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Mandibular gland proteomics of the Mexican alligator lizard, *Abronia graminea,* and the red-lipped arboreal alligator lizard, *Abronia lythrochila*

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ABSTRACT

A useful approach to deepen our knowledge about the origin and evolution of venom systems in Reptilia has been exploring the vast biodiversity of this clade of vertebrates in search of orally produced proteins with toxic actions, as well as their corresponding delivery systems. The occurrence of toxins in anguimorph lizards has been demonstrated experimentally or inferred from reports of the toxic effects of the oral secretions of taxa within the Varanidae and Helodermatidae families. In the present study, we have focused on two alligator lizards of the Anguidae family, the Mexican alligator lizard, *Abronia graminea*, and the red-lipped arboreal alligator lizard, *A. lythrochila*. In addition, the fine morphology of teeth of the latter species is described. The presence of a conserved set of proteins, including B-type natriuretic peptides, cysteine-rich secretory proteins, group III phospholipase A_2, and kallikrein, in submandibular gland extracts was demonstrated for both *Abronia* species. These proteins belong to toxin families found in oral gland secretions of venomous reptile species. This finding, along with previous demonstration of toxin-producing taxa in both paleo- and neoanguimorpha clades, provides further support for the existence of a handful of conserved toxin families in oral secretions across the 100+ million years of Anguimorpha cladogenesis.

1. Introduction

The Anguimorpha suborder of squamate reptiles was named by Max Fürbringer in 1900 to include all autarchoglossans (scincomorphs, anguimorphs, and varanoids) closer to Varanus and Anguis than to Scincus (Fürbringer, 1900). Currently, this clade includes approx. 250 species (Uetz et al., 2017), which according to molecular data are distributed in two divergent clades rooted in the early to mid-Cretaceous, 127.1 (105.5–148.7) million years ago (Mya) (Douglas et al., 2010; Reeder et al., 2015; Zheng and Wiens, 2016): Paleoanguimorpha comprising the families Shinisauridae (Chinese crocodile lizard), Lanthanotidae (the Bornean earless monitor), and Varanidae (monitor lizards), and Neoanguimorpha (families Helodermatidae (Gila monsters and Mexican beaded lizards), Anguidae (alligator lizards, glass lizards, American legless lizards), and Xenosauridae (knob-scaled lizards) (Vidal and Hedges, 2009; Hedges and Vidal, 2009; Wiens et al., 2012; Pyron et al., 2013; but consult Cerňanský et al. (2014) regarding lack of morphological support for the topology of the proposed Neoanguimorpha clade).

Anguimorph lizards, along with iguanians and snakes, constitute the proposed monophyletic Toxicofera clade (Greek for "those who bear toxins") of all venomous reptiles (Vidal and Hedges, 2005; Fry et al., 2006; Wiens et al., 2012; Fry et al., 2012). The advocates for this hypothesis estimated that the last common ancestor of all toxicoferan reptiles lived about 170 million years ago (Mya) in the mid Jurassic period of the Mesozoic Era (Fry et al., 2006; Vidal and Hedges, 2009). The Toxicofera clade encompasses about 4600 extant species of Squamata within the suborders Serpentes, Anguimorpha, and Iguania, including all known venomous reptiles and an undefined number of related non-venomous species. However, both the definition of "venomous" and the assertions that "all toxicoferan reptiles descended from a common venomous ancestor" and that there was a "single common origin of venom at the base of the clade" (Fry et al., 2012), have been subjected to much criticism (Weinstein et al., 2012; Kardong, 2012;

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Jackson et al., 2013a, 2013b; Hargreaves et al., 2014). Also, conflicting topologies have been inferred for the placement of iguanians (iguanas, anoles, chameleons, dragons, and relatives) in the squamate reptile phylogeny (Losos et al., 2012): at the base of the tree based on morphological data (Gauthier et al., 2012), and in the Toxicofera clade, with snakes and anguimorphs, based on molecular datasets (Wiens et al., 2012). The discrepacy was resolved through integration of molecular, morphological, and paleontological data, with the retention of Iguania and the unexpected placements for two major fossil lineages (Mosasauria and Polyglyphanodontia) within Toxicofera (Reeder et al., 2015).

The occurrence of toxins in anguimorph lizards has been demonstrated experimentally or inferred from reports of the toxic effects of the oral secretions of a few taxa within the monogeneric families Varanidae (Hardwicke and Gray, 1827) and Helodermatidae (Gray, 1837; Dobson et al., 2024 and references therein). Throughout this manuscript we use the term "toxin" for proteins that exert toxic activities, regardless of whether those activities are carried out in an ecological context or not. Within genus *Varanus*, species from which proteins with characterised toxic activities have been documented include the iconic Komodo dragon, *V. komodoensis*, (Fry et al., 2009) and the monitor lizards *V. griseus*, *V. bengalensis*, *V. kordensis*, *V. albigularis*, *V. varius*, and *V. salvadorii* (Koludarov et al., 2017). Venomous species within the North American genus *Heloderma* include the Gila monsters (*H. suspectus* ssp) and the Mexican beaded lizards (*H. horridus* ssp.) (Mebs, 1968; Mebs 1969a; Mebs 1969b; Hendon and Tu, 1981; Nikai et al., 1992; Utaisincharoen et al., 1993; Huang and Chiang, 1994; Tu, 2000; Fry et al., 2010a; Reiserer et al., 2013). In a recent study (Dobson et al., 2024) demonstrated anticoagulant toxicity due to destructive cleavage of fibrinogen and procoagulant bioactivity on human and bird plasma for Varanus and Heloderma venoms, respectively. This study also examined the effects upon the cardiovascular system, including the liberation of kinins from kininogen, which contributes to hypotension induction. Helodermatid bites can produce serious symptoms such as angioedema, hypotension, cardiac ischemia, and bronchoconstriction (Amri and Chippaux, 2021), and similar effect of angioedema and hypotension have been recorded in bites from *V. griseus* and *V. komodoensis* (Ducey et al., 2016; Zima, 2019). Proteomic analyses of the mandibular glands of the Borneo earless monitor, *Lanthanotus borneesis*, confirmed studies of Koludarov et al. (2017) and Fry et al. (2006, 2009, 2010a, b) showing that kallikrein enzymes represent major components in oral gland secretions of anguimorphan lizards. More recently, Dobson et al. (2024) provided the first evidence for kinin-generating activity in *L. borneensis* oral secretion. Conversely, proteomic analysis of mandibular extract of the Chinese crocodile lizard, *Shinisaurus crocodilurus* (Calvete et al., 2023), provided no evidence of venom-derived peptides or proteins, strongly supporting the non-venomous character of this anguid lizard.

The Borneo earless monitor and the Chinese crocodile lizard are the single species of their respective subfamilies (Lanthanotidae and Shinisauridae) and, together with Varanidae, represent the sister clade of the Neoanguimorpha clade (Douglas et al., 2010; Wiens et al., 2012; Pyron et al., 2013; Zheng and Wiens, 2016). Transcriptome analysis of the mandibular gland of the Mexican alligator lizard, *Abronia graminea*, has revealed transcripts encoding putative bioactive peptides and proteins similar to those found in the oral gland secretion of the true venomous lizards *Heloderma* spp., including helokinestatin, natriuretic, celestin and cholecystokinin peptides inducing hypotension, the neurotoxic helofensin, lectins, nerve growth and vascular endothelial growth factors, as well as the enzymes kallikrein, group III phospholipase A_2 and hyaluronidase (Koludarov et al., 2012).

Alligator lizards of genus *Abronia* belong to the family Anguidae within the neoanguimorpha clade. With 87 extant species of lizards native to the Northern Hemisphere, the family Anguidae represents a group of three subfamilies found across the Northern Hemisphere, Anguinae (20 species of glass lizards), Anniellinae (5 species of American legless lizards), and Gerrhonotinae (62 species of alligator lizards), native to North and Central America (Uetz et al., 2017). The large fossil record for the Anguidae suggests that this clade probably evolved in North America during the Cretaceous before dispersing to Europe in the Paleogene, roughly 50 million years ago (Wiens and Slingluff, 2001). The 39 species of alligator lizards of genus *Abronia* listed in the Reptile Database (http://www.reptile-database.org; Uetz et al., 2017) are native to Mexico and Central America, across Guatemala, northern El Salvador, Honduras, Nicaragua, Costa Rica, and into northwestern Panama (Clause et al., 2016; Gutiérrez-Rodríguez et al., 2021; García-Vázquez et al., 2022). They are diurnal and usually live among bromeliads and other epiphytic plants high in the trees in upper to mid-elevation woodlands, particularly evergreen cloud forests and seasonally dry pine and pine-oak forests, where they are highly endangered due to habitat destruction, i.e. by deforestation and wildfire (Köhler, 2003). The first published wild dietary data for any arboreal species of *Abronia*, an analysis of faecal material from two individuals of *A. zongolica*, indicated that this species feeds on insects (Orthoptera, Coleoptera, Lepidoptera, and Hemiptera) along with other unidentified small invertebrates. (García-Vázquez et al., 2022).

In the present study, we report proteomic analyses of the

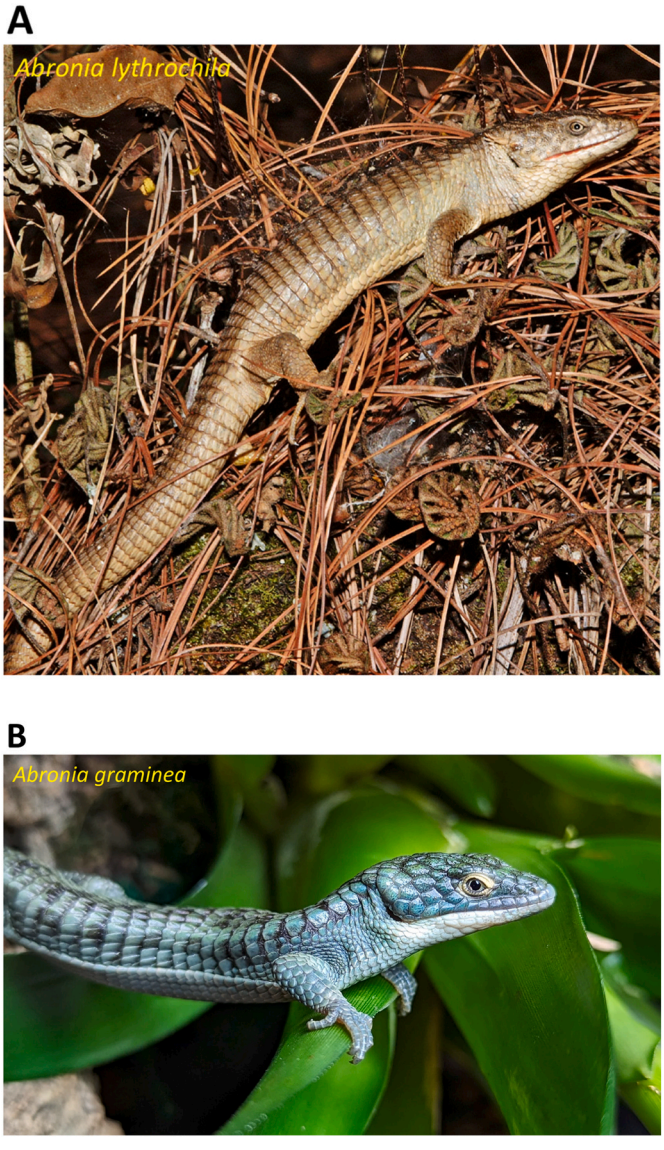

Fig. 1. Panel **A**) The red-lipped arboreal alligator lizard *Abronia lythrochila*. (Photo: Gunther Köhler); Panel **B**) The Mexican alligator lizard *Abronia graminea* (Photo: Anja Röselmaier). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

submandibular gland extracts recovered from frozen specimens of a Mexican alligator lizard, *Abronia graminea* (Fig. 1A), endemic to the Sierra Madre of the Oaxaca highlands, and from two red-lipped arboreal alligator lizards, *Abronia lythrochila* (Fig. 1B), endemic to the state of Chiapas in Mexico, to assess their putative toxin-producing activity.

2. Material and methods

2.1. Mandibular glands

The right and left mandibular glands were excised from a captive bred specimen of *Abronia graminea* (20 cm total body length) and two specimens of *A. lythrochila* (21 and 28 cm total body length), which had been kept frozen at - 20 °C for several months after their natural death. The glands were placed in separate 5 mL Eppendorf ProteinLoBind tubes and homogenized in physiological saline at 4 °C by ultrasonication. After centrifugation at 3.000 rpm at 4 °C for 15 min, the supernatants were immediately lyophilized and submitted to proteomic analysis.

2.2. Scanning electron microscopy (SEM)

The lower jaw of *A. lythrochila* was mounted on an aluminium holder, sputtered with gold, and analysed with a Hitachi S-4500 scanning electron microscope at an acceleration voltage of 5 kV (cold-field emission electron source).

2.3. Proteomic analyses

The mandibular gland extracts from both *Abronia* species were analysed using two proteomic strategies: a 'shotgun' MS/MS approach (Lomonte and Fernández, 2022) at the Proteomics Unit of Instituto Clodomiro Picado, and an RP-HPLC/SDS-PAGE decomplexation bottom-up LC-MS/MS workflow (Calvete, 2014) at the proteomics facility of Instituto de Biomedicina de Valencia. Protein concentration was quantifed spectrophotometrically using a NanoDrop™ One instrument (Thermo Scientific) and selecting a standard extinction coefficient of 1 absorbance unit at 280 nm for a 1 mg/mL protein solution measured in a 1 cm-.pathlength quarz cuvette.

For the shotgun analysis, 15 μg of total proteins extracted from the pooled left and right gland extract were diluted in 25 mM ammonium bicarbonate and subjected to reduction with 10 mM dithiothreitol (30 min at 56 °C), alkylation with 50 mM iodoacetamide (20 min in the dark), and digestion with sequencing grade trypsin (0.25 μg/sample, 37 °C, overnight). After stopping the reaction with 0.4 μL of formic acid, the tryptic peptide digests were centrifuged and separated by RP-HPLC on a nano-Easy 1200 chromatograph (Thermo) in-line with a Q-Exactive Plus® mass spectrometer (Thermo). Ten μL samples (~0.7 μg of peptide mixture) were loaded onto a C_{18} trap column (75 μm × 2 cm, 3 μm particle; Thermo), washed with 0.1% formic acid (solution A), and separated at 200 nL/min on a C_{18} Easyspray PepMap® column (75 μm × 15 cm, 3 μm particle; Thermo). A gradient toward solution B (80% acetonitrile, 0.1% formic acid) was developed for a total of 45 min (1–5% B in 1 min, 5–26% B in 30 min, 26–79% B in 6 min, 79–99% B in 2 min, and 99% B for 6 min). MS spectra were acquired in positive mode at 1.9 kV, with a capillary temperature of 200 °C, using 1 μscan in the range 400–1600 m/z, maximum injection time of 50 msec, AGC target of 1×10^6, and resolution of 70,000. The top 10 ions with 2–5 positive charges were fragmented with AGC target of 3×10^6, minimum AGC 2×10^3, maximum injection time 110 msec, dynamic exclusion time 5 s, and resolution 17,500. MS/MS spectra were processed against protein sequences contained in the UniProt database (Release, 2023_5) for Lepidosauria (625556 entries) using Peaks X® (Bioinformatics Solutions). Parent and fragment mass error tolerances were set at 15.0 ppm and 0.5 Da, respectively. Cysteine carbamidomethylation was set as fixed modification, while methionine oxidation and deamidation of asparagine or glutamine were set as variable modifications. A maximum of 2 missed cleavages by trypsin in semispecific mode were allowed. Filtration parameters for match acceptance were set to FDR<0.1%, detection of ≥1 unique peptide, and −10logP protein score ≥30.

For the bottom-up strategy, the proteins extracted from the left and right mandibular glands of *Abronia graminea* and *A. lythrochila* were separated by SDS-PAGE in 10% polyacrylamide gels run under reducing conditions. Protein bands were excised from Coomassie Brilliant Blue-stained gels and subjected to automated in-gel reduction and alkylation (as described above for the 'shotgun' MS/MS approach) on a Genomics Solution ProGest™ Protein Digestion Workstation. Tryptic digests were submitted to MS/MS analysis on a nano-Acquity Ultra-Performance LC® (UPLC®) equipped with a BEH130 C_{18} (100 μm × 100 mm, 1.7 μm particle size) column in-line with a Waters SYNAPT G2 High Definition mass spectrometer. Doubly and triply charged ions were selected for CID-MS/MS. Fragmentation spectra were submitted to MASCOT Server (version 2.6) at http://www.matrixscience.com and matched against the [bony vertebrates] taxonomy restricted dataset of the NCBI non-redundant database (release 258 of October 15, 2023). Search parameters were: enzyme: trypsin (two-missed cleavage allowed); MS/MS mass tolerance for monoisotopic ions: ±0.6 Da; carbamidomethyl cysteine and oxidation of methionine were selected as fixed and variable modifications, respectively. Assignments with significance protein score threshold of $p < 0.05$ (Mascot Score >43) were taken into consideration, and all the associated peptide ions hits were manually validated.

3. Results and discussion

3.1. Scanning electron microscopy of Abronia lythrochila teeth

Scanning electron micrographic analysis of the mandibular pleurodont teeth of the lower jaw of the red-lipped arboreal alligator lizard, *A. lythrochila* (Fig. 2) showed a conical, soft rounded and posteriorly curved apices morphology without any sign of groove, external opening or striations. This morphology bears notable resemblance with that described in the lower jaw of another anguid species, the legless lizard *Pseudopus apodus*, (Figs.5B and 11E in Klembara et al., 2014), but strongly departs from the grooved lower jaw teeth of *Heloderma* species (Shufeldt, 1891). Teeth with deep root-to-tip grooves have been found in the fossil record of both branches of the Anguimorpha clade, in *Estesia mongoliensis* (Monstersauria: Paleoanguimorpha) (Yi and Norell, 2013) and in *Varanus priscus* (Megalania: Neoanguimorpha) (Fry et al., 2009).

The fossil record indicates an Asian origin for Varanidae and a North American origin for Helodermatidae originated from an Asian monstersaur-like form (Cabezuelo Hernández et al., 2022, and references cited). In extant anguimorph taxa the teeth with deep root-to-tip groove morphology have been conserved only in genus *Heloderma*. Gila monsters and beaded lizards largely prey on eggs, a diet known to lead to reduction of venom apparatus in other reptiles (Heatwole, 1999; Li et al., 2005). The reason for Heloderma retaining the capability to produce and inject venom may lie in a functional specialization to use venom for defence promoted by natural selection in response to their relatively poor capability to escape attack by predators (Beck, 2005). This does not apply to monitor lizards, which possess powerful jaws with serrated teeth that allow them to inflict massive injuries aiding in killing large or fast prey with minimal risk of injury for the predator. Endemic to five small islands in Eastern Indonesia, the Komodo dragon (*Varanus komodoensis*) is the world's largest lizard, with adults body mass reaching up to 90 kg and a length of 3m (Jessop et al., 2006). Anatomical investigation of the *V. komodoensis* oral exocrine gland system revealed separate ducts leading from each compartment of a compound mandibular gland opening between successive serrated pleurodont teeth (Fry et al., 2009). Such dentition may aid deploying their oral secretions into the wound, through a scissoring action at the advancing junction between upper and lower teeth and by lateral gripping and compression in a slot (Abler, 1992). However, there are relatively few field

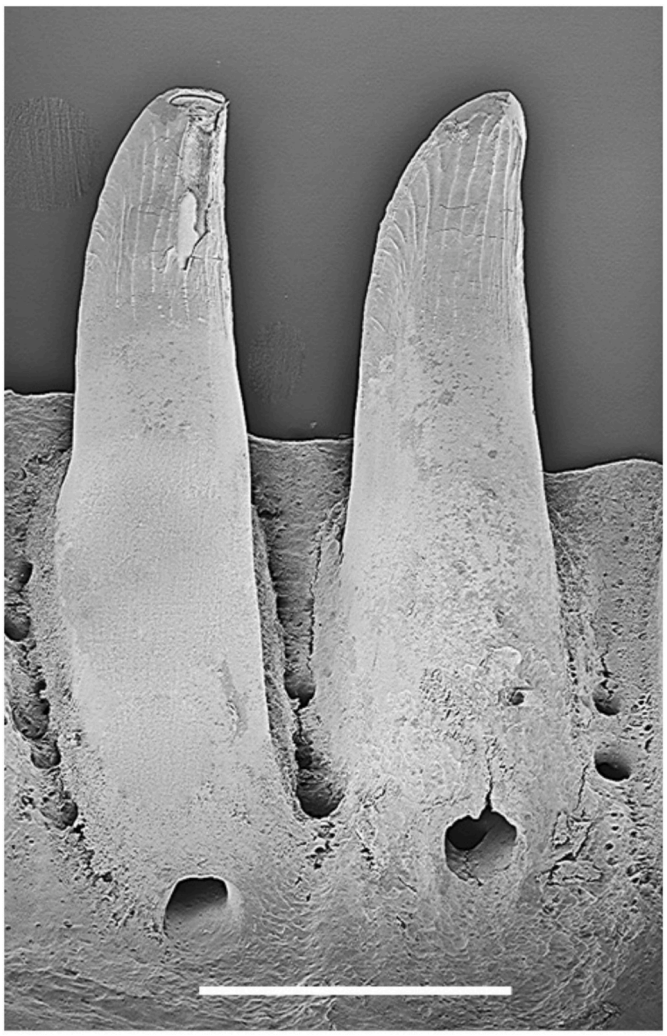

Fig. 2. Scanning electron microscopy of pleurodont teeth of *A. lythrochila* (Bar - 0.6 mm).

observations of *V. komodoensis* predating in the wild. Komodo dragons dispatch large ungulate prey (Timor deer, feral pigs, water buffalo) by biting and tearing flesh. If a prey escapes, the dragon occasionally track prey until it succumbs. Whilst persistent bleeding due to the bite inflicted injury appears to be the primary mechanism of prey subjugation, the involvement of putative life-threatening sepsis induced by oral bacteria (Auffenberg, 1981; Montgomery et al., 2002; Bull et al., 2010) and/or the anticoagulant and hipotensive effect of venom (Fry et al., 2009; Dobson et al., 2024) inoculated into the wound have been hypothesized. However, the ecological and evolutionary bases of sepsis and/or venom in Komodo prey acquisition remain experimentally unsupported. Notably, extinct *Varanus* (Megalania) *priscus* differed from extant Komodo Dragon by possessing labial and lingual grooves that run from the base toward the tip of the tooth, an ancestral morphology thought to represent evidence for venom use by one of the largest known terrestrial lizards (Fry et al., 2009).

3.2. *Proteomic analyses of Abronia graminea and A. lythrochila mandibular gland extracts*

Outcome of the bottom-up mass spectrometric assignments of the proteins extracted from the mandibular glands of *A. graminea* (Suppl. Table S1) and result of the label-free shotgun proteomic analysis of the protein extracts of *A. graminea* (Suppl. Tables S2 and S3) and *A. lythrochila* (Suppl. Tables S4 and S5) are summarized in Fig. 3. Both alligator lizards expressed identical toxin types albeit at different relative abundances. Further, in addition of structural and house-keeping proteins, the gland extract proteomes of *A. graminea* and *A. lythrochila* contain also proteins (protein disulfide-isomerase, peptidyl-prolyl cis-trans isomerase, peroxiredoxin-6, thioredoxin, glutaredoxin, and heat shock 70 and 90, at relative abundances of 0.01–0.03% of the total quantified maxillary gland extracts) thought to play a key role catalyzing the oxidative folding of venom toxins such as natriuretic peptides, PLA_2 and CRISP, whose functional molecular scaffolds are stabilized by a network of intramolecular disulfide linkages (Reeks et al., 2015). Both, the transcriptome (Koludarov et al., 2012) and proteome analyses (this work) confirm the presence of potentially toxic proteins expressed in the mandibular glands of *Abronia* species.

The set of conserved proteins found in the mandibular glands of the alligator lizards such as B-type natriuretic peptides, cysteine-rich secretory proteins (CRISP), group III phospholipase A_2 (PLA_2), and kallikrein-type serine proteinase, are present also in the gland transcriptomes, and in gland extracts of *Varanus* and *Heloderm*a species (Fry et al., 2010a, 2010b; Koludarov et al., 2017). Group III PLA_2s and kallikrein-type serine proteinases are shared across all the lizard venoms (Dobson et al., 2024). Kallikrein was identified as the only putative toxin in the mandibular gland extract of the earless monitor lizard *Lanthanotus borneensis* (Mebs et al., 2021), a sister taxon to Varanidae within the Paleoanguimorpha branch (Fig. 4). Kallikrein appears also to be the predominant enzyme in *Varanus* mandibular glands (Koludarov et al., 2017), where its dominant *in vitro* documented toxic actions are the cleavage of the alpha- and beta-chains of fibrinogen, thus disrupting the final stage of clot formation, and the liberation of kinins from kininogen (Datta and Tu, 1997; Dobson et al., 2019; Dobson et al., 2024). Inducing blood loss and hypotension may enhance a predator's chances of weakening and subjugating a prey. On the other hand, kallikrein is also known to cause intense pain in helodermatid envenomings to humans

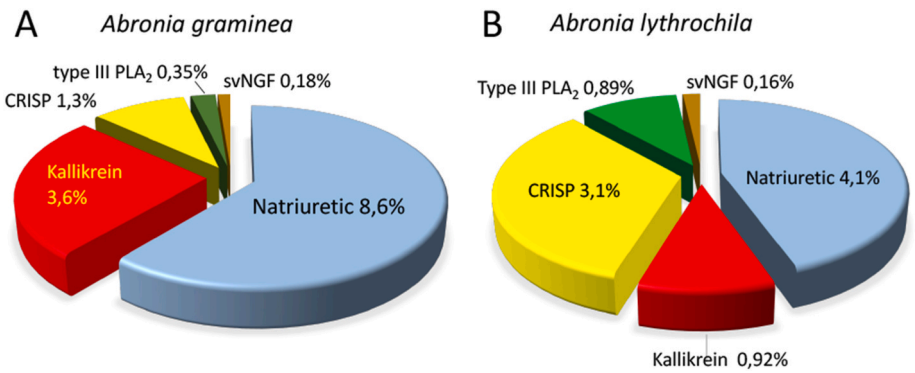

Fig. 3. Relative abundances of toxin families gathered by label-free ion-intensity shotgun MS analysis from the protein extracts of the submandibulary glands of *Abronia graminea* (panel A) and *Abronia lythrochila* (panel B).

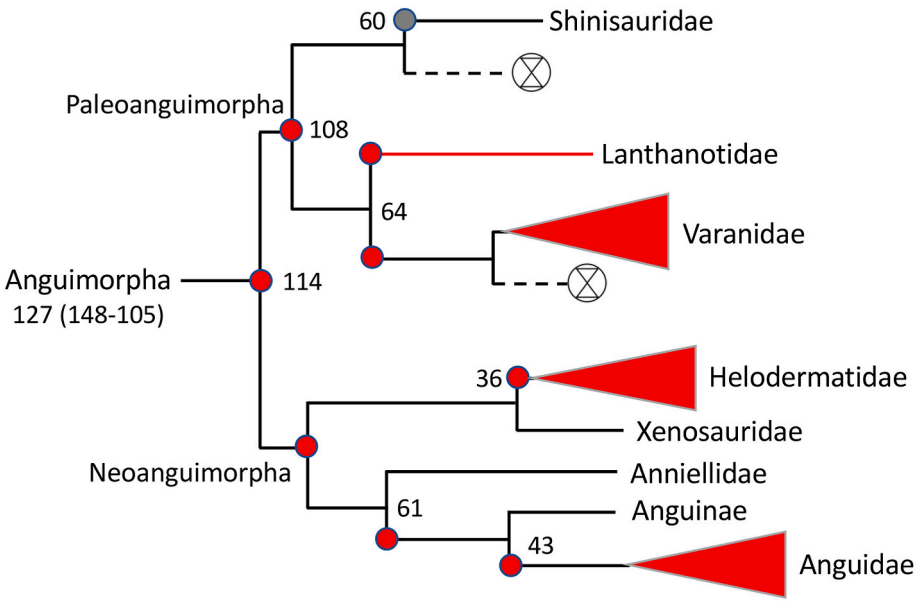

Fig. 4. Scheme of phylogenetic relationships of squamate reptiles of the Anguimorpha suborder (adapted from Douglas et al., 2010; Conrad et al., 2011; Reeder et al., 2015; Zheng and Wiens, 2016). Estimated mean divergence times for selected nodes are indicated in millions of years (mya). The most recent common ancestor (MRCA) of the Anguimorpha clade has been dated in the Early Cretaceous, 127.1 (105.5–148.7) mya; the MRCA node for Lanthanotidae has been estimated to be Mid Cretaceous (108.3 (83.3–133.2) mya); divergence time of 59.7 (17.6–100.3) million years (Early Paleocene) has been inferred for Shinisauridae. The MRCA for Varanidae was placed in the Early Eocene (48.7 (30.7–73.6) mya), and the divergence of Helodermatidae has been dated at 35.4 (29.4–41.4) mya. A broken line indicates an extinct lineage. Gray and red circles represent basal nodes for taxa lacking or expressing proteins belonging to known toxin classes in their mandibular glands, respectively. Lineages with mandibulary glands that produce oral secretions bearing toxins are highlighted in red, a line for the single species *Lanthanotus borneensis* expressing kallikrein as the only toxin and red triangles for clades including multi-toxin-bearing taxa. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Russell and Bogert, 1981; Amri and Chippaux, 2021). In an ecological context, inflicting incapacitating pain in the predator may enable a quick retreat for the perpetrator (Niermann et al., 2020). In addition, the vasodilatory action of B-type natriuretic peptides along with kinin release from kininogen by kallikrein enzymes underlie the hypotensive effect of helodermatid venoms (Mebs, 1968; Alagón et al., 1986). Although the ecological role of helodermatid kallikrein cannot be generalized to other anguid lizards, it is tempting to speculate that the extreme specialization of the earless monitor lizard towards a kallikrein-only toxic mandibular secretion might represent an adaptation for a defensive role.

Together with kallikrein, group III PLA_2 and CRISP proteins have been identified as recruited for use as toxins in lizard venoms (Fry et al., 2010b). Platelet aggregation-blocking group III PLA_2 appears to be responsible for the anticoagulant toxicity documented for varanid oral secretions (Dobson et al., 2021, 2024). CRISP molecules first appeared in reptile venoms at the base of the Toxicofera clade (Fry et al., 2006) and are widely distributed in venoms of extant snakes of family Colubridae, and in helodermatid and varanid species of lizards. CRISP molecules have been associated with the inhibition of a number of voltage-gated ion channels (Tadokoro et al., 2020; Dobson et al., 2021). The toxinological profile of these molecules may suggest ion channel neurotoxicity of helodermatid and varanid lizard venoms. However, the biological activities and ecological role of CRISP toxins in the alligator lizards, *Abronia graminea* and *Abronia lythrochila*, or in any other anguimorph taxa, remain absolutely obscure.

4. Concluding remarks

Our understanding of the adaptive relationships between dentition and the evolutionary ecology of anguimorph lizards is hindered by both, fundamental gaps in the fossil record (Evans, 2003) and the scarce multidisciplinary and multiomics studies of extant organisms. Our finding that species within the Anguidae clade of the neoanguimorpha branch express in their mandibular glands proteins homologous to toxins of venomous toxicoferan taxa, varanid and helodermatid lizards and snakes, provides further support for the presence of a handful of conserved toxin families in the oral secretions across the 100+ million years of Anguimorpha cladogenesis (Fig. 4). *In vitro* shared hypotension induction through the liberation of kinins from kininogen by Heloderma, Lanthanotus and Varanus oral secretions, might suggest that this activity was present in the last common ancestor of anguimorph lizards. Whether these ancestrally recruited proteins have evolved trophic traits retaining their toxic activity, or this function has regressed due to lack of selective ecological pressure to maintain it, awaits omics analyses of unexplored anguimorph lineages (i.e. Xenosauridae, Anniellidae, Anguinae) and functional studies on natural prey of oral secretions across Anguimorpha cladogenesis.

Ethical statement

Authors declare that international ethical guidelines for scientific papers were followed in the preparation of this manuscript.

CRediT authorship contribution statement

Juan J. Calvete: Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation, Writing – review & editing, Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Bruno Lomonte:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Formal analysis, Data curation. **Jordi Tena-Garcés:** Writing – review & editing, Formal analysis. **Michael Zollweg:** Writing – review & editing, Methodology, Formal analysis. **Dietrich Mebs:** Writing – review & editing, Writing – original draft, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.toxicon.2024.108055.

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