Endless forms most beautiful: the evolution of ophidian oral glands, including the venom system, and the use of appropriate terminology for homologous structures

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ORIGINAL PAPER

Endless forms most beautiful: the evolution of ophidian oral glands, including the venom system, and the use of appropriate terminology for homologous structures

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Abstract The differentiated serous-secreting dental glands of caenophidian snakes are diverse in form despite their developmental homology. This variation makes the elucidation of their evolutionary history a complex task. In addition, some authors identify as many as ten discrete types/subtypes of ophidian oral gland. Over the past decade and a half, molecular systematics and toxinology have deepened our understanding of the evolution of these fascinating and occasionally enigmatic structures. This paper includes a comprehensive examination of ophidian oral gland structure and (where possible) function, as well as new data on rictal glands and their associated anatomy. Following this, appropriate use of terminology, especially that pertaining to homologous structures (including the controversial "venom gland" vs "Duvernoy's gland" debate), is considered. An interpretation of the evolutionary history of the ophidian venom system, drawing on recent results from molecular systematics, toxinology and palaeontology, concludes the paper.

Keywords Snake · Venom · Evolution · Anatomy · Terminology · Function

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Introduction

The differentiated serous-secreting dental glands of caenophidian snakes (venom glands/Duvernoy's glands) are diverse in form despite their developmental homology. This variation makes the elucidation of their evolutionary history a complex task. Some authors (e.g. Weinstein et al. 2012) have suggested that their diversity of form might be indicative of a corresponding diversity of function, whereas others (e.g. Fry et al. 2012), drawing on a growing body of evidence (including their loss or reduction in numerous species), have concluded that the *primary* (not necessarily *sole*) function influencing the evolution of these glands is the production of venom. For this reason, the latter group have preferred the term "venom gland" for all variations of this organ, a choice intended to highlight both their developmental homology and their inclusion within a single function category.

The oral glands of snakes are exceptionally diverse (Fig. 1; Table 1)—some authors have identified as many as 10 oral in addition to two non-oral "cephalic glands" (Taub 1966). Not only are there a great number of types,

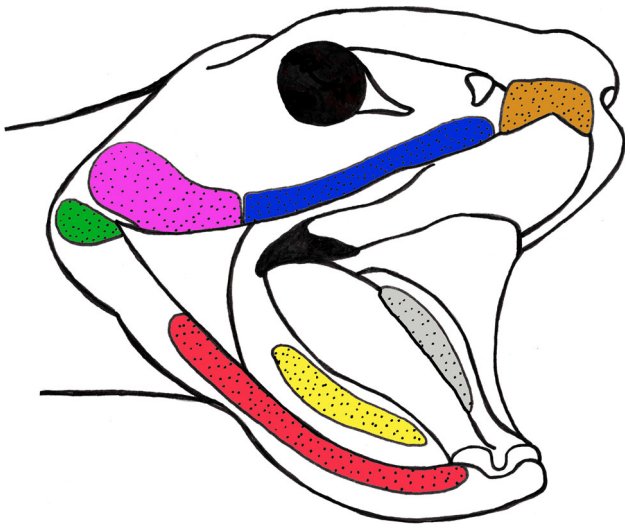

Fig. 1 Simple schematic of the oral glands of snakes (illustration by Genevieve Jackson, modified from Kochva 1978). The glands of the upper jaw include the premaxillary (*brown*), supralabial (*blue*), venom (*pink*) and rictal (*green*). The glands of the lower jaw include the infralabial (*red*), sublingual (*yellow*) and the supralingual (*grey*)

Table 1 Ophidian oral glands and their functions (putative or established)

Gland	Function
Supralabial	Lubrication
Venom/dental	Production of venom; antimicrobial(?)
Accessory	Antimicrobial(?)
Rictal	Antimicrobial(?); lubrication(?); production of venom
Premaxillary	Salt extraction (*Cerberus*)
Infralabial	Lubrication; production of venom Dipsadidae(?); mucous control(?) (Dipsadidae)
Sublingual	Lubrication; salt extraction (marine Elapidae; *Acrochordus*)
Supralingual	Unknown
Temporomandibular	Unknown

but the structure and arrangement of glands differ widely amongst snakes, sometimes even between members of the same genus (Kochva 1978; Wollberg et al. 1998; Fry et al. 2008). Considering the diversity of venom gland forms within, the broader diversity of ophidian oral glands may be instructive. Doing so may serve to highlight the homology of venom glands (the diversity of which may otherwise make this hard to appreciate) and emphasise the importance of terminological consistency in avoiding the generation of confusion. That they are part of a larger system comprised of numerous glands potentially capable of fulfilling a range of functional roles may also help to clarify the distinct function of the venom glands. For example, where several purely mucous-secreting glands are present and able to contribute their secretions to the lubrication of prey items, it is unlikely that purely serous-secreting glands exist in order to contribute to this particular functional role.

The extreme lability of ophidian oral gland arrangements makes gaining a clear understanding of the function and evolutionary history of each gland type difficult (Gans 1978), and has resulted in considerable confusion and controversy regarding terminology, with authors frequently using different names for the same structures (Taub 1966). Recently, the improvement in snake phylogenies achieved through molecular phylogenetics has facilitated further insight into the evolution of snake oral glands, in particular the venom system, including the venom gland and associated musculature and dentition (Vidal 2002; Fry et al. 2006, 2008; Vonk et al. 2008). This has not put an end to the terminological controversies, however, as researchers continue to differ in opinion regarding the most appropriate nomenclature for the various forms of the venom gland (Weinstein 2011; Fry et al. 2012).

This paper synthesises previously published data with newly acquired data in order to provide a modern perspective on the evolution of snake oral glands. After detailing the anatomy of the full range of ophidian oral glands, it concludes with a discussion regarding the use of appropriate terminology and a consideration of the evolution of the venom glands in particular. In addition, it includes previously unpublished data, including some collected by the late Garth Underwood, a pioneering researcher in the field of snake oral gland anatomy, and is dedicated to his memory.

Note on terminology

Issues relating to terminology are discussed in detail in a section devoted to that topic. Wherever possible, this manuscript follows the practice of using the single most appropriate name for homologous structures, despite the fact that these may vary in form amongst species. Thus, for example, the differentiated (from the supralabial gland) toxin-secreting dental glands of the Caenophidia are referred to as "venom glands" (cf. "Duvernoy's gland" in non-front-fanged species) and the glands of the corner of the mouth are referred to as "rictal glands" (cf. "posterior gland" or "anterior temporal gland"), except where other authors are directly quoted.

Note on snake phylogeny

The phylogeny followed in the present paper is that of Vidal et al. (2009), which was generated using 9 nuclear genes from all major snake lineages with the exception of the Xenophidiidae and broadly corroborates the results of

the morphological and molecular analyses of Lee et al. (2007), Vidal et al. (2008), Kelly et al. (2009, 2011), Pyron et al. (2011) and Zheng and Wiens (2016).

Methods

Species examined

Achalinus rufescens, *Acrochordus granulatus*, *Amblyodipsas unicolor*, *Anilios guentheri*, *Aparallactus capensis*, *Aparallactus modestus*, *Aspidelaps lubricus*, *Aspidelaps scutatus*, *Aspidites melanocephalus*, *Atractaspis engaddensis. Atractaspis microlepidota*, *Azemiops feae*, *Boa constrictor*, *Bolyeria multocarinata*, *Bungarus lividus*, *Calabaria reinhardtii*, *Calliophis bivirgatus*, *Casarea dussumieri*, *Causus resimus*, *Causus rhombeatus*, *Cerberus rhynchops*, *Cerastes cerastes*, *Chilorhinophis gerardi*, *Crotalus atrox*, *Cylindrophis ruffus*, *Dendroaspis angusticeps*, *Dendroaspis jamesoni*, *Dendroaspis polylepis*, *Dendroaspis viridis*, *Dispholidus typus*, *Elapsoidea guentherii*, *Elapsoidea laticincta*, *Elapsoidea loveridgei*, *Elapsoidea nigra*, *Elapsoidea semiannulata*, *Elapsoidea sundevallii*, *Fimbrios klossi*, *Heterodon platyrhinos*, *Homoroselaps lacteus*, *Hemachatus haemachatus*, *Hydrophis* (*Pelamis*) *platurus*, *Hypnale hypnale*, *Imantodes cenchoa*, *Indotyphlops braminus*, *Langaha madagascariensis*, *Madagascarophis colubrinus*, *Naja* (*Boulengerina*) *annulata*, *Naja annulifera*, *Naja* (*Boulengerina*) *christyi*, *Naja haje*, *Naja katiensis*, *Naja* (*Paranaja*) *multifasciata*, *Naja melanoleuca*, *Naja mossambica*, *Naja naja*, *Naja nigricollis*, *Naja nivea*, *Naja pallida*, *Naja* (*Pseudohaje*) *goldii*, *Naja* (*Pseudohaje*) *nigra*, *Pareas carinatus*, *Pareas monticola*, *Philothamnus irregularis*, *Psammodynastes pulverulentus*, *Scolecophis actrocinctus*, *Stenorhina freminvillei*, *Tachymenis peruviana*, *Trimorphodon biscutatus*, *Tropidophis haetianus*, *Walterinnesia aegyptia.*

Histology

Venom delivery systems from each species were excised from intact heads. Glands and ducts were then fixed in 10 % neutral buffered formalin for a minimum of 2 days. Biopsies were then rinsed in running water for 10 min to remove formalin. Once formalin was removed, biopsies were dehydrated in an ethanol series (70 %—45 min, 90 %—45 min, 2 changes of 100 %—45 min), cleared in 2 changes of xylene, followed by paraffin (2 × 45 min) subjecting the tissue samples to -60 psi during the second change prior to embedding. Serial transverse and longitudinal sections (10 µm thick) were taken of the ducts and salivary glands using a Hyrax M25 Rotary Microtome. The sections were then fixed to glass slides and air-dried. Once dry, the slides were then stained with Mayer's haematoxylin and eosin (Luna 1968), Puchtler's picro-sirus red (Kiernan 1999), Bielschowsky's silver stain (Luna 1968) and a variation of Masson's trichrome where aniline blue was substituted for fast green (Luna 1968). Slide viewing was carried out via a differential interference contrast microscopy.

Magnetic Resonance Imaging

MRI was used to examine the three-dimensional shape and internal anatomy of the venom glands. Formalin-ethanol fixed heads were first submersed in Fomblin (Solvay Solexis) to prevent air artefacts. Depending on head size, imaging was performed on either 9.4 T (small/medium) or 17.6 T (large) vertical 89-mm-bore systems (Bruker BioSpin, Rheinstetten, Germany) with a Bruker Micro2.5 gradient system of 1 T/m and transmit/receive birdcage radiofrequency coil with diameter of 10–30 mm. *Anilios guentheri* images were acquired on a Bruker 9.4 T wide bore system with a microimaging accessory and a 400 MHz 1H 5-mm cryoprobe for NMR microscopy. The cryoprobe was used in combination with separate Micro2.5 gradient system (1.5 T/m maximum gradient strength). Bruker ParaVision 3.0 software was used for image acquisition. Anatomical images were acquired using a 3D gradient echo sequence. The field-of-view and matrix were varied to fit the individual samples, resulting in voxel sizes between $(40)^3$ mm^3 and $(70)^3$ mm^3. Imaging parameters were: TE = 8 ms, TR = 40 ms, flip angle 20°, 4–8 averages, total scan time between 3 and 9 h per sample, depending on size and resolution. Image segmentation of the glands was performed manually in Amira 4.1 (Mercury Computer Systems Inc.), and 3D surface renderings were generated for all species.

Dissection

The heads of 30 species of elapid snake (29 African and 1 Asian) were dissected in order to examine the rictal gland and associated structures (see Table 2 for full list of species).

Results and discussion

Gland anatomy

Oral glands of the upper jaw

In the majority of snakes, the upper jaw supports two oral glands: the rictal and the sero-mucous plesiomorphic form of the supralabial. In the plesiomorphic state, the maxillary

Table 2 Rictal and supralabial gland conditions of elapid snakes determined by dissection

	Supralabial, mucous/serous	Rictal gland, presence	Supralabial overlap	Rictal gland duct/pocket	Corner of mouth, accessory lobules: serous/mucous	Cilia
Aspidelaps lubricus	.	+	.	.	.	.
Aspidelaps scutatus	m	+	+	d	s	-
Bungarus lividus	m	+	+	d	-	-
Dendroaspis angusticeps	.	+	++	.	.	.
Dendroaspis jamesoni	s	+	++	p	m	-
Dendroaspis polylepis	.	+	++	.	.	.
Dendroaspis viridis	.	+	++	.	.	.
Elapsoidea guentheri	m	+	+	d	-	-
Elapsoidea laticincta	.	+	+	.	.	.
Elapsoidea loveridgei	m	+	+	d	-	-
Elapsoidea nigra	.	+	+	.	.	.
Elapsoidea semiannulata	.	+	+	.	.	.
Elapsoidea sundevalli	.	+	+	.	.	.
Homoroselaps lacteus	m	-	-	NA	NA	-
Hemachatus haemachatus	m	+	+	p	+	-
Naja annulata	s	?	NA	NA	s	-
Naja annulifera	m	+	-	p	-	+
Naja christyi	m	+	-	p	s	-
Naja goldi	m	+	-	d	+	-
Naja haje	m	+	-	p	-	+?
Naja katiensis	m	+	+	p	+	+
Naja melanoleuca	m	+	-	p	-	+?
Naja mossambica	m	+	-	p	-	+
Naja multifasciata	m	+	+	d	+	-
Naja naja	m	+	+	p	s	-
Naja nigra	.	+	.	.	.	.
Naja nigricollis	m	+	+	p	-	+
Naja nivea	m	+	+	p	.	+
Naja pallida	m	+	-	p	+	+
Walterinnesia aegyptia	m	+	-	d	-	-

+ = present; − = absent; ? = unresolvable; . = no observation; m = mucous; s = serous; d = duct; p = pocket

glands are smaller than the mandibular glands, as in Anguimorpha and Iguania lizards (Fry et al. 2006, 2010, 2013). In the advanced snakes, upper jaw supports three oral glands: the rictal, and the plesiomorphic supralabial divided into distinct mucus and protein (venom) glands, with the upper jaw glands larger than the lower jaw glands. These three upper jaw glands have a rather characteristic pattern (Fig. 2). The predominantly mucoid supralabial gland is located along the lateral margin of the upper jaw; the predominantly serous venom gland is located along the caudal portion of the maxilla and upper jaw (frequently bordered both caudally and cranially by the supralabial) (Fig. 3a). The rictal pouch is typically located immediately medial to the caudal portion of the venom gland (and the adjacent supralabial gland); the rictal glands themselves are located in the superior portion of the pouch, often at a frontal plane above the supralabial or venom glands (Fig. 2).

Supralabial glands When reviewing the literature concerning ophidian oral glands, it can be difficult to distinguish between references to "labial" and "dental" glands. Labial glands are widely present in squamate reptiles, but dental glands proper are a synapomorphy of

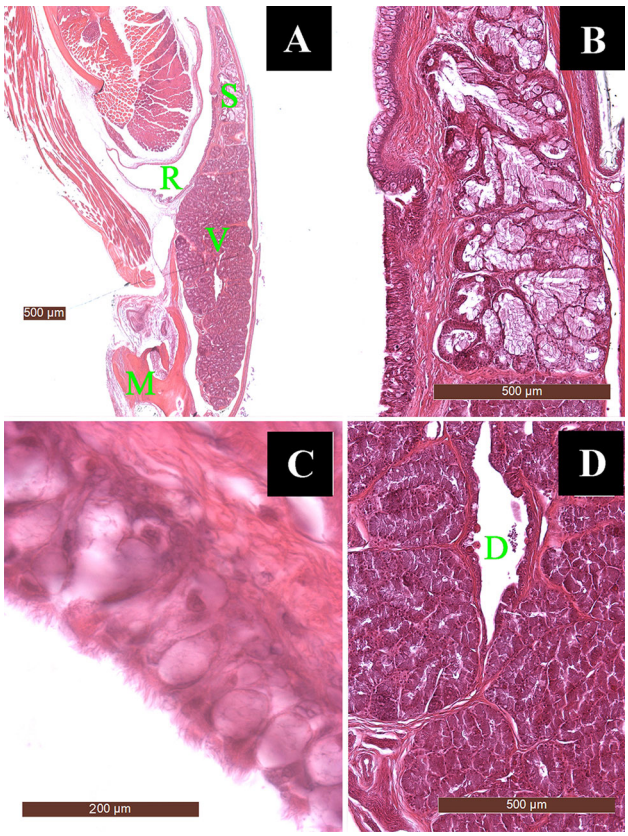

Fig. 2 Characteristic spatial relationships and histological features of the ophidian oral glands found on the upper jaw, illustrated with frontal sections through *Langaha madagascariensis* (all stained with H&E). **a** Low magnification showing the venom gland abutting the supralabial gland posteriorly and the maxilla medially; medial to the venom and supralabial glands is the rictal pouch, which, in it's superior portion, will support the rictal gland (*scale bar* 500 µm). **b** The supralabial gland demonstrating the abundance of mucoid cells and the almost segmental appearance of the ducts. **c** The rictal pouch is typically lined with a ciliated epithelium rich in unicellular mucoid glands. **d** The serous venom gland with the well-demarcated central duct. *D* duct, *M* maxilla; *R* rictal pouch; *S* supralabial glands; *V* venom gland

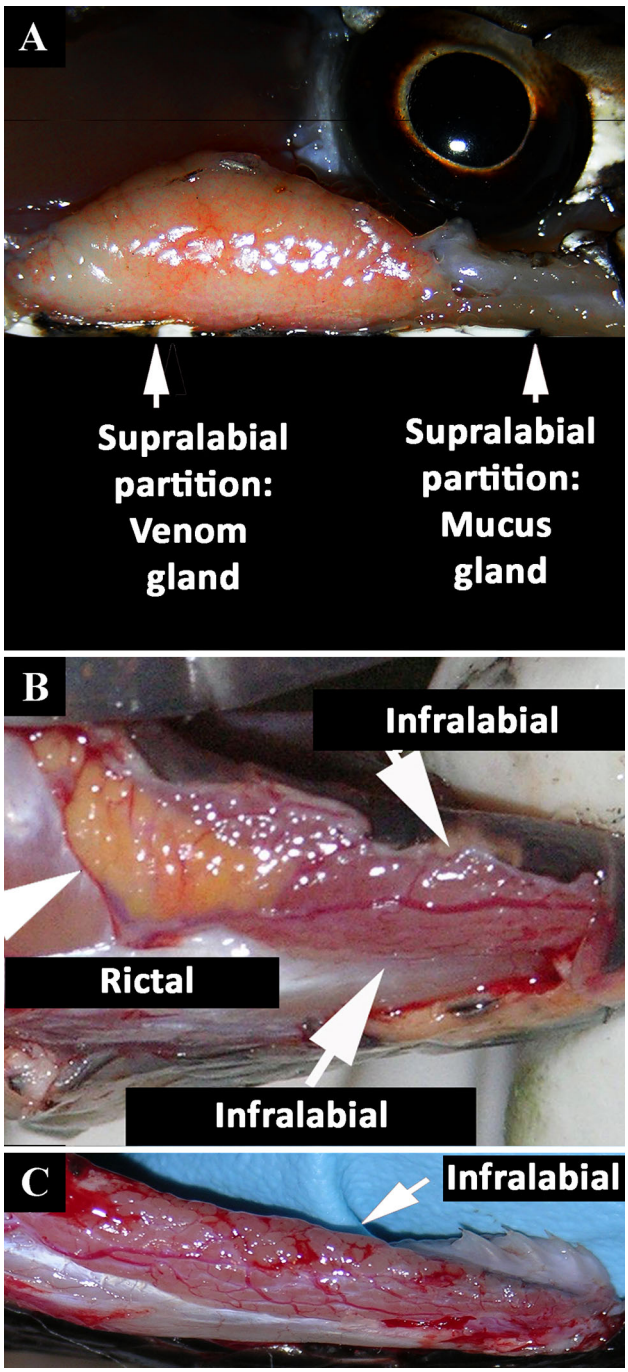

Fig. 3 Dissections of **a** *Malpolon monspessulanus*, **b** *Cylindrophis ruffus* and **c** *Aspidites melanocephalus*

the Toxicofera (Kochva 1978, 1987; Fry et al. 2013). Taub (1967) considered all supralabial glands to be composed of purely mucous cells and thus "easily distinguishable" from the serous-secreting dental ("Duvernoy's") glands (Fig. 3a). More recently, the simplicity of this distinction has been undermined by the demonstration that the labial glands of many snakes contain serous as well as mucous cells (Underwood 2002). In fact, several earlier authors had described mixed sero-mucous labial glands (Kochva 1978), and Taub himself had noted their occurrence in numerous species in the very same paper in which he claimed all labial cells were purely mucous (Taub 1967)! In snakes, the term "dental gland" is often reserved for the specialised venom or "Duvernoy's gland" of the Caenophidia (Underwood 2002); however, the "labial" glands of iguanian lizards are also referred to as "dental" glands (Kochva 1978, 1987). Polystomatic dental glands such as those of the Iguania are absent in snakes. Most properly, labial glands and dental glands can be differentiated by the location of their duct openings—the ducts of supralabial glands open into the space between the maxilla and the supralabial scales, whilst the ducts of dental glands open close to the bases of the teeth (Kochva 1978). Unfortunately, this neat distinction is

further convoluted by the variation that occurs in the passage of the duct of the venom gland in caenophidian snakes—the ducts lead straight to the base of the (front or rear) fangs in many species, but in some non-front-fanged snakes it may open directly into the buccal cavity (Fry et al. 2008). The ducts of labial glands are also notoriously difficult to observe (Underwood 1997). As is often the case when trying to split two ancestrally homologous structures into discrete groups, nature refuses to cooperate with our desire for quantisation and various intermediate forms confound easy classification. In trying to avoid the trappings of overly complex terminology, some recent authors addressing the evolution of the venom system of toxicoferan reptiles have preferred the terms "mandibular venom gland" and "maxillary venom gland" for the labial/dental glands of the lower and upper jaw (Fry et al. 2006, 2012, 2013, 2015).

Typical ophidian supralabial glands are predominantly composed of mucous cells and exist in a continuous strip of glandular tissue (small glands in rows) along the upper jaw (Figs. 4, 8a–f, 10a). The glands are generally polystomatic (possessing multiple ducts), indicative of their plesiomorphic condition (Taub 1966; Kochva 1978). Underwood (2002), however, recorded considerable variation in the structure of the supralabial glands, both in their cellular composition and their size and extension along the jaw. The large supralabial gland of *Cylindrophis ruffus* (Uropeltoidea) contains serous tubules that feed into mucous tubules with ducts opening along the margin of the lips. It also exhibits a peculiar arrangement in which the posterior portion of the

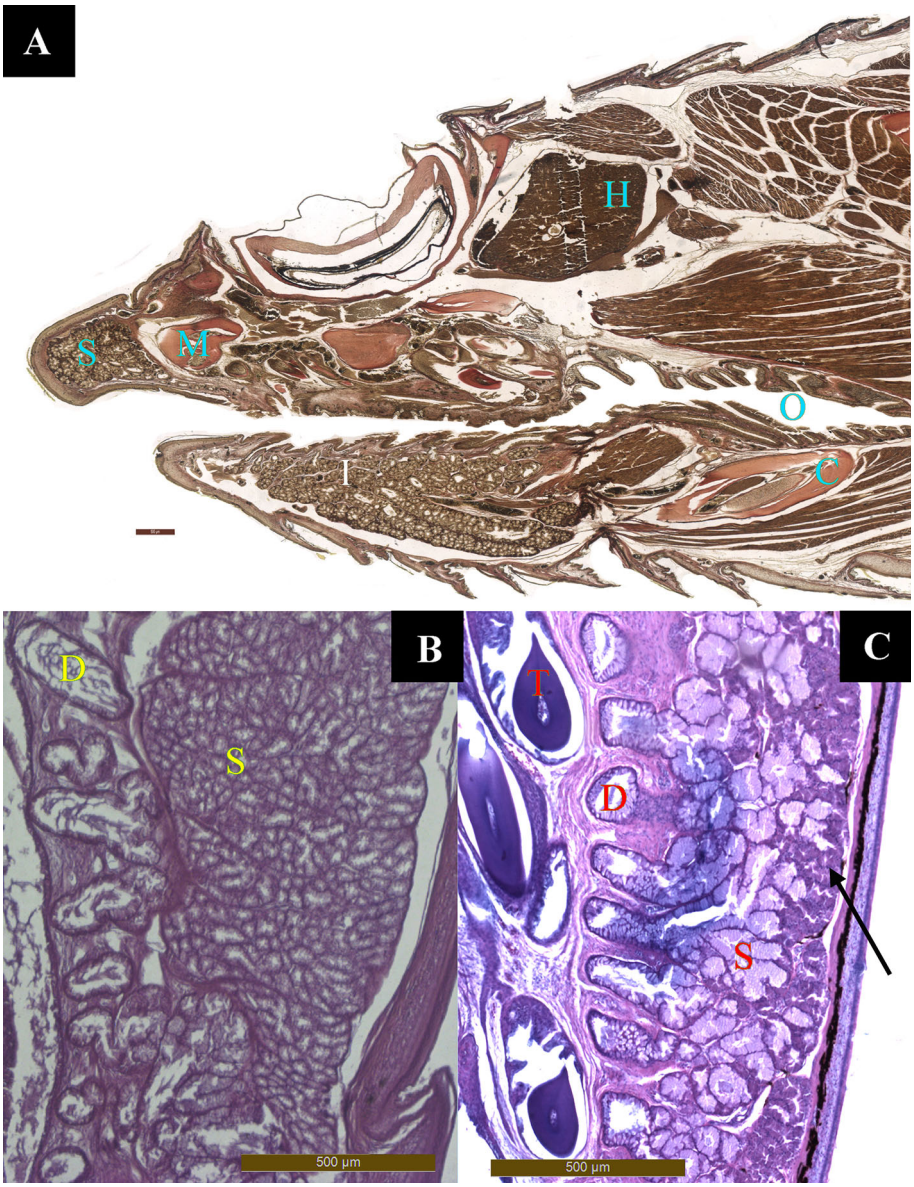

Fig. 4 Supralabial glands. **a** Silver stain of the sagittal section through the head of *Heterodon platyrhinos* showing the relationship of the supralabial (S) and infralabial (I) glands to the maxilla (M) and compound (C) bones (respectively). **b** Modified Masson's trichrome stain of a frontal section through the supralabial gland of *Trimorphodon biscutatus* showing the repeated, almost segmental, nature of the supralabial gland (S) ducts (D). **c** Modified Masson's trichrome of a frontal section through the supralabial gland of *Philothamnus irregularis* in which the typically mucoid supralabial glands includes an abundance of serous cells (*arrow*)

gland (situated beneath the superior rictal gland) is segregated from the anterior majority by a "curtain of connective tissue". Additional species in which Underwood (2002) observed mixed sero-mucous labial glands are *Casarea dussumieri*, *Bolyeria multocarinata* (both Bolyeriidae) *Tropidophis haetianus* (Tropidophiidae), *Achalinus rufescens*, *Fimbrios klossi* (both Xenodermatidae) (Fig. 4). The labial glands of *Pareas carinatus* and *Pareas monticola* (Pareatidae) also exhibit an interesting structure—the distinction between serous and mucous cells was particularly distinct within these glands and an additional row of lobules was present in the supralabial which indented the margin of the main (supralabial) gland behind the eye. Underwood (2002) suggested that this portion of the gland might have been misinterpreted as the venom gland by Taub, who speculated that mixed sero-mucous supralabial glands might be the precursor condition to the full segregation of the venom gland (Taub 1967).

Taub (1967) reported a "strikingly different arrangement of the supralabial glands" in *Xenodermus javanicus*, in which the "serous cells are arranged in cords of cells which alternate with cords of mucous cells along the entire supralabial region". He also observed a similar pattern in the related *Fimbrios klossi* and suggested that this unique arrangement lent support to the consideration of Xenodermatinae as a monophyletic assemblage. Although Underwood (2002) was unable to confirm the presence of alternating rows of cells in *Achalinus* and *Fimbrios*, finding only typical sero-mucous supralabial glands, the monophyly of the group is now well established and it has long since been elevated to family level (Xenodermatidae—Vidal et al. 2007a, b). Xenodermatidae are the next most basal group of caenophidian snakes after the Acrochordidae (Vidal et al. 2009; Wang et al. 2009). Acrochordid snakes possess only mucous-secreting labial glands that are atrophied considerably (this study and also Underwood 2002). This suggests they have no need of venom, an inference consistent with reports that they swallow their fish prey live or asphyxiate it by wrapping around its gills (Lillywhite 1996; Fry personal observations of *Acrochordus granulatus*). As they are feeding upon a prey item covered in a layer of low-friction slime, *Acrochordus* species have no need to expend their own energy to provide sufficient lubrication for swallowing. Thus, further investigation of the oral glands of the next lineage to diverge from the stem snakes (the Xenodermatidae snakes, an assemblage now containing at least 6 genera and 18 species—Teynie et al. 2015) may shed considerable light on the evolution of the sophisticated venom apparatuses of later diverging other caenophidian snakes.

Premaxillary glands Although the mucous-secreting premaxillary gland is clearly discernible as a segregated secretory structure in histological examinations of various snake species (e.g. Kochva 1978), Taub (1966) considered them to be an "anterior expansion of the supralabial glands". Burns and Pickwell (1972) concurred, finding the premaxillary portion of the supralabial gland to be enlarged and expanded in both hydrophiine sea snakes and *Laticauda*, a state that presumably evolved convergently in these two independently marine elapid snake lineages. These authors suggested that damage inflicted by skinning the head in preparation for histological examination might either remove this portion or obscure its continuity with the rest of the supralabial gland, although they commented on the separation of the anterior and posterior regions of the supralabial gland in some species.

The "premaxillary gland" has evolved into a specialised salt extraction gland in the homalopsid snake *Cerberus rhynchops*, and the structure of this gland is "distinctively different from that of the supralabials" in this species (Dunson and Dunson 1979). This role for premaxillary gland is in contrast to the specialisation of the posterior sublingual gland for this function in marine elapid snakes and *Acrochordus granulatus* (see below). The enlargement of the premaxillary secretory tissue in sea snakes reported by Burns and Pickwell (1972), however, suggests that this tissue may have a supplemental role in salt extraction in these snakes. Indeed, the premaxillary region of the supralabial gland of *Laticauda colubrina* was considered the "natrial" (salt) gland until its considerable reduction in size in *Hydrophis* (*Pelamis*) *platurus* was documented (Dunson et al. 1971; Burns and Pickwell 1972). The premaxillary gland of *C. rhynchops* is far less efficient at salt excretion than the posterior lingual gland of the marine elapid snakes, but is nonetheless likely to be an important adaptation facilitating the specialised estuarine ecology of this species (Dunson and Dunson 1979).

Rictal glands Rictal glands have previously been referred to as "posterior" glands (Kochva 1978) and "anterior temporal glands" (Phisalix 1922), but McDowell (1968) introduced the term "rictal gland" and recent authors have preferred this (Underwood and Kochva 1993; Wollberg et al. 1998; Underwood 2002; Fry et al. 2013). Confusingly, McDowell (1986) recommended the use of "rictal gland" for the "invagination of the skin…at the corner of the mouth", even when no glandular tissue is present. Subsequent authors have not followed this suggestion. Although Phisalix and Caius (1918) reported that secretory products of the rictal glands of *Eryx conicus* (Erycinae) and several species of uropeltoid snake were toxic to birds, almost a century passed before experimental confirmation of their expression of venom toxins (Fry et al. 2013).

McDowell (1986) conducted a detailed examination of the rictal glands of many species of caenophidian snake,

identifying 10 different states of development. He mistakenly believed that the venom gland might develop from the rictal gland, despite noting its co-occurrence in numerous species with the "Duvernoy's gland", which had previously been identified as homologous with the venom gland (Kochva 1978). In addition, rictal glands often co-occur with what has been occasionally (and misleadingly—see discussion on terminology in this article) referred to as the "true" venom gland of front-fanged snakes (Kochva 1978; Wollberg et al. 1998).

Rictal glands are one of the most variable structures within the hyper-labile assemblage of ophidian oral glands, sometimes differing markedly between species within the same genus (Underwood and Kochva 1993; Wollberg et al. 1998; Table 2). They are also apparently unique in that glands made up of purely serous lobules may drain directly into the mouth, without the secretion passing first through mucous lobules—all other serous ophidian oral glands have associated mucous tubules (Kochva 1978). Rictal glands may occur exclusively on the top jaw (*superior* rictal gland), bottom jaw (*inferior* rictal gland) or on both jaws, or be absent entirely (Wollberg et al. 1998; Underwood 2002; Fry et al. 2013). The inferior portion of the rictal pouch (the superior invagination of the corner of the mouth) is frequently lubricated by secretions from the posterior supralabial glands (Fig. 5). More superiorly, as the rictal epithelium thickens and becomes ciliated, glands develop along the margins of the rictal pouch (Fig. 5). It is at the terminal (superior) portion of the rictal pouch where the greatest variation in glandular morphology is observed, ranging from no glands, through smaller diffuse glands, to large (particularly serous) glands (Fig. 5).

Wollberg et al. (1998) conducted a survey of the rictal glands of a wide variety of caenophidian snakes and uncovered considerable variation. The condition they describe as "most widespread" is the presence of a superior rictal gland with a duct opening at the corner of the mouth into the groove between upper and lower lips.

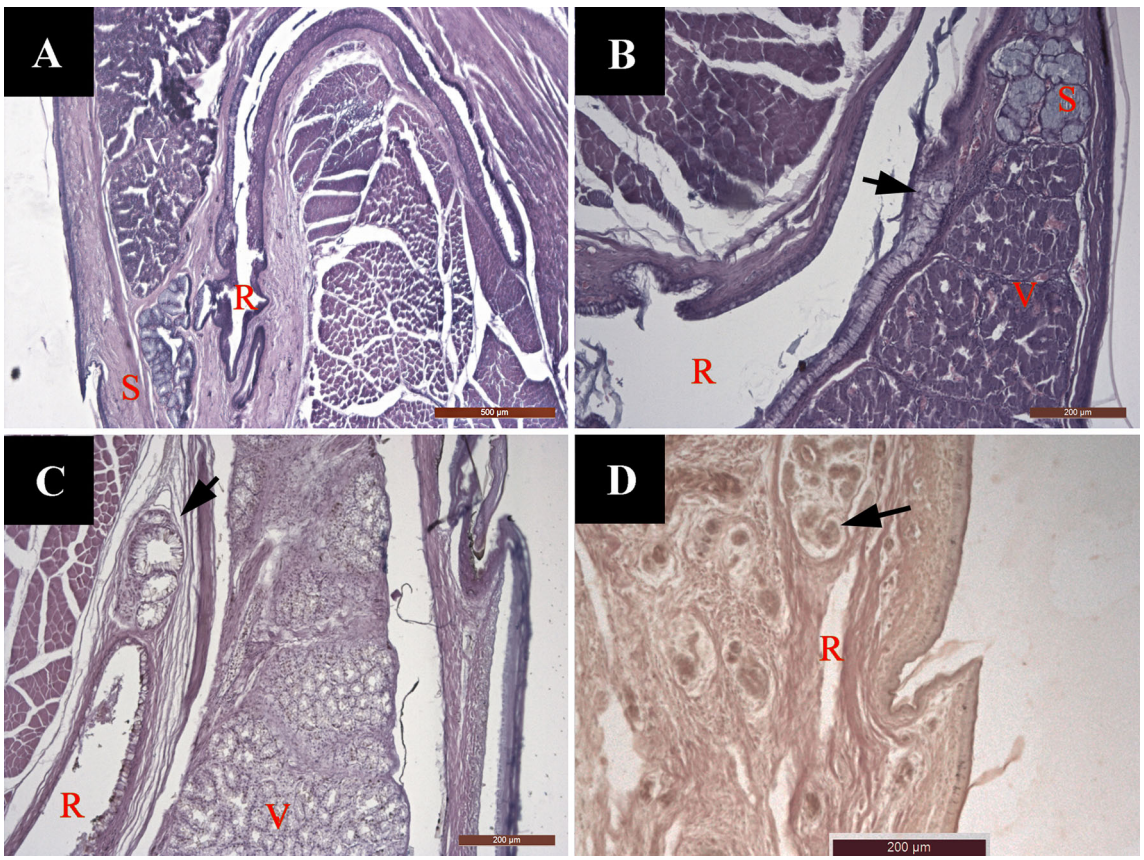

Fig. 5 Rictal pouch and glands. **a** Modified Masson's trichrome of a frontal section through the rictal pouch of *Stenorhina freminvillei* showing the posterior supralabial glands emptying into the rictal pouch. **b** Modified Masson's trichrome of a frontal section through the rictal pouch of *Imantodes cenchoa;* note how the thickened lining epithelium (*arrow*) is morphologically distinct from both the venom and supralabial glands. **c** Modified Masson's trichrome of a frontal section through the rictal pouch of *Trimorphodon biscutatus* the anterior lining of the pouch has formed into a series of glandular pockets (*arrow*). **d** Van Gieson's stained frontal section through the rictal "gland" of *Hydrophis* (*Pelamis*) *platurus* where a diffuse group of (mainly serous) glands (*arrow*) occur at the apex of the rictal pouch. *R* rictal pouch; *S* supralabial glands; *V* venom gland

There was no inferior rictal gland in this condition. A number of the lamprophiid snakes they examined possessed both superior and inferior rictal glands, with the superior glands typically being more developed, although in some (e.g. *Poecilopholis*) the inferior gland is larger. Some species of lamprophiid snake (e.g. *Buhoma* and *Polemon*) possess a serous rictal gland larger than their venom gland, and in a majority of species in this family (including the front-fanged *Atractaspis*) a rictal gland co-occurs alongside a venom gland. Amongst the Lamprophiidae, *Amblyodipsas*, *Hypoptophis*, *Micrelaps* and *Xenocalamus* apparently lack rictal glands altogether. In the present study, we observed considerable variation in rictal gland condition amongst species within the Elapidae (Table 2). The condition of rictal glands also varies considerably amongst the Viperidae—pit vipers (Crotalinae) appear to lack rictal glands, but "true" vipers (Viperinae) may have one, both or no rictal glands. Within the genus *Causus* (Viperinae), species may either have a superior rictal gland or no evidence of rictal glands, and this variation in condition does not seem to correlate with the possession of either a short or long venom gland (see section on venom glands).

Only recently has work been undertaken to investigate the secretory products of the rictal gland (Fry et al. 2013). The products are identical from both the upper and lower rictal glands and include toxins homologous to those made by classic venom glands. Sequences of 3-finger toxins recovered from the upper and lower rictal glands exhibited 100 % similarity to those from the venom/dental gland in the species studied (*Cylindrophis ruffus*). This was interpreted as evidence of the rictal gland evolving from the same ancestral supralabial/dental gland as the venom gland and that the expression profiles of the venom and rictal glands remain under the same genetic control.

Venom glands The ophidian venom gland is a dental gland that develops from a primordium at the posterior end of the dental lamina. The venom glands of all caenophidian snakes (including the front-fanged Viperidae, Elapidae and Atractaspidinae as well as non-front-fanged species) develop from this same primordium, from which the associated venom delivery teeth also derive (Kochva and Gans 1970; Vonk et al. 2008). In addition, all caenophidian venom glands are innervated by the same cranial nerve (maxillary branch V2 of the trigeminal nerve) and supplied with blood by vessels branching from the internal carotid artery (Kochva 1965; Taub 1966). The glands of Elapidae, Atractaspidinae and non-front-fanged snakes share a similar pattern of differentiation and branching in the later stages of development, whilst the glands of Viperidae exhibit a developmental pattern distinct to the rest (Kochva and Gans 1970). These developmental relationships of the venom glands reflect the phylogenetic relationships of the taxa (Vidal et al. 2009).

Venom glands have received more specific research attention than any other ophidian oral gland, and they differ considerably in form amongst taxa, sometimes even between species in the same genus. Within the two larger families of front-fanged snakes, Elapidae and Viperidae, the gland exhibits the same basic structure in all species (although its gross anatomy may differ considerably—see below), but within other families the structure may vary, with intrafamilial diversity reaching a peak in the Lamprophiidae (Vidal et al. 2008). Above the familial level, it is impossible to combine venom glands into meaningful groups and trying to group the venom glands of front-fanged species together on the basis of their being "high-pressure systems" is especially arbitrary, as in many ways the glands of the Elapidae are more similar to those of non-front-fanged snakes than those of the Viperidae (see below).

As well as differing in their internal structures, venom glands differ amongst taxa in their associated musculature and dentition (Fry et al. 2008). Jackson (2003) reviewed the terminology used by various authors to classify the musculature associated with the venom delivery system, and her terminological recommendations are followed below.

Venom glands of non-front-fanged snakes The "non-front-fanged" snakes (NFFs) are a huge, non-phylogenetic assemblage of species with modified venom delivery teeth associated with the venom gland. As the name for the group suggests, the fang(s) may be anywhere on the maxillary other than the "front" (as it is in the front-fanged clades Atractaspidinae, Elapidae and Viperidae). Fangs may also be grooved, semi-tubular or ungrooved (Fry et al. 2012). The fangs, as well as the venom glands, of NFFs are developmentally homologous with those of front-fanged species, and the fangs and venom glands of all venomous snakes develop from a common primordium (Kochva and Gans 1970; Vonk et al. 2008). The assemblage includes members of several families, most of which were formerly grouped in the polyphyletic "Colubridae". NFFs members of the Lamprophiidae are discussed below, in the separate section devoted to that family. The venom glands of this group were termed "Duvernoy's gland" by Taub (1966). This term is inappropriate, however, for reasons discussed in detail in the section on terminology below.

The largest studies of the venom glands of NFFs remain those conducted by Taub (1967) and McDowell (1986). Fry et al. (2008) also conducted a fairly broad survey of NFFs venom systems. The present manuscript does not attempt to report the findings of these studies comprehensively but will provide an overview of some of their more

salient details, reinterpreted in light of currently available data. Researchers are directed to the original studies for additional information.

The venom glands of NFFs are highly variable and may be entirely composed of serous-secreting cells or contain a mixture of both serous- and mucous-secreting cells (Figs. 6, 7). Purely serous glands were by far the more common arrangement in species examined by Taub (1967). The largest and most anatomically distinct NFFs venom gland is that of the boomslang, *Dispholidus typus* (Taub 1967; Kochva 1987; Fry et al. 2008), which has been responsible for multiple human fatalities. As Taub (1967) comments, "Multiple invaginations and evaginations of the lobule wall provide enormous secretory surface and storage space". Indeed, the central lumen of the *D. typus* venom gland is larger than that of the homologous gland of most elapid snakes (Fry et al. 2008). The gland of the closely related *Thelotornis kirtlandii*, another species of NFFs that has been responsible for fatal bites to humans, also possesses an appreciable central lumen (Kochva 1978). Concentric layers of connective tissue, as well as striated muscle fibres of the adductor externus superficialis, attach to the venom gland of *D. typus*, and to a lesser extent that of *Thelotornis capensis*, suggesting that this clade has independently evolved a "rudimentary" high-pressure venom system (Kochva 1978; Fry et al. 2008).

Another distinct NFFs venom gland anatomy is that possessed by *Gonionotophis* (*Mehelya*) *capensis* (McDowell 1986; Kochva 1987; Fry et al. 2008). McDowell (1986) argued that there were four main patterns of venom gland and associated anatomy: the Elapidae pattern; the Viperidae pattern; the *Atractaspis* pattern; and the *Gonionotophis* (*Mehelya*) pattern. The *Gonionotophis* venom gland is not as large as that of some other NFFs (e.g. *D. typus*, *Micrelaps*, *Rhamphiophis*—Kochva 1978), but has a wide central lumen (again larger than that of a typical elapid snake venom gland, Fig. 6) and directly attached muscle fibres of the pterygoideus, representing another independent evolution of compressor musculature (Kochva 1987; McDowell 1986; Fry et al. 2008).

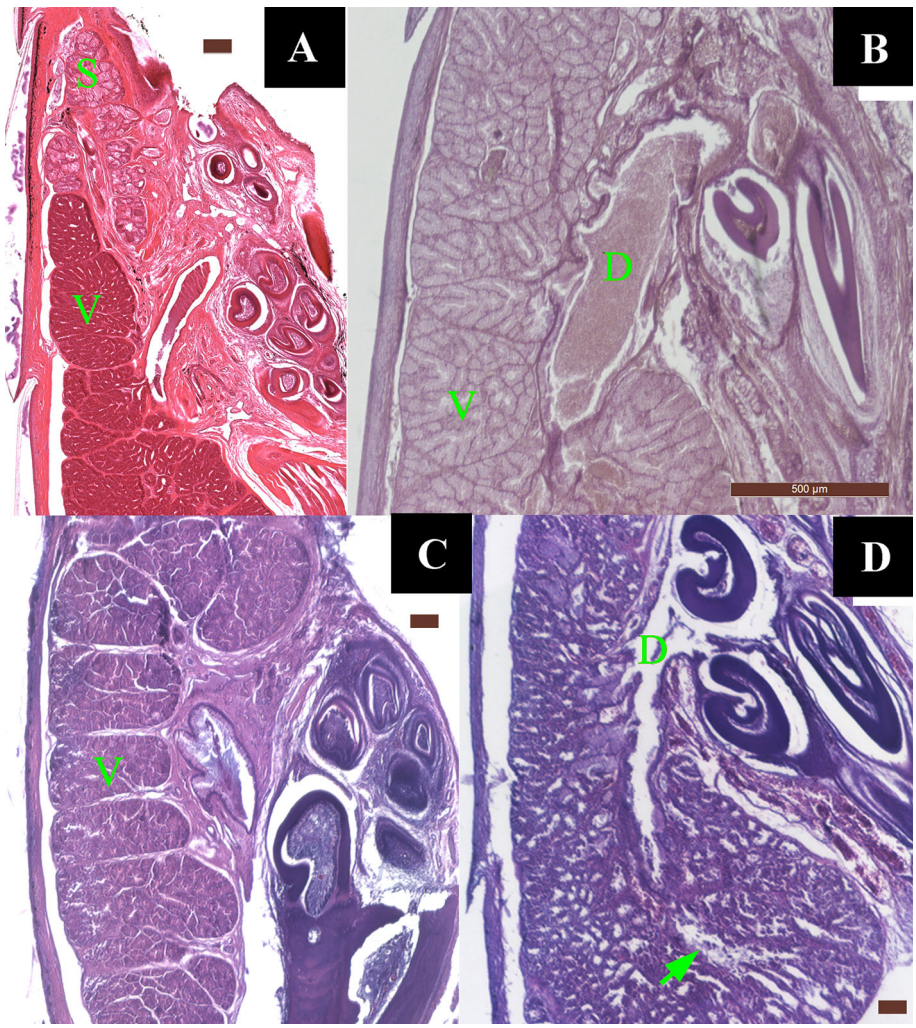

Fig. 6 Diversity of the venom gland and duct in non-front-fanged species. **a** H&E-stained frontal section through *Scolecophis actrocinctus* showing the marked distinction between the serous venom gland and the mucoid supralabial gland (*scale bar* 100 µm). **b** Modified Masson's trichrome of a frontal section through *Tachymenis peruviana* showing the relatively large venom duct which can function in venom storage. **c** Modified Masson's trichrome of a frontal section through the lobate venom gland of *Madagascarophis colubrinus* (*scale bar* 100 µm). **d** Modified Masson's trichrome of a frontal section through *Psammodynastes pulverulentus* (*scale bar* 100 µm) where the venom gland supports an elongate central lumen (*arrow*). *D* venom duct; *S* supralabial gland; *V* venom gland

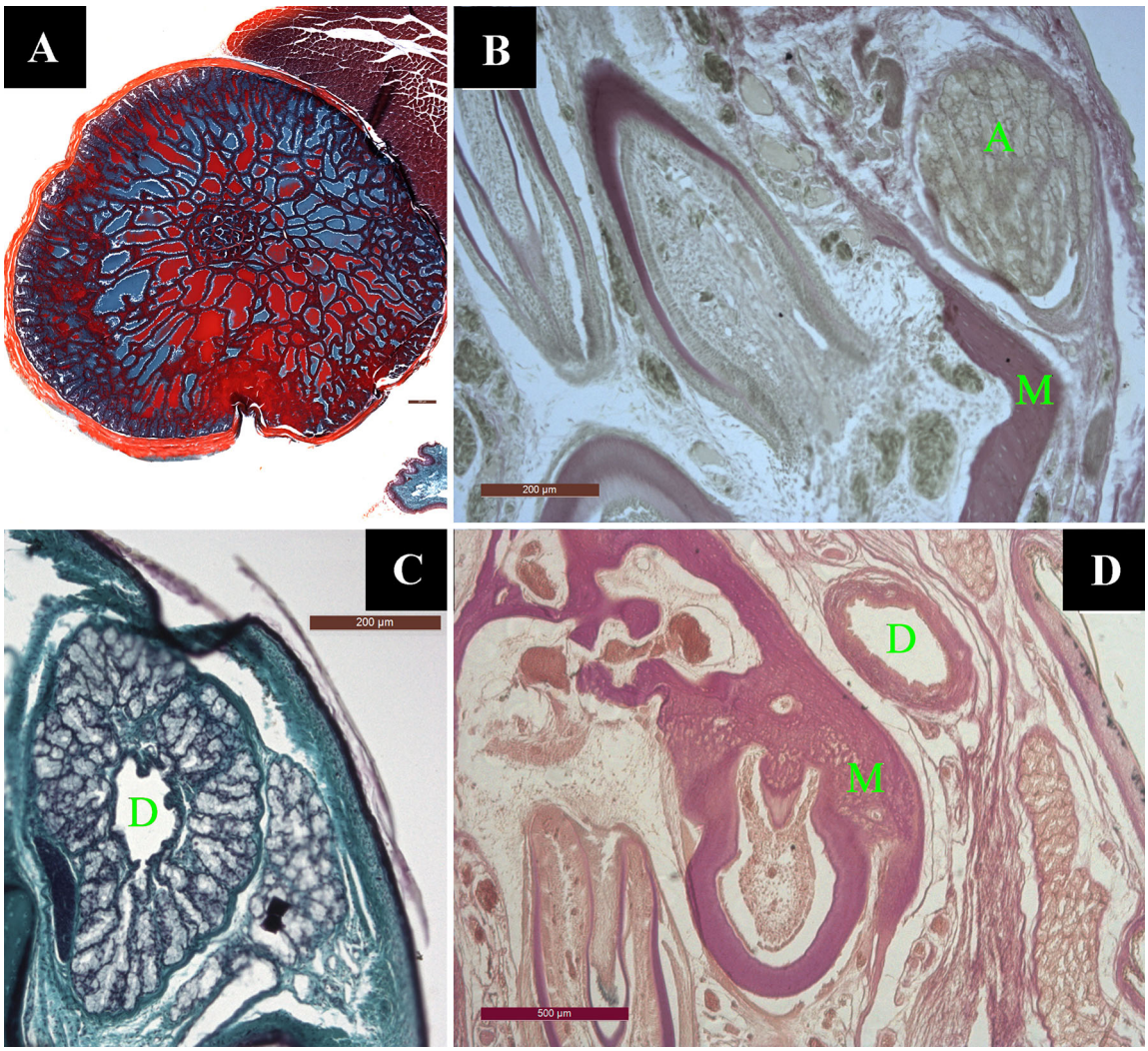

Fig. 7 Elapid and viperid venom delivery system. **a** Modified Masson's trichrome of a transverse section through the venom gland of *Walterinnesia aegyptia* showing the characteristic parenchyma and subdivided small central lumen characteristic of elapids (*scale bar* 200 µm). **b** Van Gieson's stain of the accessory venom gland of *Cerastes cerastes* which, like most viperid accessory venom glands, has an abundance of mucoid cells. **c** Modified Masson's trichrome of the venom duct of *Hemachatus haemachatus*, which is lined with a mucoid epithelium. **d** Van Gieson's stain of the venom duct of *Crotalus atrox* showing the relatively thick wall and absence of mucoid lining. *A* accessory venom gland; *D* duct; *M* maxilla

It should be noted that danger to humans is not always correlated with development of the venom gland. The natricid snake *Rhabdophis tigrinus*, which (along with its congener *R. subminiatus*) has been responsible for multiple life-threatening bites (including fatalities) to humans (Smeets et al. 1991; Hifumi et al. 2014), has a largish but otherwise unremarkable venom gland without an appreciable lumen or any direct muscular attachment (McDowell 1986; Fry et al. 2012). Its fangs are also smooth and ungrooved (Fry et al. 2008).

Venom glands of the Elapidae Due to their similarity to the so-called "Duvernoy's gland" of non-front-fanged snakes, the venom glands of elapid snakes were previously considered plesiomorphic in relation to those of viperid snakes (Kochva 1987). In contrast to the latter, the venom glands of elapid snakes typically have a small central lumen or lack one entirely (Fry et al. 2012). The venom is primarily stored in the secretory granules of the cells (Kochva 1978). The fine structure of the secretory cells of elapid snake venom glands is also more similar to that of non-front-fanged snake venom glands than it is to those of viperid snakes, as may be expected given the nested phylogenetic placement of the Elapidae within Caenophidia. The duct of the venom gland, which terminates at the base of the tubular maxillary fang, passes through a predominantly mucous-secreting accessory gland. The function of the accessory gland (Fig. 7) remains unclear, although its reduction in sea snakes, which have venom glands

otherwise similar to those of other elapid snakes (Gopalakrishnakone and Kochva 1990a), may provide a clue.

The venom gland, located suborbitally, varies in size considerably amongst species (Fry et al. 2012). The main gland consists of branched secretory tubules of varying length and a single duct leads from this region through the accessory gland, which is considerably larger in relation to the main gland than that of viperid snakes (Kochva 1987). After passing through the accessory gland, the duct terminates in a "venom vestibule" at the base of the fang sheath (Fry et al. 2008).

Although there is generally little variation in either the gross- or fine-level structure of the venom gland throughout the Elapidae, an exception to this is the elongate glands of the Asian coral snakes *Calliophis bivirgatus* and *C. intestinalis* (Fig. 12), and the little-known fossorial species from New Guinea, *Toxicocalamus buergersi* (Yang et al. 2016). *Calliophis* and *Toxicocalamus* are not closely related genera, and it is thus clear that their unusual venom glands have evolved independently. Indeed, both genera are phylogenetically nested within clades otherwise populated by snakes with typical venom glands.

The elongate venom glands of *T. buergersi* extend posteriorly from the head inside the snake's body cavity almost as far as the heart (McDowell 1969). The arrangement of muscles attached to the gland follows the standard elapid snake pattern, but McDowell (1969) reports the presence of a "broad and fleshy cutaneous muscle" that covers the cephalic portion of the gland. Further investigation of this unique anatomy is desirable. Why *T. buergersi*, uniquely amongst the 12 currently described species of *Toxicocalamus*, should have such unusual venom glands is mysterious. Little is known about the natural history of the genus, but it is assumed, based on stomach contents recovered from specimens of several species, that all feed primarily or exclusively on giant megascolecid earthworms (Shine and Keogh 1996; O'Shea et al. 2015). McDowell (1969) speculated that *T. buergersi* may possess a unique feeding ecology that accounts for the evolution of its elongate venom glands, but this possibility remains to be investigated. The venom of one species in the genus, *T. longissimus*, has recently been investigated and the composition appears to be fairly typical of small Australasian elapid snakes—dominated by 3-finger toxins (3FTx), which are typically postsynaptic neurotoxins (Calvete et al. 2012).

Due to their unusual venom glands, *Calliophis bivigatus* and *C. intestinalis* were formerly classified in the genus *Maticora*. Based on molecular and morphological characters, however, Slowinski et al. (2001) synonymised *Maticora* with *Calliophis*. The venom gland of *C. bivirgatus* was described in detail by Gopalakrishnakone and Kochva (1990b) and Yang et al. (2016): it extends up to 1/4 of the snakes body length, inside the body cavity. The posterior one-third of the gland contains the majority of secretory cells, and the duct extends forward to the head. Compressor musculature encircles the posterior half of the gland. As with most elapid snake venom glands, the main secretory section of the gland does not have a lumen and the venom is stored in the cell's secretory granules. As with *T. buergersi*, the evolutionary "justification" for this spectacular arrangement remains something of a mystery—although the elongation of the venom glands appears to facilitate a far higher venom yield than that of closely related species with normal venom glands (Fry, personal observation). The two long-glanded species of *Calliophis* do not differ dramatically from their congeners in feeding ecology, although they are reported to include those very congeners themselves in their diet (Stuebing and Inger 1993). They are, however, the largest of the genus and a novel 3-finger toxin that acts as a sodium channel agonist was recently discovered in the venom of *Calliophis bivirgatus* and linked to their predilection for feeding on other venomous elapid snakes (Yang et al. 2016). It is conceivable that their increased venom yield (facilitated by the elongation of their venom glands) has evolved under the same selection pressure (i.e. as part of an arms race between predator and prey that would otherwise be similarly matched); however, this interesting conjecture should be investigated further prior to general acceptance.

In elapid snakes, venom gland compressor musculature derives from the adductor externus superficialis (AES), as in the NFFs (where compressor musculature is present). In the elapid snake arrangement, the AES extends dorsally from the postorbital bone to attach to the dorsal and caudal surfaces of the gland and ventrally from the lower jaw to the ventral surface of the gland (Kochva 1962; Jackson 2003).

Venom glands of the Viperidae The anatomy of the viperid snake venom gland is fairly consistent throughout the family, although there are some small differences between the glands of Crotalinae (pit vipers) and Viperinae ("true" vipers), with the enigmatic *Azemiops* (Azemiopinae, sister group to Crotalinae) possessing glands that follow the crotaline snake pattern (Kochva and Gans 1965; Kochva 1978; Mackessy and Baxter 2006). Two species in the genus *Causus*—*C.resimus* and *C. rhombeatus*—possess elongate venom glands that extend beyond the head but are otherwise similar in structure to those of other viperid snakes. Unlike those of the long-glanded elapid snakes, the elongate venom glands of these two species of *Causus* do not enter the rib cage (this study and Kochva and Gans 1970) (Fig. 12).

Kochva and Gans (1965) examined the venom gland of *Daboia palestinae* in detail: the gland is large, extending from beneath the eye backwards to the corner of the mouth, and is surrounded by a "thick capsule of connective tissue" to which ligaments and compressor musculature attach. For the purposes of discussion, it may be divided into several parts: the main secretory section at the rear of the gland, the primary duct, the accessory gland and the secondary duct that feeds venom to the fang sheath (Fig. 7). The main gland is composed of repeatedly blanching secretory tubules and is subdivided into multiple (up to 8) lobules by connective tissue. Secreted venom is primarily stored in a wide lumen, which takes up much of the anterior two-thirds of the main gland. During a bite, venom is propelled forward into the primary duct by the action of the compressor musculature, after which it passes through the accessory gland and then to the fang sheath via the secondary duct.

Mackessy and Baxter (2006), in their examination of the venom gland of the pit vipers *Crotalus atrox*, *C. oreganus* and *C. viridis*, noted that the venom is also stored in the small "ductules" of the main gland and in the primary duct, but is absent from the accessory gland except during an active delivery sequence. A "glandular isthmus" prevents venom flowing forward from the primary duct to the accessory gland when the apparatus is at rest.

The accessory gland contains six (Sakai et al. 2012) or seven (Mackessy 1991; Mackessy and Baxter 2006) cell types, including serous- and mucous-secreting cells. Mackessy and Baxter (2006) noted the similarity of the cell arrangement of the accessory gland, in which a serous-secretory section is followed by a mucous-secretory section, to that of mammalian gastric glands. The function of this gland remains mysterious—the hypothesis that it contributes to the "activation" of venom components on their way from the main gland to the fang seems unlikely, as the secretory products of the entire venom apparatus (extracted by milking) and those extracted from the main gland only are identical in composition (Mackessy and Baxter 2006). The recent publication of the (elapid snake) king cobra (*Ophiophagus hannah*) genome revealed the expression profile of the accessory gland to be rich in lectins (Vonk et al. 2013). Snake venom lectins have multiple functions, including a possible antibacterial and antifungal role as well as coagulopathic effects upon platelet aggregation (Arlinghaus et al. 2015). It may be that the lectin-rich secretion of the accessory gland helps to protect the fleshy fang sheath from infection.

The viperid snake venom gland compressor musculature differs from that of elapid snakes in that it is derived from the *adductor externus profundus* (AEP) (Kochva 1962). The arrangement of the AEP is convoluted in that it extends from the lower jaw away from the venom gland and passes medially around the AES before turning back towards the gland and attaching directly to it (Kochva 1958; Jackson 2003).

Venom glands of the Lamprophiidae The venom glands and associated delivery mechanisms of the Lamprophiidae are the most variable of any single family of snakes (Fry et al. 2008). This intrafamilial diversity includes the small, exclusively mucous-secreting (and thus not a *functional* venom gland) gland of *Pseudaspis cana* (Taub 1967), which subdues prey with its powerful jaws (possibly in concert with constriction), much like the colubrid snakes *Drymarchon* and *Ptyas* (Fry et al. 2008); large serous-secreting glands without muscle attachment associated with enlarged fangs at the rear of the maxillary in non-front-fanged genera such as *Micrelaps*, *Malpolon*, *Psammophis* and *Rhamphiophis* (Kochva 1978; Fry et al. 2008) (Fig. 3a); serous-secreting glands associated with rudimentary compressor musculature and rear fangs in *Brachyophis* (Underwood and Kochva 1993; Fry et al. 2012); and the high-pressure, venom delivery system of the front-fanged Atractaspidinae [*Atractaspis* and *Homoroselaps* (Vidal et al. 2008)]. Such variability in the venom system has understandably been the cause of taxonomical confusion in the past: *Homoroselaps* was considered to be a member of the Elapidae, the "Colubridae", and then the Elapidae again (McDowell 1986; Underwood and Kochva 1993), and the large mobile fangs and reduced maxillary of *Atractaspis* resulted in its erroneous classification as a viperid snake (Kochva 1967).

Kochva and Gans (1970) examined the venom glands of several species of lamprophiid snake. *Aparallactus capensis* and *Chilorhinophis gerardi* have small venom glands surrounded by connective tissue with no muscular attachment. The main gland consists of branching serous tubules that open into a duct lined with mucous-secreting cells that delivers venom to the fang sheath. The duct is considerably longer in *C. gerardi* than *A. capensis*. The venom gland of *Aparallactus modestus* is reported to differ from that of *A. capensis*, but the details of this difference were not discussed in the text. The venom gland of *Amblyodipsas unicolor* is described as similar to that of the colubrid snake *Dispholidus typus*, which is often considered to have most well-developed venom gland of any NFF snake (Taub 1967; Kochva and Gans 1970; see above for description). A poorly preserved specimen of *Xenocalamus mechowii* appeared to have a venom gland similar to that of the colubrid snake *Thrasops jacksonii* which in turn has glands similar to, but less well-developed than, those of *D. typus*. No differentiated venom glands were located in the two species of *Lycophidion* examined.

The venom glands of *Atractaspis* are anatomically distinct from those of other front-fanged snakes. They have a wide central duct adjoined by radially arranged unbranched

serous-secretory tubules. Also unique amongst front-fanged snakes is the lack of a distinct accessory gland and the presence of mucous-secreting cells at the end of each serous tubule (Kochva et al. 1967). As in the other two front-fanged clades, elongate venom glands have evolved within the *Atractaspis*; these glands may be up to 12 cm long (1/5 total body length) in *A. engaddensis* and 30 cm long (1/3 total body length) in *A. microlepidota* (Kochva 1978). The fine structure of these glands is typical of *Atractaspis*. Again the reason for this special anatomy is unclear, although it likely has the consequence of facilitating an increased venom yield, as in *C. bivirgatus* (see above) (Fig. 12).

The venom gland compressor musculature of *Atractaspis* is of unknown homology, but may derive from the *adductor externus medialis*. It does not derive from the AES, which is absent in this genus (Jackson 2003). Muscle fibres attach to the venom gland in multiple places as the muscle extends from the parietal bone, passing caudally along the gland before wrapping around it and extending along its central surface to the corner of the mouth (McDowell 1968; Jackson 2003).

Temporomandibular gland Phisalix (1922—discussed in Taub 1966) described a "temporomandibular" gland in Typhlopidae (Scolecophidia—a paraphyletic group, see below), which she considered distinct from the "anterior temporal" (rictal) and "parotid" (venom) glands of other snakes. The gland is largely composed of serous cells, is separate from the supralabial gland and is located behind and below the eye (a position similar to that of the venom gland). Investigations of the oral glands of "scolecophidian" snakes have been extremely limited, and subsequent investigators have failed to identify a distinct "temporomandibular gland" (Taub 1966). Haas (1964), however, located a gland he termed the "accessory supralabial gland" in *Liotyphlops albirostris* (Anomalepididae—also part of the paraphyletic "Scolecophidia"). This gland had a single duct and a "secretion-filled wide lumen". He was uncertain whether this gland was the "temporomandibular gland" of Phisalix or "a sort of poison gland". He did not report finding a similar gland in his examination of *Anomalepis aspinosus*, another species of anomalepidid snake (Haas 1968).

Given the location of the "temporomandibular" and "accessory supralabial" glands, as well as their serous-secretory cells and single ducts, it seems likely that they are one and the same and are homologous with the venom glands of alethinophidian snakes. Our investigation of typhlopid snake oral glands via magnetic resonance imaging revealed the presence of large glands below the eye, presumably the "temporomandibular gland" of Phisalix, in addition to large glands located more distally on the upper jaw. A pair of smaller, possibly sublingual glands was evident on the lower jaw (Fig. 8g, h). The glands of the upper jaw are larger than the venom glands of many front-fanged snakes. Unfortunately, the snake's thick scales resulted in poor histological sectioning, but (following Phisalix and Haas) it seems likely that these large glands are composed of serous cells. The report by Haas of a "wide lumen" is intriguing based on their specialisation for feeding on relatively "defenceless" prey (predominantly ant and termite larvae, although adult ants, termites and ticks are also consumed—Webb and Shine 1993). It might be expected that these snakes would have little use for serous glands with significant storage capacity (i.e. glands very similar to some highly derived venom glands) and that their dental glands would be greatly reduced, as the venom glands have been in some other taxa that feed on soft-bodied, relatively immobile prey (Fry et al. 2012). Further investigation of the oral glands of scolecophidian snakes, as well as their feeding ecology, is long overdue.

Oral glands of the lower jaw

In the majority of snakes, the lower jaw is associated with two primarily mucoid glands: the infralabial gland located along the lateral margin of the lower jaw, and the sublingual glands located inferior to the tongue sheath and/or tongue although a large inferior rictal gland may be present (as in *Cylindrophis*) (Figs. 1, 3b, c).

Infralabial glands The infralabial glands are a long cord of either purely mucous or sero-mucous cells, which, like the supralabial counterpart, are drained by multiple ducts (Fig. 9) (Taub 1966; Kochva 1978; Underwood 2002). As with the supralabial glands, the plesiomorphic state are glands dominated by protein secretory cells (this study and Fry et al. 2008, 2013) (Fig. 3b). Purely mucoid cells are found only in derived lineages such as the constricting snakes discussed below. In plesiomorphic snakes like *Cylindrophis*, toxin transcripts are expressed in these glands at detectable levels (Fry et al. 2013), however, and along with the presence of serous-secreting oral glands in more basal alethinophidian snakes (e.g. *Cylindrophis*—Underwood 2002) and "scolecophidian" snakes (Haas 1964), this indicates that the possession of purely mucous-secreting oral glands is likely a derived state. This again highlights the functional plasticity of ophidian oral glands—particular anatomical structures have been repurposed multiple times within the evolutionary history of the Serpentes.

The labial (both supra- and infra-) glands of constricting snakes such as Pythonidae are large and predominantly mucous-secreting (Underwood 2002; Fry et al. 2013;

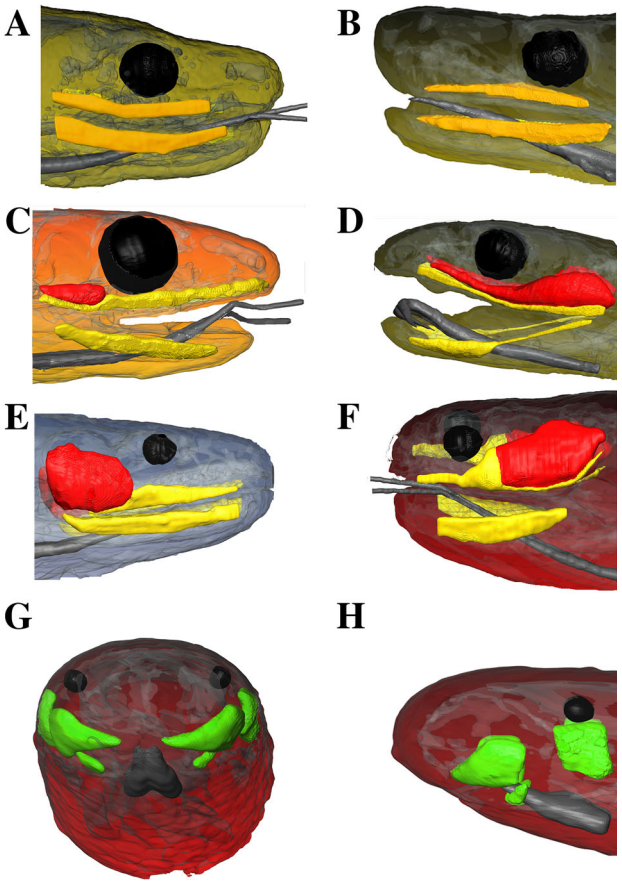

Fig. 8 Magnetic resonance imaging (MRI) of snake oral glands. *Orange* mucoid labial glands (no venom gland); *yellow* mucoid labial glands (in presence of venom gland); *red* venom gland; *green* "scolecophidian" oral glands of unknown homology (see text for discussion). **a** *Eunectes notaeus*, **b** *Python regius* with the mandibular glands exceeding the size of the maxillary as part of the exaptation for lubrication of feathered and furred prey (see Fig. 10a, b), **c** *Pantherophis guttatus*, **d** *Dendroaspis polylepis*, e *Cerberus rynchops*, **f** *Helicops leopardinus* G/H *Anilios guentheri*

Figs. 3c, 10). In these snakes, the role of these glands is obviously lubrication, facilitating the ingestion of large prey items. The mandibular gland exceeds the size of the maxillary gland (Figs. 8a, c, 10a, b). Both the maxillary and mandibular have the pachydermal folding arrangement like that of the serous labial glands.

A fascinating exception concerns the infralabial gland of some dipsadid snakes, which has apparently evolved into a functional mandibular venom gland (Laporta-Ferreira and Salomão 1991; Salomão and Laporta-Ferreira 1994). This demonstrates the remarkable functional plasticity of ophidian oral glands.

The unique development of the infralabial glands in dipsadid snakes has long been recognised (Taub 1966), and the morphology and histochemistry of three species was recently reported in detail (de Oliveira et al. 2008). In *Atractus reticulatus*, the gland is relatively small (~40 % the length of the jaw) and predominantly mucous-secreting (thus a typical ophidian infralabial gland), whereas in *Dipsas indica* and *Sibynomorphus mikanii* the gland is large (~70 % the length of the jaw) and seromucous. In *D. indica* the *levator anguli oris*, muscle of the lower jaw is particularly well developed and surrounds the gland, suggesting that it may compress the gland during biting. *D. indica* and *S. mikanii* snakes feed predominantly on soft-bodied molluscs, and the secretions of the infralabial glands (as well as the seromucous supralabial and "Duvernoy's" glands) of *Sibynomorphus neuwiedi* (Laporta-Ferreira and Salomão 1991) and *S. mikanii* (Salomão and Laporta-Ferreira 1994) have toxic effects on snails and slugs, both immobilising them and (particularly in the case of the labial gland secretions) weakening their tissues. Given the degree of anatomical specialisation and the demonstrated toxic effects of its secretions, it therefore seems reasonable (however see below) to consider the infralabial gland of certain dipsadid snakes a venom gland that facilitates prey subjugation—immobilisation of prey is an obvious function of venom and weakening the tissues of snails likely makes them easier to remove from their shells prior to ingestion (Laporta-Ferreira and Salomão 1991). These snakes use their mandible in order to remove their prey from its shell, explaining the particular development of this gland. The lack of specialised seromucous oral glands in *Atractus* may be explained by the fact that these snakes feed predominantly on earthworms.

The interpretation of a venomous function for dipsadid snake seromucous infralabial has recently been questioned (Zaher et al. 2014). Another possibility is that the primary function of these glands is "mucus control"—Zaher et al. point out that the ducts of the glands do not appear to be directly associated with the teeth of the mandible and also speculate that dipsadid snakes that feed on shelled molluscs remove them from their shells too rapidly for venom to take effect (Zaher et al. 2014). Glandular ducts that secrete well-characterised toxins do not always open directly at the base of venom delivery teeth (see above), and Zaher et al. also comment on the difficulty of locating the glandular ducts in many of the specimens they examined (Zaher et al. 2014). Although "mucus control" is certainly a plausible function for these glands, the evidence presented by Laporta-Ferreira and Salomão (1991) is not discussed by Zaher et al. nor is any evidence provided for a mechanism of "mucus control" that might act significantly faster than venom. Mucous control could in fact be achieved by at least two distinct mechanisms—either by enzymes that rapidly dissolve the mucous or by toxins that disrupt the mollusc's ability to produce the mucous; deployment of the latter might reasonably be considered "venom" in any case. In practice, it is often very difficult in "borderline cases" to determine whether or not it is appropriate to

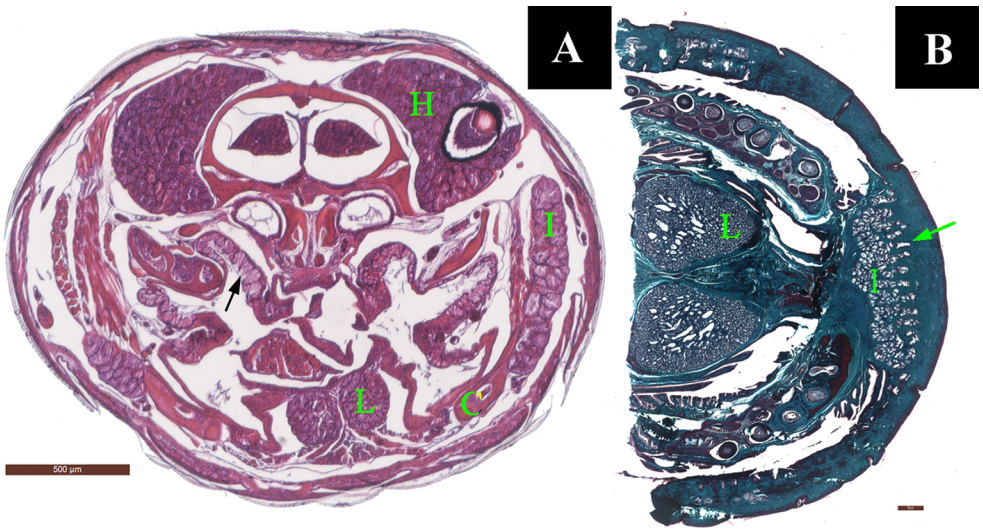

Fig. 9 Infralabial glands. **a** H&E-stained transverse section through the head of *Indotyphlops braminus* showing the infralabial gland coursing along the compound; note the prominent sublingual glands and the abundance of mucoid glands in the oral epithelium (*arrow*). **b** Modified Masson's trichrome of a frontal section through the "mandibular symphysis" of *Calabaria reinhardtii* showing the infralabial glands extend beyond the lower jaws to merge in the midline; note that here, where there are no teeth, the gland still features an almost segmental pattern of ducts (*arrow*). *C* compound bone; *H* harderian gland; I infralabial gland; *L* sublingual gland

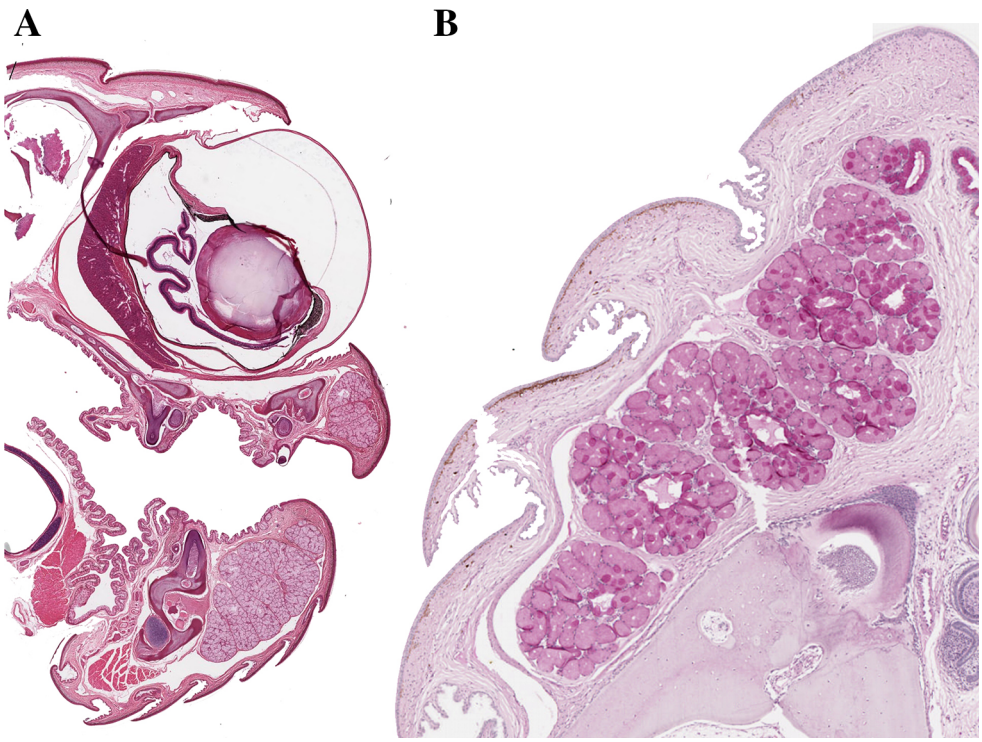

Fig. 10 **a** H&E-stained *Aspidites melanocephalus* showing the maxillary and mandibular mucus glands, with the mandibular glands exceeding the size of the maxillary as part of the exaptation for lubrication of feathered and furred prey (see Fig. 8a, b), **b** Masson's trichrome-stained *Boa constrictor* mandibular mucus glands showing dense mucus granules

consider a particular organism "venomous" (Jackson and Fry 2016) and dipsadid snakes such as *Sibynomorphus* are just one example amongst myriad "borderline cases" within the Toxicofera. Regardless, "mucus control" and venom are not mutually exclusive functions for the infralabial gland—in the world of ophidian oral glands, it is not uncommon for a single anatomical structure to exhibit multiple functions.

Sublingual and supralingual glands Ophidian sublingual glands are typically mucous-secreting, although they vary in structure (Taub 1966; Kochva 1978). In "scolecophidian" and "henophidian" snakes, the glands are paired laterally, with a single duct from each gland opening towards the anterior of the buccal cavity (Figs. 8, 9). In *Xenopeltis* (Xenopeltidae), each sublingual gland is associated with a small gland of apparently unknown function. In

caenophidian snakes, an additional posterior sublingual gland is present behind the paired lateral glands (Fig. 11) (Kochva 1978). Taub (1966) reviewed earlier work on sublingual glands and states that "confusion" caused by the widely varying descriptions may be either "the result of errors on the part of some of the workers" or "symptomatic of great variation of these glands within the Ophidia" and concludes that the "topic certainly deserves independent investigation". Unfortunately, little further investigation of the sublingual glands of the majority of snake species seems to have been conducted.

The exception amongst the general neglect of ophidian sublingual glands is the posterior sublingual of caenophidian snakes, which on at least three independent occasions has evolved into a salt extraction gland. This adaptation, which has occurred twice in the Elapidae (in *Laticauda* and in the common ancestor of hydrophiine sea snakes—Dunson et al. 1971) and once in *Acrochordus granulatus* (Dunson and Dunson 1973), is a fascinating example of convergent functional evolution.

Supralingual glands occur in the Viperinae (although apparently not in the Crotalinae—Taub 1966) and in some sea snakes (Kochva 1978). These glands are located on the tongue sheath and may surround the tongue; their function and homology is unknown, but they may be an extension of the posterior sublingual (Taub 1966).

Terminology

Research on snake oral glands has been adversely affected by terminological confusion. This confusion has primarily resulted from the extreme lability of the glands in structure and arrangement amongst species, which confounded many early attempts to establish the homology of particular glands and resulted in numerous authors coining their own names for the same structures in different snakes. Thus, we find Phisalix (1922) speaking of the "temporomandibular glands" of "scolecophidian" snakes; Haas (1964) discovering a gland in a "scolecophidian" snake he considered similar and questioning whether it should be compared to the "*anterior temporal* gland" of Phisalix; Taub (1966) insisting (*pace* Phisalix) that temporomandibular glands are limited to "scolecophidian" snakes and anterior temporal glands to "primitive" alethinophidian snakes; Kochva (1962) describing the "posterior glands" of "colubrid" and viperid snakes; McDowell (1986) referring to "rictal glands"; and Underwood and Kochva (1993) adopting this latter term for all of the above, with the possible exception of the "temporomandibular gland" of Phisalix, which remains mysterious!

Much of this confusion has been addressed in previous reviews and studies (e.g. Taub 1966; Kochva 1978; Underwood and Kochva 1993; Underwood 2002), but considerable controversy remains regarding appropriate nomenclature for the post-orbital toxin-secreting dental glands of the Caenophidia (Weinstein 2011; Fry et al. 2012). Taub (1966) coined the name "Duvernoy's gland" for this gland in NFF snakes to replace the term "parotid gland", which invited confusion with the mammalian parotid gland, a non-homologous structure. He chose the name "Duvernoy's" in honour of George Louis Duvernoy, "since he was the first to recognise their distinct character" and noted that "use of an eponym may meet with some objection but it is certainly preferable to the term parotid".

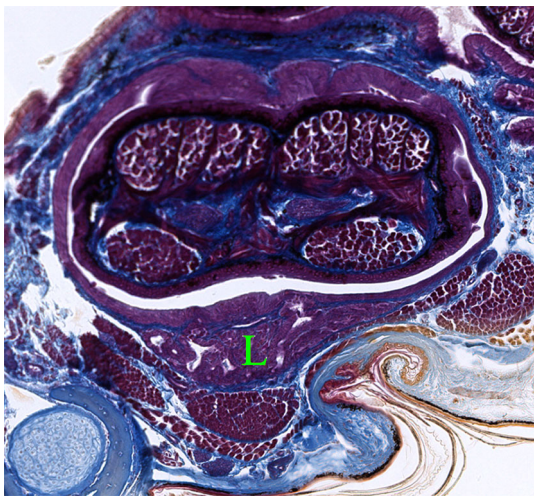

Fig. 11 Milligan's trichrome-stained transverse section through the tongue and sublingual gland (L) of *Hypnale hypnale*. In this species, the typically paired sublingual glands (see Glands 2) are continued posteriorly by a single midline structure (the posterior sublingual gland) the ducts of which can be seen opening into the lingual sheath

That the "Duvernoy's gland" of NFF snakes and the "venom gland" of front-fanged snakes are developmentally homologous had previously been established (Kochva and Gans 1970 but see earlier references within), although Taub does not comment on this. His discussion reflects a number of common confusions regarding the concept of homology, and he mentions the then prevalent (now refuted—see Fry et al. 2012 for details) idea that the venom glands might form an "evolutionary series" in which the venom glands of the Elapidae evolved into the "more advanced" venom glands of the Viperidae. The term "Duvernoy's gland" may have been erected in order to avoid confusion generated by the suggestion of homology with mammalian parotid gland, but apparently Taub did not consider the confusion its coinage might engender via the implication of *non*-homology with the venom glands of front-fanged snakes.

Taub ends his discussion of venom glands by commenting, "there is now some evidence that the two types [of venom gland] may not be homologous". Indeed, they

cannot be referred to as "homologous" without including the "Duvernoy's gland" of NFF snakes under the same classification, because they are polyphyletic with respect to the latter and their relatively superficial similarities (essentially limited to their "high-pressure" delivery of a venom bolus and their association with hollow fangs) are the result of convergent evolution. For this reason, the high-pressure venom systems of front-fanged snakes (Elapidae, Viperidae and Atractaspidinae), might be described as *homoplastic* (convergent in form and function—Currie 2014) with one another and homologous with the differentiated dental glands of non-front-fanged snakes.

In his discussion of the "Duvernoy's gland", Taub (1966) notes that "the idea has often been expressed that these glands produce venom", citing several studies going back to Phisalix (1922), and notes that because of their predominantly serous-secretory cells and their single duct associated with the posterior maxillary teeth, this idea is well supported and the glands should be considered an entity separate to the supralabial glands, which are typically mucous-secreting and polystomatic. In fact it has long been reported that NFF snakes use their toxic oral secretions to subdue their prey (see, e.g. Alcock and Rogers, 1902), and this observation has since been corroborated by a large number of experiments and observations of many different species of NFF snakes (see Fry et al. 2012; Jackson et al. 2013a, b). A toxic oral secretion produced in a specialised gland and delivered by a bite in order to facilitate the subjugation of prey is "venom" by any modern definition (Mebs 1978; Fry et al. 2009; Weinstein 2015).

Venom is a functional trait (Fry et al. 2012; Weinstein et al. 2012; Jackson et al. 2013a) and "venom glands" constitute a *function category* (Millikan 1989; Jackson and Fry 2016). Other obvious function categories include hearts, wings and eyes. The traits within a function category are not necessarily homologous, rather they are homoplastic—grouped together as a result of their shared functional role. The venom glands of hymenopteran insects, for example, are not homologous with those of viperid snakes, but they share a function: the production of venom. Function categories may be defined stipulatively, descriptively or theoretically (Millikan 1989). The members of a *stipulative* function category are grouped together for the purposes of analysis—we may not have a theory-level understanding of their functional similarities, but we know that they form a natural group that should be considered together. Stipulative function categories are especially useful for biologists studying the evolution of a specific trait, because traits often exist in a variety of forms, points on a continuum, which we may interpret as different branches of the same evolutionary tree.

Table 3 Recommended nomenclature for ophidian oral glands with multiple synonyms currently in use

Recommended nomenclature	Synonym(s)
Rictal gland	Anterior temporal gland; posterior gland
Venom gland	Duvernoy's gland
Dental gland	Incipient venom gland[a]

[a] Suitable for stipulative use in discussions of venom gland evolution

The venom glands of caenophidian snakes are homologous but diverse in form, particularly amongst NFF species. Despite this diversity of form, they may all be considered members of the same function category: those with a well-characterised venomous function (i.e. those that we are confident contribute to prey subjugation or defence against predators) are *descriptively* "venom glands" and those that are merely homologous structures (e.g. may have lost their venomous function following the evolution of effective constriction) are *stipulatively* so. Splitting them into myriad subcategories will not help us understand their evolution—such categories could never be clearly defined because discernible boundaries between the forms are not likely to exist.

In order to avoid creating further confusion regarding the development, evolution and function of ophidian oral glands, it is vitally important that each homologous structure be given a single, appropriate name. The term "Duvernoy's gland" is inappropriate—its original purpose was to avoid the implication of homology between ophidian and mammalian "parotid" glands, but it generates the equally spurious implication of *non*-homology between the venom glands of front-fanged and non-front-fanged snakes. Our modern appreciation of the homology of the toxin-secreting oral glands of non-front-fanged snakes with those of front-fanged snakes, coupled with the large number of experiments demonstrating the use of the products of these glands in prey subjugation and a sound application of the biological function concept, leaves no doubt that the most sensible name for these glands is "venom glands". Recommended nomenclature for the venom gland and related structures is provided in Table 3.

The evolution of the ophidian venom system

There are at least five possible functional roles that ophidian oral glands may fulfil. They may contribute to maintaining the condition of the mouth and teeth; may facilitate the ingestion of large prey items by providing lubrication; may

produce enzymes that aid the digestive process; may produce antimicrobial compounds (peptides and enzymes); or may produce venom. In the final category, the functions of venom may include prey subjugation, defence against predators or competitor deterrence. A single oral gland may fulfil more than one of these functional roles.

The most likely function for oral glands that are purely mucous-secreting is lubrication. This is corroborated by the fact that boid and pythonid snakes, which are powerful constrictors able to subjugate and consume large prey animals, have predominantly mucous-secreting oral glands (Fry et al. 2012). The feeding ecology of these snakes, along with their specialised anatomy, is likely a derived condition (Hsiang et al. 2015). In order to attempt a reconstruction of the evolutionary history of ophidian oral glands, it is necessary to consider the likely arrangement of these structures in both the common ancestor of all snakes and the common ancestor of all venomous reptiles.

Earlier attempts to reconstruct the evolutionary history of ophidian venom systems were stymied by a lack of knowledge regarding the phylogenetic relationships both within Squamata and within Serpentes. It is now clear that all extant venomous reptiles are part of the clade Toxicofera, which includes the iguanian and anguimorph lizards, as well as Serpentes (Vidal et al. 2009). The primary synapomorphy of this clade is the possession of serous-secreting dental glands, which, in snakes as well as helodermatid and varanoid (genera *Varanus* and *Lanthanotus*) lizards, have evolved into specialised venom glands (Fry et al. 2012).

Reconstructions of ancestral character states are achieved by utilising a combination of inference from extant species, examination of the fossil record and genetic evidence. Note that the degree of plesiomorphy identified in extant species is relative and inferred. All extant toxicoferan reptiles have been evolving for the same period of time since the most recent common ancestor (MRCA). As soft character states rarely fossilise, it is difficult to estimate the absolute degree of change in the venom system of any extant species in relation to that of the MRCA. The dental glands of iguanian lizards have been referred to as "incipient" venom glands, reflecting the fact that they may remain closest in arrangement to the putative ancestral Toxicofera state. In the anguimorph lizards, selection favoured specialisation of the mandibular dental gland, whereas in the Serpentes it was the maxillary dental gland that formed the substrate for the subsequent evolution of the advanced snake venom system (Fry et al. 2012).

The evolutionary origins of snakes are still poorly understood, although attempts have been made to reconstruct the ancestral snake phenotype. Snakes likely evolved in the Jurassic ($\sim$170 Mya) on West Gondwana (the supercontinent comprising South America and Africa) and were nocturnal, carnivorous and terrestrial or fossorial (Vidal et al. 2009; Vidal and David 2004; Vidal et al. 2009; Simões et al. 2015). A significant recent discovery is the paraphyly of "Scolecophidia" (fossorial "worm-like" small snakes with a limited mouth gape size, feeding on termites and ants) with one scolecophidian lineage (Anomalepididae) more closely related to the alethinophidians (all other ecologically diverse "typical" snakes) than to the other scolecophidians (Vidal et al. 2009; Zheng and Wiens 2016). The third most basal snake lineage, named Amerophidia (Vidal et al. 2007a, b), includes terrestrial and macrostomatan (a large mouth gape size) Tropidophiidae (genera *Tropidophis* and *Trachyboa*) and fossorial South American Aniliidae (genus *Anilius*) with a relatively limited mouth gape size. Unfortunately, the phylogenetic position of the remaining fossorial alethinophidian lineage, with a relatively limited mouth gape size, the Asian Uropeltoidea (including the genus *Cylindrophis* discussed in this paper) remains imperfectly resolved, which limits our understanding of early snake ecology, behaviour and evolutionary history.

According to a recent attempt to reconstruct the ancestral snake phenotype (Hsiang et al. 2015), the earliest snakes may have been nocturnal foragers that preyed upon small vertebrates that they encountered in their night-time refugia. These ancestral snakes are unlikely to have been constrictors and likely fed on prey not wider than their own heads. Amongst extant snakes, this description is most similar to basal members of the Alethinophidia, such as *Anilius* and *Cylindrophis*. Both genera feed upon slender prey items, and neither are effective constrictors—they subdue their prey by biting it repeatedly (Cundall 1995; Marques and Sazima 2008). These genera both lack differentiated venom glands but possess exceptionally large serous-secreting rictal glands (Underwood 2002), which may secrete 3-finger toxins (typically postsynaptic neurotoxins). In the case of *Cylindrophis* (Fry et al. 2013), this system may play a functional role in prey subjugation. This is a possibility that warrants further investigation.

Another favoured hypothesis is that the snake MRCA resembled the "Scolecophidia", which are the most basally divergent snakes, although this group is now known to be paraphyletic (Vidal et al. 2009). "Scolecophidian" snakes are highly specialised predators of invertebrates and have many phenotypic traits that are unlikely to be plesiomorphic for snakes in general (Simões et al. 2015). However, they also share numerous traits with basal alethinophidian snakes such as *Cylindrophis* and *Anilius*. What this suggests is that features of the phenotype of the snake MRCA may be recoverable by consideration of the "phenotypic intersection space" of "scolecophidian" and basal alethinophidian snakes. This intersection space includes the possession of large serous-secreting maxillary glands, preference for small prey (either vertebrate or invertebrate,

but not wider than the snake's own head), lack of effective constriction behaviour, nocturnal foraging and (semi-) fossoriality.

It has been hypothesised that the ancestral function of the serous-secreting oral glands of toxicoferan reptiles was antimicrobial and that this might have exapted them for their subsequent evolution into venom systems (Shivik 2006; Fry et al. 2013). This remains a plausible hypothesis. In the earliest days of ophidian evolution, however, it appears that snake feeding ecology was poised to branch in multiple directions. As well as possessing a rudimentary venom system, the ancestral snakes were likely rudimentary constrictors. Constriction probably began merely as a way for limbless predators to prevent their prey from escaping. Indeed, many extant venomous snakes continue to coil around their prey in order to secure it whilst their venom takes effect (Shine 1985). *Cylindrophis* and *Anilius*, whilst not effective constrictors (they release their prey multiple times during their attempts to subdue it), coil around their prey whilst repeatedly biting it (Marques and Sazima 2008). The next stage of snake evolution, as reconstructed by Hsiang et al. (2015), saw a split in prey subjugation strategies in which constriction and development of a more effective venom system were favoured in separate lineages (further specialisation for burrowing and invertebrate prey were also favoured in the lineage(s) from which the extant "Scolecophidia" descend). The precise details of this "just so story" remain mysterious, and it is important to note that the interpretation of Hsiang et al. (2015) remains speculative. In particular, conflict between the phylogeny relied upon in that study and that recovered in other studies (e.g. Vidal et al. 2009; Pyron et al. 2011; Streicher and Wiens 2016) suggest that additional data may be required to resolve these fascinating questions. Streicher and Wiens (2016) specifically criticised the preferred tree of Hsiang et al. (2015) and suggested that it be utilised in evolutionary studies only with "considerable caution". For the purposes of the present discussion, it is sufficient to say that the plesiomorphic snake condition likely included large serous-secreting dental glands and that the snake MRCA was not a constrictor. However, the oral glands (and biology in general) of all basal snakes—"scolecophidians", Aniliidiae, Tropidophiidae and Uropeltoidea—remain understudied. Investigation of these taxa may shed further light on the evolutionary pathways traversed by ophidian oral glands.

An instructive way to think of natural selection is as a process that drives the "reification of past contingency". The concept of exaptation (Gould and Vrba 1982) is also useful when considering the evolution of specific traits. It may be that the antimicrobial function of the serous-secreting dental glands of an ancestral toxicoferan reptile was co-opted for use in prey subjugation when a contingent change in the expression level of a certain active molecule resulted in a very small increase in the success rate of the reptile's predation attempts. It is important to note that "rapid prey death" is not at all necessary for the evolution of venom. All that is required is a slight increase in the efficacy of prey capture for selection to favour the further specialisation of the venom system. As soon as a trait makes a contribution to the fitness of an organism, the property of the trait responsible for that contribution may be considered its function (Godfrey-Smith 1994).

It is sometimes tempting to think of the evolution of a particular functional trait within a specific clade as travelling along a single pathway towards ever more efficient forms. In reality, evolution is a many branching path and fitness landscapes may often be "rugged"—reaching a local fitness peak may ultimately prevent access to global maxima (Gavrilets 2008). Just because the venom systems of NFFs may be "less effective" than those of front-fanged snakes does not mean that the production and delivery of venom is *not* their primary function (cf. Weinstein et al. 2012; Kardong 2012). In another twist of the evolutionary tale, related groups of taxa may diverge at one point only to later discover the same "good trick" (Dennett 1995) via a slightly different pathway, as has occurred with the three clades of front-fanged snakes.

The toxicoferan reptile venom system has undergone considerable "evolutionary tinkering", particularly amongst the snakes, and exists in myriad extant forms (Vidal 2002; Fry et al. 2012, 2015; Fig. 12). Included amongst this tinkering are multiple reversal events, including amongst the caenophidian ("advanced") snakes, in which the transition to defenceless prey or the adoption of constriction as a primary method of prey subjugation, has resulted in a reduction of the system. This extant diversity, along with the difficulty of seeing clearly into the distant evolutionary past, makes it difficult to pinpoint the precise moment at which serous-secreting dental glands acquired a venomous function. The extant diversity and propensity for reversals also make it difficult (if not impossible) to draw clear boundaries between "venomous" and "nonvenomous" species of snake. Such binary distinctions are not always present in nature, where traits exist as points along a continuum on which only the poles are clearly definable. Ultimately, such distinctions are less important than, and perhaps unnecessary for, gaining a deeper understanding of the evolution of this fascinating system.

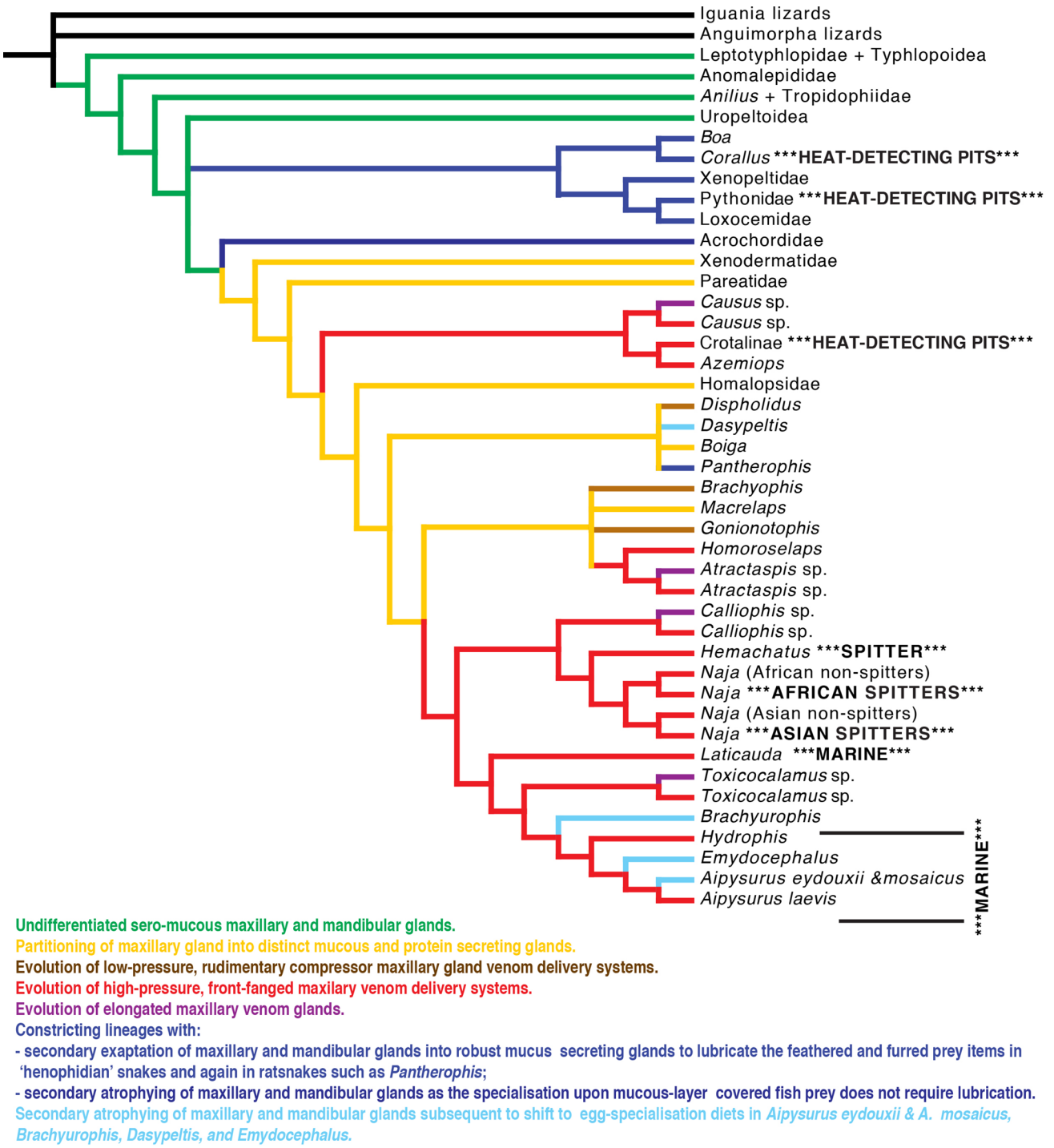

Fig. 12 Toxicofera evolutionary tree showing the timing maxillary gland diversifications relative to organismal diversification (Fry et al. 2006, 2008, 2013; Vidal 2002; Vidal and David 2004; Vidal and Hedges 2005; Vidal et al. 2009)

Conclusion

The primary goal of this paper, as well as providing a overview of ophidian oral gland anatomy and providing new data on the anatomy of rictal glands, is to clear up terminological confusion and clarify the evolutionary relationships connecting particular subtypes of glands. This is of particular importance for consideration of the evolution of the venom system. Although this has been discussed at length in the text above, in the interests of achieving the utmost clarity a summary of our interpretation concludes the paper.

"Dental glands" are a subset of labial (either supra- or infra-) glands characterised by the presence of ducts that open close to the base of the teeth. This condition is an anatomical synapomorphy of the clade Toxicofera. As dental glands have evolved the specialised function of venom production within this clade, they are also stipulatively referred to as "incipient venom glands". "Venom glands" constitute a function category that includes all those glands that have the function of venom production. Anatomically, venom glands in snakes (with the possible exception of certain species of dipsadid snake, see above) are derived from maxillary (upper jaw) dental glands. Venom glands differ morphologically both within and amongst the snake families in which they occur. For this reason, the functional (non-anatomical) designation "venom gland" is preferred for all variants and the term "Duvernoy's gland" is not recommended.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Animal rights statement This paper complied with the relevant legislation surrounding animal use, and all work was done on previously collected, preserved specimens in collections, and thus, animal ethics approval was not needed.

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