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Morphology of Duvernoy's Glands and Maxillary Teeth and a Possible Function of the Duvernoy's Gland Secretion in *Helicops modestus* Günther, 1861 (Serpentes: Xenodontinae)

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Abstract. We investigated the gross anatomy, histology and ultrastructure of Duvernoy's glands and scanning electron microscopy of maxillary teeth of *Helicops modestus,* as well as its prey-handling behavior in laboratory. We later compared this histology with other species of Hydropsini. Duvernoy's glands are located in the post-ocular region, immediately behind the supralabial gland. Each gland is connected to a pair of ungrooved rear fangs by a vestibule from which the secretion is drained. Histological analysis showed that the gland is wrapped by a layer of connective tissue and consists of a glandular body formed by prismatic cells organized in acini and a duct lined with columnar cells. The prismatic cells are positive to PAS and bromophenol blue, indicating glycoprotein content, whereas the columnar ductal cells are positive to PAS and alcian blue pH 2.5, indicating the presence of acid mucous. Transmission electron microscopy showed electron-dense, heterogeneous granules in the prismatic cells, whereas the granules of the columnar cells were electron-luscent and homogeneous. The Duvernoy's glands of *H. modestus* are more similar to those of *H. angulatus* than any other species analyzed. Observations of prey-handling behavior showed that *H. modestus* strikes and holds fish in its mouth while repeatedly carrying out bilateral raking motions with both maxillae. Ingestion starts headfirst. We observed only a single episode of constriction. Snakes usually swallowed fish alive but clearly immobilized, suggesting that the primary function of the Duvernoy's secretion is associated with the quiescence/immobilization of the fish prey.

Keywords. Dipsadidae; Hydropsini; Prey-handling behavior; Rear-fanged snakes; Water snakes.

INTRODUCTION

In snakes, the venom-delivery systems (VDSs) include a pair of venom glands, their associated muscles, and enlarged front fangs (Jackson, 2003). While viperids, elapids and some Atractaspididae are well known to possess specialized venom apparatuses used to inject toxins into prey (Greene, 1997; Vidal, 2002), the VDSs of the other advanced snakes (Caenophidia), when present, show wide morphological variation, including a pair of rear fangs and Duvernoy's glands, which are rarely associated with muscles (Kochva, 1978; Underwood, 1997; Jackson, 2003; Weinstein et al., 2010).

Morphological and physiological studies on the Duvernoy's glands of rear-fanged species (see Taub, 1967; Gabe and Saint-Girons, 1969 for details) have provided evidence on the possible evolutionary pathways of the venom apparatus in snakes (Kochva, 1987). The previous decade was rich in discussion with regards to the idea that venomousness evolved very early on within the advanced snakes and favored the massive radiation within this group (Vidal, 2002; Fry et al., 2003; Jackson, 2003, 2007; Fry and Wüster, 2004). Nevertheless, despite this progress, little is known about the morphological diversity of Duvernoy's glands and even less about the function of their secretions in rear-fanged species.

Snakes in the genus *Helicops* are part of the monophyletic tribe Hydropsini (Xenodontinae, Dipsadidae), which is widely distributed throughout South America (Vidal et al., 2000; Zaher et al., 2009). *Helicops modestus* Günther, 1861 is a viviparous, aquatic species with dorso-anteriorly positioned eyes and nostrils and curved dentition that feeds mainly on fishes and amphibians (Martins and Oliveira, 1998; Aguiar and Di-Bernardo, 2004). Salomão et al., (2003) showed that, in relation to the number of bites in humans, this species leads rear-fanged species in southeastern Brazil, with the main clinical manifestations being restricted to the bitten area and including pain, edema, abrasions, and hemorrhage.

Duvernoy's glands in Neotropical rear-fanged snakes are associated with a wide variety of features and possess secretions with complex chemical compositions and diverse functions, some of which are medically important. Therefore, we here investigated the morphology of the Duvernoy's glands and maxillary teeth as well as the prey-handling behavior of *Helicops modestus*. For comparative purposes, we also verified the histology of the Duvernoy's glands in other hydropsini snakes and addressed the

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following questions: Do the histological and histochemical characteristics of Duvernoy's glands differ among species of Hydropsini? What is the function of the Duvernoy's secretion in *H. modestus?*

MATERIALS AND METHODS

Morphology

Histology and histochemistry

Seven adult individuals of *Helicops modestus* (Fig. 1A; Appendix), from the Laboratório de Herpetologia, Instituto Butantan, Brazil, were euthanized with an intraperitoneal overdose of sodium thiopental (30 mg/Kg), preserved in formalin, and deposited in the Alphonse Richard Hoge Herpetological Collection at the Instituto Butantan (IBSP 67068, 67691, 71843, 76610, 76611, 76612, 76613). The heads of three individuals (IBSP 76610, 76612, 76613) were completely removed, fixed for 24 h in Bouin's solution, and decalcified in 4.13% aqueous EDTA, pH 7.2, for 60 days, renewed every third day and kept in constant stirring. The decalcified heads were dehydrated in ethanol, embedded in paraffin, and subjected to serial sagittal sectioning (7 µm) using a Microm HM 340 E microtome with disposable steel blades. Sections were stained with hematoxylin-eosin (HE) for general study and N.M.C. (Nylceo Marques de Castro) and Mallory trichrome (Castro and Camargo, 1951; Junqueira et al., 1979) for identification of the collagen and muscle fibers and epithelia. The glands of the other four individuals (IBSP 67068, 67691, 71843, 76611) were dissected immediately after euthanization, fixed in 4% paraformaldehyde PBS 0.1 M (pH 7.2) for 24 h, dehydrated in ethanol, and embedded in historesin (glycol methacrylate, Leica).

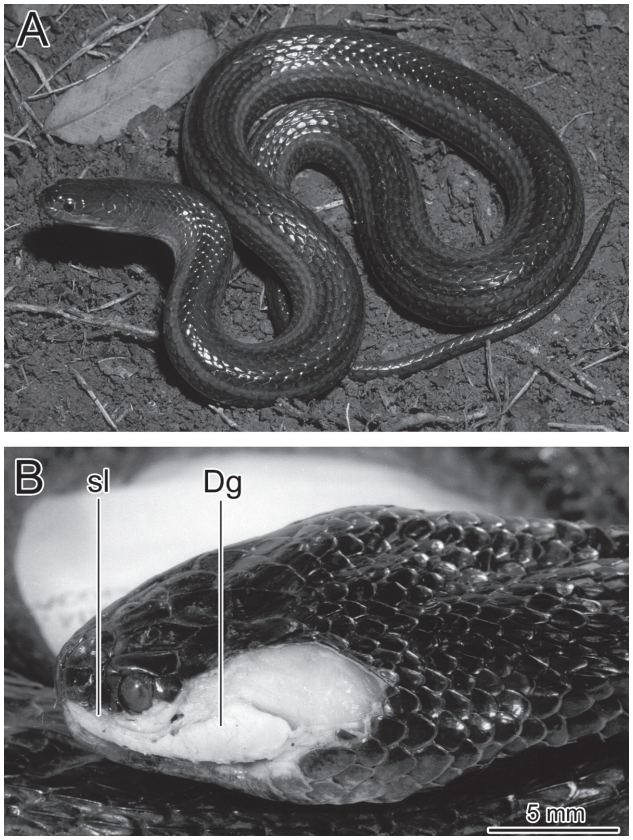

Figure 1. (A) Female *Helicops modestus* from Brazil: **São Paulo:** Jacareí (SVL = 582 mm). Photograph by Otávio A.V. Marques. **(B)** Lateral view of the dissected head of *H. modestus* showing the position of Duvernoy's and supralabial glands. *Abbreviations:* Dg, Duvernoy's gland; sl, supralabial gland.

Histological sections of Duvernoy's glands were made from preserved specimens of the following species of hydropsini: *Helicops angulatus* (Linnaeus, 1758), *H. carinicaudus* (Wied-Neuwied, 1825), *H. hagmanni* Roux, 1910 and *Hydrops triangularis* (Wagler, 1824) (Appendix). These glands were dehydrated in ethanol and embedded in historesin; additional procedures followed those applied to *H. modestus*. Sections of 2 µm were obtained in a Microm HM 340 E microtome using glass knives and stained with toluidine blue-fuchsin or submitted to the following histochemical procedures (following Bancroft and Stevens, 1996): periodic acid-Schiff (PAS), alcian blue pH 2.5, and combined PAS and alcian blue pH 2.5 (Pearse, 1985; Kiernan, 2001) to identify neutral (PAS) and acid (alcian blue) mucosubstances, as well as proteins (bromophenol blue). Photomicrographs were obtained with an Olympus BX51 microscope and Olympus SZ stereomicroscope, both equipped with a digital camera and operated with the software Image-Pro Express (MediaCybernetics). All histological slides were deposited in the herpetological collection of the Museu de Zoologia da Universidade de São Paulo (MZUSP) under the same voucher numbers of their respective specimens. Average measurements of the glandular acini were taken for 30 acini/gland in at least three different slides for each species, following Oliveira et al. (2014).

Ultrastruture

Fragments of approximately 1 mm^3 of *Helicops modestus* Duvernoy's glands were fixed in Karnovsky solution pH 7.2 (Karnovsky, 1965) for 24 h, fixed in 1% osmium tetroxide, contrasted in 2% uranyl acetate, dehydrated in ethanol, and embedded in epoxy resin. Ultrathin sections (60 nm) were obtained using a Sorvall MT 6000 ultra microtome, using 2% uranyl acetate and lead citrate for contrast, and examined with a LEO 906E transmission electron microscope (TEM) operating at 80 kV. For scanning electron microscopy (SEM) of maxillary teeth, soft tissues were removed manually, the maxilla was mounted on SEM stubs, and the sample was dried, coated with gold, and examined in a LEO 440 scanning electron microscope.

Prey-handling behavior

Individual snakes (four males, 180–345 mm SVL, 5–30 g; two females, 320 mm and 580 mm SVL, 21 g and 160 g, respectively; Appendix) were housed separately in 50 × 30 × 30 cm terrariums, with a 5 cm layer of water. Stones were placed in one side of the terrarium to provide a partial dry environment covering about 20% of the total area. Room temperature varied from 20–32°C, and humidity was never below 60%. The poeciliid *Xiphophorus* sp. and cichlid *Geophagus brasiliensis* (Quoy & Gaimard, 1824), typical fish prey of *Helicops modestus* in nature (Scartozzoni, 2010), were offered as food items.

From a total of 28 feeding episodes, 22 episodes of subduing and 20 episodes of swallowing prey were used for statistical analyses. Prey-handling behavior was described based on the "all occurrences sampling" and the "sequence sampling" methods cf. Lehner (1996), and divided into three phases. (1) Orientation towards prey, approach and strike time (time from moment fish is placed in terrarium until capture); (2) subduing time (time from moment of capture to the first mandibular movement of the snake in any direction or release of the fish); (3) swallowing time (time from beginning of ingestion until the moment when the fish could no longer be observed inside the snake's mouth). The duration of each phase is reported in the text as minutes:seconds but was converted to seconds for statistical analyses. We used relative prey mass [(mass of the prey)/(mass of the snake)] in analyses (Rodríguez-Robles, 1992; Rodríguez-Robles and Leal, 1993). ANCOVA analyses were performed according to Rodríguez-Robles and Leal (1993) to test possible differences in the subduing and swallowing times between two-prey types. Statistical tests were performed using Past software (Paleontological Statistics, version 3.02, Hammer et al., 2001).

RESULTS

General morphology

The Duvernoy's glands of *Helicops modestus* are located in the post-ocular region, behind and juxtaposed to the supralabial gland. They measure about 5 × 3 mm and are yellowish in adult specimens (Figs. 1B; 2A), lacking any visible association with muscle fibers along their exterior. They are incorporated in a capsule of connective tissue from which several septa penetrate the glandular body, dividing it into several lobules (Fig. 2C). The body itself is composed of a secretory portion and a net of internal ducts. Contiguous to Duvernoy's gland is a long supralabial gland, which consists of a series of juxtaposed, small, glandular units extending rostrally to the maxilla (Figs. 2A, B).

The rear fangs are ungrooved and clearly differentiated from the other teeth by their larger size, less curved shape, and presence of a canal between them (Figs. 3A, B). They are enveloped in the fold that forms the fang sheath, except for the fang tips, which are permanently exposed inside the mouth and from which the secretion drains into the oral cavity. They possess ridges along the entire posterior surface and anteriorly from the mid-point to the tip of the fang, most prominent on the posterior surface (Figs. 3D, E), giving the fangs a beveled bladelike appearance (Fig. 3D). The remaining maxillary teeth are characterized by ridges along their labial and lingual surfaces extending from their mid-points to their tips (Figs. 3C, D) that give them a beveled, bladelike appearance (Fig. 3C). They are separated from the rear fangs by a diastema (Figs. 3A, B). All maxillary teeth lack striations on their posterior surface.

Duct system, histology and histochemistry of Duvernoy's gland

The ductal system within the glandular body of Duvernoy's gland consists of a net of thin ducts connected to the lobules (the lobular ducts) in *Helicops modestus* (Figs. 4A, B). From these thin ducts the secretion is conducted to the major ducts, which have larger diameters (Fig. 2C) and connect to a vestibule in which the greatest amount of secretion was observed (Fig. 2D). The vestibule is an epithelial fold surrounding the rear fangs. From this fold, the secretion is released into the oral mucosa (Figs. 2E, F) and then drained into the oral cavity.

In comparison to Duvernoy's gland, the secretory system of the supralabial glands consists of a series of short ducts, each connecting the individual glandular units directly to the oral cavity along the maxilla (Fig. 2B). Instead of being directly associated with the teeth, as in Duvernoy's glands, the openings of the labial gland ducts are located away from the teeth (Fig. 2B).

The Duvernoy's gland of *Helicops modestus* comprises numerous acini composed of prismatic secretory cells full of small spherical granules and with round nuclei. The acini are separated by sheets of connective tissue and have remarkably small lumina (Figs. 4A, B). The internal duct system is lined by columnar secretory cells, particularly in the medial region of the gland (Figs. 4A, B). These columnar cells have basal, round nuclei and cytoplasm entirely filled with secretion granules that are positive to toluidine blue-fuchsin (Fig. 4B).

Histological features of Duvernoy's glands in the other species of hydropsini were similar to those described for *Helicops modestus*. Only *H. angulatus,* along with *H. modestus,* had ducts lined with mucous cells (Fig. 4G; Table 1). *Helicops modestus* and *H. angulatus* also had acini with smaller diameters compared to the other

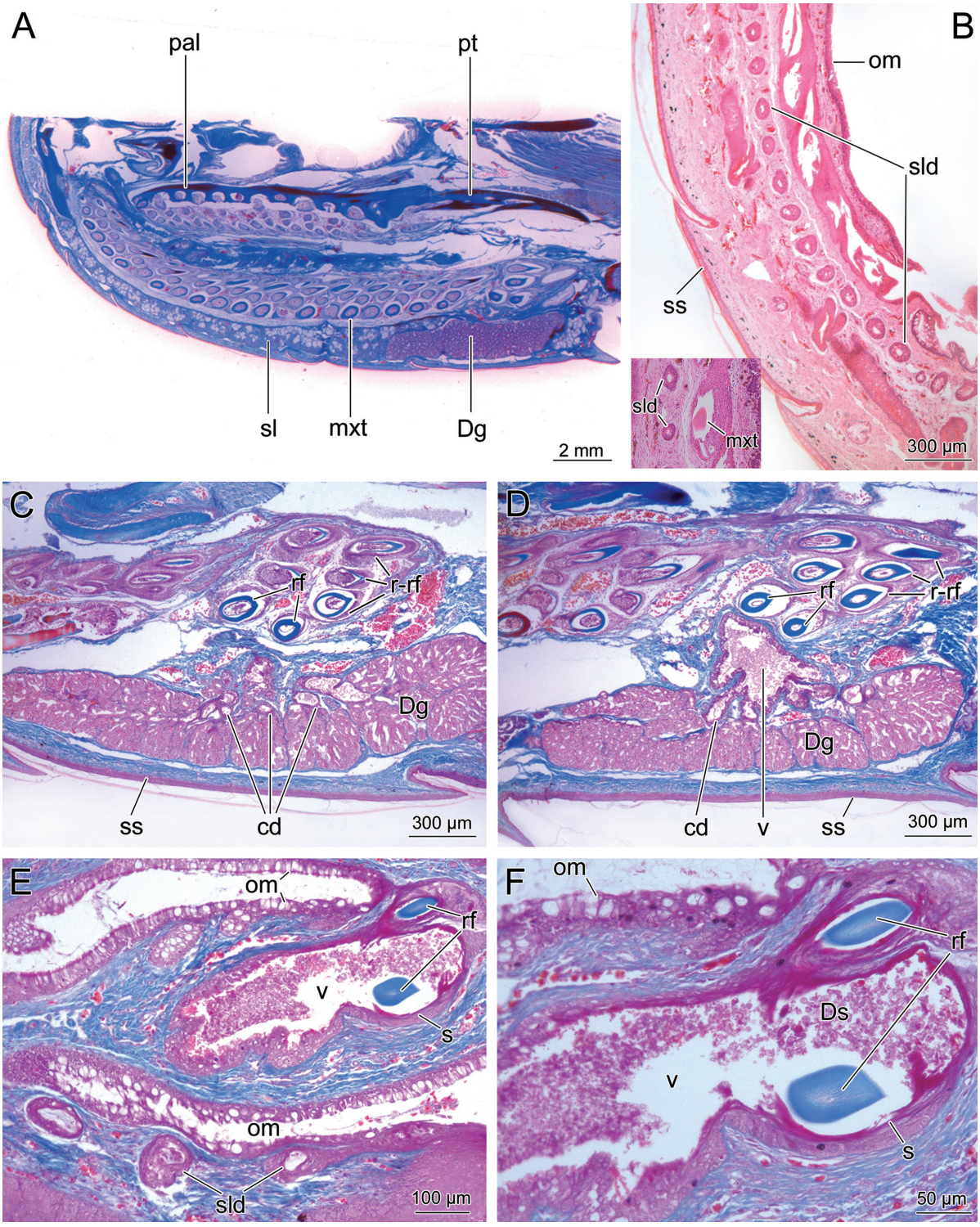

Figure 2. (A) Longitudinal section of a *Helicops modestus* head from the palate, showing Duvernoy's and supralabial glands and superior maxillary teeth. Paraffin, N.M.C. trichrome staining. **(B)** Longitudinal section of the supralabial glands showing ducts openings along the maxillary region. The insert shows the lack of connection between the maxillary teeth and the ducts of the supralabial glands. Paraffin, HE staining. **(C)** Longitudinal section showing Duvernoy's gland and the lobular common ducts running towards the central region of the gland. Paraffin, Mallory trichrome staining. **(D)** Longitudinal section of Duvernoy's gland with the lobular common ducts opening into the vestibule, which envelopes the rear fangs. Paraffin, Mallory trichrome staining. **(E)** Vestibule directly connected to the sheath, which incorporates the rear fangs. Paraffin, Mallory trichrome staining. **(F)** Higher magnification of (E) showing the transition from the vestibule (filled with Duvernoy's gland secretion) and the sheath enveloping the tip of the rear fangs. In one of the fangs it is possible to see the connection of the vestibule with the fang sheath. Paraffin, Mallory trichrome staining. *Abbreviations:* cd, lobular common duct; Dg, Duvernoy's gland; Ds, Duvernoy's secretion; mxt, maxillary teeth; om, oral mucosa; pal, palatine bone; pt, pterygoid bone; rf, rear fangs; r-rf, replacement rear fangs; sl, supralabial gland; sld, supralabial glands ducts; s, sheath; ss, supralabial scales; v, vestibule.

species, whereas *Hydrops triangularis* had the largest acini (Table 1) as well as larger intra-acinar spaces.

Within the acini of *Helicops modestus,* the cytoplasmic granules of the prismatic cells are highly positive to bromophenol blue (Fig. 4C) and PAS (Figs. 4E, F). This is characteristic of a secretion composed of neutral mucous and protein, typical of seromucous cells. The granules, however, are not homogeneous and show a heterogeneous distribution of different histochemical results (Fig. 4F). In the internal ducts, the cytoplasmatic granules of the columnar cells are highly positive to alcian blue pH 2.5 (Fig. 4D) and PAS (Figs. 4E, F) but negative to bromophenol blue (Fig. 4C). This indicates that the secretion is mainly mucous and does not contain proteins.

The supralabial glands are composed predominantly of acini with narrow lumina and are lined by mucous cells that react positively to both alcian blue pH 2.5 and PAS. Only the dorsal-most region of the gland is constituted by acini with seromucous cells that have round nuclei and cytoplasm replete with the basophilic secretory granules (Fig. 2A).

Ultrastructure of Duvernoy's gland

The ultrastructural analysis of the columnar cells of the ducts showed round nuclei and a more homogeneous cytoplasm, which is almost entirely filled with juxtaposed secretory granules. Cells are tightly adhered by multiple desmosomes. Granules in the columnar cells are quite electron-luscent and homogeneous, indicating the presence of mucous secretion (Fig. 5A). Microvilli and exocytotic secretion granules are seen at the luminal side of the epithelium (Fig. 5B). Myoepithelial cells were not observed. In contrast, the prismatic cells that compose the acini had nuclei with an irregular form and cytoplasm rich in rough endoplasmic reticulum with wide lamellae containing low electron-density material mainly along their periphery. There are also a large number of secretion granules in the central area of the cells. Electron density is heterogeneous among granules, and even within the same granule, where contents with different textures coexist (Figs. 5C, D), indicating the presence of different,

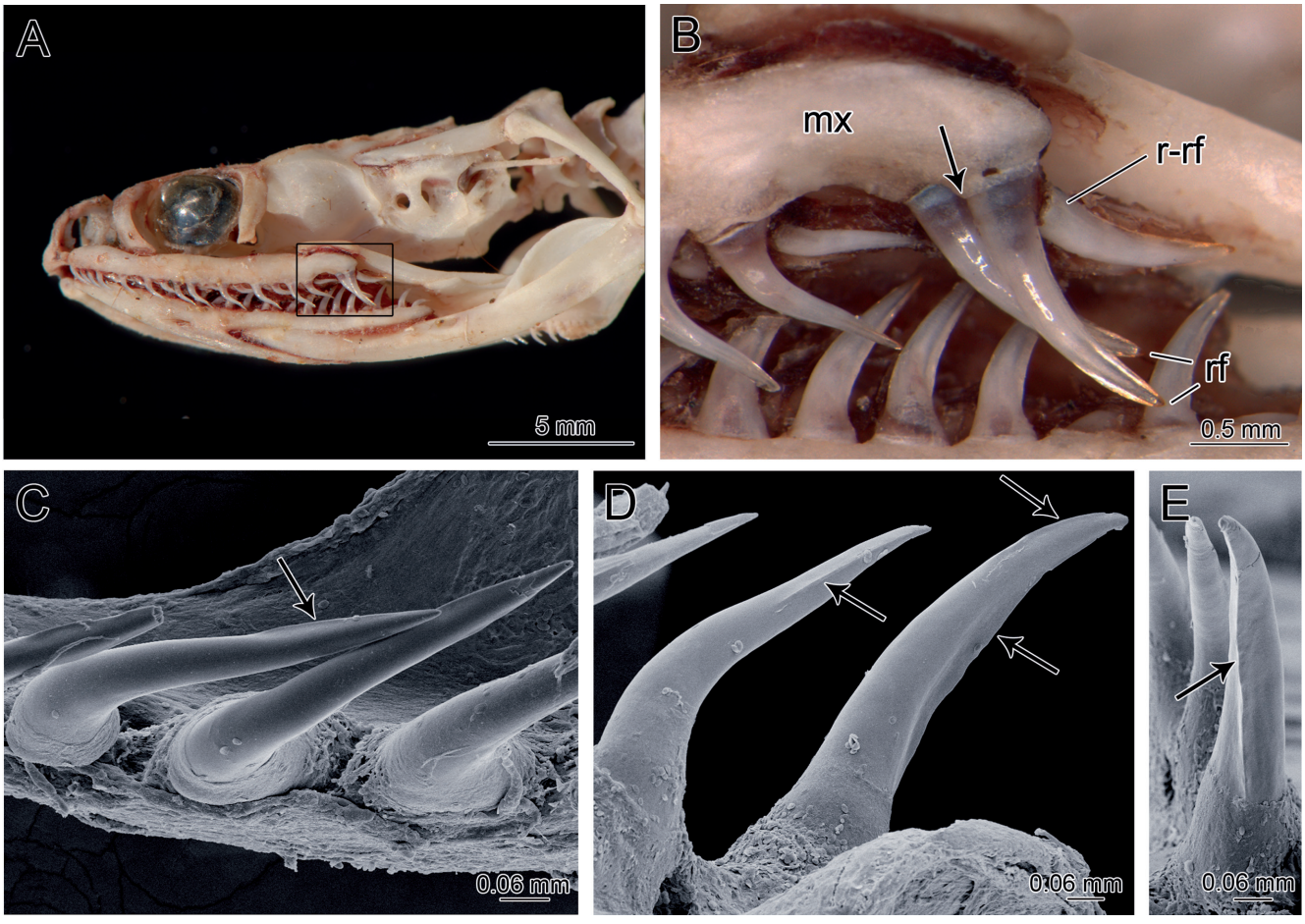

Figure 3. (A) Lateral view of *H. modestus* skull. **(B)** Higher magnification of the area outlined in (A). Note the size and curved shape of the rear fangs and the formation of a canal between them (arrow). **(C–E)** Scanning electron microscopy. **(C)** Ventral view of the maxillary bone showing anterior teeth with ridges from the mid-point to the tip on both sides of the teeth, giving a beveled, bladelike appearance. **(D)** Rear fangs showing greater prominence of the ridge on the posterior surface and less prominence on the anterior surface (arrows) and anterior maxillary teeth showing ridge (arrow) on the lingual surface. **(E)** Posterior view of the rear fangs showing the prominent ridge in detail (arrow). *Abbreviations:* mx, maxillary bone; rf, rear fangs; r-rf, replacement rear fangs.

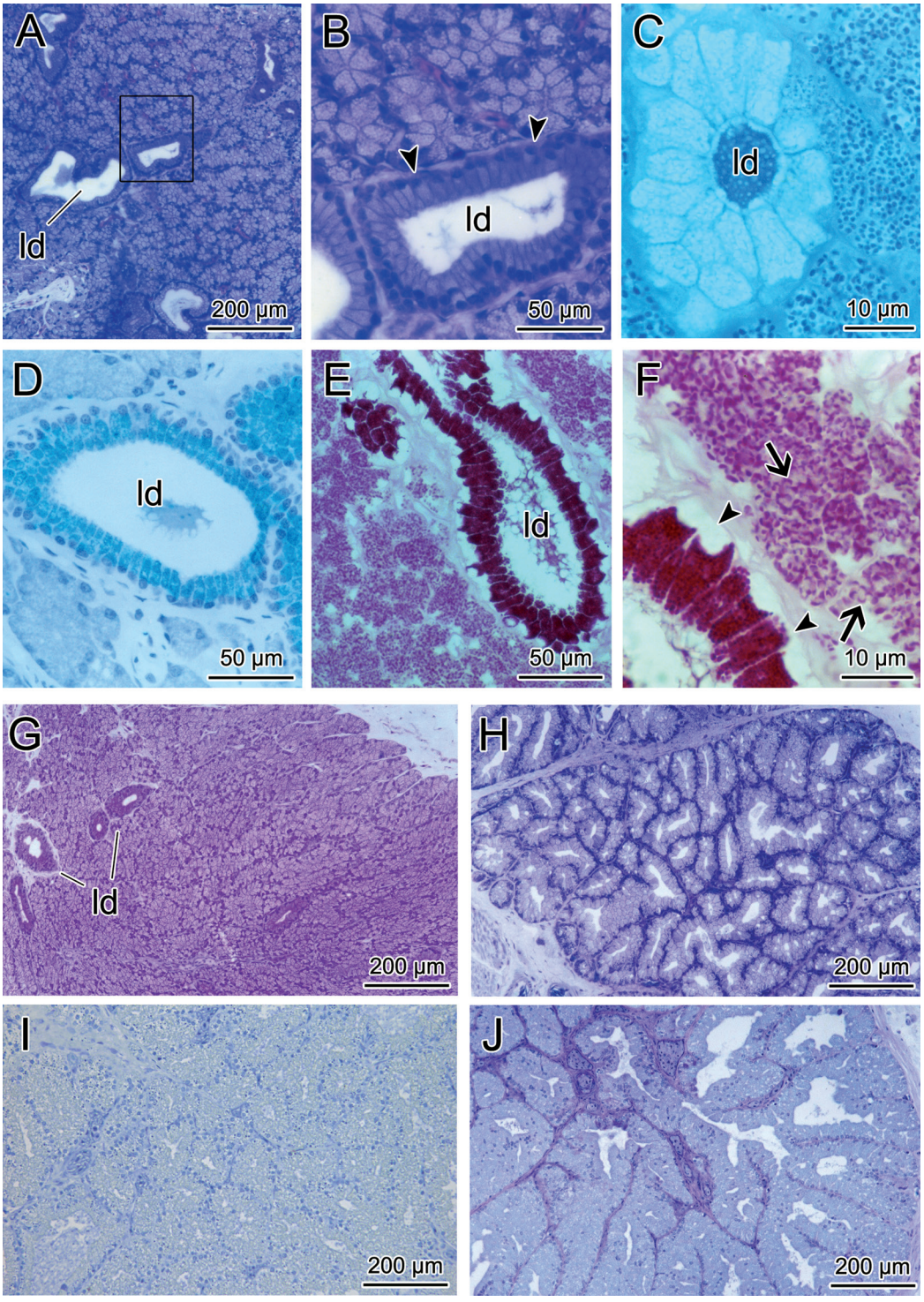

Figure 4. (A) Sagittal section showing the general structure of the Duvernoy's gland of *H. modestus,* composed of epithelial secretory acini with lobular ducts distributed throughout. Historesin, Toluidine blue-fuchsin staining. **(B)** High magnification of the region outlined in (A), showing the acini delimited by septa of connective tissue as well as the lobular ducts, lined with columnar mucous cells with basal and round nuclei (arrowheads). Historesin, Toluidine blue-fuchsin staining. **(C)** Bromophenol blue histochemical method, showing two clearly distinct regions in the granular body: a positive region, constituted by the seromucous cells of the acini and a negative region, constituted by the cells lining the ducts. Historesin. **(D)** Method of alcian blue pH 2.5, showing a positive result for the mucous cells lining the duct, and a contrasting negative result for the seromucous cells of the acini. Historesin, nuclear staining with haematoxylin. **(E)** Periodic acid-Schiff (PAS) method, showing positive results in all cell types (columnar mucous cells of the duct and seromucous cells of the acini), although the cells of the duct react more intensely than cells of the acini. Historesin. **(F)** Higher magnification of the anterior figure showing the reaction of the secretory granules. Note the heterogeneous distribution of the positivity in granules in the acini (arrows). Historesin, Periodic acid-Schiff reaction. **(G–J)** Sagittal sections showing the general structures of the Duvernoy's glands of *Helicops angulatus* **(G),** *Helicops carinicaudus* **(H),** *Helicops hagmanni* **(I)** and *Hydrops triangularis* **(J).** Historesin, Toluidine blue-fuchsin staining. *Abbreviation:* ld, lobular ducts.

non-mixed substances (probably mucous and protein) in the secretion.

Prey-handling behavior

When fish were placed in the *Helicops modestus* terraria, tongue-flicking movements (tfm) began immediately (18–52 tfm/min; mean = 32.6; *n* = 5) while the snake faced and approached the fish. Movements of the fish's fins seemed to facilitate prey location. The submersed snake progressively approached the fish by repeated lateral undulations, resulting in irregular sinusoidal flexions that pushed the body forward, typical of pre-striking behavior. Strikes were followed by immediate bites on the fish's head (*n* = 10; 36%), mid-regions (*n* = 7; 25%) or tail (*n* = 11; 39%).

Subduing time varied from 0:25 to 17:37 (Table 2) and was positively correlated with prey mass (R^2 = 0.22; F (1.20) = 5.59, $p < 0.05$; *n* = 22; Fig. 6). Prey:snake mass ratio was 0.04–0.5 (mean = 0.18). While immobilizing prey, *Helicops modestus* carried out repeated bilateral raking motions of both maxillae. Constriction was observed only once, by a juvenile female snake (180 mm in SVL, and 5 g) that formed two coils while subduing a fish with a mass ratio of 0.4. Adult snakes (SVL > 345 mm) dealing with prey that had a similar mass ratio did not use constriction. The snakes sometimes kept the prey pinned against the terrarium wall using irregular sinusoid flexions that did not form proper coils. In some cases, after the fish was

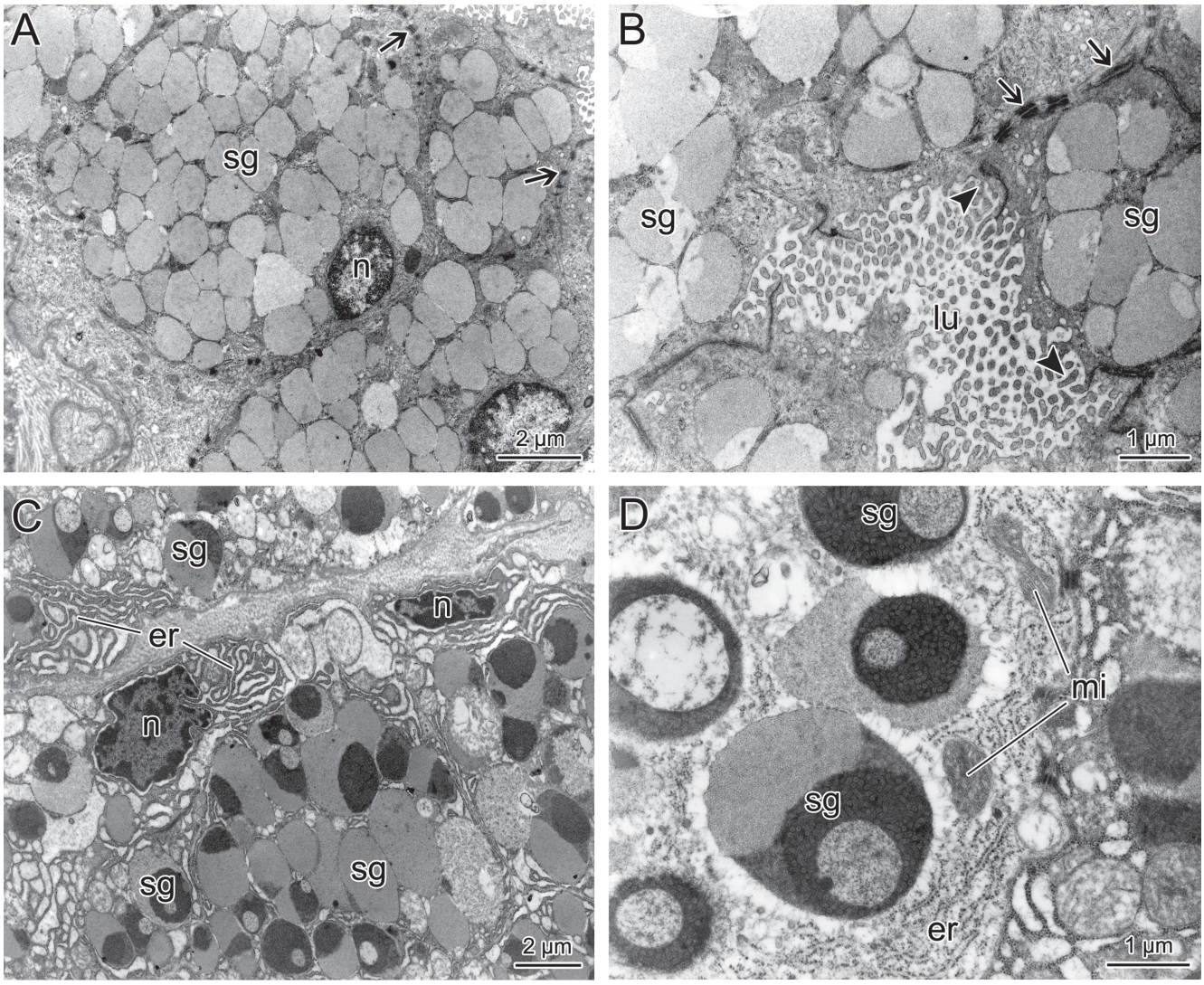

Figure 5. Transmission electron microscopy of the Duvernoy's gland of *H. modestus.* **(A)** General nature of the columnar ductal cells, with a cytoplasm packed with low electron-dense heterogeneous granules. Arrows indicate desmosomes. **(B)** Higher magnification of columnar cells showing microvilli (arrowheads) in the apical membrane. The lumen is filled with granules of low electron density and homogeneous structure. Arrows indicate desmosomes. **(C)** Secretory cells of the acini showing the more prominent endoplasmic reticulum, mainly in the regions around the nuclei. The cytoplasm is replete with secretory granules, some of which have electron-dense heterogeneous cores. **(D)** Higher magnification of the previous figure showing the distinction of electron-densities within the granules. *Abbreviations:* er, endoplasmic reticulum; lu, lumen; mi, mitochondria; n, nuclei; sg, secretory granules.

Table 1. Descriptive statistics of histochemistry and acinar measurements of Duvernoy's glands in five species of Hydropsini.

	Histochemistry			Acinar diameter (μm)		
	PAS	**AB**	**BB**	**Range**	**Mean**	**SD**
Helicops angulatus	+	+*	+	31.56–78.99	47.78	9.79
Helicops carinicaudus	+	-	+	48.21–103.65	68.38	16.3
Helicops modestus	+	+*	+	23.84–54.80	38.66	7.3
Helicops hagmanni	+	-	+	44.34–92.82	65.04	10.7
Hydrops triangularis	+	-	+	36.38–129.93	73.57	27.46

Abbreviations: AB, alcian blue; BB, bromophenol blue; PAS, periodic acid Schiff.
* Cells restricted to the lining of the ducts.
(+) Positive to the reaction.
(-) Negative to the reaction.

Table 2. Differences in the times taken to subdue and swallowing *Geophagus brasiliensis* and *Xiphophorus* sp. fish prey species by *Helicops modestus*. ANCOVA analyses were performed using time in seconds with relative prey mass as the covariate.

	(Mean ± SD)	**Range**	***n***
	Subduing time (min:s)		
Total	5:27 ± 4:34	0:25–17:37	22
Geophagus brasiliensis	7:11 ± 5:01	1:45–17:37	12
Xiphophorus sp.	3:21 ± 2:59	0:25–8:30	10
	ANCOVA, F = 1.906, df = 1, *P* = 0.18.		
	Swallowing time (min:s)		
Total	16:29 ± 16:08	0:36–45:20	20
Geophagus brasiliensis	27:5 ± 15:03	7:00–45:20	10
Xiphophorus sp.	5:14 ± 6:67	0:36–17:53	10
	ANCOVA, F = 12.84, df = 1, *P* = 0.002.		

immobilized it was released, either dead (*n* = 5) or alive (*n* = 3). All episodes of ingestion began headfirst, with the fish either dead (*n* = 5; 23%) or alive (*n* = 17; 77%, as evidenced by opercular movements) but always immobilized. The mean swallowing time of *H. modestus* was 16:29 and differed between the two prey-types (Table 2).

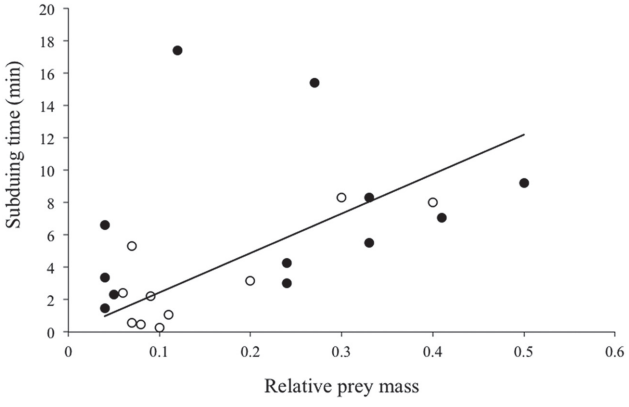

Figure 6. Regression between relative mass of *Helicops modestus* prey and the subduing time: the heavier the prey, the longer the time taken to immobilize it. Black dots: *Geophagus brasiliensis;* white dots: *Xiphophorus* sp.

DISCUSSION

Extensive variation in dentition size and morphology among rear-fanged snakes has been described, particularly within the subfamily Xenodontinae (Anthony, 1955; Vaeth et al., 1985; Kochva, 1987; Young and Kardong, 1996; Vidal et al., 2000). This has led to the present consensus that grouping taxa according to parameters such as aglyphous or opistoglyphous does not help the recognition of natural lineages. Several authors have even suggested abandoning the use of such terms altogether (Vidal et al., 2000; Fry et al., 2003), as we have done in this paper. Our results clearly demonstrated the presence of numerous, highly curved or recurved teeth in *Helicops modestus,* both characters regularly associated with piscivory (Savitzky, 1983). The rear fangs were also ungrooved, similar to the condition described by Mckinstry (1983). The presence of dental ridges and striations on the maxillary teeth has been associated with species that consume either fish or soft-bodied prey (Vaeth et al., 1985). Their main function is suggested to be to improve the grip, during manipulation and swallowing, on slippery prey items (Wright et al., 1979) such as fish, which form the majority of the diet of *H. modestus* (see Scartozzoni, 2010 and references therein). In terms of the distinction between posterior and anterior maxillary teeth, the location of ridges along the posterior and anterior surfaces of the former differentiated them from their presence on the labial and lingual surfaces of the latter, even when the diastema separating them was narrow (Jackson and Fritts, 1995; Vonk et al., 2008).

The proximity and position of the rear fangs in *Helicops modestus* form a deep groove that seems to serve the function of delivering the secretions from Duvernoy's glands to the oral cavity, as suggested by Taub (1967) and shown by Young et al. (2011: fig. 1b). The repeated protraction of maxillary bones by *H. modestus* while subduing prey is suggested to assist in the slow, passive but continuous delivery of secretion to the bite wound (Deufel and Cundall, 2006). Other morphological aspects in *Helicops,* such as its larger head (Avila et al., 2006), longer quadrate bones and a cranial rotation during jaw opening have

also been recorded in association with piscivory and are thought to enable species to better conform to the shape of the prey during swallowing, while simultaneously maximizing gape during jaw opening (Greene, 1997).

Duvernoy's glands frequently release their contents via 1–3 main ducts that deliver the secretion to the rear fang sheath (Smith and Bellairs, 1947; Taub, 1967; Kochva, 1978; Underwood, 1997; Fry et al., 2008). From there it flows into the mouth, as described for the colubrid *Boiga irregularis* (Zalisko and Kardong, 1992). In *Helicops modestus,* the process is similar: the internal net of ductules in Duvernoy's gland aggregate to form the common lobular ducts that collect and conduct the secretion to the vestibule and, from there, to the interior of the rear fang sheath. The relationship between the vestibule and the rear fang sheath seems to be involved in directing the secretion to the apical portion of the rear fangs. In contrast, the supralabial glands show a much simpler duct system, comprising a series of short ducts, each connected to the individual glandular units in the supralabial glands.

The Duvernoy's glands of *Helicops modestus* and the other species of Hydropsini analyzed here are similar to those described in most rear-fanged species able to kill their prey by envenomation (Taub, 1967; Lake and Trevor-Jones, 1996). Their secretory portions consist only of seromucous cells. In *H. modestus* as well as *H. angulatus* true mucous cells were present only within the lining of the duct wall, whereas in *H. carinicaudus, H. hagmanni* and *Hydrops triangularis* Duvernoy's glands were composed exclusively of seromucous cells without true mucous cells on the duct wall. Both the histochemical and ultrastructural analyses of the Duvernoy's glands of *H. modestus* revealed the presence of seromucous cells, which are characterized by granules with contents that have a range of (predominantly high) electron densities. The range of electron densities observed within the same secretory granule seems to be not commonly found in snakes. However, besides the fact that this structural pattern is consistent with histochemical results, where there is a different staining affinity in the same secretory granule, to date no interpretative hypothesis has been proposed to explain this pattern. Mucous cells, in contrast, have homogeneous and weakly electron-dense granules. On the other hand, the supralabial glands had acini composed of both mucous and seromucous cells and may, thus, be considered a true mixed gland (Taub, 1967; Gabe and Saint-Girons, 1969).

In contrast to front-fanged venom glands, which have sophisticated compressor musculature and an internal reservoir where venom is stored and then delivered under high pressure (Kochva, 1987; Gopalakrishnakone and Kochva, 1990), the liberation and delivery of the Duvernoy's gland secretion is not as well understood. Although *Helicops modestus* lacks any proper musculature or fibers attached directly to the capsule of Duvernoy's gland, this condition does not exclude the possibility of involvement of indirect muscle stimulation. Jansen and Foering (1983) proposed this scenario for *Thamnophis sirtalis* (Natricidae), as the *adductor mandibulae externus superficialis* muscle is greatly enlarged in this and other species of Hydropsini (Zaher et al., 2009). Internally, the Duvernoy's gland of *H. modestus* does not show a wide central reservoir for accumulation of the secretion. Rather, acinar spaces are reduced and the Duvernoy's gland secretion was seen within the ducts and the vestibule formed by the duct assemblage. Furthermore, the ultrastructure did not show myoepithelial cells in the Duvernoy's glands of *H. modestus,* which are usually present between the basal membrane and the secretory cell membrane, as is the case of the natricids *Rhabdophis tigrinus* and *Thamnophis elegrans vagrans* (Yoshie et al., 1982; Kardong and Luchtel, 1986).

The time spent subduing fish is positively correlated with the relative prey mass (Fig. 6), which was shown to be a general trend by Mori (1998) in homalopsids and the xenodontine *Alsophis* (= *Borikenophis*) by Rodríguez-Robles (1992) and Rodríguez-Robles and Leal (1993). The propensity (approx. 80%) for *Helicops modestus* to swallow the fish while still alive but clearly immobilized (determined by a reduction of the opercular movements), suggests prey quiescence/immobilization as a possible primary function of the Duvernoy's gland secretion. Indeed, previous laboratory tests (Albolea et al., 2000) demonstrated that once injected with the Duvernoy's gland secretion of *H. modestus* fish first showed a decrease in opercular activity, followed by a period of immobilization (approximately 30 min) and finally death. In contrast, mice injected with the secretion died approximately 10 min after injection (Albolea et al., 2000). Similar results were also observed in the Duvernoy's gland secretion of *H. angulatus,* whereby mice died 6–8 min after intraperitoneal injection (Estrella et al., 2011a). In the latter case, Estrella et al. (2011b) isolated and described the CRISP helicopsin, a channel toxin and/or excitatory neurotoxin. These results are consistent with a quiescence/immobilization role of the Duvernoy's gland secretion in *H. modestus.* The differences in the time of death between fish and mice may be explained by differences in their specific susceptibility to the venom of snakes that naturally prey on either group (Heatwole and Powell, 1998).

The repeated bilateral raking motions of both maxillae exhibited by *Helicops modestus,* especially when subduing larger prey, might facilitate delivery of the Duvernoy's gland secretion into the prey. It does not seem to facilitate swallowing, even if accompanied by a shift in the position of the bite (Jansen and Foehring, 1983; Rodríguez-Robles, 1992; Mori, 1998). Furthermore, our laboratory observations suggest that constriction might only be employed by juveniles in *H. modestus*. In nature, although feeding data are scarce, constriction has been observed once in an

uncollected *H. infrataeniatus* specimen of unknown sex and maturity when subduing frogs (Martins and Duarte, 2003) and another in an adult individual of *H. hagmanni* when subduing fish (Sturaro and Gomes, 2008).

Differences in the time *Helicops modestus* individuals took to swallow the two prey types are likely due to differences in prey dimensions, as found by Rodríguez-Roblez and Leal (1993) for *Alsophis* (*Borikenophis*). *Geophagus brasiliensis* is clearly wider (i.e., greater maximum distance across the transversal plane of the body) than *Xiphophorus* sp. The longer time it took to swallow *G. brasiliensis* could also be related to the presence of dorsal and anal-fin spines in this species (Kullander, 2003), which make ingestion more difficult, increasing the swallowing time.

Besides being a specialized piscivore (Scartozzoni, 2010), *Helicops* also secondarily exploits terrestrial prey (Martins and Oliveira, 1998; Aguiar and Di-Bernardo, 2004; Ávila et al., 2006), particularly frogs and lizards (Scartozzoni, 2010). In both situations, hunting behavior seems to be the same, with both the visual and chemical systems being used to locate prey. It is also typical of water snakes to ingest prey headfirst to eliminate the risk of damage to the digestive tract by the fin-spines (Sturaro and Gomes, 2008). Tail-first ingestion has only been observed in *H. infrataeniatus* and only when prey were very small or lacked spiny fins (Aguiar and Di-Bernardo, 2004). In most cases, *H. modestus* bit the head or middle of the body and held the prey until totally immobilized, presumably to ensure immobilization before swallowing and avoiding risking the prey escaping, as also described for other water snake species, including the homalopsid *Enhydris plumbea* (Mori, 1998) and the colubrid *Rhabdophis tigrinus* (Mori, 2006).

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APPENDIX

Specimens examined

Helicops modestus [Morphological study] (*n* = 7): BRAZIL: **São Paulo:** São Paulo, IBSP 67068; BRAZIL: **São Paulo:** Jarinu, IBSP 67691; BRAZIL: **São Paulo:** Mairinque, IBSP 71843; BRAZIL: **São Paulo:** São Roque, IBSP 76610; BRAZIL: **São Paulo:** São Roque, IBSP 76611; BRAZIL: **São Paulo:** Cotia, IBSP 76612; BRAZIL: **São Paulo:** Embú, IBSP 76613); [Prey-handling behavior] (*n* = 6): BRAZIL: **São Paulo:** Santana do Parnaíba, IBSP 67401; BRAZIL: **São Paulo:** Santana do Parnaíba, IBSP 66932; BRAZIL: **São Paulo:** Jaguariúna, IBSP 64077; BRAZIL: **São Paulo:** Amparo, IBSP 66931; BRAZIL: **São Paulo:** Nazaré Paulista, IBSP 66584; BRAZIL: **São Paulo:** Rio Claro, s/n, specimen from didactic collection of Butantan Institute destroyed by fire 15 May 2010.

Helicops angulatus (*n* = 2): BRAZIL: **Tocantins:** Porto Nacional, IBSP 66875; BRAZIL: **Tocantins:** Porto Nacional, IBSP 66878.

Helicops carinicaudus (*n* = 2): BRAZIL: **São Paulo:** MZUSP 22632; BRAZIL: **São Paulo,** s/n.

Helicops hagmanni (*n* = 1): BRAZIL: **Amazonas:** Miracatu - Jaú, Field number J58.

Hydrops triangularis (*n* = 2): BRAZIL, IBSP 68262; BRAZIL: **Tocantins:** Lajeado, IBSP 65821.