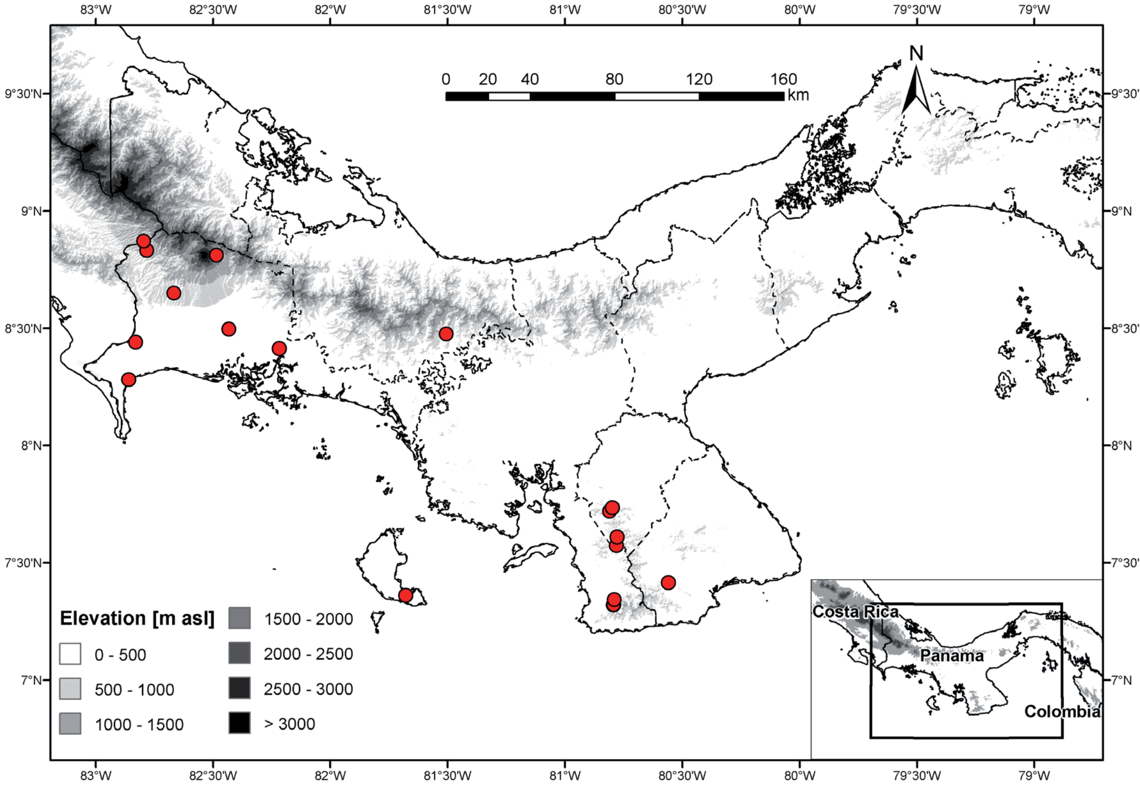

Figure 48: Collection sites of *Bolitoglossa lignicolor* (red dots) in Panama.

inhabited by *B. lignicolor* include Lowland Atlantic Wet and Moist Forest and Premontane Wet Forest. There is little knowledge on the diet of this salamander. A specimen I collected in Santa Clara choked up a relatively large spider (about 6 mm body length; HW of salamander: 7.2 mm).

Remarks: The type locality of *Bolitoglossa lignicolor* has been discussed by Hertz et al (2013b). According to that, Bauer *et al.* (1993), when examining the type material, found a label in the jar giving the collection site of the holotype as “Camarón, Province of Chiriquí”. Camarón, Spanish for “shrimp”, has been copied incorrectly by several authors (*e.g.*, Frank & Ramus 1995; Frost 2014; Köhler 2011) as “Camron”. After examining several maps three sites called Camarón in Chiriquí have been found, which all are situated within the assumed distribution area of *B. lignicolor*. One “Camarón” is on the banks of the Río Tabasará, near the provincial boundary with Veraguas in the district of Tolé. Moreover, another two “Camarón Arriba” exist. The first being located in what is now the Comarca Ngöbe-Buglé, but which had been part of Chiriquí before 1997, approximately 14 km north-east of San Lorenzo, and a second “Camarón Arriba” in the district of Bugaba. Although the collector of the type material, entomologist H. Ribbe, did not record an itinerary, it is suspected that the type locality is the second Camarón Arriba, about 10 km north of the city of Bugaba. A few years later, G. C. Champion collected insects for F. D. Godman, who mentioned this place in his notes (Godman 1915). This location is well within the known distribution range of *B. lignicolor*, is climatically suitable, and was quite accessible even back in the 19th century, as it is not far from the road to Costa Rica. This evidence gives reason to restrict the type locality to "Camarón Arriba, District of Bugaba, Province of Chiriquí".

***Bolitoglossa magnifica* Hanken, Wake, and Savage 2005**, Copeia, 2005: 228.

Holotype: MVZ 128619, by original designation.

Type locality: „Panamá, from the southeast slope of Volcán Chiriquí, 7.8 km W and 2.5 km N (airline) of Boquete, Prov. Chiriquí, 8° 47.6′ N, 82° 30.05′ W, 2400 m elevation"

Conservation Status: IUCN category: Endangered (EN). Hanken et al (2006) listed it as EN because of its small known distribution range and a general loss of habitat. The extent of occurrence is about 70 km^2. In fact, inadequate data allow only a restrained assessment of this species. Hertz *et al.* (2012b) collected four individuals only a few kilometres away from the species' type locality at PNVB while ascending from the town of Boquete to the peak by car. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 3 (forest formation) = 11.

Diagnosis: A large black *Bolitoglossa* with low counts of maxillary teeth. The tail is always much shorter than SVL (TL/SVL= 0.50–0.70%). In juveniles and subadults (SVL < 50 mm), whitish flecks are present on dorsal and lateral surfaces of tail.

Coloration in life: AH 324: Dorsal ground color Blackish Neutral Gray (82); lateral and ventral surfaces of body and head mottled with Pearl Gray (81), tail with large Pearl Gray (81) blotches on its lateral and ventral surfaces. As already mentioned by Hanken *et al.* (2005), the presence of large grey blotches on the tail seems to be ontogenetic. In my sample of four specimens, two with a SVL 44.2–45.7 mm have white blotches, while the two larger ones with a SVL 50.4–61.2 mm have not. However, none of the specimens I collected is entirely black like the holotype, an adult female of about 100 mm SVL. This supports the idea of Hanken *et al.* (2005) that this species darkens with increasing age and size.

Distribution: As currently understood, *Bolitoglossa magnifica* is endemic to the slopes around Volcán Barú (Fig. 49).

Habitat and natural history: *Bolitoglossa magnifica* has been found at intermediate elevations between 1250 and 2300 m asl around Volcán Barú, Panamas highest mountain.

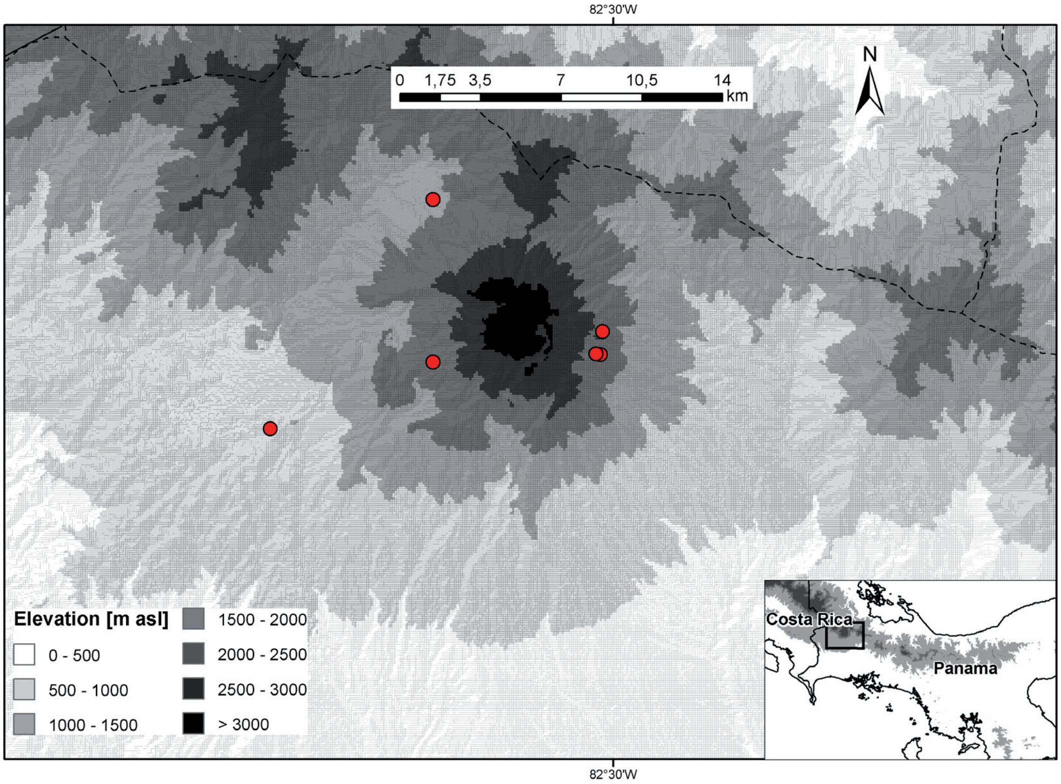

Figure 49: Collection sites of *Bolitoglossa magnifica* (red dots) on the slopes of Volcán Barú.

Mean annual temperature is between 19 °C at the lowest site and 13 °C at the highest. The annual precipitation is with about 2700 mm highest at the lowest site, while approximately 2400 mm at higher elevations. In a relatively short period of time (approximately 15 min), I found all four individuals under a pile of wood by the side of the road. I suppose individuals may aggregate at such exceptionally suitable hiding places.

Remarks: The type material, which was collected in 1975 by J. Lynch and J. Hanken, went under the name *Bolitoglossa nigrescens* for a long time (*e.g.*, Hanken & Wake 1982) until Hanken *et al.* (2005) revised the large black salamanders from Costa Rica and Panama and recognized it as a separate species. However, the morphological differences to *B. nigrescens* are minor and it might turn out that *B. magnifica* is not a valid species. Future studies will have to include molecular data. *Bolitoglossa magnifica* was not collected for 34 years until the most recent findings by Hertz *et al.* (2012b), though this does not necessarily mean it is a rare species. The type series is with 12 specimens, all collected in the year 1975, quite large and it took me only 15 minutes to find the four additional specimens. The reason why it has not been observed for so long is most likely due to the lack of herpetological field work in that area.

Bolitoglossa marmorea (**Tanner and Brame, 1961**, Great Basin Nat., 21: 23.)

Holotype: BYU 17704, by original designation.

Type locality: „Crater of Volcán Baru (Chiriquí), elevation 10,500 ft. [approx. 3200 m], Chiriquí Province, Panama".

Conservation Status: IUCN category: Endangered (EN). Solís *et al.* (2004b) classify *Bolitoglossa marmorea* as EN, because of its small extent of occurrence that is approximately 350 km^2. The species occurs in two PAs, PILA and PNVB. There is an observable degradation of habitat extent and quality in PILA due to felling of smallholder farming and occasional fires. In contrast, PNVB seems to be better protected. The species is abundant at the summit of Volcán Barú (Hertz *et al.* 2012c). However, I did not find any specimen in the crater of the volcano, just as I could not find it around Cerro Pando. However, I see not much need for a change in the conservation status of *B. marmorea* so far. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 12.

Diagnosis: The sample size of *Bolitoglossa marmorea* is extraordinary high compared to other members of the genus. The type material alone comprises 47 specimens (Tanner & Brame 1961). Savage (2002) gives the SVL of adult specimens as 59 to 66 mm in males and 60 to 72 mm in females and high tooth counts of 48 to 80 MT and 22 to 38 VT. Hands and feet are moderately webbed with the last phalange free of webbing. The tail is the same length or slightly longer than the SVL in adult specimens, but is generally shorter than the SVL in juveniles (Tanner & Brame 1961). There are 0–0.5 costal folds between adpressed limbs in males and 1.5–2.5 costal folds in females. The five specimens that I collected are juvenile to subadult females of 29.7 to 43.5 mm SVL, 1–4 PMT, 6–33 MT, and 10–20 VT. The limb interval is 2–2.5 costal folds. The relative tail length is higher in my sample with increasing SVL, with 55% in the smallest individual, to 84% in the largest.

Coloration in life: The usual color pattern in this species is a dark dorsal ground color with rusty brown to yellow stippling, which occasionally may be fused to larger blotches. I recorded the coloration of the largest specimen (AH 327) in field as follows: Ground color on body and head Dark Grayish Brown (20), marbled with Burnt Umber (22) stippling that is grading into Raw Umber (23) on the dorsal surfaces of legs and tail.

Distribution: The biggest portion of the distribution area of *Bolitoglossa marmorea* lies in Panama. Moreover, it is known to occur in adjacent Costa Rica on the western slopes of Cerro Pando (Savage 2002). In Panama, it is principally known from only two locations in

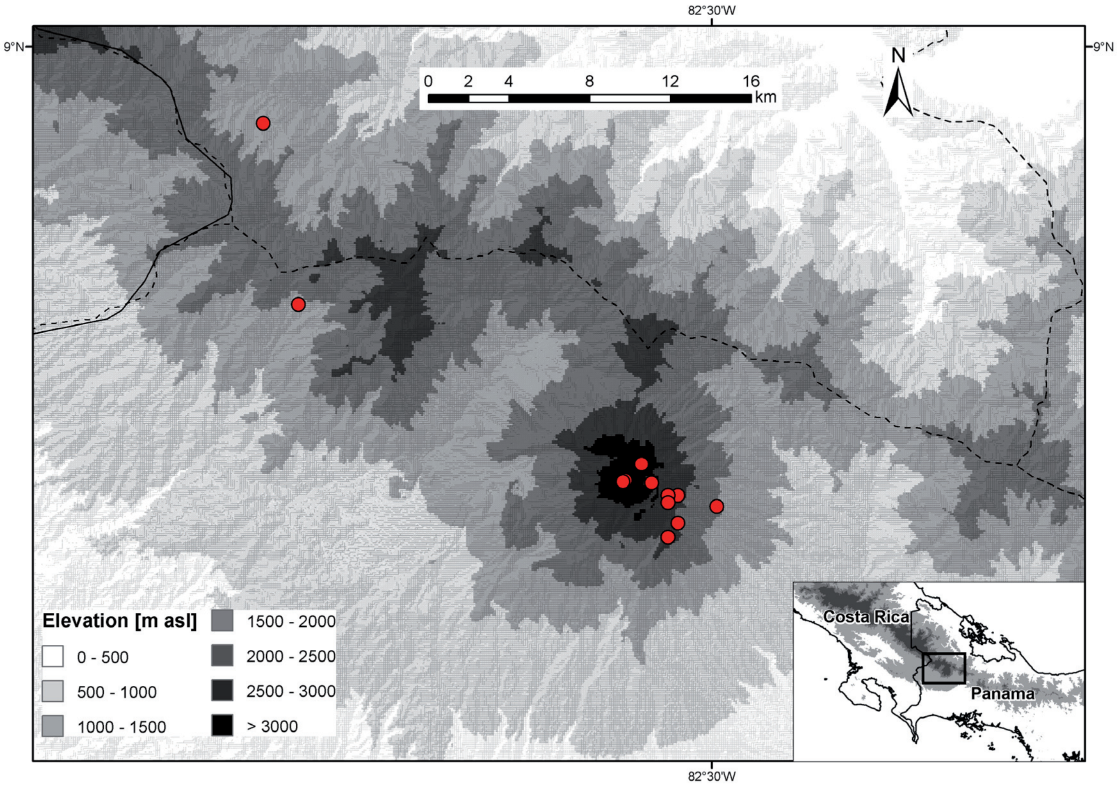

Figure 50: Collection sites of *Bolitoglossa marmorea* (red dots) in Panama.

the western Cordillera Central, Volcán Barú and Cerro Pando, albeit it has been collected at several sites at these locations (Fig. 50). A specimen photographed at Mount Totumas by Jeffrey Dietrich (pers. comm. 2012), on the Pacific side between Cerro Pando and Volcán Barú, most likely belongs to this species.

<u>Habitat and natural history:</u> This is the species with the highest vertical distribution in all Panamanian amphibian species. It is found from 1920 m on the northern slope of Cerro Pando to 3445 m, near the peak of Volcán Barú. Annual mean temperature at collecting sites ranges from 16 °C on the northern slopes of Cerro Pando and Mount Totumas, to 7 °C near the peak of Volcan Barú. The peak of Volcán Barú is also the site with the highest annual precipitation of 2800 mm, while the lowest precipitation of about 2255 mm is received by the valley of Boquete. Sites in the western Talamanca receive around 2400 mm. The habitat of *Bolitoglossa marmorea* can therefore be characterized as Lower Montane Wet Forest at lower elevations, and accordingly as Montane Rain Forest at higher elevations. Wake *et al.* (1973) reported to have found it, while it was active at night, foraging on mossy branches on Cerro Pando. The type series has been taken from under rocks in the crater of Volcán Barú (Tanner & Brame 1961). I collected all five specimens in one morning on November 6, 2009, between 7:50

and 12:00 hr, by turning rocks on the treeless summit of Volcán Barú. None of the collected specimens was active when encountered.

Remarks: When I looked at the photos of *Bolitoglossa marmorea* specimens from Cerro Pando that are published on AmphibiaWeb, I noticed differences in coloration and extent of webbing compared to the specimens I collected around the type locality. To date no tissue material from Cerro Pando specimens is available. Since some other amphibian species complexes (*e.g.*, *Bolitoglossa minutula* complex, *Craugastor podiciferus* complex) show large genetic distances between Cerro Pando and Volcán Barú populations, a comparison of *B. marmorea* populations is appropriate.

***Bolitoglossa minutula* Wake, Brame, and Duellman, 1973**, Contrib. Sci. Nat. Hist. Mus. Los Angeles Co., 248: 7.

Holotype: KU 116554, by original designation.

Type locality: „north slope of Cerro Pando, 1920 m (6298 ft) elevation, Provincia de Bocas del Toro, western Panamá“.

Conservation Status: IUCN category: Endangered (EN). Its extent of occurrence is less than 5000 km^2 and it is unable to survive in degraded habitats (Solís *et al.* 2004d). *Bolitoglossa minutula* is known to occur in two PAs, PILA and PNVB. The taxonomy of the species is not clear yet (see Remarks). A taxonomic revision is needed to reassess its conservation status. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 4 (forest formation) = 12.

Diagnosis: This is a very small species that reaches an adult maximum SVL between 36 to 37 mm in both sexes (Savage 2002; Köhler 2011). The largest specimen in my sample is an adult male from the southern slope of Cerro Pando of 38.7 mm SVL. Hands and feet are nearly fully webbed in this species, but the digits are discernible and the tips of each digit protrude slightly from the webbing. Savage (2002) gives the dentition of adult specimens as 34 to 55 MT and 15 to 40 VT. In my sample, adult specimens, as defined by a SVL of more than 29.4 mm, have 19 to 47 MT and 14 to 22 VT. The unique combination of adult size, webbing, and dentition distinguishes *Bolitoglossa minutula* from all other species of salamanders known from western Panama.

Coloration in life: Wake *et al.* (1973) have already brought up the highly variable coloration in *Bolitoglossa minutula*. The typically coloration combines various shades of brown. Some specimens show very dark brown ground coloration, while others are ocher or almost red to orange brown. The ground coloration is in most cases stippled with bluish speckles. I recorded the following specimens′ coloration in field: SMF 89854, Jurutungo: Dorsal ground color Fuscous (21); dorsal surfaces of hands and feet Buff (24); lateral and ventral surface of tail with fine Flaxflower Blue (170C) mottling; ventral surface of body Fuscous (21) grading into Buff (24) at midbody; dorsal surfaces of hands and feet Buff (24); iris Raw Umber (23).

AH 278, Jurutungo: Dorsal ground color Drab (27), suffused with Vandyke Brown (221), thighs Warm Buff (118); ventral surface Sepia (119) peppered with Flaxflower Blue (170C).

AH 270, Cerro Pando: Dorsal ground color Chestnut (32) with a suggestion of Maroon (31), laterally speckled with Flaxflower Blue (170C); dorsal surfaces of feet Amber (36); ventral surface of gular region Raw Umber (223), mottled with Sepia (119); ventral surface of body Mars Brown (223A) and of tail Sayal Brown (223C).

AH 233, Volcán Barú, Sendero Quezales: Dorsal surface of body and head Russet (34); dorsal surfaces of tail and lateral surfaces of body Flame Scarlet (15); ventral surfaces of head and body Fuscous (21); ventral surface of tail Raw Umber (23).

SMF 89853, Volcán Barú, Camp Marmecillos: Ground color Dark Grayish Brown (20), mottled with Lavender Blue (170D).

AH 336, La Nevera: Dorsal ground color Sepia (219); temporal parts of head Dark Drab (119B); a Buff (124) interorbital bar and a Buff (124) stripe reaching from the eye to the labial protuberances; hands and feet likewise Buff (124). A juvenile specimen collected at Cerro Pando under the field number AH 356 (Fig. 58 E), almost exactly resembled the coloration of the holotype in life as described by Wake *et al.* (1973). However, this coloration type is less common among the specimens I saw.

Distribution: *Bolitoglossa minutula* has been collected on all slopes around Cerro Pando near the Panama-Costa Rica border and at Volcán Barú. The finding of a juvenile at La Nevera, Comarca Ngöbe-Buglé, morphologically assigned to this species, extents the known distribution to the western slope of Cerro Santiago, approximately 80 km east of Volcán Barú (Fig. 51).

Habitat and natural history: This species inhabits dense cloud forest. The lowest elevation collection site is La Nevera at 1640 m asl, while the highest is Camp Marmecillos on the eastern slope of Volcán Barú at 2600 m asl. Most sites where *Bolitoglossa minutula* has been collected are located in the Lower Montane Rain Forest domain, but the species may also be found in Lower Montane Wet Forest and Montane Rain Forest, especially at Volcán Barú. I

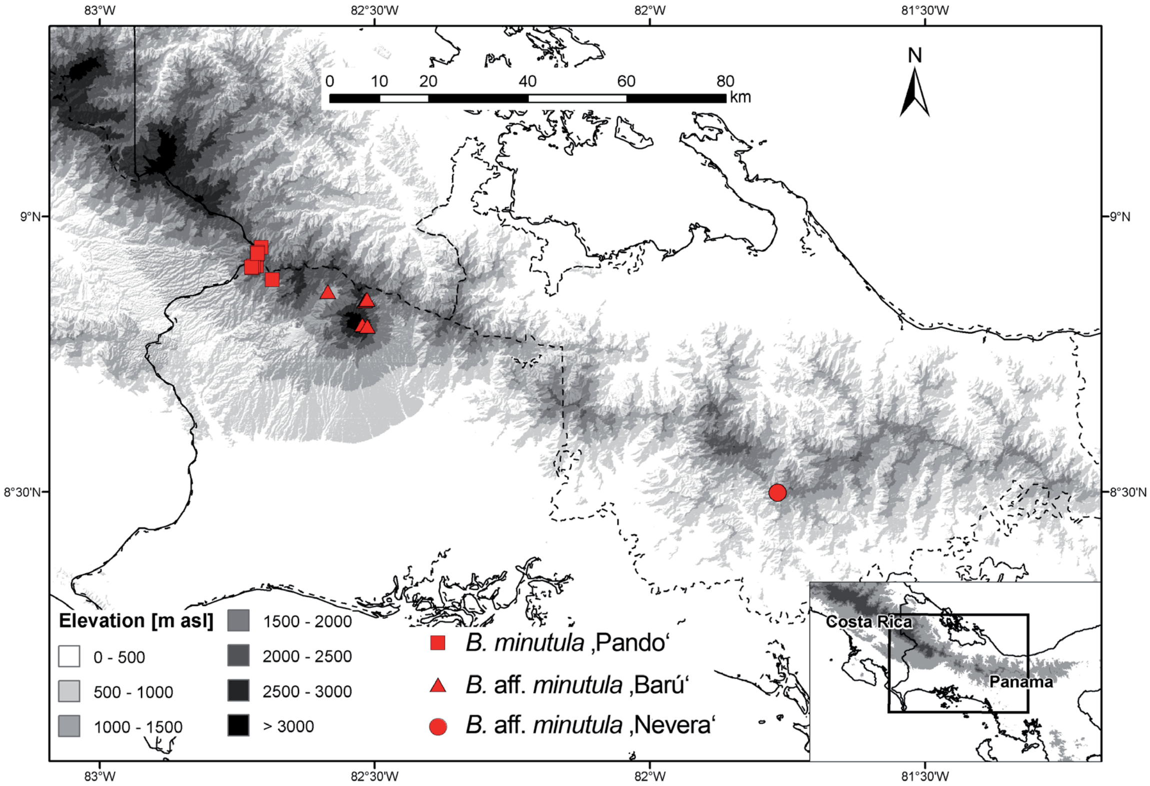

Figure 51: Collection sites of the *Bolitoglossa minutula* species complex (red dots) in Panama.

encountered all specimens on low vegetation at night. During wet nights, *B. minutula* was quite abundant at Cerro Pando. In contrast, it was much less abundant at Volcán Barú and very rare at La Nevera.

<u>Remarks:</u> I collected in total 18 specimens that are morphologically assigned to this species. Solís *et al.* (2004d) stated that the taxonomy of specimens from around Volcán Barú is questionable. Indeed, there are large genetic distances between the populations of the Cerro Pando region, Volcán Barú, and the single juvenile specimen from La Nevera in the Serranía de Tabasará. However, there is no traceable character to separate these populations morphologically. The variation is quite high and there is an overlap in every of the standard characters. The number of collected specimens is quite high compared to other *Bolitoglossa*, but it is still too small from localties other than Cerro Pando to run a profound morphological analysis. Nevertheless, the evidence available indicates that what is currently named *Bolitoglossa minutula* is likely a species complex comprised of three different genetic lineages.

Bolitoglossa nigrescens (**Taylor, 1949** Univ. Kansas Sci. Bull., 33: 282.)

Holotype: KU 23816, by original designation.

Type locality: „Boquete Camp (on highway between Millville and San Isidro del General), Costa Rica, elevation 6,000 feet.“ According to Savage (1974) a former road camp at approximately 2000 m asl between Villa Mills (Millsville) and San Isidro de El General on the west side of the Interamerican Highway.

Synonyms: *Bolitoglossa sombra* Hanken, Wake, and Savage, 2005, Copeia, 2005: 234.

Conservation Status: IUCN category: Endangered (EN). Including the populations in extreme western Panama, which were formerly treated as separated species, the extent of occurrence of *B. nigrescens* is still less than 5000 km^2. It is only known from scattered localities, thus its distribution is believed to be severely fragmented. Furthermore, the quality of its habitat is continuously declining. EVS calculation: 4 (geographic distribution) + 4 (reproductive mode) + 3 (forest formation) = 11.

Diagnosis: A large (max SVL= 70 mm in males, 84.5 mm in females; Hertz *et al.* 2011), almost completely black species of *Bolitoglossa* having no pale ring around the base of the tail. The digits on hands and feet are moderately webbed, with about two phalanges free of webbing. Adult males have 43–66 MT and 33–36 VT, adult females have 64–85 MT and

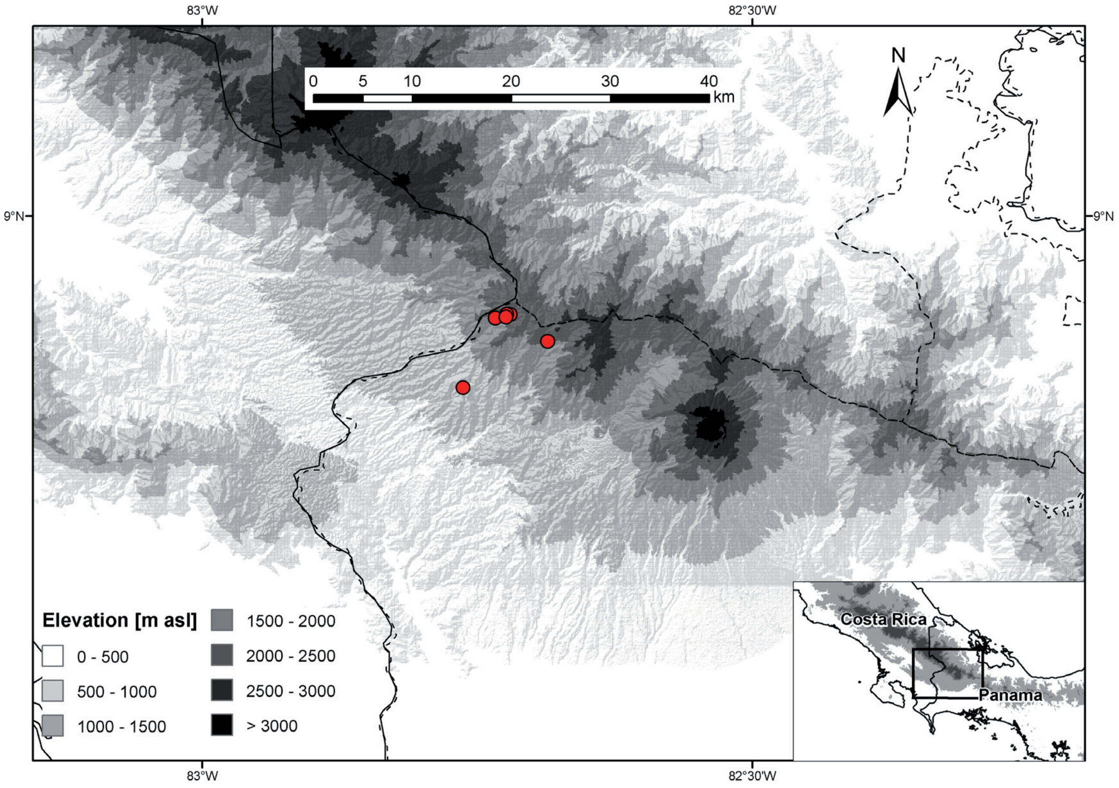

Figure 52: Collection sites of *Bolitoglossa nigrescens* (red dots) in Panama.

34–50 VT. A mental gland is present in adult males.

Coloration in life: Adult specimens are generally dull black. Subadults and juveniles often show interspersed white, cream, or brownish blotches, particularly on legs and tail. The individual coloration of a large male specimen (AH 132) was recorded as follows: Dorsal ground color between Blackish Neutral Gray (82) and Jet Black (89); posterior surface of thighs with a suggestion of Burnt Orange (116) and with irregular Warm Buff (118) blotches; labial protuberances and mental gland Pearl Gray (81), the latter with fine Blackish Neutral Gray (82) mottling; dorsal surfaces of feet Sepia (119); ventral surface of body Blackish Neutral Gray (82) with fine Pearl Gray (81) mottling, and shadings of Grayish Horn Color (91) in the surrounding of gular and cloacal regions; ventral surfaces of feet Pale Neutral Gray (86); bones of digits visible through skin from below; iris Raw Umber (223), pupil horizontal.

Distribution: Pacific slopes of the Serranía de Talamanca in Costa Rica and into western Panama (Fig. 52).

Habitat and natural history: *Bolitoglossa nigrescens* inhabits Premontane and Lower Montane Wet Forest between 1500–2300 m asl (Hanken *et al.* 2005; own data). The species was found in forest, but also in open habitat (Hertz *et al.* 2011) usually after heavy rains. One specimen that I found right after dark sitting on a leaf about 0.5 m above the ground at Mount Totumas Cloud Forest Reserve was covered with earth particles, an indication that it had spent the daytime in a ground-level hiding place.

Remarks: After having examined the specimens of *Bolitoglossa sombra* from the Jurutungo valley, I treat *B. sombra* as a junior synonym of *B. nigrescens*, as previously proposed by Boza-Oviedo *et al.* (2012). The morphological characters like tooth counts, relative limb length, and shoulder width that were used by Hanken *et al.* (2005) to define *B. sombra* as a distinct species are largely overlapping. This has become more evident since I found larger specimens for comparison at Jurutungo (Hertz *et al.* 2011). Additionally, there is a very low genetic distance in the 16S rRNA gene between a specimen from Tarbaca, Province of San José, Costa Rica, about 57 km northwest of the type locality of *B. nigrescens*, and specimens from the Jurutungo valley, which lies about 125 km southeast of the type locality of *B. nigrescens*. This indicates a continuous distribution of *B. nigrescens* along the Pacific slopes of the Serranía de Talamanca from its northern end into western Panama. However, after Taylor (1949) had collected the holotype, no additional material has been found at the type locality. Any future study that wishes to validate or disprove the taxonomic status of *B. nigrescens* and *B. sombra* proposed here should try to include topotypic specimens of *B.*

nigrescens.

Bolitoglossa pygmaea **Bolaños and Wake, 2009**, Zootaxa, 1981: 58.

Holotype: UCR 11788, by original designation.

Type locality: „Fábrega Massif (coordinates 9°07′00″ N, 82°52′40″ W) at 3100 m elevation, Provincia de Bocas del Toro, Panamá".

Conservation Status: This species is not yet assessed for the IUCN Red List, neither is there an EVS calculation in Jaramillo *et al.* (2010). Its extent of occurence is restricted to the type locality, but since this is a very remote site it seems to be well-conserved for now. I suggest to treat *Bolitoglossa pygmaea* as Vulnerable (VU) due to its small distrubution area. EVS calculation: 6 (geographic distribution) + 4 (reproductive mode) + 5 (forest formation) = 15.

Diagnosis: According to Bolaños and Wake (2009): Within the genus *Bolitoglossa* this is the smallest species known together with *B. diminuta* that is about the same size. Males reach a SVL of 23.6–32.6 mm, females of 23.5–36.8 mm.

Coloration in life: Bolaños and Wake (2009) describe *Bolitoglossa pygmaea* as almost unpigmented, basically translucent, with scattering of brownish coloration.

Distribution: Known only from the type locality at the Cerro Fábrega Massif (Fig. 53).

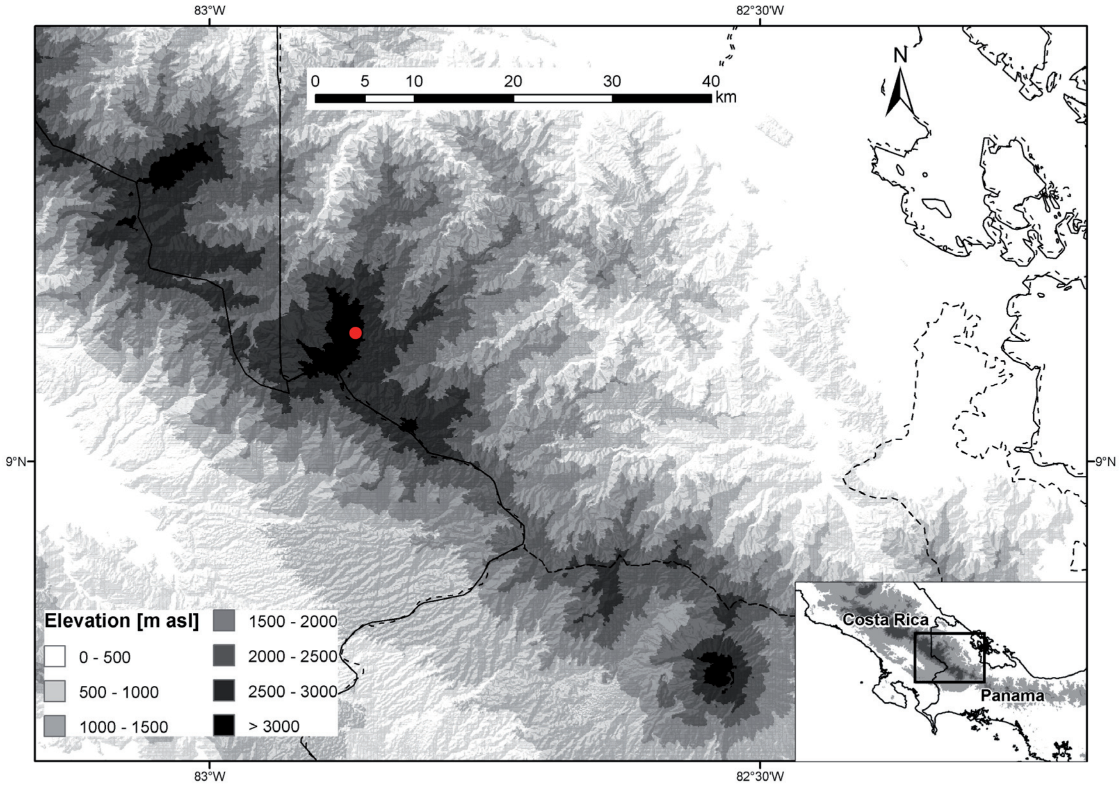

Figure 53: Only known collection site and type locality of *Bolitoglossa pygmaea* (red dot).

Habitat and natural history: The species has been found between grass tussocks on a plateau of Cerro Fábrega at an elevation of around 3000 m asl (Bolaños & Wake 2009).

Remarks: The Cerro Fábrega Massif is hardly to reach on feet. All previous expeditions were undertaken using a helicopter. Thus, I was never able to afford an expedition to this part of the Cordillera Central and therefore never collected or saw this species in life and can therefore not contribute to the knowledge of this little-known species.

***Bolitoglossa robinsoni* Bolaños and Wake, 2009**, Zootaxa, 1981: 61.

Holotype: UCR 11216, by original designation.

Type locality: „Cerro Echandi (top of Cerro 3167 m), Prov. Puntarenas, Costa Rica, latitude 9°01′50″ N (9.0306°), longitude 82°49′20″ W (-82.8222°) (3.4 km max. error, WGS84 datum), elevation 3162 m".

Conservation Status: This species is not yet assessed for the IUCN Red List. The known area of occurrence in Panama is about 20 km^2. The taxonomic affiliation of Costa Rican specimens is uncertain. Despite of the small area of occurence, the inhabited sites seem to be well-conserved due to their remote character. I suggest to list *Bolitoglossa robinsoni* as Vulnerable (VU). However, there are doubts about the taxonomic status of specimens referred

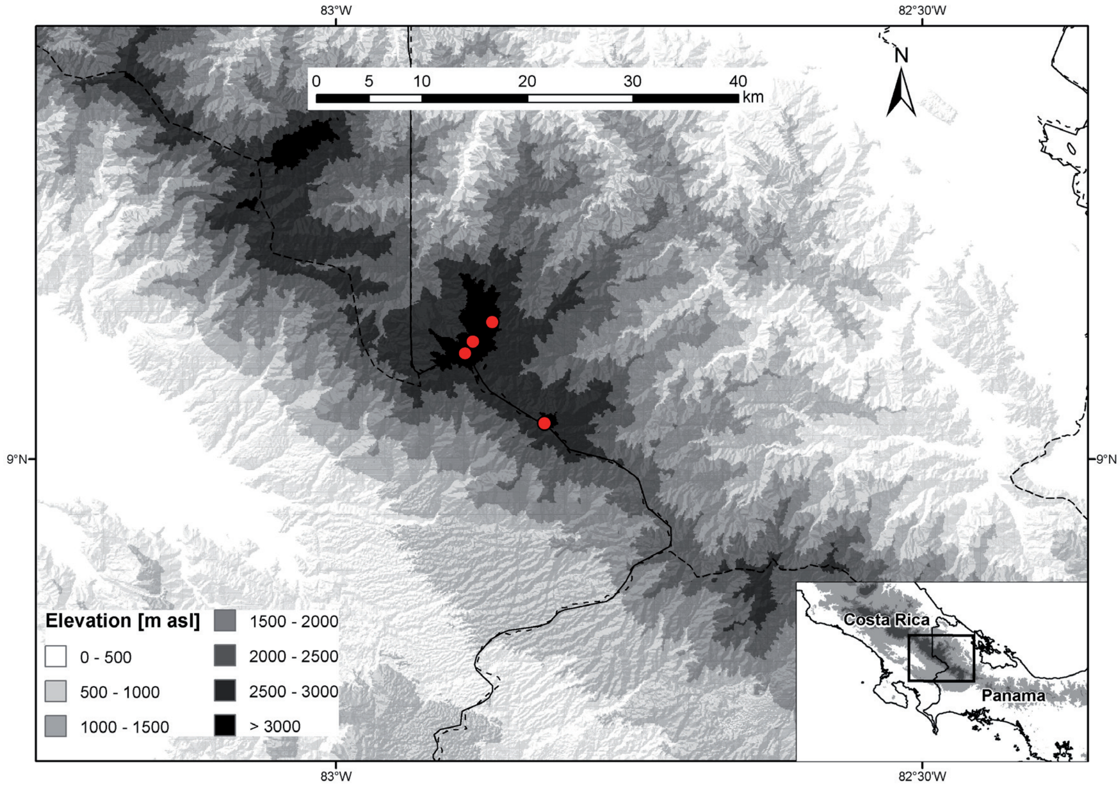

Figure 54: Collection sites of *Bolitoglossa robinsoni* (red dots) in Panama.

to as *B. robinsoni* from different localities (see Remarks). EVS calculation: 6 (geographic distribution) + 4 (reproductive mode) + 5 (forest formation) = 15

Diagnosis: *Bolitoglossa robinsoni* is a large (SVL: 45.9–63.5 mm) and robust species, with a broad head (HW/SVL≈0.12) that is well-demarcated from the body, and broad hands and feet (foot width/SVL≈0.17). It is morphological most similar to *B. cerroensis* from Costa Rica, but has more maxillary teeth.

Coloration in life: The coloration in life was not published. The alcohol coloration as provided by Bolaños and Wake (2009) is dark brownish-black dorsal ground coloration blotched with much pale spotting. Costal grooves are mostly dark, while costal folds are light colored.

Distribution: As currently understood, *Bolitoglossa robinsoni* inhabits the high peaks of more than 3000 m in the Serranía de Talamanca in the Costa Rica-Panama border region. The type series comprises specimens from Cerro Echandi and nearby Cerro Burú. Referred specimens come from other peaks in that area, namely Cerro Fábrega, Cerro Itamut, and Cerro Biné. Specimens from Valle del Silencio are morphologically different from the types and may represent different, undescribed species (see Remarks).

Habitat and natural history: There is almost nothing known on the natural history of this species. The Panamanian collection sites lie within the Montane Rain Forest domain. It has also been found in open paramo (Bolaños & Wake 2009). On Cerro Fábrega it lives sympatric with *B. pygmaea* (Boza-Oviedo *et al.* 2012).

Remarks: As mentioned before, I had never the opportunity to visit the three-thousand-meter summits in Panamas north-west. Bolaños and Wake (2009) argued that the specimens collected at six nearby locations might represent four to five different species. After he conducted further studies, David Wake (pers. comm. 2009) told me that what is called *B. robinsoni* today appears to be a species complex of as many as five species. Molecular data of one voucher specimen from Valle del Silencio, Costa Rica is available on GenBank. This locality lies nearly 20 airline kilometers away from the type locality and might represent an additional undescribed species. This specimen appeared in phylogenetic analyses in a clade together with *B. aureogularis* and *B. jugivagans* (Boza-Oviedo *et al.* 2012; Hertz *et al.* 2013a; Fig. 37 this study).

Bolitoglossa robusta (**Cope, 1894**, Proc. Acad. Nat. Sci. Philadelphia, 46: 194.)

Holotype: Called “specimen No. 226” in the species description; according to Hanken *et al.*

(2005): AMNH 5464.

Type locality: According to the original description: „Faldas of the Volcano of Irazú“; Cantón de Cartago, Provincia de Cartago, Costa Rica (in Savage 1974). Approximate coordinates according to Hanken *et al.* (2005): “ca. 9°57’ N, 83°52’ W” (9.95°, -83.86°).

Conservation Status: IUCN category: Least Concern (LC). Although its extent of occurrence is probably less than 20000 km^2 and it seems to decline, it is still regularly found and the population is estimated to be large. The decline is presumably not fast enough to qualify for a more threatened category. However, it got possibly extinct in the surroundings of San José, Costa Rica (Solís *et al.* 2004e). In Panama it is known from only a few sites, including the PAs PILA and RFLF.

Diagnosis: According to Hanken *et al.* (2005): One of the largest Central American salamanders (SVL 44.6–113.9 mm in males; 64.9–133.5 mm in females). It has a stout robust body, broad but flat head and moderate-sized eyes that protrude only slightly beyond jaw margins in dorsal view. MT count may be more than 100 in large adults. The webbing is extensive with only 1/2–1 distal phalanges of longest digits free. It is readily identified by a cream-colored, rose, or reddish ring around the base of the tail.

Coloration in life: According to Hanken *et al.* (2005), dorsal ground coloration is mostly

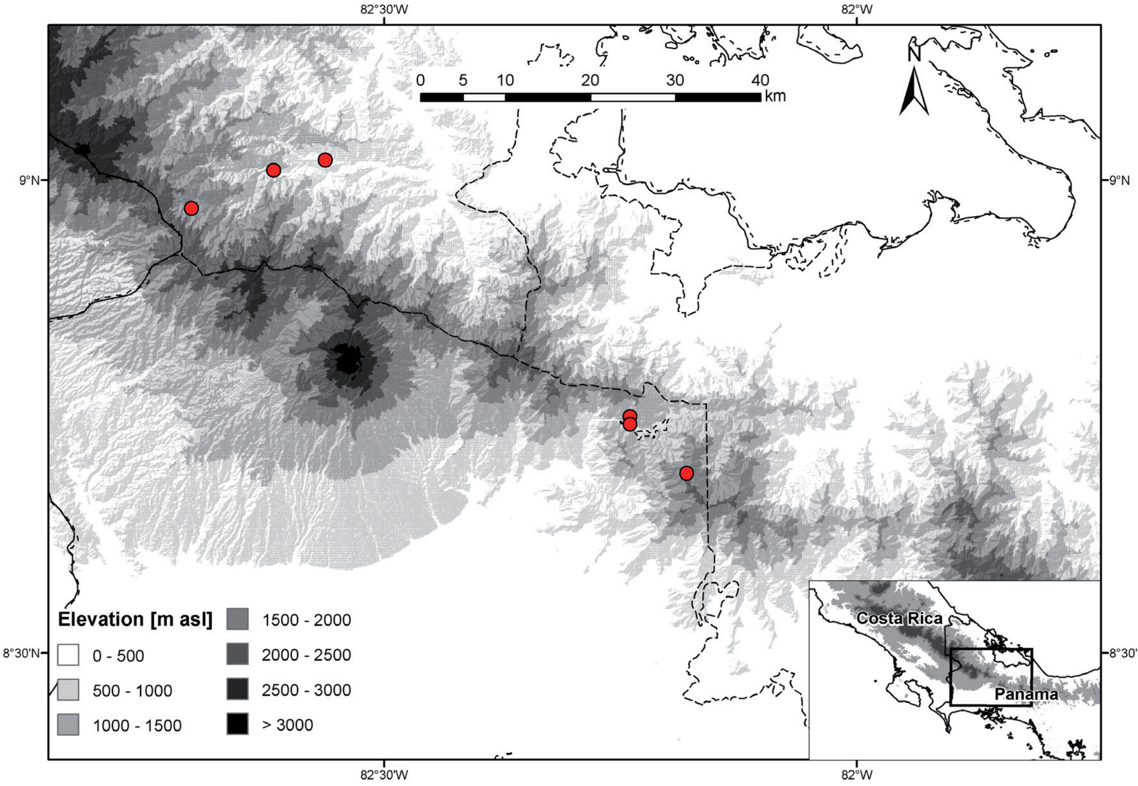

Figure 55: Collection sites of *Bolitoglossa robusta* (red dots) in Panama.

black to lead-gray, sometimes mottled with fine blue-grayish salt and pepper pattern. In particular, specimens from Panama show dirty white spots on the distal part of the body and especially on the tail. All specimens have a distinct light-colored ring around the basis of the tail.

Distribution: *Bolitoglossa robusta* is widely distributed in Costa Rica and western Panama. It occurs from Cerro Cacao in north-western Costa Rica into the Cordillera de Guanacaste and throughout the Cordillera de Tilarán and the Cordillera Central southwards into the Cordillera de Talamanca and into western Panama. In Panama it is known from sites on the Caribbean slopes of Cerro Pando and the La Fortuna Depression.

Habitat and natural history: Panamanian collection sites lie in the Premontane Wet and Rain Forest and Lower Montane Rain Forest domains. *Bolitoglossa robusta* is mainly a terrestrial salamander, but has also been found walking on bamboo as high as 0.4 m above ground. It hides under logs and thick leaf litter during the day. It is most common at elevations between 1000 and 1600 m asl (Hanken *et al.* 2005).

Remarks: Although I visited most of the Panamanian sites, where this species has previously been found (*e.g.*, Río Changena, Río Claro, and several sites in the Fortuna Depression) I never came across a specimen of *Bolitoglossa robusta*.

Bolitoglossa jugivagans **Hertz, Lotzkat, and Köhler, 2013**, Zootaxa 3636 (3): 465.

Holotype: SMF 94467 (original field number AH 314), by original designation.

Type locality: Headwaters of Río Chiriquí Malí, approximately 5 km N of the La Fortuna dam reservoir (8.7890°, -82.2154°, 1060 m), Bosque Protector Palo Seco, Comarca Ngöbe-Buglé (formerly province of Bocas del Toro).

Conservation Status: IUCN category: Not yet assessed. So far, *Bolitoglossa jugivagans* is only known from a single specimen. Thus, there is virtually no information on its extent of occurrence, population status, and ecological requirements. Therefore, it should be listed as Data Deficient (DD). Considering that the type locality and its surroundings are one of the best sampled regions in the Panamanian Cordillera Central it is supposed to be a generally rare species. EVS calculation: 6 (geographic distribution) + 4 (reproductive mode) + 5 (forest formation) = 15.

Diagnosis: (partially from Hertz *et al.* 2013a): The only specimen of *Bolitoglossa jugivagans* so far known is a small salamander with relatively short legs and a long tail. The hands and feet are moderately webbed, with the two terminal phalanges of the longest digits free of webbing. The prehensile tail is considerably longer than the body. Both, MT and VT counts are high in relation to SVL compared to morphologically similar species (Fig. 57). It can be differentiated from other small salamanders with high tooth counts from the Talamancan Mountains as follows (conditions for *B. jugivagans* in parentheses): *Bolitoglossa pygmaea* is almost unpigmented and has a short tail that never exceeds SVL (reddish-brown pigmentation and the tail much longer than SVL). *Bolitoglossa gracilis* is bright yellowish to golden tan with dark streaks and spots (reddish-brown with light streaks), has longer legs with a limb interval of 3 to 3.5 (4), and fewer counts of VT: 10–22 (27). *Bolitoglossa pesrubra* has longer legs with a limb interval of 0 to 3 folds (4), and a shorter tail with TL/SVL < 1 (=1.24). *Bolitoglossa subpalmata* has fewer tooth counts in relation to SVL: MT/SVL: 0.8–1.16 (1.63), VT/SVL: 0.32–0.73 (0.86), and a limb interval of 1 to 3 (4). *Bolitoglossa gomezi* has a shorter tail with TL/SVL: 1.15–1.19 in females (1.24), a limb interval of 1.5–3 (4), and fewer tooth counts in relation to SVL: MT/SVL: 0.97–1.13 (1.63), VT/SVL: 0.43–0.53 (0.86).

Based on molecular data, *Bolitoglossa jugivagans* is most closely related to *B. robinsoni* and *B. aureogularis* with which it forms an own clade. Both differ from *B. jugivagans* by the following characters (condition for *B. jugivagans* in parentheses): *Bolitoglossa robinsoni* is a large and stout species with a broad head with HW/SVL=0.16–0.17 (0.14) and a tail that is shorter than the TL/SVL ≤ 1 (=1.24). The Costa Rican species *B. aureogularis* is most

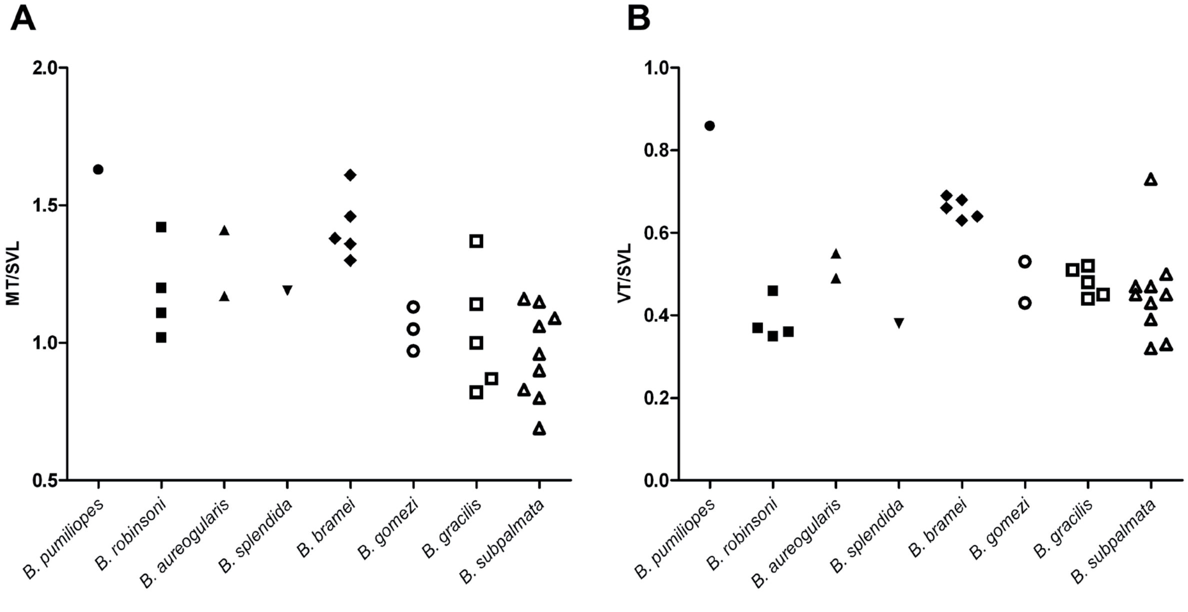

Figure 56: Tooth counts in relation to SVL of *Bolitoglossa* species resembling each other morphologically (males and females are shown together). (A) Maxillary teeth (MT) divided by snout-vent length (SVL). (B) Vomerine teeth (VT) divided by snout-vent length (SVL). From Hertz *et al.* (2013a), figure 3, page 467.

similar to *B. jugivagans* in morphometrics, tooth counts and dorsal coloration. However, *B. aureogularis* has fewer fewer tooth counts in relation to SVL: MT/SVL: 1.17–1.41 (1.63), VT/SVL: 0.49–0.55 (0.86), and much shorter legs with a limb interval of 6.5 in females and 5 in males (4 in the female holotype of *B. jugivagans*). *Bolitoglossa aureogularis* differs most markedly from *B. jugivagans* in ventral coloration, having a bright yellow gular region and a prominent midventral dark stripe on dirty white ground (gular region mustard yellow; ventral coloration of body grey to brownish speckled with blue, no conspicuous dark midvental stripe).

Coloration in life: Coloration of the holotype: Dorsal ground color Chestnut (32), interspersed with Fuscous (21) and Salmon Color (106) broken longitudinal lines. A Salmon Color (106) dorsolateral line from eye to groin separating the middorsal coloration from the lateral and ventral ground coloration; ventral ground color Sulfur Yellow (157) flecked with gray at night and Fuscous (21) at daytime; ventral surfaces speckled with Sky Blue (66); gular region, hands, and feet Sulfur Yellow (157), suffused with Fuscous (21) on limbs.

Distribution: (partially from Hertz *et al.* 2013a): This new salamander species is known only from the type locality (Fig. 56). One could speculate about the distribution when looking

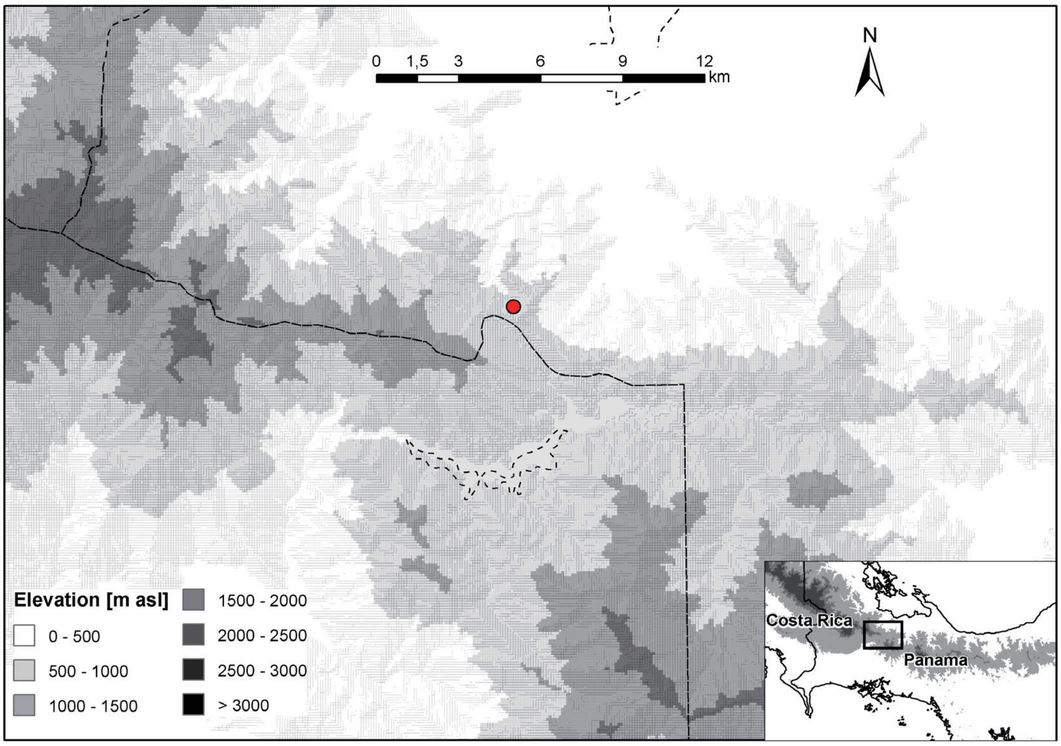

Figure 57: Type locality of *Bolitoglossa jugivagans* (red dot) in the Fortuna Depression. Dashed outline indicates the Fortuna lake.

at the distribution of other amphibians that occur in the Fortuna depression. According to that, the horizontal distribution of *B. jugivagans* may either be predominantly Caribbean or the species is endemic to the Fortuna area. Many species distributed along the Atlantic drainage enter the Pacific slopes in the Fortuna depression, while only few species with distributions along the Pacific drainage are present in Fortuna (Myers & Duellman 1982). Reptile and amphibian species that are apparently endemic to the Fortuna area and vicinity, include *Anolis*

Figure 58: Examples of *Bolitoglossa* specimens in life. **A** *B. anthracina*, male (SMF 94465), La Fortuna. **B** *B. anthracina*, female (SMF 94466), day coloration, La Fortuna. **C** Same specimen (SMF 94466) as B, night coloration. **D** Portrait of *B. colonnea*, male (SMF 94460), Río Changena. **E** *B. colonnea*, female (SMF 94464), Cerro Negro. **F** *B. compacta*, female (AH 244), Volcán Barú. **G** *B. compacta*, potential male (not collected), Cerro Totuma, photo by J. Dietrich. **H** *B. gomezi*, female (SMF 94462), Jurutungo. **I** *B. jugivagans*, female holotype (SMF 94467) **J** *B. lignicolor*, male (SMF 94459), Alto Tolica. **K** *B. magnifica*, female (AH 323), Volcán Barú. **L** *B. magnifica*, female (SMF 89848), Volcán Barú.

fortunensis Arosemena and Ibáñez, *Sphenomorphus rarus* Myers and Donnelly, *Oedipina fortunensis* Köhler, Ponce, and Batista, and in a broader sense also *Oophaga arborea* Myers, Daly, and Martínez and *O. speciosa* Schmidt. *Anolis fortunensis* and *O. fortunensis* are only known from sites on the Pacific versant of the Fortuna Depression (Mayer 2011; Hertz *et al.* 2011). *Sphenomorphus rarus* is known only from the type locality on the Atlantic versant (Köhler 2008) and *O. arborea* is found on the Atlantic slopes from the continental divide

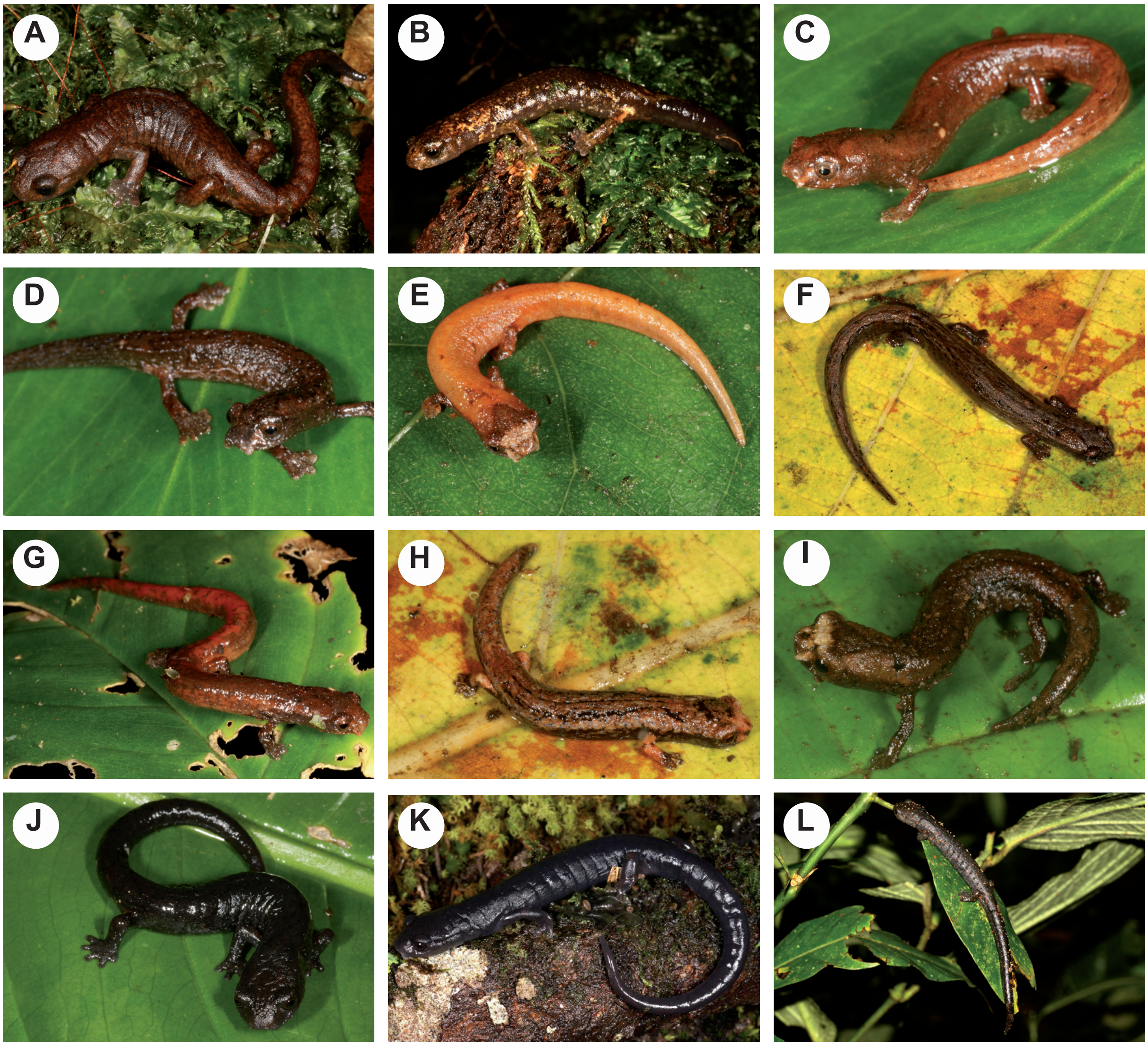

Figure 59: Examples of *Bolitoglossa* specimens in life. **A** *B. marmorea*, female (SMF 89850), Volcán Barú. **B** *B. marmorea*, juvenile (SMF 89852), Volcán Barú. **C** *B. minutula*, female (AH 275), Jurutungo, Cerro Pando. **D** *B. minutula*, male (SMF 89855), Jurutungo, Cerro Pando. **E** *B. minutula*, juvenile (AH 356), Jurutungo, Cerro Pando. **F** *B.* aff. *minutula*, female (AH 235), Volcán Barú. **G** *B.* aff. *minutula*, male (AH 233), Volcán Barú. **H** *B.* aff. *minutula*, juvenile (AH 234), Volcán Barú. **I** *B.* aff. *minutula*, juvenile (AH 336), La Nevera. **J** *B. nigrescens*, female (AH 351), Jurutungo, Cerro Pando. **K** *B. nigrescens*, male (SMF 89792), Jurutungo, Cerro Pando. **L** *B. nigrescens*, (not collected), Jurutungo, Cerro Pando.

down into the lowlands, but also enters the Pacific slopes of the Fortuna depression (Myers *et al.* 1984). The type localities of the two latter species are in the immediate vicinity of the type locality of *B. jugivagans*.

Habitat and natural history: (partially from Hertz *et al.* 2013a): The type locality is easily reachable on a paved road that serves as a maintenance access way to the Trans Panama Oil Pipeline. The river bank next to the street is an old clearing, overgrown with grass, herbaceous plants, and bushes, while the opposite bank is predominantly covered by mature forest. The holotype was encountered on October 28, 2009 at 23:00 h active on the large leaf of an Araceae plant growing out of the long grass on the cleared side of the river. That day, it had been raining in the afternoon and evening; my pluviometer registered 44.5 mm of precipitation between 16:00 and 24:00 h. The recorded temperature was 19.6 °C between 18:00 and 23:00 h, and then gradually decreased to 17.6 °C at 06:00 h. Relative humidity was constantly high at close to 100%. At this site, the mean annual precipitation is approximately 3000 mm (Fig. 12) and mean annual temperature approximately 20.6 °C (Fig. 11) what accounts for the Premontane Wet Forest life zone according to the Holdridge (1967) classification. It was the only salamander encountered at this site. Other amphibian species collected in the vicinity include *Craugastor fitzingeri*, *Diasporus* cf. *tigrillo*, *Duellmanohyla uranochroa*, *Espadarana prosoblepon*, *Incilius coniferus*, *Pristimantis cruentus*, *P. pardalis*, *Pristimantis* sp. and *Smilisca phaeota*. Additionally, the river contained tadpoles of *D. uranochroa*; the water temperature was 19.4 °C. This site is furthermore the type locality of the recently described snake species *Sibon noalamina* Lotzkat, Hertz, and Köhler. Lotzkat *et al.* (2012) give additional information on the reptile fauna of the surroundings. Members of the genus that are likely to occur in sympatry with *B. jugivagans* are *B. colonnea* and *B. robusta* and less likely, but still possible, *B. schizodactyla* and *B. anthracina*.

Remarks: Although known from only a single specimen, *Bolitoglossa jugivagans* is very well differentiated from other salamanders by both morphological and molecular data.

6.3. TAXONOMY: GYMNOPHIONA – CAECILIIDAE – *OSCAECILIA*

Caeciliidae

The taxonomy of the caecilian family Caeciliidae is a controversial issue. Recent molecular studies such as Zhang and Wake (2009) or Pyron and Wiens (2011) include very few species of the closely related genera *Caecilia*, *Oscaecilia* and *Typhlonectes* in their phylogeny. Since the monophyly of *Caecilia* and *Oscaecilia* is indisputable in both papers and *Typhlonectes* is not relevant in my study, I decided to follow the nine-family classification of Wilkinson *et al.* (2011), which proposes that the family Caeciliidae comprises only the two genera *Caecilia* and *Oscaecilia*. In total, these two genera contain 42 species to date.

Oscaecilia

According to Taylor (1968) the genus *Oscaecilia* is morphologically distinct from the genus *Caecilia* by the absence of externally visible eyes. Most species in this genus inhabit northeastern South America. Only three described species occur in Central America: *Oscaecilia elongata* (Dunn, 1942), *O. ochrocephala* (Cope, 1866), and *O. osae* Lahanas and Savage, 1992. *Oscaecilia elongata* and *O. ochrocephala* have been recorded from eastern and central Panama, while O. osae is only known from the Golfo Dulce region in southwestern Costa Rica. Only *O. ochrocephala* has been collected more frequently, the other two species are only known from very few specimens. There have been no records of the genus from western Panama prior to this study.

6.3.1. MOLECULAR ANALYSIS

The number of available specimens of the genus *Oscaecilia* and thus also tissue samples for molecular phylogenetic analysis is very scarce. I collected one specimen in Los Algarrobos near David in western Panama (AH 297) and obtained a 16S mtDNA sequence from this specimen. Later, A. Batista collected an additional specimen at Gualaca in western Panama (AB 1232) and provided me with the 16S mtDNA sequence of that specimen. In 2013, I received two living *O. osae* from R. Weixler collected at La Gamba, Costa Rica. In order to keep the specimens alive for further studies, I took blood samples from which I gained short fragments of the 16S rRNA gene, with the help of the Senckenberg Conservation Genetics group at Gelnhausen. All these sequences where supplemented by a sequence of *O. ochrocephala* on GenBank, which is the only specimen of the genus available. Additionally, nearly all available specimens of the sister genus *Caecilia* on GenBank were included in the alignment. The only exception is a specimen labelled as *Caecilia* sp. with the GenBank accession number X86297 that was not included. The sequence of this specimen is very short and was difficult to include into the alignment. To test the monophyly of *Oscaecilia* in regard to *Caecilia* I included GenBank sequences of the next relatives *Thyphlonectes natans* and *Chthonterpeton indistinctum* into the alignment. I used a GenBank 16S rRNA gene sequence

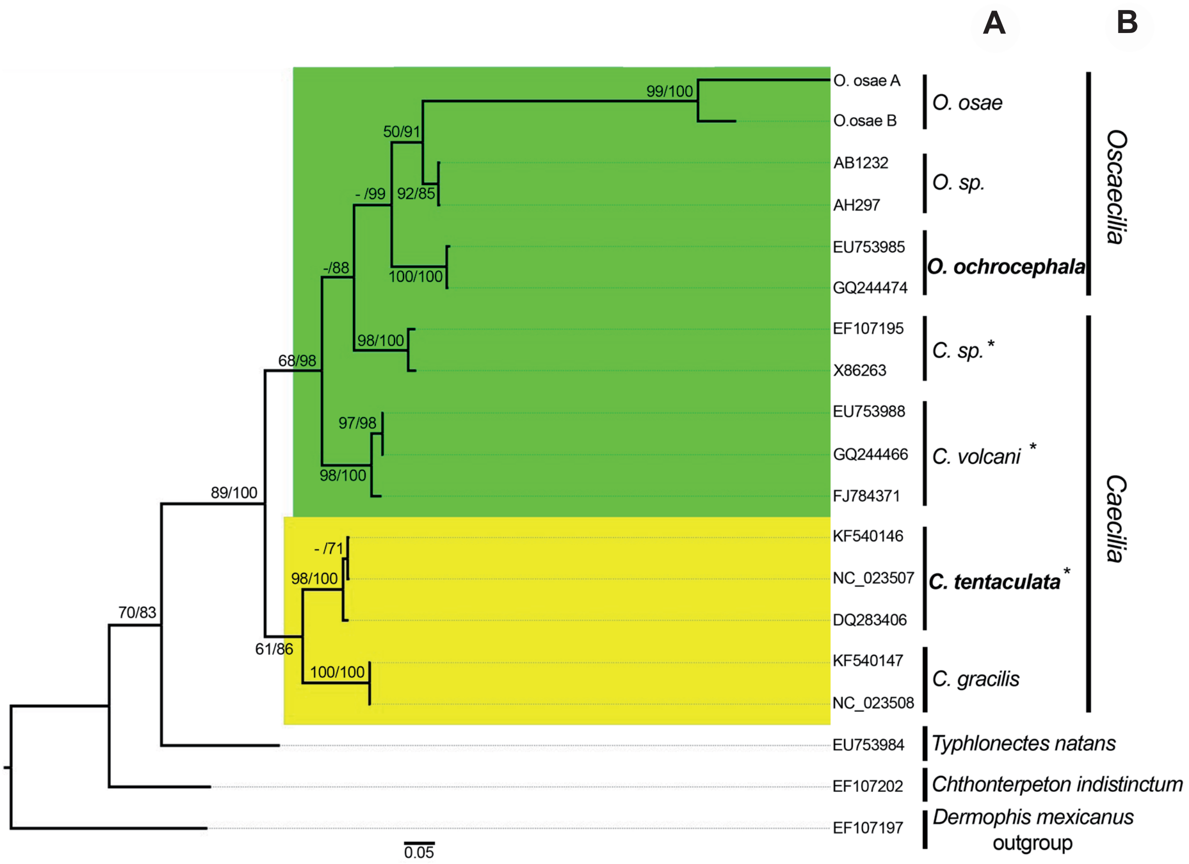

Figure 60: ML-Tree of the 16S mtDNA of all available specimens of Caeciliidae and close relatives. Numbers near nodes represent bootstrap support (before slash) and Bayes posterior propabilities multiplied by 100 (after slash). Branches are labeled with GenBank accession numbers or field numbers. The two major clades in Caeciliidae are highlighted in color. Scale bar refers to number of substitutions per site. **A** Species identification, bold names indicate type species of the genus. Asterisks indicate species with always externally visible eyes. **B** Traditional classification of genera within Caeciliidae.

of *Dermophis mexicanus* as outgroup. The final alignment contained 395 positions of which, excluding the outgroup, 195 were variable and 136 parsimony informative.

The topology of the ML and the Bayes tree are basically congruent. In both trees specimens assigned to the genus *Oscaecilia* form an own branch (Fig. 59) that receives low support in the ML analysis (bs=37), but high support in the Bayes analysis (pp=99). In both analyses, the *Oscaecilia* branch forms a subtree with *Caecilia* sp. from Ecuador and *C. volcani* (bs=68; pp=98). A second, well-supported subtree (bs=89; pp=100) contains the two species *C. tentaculata* and *C. gracilis*. Thus, *Caecilia* is paraphyletic with respect to *Oscaecilia*. The classification of *Oscaecilia* into two distinctive subgroups by the index of attenuation as proposed by Savage (2001) could not be tested with molecular data, because of

Table 11: The number of base differences per site from averaging over all sequence pairs within each lineage of Caeciliidae and relatives are shown. Standard error estimates are shown in the last column. The presence of n/c in the results denotes cases in which it was not possible to estimate distances.

Lineage	**p-distance**	**Standard error**
Oscaecilia osae	0.0765	0.0200
Oscaecilia sp.	0.0000	0.0000
Oscaecilia ochrocephala	0.0000	0.0000
Caecilia sp.	0.0059	0.0057
Caecilia volcani	0.0157	0.0076
Caecilia gracilis	0.0000	0.0000
Caecilia tentakulata	0.0000	0.0000
Typhlonectes natans	n/c	n/c
Chthonterpeton indistinctum	n/c	n/c
Dermophis mexicanus (outgroup)	n/c	n/c

the limited taxon sampling. The principle aim of this approach was the barcoding of *O. osae* in relation to *O*. sp. from western Panama. Both lineages are strongly diverging and together form an own clade that is moderately well-supported in both trees (bs=50; pp=91). The two *O*. sp. specimens are grouped together with moderatly high support in both trees (bs=92; pp=85). Likewise, the two *O. osae* specimens form a highly supported cluster (bs=99; pp=100). The p-distance between the two *O*. sp specimens is zero, while there is a high p-distance of more than 7.6% between the two *O. osae* specimens. The "between group p-distances" are 13.5%

Table 10: Estimates of p-distances over sequence pairs between lineages of Caeciliidae and close relatives. The number of base differences per site from averaging over all sequence pairs between groups are shown. Standard error estimates are shown above the diagonal.

	Lineage	1	2	3	4	5	6	7	8	9	10
1	*Oscaecilia osae*		0.025	0.027	0.025	0.028	0.031	0.029	0.031	0.032	0.033
2	*Oscaecilia* sp.	0.135		0.023	0.022	0.023	0.027	0.026	0.026	0.029	0.031
3	*Oscaecilia ochrocephala*	0.168	0.100		0.024	0.026	0.028	0.027	0.030	0.031	0.032
4	*Caecilia* sp.	0.153	0.097	0.115		0.022	0.027	0.025	0.030	0.030	0.032
5	*Caecilia volcani*	0.192	0.102	0.133	0.101		0.027	0.026	0.028	0.031	0.032
6	*Caecilia gracilis*	0.232	0.141	0.159	0.144	0.147		0.024	0.030	0.031	0.031
7	*Caecilia tentaculata*	0.209	0.129	0.147	0.129	0.133	0.106		0.029	0.028	0.029
8	*Typhlonectes natans*	0.247	0.141	0.200	0.203	0.186	0.206	0.182		0.027	0.031
9	*Chthonterpeton indistinctum*	0.274	0.188	0.218	0.206	0.220	0.212	0.171	0.153		0.027
10	*Dermophis mexicanus*	0.279	0.218	0.247	0.232	0.243	0.224	0.188	0.218	0.159	

Table 12: Morphological measurements of *Oscaecilia* specimens from Panama and Costa Rica.

Lineage	Collection Number	Sex	PG	SG	SG1	NV	SI	L	BW1PG	BWM	BWV	IA	HL	HW	LJL	SP	TND	ITD	IND	MPAT (l-r)	VPAT (l-r)	DT (l-r)	ST (l-r)
O. elongata	MCZ 215221	n/a	n/a	n/a	n/a	n/a	n/a	n/a	5.1	6.0	n/a	n/a	10.1	5.7	6.0	2.3	1.0	2.0	1.8	8-9	13-13	11-11	2-3
O. osae	LACM 138542	f	221	0	0	229	175	382	3.2	4.2	4.3	91.0	6.8	3.6	3.4	1.2	0.5	1.5	1.1	6-8	8-7	8-8	2-3
O. osae	SMF alive	n/a	221	0	0	n/a	n/a	354	n/a	4.4	n/a	80.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
O. osae	SMF alive	n/a	220	8	210	n/a	n/a	316	n/a	3.5	n/a	90.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
O. sp.	SMF 89813	m	226	0	0	224	205	554	6.2	6.6	5.5	83.9	9.7	5.9	4.2	1.2	1.2	2.1	2.0	7-8	8-9	8-8	2-2
O. sp.	MHCH 1566		223	0	0	231	204	479	4.6	5.6	4.8	85.5	6.1	4.5	2.6	1.9	0.9	1.5	1.6	7-8	9-8	9-9	2-2
O. sp.	AB 1232	m	233	0	0	n/a	n/a	564	5.3	5.8	6.1	98.1	7.9	5.0	5.5	2.4	1.0	1.9	1.9	7-7	n/a	n/a	n/a
O. sp.	Gorgas Memorial	n/a	232	0	0	n/a	n/a	558	5.6	6.6	6.2	85.1	7.8	5.1	5.0	2.3	0.9	2.1	2.0	7-7	n/a	n/a	n/a

between *O*. sp. and *O. osae*, 10% between *O*. sp. and *O. ochrocephala*, and 16.8% between *O. osae* and *O. ochrocephala*.

6.3.2. MORPHOLOGY AND CRANIAL OSTEOLOGY

External morphology in the order Gymnophiona is usually very simple, as these animals do not possess many characters and those are not difficult to measure. The problem with most species is that sample sizes are very low. Thus, the within species variation is only poorly documented. Some additional characters can be derived from x-ray and high-resolution micro-computed tomography (HRµCT) images. The genus *Oscaecilia* is characterized by having the tentacle directly under the nostril, a character that it shares with its sister genus *Caecilia*, but is distinct from *Caecilia* by having the eye covered by bone with no open socket (Taylor 1968). Both characters were found in all examined specimens of *Oscaecilia*. In total, I measured eight specimens representing the species *O. elongata*, *O. osae*, and *O*. sp.. Additionally, I examined *Oscaecilia* specimens from South America and specimens of *Caecilia* for comparison. The *O. elongata* specimen consists only of head and neck and thus does not show most of the crucial characters. Most distinctive characters presented here are derived from a HRµCT of the head (Fig. 62). The holotype of *O. osae* was reexamined taking external measurements, x-ray of the whole specimen, and HRµCT of its head. The data of the two additional specimens from La Gamba were taken from photographs of the living animals, but body length and body width could not be measured as accuratly as in preserved specimens. Some characters could not be obtained from the living specimens (n/a in Tab. 12). However, the counts of annuli in both specimens are exact. All four specimens of *O*. sp. were examined externally. Additionally, I took x-ray and HRµCT data of the specimens AH 297 and MHCH 1566. An overview of all collected data is given in Table 12.

At a brief glance, *Oscaecilia osae* and *O*. sp. appear very similar. Both are very attenuate forms expressed by an IA of 80.5–91.0 (n=3) in *O. osae*, and 83.9–98.1 (n=4) in *O*. sp.. Tooth counts are also in a similar range. However, several morphological differences were found, which distinguish *O. osae* specimens and *O*. sp. specimens from western Panama. The *O. osae* specimens are with 280–385 mm (n=3) L smaller than the western Panamanian *O*. sp. that are between 479–564 mm (n=4). Further, primary annuli count for *O. osae* is 220–221 (n=3) and 223–232 (n=4) for *O*. sp.. In the smaller living specimen of *O. osae* from La Gamba are eight secondary annuli detectable none of which are complete. Secondaries begin at primary 210. The holotype of *O. osae* and the second specimen from La Gamba lack secondary grooves. Equally, all specimens of *O*. sp. from western Panama lack secondaries. Another difference is found in the scale inception that is at primary annuli 175 in the holotype of *O. osae*, but at annuli 204–205 (n=2) in *O*. sp. as seen on the x-ray images (Fig. 70).

The only, incomplete specimen referred to *Oscaecilia elongata* is distinguished from both before mentioned groups by several cranial characters. Tooth counts of vomero-palentine and dentary teeth are conspicuously higher than in *O. osae* and *O*. sp., the snout is pointed while rounded in the other two, and there is a well-visible ethmoid bone in MCZ 21521 (Fig. 62) that is partly to completely covered by the frontals in all examined specimens of *O. osae* and *O*. sp. (Fig. 66, Fig. 68). I further examined morphologically the specimen USNM 16417 labeled as *O. elongata* from 10 km south-west of Giron, Azuay, Ecuador. This specimen is 474 mm in length and the IA is 72.36. It has 213 primary grooves and no secondaries and eyes in open sockets. The grooves are darker colored than the base color of the body; the last ten grooves were cut in, probably to look for scales. I examined these incisions, but could not find any scales. The well-sampled *O. ochrocephala* is very distinct from its western congeners (data taken from Savage & Wake 2001) by a count of 169–198 primary annuli and 7–31 secondary annuli, and an index of attenuation of 40–60.

6.3.3. CONCLUSIONS

In my phylogeny using novel sequences, the genus *Caecilia* is clearly paraphyletic. The type species of *Caecilia* is *C. tentaculata* that together with *C. gracilis* forms a sister clade to the examined specimens of *Oscaecilia* and *C. volcani*. The only putative morphological synapomorphy of the genus *Oscaecilia* as proposed by Taylor (1968) is that the eye is covered by bone. However, this character is obviously a homoplasy if compared with the results of the molecular analysis. In *C. gracilis* the eyes may be in an open socket or covered by bone (Nussbaum & Wilkinson 1989). Maciel and Hoogmoed (2011b) even found both character states in a single specimen of *C. gracilis* with one eye covered by bone and the other not. In contrast, all examined specimens of *C. volcani* have external eyes in an open socket. In *O. bassleri*, for which no molecular data are available, the eyes are not in an open socket, but are visible through the bone (Fig 61), what is not the case in other *Oscaecilia* species that I have examined. Anyway, as a taxonomic consequence from my phylogenetic results, I restore the monophyly of *Caecilia* by synonymizing *Oscaecilia* with *Caecilia* until further data become available.

In view of the small sample size it is not easy to distinguish between species without

Figure 61: Paratype of *Oscaecilia bassleri* (AMNH 42840) from Peru. Skin around the eyes has been removed. Note visible eyes through bone (white arrow). Scale bar 10 mm.

externally visible eyes of *Caecilia* from Panama. It seems clear that neither *Caecilia osae* nor *C*. sp. from western Panama is conspecific with *C. ochrocephala* from central and eastern Panama. *Caecilia ochrocephala* is distinguished from both forms by several morphological characters and by a large p-distance in the 16S rRNA gene. The case of *C. elongata* is much more complicated since the type material is lost, there is no exact type locality, the original description does not allow for an unambiguous assignment, and Dunn (1942) is the only one who ever saw the type material. Taylor (1968) examined the head and neck piece (MCZ 21521) and doubted that this is conspecific with *C. elongata* as described by E.R. Dunn. The only reason for Taylor´s doubts seemed to be that he, according to his own records, found subdermal scales in the connective tissue of MCZ 21521, whereas Dunn (1942) states that there are no scales in *C. elongata*. However, when I examined the specimen I could not find any scales. Wake (1975) also stated that she could not observe such subdermal scales in any of the taxa Taylor (1968) mentioned. According to his own records, E.R. Dunn had not always much experience in finding subdermal caecilian scales. Dunn (1924) originally described *Dermophis parviceps* as scaleless, but later stated that his "lack of knowledge of South American forms and of the correlations of scales and secondaries in Caecilians" led him to overlook the scales (Dunn 1942, page 479), therefore I would expect that he was very well-experienced in finding scales at the moment that he wrote these words. However, the assumption that the absence of secondaries always indicates the absence of scales as proposed by Dunn (1942) and also Wake (1975) is obviously not true. At least not in the forms without external eyes in the genus *Caecilia* (Lahanas & Savage 1992; this work). Instead, the correlation of secondaries and scales has been correctly noted by Taylor (1968), as secondaries always indicate the presence of scales, while there might be scales without secondaries in some species. Taylor (1968, 1972) discriminated between two types of scales (*i.e.* "scales in folds" and "subdermal scales"). In his account for *C. elongata,* he stated: "However, while the diagnosis [Dunn 1942] states "no scales," the fragmentary paratype does have subdermal scales in the connective tissue" (Taylor 1972, page 1026). I suspect that the lack of scales addressed by

Dunn (1942) refer to what Taylor (1972) calls "scales in folds" and not "subdermal scales", thus both authors refer to different characters. Despite the fact that *C. elongata* would be the only one among externally eyeless *Caecilia* that has no scales in folds, I see no provable evidence that Dunn (1942) had just overlooked these scales in the type material. Although, Dunn (1942: page 527) states that "the head-and-neck from Yavisa looks quite different from *ochrocephala*" I found some similarities when comparing cranial characters of MCZ 21521 with the data for *C. ochrocephala* as given by Taylor (1969). MCZ 21521 meets the description of *C. ochracephala* in Taylor (1969) in terms of tooth counts, the presence of a visible ethmoid bone, and shape and projection of the snout. Additionally, the body grooves are slightly darker than the ground coloration in MCZ 21521, a character found also in *C. ochrocephala*. It cannot be finally said whether the specimen MCZ 21521 is conspecific with the lost type material of *C. elongata* or with *C. ochrocephala*, but it is absolutely certain that it is not conspecific with *C. osae* or *C*. sp. from western Panama. The specimen USNM 16417 from Ecuador listed in HerpNet 2 (2012) and mentioned by Solís *et al.* (2004g) as *"Oscaecilia elongata"*, has not much in common with *C. elongata* as described by Dunn (1942). This specimen has fewer primaries, is stouter, and has dark grooves. Most notably, it has well-visible eyes in open sockets. A pencil note of an unknown author in the jar of USNM 16417 reads: "There is a possibility that *elongata* belongs in *Caecilia* rather than in *Oscaecilia* if so this is *elongata*". However, this specimen corresponds most highly with the description for *Caecilia occidentalis* by Taylor (1968) from Colombia, but it is also possible that USNM 16417 represents an undescribed species. After having personally examined every available specimen referred to as *C. elongata* in databases or publications, the identity of this species remains unclear. On that account, current knowledge obliges me to declare *C. elongata* a *nomen dubium* after the definition of Mayr (1969). Furthermore, I treat *Caecilia* sp. from western Panama as Unconfirmed Candidate Species (Vieites *et al.* 2009). This assignment is limited at this point, since the sequences of the 16S mtDNA that were taken from the blood of the two living *C. osae* specimens are short and include many ambiguous positions. Anyway, the topology of the subbranch holding both taxa is dissolved in both trees with high support (Fig. 59), but there are no further molecular data available by now. I have, however, not too much confidence in the calculated p-distance. A re-analysis will be necessary to confirm if *C*. sp. and *C. osae* represent distinct species, and the assumptions made here can only be preliminary. Regardless of the barcoding results morphological differences as described before render additional evidence that both forms are distinct. When counting primary folds in the holotype of *C. osae*, I did not get the same result as Lahanas and Savage (1992). I counted several times and additionally let two colleagues count them independently again, but always with the same result. It seems that the count given by Lahanas and Savage (1992) deviates from the actual value by eleven grooves (Tab. 12). Obviously this was never checked again as the indicated value of 232 primary grooves is also found in Savage and Wake (2001) and Savage (2002). However, regarding count of vertebra and scale interception I come to the same results as Lahanas and Savage (1992).

The problem remains that it is not completely sure that the *Caecilia osae* specimens from La Gamba, that is not located on the Osa Peninsula itself, are conspecific with the *C. osae* holotype. To solve this problem, additional material from the Osa Ppeninsula, preferably from near the type locality, would be needed.

6.3.4. *OSCAECILIA* SPECIES ACCOUNTS

***Caecilia elongata* Dunn, 1942**, Bull. Mus. Comp. Zool., 91: 527.

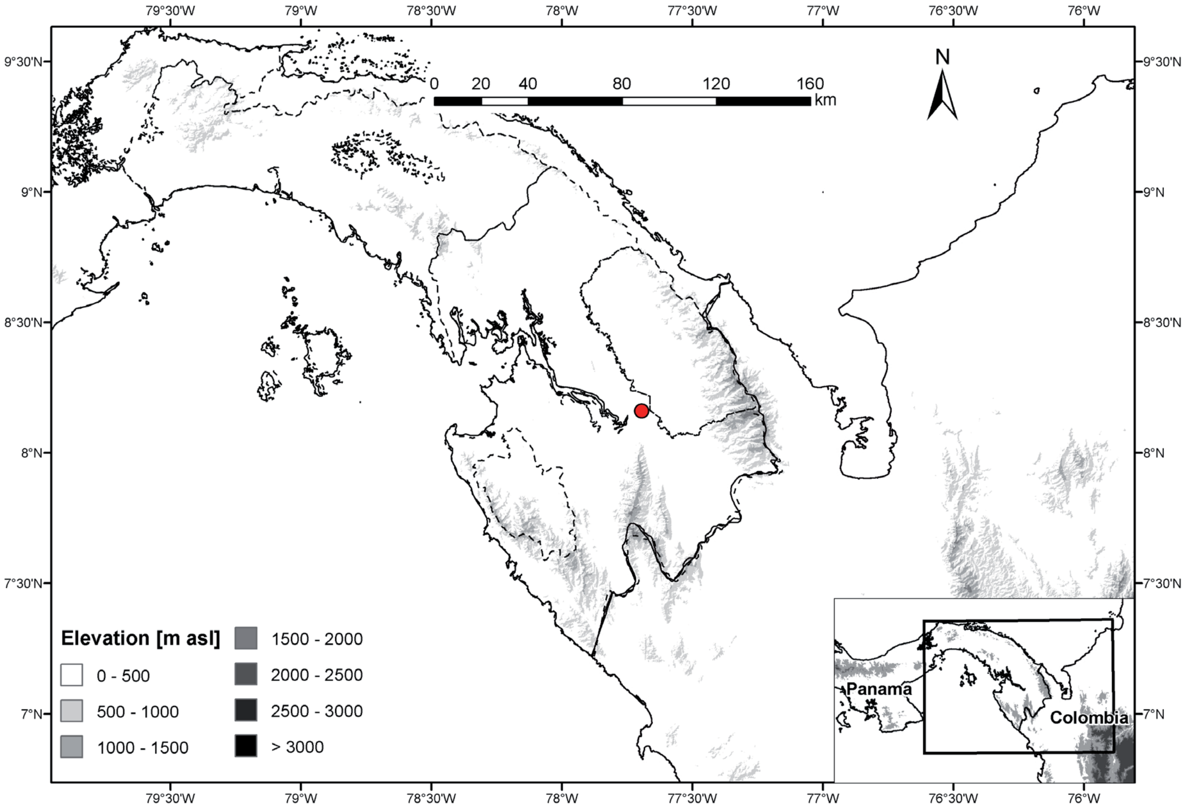

Figure 62: Position of Yaviza, Darién, collection site of the specimen MCZ 21521, referred to *Caecilia elongata*.

Holotype: ZSM 1327/0; holotype and the topotypic paratype (ZSM 1324/0) were destroyed during World War II according to Taylor (1968) and Glaw and Franzen (2006). A referred specimen from Yavisa, Darien, Panama (MCZ 21521), that was additionally used in the species description, consists of head and neck only, but may not be conspecific with the types.

Type locality: "Panamá" without further details.

Conservation Status: IUCN Category: Data Deficient (DD). There is no information on the distribution range, habitat preferences, and possible threats. An evaluation of the species conservation status is impossible at the current state of knowledge.

Diagnosis: From Dunn (1942): "A *Caecilia* without secondaries; primaries 226–231; 1/d [=IA] 83–89; no scales; eye invisible; no markings; 500–620 mm."

Coloration in life: Dunn (1942) states "no markings" without further comments on coloration.

Distribution: Contrary to the widely-believed view *Caecilia elongata* would be only known from Yavisa, Darien, Panama (Fig. 62) the collection site of the type and paratype is unknown. It is not possible to verify if the fragmentary paratype from Yavisa meets any of the characters Dunn (1942) describes. Thus, the distribution of *O. elongata* must be considered as unknown.

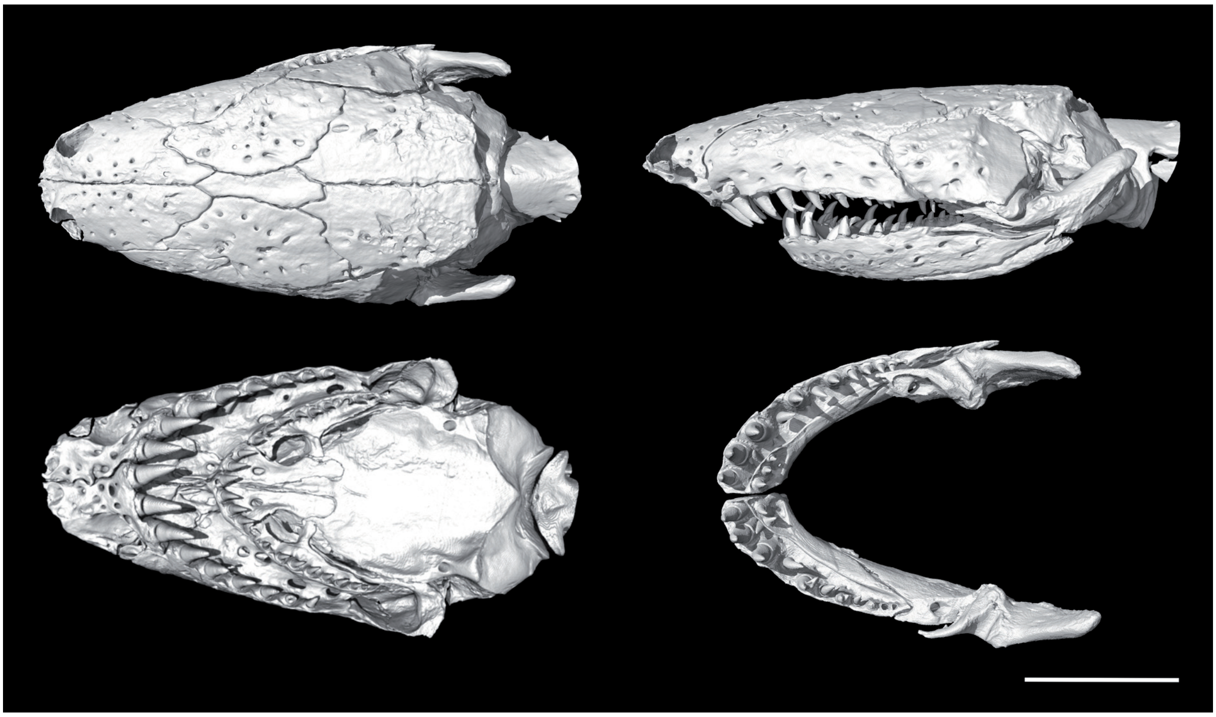

Figure 63: Volume reconstruction of synchrotron-based X-ray micro CT imaging (HRμCT) data showing the skull of *Caecilia* aff. *elongata* (MCZ 215221). Left side from top to bottom: cranium in dorsal and palatal view. Right side from top to bottom: skull in lateral view, mandible in dorsal view. Scale bar =1mm.

Habitat and natural history: Nothing is known about the species habits. Like other species in the genus I believe it to be fossorial (Köhler 2011).

Remarks: The formal conditions for a valid taxon name are fulfilled. However, the correct application of the name to a particular species is not possible. Thus, the name *Caecilia elongata* is declared a *nomen dubium*.

***Caecilia ochrocephala* Cope, 1866**, Proc. Acad. Nat. Sci. Philadelphia, 18: 132.

Holotype: USNM 29764, according to Cochran (1961).

Type locality: “Panama”; restricted to the “Atlantic side of the Isthmus of Panama (Darién)” by Taylor (1968).

Synonyms: *Caecilia sabogae* Barbour, 1906, Bull. Mus. Comp. Zool., 46: 228.

Conservation Status: IUCN Category: Least Concern (LC). It seems to be more frequently found than other species in the genus. Lynch *et al.* (2004) reported that it shows some tolerance to anthropogenic disturbance and that it has been found occasionally in urban areas. It is usually killed on sight by uneducated people, due to its snake-like appearance (Evans 1947). Swanson (1945, p. 211) reported on the superstition of laborers “that it would crawl up the rectum while its victim was defecating.” The classical amphibian EVS is not easy to calculate for *Caecilia ochrocephala*. Since it is believed to be dangerous and there

Figure 64: Holotype of *Caecilia ochrocephala* (USNM 29764) in preservative. Scale bar 10 mm.

is not much known about its reproductive biology, I calculate the EVS as if it would be a reptile. EVS calculation: 2 (geographic distribution) + 2 (persecution by humans) + 2 (forest formation) = 8.

Diagnosis: Data taken from Savage & Wake (2001): A stout *Caecilia* (IA= 40–60) of moderate length (L= 617 mm); 169–198 PG and 7–31 SG. The grooves of the primary folds are darker than the body's ground coloration.

Coloration in life: From Taylor (1968, p. 613): "Dorsally grayish-olive, the sides and venter grayish-white, extending on entire undersurface. The grooves are dark olive and the specimens are ringed with black from the collars. Head lighter, clouded yellowish-olive, generally with lighter areas on sides, tip of snout and upper lip; lower lip and jaw yellowish; the area surrounding vent, cream."

Distribution: *Caecilia ochrocephala* is primary known from central and eastern Panama. Most records concentrate around the Panama Canal and Panama City. It has also been found on the Las Perlas Archipelago. The easternmost record is a single specimen from Turbo, Colombia. West of the Canal it has been found mainly in the provinces of Panamá Oeste and

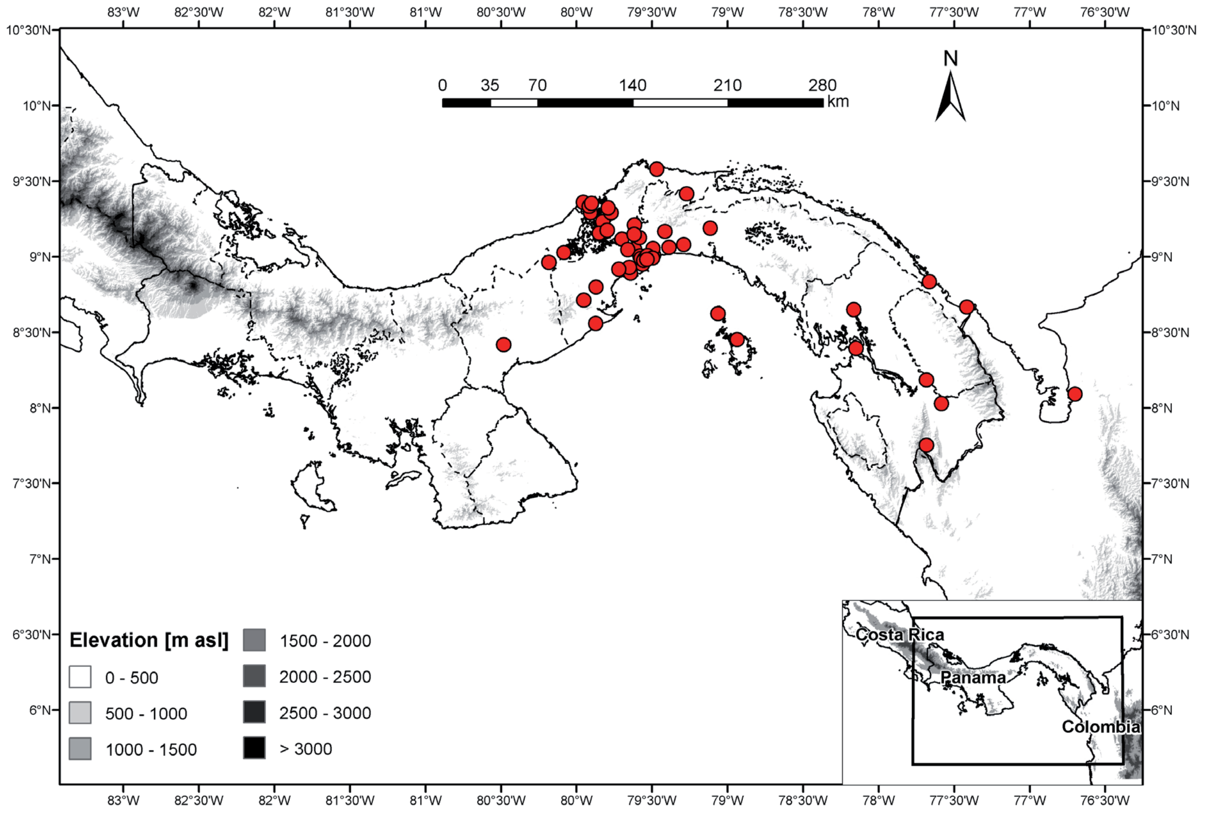

Figure 65: Collection sites of *Caecilia ochrocephala*.

Colón. The westernmost record is Rio Grande, Coclé (Fig. 64).

Habitat and natural history: Most collection sites lie within the Tropical Moist and Wet Forest and Premontane Wet Forest domains. The two southwesternmost localities lie within the Tropical Dry Forest domain. *Caecilia ochrocephala* is a lowland species that has been collected between 0–600 m asl. Most specimens have been found while digging deeper holes. Evans (1947) reported on the finding of three individuals about 1.2 m deep in the ground while drainage ditches where dug. Ibáñez *et al.* (1999) stated that they are occasionally found on Barro Colorado Island when excavations, such as graves are made. Lynch *et al.* (2004) mentioned findings to a depth of 10 m during building construction. The reproduction biology is unknown for all species in the genus. From examination of oviductal fetuses, Wake (1992) suspected *C. ochrocephala* to lay eggs that undergo direct development.

Remarks: Although *Caecilia ochrocephala* is the most sampled and best known species in the genus, there is virtually nothing known on its life history. Most collection sites are in the surroundings of the Panama Canal and in Panama City; thus, in regions with a high construction activity. I assume that this is not a rare species, but is just seldomly coming to the surface.

***Caecilia osae* (Lahanas and Savage, 1992**, Copeia, 1992: 703.**)**

Holotype: LACM 138542, by original designation.

Type locality: “the airstrip at La Sirena, Península de Osa, Canton de Osa, Puntarenas Province, Costa Rica (approx. 3 m [asl])”

Conservation Status: IUCN Category: Data Deficient (DD). Almost nothing is known on distribution range, habitat preferences, and possible threats. At the current state of knowledge the EVS calculation would be: 5 (geographic distribution) + 2 (persecution by humans) + 5 (forest formation) = 12.

Diagnosis: Based on the three specimens I examined, I provide the following diagnosis: A small (L: 316–382 mm) and very slender (IA: 80.5–91.0) caecilian, having 220–221 PG and 0–8 SG. Secondaries were found in only one specimen and the first appears at primary 210. Scale inception is at primary 175 in the holotype as seen on x-ray.

Coloration in life: The coloration in life of one specimen of *Caecilia osae* (A) that was not preserved was recorded (both specimens show the same coloration): Head Salmon Color (6), suffused with Grayish Horn Color (91); ventral surface and middle part of body indigo (73), anterior part of venter Rose Pink (108D), posterior part of venter Lavender Blue

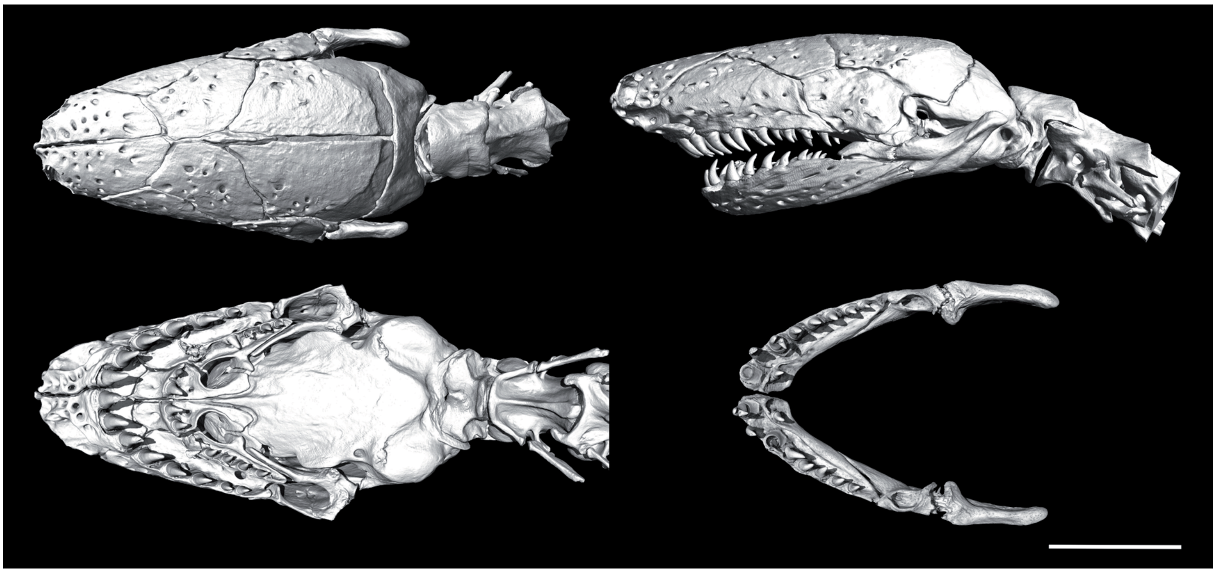

Figure 66: Volume reconstruction of synchrotron-based X-ray micro CT imaging (HRµCT) data showing the skull of the holotype of *Caecilia osae* (LACM 138542). Left side from top to bottom: cranium in dorsal and palatal view. Right side from top to bottom: skull in lateral view, mandible in dorsal view. Scale bar =1mm.

(170D); surroundings of cloaca Salmon Color (6); equally distributed Pratt´s Payne´s Gray 88 punctation over whole body, but more intensive on ventral and lateral surfaces.

Distribution: Endemic to the Golfo Dulce region of Costa Rica. Only known from two sites on the Osa Peninsula and from the surroundings of the La Gamba Research Station that is operated by the University of Vienna, Austria.

Habitat and natural history: Only known from Pacific Tropical Wet Forest. Almost nothing is known about the life history of *Caecilia osae*. Like other members of the genus it is believed to be largely fossorial, but it is occasionally encountered above the surface during heavy rains (Höbel 2008). Huber and Hödel (2010) reported on predation of *Micrurus alleni* on *C. osae*. In June, 2013 I received two living specimens collected by R. Weixler from La Gamba, which I am observing in captivity since then. I keep them in a mixture of *Sphagnum* moss and soil, which I keep very wet. The container is slightly sloped so that water accumulates at the lower end. Usually both individuals are lying on the ground of the container in the wetter corner, with the body completely in the water. In this posture, the head is above the water line or inside an air bubble, but never above the soil. I never observed them completely exposed on the surface of the soil, but cannot say for sure that theydo not get out at night. Although the eyes are covered by skin and bone, they react on exposure to light and are usually lingering on the side of the container away from the light. They readily accepted some types of earthworms (*e.g.*, *Lumbricus*) whereas they refuse others (*e.g.*, *Dendrobaena*, *Eisenia*). Large earthworms that are thicker than the diameter of the caecilian are not attacked.

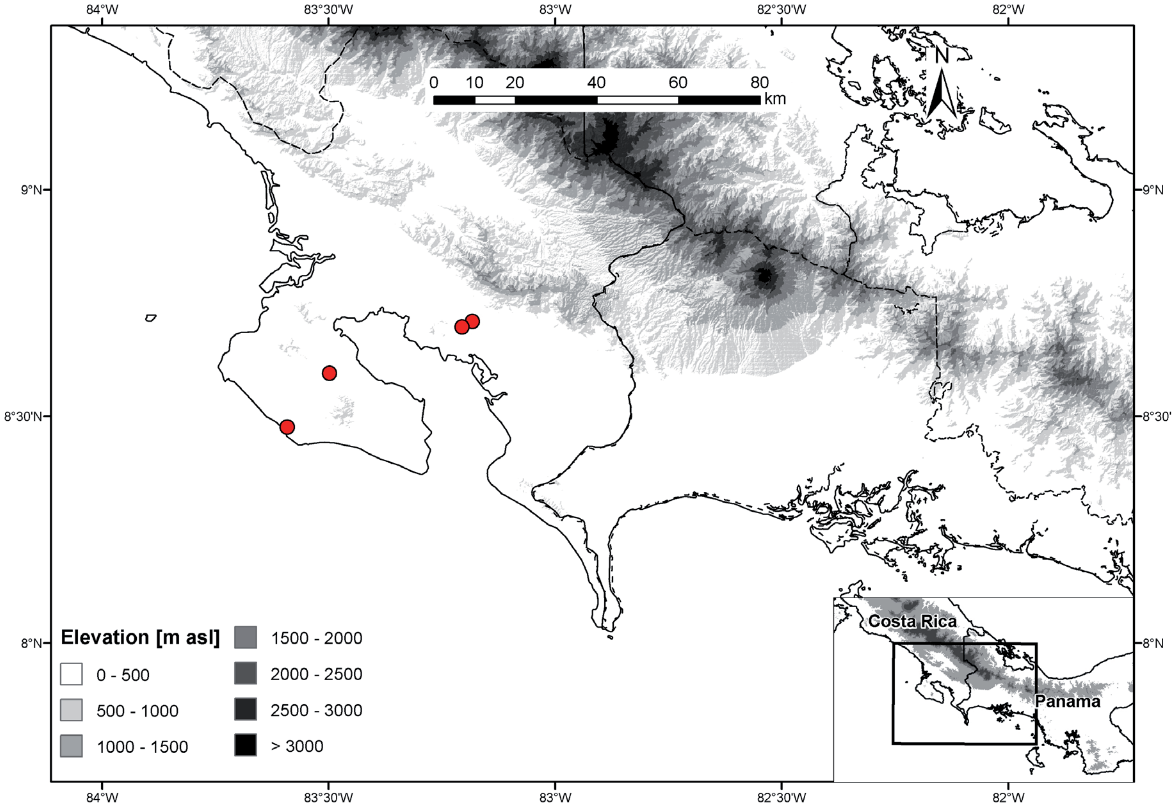

Figure 67: Collection sites of *Oscaecilia osae.*

Joseph Vargas (pers comm. 2013) observed them feeding on small crickets.

Remarks: Many questions regarding the taxonomic status of *Caecilia osae* remain unresolved. For sure it is a valid species, but thequestion wether specimens from the Osa Peninsula are really conspecific with those from La Gamba could not be investigated to date. This is mainly because no specimens exist in collections besides the holotype and the two living specimens I am keeping. There is no information concerning the whereabouts of a dehydrated specimen found dead near Los Patos (Plate 24 in Savage 2002). The specimen from La Gamba mentioned by Huber and Hödel (2010) was not collected (Walter Hödel pers. comm. 2012).

Candidate species

The following candidate species is distinct by some morphological characters and by preliminary DNA barcoding. Anyway, the data are too sparse to qualify for the Confirmed Candidate Species concept of Vieites *et al.* (2009). Nevertheless, I decided to introduce the candidate species here in order to emphasize the differences between *C. osae* and *C.* sp..

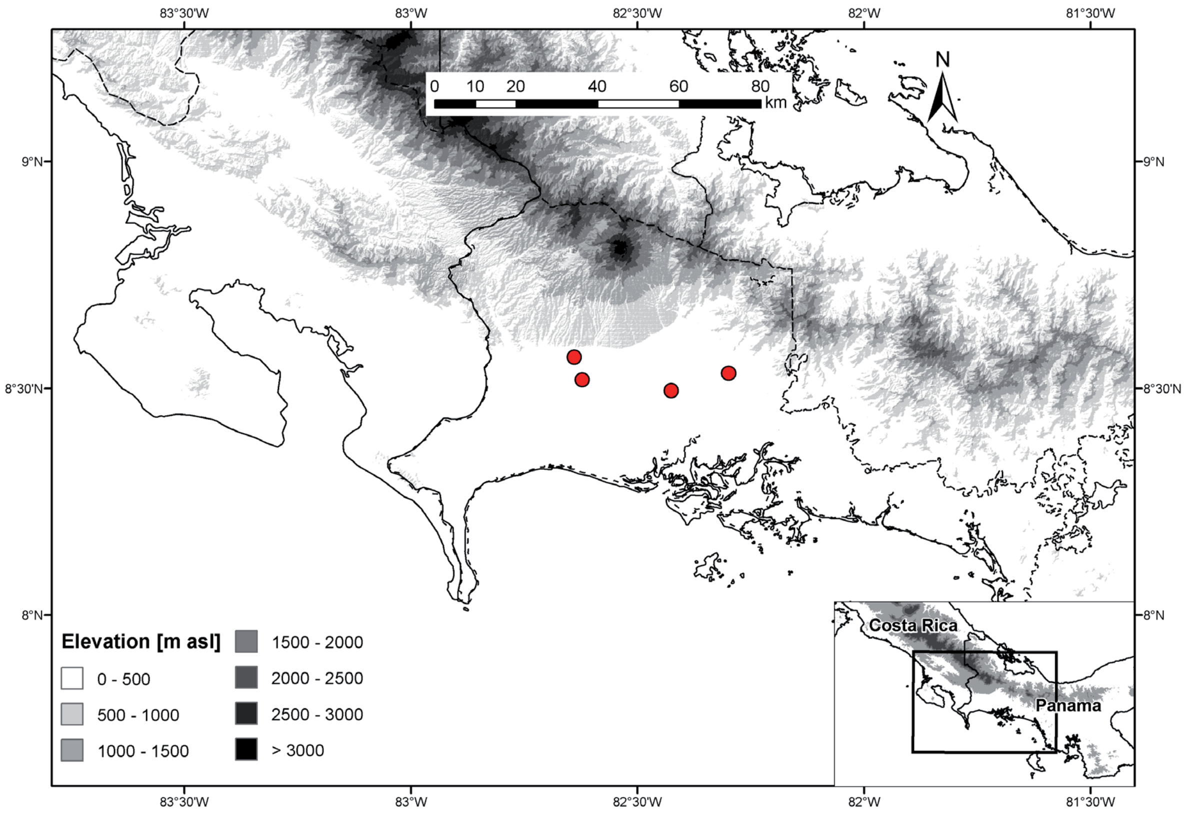

Figure 68: Collection sites of *Caecilia* sp. from western Panama.

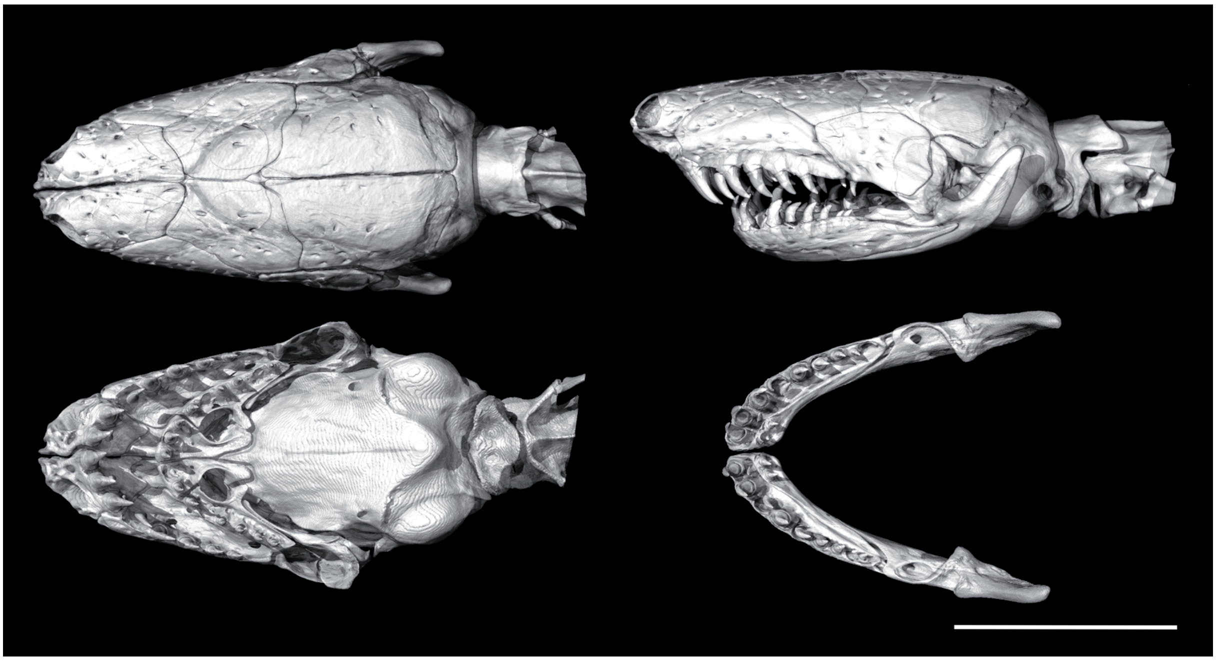

Figure 69: Volume reconstruction of synchrotron-based X-ray micro CT imaging (HRµCT) data showing the skull of *Caecilia* sp. (MHCH 1566). Left side from top to bottom: cranium in dorsal and palatal view. Right side from top to bottom: skull in lateral view, mandible in dorsal view. Scale bar = 1mm.

***Caecilia* sp.**

Conservation Status: IUCN Category: Data Deficient (DD). The species seems to be tolerant to habitat modification. Specimens are occasionally found in suburban situations. At the current state of knowledge the EVS calculation would be: 5 (geographic distribution) + 2 (persecution by humans) + 4 (forest formation) = 11.

Diagnosis: The species can be characterized on the basis of four specimens as follows: A medium sized caecilian (L: 479–564 mm) having a very slender body (IA: 83.9–98.1) and 223–233 PG and no SG. Scales are present in the two x-rayed specimen. Scale inception is at primary 204–205 (Fig. 70).

Coloration in life: AH 297: Color of head Pale Pinkish Buff (121D); dorsal color Blackish Neutral Gray (82), marmorated with Light Neutral Gray (85), particularly on the lateral surfaces; ventral coloration like dorsal coloration, surroundings of cloaca Pearl Gray (81).

Distribution: Apparently endemic to the lowlands and mid-elevations on the Pacific versant in the Province of Chiriquí.

Habitat and natural history: It inhabits the Tropical Moist and Premontane Wet Forest

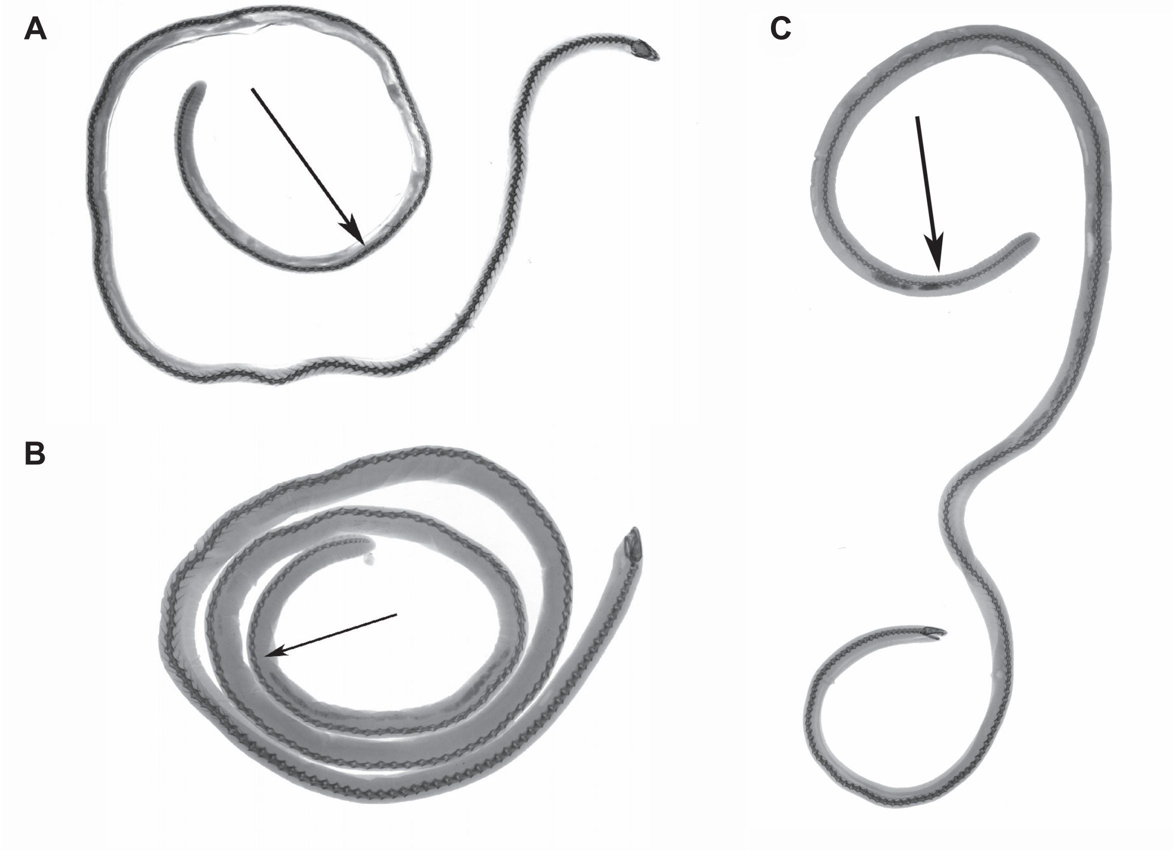

Figure 70: Radiographes of *Caecilia* specimens. Arrows indicate point of scale inception. **A** Holotype of *Caecilia osae* (LACM 138542). **B** *Caecilia* sp. (SMF 89813) from Los Algarrobos, Chiriquí. **C** *Caecilia* sp. (MHCH 1566) from San Vicente, Bugaba, Chiriquí.

domains. The whole known distribution area is agriculturally used land.

Remarks: The specimen AH 297 appears in Köhler (2011; Fig. 28+29) as *Oscaecilia elongata.*

6.4. CONSERVATION

Since the last published amphibian species list (Hertz *et al.* 2012c), the number of amphibian species known to occur in Panama has increased from 206 to 214 species. The additional eight species came from new species descriptions. Five new species (*i.e.*, *Diasporus citrinobaphaeus*, *D. igneus, Bolitoglossa jugivagans*, *Ecnomiohyla bailarina*, and *E. veraguensis*) have been described as part of this project (Hertz *et al.* 2012a; Batista *et al.* 2012; Hertz *et al.* 2013a; Batista *et al.* 2014a). In addition, two endemic Panamanian species of a genus new to Panama *Anomaloglossus astralogaster* and *A. isthminus* have been discovered (Myers *et al.* 2012). Moreover, a toad (*Incilius majordormus*) has been described from museum material (Savage *et al.* 2013). I further included the worm-salamanders *Oedipina savagei* to the list, because it is mentioned by several authors to occur in western Panama (Savage 2002; Frost 2014) and the specimen ANSP 21663 collected in Chiriquí is

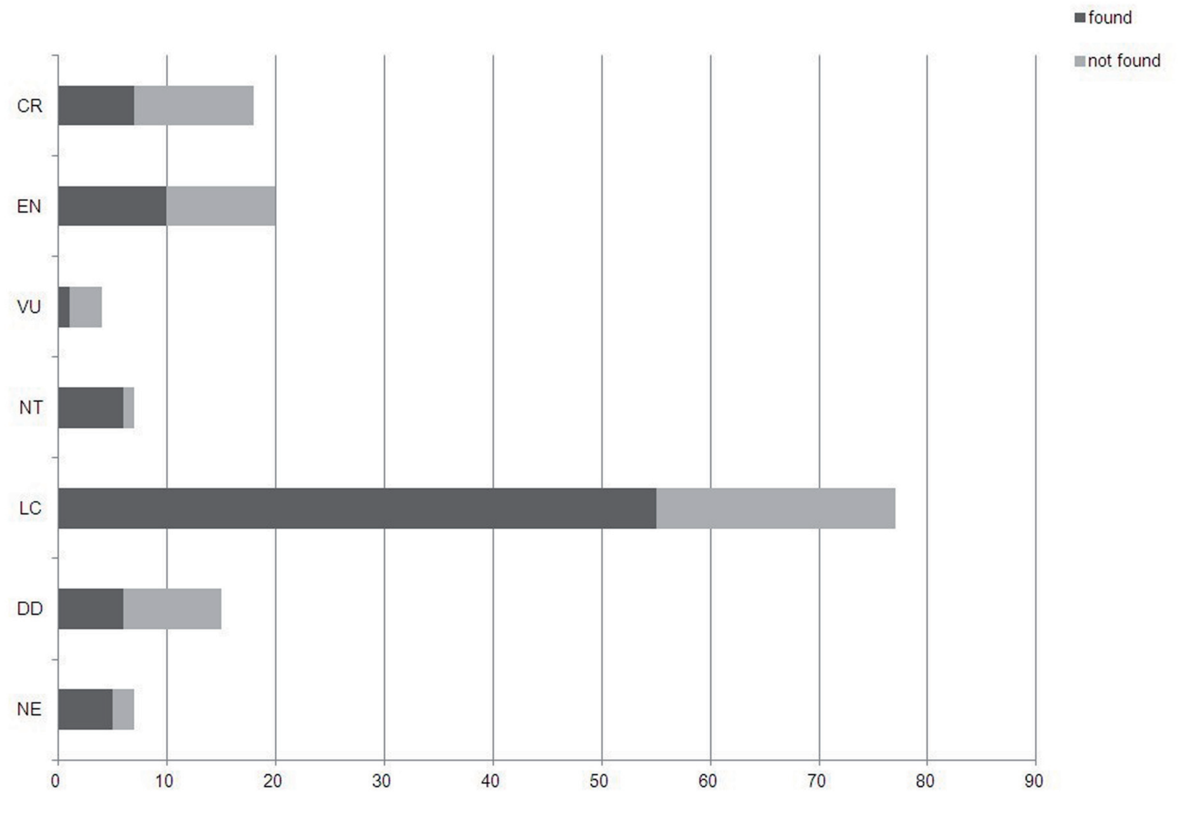

Figure 71: Number of Panamanian amphibian species found during this study in relation to the total number of Panamanian amphibian species in the respective IUCN Red List category .

assigned to this species according to HerpNet2. Species that are suspected to occur in Panama, but whose records have not yet been supported by a publication or a voucher specimen were not included in the list. Further, I follow the implementation of Lavilla *et al.* (2010) in changing the name *Trachycephalus venulosus* to *T. typhonius*. In consequence of my taxonomical results in this work, I synonymized *Bolitoglossa sombra* with *B. nigrescens* (see Chapter 1.2.3.). Additionally, I excluded *Pristimantis educatoris*, a name that is not applicable to a genetic lineage of the *P. caryophyllaceus*-complex at this point and therefore treated as a junior synonym of *P. caryophyllaceus* (Batista *et al.* 2014b). In total, 48 (22.4%) of the 214 species, are endemic to the state of Panama. The amphibian families containing the most endemic species are in descending order (species numbers in parentheses): Craugastoridae (11), Plethodontidae (10), Bufonidae (8), Hylidae (7), Dentrobatidae (6), Anomaloglossinae (2), Caeciliidae (2), Eleutherodactylidae (2). More than half (26; 54.2%) of the endemic Panamanian amphibian species are known to occur in the investigated area. Plotting the sites I visited on current species distribution maps (Köhler 2011; IUCN 2013) I achieve 148 species (69.2% of all Panamanian amphibians) that I potentially could have found at least at

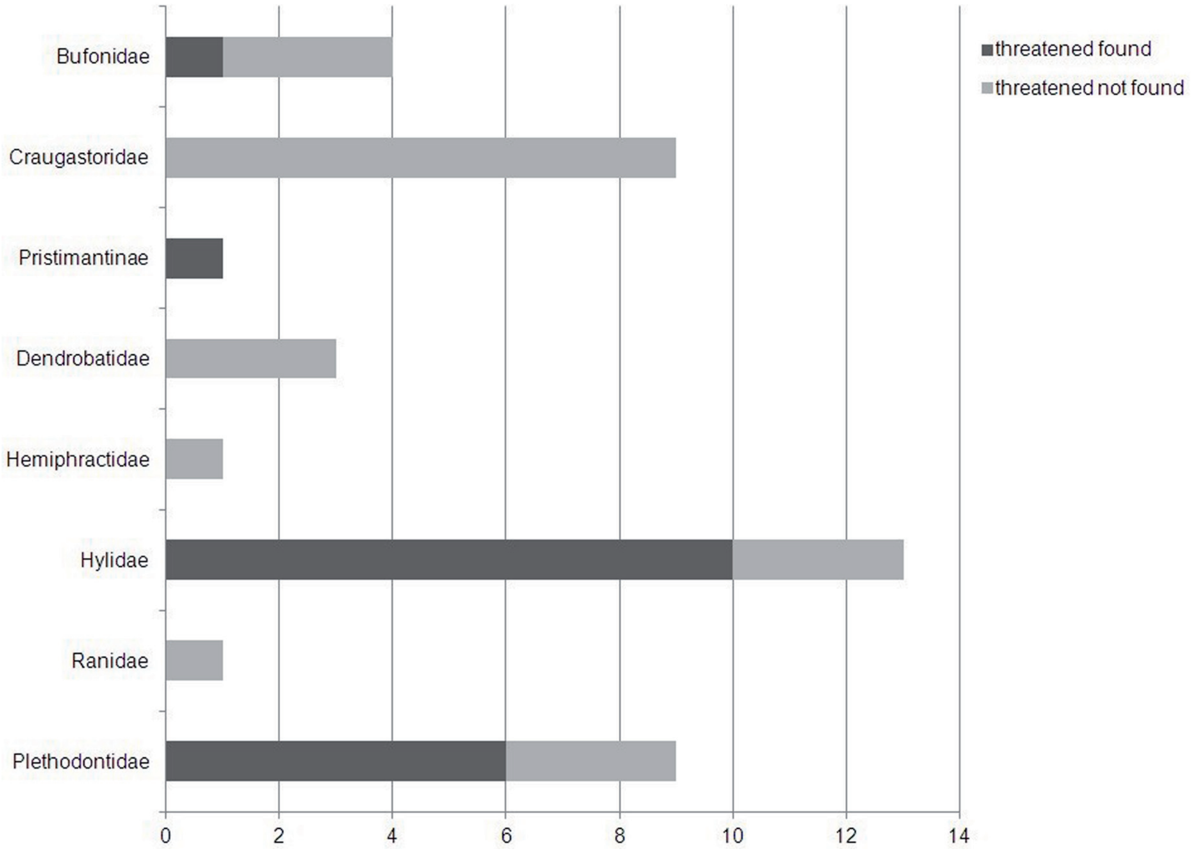

Figure 72: Number of threatened Panamanian amphibian species found during this study in relation to the total number of threatened Panamanian amphibian species arranged in families.

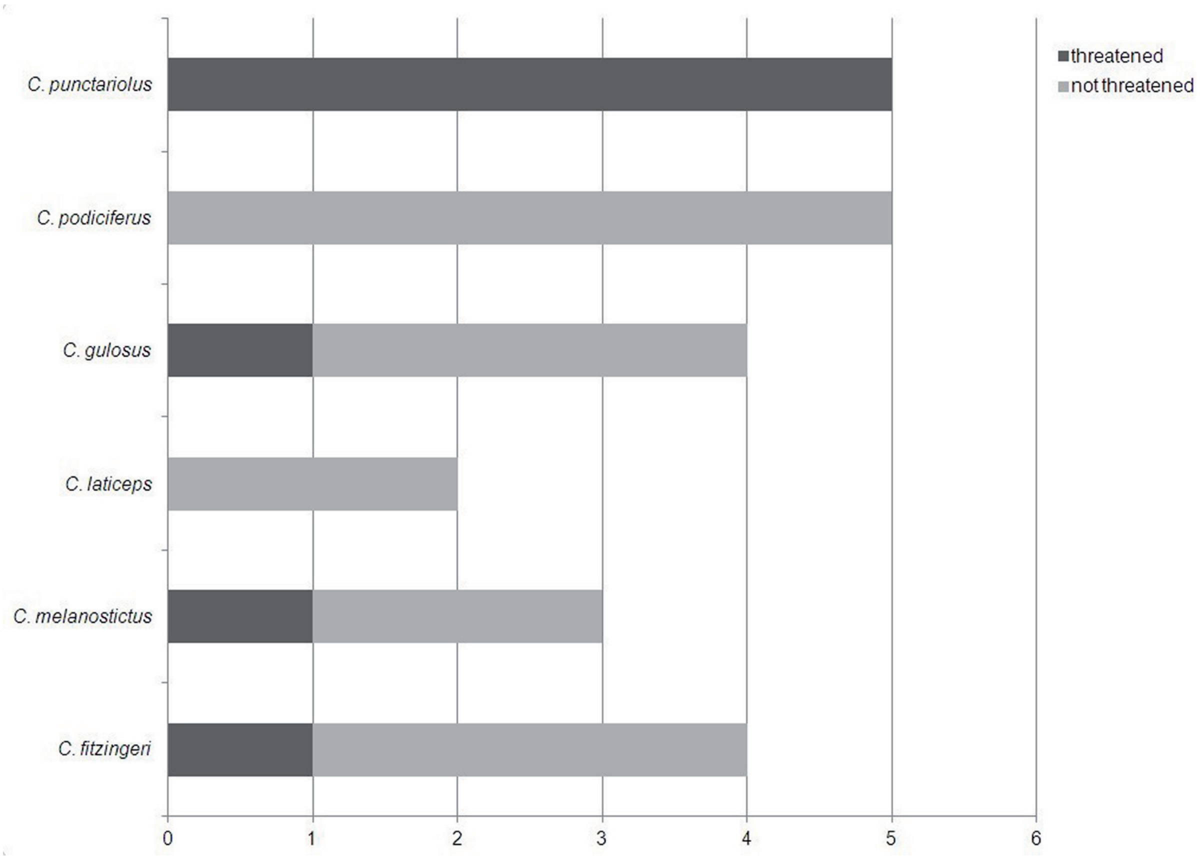

Figure 73: Number of threatened Panamanian *Craugastor* species in the respective Species Series and Species Groups sensu Hedges et al. (2008).

one of the visited sites. All species that I found in the investigated area for the first time and those that I described as new to science have been added to this list. On the contrary, I did not include candidate species that are pending formal species description. Of the resulting 148 species that potentially could have been found, I encountered 90 (60.8%), of which 7 (7.8%) were classified as Critically Endangered (CR) by the IUCN Red List (IUCN 2013), 10 (11.1%) as Endangered (EN), one species (1.1%) as Vulnerable (VU), 6 (6.7%) as Near Threatened (NT), 55 (61.1%) as Least Concern, and 6 (6.7%) as Data Deficient (DD). The remaining 5 (5.6%) were Not Evaluated (NE) yet.

The other 58 species are known from my study area, but were not found in the course of my field work. These include 11 plethodontids (2 EN, 1 VU, 4 LC, 4 DD), 9 craugastorids (4 EN, 5 CR), 8 dentrobatids (2 EN, 1 VU, 4 LC, 1 DD), 8 hylids (2 CR, 1 EN, 1 VU, 4 LC), 6 caeciliids (2 LC, 4 DD), 6 bufonids (3 CR, 2 NE, 1 LC), 4 centrolenids (4 LC), 3 hemiphractids (1 EN, 1 NT, 1 LC), 1 ranid (CR), 1 micohylid (LC), and 1 eleutherodactylid (LC). Figure 72 gives an overview about the ratio of found species in the respective IUCN categories. To get a more precise idea about which species that I have not detected are actually of major conservation concern, I set aside species listed as LC, DD, and NE, which are assumed to be of minor conservation concern or which lack adequate data to be evaluated. The only species on the "early warning stage", Near Threatened (NT) is *Hemiphractus fasciatus* on which I comment in the conservation species accounts below. However, NT is not a threatened category after the definition of the IUCN (2014). By taking a closer look on the remaining 24 species that are listed in the three threatened categories, it is noteworthy that they are not evenly distributed over the taxonomic entities. Figure 71 shows all amphibian species that I found and in relation to the total number of threatened species in the respective category known from the investigated area. Findings are distributed more than less evenly over the IUCN Red List categories. Figure 72 shows all threatened amphibian species in the study area in relation to the number of endangered species I have found. The highest total number of species in the respective threatened category is found in the family Hylidae, but of the 13 threatened species ten were rediscovered by myself in post-decline situations. A similar pattern can be observed in the family Plethodontidae, with six findings out of ten species. The pattern is different, if one looks at the family Bufonidae, with only one finding out of four threatened species, and even more in the family Craugastoridae, in which none of the nine endangered species could be found. Moreover, in the genus *Craugastor* threatened species are not equally distributed over the species series sensu Hedges *et al.* (2008) (Fig. 74). While all species in the *C. punctariolus* species series that occur in the investigated area are threatened, there are no threatened species in the *C. podiciferus* species group and in the *C. laticeps* species series. The other three species groups contain one threatened species each. Furthermore, the only endangered species in the family Ranidae was not detected (Fig. 72). As explained more in detail in the conservation species accounts (Chapter 1.4.2.), the three species of dentrobatids and the one hemiphractid were less likely for me to find, as they are more common at lower elevations or are rare canopy dwellers. Therefore, of major conservation concern and with the highest probability of being extinct in Panama are the species of Bufonidae, Craugastoridae, and the ranid *Lithobates vibicarius* that could not be found. Additionally, the absence of the hylid frog *Isthmohyla calypsa* from historical sites gives reason for concern. Whereas, the other hylids that were not found have in general always been rarely seen or collected. All threatened Plethodontids that could not be found belong to the very secretive, semifossorial genus *Oedipina*.

The percentage of species listed in threatened categories among the species that I have found during my field work plotted on different regions on a map of western Panamas protected areas, shows which parts of western Panama are of increased importance with regard to amphibian conservation (Fig. 74). It is obvious that species that qualify for threatened categories were primarily found in protected areas. The highest percentage of endangered species is found in the Volcán Barú National Park where five (56%) of the nine encountered species are endangered. This is particularly because there is a high diversity of endangered *Bolitoglossa* species, and the rare, listed as Vulnerable, *Ecnomiohyla fimbrimembra* was found. The total number of different species that I found in the International Park La Amistad is 23 species of which 7 (30%) are listed in a threatened category, which makes it the protected area with the highest total number of encountered endangered species. In Palo Seco Protection Forest and the La Fortuna Forest Reserve, *Duellmanohyla uranochroa* is the only Critically Endangered species (after 2013 it was listed as Endangered by NatureServe & IUCN SSC Amphibian Specialist Group 2013). Furthermore, the endangered *Pristimantis museosus* was found in the Palo Seco Protection Forest. With 34 species, the highest number of different species in a single area

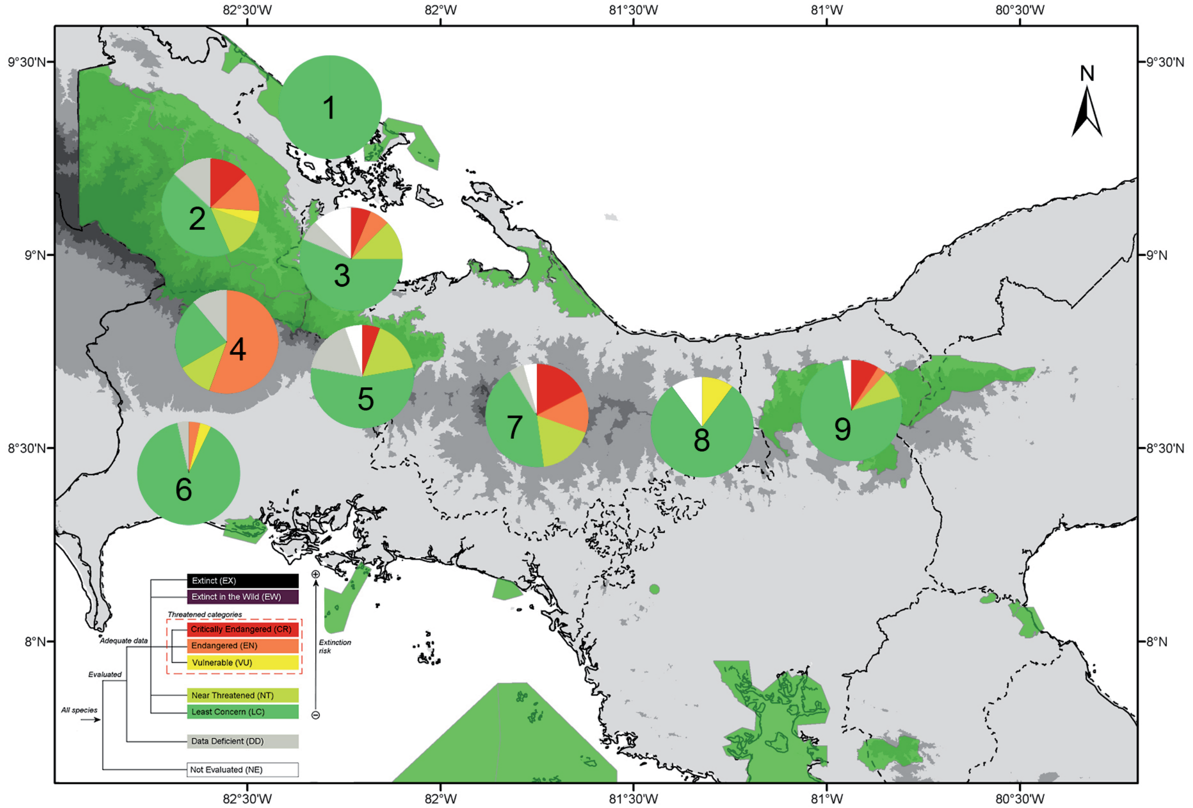

Figure 74: Percentage of amphibian species in the respective IUCN categories among the species found during this study, plotted on geographic regions. Protected areas are shaded in green. 1 Caribbean Lowlands. 2 La Amistad International Park. 3 Palo Seco Protected Forest. 4 Volcán Barú National Park. 5 La Fortuna Forest Reserve. 6 Pacific Lowlands. 7 Cerro Colorado area. 8 Eastern Comarca Ngöbe-Buglé. 9 Santa Fé National Park.

was encountered in the Santa Fé National Park. Three of these species (*Agalychnis lemur*, *Atelopus varius*, and *Hyloscirtus colymba*) are listed as Critically Endangered and one (*Pristimantis museosus*) as endangered. Although this is a relatively high total number of endangered species it represents only 11.8% of the 34 species found, or in other words, the high amphibian diversity in the Santa Fé National Park narrows the share of endangered species. However, this does not degrade the conservation importance of the Santa Fé National Park, as the three Critically Endangered species have been found only there. Generally spoken, unprotected areas have lower shares of endangered species. In the unprotected Chiriquí lowlands and midelevations, the only species listed as Endangered is *Ptychohyla legleri* based on the finding of a single juvenile specimen. The second threatened species is the Vulnerable *Bolitoglossa lignicolor* that I consider to be less threatened than originally anticipated (see individual species account on page 112–113). Although less exhaustively sampled, all eleven species detected in the Caribbean lowlands are considered to be Least Concern, even though this count includes also species collected in the protected area of the San San Pond Sak

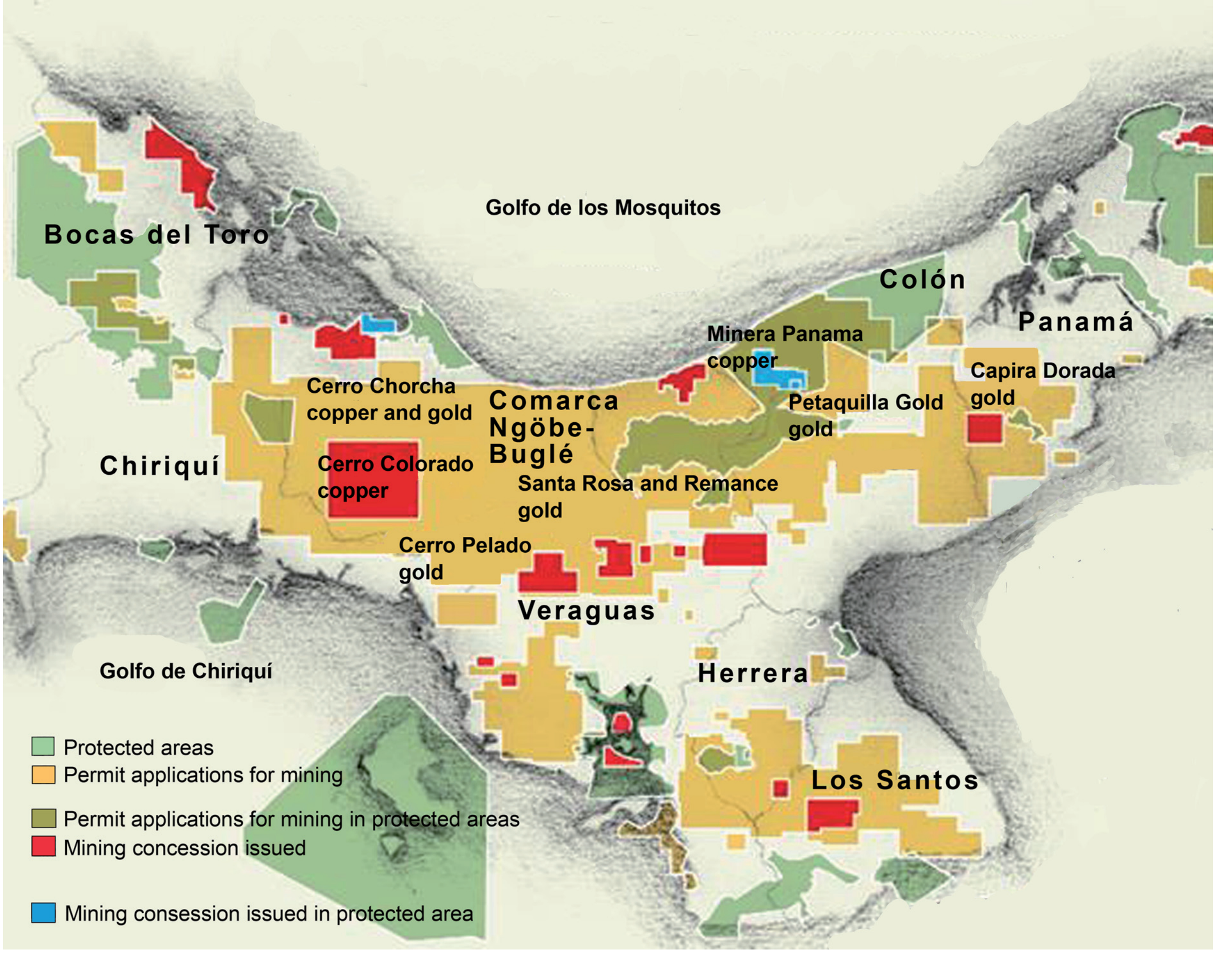

Figure 75: Excisting and planned mining projects in western Panama. Modified after an article in the Panamanian newspaper „La Prensa“ (August 27, 2010).

wetlands. The eastern part of the Comarca Ngöbe Buglé includes only one threatened species (*i.e.*, *Bolitoglossa lignicolor*) out of ten. Furthermore, here is the type locality of the recently described *Diasporus citrinobapheus*, which I however do not consider to be threatened at the moment (see individual species account on page 64). An exceptionally great amount of endangered species was found in the Cerro Colorado region in the central Comarca Ngöbe-Buglé. Here I found four Critically Endangered hylid species (*Isthmolyla debilis*, *I. graceae*, *I.* cf. *rivularis*, and *I.* cf. *tica*), including all three species of *Isthmohyla* that were on the Lost Frogs list of the IUCN SSC Amphibian Specialist Group in 2012 (Hertz *et al.* 2012d). Furthermore, I found three Endangered species here (*i.e.*, *Agalychnis annae*, *Bolitoglossa* cf. *minutula*, and *Pristimantis museosus*). Thus, the number of threatened species in the Cerro Colorado region is with 7 of 23 (30%) the same as in the International Park La Amistad, but without being a protected area. Three of the threatened species at Cerro Colorado (*Agalychnis annae*, *I. debilis*, and *I. graceae*) have been found only here in recent years. The not yet evaluated species *D. igneus* is believed to be endemic to the Cerro Colorado area (Batista *et al.* 2012). I swapped six specimens from the Cerro Colorado area (four *I. graceae*, one *I. picadoi*, and one *Hylanibatrachium talamancae*) for the presence of *Bd*. All were tested negative, except one specimen of *I. graceae* tested with equivocal result (Hertz *et al.* 2012c). As a general conclusion of my work in the Comarca Ngöbe-Buglé, the accumulation of findings of threatened amphibian, particularly in the Cerro Colorado area highlights the importance of that region for amphibian conservation. The mining plans of the Panamanian government, which encompass virtually the whole territory of the Comarca Ngöbe-Buglé and thus the entire Serranía de Tabasará (Fig. 75) are currently the biggest threat for many already endangered amphibian species.

6.4.1. CONSERVATION ACCOUNTS: ADDITIONAL SPECIES RECORDED 2008–2013

Family Bufonidae
LC – Least Concern

Incilius coniferus (Cope, 1862)

I found this species at several sites on the Atlantic slopes and in Santa Clara, Chiriquí on the Pacific slopes of western Panama. All these sites are under agricultural use. While adults are less common, juveniles are frequently seen. On July 29, 2010 between 3 and 4:30 am, I found several adults in puddles near Río Hacha, Comarca Ngöbe-Buglé that were neither calling nor showing any other indication of mating. I consider *Incilius coniferus* to be not threatened at the moment.

Incilius epioticus (Cope, 1876)

There is still very scarce information on the population status and natural history of this species. In 2010, several specimens crossed our camp at Río Changena, Bocas del Toro, 1650 m, between 8 and 10 am. It seems not to be rare in appropriate habitat. There is no evidence for a direct threat like *Bd*, but since this is a species of deep primary forest, it is surely affected by habitat destruction. Oestreicher *et al.* (2009) reports on ongoing deforestation in the La Amistad International Park. I assume this species is more threatened than currently expected.

Rhaebo haematiticus Cope, 1862

I found adult specimens to be relatively rare in western Panama. However, juveniles can be locally abundant. On July 23, 2008, I found several juveniles on the banks of Río Escarrea in the Chiriquí lowlands. Populations at upland sites seem to be declining, while the species persists at lowland sites. Declining has been reported from La Fortuna (Lips 1999), where I did not find a single specimen. All places where I recorded *Rhaebo haematiticus* are between 30 and 700 m asl on both slopes. It is a forest species that is not encountered in open habitats. Especially on the Pacific side its habitat is affected by agriculture. At least in Panama it might be more threatened than previously anticipated.

Rhinella marina (Linnaeus, 1758)

It is very common in western Panama and occurs in all kinds of habitat, from urban areas to pristine forests. I found it on the Pacific as well as on the Caribbean slopes between sea level and 1380 m asl. There is no evidence for a decline so far.

CR – Critically Endangered

Atelopus varius (Lichtenstein & Martens, 1856)

Atelopus varius is regularly found at Cerro Negro, Veraguas, but usually in low numbers

(Hertz *et al.* 2012c). From 2012 to 2013, Perez *et al.* (2014) surveyed 16 suitable habitats in western Panama, of which they found *A. varius* at three sites, including Cerro Negro. Since the taxonomy of this species remains questionable (Richards & Knowles 2007), it is noteworthy that Cerro Negro is near the type locality of *A. varius* (Lötters *et al.* 1998). The number of living individuals at Cerro Negro is unknown and survey work is needed. Although situated in a protected area, the population at Cerro Negro is threatened by illegal logging for cattle farming. The future of the species depends very much on the effective protection of its habitat.

Family Centrolenidae

DD – Data Deficient

Hyalinobatrachium vireovittatum (Starrett & Savage, 1973)

The validity of this species is doubted (Kubicki 2007; own data). There are some specimens from La Fortuna Forest Reserve that show the uniform yellow paravertebral stripes described by Kubicki (2007) as distinguishing character to *H. talamancae*. However, my preliminary barcoding revealed that the 16S genetic p-distance is only 1% to other specimen from Panama identified as *H. talamancae* (Appendix 10.2.1.). However, I did not include specimens of populations from either Costa Rica or Valle de Anton in my studies, which are considered to meet the original species description best (Kubicki 2007). Until the taxonomy is not clarified, an evaluation of its conservation status in Panama is not possible.

LC – Least Concern

Cochranella granulosa (Taylor, 1949)

This species is not uncommon in western Panama, but most calling males sit very high above the streams and are not easy to get. I found it in agricultural and suburban situations only. I recorded it on the Burica Peninsula and heard it calling in Los Algarrobos, Chiriquí near David (Lotzkat & Hertz 2011), where it seems to be abundant. I tested a specimen from Santa Clara at 1200 m asl positive for *Bd* (Hertz *et al.* 2012c), but it showed no obvious symptoms.

Espadarana prosoblepon (Boettger, 1892)

Espadarana prosoblepon is the most abundant glass frog species in mountainous western Panama between 900 and 2050 m asl. It is still found at mountain streams were other frogs have disappeared; it breeds even in modified habitats with polluted rivers. It does not need trees, but at least bushes overhanging the water and therefore it would probably not survive in completely deforested habitats.

Hyalinobatrachium fleischmanni (Boettger, 1893)

This species is relatively abundant at low- and mid-elevations, especially in degraded habitat. Near Río Sereno, Chiriquí, I found it in the extensive coffee growth of Finca Eleta at 1440 m asl, where it occurs sympatrically with *Espadarana prosoblepon*. There, I found both species on the banks of Río Candela that was polluted with different kinds of garbage including rusty batteries. Probably the water is contaminated. At the cattle farm Finca La Providencia (40 m asl) near Ponuga it was the only glass frog species.

Hyalinobatrachium talamancae (Taylor, 1952)

I found this species to be very abundant in streams on both slopes and at every place I visited in the Serranía de Tabasará between La Fortuna and Paredón. Its known distribution area is fragmented and my records from the Comarca Ngöbe-Buglé were new (Hertz *et al.* 2011). Paredón is the lowest location with 786 m asl, while all other places are between 1430 and 1864 m asl. I have never found it west of La Fortuna. The taxonomic status of *H. vireovittatum* in relation to *H. talamancae* is questioned (Kubicki 2007; own data; see also *H. vireovittatum*).

Sachatamia albomaculata (Taylor, 1949)

This species is abundant in appropriate habitat. I found it on the Caribbean side at Willie Mazú, Protected Forest Palo Seco, Comarca Ngöbe-Buglé, and on the Pacific side in the eastern part of my study area near Paredón, Comarca Ngöbe-Buglé, as well as in the Santa Fé National Park. I noticed no declines in areas where *Bd* has possibly become enzootic.

Teratohyla pulverata (Peters, 1873)

In 2010, I found a single specimen of this species at Willie Mazú, Protected Forest Palo Seco, Bocas del Toro at 650 m asl. I would consider it to be rather rare in Panama. This is also evidenced by the number of preserved specimens from Panama in collections. There are no specimens neither in the collection of the MVUP nor in the collection of the Círculo Herpetológico de Panamá. Two specimens are traceable via HerpNet2, both from the Darién (*i.e.*, KU 116493 and USNM 151108). Kubicki (2007) stated that *Teratohyla pulverata* inhabits also the canopy of larger trees and might therefore be found less frequently. *T. pulverata* is certainly affected by deforestation, although not immediately threatened in view of its large distribution area.

Family Craugastoridae
Subfamily Craugastorinae

DD – Data Deficient

Craugastor monnichorum (Dunn, 1940)

Craugastor monnichorum is relatively common around Volcan Barú (Köhler *et al.* 2008; own observations). I collected it at Bajo Mono, Boquete, only about 3 km from Finca Lerida, its type locality. The extent of occurrence is larger than previously thought (Hertz *et al.* 2011), extending far into the Serranía de Tabasará. However, in the 16S barcoding a specimen from Bajo Mono and a specimen from La Fortuna build a well-supported cluster, but there is a p-distance of 3.2% between both specimens (Appendix 10.2.2.). There are no distinct morphological differences traceable. A deeper analysis of specimens referred to as *C. monnichorum* would be needed. Specimens from the Serranía de Tabasará should be included in any future study. I found it in pristine forests only. Probably it is threatened by habitat loss.

LC – Least Concern

Craugastor bransfordii (Cope, 1886)

Craugastor bransfordii is probably a species complex with unresolved taxonomy. Since the type locality of *C. bransfordii* is Nicaragua, populations in Panama represent at least one undescribed species (Crawford & Smith 2005). I could find it at mid-elevations (700–1260 m asl) on the Caribbean slopes of the Cordillera Central. At the La Fortuna Forest Reserve it occurred also on the Pacific drainage, where it lives sympatrically with *C. podiciferus*, albeit *C. bransfordii* is the less frequently found species.

Craugastor crassidigitus (Taylor, 1952)

Craugastor crassidigitus is an abundant species in all habitats, especially between 1000 and 1600 m asl. I found it in closed forest as well as on cow meadows. At many deforested upland sites it is the only abundant frog species. According to my own preliminary barcoding results, genetic p-distances between specimens from western Chiriquí, the Comarca Ngöbe-Buglé, and El Copé are around 3% respectively (Appendix 10.2.2.).

Craugastor fitzingeri (Schmidt, 1857)

Craugastor fitzingeri is a very common species, even in disturbed habitats, but rather in lowland situations below 1000 m asl. It is found in all kind of habitats, including gardens in suburban areas (Lotzkat & Hertz 2011).

Craugastor gollmeri (Peters, 1863)

I found *Craugastor gollmeri* infrequently but regularly on the Atlantic slopes in western Panama and also on the Pacific slopes of the Fortuna depression. All findings have been made in pristine forest.

Craugastor jota (Lynch, 1980)

I found this species only on a field trip to Río Changena on the northern slopes of Cerro Pando between 1700 and 1820 m asl, where it was quite abundant. The distribution area is apparently relatively small and there is ongoing deforestation in the La Amistad International Park. I saw huge clearings used as pasture for cattle, where pristine forest used to be in 1966 (William Duellman pers. comm. 2010). My preliminary barcoding results, revealed a p-distance of more than 3% in the 16S rRNA gene to its nearest congeners within the *C. podiciferus* complex. This confirms the validity of this species (Appendix 10.2.2.).

Craugastor megacephalus (Cope, 1876)

I found *Craugastor megacephalus* from time to time in the Santa Fé National Park, Veraguas between 450 and 1200 m asl, but it is a rather uncommon species there. So far, I found it in relatively undisturbed forests only. It is probably susceptible to habitat destruction. Crawford *et al.* (2010) reported a decline of this species following *Bd* arrival at El Copé.

Craugastor melanostictus (Cope, 1876)

This species is relatively common in well-conserved montane forests. I found no evidence for a *Bd* driven decline in this species. However, the taxonomy is unclear. Probably more than one species are currently combined under this name as discovered by DNA barcoding. In a preliminary NJ tree, specimens morphologically assigned to *C. melanostictus* are paraphyletic (Appendix 10.2.2.). A taxonomic revision is needed and a subsequent evaluation of the conservation status of the different clades.

Craugastor noblei (Barbour & Dunn, 1921)

Within my study area, A. Carrizo and L. Stadler detected *Craugastor noblei* at different sites in Santa Fé National Park, Veraguas (Carrizo 2010; Stadler 2010). It is not a very common species, but can be regularly found. *Craugastor noblei* inhabits relatively undisturbed forest, so I suspect it to be susceptible to habitat loss.

Craugastor stejnegerianus (Cope, 1893)

In Panama, the distribution of *Craugastor stejnegerianus* is restricted to the humid southwestern portion. In the investigated area I found it frequently near Santa Clara, Chiriquí, but not on the Buríca Peninsula where it should also occur. To a certain extent it seems to be adaptable to habitat modification. In Santa Clara it is commonly found, although this is an agricultural area with no forests left. However, the species needs some shadow in the form of plantations to survive. Preliminary genetic barcoding revealed a p-distance of 4.5–4.7% between the Panamanian specimens that I collected and a specimen on GenBank, collected approximately 10 km south of San Isidro de El General, Costa Rica (Appendix 10.2.2.).

Craugastor talamancae (Dunn, 1931)

I detected only two specimens of *Craugastor talamancae*, both found in Atlantic lowland forests at 30–460 m asl, a habitat type in which I spent less sampling time. The overall population status in Panama is estimated to be good (Solís *et al.* 2010b).

NT – Near Threatened

Craugastor podiciferus (Cope, 1876)

My own preliminary genetic barcoding and the analysis of bioacoustic data revealed that up to four Panamanian species may be combined under this name with additional ones in Costa Rica (Appendix 10.2.2.). A revision is needed to allow for a protection of all potential species. However, in intact habitats members of the *C. podiciferus* complex are usually very common inhabitants of the leaf litter. An exception is the putative candidate species from Cerro Pando, which is only occasionally found. Its uncorrected p-distance in the 16S rRNA gene is not less than 7.8% to its closest relatives.

EN – Endangered

Craugastor azueroensis (Savage, 1975)

This is the only species included here that does occur outside of my study area. Although I have no personal experience with this species in the field, I participated in an evaluation of its conservation status (Köhler *et al.* 2012). Köhler *et al.* (2012) found 12 individuals on a 100 m transect at Cerro Hoya, thus it seems to be still common. *Bd* has not been detected in seven specimens that were tested. The isolated mountains on the western Azuero Peninsula might serve as a refuge from *Bd* (Köhler *et al.* 2012). However, the populations of *C. azueroensis* should be closely monitored and *ex-situ* populations should be established.

Subfamily Pristimantinae

LC – Least Concern

Pristimantis cerasinus (Cope, 1876)

Pristimantis cerasinus is common on the humid Atlantic versant below 1320 m asl in western Panama. I also found it on the Pacific slopes in Veraguas where the effect of the Cordillera as a climatic barrier is minor. I only found it in closed forest. In Veraguas, where deforested areas are larger, I found it also in riparian forests, but never in open habitats. Preliminary barcoding revealed that specimens currently operated under the name *P. cerasinus* form a monophyletic clade (Appendix 10.2.3.). However, large genetic p-distances within this clade point out the need for a taxonomic reevaluation.

Pristimantis moro (Savage, 1965)

There is very little information on the species population size, taxonomy, and possible threats. I saw *Pristimantis moro* only once at Alto de Piedra, Veraguas, where an amplectant pair was found by L. Stadler (Hertz *et al.* 2011). I think the information on this species is too scarce to list it as Least Concern.

Pristimantis cruentus (Peters, 1873)

Pristimantis cruentus is regularly found, but not really common. I found specimens only in forests. On the Pacific slopes of western Panama that are more effected by agriculture, I found it also in riparian forest patches, but never in open habitats. I conducted a preliminary taxonomic approach on all specimens I collected under the name *P. cruentus*. Specimens from the Pacific and the Caribbean slopes do not vary much in morphology and have a within group p-distance in the 16S rRNA gene of 0.9%. These specimens match best the original description of *P. cruentus*. Whereas specimens from the higher elevations of the Serranía de Tabasará differ in morphology, male advertisement call, and 8.4% 16S mtDNA p-distance from *P. cruentus*. Thus, they represent an undescribed species. Photos of specimens of this undescribed species are shown in Hamad (2009) under the name *P. cruentus* and *P. cerasinus* (Figs. 35–37). After taxonomy has been resolved a revision of the conservation status needs to be done.

NT – Near Threatened

Pristimantis caryophyllaceus (Barbour, 1928)

Pristimantis caryophyllaceus can be abundant locally, while it is infrequently found at other places. I found it at elevations between 700 and 1970 m asl, mostly on the Atlantic slopes of the Cordillera Central, between the northern slope of Cerro Pando and PN Santa Fé, Veraguas. In the La Fortuna Forest Reserve, Chiriquí and Alto de Piedra, Veraguas, I could also find it on the Pacific versant. Based on a new molecular approach that resulted from this study, the assumed sister species *P. educatoris* is not valid (Batista *et al.* 2014b) and is therefore treated under the name *P. caryphyllaceus* here.

Pristimantis pardalis (Barbour, 1928)

Pristimantis pardalis can be a common species in some places. I found it at elevations between 600 and 1670 m asl. Males call from very dense vegetation. It is at least locally affected by habitat loss.

Pristimantis ridens (Cope, 1866)

I found *Pristimantis ridens* more frequently in disturbed than in pristine habitats. However, it needs at least small trees, bushes, or hedges then it lives also in the yard of houses in suburban situations. I never found it on the ground in open habitats like pastures. Apart from that it seems to be quite adaptable. A taxonomic revision should be subject of further studies, since preliminary barcoding revealed distinct genetic lineages among populations of *P. ridens* (Appendix 10.2.3.).

Pristimantis taeniatus (Boulenger, 1912)

Pristimantis taeniatus is not uncommon but not always easy to encounter. The taxonomy is not clear yet. Within my study area, I found *P. taeniatus* only at Santa Clara in extreme western Panama, a locality not yet reported for this species. Preliminary examination of different Panamanian populations showed differences in the 16S mtDNA sequences (Appendix 10.2.3.) and bioacoustics of the Santa Clara population to populations further east. Since the type locality of *P. taeniatus* is in Colombia, the Santa Clara population likely represents an undescribed species. In Santa Clara it occurred syntopic with *P. ridens* that is superficially similar looking. A taxonomic revision is needed, as some lineages might be more threatened than others.

EN – Endangered

Pristimantis museosus (Ibáñez, Jaramillo, & Arosemena, 1994)

Pristimantis museosus is not common, but regularly found throughout its distribution area (Hertz *et al.* 2012c). I found it also on the northern slope of Cerro Pando near the border to Costa Rica, so it might even be found in Costa Rica in the future. The Cerro Pando specimen, however, shows a remarkable p-distance in the 16S rRNA gene of approximately 4% to specimens collected further east (Appendix 10.2.3.). Thus, a taxonomic reevaluation would be appropriate. It inhabits pristine forests with little disturbance and seems to be susceptible to forest degradation. However, it is found in several protected areas in western Panama. I assume *P. museosus* to be less threatened than previously thought.

Family Dentrobatidae

LC – Least Concern

Dendrobates auratus (Girard, 1855)

The number of individuals of *Dendrobates auratus* to find is usually small, but the species is widespread. I found it regularly in lowland situations on both, the Caribbean and Pacific sides. At Santa Fe it was encountered up to 1010 m asl on the slopes of Cerro Mariposa (Stadler 2010). It can survive in degraded habitat if forest patches and/or riverine forests persist and arboreous corridors make these forest patches accessible. It is found in Los Algarrobos (Lotzkat & Hertz 2011) where the Pacific blue and black color morph is common, whereas it is not reported from the botanical garden of the UNACHI in David (Batista & Ponce 2011).

Oophaga pumilio (Schmidt, 1857)

I found *Oophaga pumilio* to be very abundant on Bastimentos Island, even in the town of Bastimentos. It is generally common on the islands of the Bocas del Toro Archipelago, where several color morphs exist (Batista & Köhler 2008). Moreover, Carrizo (2010) reports it from Cerro Narices, Veraguas were it was less abundant. Some island populations might be threatened by over-collection for the pet trade and by habitat destruction for tourism (Solís *et al.* 2010d).

NT – Near Threatened

Silverstoneia nubicola (Dunn, 1924)

I could find this species only on the banks of Río La Gloria on the Caribbean coast between Miramar and Las Cañas at 30 m asl were it was abundant. According to literature it inhabits elevations between 200 and 1600 m (Köhler 2011), where I could not find any similar specimens. However, it is considered a species complex composed of several undescribed species (Bolaños *et al.* 2004a).

Family Hylidae

DD – Data Deficient

Isthmohyla infucata (Duellman, 2001)

I found only a single adult specimen at Río Changena on the northern slope of Cerro Pando, but tadpoles where relatively abundant. Although a morphological tadpole description is still lacking, I could identify the tadpoles (AH 558) by DNA barcoding (Appendix 10.2.4.). There is at least a reproductive population that seems to persist on the Atlantic slopes of extreme western Panama. There are no *Bd* related declines reported. However, its extent of occurrence is very small and there is ongoing deforestation in the International Park La Amistad and the Palo Seco Protected Forest, so it is likely that it qualifies for a threatened category.

LC – Least Concern

Agalychnis callidryas (Cope, 1862)

In western Panama, I found *Agalychnis callidryas* to be relatively common on the Burica Peninsula and Alto de Piedra in Veraguas on the Pacific side. On the Atlantic side, I found it in the San San Pond Sak wetlands, Bocas del Toro, and between Chiriquí Grande and Almirante. Some populations might be more threatened than others.

Dendropsophus ebraccatus (Cope, 1874)

I found *Dendropsophus ebraccatus* to be relatively common on the Burica Peninsula, where I found breeding congregations on flooded cattle meadows. It seems to be adaptable to habitat modification, as there is almost no natural habitat left on the Buríca Pensinsula.

Dendropsophus microcephalus (Cope, 1886)

This is a very common species in the lowlands of western Panama. I saw large breeding aggregations whenever a cow meadow was flooded.

Dendropsophus phlebodes (Stejneger, 1906)

I found *Dendropsophus phlebodes* only once in the San San Pond Sak wetlands, where I consider it to be rather uncommon. This might be also due to relatively high salinity in these coastal swamps. Generally, it is considered to have stable populations within its large distribution area (Solís *et al.* 2010e).

Hyloscirtus palmeri (Boulenger, 1908)

Carrizo (2010) collected one male and one female at Rio Mulaba, Santa Fe National Park. This is the westernmost population known in Panama. Carrizo (2010) did not give an estimation of the population size at this site, but throughout its distribution area it is not immediately threatened (Bolívar *et al.* 2008).

Hypsiboas pugnax (Schmidt, 1857)

I found *Hypsiboas pugnax* near Ponuga, Veraguas, on the farm La Providencia owned by the family Carrizo-Diaz, where it was relatively numerous around breeding ponds. In Panama its distribution is only poorly known. There, it seems to be less common than the at first glance very similar *H. crepitans*. However, the analysis of call recordings I made of the collected specimen and its morphology identify the population at Ponuga as *H. pugnax*.

Hypsiboas rosenbergi (Boulenger, 1898)

Hypsiboas rosenbergi is common in southwestern Panama. I found *H. rosenbergi* in pastureland on the Buríca Peninsula, La Concepción, and Los Algarrobos, all these sites are in the province of Chiriquí. This species was abundant at all these sites and I would say it is a typical species of extensively used agricultural land. I consider it not to be threatened right now.

Hypsiboas rufitelus (Fouquette, 1961)

Hypsiboas rufitelus seems to be abundant in the Carribbean lowlands in appropriate habitat. I found many individuals in the San San Pond Sak wetlands, where they gathered around breeding waters in swamp forest. The breeding sites were about 3 km upstream from the mouth of Río San San, while I found single individuals only 100 m from shoreline. I found *H. rufitelus* in the well-preserved San San Pond Sak wetlands only and suspect it to be susceptible to habitat destruction. In view of its large distribution area, I consider it not to be threatened at the moment.

Isthmohyla lancasteri (Barbour, 1928)

I found an apparently strong population of this species at Rio Clarito, Atlantic slopes of western Panama, between 1300 and 1600 m asl. *Isthmohyla lancasteri* is obviously not endangered so far. However, I could not reconfirm findings of this species in 2006 (SMF 85380–81) from the continental divide in the Fortuna depression. It might have been locally declined.

Scinax altae (Dunn, 1933)

Scinax altae is very common at Los Algarrobos near David (Lotzkat & Hertz 2011). It is the only amphibian species that I frequently found inside houses. It seems to be very adaptable to habitat modifications. The surroundings of Los Algarrobos are characterized by extensive cattle farming.

Scinax boulengeri (Cope, 1887)

This species was relatively abundant on a flooded cow meadow on the Burica Peninsula. I did not find it on the Caribbean side. *Scinax boulengeri* is wide-ranging and seems to be adaptable to habitat modifications. Thus, it is not facing serious threats at the moment.

Scinax elaeochrous (Cope, 1876)

Scinax elaeochrous is fairly common around David. I found it on cow meadows and other extensively used agricultural sites, where males were calling from solitary trees and fence posts.

Smilisca phaeota (Cope, 1862)

Smilisca phaeota is a very common species, probably one of the most common frog species in western Panama. I found it in almost all habitats from forest to cow pasture between 90 and 1600 m asl. It is obviously neither affected by habitat modifications nor *Bd*.

Smilisca sila Duellman & Trueb, 1966

Smilisca sila is a relatively common species. I found it on the Pacific slopes at all visited sites between Santa Clara, in the province of Chiriquí, and Santa Fe National Park, in the province of Veraguas between 150 and 1630 m asl. At La Fortuna Forest Reserve it is one of the few surviving stream breeders following chytridiomycosis outbreak. Dam projects may be a threat as I only found it along unspoiled rivers so far. Anyway, it seems not to be affected by *Bd*. In my preliminary barcoding analysis, distinct differences between a 16S mtDNA sequence of my specimen (AH 435) and comparative sequence of a specimen from Costa Rica on GenBank, assigned to the same species, become apparent (Appendix 10.2.4.).

Smilisca sordida (Peters, 1863)

I found only two specimens that I would assign to *Smilisca sordida* on the basis of morphological characters. One specimen was collected in Santa Clara and the other in the La Fortuna Forest Reserve, both in the province of Chiriquí. The latter site would be outside of the documented range for this species (Köhler 2011). I did not collect tissue samples of these two specimens nor did I obtain call recordings that could prove or disprove the morphological designation.

Trachycephalus typhonius (Linnaeus, 1758)

Trachycephalus typhonius is common throughout the Pacific lowlands of western Panama. I found it at two sites that are not plotted in the current IUCN map. These are Los Algarrobos, Chiriquí (Lotzkat & Hertz 2011), and near Ponuga, Veraguas. Both are extensively used agricultural areas. It seems to be very adaptable.

NT – Near Threatened

Isthmohyla picadoi (Dunn, 1937)

This species is not really rare, but in most cases difficult to reach due to its canopy dwelling life style. Trees inhabited by *Isthmohyla picadoi* can be located by following the male advertisement call. Tadpoles are frequently found in larger bromeliads. In Jurutungo, *I. picadoi* tolerates habitat modifications and is found on pasture land provided that some large trees with bromeliads are present. It is more widely distributed than previously thought. Its distribution in Panama is not only restricted to extreme western Panama, but also reaches far into the Serranía de Tabasará (Hertz & Lotzkat 2012). I found it only above 2000 m asl. Thus, east of the Fortuna depression where peaks of 2000 m elevation are insular, single populations might be endangered by habitat destruction. A taxonomic comparison is needed to evaluate whether the disjunct populations on the various insular high peaks in its distribution area belong all to the same species. At least the two specimens that I barcoded (*i.e.*, AH 484 and HAU 030) show large p-distances between each other (Appendix 10.2.4.).

Isthmohyla zeteki (Gaige, 1929)

Due to the canopy dwelling habits, *Isthmohyla zeteki* is not easy to survey. At one site it is found in terrestrial bromeliads (Köhler *et al.* 2008; Hertz *et al.* 2012a) where it is easier to observe. At this site, Hertz *et al.* (2012a) recorded and subsequently described the advertisement call what now allows for audio transect surveys (Lips *et al.* 2001) at other sites. Since the species is more widely distributed than previously known and breeds also in large terrestrial bromeliads in open habitat, it is probably not so much susceptible to habitat loss. However, to which threats *I. zeteki* is finally exposed needs to be evaluated. Additionally, my own preliminary barcoding results suggest that specimens from the Serranía de Tabasará may represent a distinct species (Appendix 10.2.4.). Unfortunately, the site at La Nevera where the species lived in terrestrial bromeliads has recently been destroyed by road construction (own observation 2013).

EN – Endangered

Agalychnis annae Duellman, 1963

A single female specimen of *Agalychnis annae* was found in 2008 in the Cerro Colorado region, Comarca Ngöbe-Buglé (Hertz *et al.* 2011; 2012c). Since then no additional specimens have been found. Thus, there is no information on a population in Panama. However, the whole Cerro Colorado area has no protection status and there is an ongoing pressure from mining and road construction. In July 2013, an expedition to Cerro Colorado yielded no additional specimens. I suspect *A. annae* to breed in potholes in the gravel road, but a great part of the road from Hato Chamí to Hacha has been paved in 2012 (own observation 2013).

Duellmanohyla uranochroa (Cope, 1876)

Since I found the first tadpoles in 2008 the number of specimens in the La Fortuna Forest Reserve seems to have increased. Between 2008 and 2010 tadpoles were found frequently, but despite intensive search efforts only two adults were found (Hertz *et al.* 2012c). In the night of July 27, 2013 my team and I found between 3–5 calling males in each of three surveyed creeks in the La Fortuna Forest Reserve. Tadpoles were abundant and easy to find, but all of them lacked keratinized mouthparts. We would need to know if the population at

La Fortuna Forest Reserve and maybe others are really increasing and why. However, the number of individuals is still low and I would not say the species is out of danger. Although the prediction for the survival of the species seems to be good, I think it was a premature conclusion that *D. uranochroa* has been recently downgraded from CR to EN in the IUCN Red List .

Ecnomiohyla fimbrimembra (Taylor, 1948)

There is no data on the population status of *Ecnomiohyla fimbrimembra*, but its canopy habitat is not easy to survey. By chance I found a single male specimen in 2009 on the ground (Hertz *et al.* 2012c; Batista *et al.* 2014a). It would be good to have audio samples of its advertisement call so that one could run audio transects. That would help to identify trees with breeding holes, but until now the call is unknown. In consideration of its canopy life style it should be susceptible to habitat loss.

Ptychohyla legleri (Taylor, 1958)

I found a single froglet at Santa Clara, Chiriquí, the first and so far only locality in Panama where *Ptychohyla legleri* has been reported to occur (Duellman 2001). Following expeditions to this place revealed no additional specimens (Hertz *et al.* 2012c). Its extent of occurrence is small and populations are fragmented. It is said to be more common at other sites in Costa Rica (Santos-Barrera *et al.* 2008).

CR – Critically Endangered

Agalychnis lemur (Boulenger, 1882)

A single specimen has been found in 2008 in Santa Fé National Park (Carrizo 2010; Hertz *et al.* 2012c). Woodhams *et al.* (2006) found that the antimicrobial skin peptides of *Agalychnis lemur* are very effective against *Bd* zoospores. Maybe this is why the species persists in low numbers at a few sites. However, all Panamanian populations are weakened and ongoing deforestation might lead to the extinction of this species.

Hyloscirtus colymba (Dunn, 1931)

On repeated field trips to Alto de Piedra I noticed an increasing abundance of specimens. In 2008, student collaborators and I found one adult and some tadpoles in a 36 days field work period (Stadler 2010), in 2009, one adult was found in one week, and 2010 I found four adults and several tadpoles in two days (Hertz *et al.* 2012c). However, I think the risk of extinction is still high since all population suffered from severe declines and it is only little known about its population dynamics. *Bd* has been found to be enzootic within the population (Hertz *et al.* 2012c). There is a notable p-distance of about 2% between 16S mtDNA sequences of one specimen from my own collection and two specimens on GenBank (Appendix 10.2.4.). This needs to be further adressed in upcoming studies.

Isthmohyla debilis (Taylor, 1952)

I found *Isthmohyla debilis* regularly around Cerro Santiago, Comarca Ngöbe-Buglé. Males can easily be located by following their cricket-like calling. I saw no amplectant pairs, nor females, or tadpoles. However, searching more focussed for tadpoles of this particular species could be successful. At present, Cerro Santiago is the only place where specimens have been recorded in the last five years (Hertz *et al.* 2012c). There are no protected areas in the region, there is ongoing deforestation in the whole area, new streets have been built, and there are mining plans. All this threatens the so far last known population of *I. debilis*.

Isthmohyla graceae (Myers & Duellman, 1982)

I found the first adult specimens and tadpoles of *Isthmohyla graceae* in 2010 near the type locality at Cerro Colorado, Comarca Ngöbe-Buglé (Hertz *et al.* 2012c). I visited another nearby location in 2013 where the species was moderately abundant too, with several calling males. Just as at the other site I could not find females, but tadpoles were present. Additionally, I found an egg clutch attached to an aquatic plant in a puddle, presumably belonging to this species. At present, there are no indications for a decline of the population at Cerro Colorado. However, it is the only known population of *I. graceae* that persists after severe *Bd* related declines throughout its range, the area is not protected, and there are plans for open pit mining in the area (Fig. 74). If the mining plans are implemented it is likely that this population goes extinct.

Isthmohyla rivularis (Taylor, 1952)

At Cerro Pando, Chiriquí, I regularly found calling males of *Isthmohyla rivularis* in 2008 and 2009, a single female could also be found at this site (Hertz *et al.* 2012c). In 2013, I found one calling male at Mount Totumas Forest Reserve, but did not visit the sites at Cerro

Pando again. In 2008, I collected a specimen at Cerro Colorado (AH 055) that I compared to another specimen from that locality (AMNH 107965; Myers & Duellman 1982). I consider both to be conspecific, but doubts remain if these are conspecific with *I. rivularis* from further west. My preliminary DNA barcoding approach revealed a paraphyly in the two specimens assigned to *I. rivularis* (Appendix 10.2.4.). The specimen from GenBank (*i.e.*, MVZ 149750) was collected at Volcán Barva in the Cordillera Central of Costa Rica, approximately 20 km from the type locality at Volcán Poás. Thus, it is likely that the Panamanian Cerro Colorado population represents an undiscribed species. Unfortunety, I did not collect tissue samples of the Cerro Pando population for comparison. There are no other records from Panama, but at least the population at Cerro Pando seem to increase. However, the likelihood of this species going extinct depends on how well the sites where the species revovers will be protected from habitat destruction in the future. Monitoring work is also needed to assess the population status at several sites.

Isthmohyla tica (Starrett, 1966)

I found a calling male of *Isthmohyla tica* (AH 496) and heard a second one while collecting at Río Changena for one night. I guess searching the river thoroughly could result in the discovery of additional specimens. My call recordings of the Río Changena specimen matches the published call description of a specimen from Tapantí, Costa Rica (Duellman 2001) in most measured values. Additionally, I collected a female specimen (AH 529) at Río Hacha, Comarca Ngöbe-Buglé (Hertz *et al.* 2012c). My preliminary DNA barcoding analysis proofed the close relationship of both specimens, but with a noticeable p-distance of 1.9% between them (Appendix 10.2.4.). Further, *I. tica* is paraphyletic in my analysis with respect to a specimen on GenBank (*i.e.*, MVZ 207211). This specimen, however, was collected at Volcán Cacao in the very far northwest of Costa Rica. This site is outside the known distribution range of *I. tica*. I examined photographs of MVZ 207211, showing that the snout is clearly protruding, a character that is typical for *I. rivularis* and *I. debilis*, but not for *I. tica*. The type locality of *I. tica* is Volcán Turrialba in Costa Rica, about 160 km northwest of Río Changena and about 270 km northwest of Río Hacha. Volcán Cacao lies more than 200 km northwest of Volcán Turrialba. A taxonomic revision would be needed to evaluate how many species are currently united under this name and how their conservation statuses are.

Family Leiuperidae

LC – Least Concern

Engystomops pustulosus (Cope, 1864)

Engystomops pustulosus is the only amphibian species I know, that breeds in the inner city of David in roadside ditches and the canalization, where the water is usually heavily polluted with oil, petrol, and chemicals. It is abundant in all kinds of open habitat. For now I would consider it to be the least endangered amphibian species in Panama.

Family Leptodactylidae

Leptodactylus fragilis (Brocchi, 1877)

This species is very common at many Pacific lowland sites. It is adaptable to open habitats and often encountered on pasture land.

Leptodactylus insularum Boulenger, 1898

Leptodactylus insularum is common at rural sites in and around David (Batista & Ponce 2011; Lotzkat & Hertz 2011, as *L. bolivianus*). I also found it to be common at Finca La Providencia near Ponuga, Veraguas. Between these two sites, there is a large gap in the known distribution of this species (Heyer & Heyer 2013). It is frequently found in open pasture land and seems not to be endangered at this point. As *L. bolivianus*, it has been evaluated as Least Concern in the IUCN Red List of Threatened Species Version 2013.2 (Solís *et al.* 2008b). However, Heyer and de Sá (2011) found the Panamanian populations to belong to a separated species. Until the next evaluation, Heyer and Heyer (2013) recommend to list *L. insularum* as Least Concern.

Leptodactylus melanonotus (Hallowell, 1861)

I found *Leptodactylus melanonotus* in Los Algarrobos, Chiriquí (Lotzkat & Hertz 2011) on the Pacific side, where it inhabits open, extensively used grassland and in the San San Pond Sak wetlands on the Atlantic side, where it lives in pristine swamp forests. It is very abundant at both sites. This demonstrates that it is adaptable to a wide variety of different habitats throughout its large distribution area. Thus, it is unlikely to qualify for a threatened category in the near future.

Leptodactylus savagei Heyer, 2005

Leptodactylus savagei is a very common species. It is especially abundant in agricultural areas, but I also found it in primary forests. Its adaptability to a great variety of habitats makes it unlikely to decline in the near future.

Family Microhylidae

LC – Least Concern

Elachistocleis pearsei (Ruthven, 1914)

In June 2010, directly after the first heavy rains in the year I found *Elachistocleis pearsei* to be locally abundant on flooded pasture land around Los Algarrobos, but breeding was only observed over a period of one week, and then all specimens disappeared again. The flooded meadow contained also ant nests in high quantities, with which it might be ecologically linked (Solé *et al.* 2002; Mebs *et al.* 2010). The species identity is not fully clarified, as also *E. ovalis* appears on most lists of the amphibians of Panama. However, Caramaschi (2010) considered *E. ovalis* a *nomen dubium*. Which names apply to Panamanian species is unclear. A taxonomic revision of the genus in Panama is needed. Preliminary genetic barcoding of the specimen I collected in Panama, and sequences on GenBank of specimens from Colombia, where the type locality of *E. pearsei* is located, as well as from central Panama built a cluster (Appendix 10.2.5.). The within-group p-distance of this lineage is 0.3%. Although little is known about the species habitat preferences it survives at least in extensive agricultural land and there is no evidence of *Bd*-driven declines.

Family Ranidae

LC – Least Concern

Lithobates taylori (Smith, 1959)

Usually a breeding pond is occupied by many individuals, but not every adequate pond contains this species. The *Lithobates pipiens* complex species in western Panama needs to be revised to clarify its taxonomic status (Savage 2002). I never found it in the Serranía de Talamanca, but only in the Serranía de Tabasará. In 2009, I found several dead and dying, recently metamorphosed frogs of that species in Llano Tugrí, Comarca Ngöbe-Buglé, at 1300 m asl. The symptoms suggest an outbreak of chytridiomycosis, but microscopical examination of skin scrapings provided no positive results. In Alto de Piedra, Veraguas, at 870 m asl all examined individuals seemed to be healthy.

Lithobates vaillanti (Brocchi, 1877)

I did not visit many sites within the species distribution area, but in the San San Pond Sak wetlands it was abundant in the mangroves.

Lithobates warszewitschii (Schmidt, 1857)

Lithobates warszewitschii is common on the Atlantic versant, especially at lower elevations. In 2013, I saw *L. warszewitschii* in the La Fortuna Forest Reserve on the banks of Río Hornito at 1300 m asl, after not having seen a single specimen there between 2008 and 2010. Upland populations might have been declined due to chytridiomycosis. I never found *L. warszewitschii* in open habitats. Supposedly, it needs at least forest patches to survive.

Order Caudata

Family Plethodontidae

LC – Least Concern

Oedipina complex (Dunn, 1924)

I found only two specimens of *Oedipina complex* near Santa Fé, Verguas. A preliminary barcoding approach revealed It might be more common further east. It has a relatively large extent of occurrence and different genetic lineages might represent different species (Appendix 10.2.6.). I found it in pristine and secondary forests. It presumably does not survive in completely deforested areas.

NE – Not Evaluated

Oedipina fortunensis Köhler, Ponce, & Batista, 2007

Oedipina fortunensis has been rarely encountered to date. It has not been assessed by the IUCN Red List yet. The species is only known from three individuals, collected at the type locality and two nearby sites in the La Fortuna Forest Reserve (Köhler *et al.* 2007; Hertz *et al.* 2011; Himes & Enge 2013). In contrast to the statement in Himes and Enge (2013) “first report from the Pacific side of the Continental Divide” all three localities are on the Pacific side. According to my preliminary barcoding results, it is well-distinct in the 16S mtDNA sequence from *O. complex* and *O. savagei*, which share some morphological characteristics with *O. fortunensis* (Hertz *et al.* 2011).

6.4.2. CONSERVATION ACCOUNTS: SPECIES NOT RECORDED 2008–2013

Order Anura
Family Bufonidae

CR – Critically Endangered

Atelopus chiriqiensis Shreve, 1936

I surveyed most of the places in western Panama where *Atelopus chiriquiensis* has been known to occur, plus several sites that meet the species habitat requirements, but I could not find a single specimen. Lips *et al.* (2010) considered it to be extinct in Costa Rica and noted that it has probably declined in Panama, too. After my own endeavours to relocate surviving populations were without success I fear that the species might have become extinct.

Incilius fastidiosus (Cope, 1876)

In his field notes, William Duellman (pers. comm. 2010) mentions that he collected several *Incilius fastidiosus* on the Atlantic slope of Cerro Pando on May 9, 1966. Although I followed the route of Duellman´s expedition, I could not find a single specimen, neither adult nor larva. However, I stayed at the indicated elevation only one night on Jule 13, 2010. In view of the species hidden, presumably fossorial lifestyle and the fact that it is seen mostly in the reproductive season between February and March (Lips & Krempels 1995). Thus, I would not conclude that it means the species has disappeared just because I did not find it. Further survey work would be needed.

Incilius peripatetes (Savage, 1972)

This species is only known from the holotype (UMMZ 58430) collected on the trail from

Boquete to Almirante at 1500 m elevation. Additional specimens from Cerro Bollo formerly referred to as *Incilius peripatetes* have been recently described as a different species, *I. majordomus* (Savage *et al.* 2013). I visited the slope on the opposite side of the Almirante trail, an area in Boquete called Bajo Mono, approximately 4.5 km from the type locality and could not find any toad species there. However, it cannot be finally said if *I. peripatetes* has disappeared, but since many highland toads have declined considerably this is possibly also true for *I. peripatetes*.

Family Craugastoridae

EN – Endangered

Craugastor gulosus (Cope, 1875)

I visited some of the historical collection sites of this species like Río Claro and several sites in the La Fortuna Forest Reserve. I did not find any species of this group there. However, it is considered to be a very rare species (Solís *et al.* 2010c). Thus, I do not draw any conclusions from the fact that I did not find it.

Craugastor obesus (Barbour, 1928)

Craugastor obesus is a *Craugastor* species with extensive toe webbing like other species in the *C. punctariolus* Species Series. It used to be common in the La Fortuna Forest Reserve. However, I did not find any specimen that is similar to the species descriptions in the literature.

Craugastor punctariolus (Peters, 1863)

Within the investigated area, *Craugastor punctariolus* might be found in the Serranía de Tabasará. I examined two specimens from Fortuna that are in the collection of the Universidad de Panamá (MVUP 504, 515) for comparison. However, among the specimens I found are no species of *Craugastor* having such extensive foot webbings.

Craugastor rhyacobatrachus (Campbell & Savage, 2000)

Although, I visited several sites in the Panamanian distribution area on the southern slopes of the Serranía de Talamanca between Cerro Pando and Volcán Barú, I did not find a single

Craugastor with extensive toe webbings. Thus, I can with reasonable certainty exclude that the species is among the specimens I found.

CR – Critically Endangered

Craugastor catalinae (Campbell & Savage, 2000)

I visited some of the sites mentioned by Campbell and Savage (2000), but could not find any species of *Craugastor* that would match the species description.

Craugastor emcelae (Lynch, 1985)

Craugastor emcelae is morphologically very close to *Craugastor monnichorum* (see account on *C. monnichorum*). Although I visited Río Claro, the type locality of *C. emcelae*, I found no specimen that resembled the species descriptions of neither *C. emcelae* nor *C. monnichorum*.

Craugastor ranoides (Cope, 1886)

I did not find any *Craugastor* specimen that met the description of *C. ranoides*, although I visited several sites where the species has been collected historically.

Craugastor tabasarae (Savage, Hollingsworth, Lips, & Jaslow, 2004)

The eastern end of my study area is at the western margin of the known distribution area of *Craugastor tabasarae*. There is a record from the Santa Fé district in Veraguas that was made in 2008 (Medina *et al.* 2010). There I could not find any specimen that matched the original species description. The record from La Nevera, Comarca Ngöbe-Buglé, (Köhler *et al.* 2008) is based on a misidentified *C. melanostictus*, to be recognize by the well-visible large heel tubercle that is lacking in *C. tabasarae* according to the original description. However, two specimens without heel tubercle that I collected in 2013 at La Nevera (*i.e.*, AH 569 and AH 574) are placed close to *C. tabasarae* in the preliminary barcoding approach. These two specimens share also some morphological characters with *C. tabasarae*, but likely represent an undiscribed species.

Craugastor taurus (Taylor, 1958)

In Panama this species has been collected at Puerto Armuelles on the base of the Buríca

Peninsula (Campbell & Savage 2000). Solís *et al.* (2008a) reported on the finding of a single specimen. M. Ponce photographed *C. taurus* at Las Mellizas on the Buríca Peninsula (Köhler 2011, Fig. 430). Not far from there, I collected for one day (31.05.2010) in the surroundings of the village Limones and found no specimen that met the characteristics of *C. taurus*. However, the Buríca Peninsula is heavily deforested and there might be a better chance to relocate *C. taurus* in the poorly studied Chorogo region that contains some of the last remnant of Tropical Pacific Wet Forest in Panama (Arauz 1999).

Family Dentrobatidae

VU – Vulnerable

Oophaga granulifera (Taylor, 1958)

Oophaga granulifera is known from a single specimen collected at Chorogo at the base of the Burica Peninsula (Ibáñez *et al.* 2007). Thus, there was a theoretical chance for me to find it. However, the vegetation at the collection site is almost natural while the Burica Peninsula itself, where I collected, is mostly deforested. Habitat loss is considered to be a major threat for *O. granulifera* in Panama.

EN – Endangered

Oophaga arborea (Myers, Daly, & Martínez, 1984)

The type locality of *Oophaga arborea* is in the vicinity of a site on the continental divide that I repeatedly visited for collecting, the high valley of Río Chiriquí Malí (Lotzkat *et al.* 2012; Hertz *et al.* 2013a). However, I found no specimens of *O. arborea* neither did I notice any *Oophaga* calls. Since it occurs also at lower elevations on the Atlantic slope (Myers *et al.* 1984) I could have found it also at Willie Mazú, but there I also did not find any evidence for this species. *Oophaga arborea* is a high canopy species and therefore not easy to survey.

Oophaga speciosa (Schmidt, 1857)

The known distribution of the Panamanian endemic, *Oophaga speciosa* is almost identical with that of *O. arborea*. Thus, as stated in the account of the latter species, there would have been a chance for me to find it either at the headwaters of Río Chiriquí Malí or at Willie Mazú.

Nevertheless, I did not find it at any of these sites albeit this is a terrestrial species, in contrast to *O. arborea*. According to Solís *et al.* (2010a) habitat loss and collecting for the pet trade are major threats. However, the sites that I visited in the species´ distribution area are covered with forest and are less frequented by humans. Further research is needed to evaluate the population status of *O. speciosa* and to identify actual threats.

Family Hemiphractidae

NT – Near Threatened

Hemiphractus fasciatus Peters, 1862

Among the species inhabiting my study area, *Hemiphractus fasciatus* is the only species listed as Near Threatened that I could not find. Anyway, although this is not a threatened category, I decided to discuss this species here. *Hemiphractus fasciatus* is widely distributed in Colombia and Ecuador, while its distribution in Panama is poorly known. It has been collected at several sites that I visited intensively including Cerro Pando, the La Fortuna Forest Reserve and the Cerro Colorado area (Duellman 2001). However, I did not find it at none of these sites. Crawford *et al.* (2010) reported on a 100% decline in relative abundance of this species at El Copé, Coclé, after the arrival of *Bd*. I suggest it to be much more threatened in western Panama than in other parts of its distribution area. Since other frogs are a main prey item of *H. fasciatus* (Coloma *et al.* 2004a) it might also be indirectly affected by the loss of amphibian diversity in its habitat.

EN – Endangered

Gastrotheca cornuta (Boulenger, 1898)

This species inhabits lowland and premontane elevations on the Caribbean slopes of my study area, below 1000 m asl (Köhler 2011). I could not find *Gastrotheca cornuta*, but I visited relatively few spots with suitable habitat in the investigated area. I would have expected it to occur at Willie Mazú, where I searched only four nights. Since *G. cornuta* is a canopy species, there was only little chance to find it. However, I also could not hear any vocalizations of this species. Lips *et al.* (2006) reported on a dead individual at El Copé, Coclé, that was tested positive for *Bd*. Habitat loss is also considered a major threat (Coloma *et al.* 2004b).

Family Hylidae

VU – Vulnerable

Ecnomiohyla miliaria (Cope, 1886)

Like other species in the genus, *Ecnomiohyla miliaria* is not easy to survey as it is a high canopy species that is rarely found on the ground. A specimen which I found in the Santa Fé National Park, Veraguas and which I first identified as *E. miliaria* turned out to be an undescribed species, now described as *E. veraguensis* (Batista *et al.* 2014a). This specimen, the holotype of *E. veraguensis*, appears as *E. rabborum* in Köhler (2011; Fig. 537). Since the taxonomy of *E. miliaria* is complicated (Savage & Kubicki 2010), a revision of the specimens currently referred to as *E. miliaria* is needed before its conservation status can be assessed. However, so far the lack of material hinders deeper taxonomical anlayses.

EN – Endangered

Duellmanohyla lythrodes (Savage, 1968)

I included this species in the list, because there was a small opportunity that I might find it at one of the visited places in the Caribbean lowlands. However, *D. lythrodes* is generally a rare species and I did not visit Quebrada El Guabo above Río Changuinola, the only place where this species has been previously collected in Panama (Duellman 2001).

CR – Critically Endangered

Isthmohyla angustilineata (Taylor, 1952)

In 2006, a single adult specimen of *Isthmohyla angustilineata* has been encountered at Cerro Horqueta, in the La Amistad International Park (Hertz *et al.* 2012c). There have been no following expeditions to this site to evaluate its population status. It is generally rare and might be threatened by various factors, including *Bd*.

Isthmohyla calypsa (Lips, 1996)

In several subsequent years I visited most of the Panamanian sites, where *Isthmohyla calypsa* was known to occur, but never found a single specimen. *Isthmohyla lancasteri* occurred at lower elevations, but these specimens had no long spiny projections as described for *I. calypsa* (Trueb 1968; Lips 1996). I assume that *I. calypsa* has disappeared from the historical Panamanian sites. However, the *I. lancasteri* group needs a taxonomic revision to evaluate the conservation status of the particular species.

Family Ranidae

CR – Critically Endangered

Lithobates vibicarius (Cope, 1894)

I visited several historical collection sites within this species Panamanian distribution range. However, I did not find any specimen of *Lithobates vibicarius* and not even another species of *Lithobates* in the Serranía de Talamanca.

Order Caudata
Family Plethodontidae

VU – Vulnerable

Oedipina alfaroi Dunn, 1921

According to the distribution map of *Oedipina alfaroi* by Solís *et al.*, (2010f) there would have been a small opportunity for me to find it in lowland Bocas del Toro. However, *O. alfaroi* is an uncommon species and I have spent little time around Almirante and in addition, search was restricted to mostly degraded habitats. In the San San Pond Sak wetlands I stayed close to the shore line. Despite of well-preserved forests, the relatively high salinity of the water is probably responsible for the reduced amphibian diversity.

EN – Endangered

Oedipina gracilis Taylor, 1952

Most authors give the humid Atlantic lowlands of Costa Rica and adjacent northwestern Panama as distribution of *Oedipina gracilis* (Savage 2002; Bolaños *et al.* 2004b; Köhler 2011; Frost 2014). I did not find a specimen in a collection or in a publication that shows the species is present in Panama. According to the IUCN distribution map and elevation data (Bolaños *et al.* 2004b) I would have been able to find it at Willie Mazú or in the Caribbean lowlands.

Oedipina grandis Brame & Duellman, 1970

The extent of occurrence of *Oedipina grandis* is small. It has been collected at two sites only, the surroundings of Las Tablas, Costa Rica, and the slopes of nearby Cerro Pando in Panama. It was frequently found at Las Tablas in the early 1990s (Savage 2002), but then declined in abundance for unknown reasons (Lips 1998). The last record from Panama was made in 2006 (Hertz *et al.* 2012c).

7. DISCUSSION

7.1. CRYPTIC AMPHIBIAN DIVERSITY AND INTEGRATIVE TAXONOMY

7.1.1. *DIASPORUS*

It is astonishing that there has been so little taxonomic work conducted on the genus *Diasporus*. The members of this genus are abundant throughout their range and relatively easy to collect, if not necessarily always easy to locate. Further, most species and populations are not immediately threatened so that taking a few specimens per locality does not affect the integrity of the population. Moreover, calls are very easy to record, as typical causes that would normally interfere anuran vocalization, like careless approaching or torch light, do not stop *Diasporus* males from calling. It is even possible to collect a noncalling male that will later call from the collection bag once it is brought back to the camp. As an additional advantage, *Diasporus* calls are very simple in their structure and thus very easy to analyze. Despite all these advantages, only nine species had been described previous to this study. Now, the total number of species has increased to eleven. It is certainly a problem that *Diasporu*s species are morphologically very similar to each other and that the actual species diversity cannot be revealed by morphology alone, but only by integrative approaches. However, Hertz *et al.* (2012b) were the first to include a molecular phylogeny in a *Diasporus* species description, while most other *Diasporus* species were described by morphological traits only. Only, Chaves *et al.* (2009) presented the results of a BLAST search conducted with a short fragment of the nuclear Rag 1 gene. An evident problem in my 16S mtDNA phylogeny presented in Chapter 1.1 is that *D. hylaeformis*-like specimens are grouped together with *D. vocator*-like specimens although both are well-distinguishable by both morphology and male advertisement call. There is only one specimen of *D*. aff. *vocator* 'Caribbean', represented by a sequence that has about 16% missing data compared to the rest of the alignment. In most cases, including sequences with missing data increases the phylogenetic accuracy of the entire tree (Wiens 1998), but a lack of data is, at the same time, a lack of differences and thus, relationships of more closely related taxa cannot be resolved. Another major limitation on the usage of a single mitochondrial marker for phylogenetic tree inference is that past or present interspecific hybridization among closely related taxa and mitochondrial introgression may result in the lumping of different species in a single clade (Funk & Omland 2003). To test if this explains the lumping of *D. vocator* and *D. hylaeformis* specimens in my 16S mtDNA phylogeny, a nuclear gene tree is necessary. However, this approach was not further pursued here. Furthermore, incomplete taxon sampling may have a great impact on the phylogeny, as various studies have shown how increased taxon sampling largely improves phylogenetic inference (*e.g.*, Hillis 1996; Graybeal 1998; Poe & Swofford 1999; Pollock *et al.* 2002; Zwickl & Hillis 2002; Heath *et al.* 2008). While the taxon sampling presented here is by far the densest compiled so far for the genus *Diasporus* and there are still more lineages revealed than nominal taxa are available, one could assume that, in turn, the actual species diversity within the genus is vastly underestimated. Thus, the inclusion of more taxa or genetic lineages to resolve a more accurate phylogeny would require *Diasporus* spp. to be more exhaustively collected across political borders. The underestimation of species in the genus *Diasporus* is further corroborated by the accumulation of type localities in Costa Rica, Panama and Colombia, what rather reflects localities where most taxonomists concerned with this genus have worked (Fig 14) than diversity pattern. Moreover, I found up to three syntopic species in certain areas (*e.g.*, Cerro Colorado). Considering that my studies covered only a small part of the total area inhabited by members of the genus, the eleven described species likely represent only the tip of the iceberg. The area I studied covers approximately 28 000 km2 were I count at least eight different species, including some undescribed ones. The area occupied by the genus covers roughly 800 000 km2, so it is around 28 times larger than my study area, suggesting that a lot of additional species in the genus remain to be described. However, at the current state of knowledge the center of speciation is most probably the central mountain range of Lower Central America. Hedges *et al.* (2008) assumed that allopatric speciation in isolation, like on islands and upland areas, is probably the main driver of speciation in Terrarana. It is still very likely that more species will be discovered in other parts of the destribution area of the genus *Diasporus*. Anyway, describing the eleven species of *Diasporus* known to date took 139 years and it does not look as if we can significantly increase this rate in view of further reduction of jobs in taxonomy.

The obviously best way to distinguish *Diasporus* species, especially if they occur in syntopy, is the male advertisement call. The acoustic niche hypothesis is a widely excepted model in the bioacoustics theoretical framework (Farina 2014). Syntopic species that could be distinguished in the field by their vocalizations have invariably turned out to form divergent genetic lineages in the subsequent DNA barcoding and are usually also distinguishable by morphological traits. Accordingly, *Diasporus* males that live in sympatry have their own acoustic niches to prevent mismating under competition. Since the advertisement calls of *Diasporus* species are not very complex in structure, consisting of a single note with no pulses, there are basically three possibilities for acoustic niche partitioning that would require only few evolutionary steps:

1. Temporal partitioning, *i.e.*, different species in sympatry call at different times of the day or the year. This is obviously not the case, as all *Diasporus* species call throughout the year between dusk and dawn, with a slightly reduced activity during the dry season in all observed species. Most recordings have been made between 20:00 h and 24:00 h, and usually different species have been heardat the same time.
2. Frequency partitioning, *i.e.*, different species in sympatry call at different dominant frequencies. This is observable in all *Diasporus* species found in sympatry (Fig. 18).
3. Call length partioning, *i.e.*, the length of the call varies between species in sympatry. Along with frequency partitioning this is evident in syntopic *Diasporus* species (Fig. 18).

There is also evidence for character displacement in the vocalizations of *Diasporus* species in syntopy, a phenomenon that has been described in several other anurans (*e.g.*, Blair 1974; Loftus-Hills & Littlejohn 1992; Gerhardt & Huber 2002; Höbel & Gerhardt 2003; Lemmon 2009). This is suggested by the comparison of *D.* cf. *hylaeformis* with *D.* sp. ‚Colorado‘ (see chapter 1.1.2). If I compare *D.* sp. ‚Colorado‘ with all specimens of *D.* cf. *hylaeformis* collected at several sites throughout the main ridge of the Cordillera Central there is no significant difference in neither call length nor dominant frequency observable (Tab. 4). However, if I compare *D.* sp. ‚Colorado' only with a syntopic specimen of *D.* cf. *hylaeformis* both parameters are significantly different (Fig. 18). This effect in *Diasporus* species needs to be further studied, since I can only compare two individuals so far. However, character displacement in anuran vocalizations is an experimentally well-investigated hypothesis and my results give evidence to assume character displacement in advertisement calls of syntopic *Diasporus* species. Further studies of vocalizations of *Diasporus* species are surely a worthwhile subject for future research.

In contrast, morphological characters seem to be conserved within and among anuran species in general (*e.g.*, Vences *et al.* 2005a; Lougheed *et al.* 2006) and, as expected, this seems to be also the case in *Diasporus*. For that reason, it is difficult to distinguish species by morphology alone, since most morphometric characters are overlapping between species (Tab. 5). Form and extent of digital disk covers and pads have been used as the main characters to distinguish among *Diasporus* species for a long time (Savage 1997; Lynch 2001; Savage 2002). However, since the toe pad morphology is recognized to be an adaptation to a particular lifestyle of a frog (Emerson & Diehl 1980), this character is likely to be a homoplasy. Accordingly, *Diasporus* species that share particular digital disk features are not necessarily close relatives, thus this character is less suitable to define monophyletic groups. The most recent taxonomical papers on *Diasporus* described finger and toe disks as distinguishing feature, but this can only be used in combination with other characters to distinguish between species. On the other hand, dorsal and ventral coloration became more important and the three more recently described species even carry coloration features in the specific epithet (Chaves *et al.* 2009; Batista *et al.* 2012; Hertz *et al.* 2012b). In turn, coloration in amphibians has often evolved to be either cryptic or aposematic (Duellman & Trueb 1986) what makes it also susceptible to convergence. Usually, a combination of morphological characters can still be used to distinguish *Diasporus* species if the sample size of specimens examined is large enough. In a recent paper, Padial *et al.* (2014) questioned the validity of *D. citrinobapheus* with respect to *D. tigrillo* on the grounds that Hertz *et al.* (2012b) gave allegedly no morphological characters to distinguish between both species and included no DNA sequence data of *D. tigrillo*. This, however, is not correct since Hertz *et al.* (2012b) provided several diagnostic morphological characters that allow to differentiate between *D. citrinobapheus* and *D. tigrillo* on the basis of the material available at that time. The most distinctive differences between the two species are skin texture and relative shank length.

In the present study, I demonstrated that the specimens assigned to *D. tigrillo* on the basis of the morphological characters described by Hertz *et al.* (2012b) form a well-supported clade in the 16S mtDNA phylogeny. Although, *D.* aff. *tigrillo* appears as a sister taxon to *D. citrinobapheus*, both are separated by more than 4% p-distance. However, the problem remains that there is no genetic material of *D. tigrillo* from near the type locality available to date.

However, the future of taxonomic work on *Diasporus* can only be integrative. In contrast to morphological and molecular identification, acoustic traits can help to identify divergent lineages directly in the field. In most cases, the parameters frequency and call length are sufficiently distinct to identify different syntopic species simply by careful listening. Morphological and molecular examination can then be conducted in the laboratory. Future large-scale studies should collect specimens of different call types from a variety of habitats throughout the distribution area of the genus. Since six different countries would be involved in such a study, this might be a task not only for a single person, but for a consortium of researchers that are familiar with the genus.

7.1.2. *BOLITOGLOSSA*

The genus *Bolitoglossa* has developed a tremendous species richness in the central mountain ranges of Lower Central America, and especially in the Serranía de Talamanca. It is particularly the merit of the research group led by D. Wake that we have a fairly accurate idea of the actual species diversity nowadays. However, as I showed in this study many taxonomic problems remain and there are still undescribed species to discover, even in comparatively well-investigated areas like the Fortuna Depression. Anyhow, expeditions and collecting activity with the intense to discover new *Bolitoglossa* species is in a sharp decline. Most of the species described after the turn of the millennium have been discovered among museum material collected a long time ago (Brame *et al.* 2001; Hanken *et al.* 2005; Wake *et al.* 2007). Therefore, due to the lack of fresh material, even more recently described species were diagnosed using morphological characters only. Even the holotype and so far only known specimen of *B. copia*, that has been collected in 2002, but the species description is based on morphology only (Wake *et al.* 2005). An exception is the work of Boza-Oviedo *et al.* (2012) that included a relatively large set of sequence data of several *Bolitoglossa* species and a phylogeny from the Costa Rican part of the Serranía de Talamanca. Parra-Olea *et al.* (2004) were the first to present a phylogenetic hypothesis for the genus based on the mitochondrial cyt b and 16S markers and defined subgenera for the revealed clades. I combined the 16S mtDNA data of Panamanian *Bolitoglossa* species of Boza-Oviedo *et al.* (2012) and Parra-Olea *et al.* (2004) with the sequences that resulted from my own fieldwork and obtained the largest phylogeny for Panamanian salamanders so far calculated. It is probably due to the large taxon sampling that most clades received high statistical support, especially in the Bayes tree. Furthermore, these clades reflect the results of Parra-Olea *et al.* (2004) regarding subgenera and species groups (Fig. 37). I am therefore confident that the species that have been included here for the first time have been correctly placed in their respective group. In this respect, it was a surprise that *B. anthracina* is nested in the *B. subpalmata* group and not in the *B. schizodactyla* group like other large black *Bolitoglossa*. The assumption of Hanken *et al.* (2005) that the large black salamanders of the central mountain ranges of Costa Rica and Panama form a monophyletic group is obviously not true, at least not in the case of *B. anthracina*. Unfortunately, for many species in the group there are still no molecular sequence data available. It would be interesting to know if *B. anthracina* is the only species of large black salamander that does not belong to the *B. schizodactyla* group. *Bolitoglossa copia* from El Copé was considered to be a sister taxon of *B. anthracina* (Hanken *et al.* 2005), an assumption that would be worthwhile to test with molecular data once these become available. By using a denser taxon sampling of molecular and morphological data, I showed that *B. sombra* is a synonym of *B. nigrescens*. This was also suspected by Boza-Oviedo *et al.* (2012), but they received only low statistical support in their ML analysis and had no additional morphology data so they refrained from drawing taxonomic conclusions.

The highest diversity of *Bolitoglossa* species in Panama is observed in the Panama-Costa Rica border region above 1000 m asl (Hertz *et al.* 2013a, p. 473, Fig. 8). This might be a misinterpretation that comes from increased research activity in the Serranía de Talamanca in comparison to the Serranía de Tabasará. Especially the highest peak of the Serranía de Tabasará, Cerro Saguí, is virtually unexplored. It is nevertheless conspicuous that my own collecting over longer periods in the higher portions of the Comarca Ngöbe-Buglé yielded only a single specimen of *B*. cf. *minutula*. The same collection effort in the Serranía de Talamanca and on Volcán Barú produced many more specimens of more *Bolitoglossa* species. However, salamander specimens found in the Comarca Ngöbe-Buglé are more likely to represent distinct lineages or even undescribed species as the examples of *B. jugivagans* and *B*. cf. *minutula* show.

Up to now, modern salamander taxonomy integrated morphological characters with genetic markers. However, the tropical plethodontids in the tribe Bolitoglossini have the highest species diversification rate in all salamanders with *Bolitoglossa* being the most species-rich salamander genus in the world (Wiens 2007). Probably, many species are still undiscovered. Adams *et al.* (2009) could show that species diversification in plethodontids is not correlated with morphological changes, what suggests that the number of cryptic species is high. Especially, the most useful morphological characters are likely to be the result of convergent evolution. Foot webbing is likely to be a paedomorphic character influenced by the degree of arboreality (Alberch 1981). Furthermore, the number and shape of teeth is probably linked to different prey items and general nutrition of the respective species. Besides, tooth number changes during ontogeny, this character is sexually dimorphic (Ehmke & Clemen 2000). For those reasons, in most cases sample sizes have to be relatively large to retrieve taxonomic information from morphology alone. In future studies, it might be useful to include a species recognition criterion in addition to morphology and barcoding markers. In salamanders sexual communication is mediated by proteinaceous pheromones and it is probably possible to retrieve phylogenetic information from the genes or RNA that codes these pheromones (Palmer *et al.* 2005; Palmer *et al.* 2010).

7.1.3. *CAECILIA*

Among zoologists it is unanimously agreed that caecilians are the least investigated group of terrestrial vertebrates. There are even many biologists who never heard about this exclusively tropical amphibian order (Himstedt 1996). Unexpectedly, ongoing taxonomic research raised the number of species known to science from 176 (Zhang & Wake 2009) to 200 (AmphibiaWeb 2014) in only five years. However, there are still only a handful of taxonomists concerned with caecilians so species descriptions come from the geographical regions where these few taxonomists are working in. In recent years, this was mainly India (*e.g.*, Giri *et al.* 2011; Kotharambath *et al.* 2012; Agarwal *et al.* 2013; Kamei *et al.* 2013) and Brazil (*e.g.*, Maciel & Hoogmoed 2011a,b; Maciel & Hoogmoed 2013). *Caecilia* is on the one hand the most species rich genus in Gymnophiona, but on the other also one of the least known genera within this order of amphibians. However, caecilians are often not collected by fiel herpetologists, what keeps the numbers of specimens in scientific collections low. For example, the holotype was the only known specimen of *C. osae* since its description in 1992. In 2009, Huber and Hödel (2010) found a caecilian specimen that most probably belongs to that species. Although, the specimen was attacked by a coral snake (*Micrurus alleni*) and almost dead when it was found, it was not collected, but left in the forest (Walter Hödel pers. comm. 2012). This case shows that even experienced herpetologists pay not much attention to these strange amphibians.

The value of the only character used by Taylor (1968) as a synapomorphy in the genus *Oscaecilia*, the eye covered with bone or not, has been discussed by several authors. Nussbaum and Wilkinson (1989) suggested that *Caecilia gracilis* might be more closely related to *Oscaecilia*, because some specimens have the eyes covered with bone, but others not. Later, the same authors (Wilkinson & Nussbaum 2006) doubted the phylogenetic value of this character at all. Recently, molecular phylogenies revealed the paraphyly of *Caecilia* with respect to *Oscaecilia* (Zhang & Wake 2009; Pyron & Wiens 2011), but did not dare to make taxonomical changes due to the reduced taxon sampling. In the phylogeny of Zhang and Wake (2009) *C. volcani* and *C. tentaculata* cluster together, but the node receives only low statistical support. In their study, this subbranch forms a sister clade to *C. ochrocephala* and *C*. sp. from Ecuador. In contrast, the analysis of Pyron and Wiens (2011) as well as my own analysis found *C. volcani* in a clade together with *C. ochrocephala*. Likewise, in the phylogeny of Pyron and Wiens (2011) and my own, *C. tentaculata* appears in a subbranch neighboring the *C. volcani*-*ochrocephala* clade. Additionally, in my analysis, *C. tentaculata* is grouped together with *C. gracilis*; the latter species was not included in the study of Pyron and Wiens (2011). According to my results, one could restore the monophyly of *Caecilia* and *Oscaecilia* by transferring *C. volcani* and *C*. sp. from Ecuador to *Oscaecilia* because the type species of *Caecilia* and *Oscaecilia* are nested in other well-separated subbranches, respectively. However, for now I prefer to include *Oscaecilia* in the synonymy of *Caecilia*, because my phylogeny comprises only seven species, two of which have not been described yet, while the Caeciliidae count 42 species to date (AmphibiaWeb 2014). Additionally, I did not find a morphological autapomorphy for the respective clade at this point. Therefore, species which have not been included in the molecular analysis cannot be assigned toone of the recovered clades. A larger phylogeny that includes more taxa of Caeciliidae, now a monotypic family, is certainly needed. This should go along with a profound morphological study to identify characters that can be used to define the molecular clades. Probably, as a side effect, this will also lead tothe discovery of additional undescribed species.

7.2. BIOGEOGRAPHIC IMPLICATIONS

Darwin (1859) explained the absence of amphibians from volcanic islands located far from continental mainland by the susceptibility of amphibians to salt water. This sounds plausible, so most scientists assumed amphibians to be generally poor over sea dispersers. This assumption plays a key role in the reconstruction of the colonization history of the Central American land bridge by amphibians. However, recent biogeography studies showed that over sea dispersal of amphibians is possible under certain conditions (Vences *et al.* 2003, 2004; de Queiroz 2005). Heinicke *et al.* (2007) assumed that Terrarana arose in South America and that the ancestors of the Eleutherodactylidae diverged from their South American relatives about 47 Ma and began diversification 29 Ma. In the time tree of Heinicke *et al.* (2009), the South American genus *Adelophryne* diverged from the branch leading to *Eleutherodactylus* and *Diasporus* in the early Mid-Paleogene (around 50 Ma) what supports the assumption of a South American origin of Eleutherodactylidae. Hedges *et al.* (2008) included *Diasporus diastema* in their phylogeny and revealed a close relationship to the predominantly Caribbean *Eleutherodactylus*. Thus, they expected that the dispersal of *Diasporus* occurred late in the interval 47–29 Ma. Later, this was more precisely defined to the mid-Cenozoic, about 32 Ma, by Heinicke *et al.* (2009). However, both the colonization of the Antilles through ancestors of Eleutherodactylidae from South America as well as the colonization of Central America from the Antilles through the *Eleutherodactylus* subgenus *Syrrhophus* and the *Diasporus* ancestors must have occurred over sea water. Heinicke *et al.* (2007) included two species of the subgenus *Syrrhophus* in their time tree. According to that, *Syrrhophus* probably originated by dispersal from Cuba to Central America and began radiation in southern North America and northern Middle America around 20 Ma. Likewise, but earlier, *Diasporus* reached Lower Central America around 32 Ma from not yet identified Caribbean islands. The different timing of radiation in the *Syrrhophus* and *Diasporus* clades and the high genetic distance as indicated in recent phylogenetic studies (Hedges *et al.* 2008; Pyron & Wiens 2011; Padial *et al.* 2014) give reason for the assumption of two independent colonization events with *Diasporus* being the older clade of Eleutherodactylidae in mainland Central America. In contrast to the *Eleutherodactylus* subgenus *Syrrhophus* which has close relatives among *Eleutherodactylus* on Cuba, *Diasporus* is well-separated from all other genera of Eleutherodactylidae with no close relatives among living Caribbean forms. Thus, the origin of *Diasporus* is still unclear. Today, the highest species diversity of *Diasporus* is found in Lower Central America. It seems likely that *Diasporus* dispersed from Caribbean islands into Lower Central America and then spread to the north as far as southeastern Honduras and to the south into South America probably after the closure of the Panama Portal. There, the already lifted Andes formed a barrier that led to the present day distribution of *Diasporus* in South America that is exclusively west of the Andes. A time tree including all genera in the family could probably decipher the complex chronological sequence of dispersal in Eleutherodactylidae and the role of over sea dispersal in these frogs. At the current stage of knowledge, I consider it possible that over sea dispersal in the Eleutherodactylidae occurred. Hedges *et al.* (2008) pointed out that Eleutherodactylidae have a small body size in common and usually exhibit expanded terminal digits with pads. I argue that small frogs with an improved climbing ability are probably better rafters on driftwood than large and heavy terrestrial frogs. In addition, sea ways were not very wide between the islands of the proto-Antillen island chain (see Introduction 4.4).

The ancestors of the plethodontid genera *Bolitoglossa* and *Oedipina* are clearly of North American origin as derived from the generally high diversity of salamanders in the temperate zones of North America, Europe, and Asia. Accordingly, there is common agreement that the salamanders of Panama came from the north. However, the dispersal of salamanders from Central to South America is discussed controversely because South American members of *Bolitoglossa* appear to be older than the final closure of the Panama Portal (Hanken & Wake 1982). Hanken and Wake (1982) gave two possible scenarios to explain this pattern. Either the different South American lineages evolved on the southern part of the Central American peninsula and entered South America after the closure of the Panama Portal in the late Pliocene, or they entered South America before the closure of the Panama Portal and differentiated in South America. Parra-Olea *et al.* (2004) from their own results, suggested that the South American invasion of *Bolitoglossa* through the subgenus *Eladinea* occurred 10–13 Ma, thus well before the final closure of the Panama Portal. Species with predominantly South American distribution that are found in eastern Panama may have reentered Central America after closure of the Panama Portal. Unfortunately, there have been no additional recent studies on the South American invasion of *Bolitoglossa*. The latest contribution by Coates and Stallard (2013) on the geological formation of the Isthmus of Panama found evidence that 12–15 Ma widespread shallowing and oroclinal bending created few narrow but deep marine passages. This explains on the one hand the trans-isthmus marine fossil record, but on the other the sea ways might have been narrow enough to have been occasionally crossed by *Bolitoglossa* species. However, this will need to be further examined. I share the view of Parra-Olea *et al.* (2004) that the colonization of Central America by salamanders from the north happened long before any entry into South America. Thus, there has been a species diversification on the Central American peninsula that is also supported by the decrease of diversity among salamanders from north to south. The subgenus *Eladinea* to which most of the Panamanian salamanders belong has an exclusively Lower Central American and South American distribution with the Nicaragua depression as the northern limit (Parra-Olea *et al.* 2004). The species diversity of *Eladinea* is extraordinary high in the Serranía de Talamanca (Boza-Oviedo *et al.* 2012). Therefore, I conclude that the formation and radiation of *Eladinea* was autochthonous in Lower Central America. As the marine corridors narrowed 12–15 Ma occasional over sea crossing of salamanders to South America may have happened. This timing fits also very well the molecular clock calculations (Hanken & Wake 1982; Parra-Olea *et al.* 2004). Probably, over sea dispersal happened several times what explains the large genetic distances among South American salamanders, but also the low species diversity in South America compared to Central America. Accordingly, the invasion of South America by the salamander genus *Bolitoglossa* is a combination of both hypotheses proposed by Hanken and Wake (1982). However, this also indicates that *Bolitoglossa* salamanders are less good over sea dispersers than frogs of the family Eleutherodactylidae, as they crossed only narrow marine barriers and never reached remote Caribbean islands.

Another question that remains is why there are so few salamander species in the Serranía de Tabasará if there are so many in the neighboring Serranía de Talamanca. One explanation may be that the Serranía de Talamanca was uplifted by subduction of the Cocos Ridge in the Pliocene, while the Serranía de Tabasará arose from Quarternary volcanism and, accordingly, is substantially younger (Marshall 2007). The colonization of the Serranía de Tabasará by montane *Bolitoglossa* species happend from the Serranía de Talamanca. This is evident from the fact that the closest relatives of *Bolitoglossa* species in the Serranía de Tabasará are found in the Serranía de Talamanca and not in the surrounding lowlands (*e.g.*, *B*. aff. *minutula* from La Nevera and *B. jugivagans*). The crossing of the Fortuna Depression might have happend during Quarternary glacial periods. The time for diversification in salamanders in the Serranía de Tabasará might have been too short to develop more species diversity. As opposed to that, a specimen of the lowland species *B. lignicolor* from the Pacific slopes of the Serranía de Tabasará showed low distances in the 16S rRNA gene and morphology to conspecifics from other sites (Hertz *et al.* 2013b), what accounts for a recent colonization and the lack of climatic barriers in the moist Pacific lowlands. On the other side, in the Caribbean lowland species *B. colonnea* clear differences in tooth counts between specimens from the slopes of the Serranías de Talamanca and Tabasará were perceived (Hertz *et al.* 2013b). However, until now, phylogenetic relationships among the various populationes referred to this species could not be tested using molecular data. *Bolitoglossa* aff. *minutula* from La Nevera seems to be an independently evolving lineage, albeit with close relationships to *B*. aff. *minutula* from Volcán Barú. Anyway, the taxonomy of the *B. minutula*-like lineage from Volcán Barú is also unclear, since the type locality of *B. minutula* is Cerro Pando and the specimens from there form another genetic lineage. *Bolitoglossa copia* and *B. jugivagans*, both known from a single individual respectively, seem to be endemic to the Serranía de Tabasará (Wake *et al.* 2005; Hertz *et al.* 2013a). It was obvious during field work that in the high parts of the Serranía de Tabasará, salamanders are rarely encountered compared to the same altitudes in the Serranía de Talamanca. However, it must be noted that the Serranía de Tabasará is one of the least investigated areas in Panama. Especially the highest mountains, Cerro Saguí and Cerro Santiago, are mostly unexplored. Therefore, I assume that species diversity of salamanders is probably lower in the Serranía de Tabasará than in the Serranía de Talamanca, but still higher than currently known. I suppose that field work in the Serranía de Tabasará, especially on its highest peaks, will uncover more unnamed species.

Most of the few recent biogeographic studies on caecilians have been concerned with the origin of Asian caecilians (*e.g.* Hedges *et al.* 1993; Gower *et al.* 2002; Nishikawa *et al.* 2012). Biogeographic hypothesis with a regional focus on Central America discussed the evolution of *Dermophis* and *Gymnopis*, but remained vague concerning *Oscaecilia* and *Caecilia* (Savage & Wake 2001; Wake *et al.* 2005; Zhang & Wake 2009). The closest relative of the *Dermophis*-*Gymnopis* clade is the African genus *Schistometopum* (Pyron & Wiens 2011). According to the evolutionary time tree of Zhang and Wake (2009) split of *Dermophis*-*Gymnopis* and *Schistometopum* happened around 105 Ma, when Africa and South America finally separated. The *Dermophis*-*Gymnopis* stock entered Central America in the Paleocene during the first Central American land bridge, became extinct in South America, and diversified in Central America. The split of *Dermophis* from *Gymnopis* is calculated to have happened around 61 Ma. In Zhang and Wake (2009) the split of Caeciliidae from its sister family Typhlonectidae dates back to more than 100 Ma in the middle Cretaceous. In the time tree of Roelants *et al.* (2007), the split between both families is estimated to have occurred in the early Paleocene about 60 Ma. However, in both cases the Caeciliidae have a long independent evolutionary history. Zhang and Wake (2009) calculated the divergence of *Caecilia*-*Oscaecilia* to around

60 Ma, but in view of the paraphyly of *Caecilia* and the limited taxon sampling this is hypothesis is questionable.

In Central America, seven described species of *Caecilia* are known to occur. The distribution of *Caecilia* in Central America is restricted to the southernmost geological blocks, what accounts for a relatively recent South American origin of the genus (Savage & Wake 2001). However, we still do not have a good time estimate of the point when the ancient South American stock entered Central America. It is remarkable that in my phylogeny the Central American species form one clade, while the other clade contains the two South American Caecilians. The only exception is *C*. sp. from Ecuador that is nested within the Central American *Caecilia*. The only explanation for this is that the two clades got separated by the uplift of the Andes, as *C. gracilis* and *C. tentaculata* are distributed east of the Andes in the Amazonas basin and the other species including *C*. sp. from Ecuador west of the Andes. This agrees also with the divergence time between the two clades calculated by Zhang and Wake (2009) of 60 Ma that fits well with the beginning of the uplift of the Andes (Hoorn *et al.* 2010). Future studies that include more taxa and additional nuclear genetic marker should test if there is a general phylogeographic pattern with two clades of *Caecilia*, one in Central America and northern South America west of the Andes, and another in South America east of the Andes. If this is true, the name *Oscaecilia* could be resurrected for the former clade.

Most known species of *Caecilia* in Central America inhabit the Chocó block. The species that exclusively inhabit the Chocó block are *Caecilia isthmica*, *C. leucocephala*, and *C. nigricans*. *Caecilia ochrocephala* is mostly distributed over the Chocó block, but enters the southeastern end of the Choroteca block. The distribution of *C. elongata* remains unknown. *Caecilia osae*, *C. volcani*, and *C*. sp. from western Panama inhabit exclusively the Choroteca block. The Choroteca species are allopatric: *C. volcani* is found on the Atlantic slopes, *C*. sp. on the Pacific slopes of western Panama and *C. osae* in the Golfo Dulce region of southern Costa Rica. *Caecilia osae* is so far the only caeciliid species reported from Costa Rica, but it seems likely that additional species will be discovered. It is, for example, quite conceivable that *C. volcani* enters southeastern Costa Rica. From what I can see about the position of the tentacle in relation to the eye, the photos of two specimens of caecilians in Köhler (2011, pp. 31–32, Fig. 20, 22) referred to as *Dermophis parviceps* from Costa Rica, show actually members of the genus *Caecilia*.

At the current state of research I cannot say with certainty that *Caecilia osae* and *C*. sp. from western Panama are different species. This needs to be further investigated. However, assumed that they represent different species, they are obviously close relatives. This is evident from both, the molecular phylogeny and the morphological similarities. It is therefore assumed that both species share a common ancestor and might have evolved in allopatry by vicariance. A speciation scenario similar to that of *Anolis osae* as described by Köhler *et al.* (2010) is imaginable. According to that, the Osa peninsula has been an island or group of islands during interglacial times in the Pleistocene (Bagley & Johnson 2014). This might have isolated a founder population on the Osa peninsula from the ancestral mainland populations. However, this would require a very rapid speciation since interglacials did not exceed 0.2–0.4 Ma, a fact that does not support the two species hypothesis. Usually, speciation processes in vertebrates take on average 2.0 Ma, although faster species radiation is known for example in fishes in lacustrine environments (Avise *et al.* 1998). It might also be that after reconnection of the Osa peninsula and the main land the two subpopulations were not reconnected. Indeed, there is a gap of approximately 60 km between the eastern most collection site of *C. osae* and the western most collection site of *C.* sp.. Thus, there is no known contact zone between both populations. In this area a climatic barrier separates the wet Golfo Dulce region from the dryer western Pacific lowlands around David (Crawford *et al.* 2007; Wang *et al.* 2008; Gutierrez-García & Vázquez-Domínguez 2013). This barrier exists for at least 4 Ma (Graham & Dilcher 1995; Wang *et al.* 2008), thus the wet forest dispersal corridor had already disappeared during the closure of the Panama Portal. This indicates that the ancestor of *C. osae* might have been drought-tolerant, like *C.* sp. from western Panama is today. During an inundation event of the Osa neck in the Pleistocene the Osa population got isolated and evolved for some 100,000 years independently. After reconnection of the Osa and the main land the Osa population reentered the main land, but was limited to the wet Golfo Dulce surrounding. Alternatively, the ancestral *Caecilia* stock entered the Choroteca block before the final closure of the Canal Corridor and before disappearance of the western Panamanian wet forest corridor. This would require an over sea dispersal that is rather uncommon in Caecilians. Although it is possible, for example over sea dispersal most likely happened in the case of the Sao Tomé island endemic *Schistometopum thomense* (Zhang & Wake 2009).

7.3. THE FUTURE OF INTEGRATIVE TAXONOMY

It seems that wherever modern taxonomical approaches are applied to tropical amphibian faunas cryptic diversity is revealed (*e.g.*, Padial & de la Riva 2009; Vieites *et al.* 2009; Jansen *et al.* 2011; Crawford *et al.* 2013). In this context, it is also notworthy, that most species that have been described using exclusively morphological characters do stand up to verification by integrative taxonomy. The only case revealed in this study of an invalid taxon on the species level is that of *Bolitoglossa sombra*, a junior synonym of *B. nigrescens*. However, as I could show with classical morphometrics and especially with toothcounts there was actually no need for molecular data to detect this synonymy; the sample size in previous studies was too small to reflect actual intraspecific variation.

Large scale taxonomical approaches, like the above mentioned studies, are useful to reveal cryptic lineages among amphibian assemblages, but it is to be seen as problematic that the identified lineages are usually not named for a long time. This is not the fault of researchers conducting such studies, but rather the fault of the current scientific system. Studies presenting large-scale phylogenies can usually be placed in journals with high impact factors (IF) between 2.6 and 9.74, whereas journals publishing species descriptions and solutions to basic taxonomical problems usually have an IF that is below 1.00, a fact that has been repeatedly criticized by taxonomists (Krell 2002; Werner 2006). Nowadays, however, scientific output is measured largely by the IF alone and a high personal IF is crucial to get a permanent position as a scientist. At the same time, there is a lack of well-trained taxonomists doing high quality species descriptions. Padial *et al.* (2010) calculated that at an estimated total number of 10 million eukaryote species, we would need another 400 years at the current pace to complete the inventory of life on earth. However, this is not a reasonable timeframe when one considers that extinction rates are estimated to be 100 to 1000 times higher than in pre-human times (Pimm *et al.* 1995). Another problem with genetic lineages remaining unnamed for prolonged periods of time is that self-appointed taxonomists may name these lineages without adequate background knowledge and without giving a substantiated diagnosis of the taxon described, what causes even more work in the end (Kaiser *et al.* 2013).

When talking about DNA barcoding for species identification, it must be pointed out that the identification of the organism from which a barcoding gene has been isolated, is still performed using classical methods. Nowadays, when a new species is described, DNA barcodes of the holotype and additional specimens are usually submitted together with the original species description. Anyway, many species were described in earlier times and the correct assignment of fresh material to a known species requires experienced taxonomists. In other words, the DNA barcoding sequence on a database as a reference for species identification is only as good as the underlying taxonomic conclusion. If one uncritically uses a GenBank sequence of an organism that has been initially misidentified all following studies (*e.g.*, in taxonomy, systematics, and conservation biology) might lead to wrong conclusions.

The public is not aware of this, but as any discipline in biology, taxonomy and systematics are also affecting many aspects of our daily lives. A worldwide standardized nomenclature for organisms is needed for example to unambiguously list ingredients of foods, or in legal matters whenever lists are created that contain organisms that are prohibited to possess or are subject to specific protection. For all these reasons it is indispensable that taxonomical work receives more public recognition and that well-qualified taxonomists, trained over many years through public funds, no longer switch to careers with better opportunities for personal development.

7.4. CONSERVATION

This text has been updated and modified from Hertz *et al.* (2012c): The main problem in assessing the conservation status of Panamas amphibian fauna is the inconsistence in the taxonomy of species and species groups and the general lack of information on habitat preferences and natural history of single species. Among the 214 amphibian species that I count for Panama are 44 (20.6%) that have not been officially evaluated or lack adequate data. In these species the conservation status should not be underestimated until further data become available. However, on the basis of already evaluated species and total species numbers it is possible to identify areas of high conservational value, which should play a major role in conservation efforts. Generally spoken, 51 (23.8%) of the amphibian species inhabiting Panama qualified for an IUCN threatened category. Although the last comprehensive conservation assessment has been accomplished in 2004 and it is to be expected that nowadays, ten years later, the overall situation has probably not improved. The causes of Amphibian decline in Panama are manifold and the main reasons will be discussed below.

7.4.1. ANTHROPOGENIC ACTIVITIES

Human activities are by far the biggest threat to amphibian diversity and biological diversity in general (Kiesecker *et al.* 2001; Young *et al.* 2004; Cushman 2006; Gardner *et al.* 2007). Especially the deforestation of old-growth forest is a major problem. About 44% of Panama’s land mass is still covered by forests. Compared to other Central American countries, Panama showed a relatively low deforestation rate of 1.2% in the period between 1990 and 2000 (total Central America 1.6%), and an even lower rate of 0.4% between 2000 and 2010 (total Central America 1.2%; data taken from FAO 2011). But deforestation rates in Panama are not equally distributed, and some forests are under higher anthropogenic pressure than others. By far the highest deforestation rate among Panamanian provinces is found in the Comarca Ngöbe-Buglé (21.8%, 1992-2000) followed by the central-eastern provinces of Darién (13.9%), and Panamá (12.2%) (ANAM 2009). Unfortunately, even protected areas are not always successful in their ability to control deforestation. This is especially severe in La Amistad International Park and Palo Seco Protected Forest, where deforestation of mature forest was high, despite the protection status (Oestreicher *et al.* 2009). On a field trip to the northern slope of Cerro Pando, thus deep in the La Amistad International Park and far from any settling or road, S. Lotzkat and I crossed huge clearings for cattle where there was pure mature forest in 1966 (William Duellman pers. comm. 2010). Currently, discussions about constructing new roads through remote areas of different protected areas, including La Amistad International Park, Volcán Barú National Park, and Palo Seco Protected Forest, have come up again. The Panamanian government expects an increase of tourism and trade to be triggered by these projects. However, costs to build and maintain roads in tropical mountainous forests are usually high, making it doubtful that economic aims will be achieved (Reid & Hanily 2003). Beside other effects, these roads will accelerate deforestation by facilitating the access to formerly well-protected sites (Young 1994; Chomitz & Gray 1996; Nelson & Hellerstein 1997). It is further questionable if new roads in protected areas rather annoy tourists, who are predominantly looking for pure nature, than stimulating them to make a visit, in particular if there is no forest left to see when driving through a park. There is a general need for comprehensive management plans, better demarcation of protected areas, and year-round personnel to stop ongoing deforestation in areas that are supposed to be protected.

Although national parks and other conservation areas could be better protected from deforestation, the unprotected forests are facing a much stronger pressure. Unprotected lowland sites, which are dominated by extensive agriculture like the Chiriquí lowlands, inhabit a diverse amphibian fauna (Lotzkat & Hertz 2011; Batista & Ponce 2011). However, the share of endangered species is rather low here (Fig. 74). Anyway, land use change in the form of road construction, designation of new building development areas, and more intensified agriculture could be a future threat for species that are of less conservation concern at the moment.

Of all unprotected areas that I have surveyed in the course of this project, the Cerro

Colorado area is the most important region in western Panama with respect to amphibian conservation. Moreover, this area is largely unexplored and it is very likely that a number of species from this region still await discovery and formal description. At the same time, it is one of the largest remaining copper deposits in the world and thus most seriously impacted by anthropogenic stress. Basically, there is a lack of protected areas in the Serranía de Tabasará in comparison with the Serranía de Talamanca. The establishment of a protected area in the Cerro Colorado area of around 14,000 ha would provide protection for populations of seven Endangered and Critically Endangered amphibian species (Hertz *et al.* 2012c). Probably many other threatened organisms that were not covered by this study would also benefit from this. From all that we know, mining at Cerro Colorado will cause severe environmental damage and reduce the diversity of the Panamanian amphibian fauna once more. A few years ago, the former Panamanian government attempted to simplify the solicitation process for campanies from foreign countries to obtain concessions (Nakoneczny & Whysner 2010). Although the Cerro Colorado copper deposits are of important economic interest, mining at this site is in fact environmentally unsustainable. At present, most of the indigenous Ngöbe-Buglé, in whose autonomous territory Cerro Colorado lies, are against the mine. However, the communities surrounding Cerro Colorado have only limited access to information and are therefore easily manipulated (Simms & Moolji 2011). In the end, the future of the Cerro Colorado forest including its diverse amphibian fauna is in the hands of local communities. Anyway, from the perspective of a conservationist and amphibian specialist Cerro Colorado is of irreplaceable value.

7.4.2. CHYTRIDIOMYCOSIS

Aside from habitat loss, the emerging infectious disease chytridiomycosis caused by the pathogenic fungus *Batrachochytrium dendrobatidis* is indisputable the most acute problem amphibian populations in Panama are facing. In contrast to other parts of the world, amphibian die-offs caused by chytridiomycosis have been well-documented in Panama (Lips 1998, 1999; Lips *et al.* 2006). Thanks to this meticulous documentation, it is known when and where amphibian populations collapsed and which species were most severely affected. It is therefore interesting to visit sites where *Bd* related population declines have been observed and to evaluate the post-decline situation of such populations. Interestingly, the results of my study are not always in line with what was expected. The assumption of Lips *et al.* (2003b) that the ecology and maximum snout-vent length of amphibian species predicts *Bd* related

Table 13: Comparence of ecological traits of surviving versus non-surviving Panamanian frog species

surviving populations known						**no surviving populations known**				
Species	possible *Bd* related declines	max SVL [mm]	stream associated	elev. Range [m asl]	reencountered at [m asl]	Species	possible *Bd* related declines	max SVL [mm]	stream associated	elev. Range [m asl]
Atelopus varius	yes	32–43	stream dweller/ stream breeder	16–2150	730–1087	*Atelopus chiriquiensis*	yes	34–49	stream dweller/stream breeder	1400–2500
Incilius epioticus	no	33–40	no	1050–2040	1650	*Incilius fastidiosus*	yes	52–60	forest and dweller/stream bank breeder	760–2100
I. coniferus	no	72–94	no	0–1550	5–1177	*I. peripatetes*	yes	67–72	forest and dweller/stream bank breeder	1500–1856
Craugastor melanostictus	no	43–56	no	1150–2700	880–2491	*Craugastor catalinae*	yes	45–75	stream dweller	1220–1800
C. monnichorum	no	46–66	stream bank dweller	610–1870	1643–1872	*C. emcelae*	yes	46–70	stream bank dweller	910–1450
						C. punctariolus	yes	50–81	stream dweller	560–1800
						C. ranoides	yes	45–74	stream dweller	0–1300
						C. tabasarae	yes	34–54	stream bank dweller	600–910
Isthmohyla debilis	yes	29–32	stream breeder	910–1700	1540–1643	*Isthmohyla calypsa*	yes	36–41	stream bank dweller/ stream breeder	1500–2100
I. graceae	yes	39–41	stream bank dweller/stream bank breeder	1120–1710	1500–1712					
I. infucata	not known	43–46	no/pond, puddle breeder	830–1610	1608–1755					
I. rivularis	yes	34–36	stream dweller/ stream breeder	1210–2840	1673–2289					
I. tica	yes	34–42	stream dweller/ stream breeder	835–1920	1220–1621					
Duellmanohyla uranochroa	yes	37–40	stream dweller/ stream breeder	70–1740	1060–1420					
Hyloscirtus colymba	yes	37–43	stream dweller/ stream breeder	0–1410	876–940					
Lithobates taylori	unclear	78–88	no/pond, puddle breeder	60–1860	860–1312	*Lithobates vibicarius*	yes	73–92	no/pond, puddle breeder	1500–2700
L. warszewitschii	yes	52–63	stream bank dweller/stream breeder	0–1741	445–1300					

declines rather than taxonomic affiliation is certainly true. However, this pattern is not necessarily observable when looking at the species that are recovering following declines. The pathogen-host dynamics seem to be more complicated than previously thought. In Table 13 some of the more prominent species with good data basis from Panama affected by chytridiomycosis are compared to related species with similar ecology and maximum SVL. Highland bufonids seem to be very susceptible to chytridiomycosis and most of them show no signs of recovery so far. The two *Atelopus* species that occur in the investigated area have both suffered from severe declines. However, while no surviving *A. chiriquiensis* were found and the species is possibly extinct, *A. varius* survives in several small subpopulations (Hertz *et al.* 2012c; Gonzales-Maya *et al.* 2013; Perez *et al.* 2014). It is noteworthy that *A. varius* has a much larger historical distribution area than *A. chiriquiensis* and inhabits also lowland sites. The presence of *Bd* and subsequent amphibian population declines have been documented at sites where *A. varius* is still present (Lips *et al.* 2003a; Brem & Lips 2008). From all what we know about the physiology of *Bd*, lowland frog populations should have a higher chance of survival due to the higher ambient temperatures (Piotrowski *et al.* 2004; Andre *et al.* 2008). In contrast, surviving populations are found at mid-elevations compared to the former altitudinal distribution of the species, but still at lower elevations than the altitudinal belt where *A. chiriquiensis* was historically found. This may reflect a trade-off between host-optimal temperature, so that production of antimicrobial skin peptides works optimally (Ribas *et al.* 2009), and the pathogen-optimal temperature that is reached at higher altitudes. Like *Atelopus*, also other highland toads are affected in the same way. The mountain species of the *Incilius coniferus* group (Mendelson *et al.* 2011) have all declined, but only in *I. epioticus* no declines have been noticed. Although, there is very scarce information on the live history of *I. epioticus*, it is assumed that the eggs undergo direct development in the deep leaf litter and the species is not associated with water bodies (Vaughan & Mendelson 2007). Likewise, there are no reports on *Bd* related declines in the close relatives of *I. epioticus* (*i.e.*, *I. chompipe* and *I. guanacaste*) from Costa Rica. The other species in this group have at least an aquatic tadpole stage. *Incilius coniferus* is primarily a lowland species that I commonly found up to an elevation of 1177 m, but the other members of the *I. coniferus* group were all declining. Of those, only *I. holdridgei* has been rediscovered, but in reduced abundance compared to pre-chytridiomycosis times. In this regard, it is notable that *I. holdridgei* is clearly smaller than *I. fastidiosus* and *I. peripatetes*. So far, the predictions of Lips *et al.* (2003b) fit the observed pattern of extinctions. The conclusions change when looking at the genus *Craugastor*. While most *Craugasto*r species are not directly threatened by chytridiomycosis, the members of the *Craugastor punctariolus* Species Series (Hedges *et al.* 2008) in Lower Central America are heavily affected. All these species are strongly stream associated and spent a large part of their lives near and in streams, although they lack a free living tadpole stage. One special aspect of the declines of *Craugastor* species is that besides highland species also lowland species, like *C. ranoides* and *C. taurus* are affected by chytridiomycosis and are declining at alarming rates. Interestingly, surviving populations are found in smaller mountain ranges which are isolated from the Cordillera Central and typically surrounded by hot lowlands that receive comparatively lower precipitation. A surviving population of *C. ranoides* is known from Río Murciélago on the Santa Elena Peninsula in Costa Rica (Puschendorf *et al.* 2005; Zumbado-Ulate *et al.* 2007; Zumbado-Ulate *et al.* 2011) and the Azuero Peninsula endemic *C. azueroensis* is still abundant in its original distribution range at Cerro Hoya and the Montuoso Forest Reserve (Köhler *et al.* 2012). There is also a possibility that surviving populations of *C. taurus* persist on the Burica Peninsula. The last published sigthing of an individual was made at Las Mellizas, Peninsula de Burica by Marcos Ponce in 2008 (Marcos Ponce pers. comm. 2014; Köhler 2011, Fig. 430). The surviving populations of *C. ranoides* and *C. azueroensis* have been examined for the presence of *Bd*. While there are low concentrations of Chytrid detectable on specimens from the surviving *C. ranoides* population (Héctor Zumbado-Ulate pers. comm. 2012), there were no chytrid positive samples taken from *C. azueroensis* (Köhler *et al.* 2012). Although the number of samples taken by Köhler *et al.* (2012) is too small to conclude with certainty that the area is free of *Bd*, the isolation of the mountains on the Azuero Peninsula may have prevented the arrival of *Bd* so far. This, however, would mean that the populations of *C. azueroensis* are naïve, and thus introduction of *Bd* into the populations would likely lead to a chytridomycosis outbreak. It is therefore advisable to establish *ex-situ* populations and to monitor the area carefully and in compliance with the DAPTF Fieldwork Code of Practice protocol (Lips *et al.* 2001). Other *Craugastor* species groups in Panama are not entirely affected or not affected at all. The *Craugastor podiciferus* and *C. laticeps* Species Groups (Hedges *et al.* 2008) are not affected, while in the *C. gulosus* Species Series as well as in the *C. fitzingeri* and in the *C. melanostictus* Species Groups (Hedges *et al.* 2008), only one species is affected, respectively. In the *C. gulosus* Species Series, only *C. gulosus* is threatened and this is the only species in the series that inhabits exclusively altitudes above 1000 m (Köhler 2011). In the recent phylogeny of Padial *et al.* (2014), the only species of the *C. gulosus* Species Series sensu Hedges *et al.* (2008) included in their analysis, *C. megacephalus*, is found to be nested in the *C. punctariolus* Species Series. Accordingly, the taxonomy of both groups is in flow and a correlation between chytrid caused declines and phylogenetic lineages requires deeper analyses. Crawford *et al.* (2010) reported a 100% decline in relative abundance of *C. megacephalus* at El Copé after arrival of *Bd*. Maybe, the entire *C. gulosus* Species Series is more affected than previously anticipated. However, I found *C. megacephalus* regularly at sites where it is known to occur and I did not find indications of a decline. In the *C. fitzingeri* Species Series (Hedges *et al.* 2008), two species are obviously affected by chytrid. *Craugastor tabasarae* of the *C. fitzingeri* Species Group (Hedges *et al.* 2008) is known from a few sites in Central Panama and El Copé. The latter site is also its type locality (Savage *et al.* 2004). At El Copé, Crawford *et al.* (2010) documented the collapse of the *C. tabasarae* population after *Bd* arrival. A problem in evaluating the conservation status of species in the *C. fitzingeri* Species Series is that specimens are regularly misidentified and confused. The record (SMF 85377) by Köhler *et al.* (2008) of *C. tabasarae* from La Nevera is based on a specimen of *C. melanostictus* as indicated by the presence of an enlarged heel tubercle and alternating red and black bars on the posterior thigh surface, both lacking in *C. tabasarae*. Equally, the unequivocal identification of the only endangered species in the *C. melanostictus* Species Group of Hedges *et al.* (2008), *C. emcelae*, is not easy. *Craugastor emcelae* is most likely to be confused with *C. monnichorum* that I found from time to time between Volcán Barú and Cerro Santiago. *Craugastor emcelae* should inhabit the Cordillera Central from La Fortuna to the west. At the type locality of *C. emcelae*, the northern slope of Cerro Pando, I did not find any specimen that would resemble *C. emcelae* and I believe that it actually has declined there. *Craugastor emcelae* used to be moderately common at the type locality where 24 specimens were collected within 10 days in 1966 (Lynch 1985). It is surprising that *C. monnichorum* is obviously not declining although it is a supposed close relative of *C. emcelae* (Lynch 1985). The former species occurs at altitudes between 1640 and 1870 m asl, and, especially at Bajo Mono, was frequently found sitting close to the water of a stream (Köhler *et al.* 2008; own observation). *Craugastor* is clearly the genus in Panama that is most severely threatened by chytridiomycosis. At the same time, it is a genus in which many taxonomic problems remain.

In contrast to bufonids and craugastorids, the number of recovering species after severe declines is astonishing high in hylids. In many cases, population recovery of mountainous hylid species was observed at sites where the presence of *Bd* and the decline of these species had been documented. In Lower Central America, members of the endemic genus *Isthmohyla* have most severely declined although not all species have been affected equally. Due to the sharp decline of most *Isthmohyla* species, the published phylogenies of this genus all suffered from poor taxon sampling due to a lack of fresh tissue material, and therefore the proposed composition of the genus was largely tentative (Faivovich *et al.* 2005). My own preliminary phylogeny that includes eight species of the genus confirms the monophyly of the genus. However, the species groups, as defined by Duellman (2001) are not recovered in my analysis. The tree contains two destinct branches of *Isthmohyla*; the node, however, receives relatively low statistical support (bs= 71; Appendix 10.2.4.). One subbranch contains *I. infucata*, *I. picadoi*, *I. pseudopuma*, and *I. zeteki*, while the other contains *I. graceae*, *I. lancasteri*, *I. rivularis*, and *I. tica*. A similar phylogeny, but with reduced taxon sampling, was found by Pyron and Wiens (2011). In the context of amphibian declines, it is noticeable that *Bd*-related declines were documented only in species included in the latter clade. An exception is *I. lancasteri* of which no *Bd*-related declines were documented. On the other hand, *I. calypsa* has still declined severely (Lips 1998). Although not included in the molecular analysis, it is believed to be a close relative of *I. lancasteri* (Trueb 1968; Lips 1996). Furthermore, Lips (1998) documented the decline of *I. rivularis* at Las Tablas. That is only 5 km west of Jurutungo where a presumably increasing population of *I. rivularis* was rediscovered in the course of this project (Hertz *et al.* 2012c). Lips (1999) also documented the arrival of *Bd* and the decline of the amphibian fauna at La Fortuna. As a result, all hylids declined noticeably there. *Duellmanohyla uranochroa* seemed to survive initially at lower abundances, but continued to declined in the following years. In 2008, I did not find adult specimens, but only a few tadpoles with deformed mouthparts. In the same year, Hamad (2009) surveyed the surroundings of the Lost and Found Eco Hostel for 54 days, but did not find any additional specimens. One of the collected tadpoles was raised in the Lost and Found Eco Hostel and died a few days after metamorphosis (Andrew Bennett pers comm. 2009). In 2009, two adults were found, one near Lost and Found Eco Hostel in the La Fortuna Forest Reserve and the other at neighboring Palo Seco Protection Forest (Hertz *et al.* 2012c). In 2013, colleagues and I conducted Audio Strip Transects at four different creeks in the surroundings of the Lost and Found Hostel where we found between two and five calling males per creek (own unpubl. data). This indicates that the population at least at this place is increasing. A similar scenario as in La Fortuna was found at Alto de Piedra, but with the hylid species *Hyloscirtus colymba*. Brem and Lips (2008) reported on the *Bd* prevalence in the Santa Fé area and the decline of its amphibian fauna. During repeated visits at Alto de Piedra in subsequent years I recognized an increase in abundance of adult specimens of *H. colymba* in Alto de Piedra, but also confirmed that *Bd* and chytridiomycosis outbreaks are still present in the population (Hertz *et al.* 2012c). Other positive *Bd* results were obtained from Santa Clara, near Jurutungo, and at La Fortuna (Hertz *et al.* 2012c). It is noteworthy, that at each of these sites (*i.e.*, Jurutungo, La Fortuna, and Alto de Piedra) only a single stream-breeding hylid species was found to be recovering at the same time, although in pre-chytridiomycosis times each site was populated by several stream-breeding hylid species. There is only one visited site (*i.e.*, Cerro Colorado) where surviving populations of *Isthmohyla gracea* and *I. debilis*, as well as single individuals of *Agalychnis annae*, *I.* cf. *rivularis*, and *I.* cf. *tica* have been found. Moreover, all specimens from Cerro Colorado have been tested negative for the presence of *Bd* (Hertz *et al.* 2012c). That can be either an indication that some sites, like Cerro Colorado, are still free of *Bd* even if they are situated along the continental divide or that these species are able to completely clear *Bd* infection. To further investigate these hypotheses a more exhaustive chytrid monitoring in the Cerro Colorado is required.

Some species are known to clear *Bd* infection even at environmental conditions optimal for the pathogen. Márquez *et al.* (2010) found that *Hypsiboas creptitans* is able to clear infection within a few weeks at a pathogen-optimal temperature of 23 °C. It is further remarkable that a whole family, the Centrolenidae, have obviously entirely recovered from *Bd* related declines at least in Panama. Crawford *et al.* (2010) reported that some glass frog species have declined at El Copé among those also *Cochranella granulosa* and *Sachatamia albomaculata*, both of which I found frequently in my study area. Lips *et al.* (2003b) registered a decline in 56% of the 16 glass frog populations examined. After the ecological factors identified by Lips *et al.* (2003b) that increase the risk of *Bd*-related declines, Centrolenids in montane habitats should be susceptible to chytridiomycosis as they spent most of their lives near streams where they often sit in the splash water zone, and are stream-breeders with an aquatic tadpole stage. Two species in my area of investigation are clearly members of the riparian frog fauna at upland sites. *Espadarana prosoblepon* is very abundant at mountainous streams up to 2044 m asl., and *Hyalinobatrachium talamancae* was frequently encountered between 1400 and 1900 m asl with the lowest collection site at Paredón (786 m asl). Admittedly, Centrolenids are generally small frogs, the only argument for a possibly smaller risk of decline. Woodhams *et al.* (2006) found that innate immune defense varied significantly among species. In their tests, the skin peptid isolates with the strongest mean growth inhibiting effect on *Bd* among Panamanian amphibian species came from *E. prosoblepon*, but with large differences between individuals. In 2013, I found a single tadpole of *Hyalinobatrachium talamancae* together with several tadpoles of *Duellmanohyla uranochroa* in the same creek in the La Fortuna Forest Reserve. While there was a complete lack of keratinized mouthparts in all examined *D. uranochroa* tadpoles, denticles and beak of the *H. talamancae* tadpole appeared healthy (Fig. 76).

Generally, species that are recovering after having declined due to *Bd* may have developed some kind of resistance. Until now, very little is known about host-pathogen dynamics of *Bd* and its hosts. Kriger and Hero (2006) found the widespread and abundant Australian frog *Litoria wilcoxii* to survive and even clear *Bd* infection, what they interpreted as an immunological adaptation of adults after 25 years of *Bd* exposure. However, the mechanisms that might lead to immunity against *Bd* are poorly understood. The attempts by Stice and Briggs (2010) to stimulate the adaptive immune response of the *Bd* susceptible Mountain Yellow-legged Frog (*Rana muscosa*) via formalin-killed *Bd* cultures showed no significant immunization effect. In contrary, as various studies have shown, the skin-associated system of innate immunity apparently plays a more important role in host defence against *Bd* through antimicrobial peptides (AMPs) (*e.g.*, Woodhams *et al.* 2006; Ramsey *et al.* 2010; Conlon *et al.* 2011). This indicates that the adaptive immune system of susceptible amphibians cannot be activated by pathogen exposure, but immunization in wild populations is rather an effect of change in the allele frequency. AMPs from various frog species have been isolated and tested for antimicrobial activity, but many studies mainly focussed on the possible therapeutic potential for human medicine (Conlon 2004), or on general structure and function of the

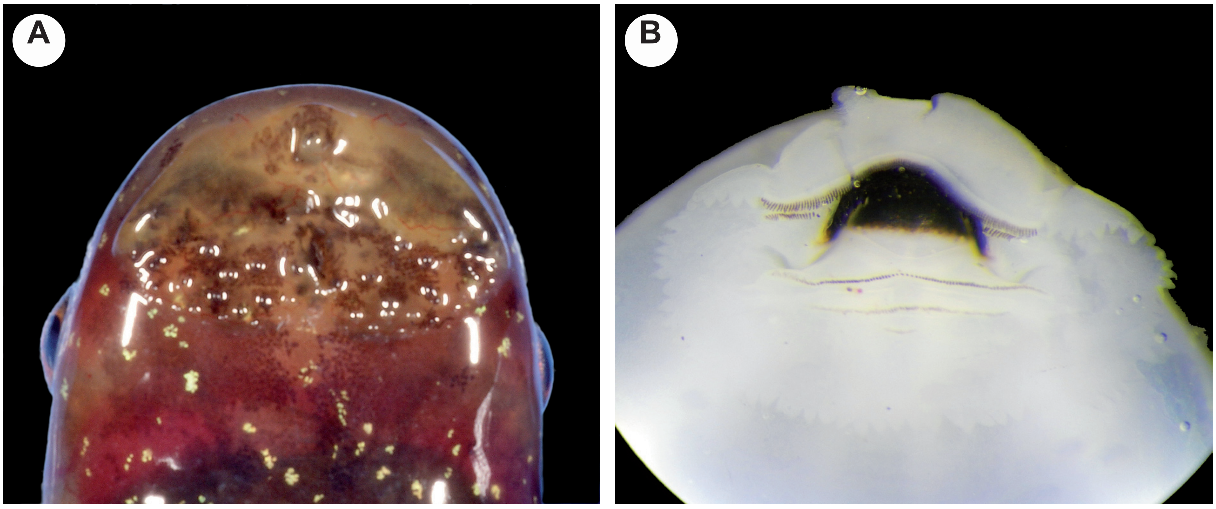

Figure 76: Ventral view of oral disks of tadpoles collected at the same creek in the same night in the La Fortuna Forest Reserve in 2013. **A** *Duellmanohyla uranochroa*, showing a complete loss of keratinized mouthparts. **B** *Hyalinobatrachium talamancae*, showing apparently normally keratinized mouthparts

peptides (Simmaco *et al.* 1998). To understand host-pathogen dynamics among amphibian species and we need to investigate wether intraspecific differences in AMP expression lead to immunological adaptation. A first allusion to this hypothesis was rendered by Tennessen *et al.* (2009) who examined intraspecific variation in the composition and activities of antimicrobial peptides expressed in the skin secretions of the Northern Leopard Frog (*Lithobates pipiens*) in conjunction with allelic differences. These authors demonstrated that individuals as well as geographically separated populations secrete different suites of peptides, that those peptides differ in their response to infectious diseases like *Bd* among others, and that these differences are reflected in genetic loci. However, it remains unclear whether this intra- and interpopulation diversity is due to natural selection, genetic drift, or phenotypic plasticity. Nevertheless, this approach is expendable and similar studies should be a main focus in future chytridiomycosis investigations. Another step towards understanding the evolutionary potential of AMPs against *Bd* in frogs has been undertaken by Woodhams *et al.* (2010). They investigated differences in AMPs among populations of the Australian treefrog *Litoria genimaculata*, and found *Bd* infected individuals from lowland populations to have lower relative intensities of AMPs compared to uninfected individuals. This relation was not found in highland populations, where infected and uninfected individuals had shown the same relative intensities in AMPs. Thus, in addition to AMP expression, environmental conditions have also an effect in the process of development of resistance against *Bd*. Neither Tennessen *et al.* (2009) nor Woodhams *et al.* (2010) had AMP samples of populations before *Bd* emergence at their disposal, and accordingly, possible effects of natural selection over time could not be examined. But in the case of the Panamanian species this should be feasible, as many *Bd* susceptible species have been brought to *ex-situ* projects before *Bd* emerged in their populations. Thus, individuals from both pre- and post-decline populations are available and should be objects of further investigation. Besides AMPs, a new approach is the selection on mucosal microbiota including increased prevalence of bacteria with antifungal capacity (Woodhams *et al.* 2014). Together, the mucosal products of host (e.g. AMPs) and microbiome are termed the mucosome. The mucosome function against *Bd* varies between different amphibian species and can predict *Bd* susceptibility.

7.5. CONCLUSIONS AND FUTURE STUDIES

Among the Panamanian amphibian species many taxonomical problems remain. We are still far from having an approximate idea of the actual number of amphibian species inhabiting the mountains of western Panama and in many species that were presumed to be single widespread species, recent studies (*e.g.*, Crawford *et al.* 2013; this work) revealed deep genealogic lineages, suggesting that amphibian diversity is largely underestimated. Especially morphologically difficult groups (*e.g.*, Terrarana) are much more diverse than originally anticipated (*e.g.*, Appendices 5.2.2.; 5.2.3.). DNA barcoding is a good tool for identification of discribed species, provided that the species identity of comparative sequences in DNA databases is correct. However, the hypothesis that a divergent genetic lineage can be called a species will always need to be tested against an integrative taxonomical background. Thus, species discovery will clearly remain a domain of taxonomy. In view of a fast developing Panama, the various threats the amphibian assemblages and the unique nature of this country are facing, a comprehensive amphibian species inventory is strongly needed. This information is essential to strike a balance between the conservation of nature and the continued successful economic development of an aspiring country such as Panama. Policy makers in Panama should take into account that the biological diversity is also a natural treasure besides precious metals like copper, gold, and silver.

Concerning chytridiomycosis and disease related amphibian decline, a high potential to understand disease dynamics comes from recovering amphibian populations. Now, almost 20 years after *Bd* arrival in Panama, data on which species are the survivors and how they are distributed is the key to learn more about the pathogen-host dynamics. With *ex-situ* populations of amphibians collected before arrival of *Bd*, we have the genetic disposition of some frog species prior to *Bd* exposure; albeit the founder effect in *ex-situ* populations likely led to a loss of genetic variation compared to the original gene pool. Since AMPs are coded by gens it should be possible to genetically compare pre-decline with post-decline populations to find out what differences exist between surviving and declining populations of particular species. It is also very interesting to further evaluate the potential of recovery against a taxonomical background. My results suggest, that if *Bd* enters a naïve amphibian comunity, ecological traits will predict which species have a higher risk of chytridiomycosis and subsequent population declines. However, my results also indicate that after epizootic stages taxonomic affiliation plays a key role in the ability to develop a resistance against chytridiomycosis with subsequent population recovery. While only single populations of the very susceptible bufonids and craugastorids survive in presumed climatic refuges, particularly centrolenids and some hylids seem to coexist with the pathogen today after having initially declined. Future research approaches could determine whether selection for disease resistance is acting on the skin mucosome function against *Bd* of different amphibian populations in western Panama and to determine whether phenotypic or developmental plasticity can affect mucosome function.

My results show that the Panamanian Cordillera Central is an area rich in amphibian species including many endemics. Much of the Serranía de Talamanca is protected by several large conservation areas. In contrast, the Serranía de Tabasará is nearly unprotected. My data show that the Serranía de Tabasará, and particularly the Cerro Colorado area, is not only an important refuge for amphibian diversity, but also a centre of endemism itself. I therefore recommend to designate additional protected areas in the Serranía de Tabasará. In consideration of the high number of endangered species found, the genetic lineages that remain taxonomically unidentified, and the relatively large remainder of primary cloud forest I identify the Cerro Colorado area as the key biodiversity area in the Serranía de Tabasará.

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10. Appendices

10.1. Appendix 1: List of the amphibian species known to occur in Panama.

Amphibian species of Panama	IUCN Red List Category (Version 2013.2)	EVS (Jaramillo *et al.* 2010)	Panama endemic	Reported from study area	Reconfirmed 2008–2013
Order Anura					
Family Aromobatidae					
Subfamily Anomaloglossinae					
Anomaloglossus astralogaster	NE	NE	X		
A. isthminus	NE	NE	X		
Subfamily Aromobatinae					
Allobates talamancae	LC	8			
Family Bufonidae					
Atelopus certus	EN	10	X		
A. chiriquiensis	CR	9		X	
A. glyphus	CR	6			
A. limosus	EN	10	X		
A. varius	CR	9		X	X
A. zeteki	CR	9	X		
Incilius aucoinae	LC	10			
I. coniferus	LC	6		X	X
I. epioticus	LC	12		X	X
I. fastidiosus	CR	10		X	
I. karenlipsae	NE	NE	X		
I. majordomus	NE	NE	X	X	
I. melanochlorus	LC	9			
I. peripatetes	CR	10	X	X	
I. signifer	LC	10	X	X	
Rhaebo haematiticus	LC	5		X	X
Rhinella acrolopha	DD	12?			
R. alata	DD	7			
R. centralis	NE	NE	X	X	
R. margaritifera	LC	7			
R. marina	LC	5		X	X
Family Centrolenidae					
Subfamily Centroleninae					
Cochranella euknemos	LC	8		X	
C. granulosa	LC	7		X	X
Espadarana prosoblepon	LC	7		X	X
Sachatamia albomaculatum	LC	8		X	X
S. ilex	LC	8			
Teratohyla pulverata	LC	9		X	X
T. spinosa	LC	8		X?	
Subfamily Hyalinobatrachinae					
Hyalinobatrachium aureoguttatum	NT	10			
H. chirripoi	LC	9			
H. colymbiphyllum	LC	8		X	

H. fleischmanni	LC	7		X	X
H. talamancae	LC	NE		X	X
H. valerioi	LC	8		X	
H. vireovittatum	DD	11		X	X?
Family Craugastoridae					
Subfamily Craugastorinae					
Craugastor azueroensis	EN	13	X		
C. bransfordii	LC	9		X	X
C. catalinae	CR	12		X	
C. crassidigitus	LC	9		X	X
C. emcelae	CR	14	X	X	
C. evanesco	NE	NE	X		
C. fitzingeri	LC	8		X	X
C. gollmeri	LC	12		X	X
C. gulosus	EN	13		X	
C. jota	DD	15	X	X	X
C. longirostris	LC	10			
C. megacephalus	LC	9		X	X
C. melanostictus	LC	12		X	X
C. monnichorum	DD	13	X	X	X
C. noblei	LC	9		X	X
C. obesus	EN	12		X	
C. opimus	LC	9			
C. podiciferus	NT	12		X	X
C. polyptychus	LC	12			
C. punctariolus	EN	12	X	X	
C. raniformis	LC	8			
C. ranoides	CR	9		X	
C. rhyacobatrachus	EN	13		X	
C. rugosus	LC	12			
C. stejnegerianus	LC	13		X	X
C. tabasarae	CR	13	X	X	
C. talamancae	LC	9		X	X
C. taurus	CR	13		X	
Subfamily Pristimantinae					
Pristimantis achatinus	DD	11			
P. adnus	NE	NE	X		
P. altae	NT	13			
P. caryophyllaceus	NT	8		X	X
P. cerasinus	LC	9		X	X
P. cruentus	LC	8		X	X
P. gaigei	LC	9			
P. moro	LC	8		X	X
P. museosus	EN	13	X	X	X
P. pardalis	NT	12		X	X
P. pirrensis	DD	14	X		
P. ridens	LC	9		X	X
P. taeniatus	LC	7		X?	X
Subfamily Strabomantinae					
Strabomantis bufoniformis	LC	9			
S. laticorpus	DD	10	X		

Family Dentrobatidae					
Subfamily Colostethinae					
Ameerega maculata	DD	15?	X	X?	
Colostethus latinasus	DD	13	X		
C. panamansis	LC	9		X	
C. pratti	LC	9		X	
Silverstoneia flotator	LC	12		X	
S. nubicola	NT	8		X	X
Subfamily Dentrobatinae					
Andinobates claudiae	DD	15	X		
A. fulguritus	LC	9			
A. minuta	LC	9			
Dendrobates auratus	LC	8		X	X
Hyloxalus chocoensis	DD	11			
Oophaga arborea	EN	13	X	X	
O. granulifera	VU	12		X	
O. pumilio	LC	9		X	X
O. speciosa	EN	14	X	X	
O. vicentei	DD	13	X		
Phyllobates lugubris	LC	10		X	
Subfamily Hyloxalinae					
Hyloxalus chocoensis	DD	11			
Family Eleutherodactylidae					
Subfamily Eleutherodactylinae					
Diasporus citrinobapheus	NE	NE	X	X	X
D. diastema	LC	7		X?	
D. igneus	NE	NE	X	X	X
D. hylaeformis	LC	13		X	X
D. quidditus	LC	10			
D. vocator	LC	10		X	X
Family Hemiphractidae					
Gastrotheca cornuta	EN	10		X	
G. nicefori	LC	10		X	
Hemiphractus fasciatus	NT	9		X	
Family Hylidae					
Subfamily Hylinae					
Anotheca spinosa	LC	11		X	
Dendropsophus ebraccatus	LC	5		X	X
D. microcephalus	LC	5		X	X
D. phlebodes	LC	5		X	X
D. subocularis	LC	7			
Duellmanohyla lythrodes	EN	10		X	
D. uranochroa	EN	9		X	X
Ecnomiohyla bailarina	NE	NE	X		
E. fimbrimembra	EN	15		X	X
E. miliaria	VU	11		X	
E. rabborum	CR	17	X		
E. thysanota	DD	17?	X		
E. veraguensis	NE	NE	X	X	X
Hyloscirtus colymba	CR	10		X	X
H. palmeri	LC	6		X	X

Hypsiboas boans	LC	7			
H. crepitans	LC	5			
H. pugnax	LC	6		X	X
H. rosenbergi	LC	6		X	X
H. rufitelus	LC	6		X	X
Isthmohyla angustilineata	CR	10		X	
I. calypsa	CR	12		X	
I. debilis	CR	10		X	X
I. graceae	CR	10	X	X	X
I. infucata	DD	12	X	X	X
I. lancasteri	LC	9		X	X
I. picadoi	NT	14		X	X
I. pseudopuma	LC	10		X	
I. rivularis	CR	10		X	X
I. tica	CR	9		X	X
I. zeteki	NT	15		X	X
Ptychohyla legleri	EN	10		X	X
Scinax altae	LC	10	X	X	X
S. boulengeri	LC	6		X	X
S. elaeochrous	LC	7		X	X
S. rostratus	LC	6			
S. ruber	LC	5			
Smilisca phaeota	LC	5		X	X
S. sila	LC	5		X	X
S. sordida	LC	7		X	X
Trachycephalus typhonius	LC	6		X	X
Subfamily Phyllomedusinae					
Agalychnis annae	EN	NE		X	X
A. callidryas	LC	7		X	X
A. lemur	CR	9		X	X
A. spurelli	LC	8		X	
Cruziohyla calcarifer	LC	8		X	
Phyllomedusa venusta	LC	11			
Family Leptodactylidae					
Subfamily Leiuperinae					
Engystomops pustulosus	LC	6		X	X
Pleurodema brachyops	LC	8			
Subfamily Leptodactylinae					
Leptodactylus fragilis	LC	6		X	X
L. fuscus	LC	8			
L. insularum	LC	7		X	X
L. melanonotus	LC	6		X	X
L. poecilochilus	LC	5			
L. savagei	LC	6		X	X
Family Microhylidae					
Subfamily Gastrophryninae					
Ctenophryne aterrima	LC	6		X	
Elachistocleis ovalis	LC	5			
E. panamensis	LC	6			
E. pearsei	LC	NE		X	X
Family Pipidae					

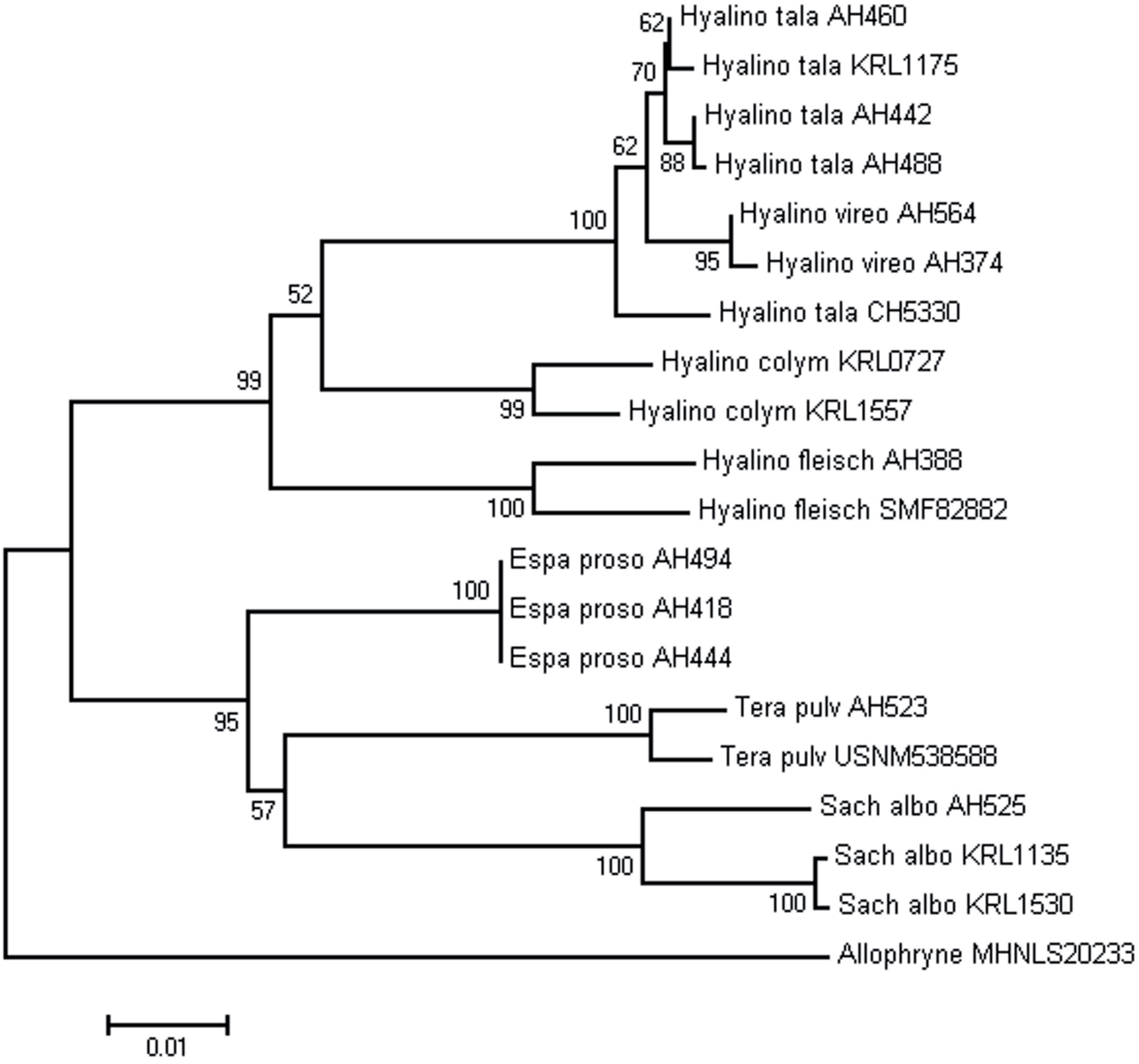
Pipa myersi	EN	15			
Family Ranidae					
Lithobates taylori	LC	9		X	X
L. vaillanti	LC	7		X	X
L. vibicarius	CR	9		X	
L. warszewitschii	LC	6		X	X
Order Caudata					
Family Plethodontidae					
Subfamily Hemidactyliinae					
Bolitoglossa (Eladinea) anthracina	DD	13	X	X	X
B. biseriata	LC	9			
B. bramei	DD	13		X	
B. colonnea	LC	12		X	X
B. compacta	EN	13		X	X
B. copia	DD	15	X		
B. cuna	DD	14	X		
B. gomezi	DD	13		X	X
B. jugivagans	NE	NE	X	X	X
B. magnifica	EN	13	X	X	X
B. marmorea	EN	13	X	X	X
B. medemi	VU	9			
B. minutula	EN	13		X	X
B. nigrescens	EN	NE		X	X
B. phalarosoma	DD	11			
B. pygmaea	DD	NE	X		
B. robinsoni	DD	NE		X	
B. robusta	LC	12		X	
B. schizodactyla	LC	12			
B. taylori	DD	11	X		
Bolitoglossa (Bolitoglossa) lignicolor	VU	12		X	X
Oedipina (Oedipina) alfaroi	VU	13		X	
O. collaris	DD	9		X	
O. cyclocauda	LC	12		X	
O. gracilis	EN			X	
O. grandis	EN	12		X	
O. pacificensis	LC	13		X	
Oedipina (Oedopinola) alleni	LC			X	
O. complex	LC	9		X	X
O. fortunensis	NE	15	X	X	X
O. maritima	CR	15	X		
O. parvipes	LC	9			
O. savagei	DD	NE		X	
Order Gymnophiona					
Family Caeciliidae					
Caecilia elongata	DD	16	X	X?	
C. isthmica	DD	13?			
C. leucocephala	LC	13			
C. nigricans	LC	13			
C. ochrocephala	LC	10			
C. volcani	DD	14	X	X	
Family Dermophiidae					

Dermophis glandulosus	DD	13		X	
D. gracilior	DD	14		X	
D. parviceps	LC	13		X	
multiplicata	LC	10		X	
Total: 214			**48**	**148**	**90**

10.2. APPENDIX 2: PRELIMINARY BARCODING RESULTS

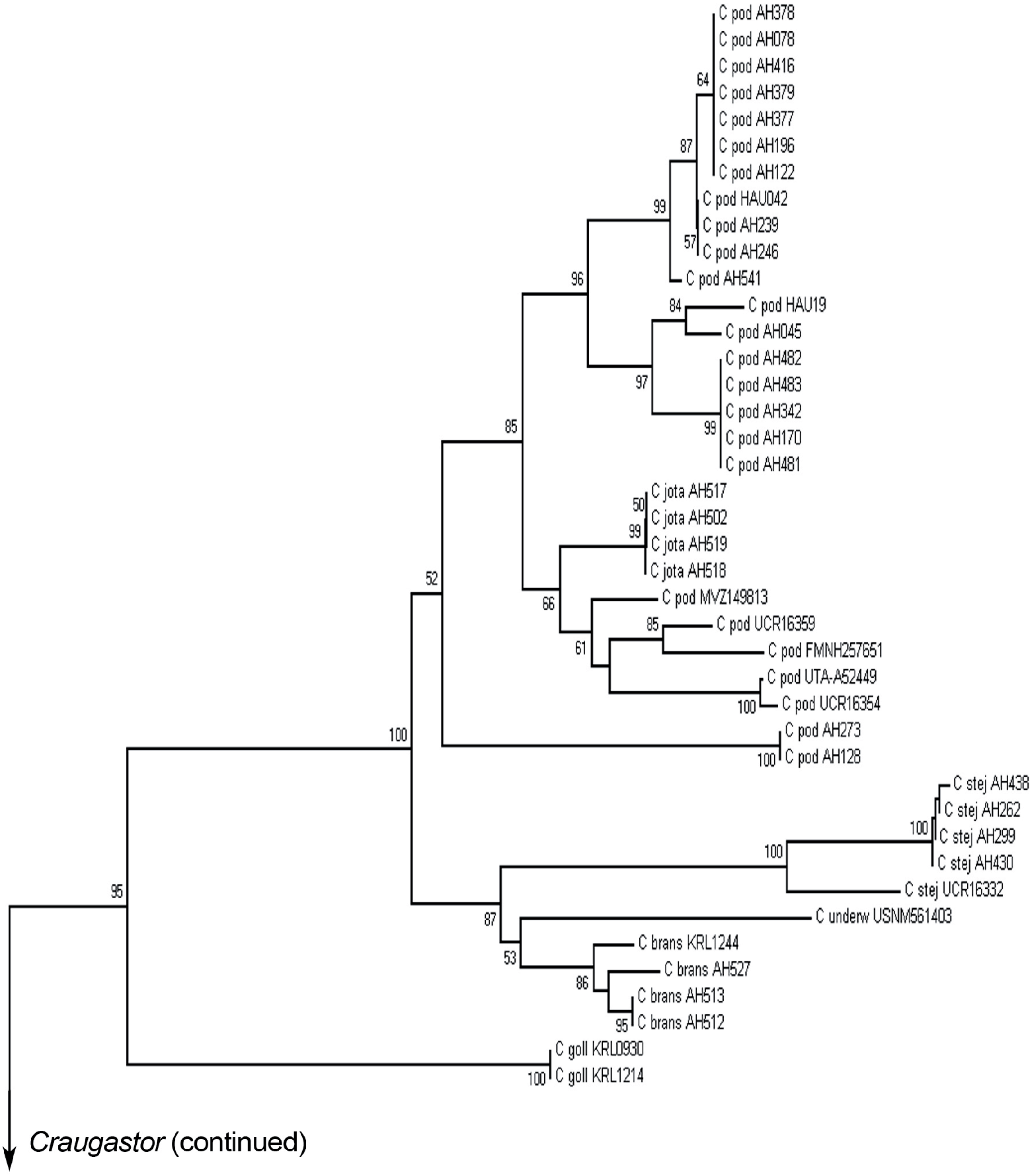
Preliminary 16S mtDNA barcoding results of supplementarily processed Panamanian amphibian taxa. Phylogenies were inferred using the Neighbor-Joining method (NJ). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (10000 replicates) are shown next to the nodes. Trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method and are in the units of the number of base differences per site. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. Evolutionary analyses were conducted in MEGA 5.1.

10.2.1. ANURA; CENTROLENIDAE

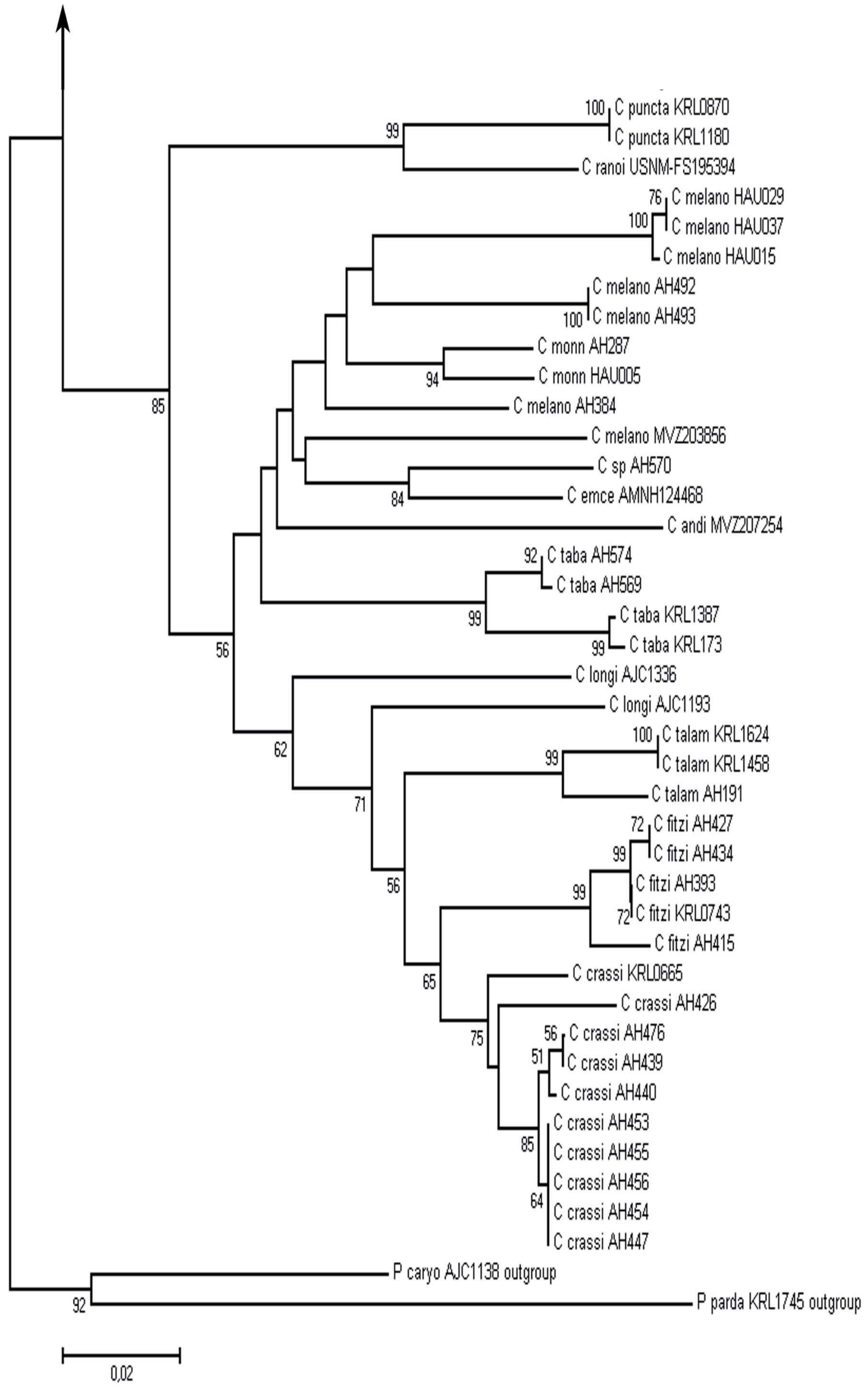


NJ tree of Centrolenidae (outgroup *Allophryne ruthveni*): The optimal tree is shown. The analysis involved 20 nucleotide sequences. There were a total of 530 positions in the final dataset.

10.2.2. Anura; Craugastoridae; *Craugastor*

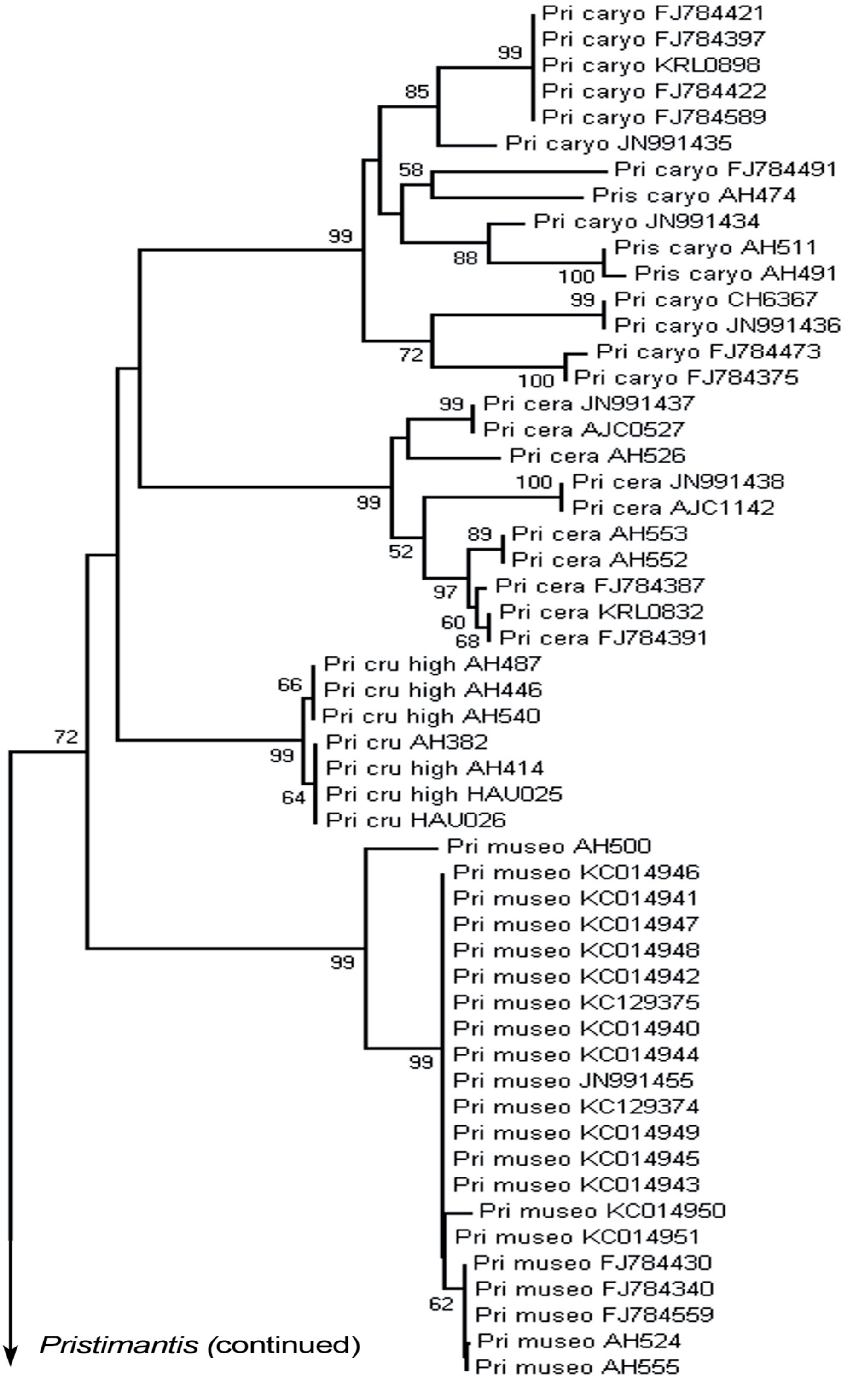


NJ tree of Craugastorinae (outgroup *Pristimantis caryophyllaceu*s and *P. pardalis*): Subtree containing specimens of the *Craugastor podiciferus* Species Group and *Craugastor gollmer*i. The optimal tree is shown. The analysis involved 82 nucleotide sequences. There were a total of 527 positions in the final dataset.



NJ tree of Craugastorinae continued: Subtree containing specimens of the *Craugastor punctariolus* and *C. fitzingeri* Species Series.

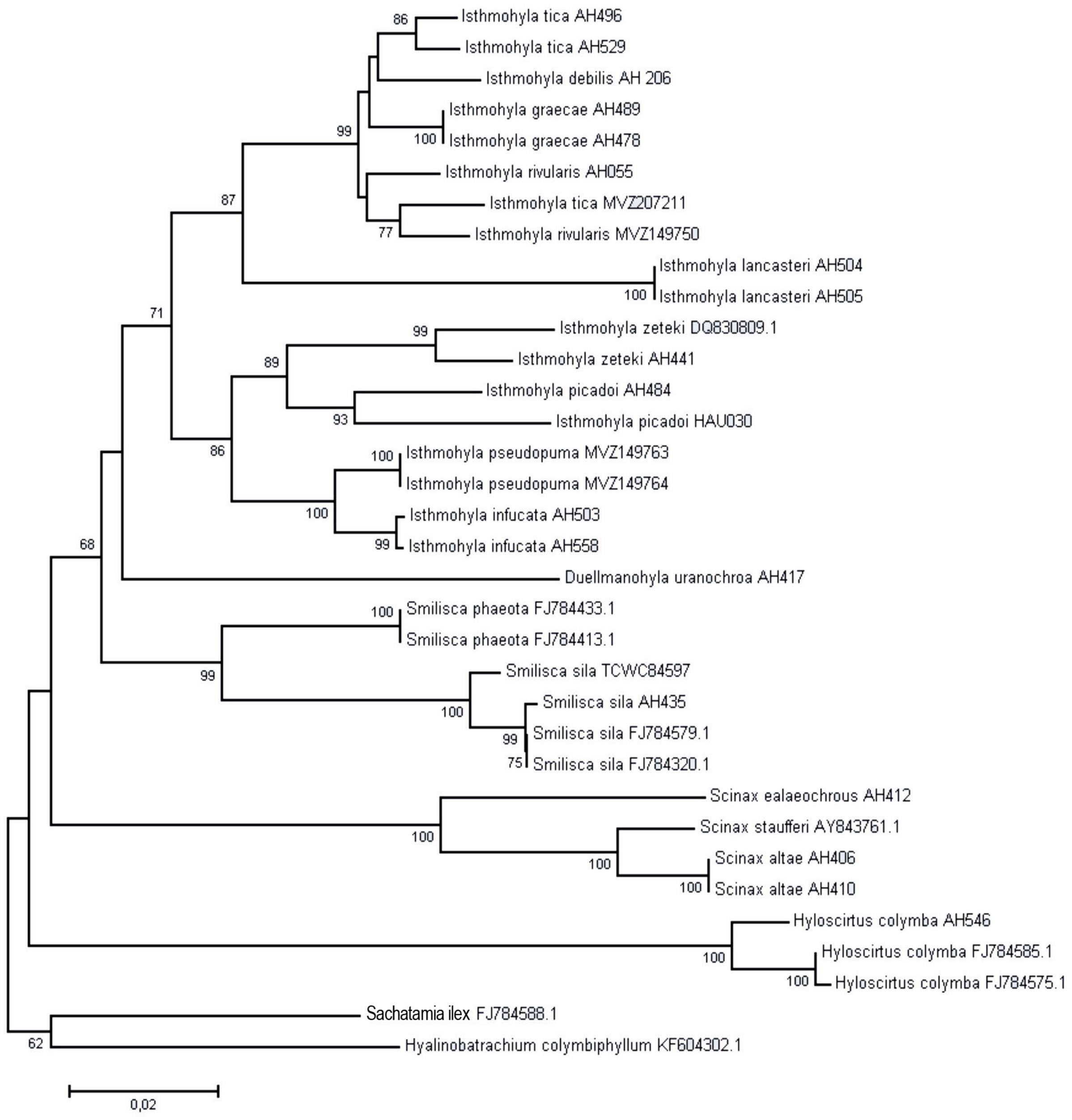
10.2.3. ANURA; CRAUGASTORIDAE; *PRISTIMANTIS*



NJ tree of Pristimantinae (outgroup *Oreobates barituensis* and *O. quixensis*): Subtree containing specimens of the *Pristimantis ridens* Species Series and *Pristimantis cerasinus*. The optimal tree is shown. The analysis involved 112 nucleotide sequences. There were a total of 509 positions in the final dataset.

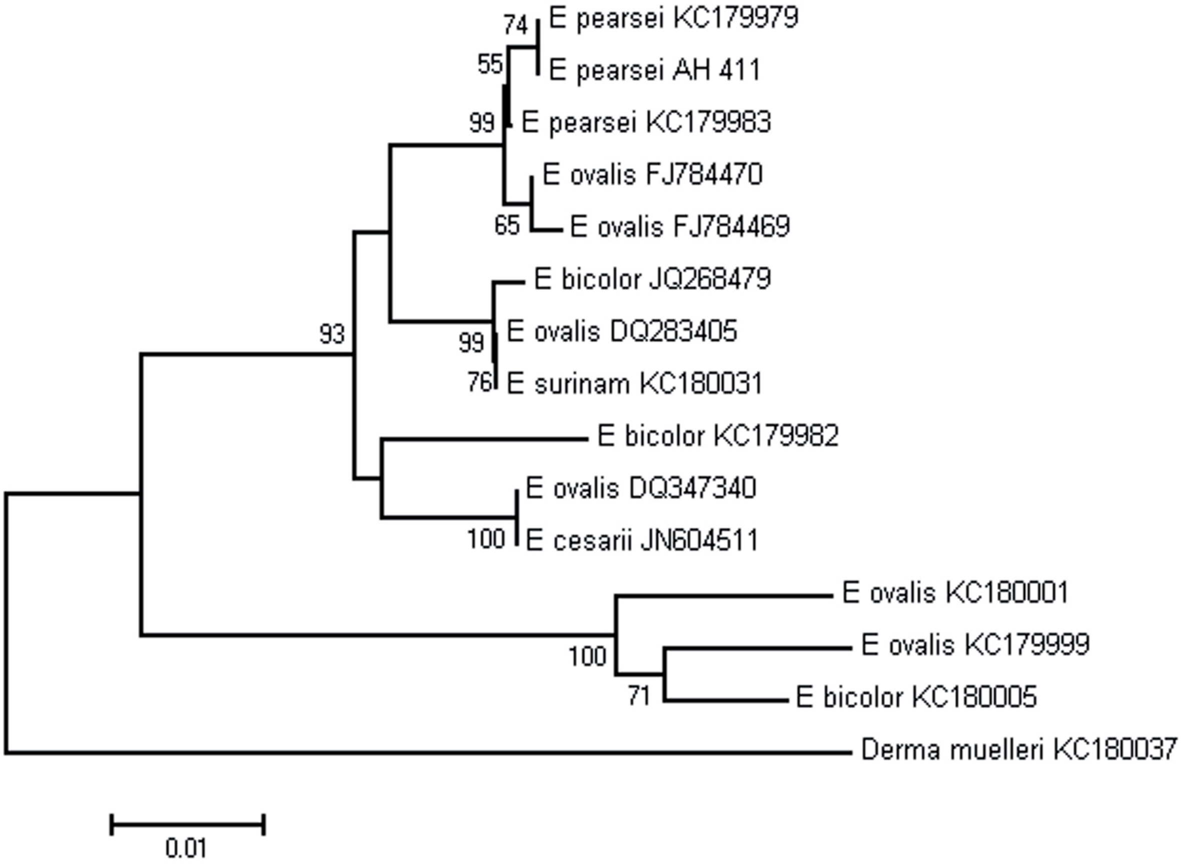
NJ tree of Pristimantinae continued: Subtree containing specimens of the *Pristimantis ridens* Species Series and *Pristimantis taeniatus*.

10.2.4. ANURA; HYLIDAE



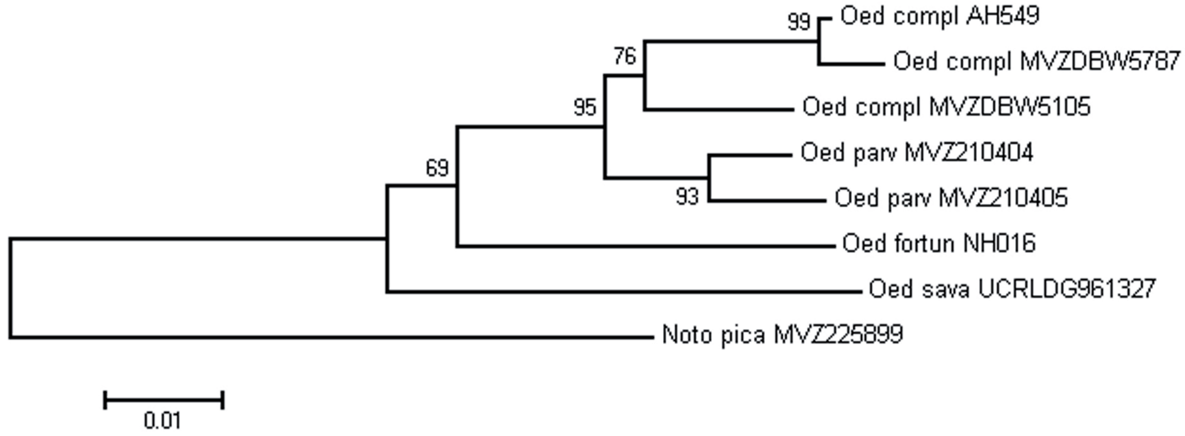
NJ tree of Hylidae (outgroup *Sachatamia ilex* and *Hyalinobatrachium colymbiphyllum*): Subtree containing specimens of the genera Duellmanohyla, Hyloscirtus, Isthmohyla, Scinax, and Smilisca. The optimal tree is shown. The analysis involved 34 nucleotide sequences. There were a total of 532 positions in the final dataset.

10.2.5. ANURA; MICROHYLIDAE; *ELACHISTOCLEIS*



NJ tree of *Elachistocleis* (outgroup *Dermatonotus muelleri*) comparing AH 411 (SMF 89808) from Panama with GenBank sequences. The optimal tree is shown. The analysis involved 15 nucleotide sequences. There were a total of 500 positions in the final dataset.

10.2.6. CAUDATA; PLETHODONTIDAE; *OEDIPINA*



NJ tree of *Oedipina* (outgroup *Nototriton picadoi*): comparing AH 411 (SMF 89808) from Panama with GenBank sequences. The optimal tree is shown. The analysis involved 8 nucleotide sequences. There were a total of 517 positions in the final dataset.

10.3. OWN CONTRIBUTIONS TO RESULTS PUBLISHED IN ADVANCE

Some of the results incorporated in this doctoral thesis have been published in advance. In all papers I have been strongly involved in the publication process, including several rounds of proofreading. The papers as originally published can be viewed in the digital appendix (SF 1).

Batista, Ponce, and Hertz. 2012. *Zootaxa* 3410:51–60.: I collected one paratype and took measurements of that specimen. I played a major role in the interpretation of the results, created the map, and wrote parts of the manuscript.

Batista, Hertz, Mebert, Köhler, Lotzkat, Ponce, and Vesely. 2014a. *Zootaxa* 3826: 449–474.: I collected the holotype of Ecnomiohyla veraguensis and the examined specimen of E. fimbrimembra together with S. Lotzkat. I took all the measurements and photos of these two specimens, and realized that SMF89877 (AH 210) is new to science. I wrote large parts of the manuscript that are concerned with E. veraguensis.

Batista, Hertz, Köhler, Mebert, and Vesely. 2014b. *Salamandra* **50 (3): 155–171.:** I collected large parts of the specimens from western Panama and did parts of the molecular laboratory work. I played a major role in the interpretation of the results and wrote parts of the manuscript.

Hertz, and Lotzkat. 2012. *Herpetology Notes* 5: 37–39.: I collected all specimens together with S. Lotzkat, performed the literature research, gathered the locality data for the map, wrote the manuscript, and created the figures.

Hertz, Lotzkat, Stadler, Hamad, Carrizo, and Köhler. 2011. *Herpetological Review* 42 (2): 245–250.: I collected all the material together with my coauthors. I examined and identified the species, took most of the photos, took all measurements, did the literature research, created the map, and wrote the manuscript.

Hertz, Batista, and Köhler. 2012a. *Herpetology Notes* 5: 355–359.: I took the recording and collected the voucher specimens. I ran the sound analysis, took the measurements and made the call description. I created all figures except Fig. 1. and wrote the manuscript.

Hertz, Hauenschild, Lotzkat, and Köhler. 2012b. *ZooKeys* **196: 23–46.:** I collected the type material together with S. Lotzkat, made the call recordings, and realized that the species is new to science. I took all the morphological measurements, did parts of the molecular laboratory work, ran the molecular and call analyses. I created all figures and tables and wrote the manuscript.

Hertz, Lotzkat, Carrizo, Ponce, Köhler, and Streit. 2012c. ***Amphibian and Reptile Conservation*** **6: 9–30.:** I collected most of the material together with S. Lotzkat. I examined and identified the specimens, took most of the photos, ran the GIS analysis, and made the calculations. I made the literature research and wrote the manuscript.

Hertz, Lotzkat, and Köhler. 2013a. *Zootaxa* **3636: 463–475.:** I collected the holotype together with S. Lotzkat. I did parts of the molecular laboratory work, took the morphological measurements, and performed the molecular and morphological analyses. I took all photos and radiographs, created all figures, and wrote the manuscript.

Hertz, Lotzkat, and Köhler. 2013b. ***CheckList*** **9(1): 83–91.:** I collected parts of the examined material together with S. Lotzkat and took most of the photos. I took the morphological measurements and did parts of the molecular laboratory work. I performed the molecular and morphological analyses, created all figures and wrote the manuscript.

Köhler, Batista, Carrizo, and Hertz. 2012. ***Herpetology Notes*** **5: 157–162.:** I discussed the results with the other authors, did the literature research on disease related population declines, and wrote large parts of the discussion chapter in the manuscript.

Lotzkat, and Hertz. 2011 "2010". ***Puente Biológico*** **3: 89–99.:** I collected all the material together with S. Lotzkat. I examined and identified all amphibian specimens and wrote the parts of the manuscript concerned with amphibians.

10.4. EIDESSTATTLICHE VERSICHERUNG

Ich erkläre hiermit an Eides Statt, dass ich die vorgelegte Dissertation

INTEGRATIVE TAXONOMY AND CONSERVATION STATUS OF AMPHIBIANS IN WESTERN PANAMA WITH AN EMPHASIS ON THE HIGHLANDS OF THE CORDILLERA CENTRAL

selbständig angefertigt und mich anderer Hilfsmittel als der in ihr angegebenen nicht bedient habe, insbesondere, dass alle Entlehnungen aus anderen Schriften mit Angabe der betreffenden Schrift gekennzeichnet sind.
Ich versichere, die Grundsätze der guten wissenschaftlichen Praxis beachtet, und nicht die Hilfe einer kommerziellen Promotionsvermittlung in Anspruch genommen zu haben.

Frankfurt am Main, den ....
(Unterschrift)