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Research Article

Phylogenetic and morphological evidence reveals the association between diet and the evolution of the venom delivery system in Neotropical goo-eating snakes

LEONARDO DE OLIVEIRA[1,2], FELIPE GOBBI GRAZZIOTIN[3], PAOLA MARIA SÁNCHEZ-MARTÍNEZ[1], MAHMOOD SASA[4,5], OSCAR FLORES-VILLELA[6], ANA LÚCIA DA COSTA PRUDENTE[7] & HUSSAM ZAHER[1]

[1]Museu de Zoologia da Universidade de São Paulo, Avenida Nazaré, São Paulo, 04263-000, Brazil
[2]Laboratório de Toxinologia Aplicada, Instituto Butantan, Avenida Vital Brasil, São Paulo, 05503-900, Brazil
[3]Laboratório Especial de Coleções Zoológicas, Instituto Butantan, Avenida Vital Brasil, São Paulo, 05503-900, Brazil
[4]Instituto Clodomiro Picado, Facultad de Microbiología, Universidad de Costa Rica, San José, Costa Rica
[5]Museo de Zoología, Centro de Investigaciones en Biodiversidad y Ecología Tropical, Universidad de Costa Rica, San José, Costa Rica
[6]Museo de Zoologia 'Alfonso L. Herrera', Faculdad de Ciencias, Universidad Nacional Autónoma de México, Mexico City, 04510, Mexico
[7]Museu Paraense Emílio Goeldi, Avenida Perimetral, Belém, Pará, 66040-170, Brazil

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Advanced endoglyptodont snakes share a complex but homologous venom delivery system associated with the upper jaw and its dentition. Recently, a remarkable novel lower jaw venom delivery system was described for the Neotropical dipsadine radiation of goo-eating snakes. While most dipsadines are opistoglyphous and exhibit large, mainly serous venom glands associated with the upper jaw and supralabial glands, goo-eating dipsadine snakes are aglyphous and lack serous upper labial venom glands. Here, we provide new morphological and histological information on the oral glands and maxillary dentition of representatives of the major lineages of dipsadines that help trace the evolutionary steps that shaped the venom delivery system of dipsadines. We performed a maximum likelihood analysis on a molecular dataset that includes 443 terminals and seven loci. Our results show that goo-eating dipsadines form a monophyletic assemblage that includes the genus *Adelphicos* for the first time, along with *Geophis*, *Atractus*, *Ninia*, *Chersodromus*, *Tropidodipsas*, *Sibon*, and *Dipsas*. We also provide the first evidence of a complete shift from an upper jaw to a lower jaw venom delivery system associated with their specialized feeding behaviour. Unlike other dipsadines who exhibit typical endoglyptodont anteroposteriorly ridged posterior maxillary teeth, goo-eating dipsadines have uniform lateromedially ridged teeth throughout their maxilla. Our results indicate that the loss of the endoglyptodont venom delivery system occurred in the most recent common ancestor of goo-eating dipsadines, probably resulting from the loss of the embryonic posterior maxillary lamina responsible for the development of the venom delivery system.

Key words: Dipsadidae, Duvernoy's glands, goo-eaters, maxillary teeth, non-front-fanged snakes, venom glands

Introduction

Snakes constitute a group of carnivorous tetrapods known to consume a large array of prey, including invertebrates (annelids, crustaceans, terrestrial arthropods, molluscs) and vertebrates (fish, amphibians, reptiles, birds, and mammals) (Greene, 1997). Many morphological specializations have been associated with specific types of prey, including those related to the feeding mechanisms (Cundall & Greene, 2000; Moon et al., 2019; Savitzky, 1983). Cranial adaptations to large prey size leading to increasing levels of hyperkinesis have been suggested to be present since the early evolution of Pan-Serpentes (Zaher et al., in press), whereas advanced groups of snakes present specific adaptations associated with specialized feeding mechanisms (e.g. marked skull modularity in aquatic-foraging

Correspondence to: Leonardo De Oliveira. E-mail: leooliveira.herpeto@gmail.com

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snakes and allometric fang length evolution in vipers; Holding et al., 2022; Rhoda et al., 2021). Although the components of the venom delivery system—constituted by venom glands, their associated muscles, and specialized maxillary dentition—are part of the feeding system of snakes, to date little is known about the association between types of prey and the emergence of these structures (Jackson et al., 2019). Recently, Zaher et al. (2019) demonstrated that the venom delivery system of caenophidian snakes arose in the most recent common ancestor of the clade Endoglyptodonta, which includes viperids, homalopsids, colubroids and elapoids.

In endoglyptodont snakes, the glands of the upper jaw include the premaxillary, supralabial, venom, Duvernoy's, and superior rictal (Jackson et al., 2017; Kochva, 1978; Smith & Bellairs, 1947). Supralabial, venom, and Duvernoy's glands are among the most studied glands, particularly because they are associated with the venomous function in snakes. The supralabial glands are located along the lateral margin of the maxilla and they are primarily mucous, developing from many serial and non-compound epithelia thickening along the upper lips (Oliveira et al., 2017; Taub, 1966). The venom and Duvernoy's glands are located along the caudal portion of the maxilla, and they are primarily serous, developing from a single invagination on the caudal portion of the dental lamina of the maxillary bone (Kochva, 1963; Oliveira & Zaher, 2022; Shayer-Wollberg & Kochva, 1967; Vonk et al., 2008).

Although considered homologous, venom and Duvernoy's glands exhibit significant morphological differences and their nomenclature have recently been the focus of an intense debate (see Jackson et al., 2017; Weinstein et al., 2010 for details). Traditionally, only front-fanged snakes of the families Atractaspididae, Viperidae, and Elapidae were considered to bear venom glands, while Duvernoy's glands were present in a vast number of non-front-fanged endoglyptodont snakes (Kardong, 2002; Taub, 1967; Vidal, 2002; Weinstein et al., 2010). However, considering the embryological evidence supporting the homology of these glands, some authors proposed synonymizing the term "Duvernoy's glands" with venom glands (Fry et al., 2012; Jackson et al., 2017).

Recent studies indicate that the upper jaw venom delivery system of endoglyptodont snakes arose as a morphofunctional adaptive novelty that triggered the radiation of this group during the Cenozoic (Fry et al., 2012; Jackson, 2003; Vidal, 2002; Vonk et al., 2008; Zaher et al., 2019). Although not always the case, reduction or loss of the venom delivery system in endoglyptodonts is often observed in species that use constriction as their preferred method of prey capture, or developed a specialized diet such as fish eggs, slugs, and earthworms (Fry et al., 2008; Jackson, 2007; Taub, 1967; Underwood & Kochva, 1993; Vidal, 2002). The terrestrial Australian elapid *Brachyurophis* and sea-snakes *Emydocephalus* and *Aipysurus* are remarkable examples of front-fanged snakes with significantly reduced venom delivery systems that are specialized in eating fish eggs (Fry et al., 2008; Gopalakrishnakone & Kochva, 1990; McCarthy, 1987). Another extreme example are the goo-eating dipsadines that feed exclusively on invertebrates and appear to have lost their endoglyptodont venom delivery system (Cadle & Greene, 1993; Fernandes, 1995; Fry et al., 2008; Oliveira et al., 2014, 2017; Taub, 1967; Vidal, 2002; Zaher et al., 2014). Although the anatomical peculiarities related to feeding in goo-eating dipsadines were documented early in the literature (e.g. Haas, 1931), little is known about how they originated in the group.

Cadle and Greene (1993) were the first to suggest that goo-eating dipsadines formed a monophyletic group composed by two subclades: one including *Geophis*, *Adelphicos*, *Ninia*, and *Atractus* who prey almost exclusively on earthworms and occasionally molluscs; and another including *Sibon* and *Dipsas* who feed almost exclusively on molluscs. Recent works failed to recover Cadle and Greene's goo-eating dipsadines as a monophyletic assemblage, with the cryptozoic *Adelphicos* clustering invariably outside the clade formed by the remaining goo-eaters (Pyron et al., 2011; Zaher et al., 2014, 2019).

Here, we provide a comprehensive phylogenetic analysis including representatives of almost all goo-eating genera (only the rare *Chapinophis* and *Omoadiphas* are not represented). We also conduct a comparative study on the general morphology and histology of the upper jaw glands (i.e. venom and supralabial glands) and the ultrastructure of the maxillary dentition in representatives of all available dipsadine genera. Our study aims to answer the following questions: Are goo-eating dipsadines monophyletic? Is the endoglyptodont venom delivery system of goo-eating dipsadines vestigial or was it lost altogether? Is there any correlation between the morphology of the upper jaw and associated glands of dipsadines and their specialized diet?

Materials and methods

DNA sequencing, alignment, and data partitioning

We downloaded the whole alignment available from Zaher et al. (2018) for the mitochondrial genes *12S* (small subunit ribosomal RNA), *16S* (large subunit

ribosomal RNA), and *cytb* (cytochrome b), and the nuclear genes *bdnf* (brain-derived neurotrophic factor), *c-mos* (oocyte maturation factor Mos) and *nt3* (Neurotrophin-3). The alignment for the mitochondrial gene *nd4* (NADH dehydrogenase subunit 4) was downloaded from the molecular dataset available from Zaher et al. (2019). The *nd4* alignment was first reduced to match the terminals in Zaher et al. (2018). After removing the sites composed only by missing data, we added 84 additional *nd4* sequences available at GenBank (Supplemental Material – Table S1). To properly test the monophyly of goo-eating dipsadines, we sequenced all four mitochondrial genes for the following seven key taxa: *Adelphicos latifasciatum*, *Adelphicos quadrivirgatum*, *Chersodromus liebmanni*, *Geophis brachycephalus*, *Rhadinaea decoratus*, *Tretanorhinus mocquardi*, *Tretanorhinus nigroluteus*, *Trimetopon pliolepis,* and *Urotheca guentheri*. The molecular protocol to extract DNA, amplify and sequence the PCR fragments followed Zaher et al. (2019).

We also downloaded from GenBank the sequences of dipsadines generated by Sheehy (2012) and Arteaga et al. (2018). Before incorporation of any GenBank sequence to our dataset, we checked for misidentified, mislabelled, or misassembled sequences by estimating preliminary gene trees through Maximum likelihood (ML) phylogenetic analyses, using the same parameters set for the analyses of the complete dataset (see below). Since Sheehy (2012) and Arteaga et al. (2018) included more than one individual per species in their analyses, we selected only the individual with the best representation of genes and sequence data after the preliminary gene tree analyses (Supplemental Material – Table S1).

The additional sequences were included in the original alignments of Zaher et al. (2018) and Zaher et al. (2019) by using the "*–add*" command in the online version of MAFFT v. 7 (Katoh et al., 2019) with default parameters. The complete concatenated matrix includes 443 terminals (240 outgroups; 203 dipsadines from which 138 are goo-eaters) and 4,652 base pairs, being available as a Nexus file at *figshare* (10.6084/m9.figshare.19601653).

Phylogenetic analysis

We used RAxML 8.2.3 (Stamatakis, 2014) to analyse the partitioned concatenated matrix. We used PartitionFinder2 v.2.1.1 (Lanfear et al., 2012) to define the best scheme of partitions, allowing only GTR + G as the model of molecular evolution without any correction for the proportion of invariant sites, as recommended in the RAxML's manual. We divided our matrix into 17 initial partitions (each protein-coding gene was partitioned by codon position and each rRNA was analysed as a separate partition). The Akaike Information Criteria with correction (AICc) was used as the selection criteria, and we set the "greedy" searching algorithm and the "unlinked" option for branch-lengths in PartitionFinder2. We performed the phylogenetic analysis by running 1000 pseudoreplicates of non-parametric bootstrap (BS) using the rapid bootstrap algorithm implemented in RAxML (-fa). This approach performs 200 complete searches for the best-scoring ML tree using each 5th bootstrap tree as a starting tree for the rapid hill-climbing search.

Species studied and gross anatomy

A total of 44 species of the subfamily Dipsadinae were dissected, including representatives of all known goo-eating genera except for the rare *Chapinophis* and *Omoadiphas*. Specimens were studied using one or more of the following techniques: gross anatomy (dissection), histology and histochemistry, scanning electron microscopy of the maxillary teeth, and computed tomography (see Supplemental Material – Data). We dissected the head muscles and glands of individuals kindly made available for study (Supplemental Material – Data). Dissections were made under a stereomicroscope Olympus SZX 12 equipped with a camera lucida. Glandular terminology followed Jackson et al. (2017) and maxillary dentition terminology followed Vaeth et al. (1985). The terminology for the external mandibular adductor muscles is still in dispute among authors (see Daza et al., 2011; McDowell, 1986; Zaher, 1994); for this reason, we followed Zaher (1994). We followed Fry et al. (2012) and Jackson et al. (2017) and used the term "venom gland" to refer to all oral glands that develop from a single invagination on the caudal portion of the dental lamina of the maxilla, including the traditional "venom glands" of the front-fanged snakes and "Duvernoy's glands" of non-front-fanged snakes.

Histology and histochemistry

We performed all histological sections on specimens belonging to scientific collections. We used entire heads, dissected glands, or both for histological analyses. Heads were skinned from nostril to neck and removed from the specimens at the level of the first cervical vertebra. After removal of the skin and disarticulation of the neck, the entire heads were submitted to decalcification in 4.13% aqueous EDTA pH 7.2 renewed every 3 days, constantly stirred for 60 days. The decalcified heads were then sagittally divided into two halves, dehydrated in ethanol, embedded in paraffin, and

submitted to serial sagittal or transversal sectioning. We performed the sections (7 μm) on a Microm HM 340 E microtome with disposable steel blades. We submitted them to haematoxylin-eosin (HE) staining, for the general study of the tissues, and to Mallory trichrome staining (Kiernan, 2015), for the identification of the collagen and muscle fibres and epithelia. Sections were still subjected to the following histochemical staining procedures (according to Bancroft & Stevens, 1996): periodic Acid-Schiff (PAS), alcian blue pH 2.5 (AB), combined alcian blue (pH 2.5), and PAS (Kiernan, 2015), and bromophenol blue (BB). We applied PAS and AB for identifying neutral and acid mucosubstances, respectively, and BB for identifying proteins. We distinguish the different cell types encountered in mucous and serous cephalic glands, according to Underwood (1997), grouping in the serous category those cells that produce secretion rich in proteins, regardless of whether they are positive for PAS. We took the photographs with a DFC425 digital camera on a Leica M205a stereoscopic microscope and a Leica M2500 microscope using Leica Application Suite software (Version 3.8).

Scanning electron microscopy (SEM)

We examined the maxillae of 12 species of dipsadine snakes (Supplemental Material – Data). Maxillae were extracted and stored separately in a 70% ethanol solution, and maxillary teeth were prepared for scanning electron microscopy, removing all soft tissue manually. After cleaning was complete, the entire maxillae were dehydrated in an increasing series of ethanol and, posteriorly, acetone solution, and dried in a critical point dryer using CO_2 as an intermediate medium. The maxillae were then coated with gold, mounted on the sputtering device, and examined in an LEO 440 scanning electron microscope.

Tomography

The soft tissue anatomy and maxillary dentition of some specimens (Supplemental Material – Data) were further examined through images generated on a high-resolution X-ray microCT scan. The heads of the specimens were stained in 1% iodine–ethanol solution for about one week to increase the tissue x-ray contrast following the procedure proposed by Metscher (2009). According to their sizes, we mounted the specimens individually in falcon tubes with alcohol to keep them hydrated and immobile while the scanning was conducted. Specimens were scanned using a Phoenix v|tome|x m (General Electric Company) housed at the Microtomography Laboratory of the Museum of Zoology of the University of São Paulo. To acquire the scans, a microfocus tube 300 was used, and different settings were applied to optimize the scan quality. We used the Datos Reconstruction software (GE Company) to reconstruct the image set. We conducted the rendering and analysis of the datasets using the software VG Studio Max Version 2.2.3.69611 64 bits. We visualized the soft tissue and bones by adjusting the histogram/threshold density settings of the greyscales.

Character evolution

We estimated character evolution by plotting ancestral state reconstructions parsimoniously in Mesquite v. 3.70 (Maddison & Maddison, 2021) a pruned version of our estimated maximum-likelihood phylogeny, in which we only retained the terminals with available morphological information. Although not optimal, this strategy allowed us to assume a more conservative approach regarding the uncertainties related to the lack of information for some of the terminals.

We scored the presence (0) or absence (1) of venom glands and maxillary dentition (Supplemental Material – Tables S1 and S2). We also scored the diet of each terminal taxon based on data from the literature, dividing it as being composed mainly by vertebrates (0) or exclusively by invertebrates (1) (additional information on diet provided in (Supplemental Material – Table S4).

We considered venom glands to be absent when they could not be distinguished from the supralabial glands on dissection or histological sections. The maxillary dentition of dipsadines is also highly variable, with grooved or ungrooved posterior maxillary teeth and diastema between the anterior and posterior teeth present or not (Mulcahy et al., 2011). We considered the size of the tooth and presence of anterior and posterior ridges as indications of the presence of posterior maxillary teeth derived from the embryological posterior maxillary lamina and distinct from the typical anterior maxillary teeth (Supplemental Material – Table S3).

Results

Phylogenetic relationships

Besides corroborating several previously hypotheses of relationship among Caenophidia (e.g. Grazziotin et al., 2012; Zaher et al., 2009, 2019), the results of our phylogenetic analysis shown for the first time that goo-eating dipsadines form a monophyletic assemblage that includes the genus *Adelphicos*, along with *Geophis*, *Atractus*, *Ninia*, *Chersodromus*, *Tropidodipsas*, *Sibon*, and *Dipsas*.

Our molecular matrix was mostly derived from Zaher et al. (2018) dataset, resulting in very similar general tree topologies recovered in both ML analyses. All caenophidian families and most higher-level clades previously defined by Zaher et al. (2009, 2018) were recovered with high bootstrap values (e.g. Colubroides, 84%; Colubroidea, 100%; Elapoidea, 100%) (Supplemental Material – Fig. S1), except for one main difference. In our analysis, Viperidae and Homalopsidae were resolved as sister groups of Pareidae (74%) and Elapoidea (<70%), respectively, instead of successive sister groups to a clade formed by Elapoidea and Colubroidea. However, this topology received relatively low bootstrap support and should be regarded with caution (Zaher et al., 2019).

Our ML tree also recovered the three dipsadid subfamilies Carphophiinae, Dipsadinae and Xenodontinae as monophyletic, the latter two with moderate bootstrap values (79% and 81%, respectively). Similarly, all their tribes described or redefined by Zaher et al. (2009, 2018) Grazziotin et al. (2012), and Pyron et al. (2015, 2016) were also resolved as monophyletic (Supplemental Material – Fig. S1). The North American genera *Heterodon* and *Farancia* were recovered along with the other members of the subfamily Carphophiinae (Zaher et al., 2009), although with low bootstrap values. The Asian genera *Thermophis* and *Stichophanes* were resolved as the successive sister groups of all other dipsadids.

The topology of the recovered tree within Xenodontinae remained the same as in Zaher et al. (2018), with the only main difference being the position of Conophiini as the sister group of Hydrodynastini. On the other hand, the addition of new sequences generated by us, Arteaga et al. (2018) and Sheehy (2012) significantly altered the topology within Dipsadinae.

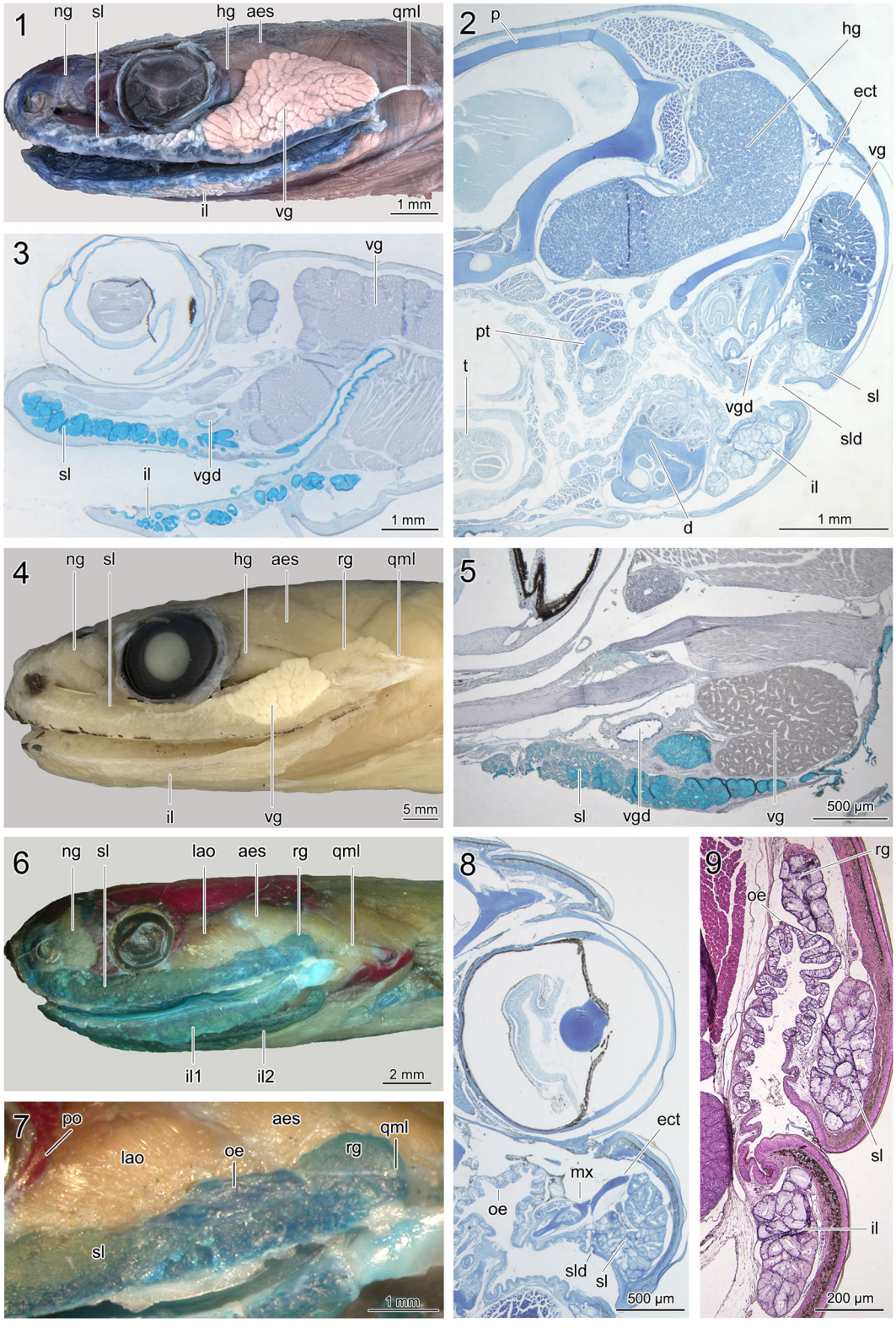
Our ML tree recovered the following 13 main clades (Supplemental Material – Fig. S1), some of which are also supported by morphological evidence: Clade C1 (78% of bootstrap), including all sampled species of *Dipsas*, except *Dip. gaigeae*; Clade C2 (<70%), mainly formed by the species of *Sibon* and *Tropidodipsas* sampled in this study, but also including *Dip. gaigeae* and the sampled species belonging to the *chalybeus* and *omiltemanus* species groups of *Geophis* (*sensu* Downs, 1967); Clade C3 (<70%), representing the tribe Dipsadini (*sensu* Zaher et al., 2014), composed by clades C1 and C2; Clade C4 (82%), composed by clade C3 and a moderately supported clade (79%) formed by the genera *Ninia* and *Chersodromus*; Clade C5 (<70%), composed by all the sampled species of *Atractus* (<70%) and members of the *championi*, *dubius*, *latifrontalis*, and *sieboldi* species groups of *Geophis* (*sensu* Downs, 1967); Clade C6 (86%), formed by clades C4 and C5; Clade C7 (<70%), composed by all species defined as goo-eating dipsadines by Cadle and Greene (1993), including the genus *Adelphicos* (97%); Clade C8 (<70%), encompassing clade C7 and two successive sister groups represented by the genus *Cryophis* and a highly supported clade (100%) composed by *Hydromorphus* and *Tretanorhinus*, respectively; Clade C9 (<70%), comprising clade C8 and three successive sister groups consisting of a clade formed by *Imantodes* and *Leptodeira* (tribe Imantodini; 96%), the genus *Nothopsis*, and a clade formed by *Hypsiglena* and *Pseudoleptodeira*; Clade C10 (<70%), composed by the sampled species of the genera *Rhadinaea*, *Pliocercus*, *Coniophanes*, *Trimetopon*, *Urotheca*, and *Amastridium*; Clade C11 (<70%), comprising two robustly supported sister clades formed by *Tantalophis*, *Trimetopon gracile*, and *Rhadinophanes* on the one hand, and *Enulius* and *Enuliophis*, on the other hand; Clade C13 (91%), composed by all non-diaphorolepidine dipsadines, including clades C9, C10 and C11; and Clade C12 (100%), the sister group of clade C13, formed by *Diaphorolepis* and *Synophis* (Supplemental Material – Fig. S1).

Glands of the upper jaw

Gross anatomy. Venom glands of dipsadines have a wide morphological variation, being conspicuously well-developed in some species and absent in others (Supplemental Material – Table S2). In species that feed on vertebrates, the venom gland is in the postorbital region and frequently reaches the corner of the mouth posteriorly (Figs 1.1–1.5, 2). No muscle fibres are associated with the venom gland in dipsadines.

Among the species analysed, we observed a differentiated venom gland in *Coniophanes fissidens*, *Cryophis hallbergi*, *Enulius flavitorques*, *Hydromorphus concolor*, *Hypsiglena torquata*, *Imantodes cenchoa*, *Leptodeira annulata*, *Rhadinaea decorata*, *Rhadinella hannsteini*, *Rhadinella montecristi*, *Tretanorhinus variabilis*, *Tretanorhinus nigroluteus*, *Trimetopon pliolepis*, and *Pliocercus elapoides*. The gland is characteristically positioned along the posterior region of the upper jaw, on the temporal region and corner of the mouth, and does not extend anteriorly beyond the posteroventral margin of the orbit.

Leptodeira annulata has the largest dorsally expanded venom gland (Fig. 1.1) that covers most of the temporal region, reaching the level of the upper margin of the orbit dorsally and covering the whole region of the upper lip posteriorly (Fig. 1.1). Like *Lep. annulata*, *Hyp. torquata* and *Cry. halbergi* have a large venom gland that projects dorsally on the temporal region.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However, it is less developed on its ventral margin, being bordered by the supralabial gland that expands dorsoventrally on the rictal region (Fig. 2.3). The venom gland is less developed in *Con. fissidens*, *Rha. decorata*, *Rhadinella* (*Rhl. hannsteini* and *Rhl. montecristi*), *Tri. pliolepis*, *Ima. cenchoa*, *Pli. elapoides*, and *Cry. hallbergi*, being mostly continuous with the dorsal margin of the supralabial gland, although a wide variation is observed between them (Figs 1, 2). In *Con. fissidens*, *Rha. decorata*, *Rhl. hannsteini*, *Rhl. montecristi*, *Tri. pliolepis*, *Ima. cenchoa*, and *Pli. elapoides*, the venom gland is mainly restricted to the lower temporal region, from the posteroventral level of the orbit to the anterior margin of the corner of the mouth (Figs 1, 2). In *Tre. variabilis*, *Tre. nigroluteus*, and *Hyd. concolor*, the venom gland is characteristically very reduced and restricted to the posterodorsal extremity of the supralabial gland, just above the rictal region, a peculiar morphology shared by these two genera (Fig. 2.4).

A differentiated venom gland is absent in all goo-eating dipsadines analysed, which included representatives of the genera *Adelphicos*, *Atractus*, *Chersodromus*, *Dipsas* (including *Sibynomorphus*), *Geophis*, *Sibon*, and *Tropidodipsas* (Supplemental Material – Data). Instead, the supralabial gland of goo-eating dipsadines extends from the posterior portion of the premaxillary gland to the level of the corner of the mouth, with no observable macroscopic modifications in their shape or coloration that reveal the presence of a venom gland (Fig. 3; (Supplemental Material – Table S2). The supralabial gland may taper posteriorly at the level of the corner of the mouth (e.g. *Dipsas mikanii* and *Dip. neuwiedi*; Figs 1.6, 1.7) or expand slightly (e.g. *Dip. williamsi*, *Dip. vagus*, *Dip. schunki*) or strongly dorsally (e.g. *Adelphicos quadrivirgatum*, *Geophis brachycephalus*, *Geo. nasalis*) just behind the orbit, but there is never any evidence of a differentiated venom gland posteriorly to the orbit.

Rictal glands are highly variable in dipsadines, being well-developed in some species and lacking in others, even within the same genus (e.g. *Dipsas*, *Rhadinaea*; Figs 1–3; (Supplemental Material – Table S2). When present, the rictal gland occupies the most posterior position at the corner of the mouth, medially to the quadrato-maxillary ligament, where it opens through a simple duct (Figs 1–3). The largest rictal glands were observed in *Pli. elapoides*, *Tri. pliolepis*, and *Con. fissidens* (Fig. 1.4). With a similarly granulated surface, the rictal gland of these three species reaches the same size as the adjacent venom gland. In *Im. cenchoa*, *Hyd. concolor*, and *Tre. nigroluteus*, and representatives of the goo-eating genera *Atractus*, *Dipsas*, *Ninia*, *Tropidodipsas*, and *Sibon* the rictal gland is reduced or barely visible on the rictal region, being either lightly granulated or smooth (Figs 1–3; (Supplemental Material – Table S2). No clear evidence of a distinct rictal gland was observed in the specimens of *Chersodromus* and *Adelphicos* analysed here. *Ade. quadrivirgatum* shows a slightly distinct gland arranged along of posterodorsal surface of the supralabial gland that extends to the posterior surface of the AES. Although by dissection we were unable to find its position with respect to the quadrato-maxillary ligament, results from the histological analysis provided support for its identification as a rictal gland (see below).

Histology and histochemistry. In *Lep. annulata*, *Ima. cenchoa*, *Con. fissidens*, *Hyp. torquata*, and *Pli. elapoides*, the venom gland is organized in acini or tubules constituted by serous cells (Figs 1.2, 1.3, 1.5). The acini or tubules are directed towards the anteromedial region of the gland, where they open into a single large duct (Figs 1.2, 1.5). The ducts of venom gland are covered with mucous cells, positive to alcian blue (pH 2.5) (Figs 1.2–1.3, 1.5), and extend from the anteromedial region of the gland to the basis of the posterior maxillary teeth, from which the secretion is liberated into the mouth. In dipsadines that feed on vertebrates, the supralabial gland is predominantly constituted of mucous cells, although

Fig. 1. Overview of the glandular morphology and associated structures in dipsadine snakes. (1–3) *Leptodeira annulata.* 1, lateral view of the dissected head stained with alcian blue and alizarin red showing cephalic glands. 2, transversal section of the post-ocular level of the head stained by a reaction of bromophenol blue, showing venom and harderian glands positivity and the opening of the venom and supralabial glands. 3, histochemical reaction of the alcian blue (pH 2.5) in a sagittal section of the head showing positivity of the supra and infralabial glands. Nuclear staining with haematoxylin. (4–5) *Coniophanes fissidens*. 4, lateral view of the dissected head. 5, sagittal section of the head evidencing the positivity of the supralabial gland to the alcian blue (pH 2.5) and showing the venom gland and the duct of venom gland. (6–9) *Dipsas mikanii*. 6, lateral view of the dissected head stained with alcian blue and alizarin red showing cephalic glands. 7, detail showing upper jaw glands. 8, transversal section of the ocular level stained by bromophenol blue reaction, showing supralabial glands. 9, transversal section showing glands and oral epithelium of the rictal region. Abbreviations: aes, *muscle adductor mandibulae externus superficialis*; d, dentary; ect, ectopterygoid; hg, harderian gland; il, infralabial gland; lao, muscle *levator anguli oris*; ng, nasal gland; oe, oral epithelium; p, parietal; po, postorbital; pt, pterygoid; qml, quadrato-maxillary ligament; sl, supralabial gland; sld, supralabial duct; t, tongue; rg, superior rictal gland; vg, venom gland; vgd, venom gland duct.

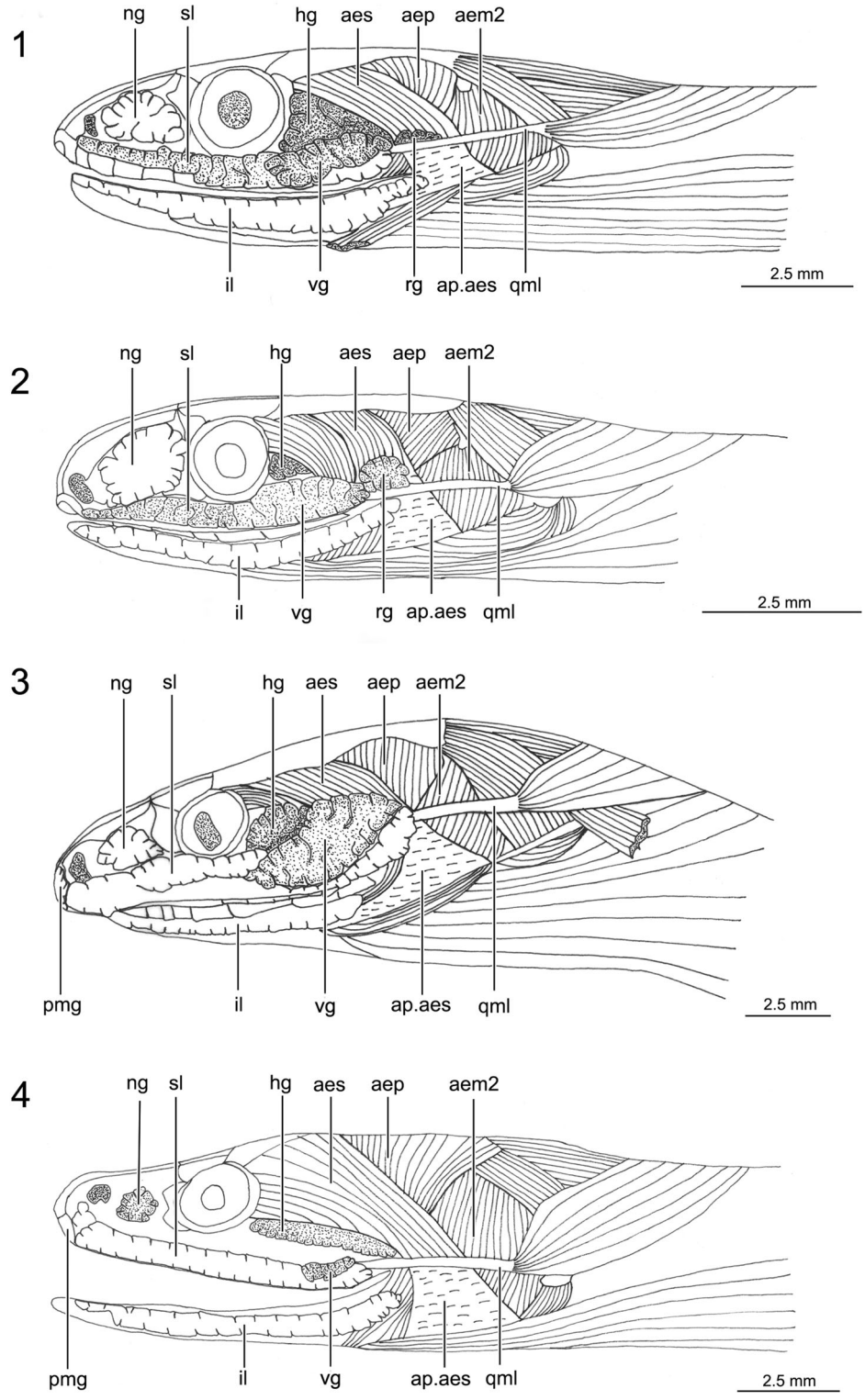

Fig. 2. Overview of the glandular morphology and associated structures in dipsadine snakes that feeding on vertebrates. 1, lateral view of the head of *Rhadinella montecristi*. 2, lateral view of the head of *Trimetopon pliolepis*. 3, lateral view of the head of *Hypsiglena torquata*. 4, lateral view of the head of *Tretanorhinus variabilis*. Abbreviations: aem2, muscle *adductor mandibulae externus medialis pars posterior*; aep, muscle *adductor mandibulae externus profundus*; aes, *muscle adductor mandibulae externus superficialis*; ap.aes, aponeurose of muscle *adductor mandibulae externus superficialis*; hg, harderian gland; il, infralabial gland; ipp, muscle intermandibularis posterior pars posterior; ng, nasal gland; pmg, premaxillary gland; qml, quadrato-maxillary ligament; sl, supralabial gland; rg, superior rictal gland; vg, venom gland.

some serous cells are also observed, especially in *Pli. elapoides* (Supplemental Material – Table S2). The supralabial gland invariably opens into the mouth through several short ducts located between supralabial scales and the oral epithelium (Fig. 1.2).

In all goo-eating dipsadines studied, the posterior third of the supralabial gland is virtually uniform, lacking any histological and histochemical difference that could reveal the presence of a venom gland (Supplemental Material – Table S2). Additionally, no ducts associated with the posterior maxillary teeth were found, further supporting the lack of a venom gland. In *Atr. reticulatus* and *Atr. pantostictus*, the supralabial gland is constituted by mixed acini formed mainly by mucous cells but also by serous cells distributed throughout the gland. In *Nin. sebae* and *Nin. hudsoni*, *Dip. indica*, *Dip. mikanii*, *Dip. neuwiedi*, and *Sib. nebulatus*, the supralabial gland is mostly constituted by serous cells, with mucous cells being restricted to the ducts and in the acini around the ducts (only in *Dipsas* and *Sibon*). In *Tro. sartorii*, the supralabial gland is mainly constituted by mucous cells, while in *Adelphicos quadrivirgatum* it is constituted by two types of mucous cells that are distinguished by their histochemistry reaction, as follow: (1) positive to alcian blue (pH 2.5), (2) strongly positive to the conjugated reaction of alcian blue (pH 2.5) and PAS. The rictal gland of *Ade. quadrivirgatum* differs from the supralabial gland in having mixed acini composed of mucous and serous cells. In *Chersodromus liebmanii*, the supralabial gland is mostly composed by serous cells, although a few mucous cells are also observed in association with their ducts.

Morphology of the maxillary teeth

Dipsadines that feed on vertebrates show significant morphological variation in their maxillary dentition, including grooved or ungrooved posteriormost teeth that may be continuous or separated by a diastema from the rest of the tooth row. In these species, the posteriormost teeth are often larger than the anterior row of teeth and always bear anterior and posterior ridges in opposition to the anterior teeth that bear medial and lateral ridges on their surface (Figs 3, 4; (Supplemental Material – Table S3).

On the other hand, all examined goo-eating dipsadines have an aglyphous maxillary dentition (i.e. without diastema or grooves posteriorly) and posteriormost teeth that tend to be smaller than the more anterior ones. More notably, instead of having anterior and posterior ridges, goo-eating posteriormost teeth bear lateral and medial ridges like those ornamenting the anterior teeth (Fig. 4). Smaller or undifferentiated posteriormost maxillary teeth with lateral and medial ridges are present in all goo-eating dipsadines analysed (Figs 3, 4; (Supplemental Material – Table S3).

A fluted enamel is present in the maxillary teeth of the goo-eating dipsadines *Dip. albifrons*, *Dip. mikanii*, *Sib. nebulatus*, *Geo. nasalis*, and *Nin. sebae*, in the form of densely packed shallow ridges concentrated in the lingual, labial, and posterior surfaces of the teeth (the anterior surface is mainly smooth) (Fig. 4). In *Adelphicos quadrivirgatum*, a fluted enamel is present around the base of the teeth, while the distal two third of the teeth is smooth except for the presence of the medial and lateral ridges (Fig. 4). The remaining species analysed in this study lack enamel fluting in their teeth (Fig. 4).

Discussion

Phylogenetic affinities within Dipsadinae

Our phylogenetic analysis represents the most complete sampling of goo-eating dipsadines (Cadle & Greene, 1993) published so far, which allowed a proper test of its monophyly. Our results indicate that *Adelphicos* is more closely related to *Ninia*, *Chersodromus*, *Atractus*, *Geophis*, *Sibon*, *Tropidodipsas*, and *Dipsas* than to any other dipsadine (Fig. 5), therefore corroborating the monophyly of the group for the first time in a molecular analysis. Although this clade was recovered with a weak bootstrap support, it is independently supported by at least one morphological trait – a fully individualized muscle *levator anguli oris* that curves around the corner of the mouth and attaches to an enlarged and partially seromucous infralabial gland or projects anteriorly to insert via a tendinous aponeurosis on the lateral surface of the anterior tip of the dentary (Zaher et al., 2014; Zaher & Prudente, pers. obs.). This character was recorded by Zaher et al. (2014) as a synapomorphy of a clade formed by all goo-eating dipsadines, except *Adelphicos* which was not retrieved in their goo-eating clade (Zaher et al., 2014, fig. 14). The present analysis resolves this inconsistency. Other dipsadines may possess a differentiated muscle *levator anguli oris* (e.g. *Rhadinaea*, *Urotheca*, *Hydromorphus*, *Synophis*, *Xenopholis*, *Enulius* and *Enuliophis*), but none of them display a specialized bundle that curves around the corner of the mouth and attaches on the fascia of the infralabial gland or on the tip of the dentary (Zaher et al., 2014). Rather, the *levator anguli oris* in these taxa attaches to the epithelial tissue of the corner of the mouth.

Even though our molecular analysis supports Cadle and Greene (1993) goo-eating clade for the first time, it

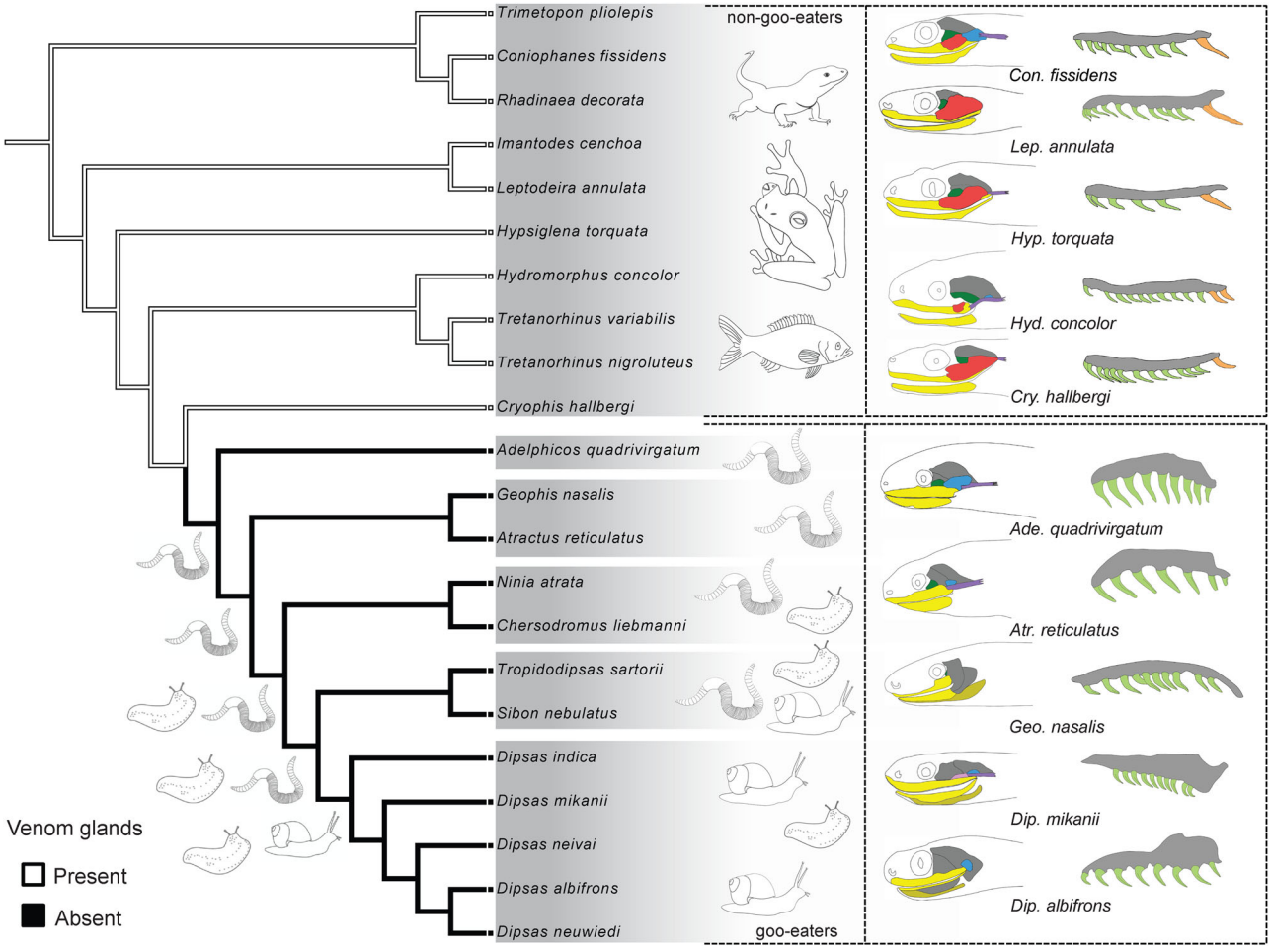

Fig. 3. Maximum parsimony optimization of morphological traits of dipsadines mapped on the pruned topology of our Maximum likelihood tree. The tree represents two main lineages of the subfamily Dipsadinae (Goo-eaters and non-Goo-eaters) and the most common items present in their diets. Inside the lateral boxes, the following morphological structures are represented: blue, superior rictal gland; dark green, harderian gland; grey, muscles *adductor mandibulae externus superficialis* and *levator angulis oris*; purple, quadrate-maxillary ligament; red, venom gland; yellow, labial gland, while the following structures are evidenced on the upper jaws: light green, anterior maxillary teeth; orange, posterior maxillary teeth.

did not recover their original hypothesis of two putative goo-eating sister clades with distinct feeding strategies: one mainly lumbriscivore, feeding almost exclusively on earthworms (i.e. *Adelphicos*, *Atractus*, *Geophis*, *Chersodromus*, and *Ninia*), and the other almost exclusively molluscivore, feeding on slugs and snails (i.e. *Sibon*, *Dipsas*, *Sibynomorphus*, *Tropidodipsas*). The putative clade of lumbriscivorous goo-eaters was retrieved as polyphyletic in our analysis, with *Adelphicos*, *Chersodromus*, *Atractus*, and *Ninia* scattered separately along the goo-eating dipsadine clade (C7). *Adelphicos* was recovered as the sister-group of a strongly supported clade (C6 with bootstrap of 86%) that comprises all the other goo-eating dipsadines, suggesting that lumbriscivory might have evolved first in the common ancestor of all goo-eating dipsadines. Importantly, the rare *Chapinophis* and *Omoadiphas*, which are not sampled here and are thought to be related to *Adelphicos*, seem to represent a separate lineage of lumbriscivorous dipsadines (Sheehy, 2012). However, nothing has been reported on the feeding behaviour of *Chapinophis* and *Omoadiphas*, and the loss of their diacranterian tooth condition might simply not reflect an invertebrate diet (Campbell & Smith, 1998; Villatoro-Castañeda & Ariano-Sánchez, 2017). *Chapinophis* was only recently sequenced, and its molecular affinities was loosely recovered in a ML analysis of dipsadines as the sister group of *Amastridium*, far from *Adelphicos* and the other goo-eaters (Sheehy, 2012). *Chapinophis* and *Omoadiphas* seem to share some important hemipenial features (Campbell & Smith, 1998; McCranie, 2006). Unlike *Adelphicos* in which the

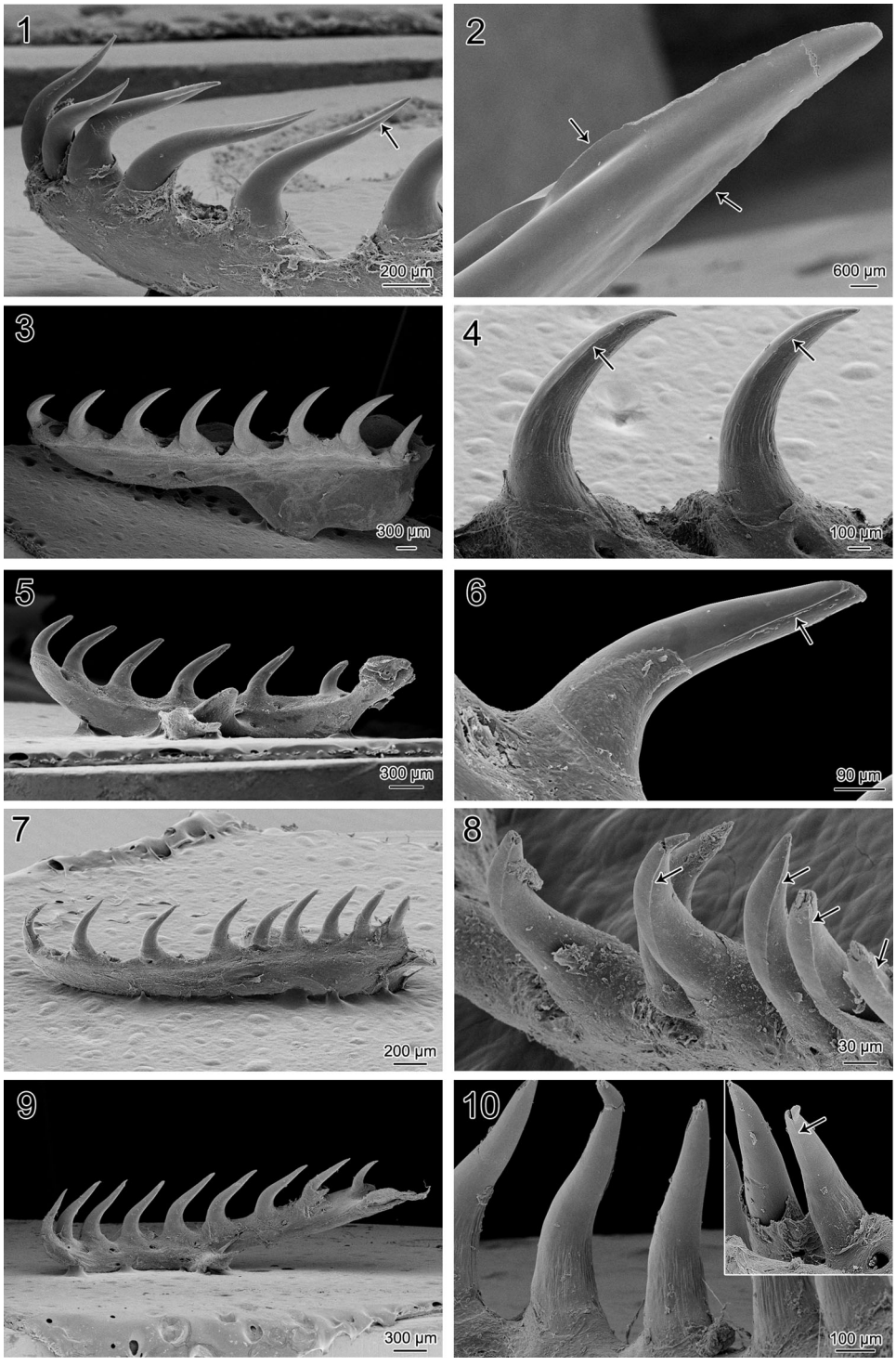

Fig. 4. Scanning electron microscopy of the maxillary teeth. (1–2) Left maxilla of *Leptodeira annulata*. 1, lateral view of the anterior part evidencing lateral ridges restricted to the apical portion of the teeth (arrow). 2, detail of the lateral view showing rear-fang with anterior and posterior ridges (arrows). (3–4) Left maxilla of *Dipsas albifrons*. 3, lateral view. 4, detail of the ridges in the medial view (arrows) and accessory ridges in the posterior surface of the teeth. (5–6) Right maxilla of *Atractus pantostictus*. 5, medial view. 6, detail showing a ridge (arrow) in the medial surface of the tooth. (7–8) Left maxilla of *Ninia sebae*. 7, lateral view. 8, detail showing lateral ridges. (9–10) Right maxilla of *Adelphicos quadrivirgatum*. 9, medial view. 10, detail showing accessory ridges in the posterior base of the teeth. The insert shows lateral ridges on the two posteriormost teeth.

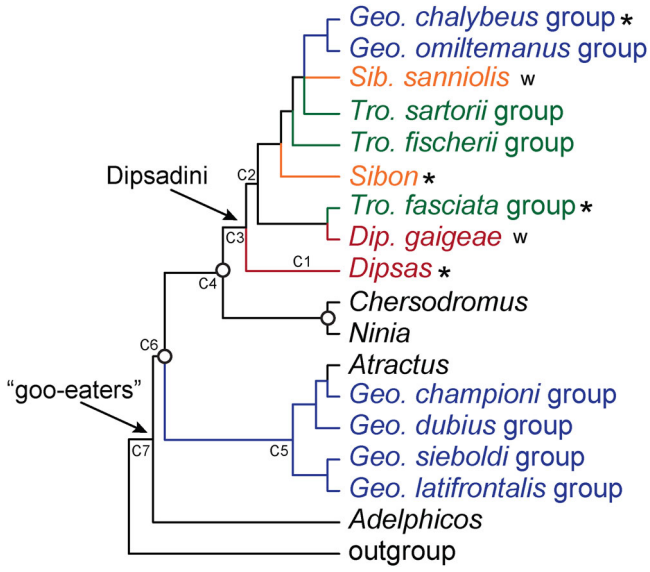

Fig. 5. Summary tree based on the topology of our Maximum likelihood tree, showing relationships among goo-eating dipsadines. An asterisk indicates the position of the type species for each non-monophyletic genus: blue, *Geophis*; orange, *Sibon*; green *Tropidodipsas*; red, *Dipsas*. Rogue taxa are indicated by a "w". C1–C7 correspond to the main clades in our Maximum likelihood tree as defined in the text.

hemipenis is unilobed and unicapitate with an undivided or slightly expanded sulcus spermaticus (*A. quadrivirgatum* and *A. veraepacis*; Campbell et al., 2018; Prudente & Zaher, pers. obs.), *Chapinophis* and *Omoadiphas* share distinctly bilobed and bicapitate organs with a sulcus spermaticus that bifurcates well within the hemipenial body. Hemipenial morphology suggests that *Chapinophis* and *Omoadiphas* are more closely related to each other than to *Adelphicos* and the other goo-eating dipsadines, corroborating Sheehy's (2012) molecular results with *Chapinophis*. Unfortunately, nothing is known about their head muscle and gland morphology.

Like most recent studies (Grazziotin et al., 2012; Grünwald et al., 2021; Pyron et al., 2011; Sheehy, 2012; Zaher et al., 2014, 2019), our analysis divided the remaining genera of goo-eaters (clade C6) in two sister clades, one composed by *Atractus* and the *championi, latifrontalis, dubius,* and *sieboldi* groups of *Geophis* (clade C5), and the other comprising *Chersodromus*, *Ninia*, *Tropidodipsas*, *Dipsas*, and the *omiltemanus* and *chalybeus* groups of *Geophis* (clade C4). Our analysis does not support the monophyly of the genera *Dipsas*, *Geophis*, *Sibon*, and *Tropidodipsas*. The highly polyphyletic nature of *Geophis*, already suggested in previous molecular analyses (Grazziotin et al., 2012; Zaher et al., 2014, 2018) and evidenced in Sheehy's (2012) study, illustrates the unstable taxonomic status of the whole goo-eating clade (Fig. 5). However, some deeper nodes recovered here are statistically well supported. These include the strongly supported (82%) clade C4, composed by the genera *Ninia*, *Chersodromus*, the tribe Dipsadini, and the *chalybeus* and *omiltemanus* groups of *Geophis*. This arrangement will likely be further corroborated in future analyses and calls for a comprehensive study of the morphology of *Geophis*. Non-monophyly of *Geophis* was already pointed out in morphological studies that commented on the striking differences observed in the *chalybeus* and *omiltemanus* groups, on the one hand, and the other species groups of *Geophis*, on the other hand (Boulenger, 1894; Downs, 1967). Boulenger (1894) stressed these morphological differences by erecting a new genus (*Dirosema*) to accommodate *Geo. chalybeus* and *Geo. omiltemanus* (along with *Geo. bracycephalus* and *Ninia psephota*), stating that these species were morphologically intermediates between *Tropidodipsas* and *Geophis*. Downs (1967) also considered the *chalybeus* and *omiltemanus* groups to be substantially distinct and less specialized in a borrowing lifestyle than the other groups of *Geophis*. Dunn (1935), for his part, noted the similarities between the "least modified members of *Geophis*" and *Ninia*. Their comments seem to corroborate the affinities of both *chalybeus* and *omiltemanus* species groups with the Dipsadini.

Within clade C4, *Tropidodipsas* also appears as a polyphyletic assemblage, with members of the *sartorii* and *fischeri* groups nested separately within *Sibon* while the *fasciata* group clusters with *Dipsas gaigeae* outside the genus *Dipsas* (Fig. 5; Sheehy, 2012). Similarly, the paraphyletic nature of *Dipsas* in respect to *Sibynomorphus* has been shown in several previous studies, which suggested that the latter should be

synonymized with the former (Fernandes, 1995; Grazziotin et al., 2012; Zaher et al., 2014, 2018). Based on these authors' previous results, Arteaga et al. (2018) and Grünwald et al. (2021) synonymized the genus *Sibynomorphus* with *Dipsas* and transferred the species *Tropidodipsas annulifera*, *Tro. sartorii*, and *Sibon sanniolus* to *Geophis*. The clade formed by *Dipsas* + *Sibynomorphus*, but excluding *Dipsas gaigeae*, is retrieved with moderate support (clade C1; 78%) and is supported by at least one conspicuous morphological synapomorphy: the presence of a divided infralabial gland formed by a reduced il1 and a well-developed il2 that opens mainly through a large duct on the floor of the mouth at the level of the intermandibular raphe (Zaher et al., 2014; Zaher & Prudente, pers. obs.). Regarding the four remaining species groups of *Geophis* sampled in previous analyses (Pavón-Vázquez et al., 2013; Pyron et al., 2011; Sheehy, 2012; Zaher et al., 2009, 2018), they invariably cluster together paraphyletically as successive sister groups to a monophyletic genus *Atractus* (retrieved here as clade C5 with the same topology and no significant statistically support). Grünwald et al. (2021) did not provide a taxonomic solution for that clade, leaving the genus *Geophis* polyphyletic. We also refrain to propose any taxonomic change to the components of clade C5 since many species of *Geophis* have not been yet included in any morphological or molecular analysis (only 12 of the 49 recognized species of *Geophis*, belonging to six out of seven groups, were sampled in molecular analyses; Wilson & Townsend, 2007).

Despite Grünwald et al.'s (2021) allocation of *Sibon sanniolus*, *Tro. sartorii*, and *Tro annuliferaus* in *Geophis*, polyphyly of *Sibon* and *Tropidodipsas* persists within clade C2, although with weak support. This result was already discussed by Sheehy (2012), who suggested the recognition of two new genera to allocate the *sartorii* and *fischerii* species groups, respectively, since *Tro. fasciata* is the type species of the genus *Tropidodipsas* Günther, 1858 and clustered as a separate subclade within clade C2. The topology of our clade C2 does not confer with the results presented by Sheehy (2012) and Grünwald et al. (2021), with *Dip. gaigeae* and *Sib. sanniolus* representing two rogue taxa with very unstable positions in independent gene trees as well in the topologies of some exploratory analyses in which we tested the impact of our taxon sampling (data not shown). These results reflect the state of flux that persists among members of clade C2, suggesting a more cautious position when considering taxonomic changes within that group.

The genera *Chersodromus* and *Ninia* are retrieved as a strongly supported clade (79%) clustering as the sister-group of the tribe Dipsadini within clade C4. *Chersodromus* and *Ninia* have long been known for their close affinities (e.g. Downs, 1967; Dunn, 1935), and Fernandes (1995) provided the first cladistic analysis corroborating their sister group affinities. They share hypapophyses on all trunk vertebrae, hemipenes with a sulcus bifurcating proximally, and keeled dorsal scales, all features absent in the other goo-eating snakes but known to be present in other unrelated groups of dipsadines (e.g. the "niniiforms" of Savitzky, 1974). Both genera also share hypertrophied Harderian glands that reach the muscle *adductor mandibulae externus medialis pars posterior* (Zaher & Prudente, pers. obs.). Expanded Harderian glands are also known to occur in *Amastridium*, *Enulius*, *Enuliophis*, *Pliocercus*, *Urotheca*, *Tretanorhinus*, and some species of *Coniophanes*, *Dipsas*, *Geophis*, and *Rhadinaea* (Zaher & Prudente, pers. obs.).

Phylogenetic trees only based on mitochondrial genes effectively represent a single gene history, since mitochondrial loci sort as a single lineage (Avise, 2000). Mitochondrial signals have been shown as misleading in some cases, including in some snake groups (e.g. Mason et al., 2019) and several studies have shown the general improvement in phylogenetics derived from using multilocus evidence (reviewed in Edwards, 2009). Although our analysis has been based on three nuclear and four mitochondrial genes, for some key terminals we manly used mitochondrial evidence (e.g. *Adelphicos*, *Chersodromus*, and *Urotheca*). To test the concordance between the mitochondrial tree and the tree generated based on the concatenated matrix, we provided an additional analysis using the mitochondrial data only, applying the same methods used for the main phylogenetic analysis. Our results indicate that, concerning dipsadines, the topology of both trees is mainly concordant (Supplemental Material – Fig. S2), which may suggest the mitochondrial evidence biased our main analysis, or that the lack of nuclear sequences for these key taxa may not have a substantial influence in the relationship among the goo-eating dipsadines. We acknowledge that to sort these hypotheses out it is necessary a more comprehensive sampling of other nuclear loci.

The evolution of soft-bodied invertebrate feeding in dipsadine snakes

Goo-eating dipsadine snakes have long been known for their molluscivorous and/or lumbriscivorous diet and, although several unusual osteological and muscular features were identified as specializations for the consumption of soft-bodied invertebrates (e.g. Brongersma, 1958; Haas, 1931; Vaeth et al., 1985), little is known about the morphology and function of their venom gland and associated posterior maxillary teeth. Moreover, until

recently our poor knowledge of the phylogenetic affinities within dipsadines hampered any attempt to better define the sequence of morphological modifications that led to the specialized goo-eating condition in the group.

Despite some important gaps in taxon sampling, our molecular phylogenetic analysis provides a strong basis to unveil key evolutionary changes that allowed goo-eating dipsadines to become one of the most successful Neotropical radiation of snakes. Our study provides the first evidence that the most recent common ancestor of all goo-eating dipsadines lost the upper jaw venom delivery system in association with the appearance of their specialized soft-bodied feeding behaviour.

Loss of the upper jaw venom delivery system associated with soft-bodied feeding. According to Underwood (1997), critical anatomical traits of oral glands such as duct systems and cellular types are only accessible through histological serial sections. By combining observations based on macroscopic dissections, histology, and tomography, we were able to confirm the presence of venom glands (i.e. the occurrence of isolated serous cells in the upper jaw glands associated with a single duct directed to the posterior maxillary teeth) in 14 dipsadine snakes, all species that prey predominantly on vertebrates (Figs 1–3; (Supplemental Material – Table S2). We also observed the presence of a diastema and/or grooves on the posterior teeth as an additional criterion for the presence of venom glands (Figs 3, 4.1–4.2; (Supplemental Material – Table S3). On the other hand, we did not confirm the presence of venom glands, diastema, and grooved posterior teeth in any of the 17 goo-eating dipsadines analysed (Supplemental Material – Tables S2 and S3).

Venom glands were already reported to be either absent (Harvey et al., 2008; Lima & Prudente, 2009; Passos et al., 2016; Phisalix, 1922; Savitzky, 1974; Taub, 1967; Zaher et al., 2014) or present in the posterior portion of the upper jaw of goo-eating dipsadines (Contrera et al., 1983; Laporta-Ferreira & Salomão, 1991; Salomão & Laporta-Ferreira, 1994; Taub, 1967). However, unlike the latter reports, we did not find in the studied goo-eating dipsadines any evidence of differentiated cells in the posterior portion of the upper jaw glands that could reveal the presence of venom glands, or the presence of any duct linking these glands to the posterior maxillary teeth. Our histological analyses failed to reveal the presence of venom glands even in species with posteriorly enlarged supralabial glands (e.g. *Dip. williansi* and *Dip. vagus*). Embryological evidence from *Dip. mikanii* further supports the absence of venom glands in goo-eating dipsadines (Oliveira et al., 2017).

On the other hand, our results reveal the presence of rictal glands in most studied dipsadines (Fig. 3; (Supplemental Material – Table S2), including previously unreported superior rictal glands located posterodorsally to the supralabial glands in *Dip. mikanii* and *Dip. neuwiedi*. These glands can be distinguished from venom glands by their medial position in respect to the quadrato-maxillary ligament (Fig. 1.6; McDowell, 1986; Underwood, 2002). The superior rictal glands of *Dip. mikanii* and *Dip. neuwiedi* might have been erroneously identified as venom glands since reports on their presence in these two species indicate reduced serous or seromucous glands located behind the supralabial glands, at the level of the corner of the mouth (Contrera et al., 1983; Laporta-Ferreira & Salomão, 1991). These are characteristics of typical of rictal glands (Underwood, 1997).

A similar mistake might have occurred with pareids, a family of basal Colubroides (*sensu* Zaher et al., 2019) also specialized in feeding on molluscs. Unlike Fry et al. (2008) who reported the presence of venom glands in *Pareas carinatus*, Underwood and Kochva (1993) pointed out that there is no evidence of venom glands in the genus *Pareas* (Underwood, 2002). The absence of venom glands in acrochordids, xenodermids, and pareids (Jackson et al., 2017; Underwood & Kochva, 1993; Zaher et al., 2019) is consistent with the presence of undifferentiated diacranterian posterior maxillary teeth in these families and suggests that the acquisition of a complex upper jaw venom delivery system occurred in the more recent common ancestor of the Endoglyptodonta within Caenophidia (Zaher et al., 2019). Underwood (1997) already pointed out an intimate connection between the presence of modified posterior maxillary teeth and venom glands. Results with dipsadines do support Underwood's observations, since modified and enlarged posterior maxillary teeth are only present in species with observable venom glands, while simple and often reduced posterior maxillary teeth are associated with species lacking venom glands (Fig. 3; (Supplemental Material – Table S3).

Our results show that only the species of dipsadines that feed on vertebrates retain venom glands and differently ridged maxillary teeth, with the more posterior teeth exhibiting anterior and posterior ridges while the anterior teeth bear lateral and medial ridges. This includes *Cryophis* and *Tretanorhinus* + *Hydromorphus*, the two successive sister-groups of the goo-eating clade (Fig. 3).

In goo-eating dipsadines, the diastema is always absent and there is no distinction between anterior and posterior maxillary teeth, except for the more accentuated reduction of size posteriorly (Fernandes, 1995;

Mulcahy et al., 2011; Peters, 1960). More importantly, all the maxillary teeth of goo-eating dipsadines exhibit lateral and medial ridges (Jackson & Fritts, 1995), including the most posterior ones, suggesting that the embryonic posterior maxillary lamina responsible for the development of both fangs and venom gland (Vonk et al., 2008) was lost in the most recent common ancestor of the group. Consequently, the adult maxilla of goo-eating dipsadines originates solely from the embryonic anterior maxillary lamina while their entire endoglyptodont venom delivery system fails to develop, a hypothesis that also accounts for the lack of a diastema in the group.

Our results also reveal a close association between diet and the loss of the upper jaw venom delivery system in the most recent common ancestor of goo-eating dipsadines (Fig. 3). These findings suggest that the embryological changes associated with the secondary loss of the upper jaw venom delivery system were responsible for triggering the morphological constraints that drove the appearance of a novel lower jaw venom delivery system adapted for a specialized diet based on soft-bodied invertebrates (Oliveira et al., 2008, 2014; Zaher et al., 2014). However, although our results based on observations of adult morphology are compelling, this hypothesis still needs to be tested by additional embryological evidence.

Lumbriscivory as the ancestral state in goo-eating dipsadines. The goo-eating clade is divided in five major lineages with a specialized diet mainly restricted to either earthworms or molluscs or comprising both kinds of prey (Fig. 3). While *Chersodromus* and *Ninia* prey on both, earthworms and molluscs, *Adelphicos*, *Atractus*, and *Geophis* (*sensu lato*) are earthworm specialists, and *Sibon*, *Tropidodipsas*, and *Dipsas* feed mainly on snails and slugs (Supplemental Material – Table S4). On the other hand, all the other dipsadine lineages seem to feed mainly on vertebrates instead.

As already suggested by Sheehy (2012), the topology of our ML tree implies that a lumbriscivorous diet appeared first in the most recent common ancestor of all goo-eating dipsadines, being retained in the lineages leading to *Adelphicos* and *Atractus* (Fig. 3). Molluscivory appeared for the first time in the most recent common ancestor of the clade formed by *Chersodromus*, *Ninia*, and the Dipsadini, with a variable soft-bodied diet including both earthworms and mollusc persisting in *Chersodromus*, *Ninia*, *Tropidodipsas*, and *Sibon*. The restricted molluscivorous diet only appeared in the most recent common ancestor of the genus *Dipsas* (clade C1). The present scenario predicts that, like *Sibon* and *Tropidodipsas*, *Dip. gaigeae* and its closest relatives would likely feed on both earthworms and molluscs (Sheehy, 2012). The clear preference of *Tro. philipii* and *Tro. fischeri* for earthworms (Sheehy, 2012) and the phylogenetic position of the lumbriscivorous *chalybeus* and *omiltemanus* species groups of *Geophis* appear to corroborate the variable ancestral diet for the Dipsadini clade (Fig. 3).

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Author contributions

LO, FG, and HZ conceived the work and wrote the manuscript. LO and FG performed the morphological experiments, conducted the phylogenetic analyses, edited the figures, and contributed equally for this study. LO, AP, and HZ dissected the specimens and conducted the gross anatomical studies. All authors contributed to data collection and to the construction of the dataset. All authors read, critically edited, and approved the final manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Supplemental material

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ORCID

Leonardo De Oliveira http://orcid.org/0000-0003-1768-603X
Felipe Gobbi Grazziotin http://orcid.org/0000-0001-9896-9722
Paola Maria Sánchez-Martínez http://orcid.org/0000-0002-7791-1326
Mahmood Sasa http://orcid.org/0000-0003-0118-5142
Oscar Flores-Villela http://orcid.org/0000-0002-2849-6912
Ana Lúcia Da Costa Prudente http://orcid.org/0000-0002-4164-6815
Hussam Zaher http://orcid.org/0000-0002-6994-489X

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