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Analyzing and comparing the buccal anatomy of European colubroid snakes: A reassessment of dentition models

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Abstract

Analyzing and comparing the buccal anatomy of European colubroid snakes: A reassessment of dentition models. Anatomical analyses reveal the presence of two rear-fanged models among European colubroids, categorized by different types of venom glands and rear maxillary fangs and differences in the features of the maxillary bones. The first model, opisthoglyphous, is characterized by the presence of purely serous venom glands, from which the secretions produced flow in the grooved posterior maxillary fangs. The posterior fangs are separated from the anterior ones by an alveolar diastema and a deviation of the maxillary bone. The second model, blade-fanged, has a venom gland composed of seromucous cells, anatomically positioned in a more recessed position compared to the opisthoglyphous model. In this model the posterior maxillary fangs are enlarged but have distal and mesial keels instead of grooves, but are not separated from the anterior teeth by diastemas or deviations of the maxillary bone. The European group of colubroids, previously composed almost exclusively of snakes considered "aglyphous," must be reconsidered in light of a system that consists mostly of opisthoglyphous snakes (predominantly the large group of Whip snakes, in addition to some species of Colubridae and Lamprophiidae), "aglyphous" snakes (exclusively Colubridae, including Ratsnakes and Dwarf snakes), and blade-fanged snakes (Natricidae, including Grass snakes and Water snakes). This reassessment is justified and supported by a comparative analysis of various European and non-European species, which confirms that "aglyphous" colubrids are residual forms of a rear-fanged model.

Keywords: Blade-fanged, Opisthoglyphous, Rear-fanged, Serpentes, Venom glands.

Resumo

Analisando e comparando a anatomia bucal de serpentes colubróides europeias: uma reavaliação dos modelos de dentição. Análises anatômicas revelam a presença de dois modelos com presas posteriores entre os colubróides europeus, categorizados por diferentes tipos de glândulas de veneno e presas maxilares posteriores e diferenças nas características dos ossos maxilares. O primeiro modelo, opistóglifo, é caracterizado pela presença de glândulas de veneno puramente serosas, das quais as secreções produzidas fluem nas presas maxilares posteriores sulcadas. As presas

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posteriores são separadas das anteriores por um diastema alveolar e um desvio do osso maxilar. O segundo modelo, presas em forma lâminas, apresenta uma glândula de veneno composta de células seromucosas, anatomicamente posicionadas em uma posição mais recuada em comparação ao modelo opistóglifo. Neste modelo, as presas maxilares posteriores são aumentadas, mas têm quilhas distais e mesiais em vez de sulcos, mas não são separadas dos dentes anteriores por diastemas ou desvios do osso maxilar. O grupo europeu de colubróides, anteriormente composto quase exclusivamente por serpentes consideradas "áglifas", deve ser reconsiderado à luz de um sistema que consiste principalmente de serpentes opistóglifas (predominantemente o grande grupo de cobras-chicote, além de algumas espécies de Colubridae and Lamprophiidae), serpentes "áglifas» (exclusivamente Colubridae, incluindo Cobras-rato e Cobras-anãs) e serpentes com presas em forma de lâminas (Natricidae, incluindo cobras-da-grama e cobras-d'água). Esta reavaliação é justificada e apoiada por uma análise comparativa de várias espécies europeias e não-europeias, o que confirma que os Colubridae «áglifos» são formas residuais de um modelo com presas posteriores.

Palavras-chave: Glândulas de veneno, Opistóglifa, Presas em forma de lâmina, Presas posteriores, Serpentes.

Introduction

To date, most of the European colubroid species have been considered "aglyphous," that is, equipped with non-grooved teeth (aglyphous = without glyphs), and consequently lacking structures suitable for the inoculation of venom during a bite (Kreiner 2007, Sindaco *et al.* 2013). The remaining small number of these snakes (5 of at least 37 species of colubroids) is considered "opistoglyphous," i.e. having furrowed or grooved teeth suitable for conveying mixed oral secretions. As indicated by the word itself (opisthoglyphous = glyphs behind), these traits occur mainly in the posterior area of the maxillae of these snakes, and in some cases in a more advanced position, but never in the anterior area, as happens in proteroglyphous (Elapidae) and solenoglyphous (Viperidae) snakes (Weinstein *et al*. 2011). The grooved teeth alone, however, are not sufficient to determine the opisthoglyphous nature of a species. In fact, snakes that share this condition have a venom gland located beneath the supralabial scales, known as "Duvernoy's gland." This gland is named after the French anatomist George Louis Duvernoy, who first described it in 1832 (Taub 1966, 1967). Within this category of ophidians, the morphology of the teeth, the posterior maxillary fangs, and the structure of the maxillary bones vary in size and composition. In some species these traits can barely be seen, whereas in others the structures are more defined and developed, leading to the possibility that the bite of certain species can cause clinical consequences in humans that may vary from mild localized symptoms to death (Weinstein *et al*. 2011).

Recent studies have demonstrated that a group of snakes, including whip snakes and racers of the western Palearctic region, that have been considered aglyphous are actually opisthoglyphous (Paterna 2023, Paterna and Grano 2024). This study investigated the buccal anatomy of various European colubroids currently considered aglyphous and opisthoglyphous and other species considered opisthoglyphous to verify whether these conditions are still attributable to these species.

Materials and Methods

An adult male *Hierophis viridiflavus carbonarius* (Lacépède, 1789), an adult male *Zamenis longissimus* (Laurenti, 1768), and two adult females *Natrix helvetica* (Linnaeus, 1758), all recent road-killed specimens, were collected in the province of Teramo, Abruzzo, Italy. The four specimens, all in excellent condition, were transported to OPHIS Museo Paleontologico e Centro Erpetologico, where dissection and specimen preparation were carried out. Dissection was performed with surgical tools, and specimen preparation used sodium hypochlorite. During the different steps of each phase, photographs were taken using a Nikon Coolpix P510 camera. The prepared bones were subjected to microscopy at the laboratories of the Faculty of Veterinary Medicine of the University of Teramo, using a Nikon SMZ1500 stereomicroscope. The anatomy of the palatomaxillary arches of different colubroid species and genera was examined in vivo and specimens were compared with each other. Photographic details of the buccal anatomy of the examined individuals were acquired with the same camera model previously reported. *Elaphe quatuorlineata* (Bonnaterre, 1790), *Hemorrhois hippocrepis* (Linnaeus, 1758), *Hierophis viridiflavus, Natrix helvetica, Thamnophis marcianus* (Baird and Girard, 1853), *Zamenis longissimus,* and *Zamenis scalaris* (Schinz, 1822) were photographed at OPHIS, while specimens of *Boiga dendrophila divergens* (Boie, 1827), *Coronella austriaca* (Laurenti, 1768), *Dolichophis caspius* (Gmelin, 1789), *Hemorrhois ravergieri* (Ménétries, 1832), *Heterodon nasicus* (Baird and Girard, 1852), *Philodryas baroni* (Berg, 1895), and *Platyceps najadum* (Eichwald, 1831) were photographed in private collections.

Results

Anatomical Analysis

In *Hierophis viridiflavus* the venom gland is clearly distinguished from the supralabial salivary gland (Figure 1A). The venom gland consists of small pinkish lobules and occurs laterally from the posterior portion of the fifth supralabial to the entire seventh. Dorsally this gland reaches the upper border of the supralabial scales, while ventrally it reaches the lower limit of such scales only in the region of the enlarged posterior maxillary teeth. This point corresponds to the lowest point ventrally reached by the lower edge of the supralabial scales (Figure 1A). The venom gland is located immediately beneath the supralabial scales and the wall of connective tissue that covers it. Ventromedially the gland is in contact with the cuff/sheath that covers the posterior maxillary teeth (Paterna 2023). Medially, the gland is in contact with the lateral wall of the maxillary bone, where, during one of the sample preparation steps, it was possible to preserve the venom gland to observe its medial extension (Figure 1B). The posterior extension reaches the posterior limit of the maxillary bone and culminates at the adductor *mandibulae externus profondus.* Anteriorly, the most voluminous portion of the gland culminates at the alveolar diastema and slightly more anteriorly with the lateral nutritive foramen, while the most anterior limit reaches the space between the last and the penultimate anterior tooth. As previously described (Paterna 2023), in addition to the posterior teeth, these anterior teeth also have grooves (Figure 1B, C). The traits observed agree with the histological analysis of Taub (1967) in which, regarding the group in which the former genus *Coluber* (actually *Hemorrhois*) is included, it is stated: "the supralabial and Duvernoy's glands are easily distinguishable in section, as they are composed of two markedly different types of cells. Duvernoy's glands are encased in a capsule of connective tissue and are composed primarily of serous cells. There is a small lumen in the center of each tubule or cord, a lumen that generally leads into a secondary collecting duct. These secondary ducts join to form a collecting or primary duct, which is lined with a mucous epithelium and leads into the mucous epithelial sheath of the posterior maxillary teeth."

In *Natrix helvetica* the venom gland is relatively larger than that present in *H. viridiflavus.* This gland is developed laterally and formed of white/cream lobules (Figure 2A), analogous to that described in *Natrix tessellata* (Akat *et al*. 2011). Compared to *H. viridiflavus,* this gland is less distinguishable from the

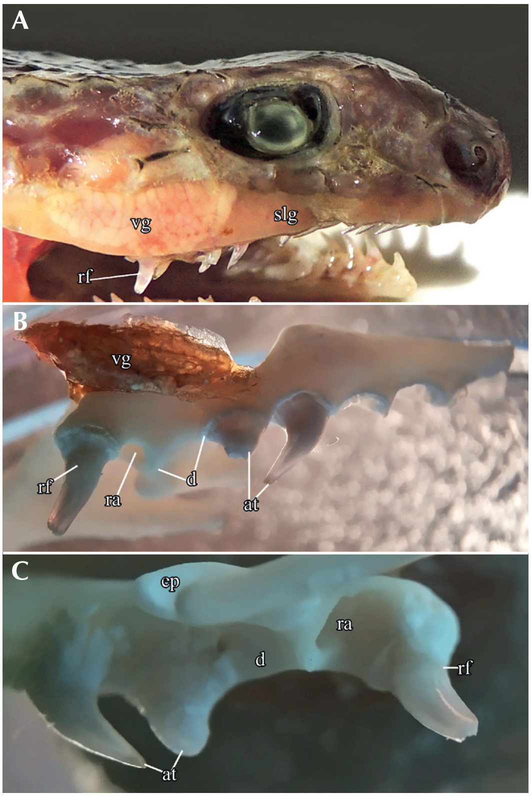

Figure 1. Adult male *Hierophis viridiflavus carbonarius* from Abruzzo, Italy. (**A**) Dissected head in right lateral view. (**B**) Prepared right maxilla in lateral view. (**C**) Prepared right maxilla in medial view. Abbreviations: at, anterior maxillary teeth; d, diastema; ep, ectopterygoid process; slg, supralabial salivary gland; ra, rear alveolus; rf, rear fang; vg, venom gland.

anterior supralabial salivary glands, which have a lobular structure of similar color. The venom gland is located below the sixth, seventh, and eighth supralabials, which delimit the gland extension both anteroposteriorly and dorsalventrally. In contrast to *H. viridiflavus,* the center of this gland is not found concomitantly with the enlarged posterior maxillary teeth, but these occur in the most anterior part of the gland (Figure 2B). These teeth do not have grooves, but they have a distal carina and a smaller mesial carina, which forms a blade shape. The anterior maxillary teeth are undulated, suitable for providing grip in holding viscous prey (Figure 2B; Paterna 2023). The venom gland is therefore located further back in this species and extends far beyond the posterior end of the maxillary bone and the adductor *mandibulae externus profondus*. Different from that observed in whip snakes, in *N. helvetica* the cuffs/sheaths of the posterior maxillary teeth are not distinct in coloration from the surrounding mucosa. The lateral wall of these cuffs/sheaths is hemispherical, and clearly distinguishable due to a depression produced by the medial contact with the venom gland. The orifices for the rear fangs are visible.

As a comparison species, similarly to the previous study (Paterna 2023), a colubrid was chosen that is sympatric with European whip snakes and grass snakes, the Aesculapian Snake *Zamenis longissimus.* In lateral view of the prepared specimen, the venom gland is absent and the supralabial salivary glands are present; these glands track the size and extension of the maxillary bone (Figure 2C). At the posterior extremity of the maxilla, behind the last maxillary tooth, the salivary gland is more dorsoventrally compressed and a different color and consistency compared to the more anterior portion. This could conceivably be a remnant of a possible Duvernoy's gland/venom gland. The last trait is identifiable in a deeper stage of preparation than the one illustrated, although it is visible in Figure 2C. This eventual remnant is positioned just below the Harderian gland, which is located posterior to the postorbital bone and adjacent to the temporal scales (Figure 2C). The supralabial salivary glands, together with the infralabial ones, have the function of lubricating the prey during swallowing through the secretion of mucus (Kochva 1978). In this species ("aglyphous") the reverse condition of the opisthoglyphous model is notable, whereby the anterior maxillary teeth are longer than the posterior ones (Figure 2C; Paterna 2023).

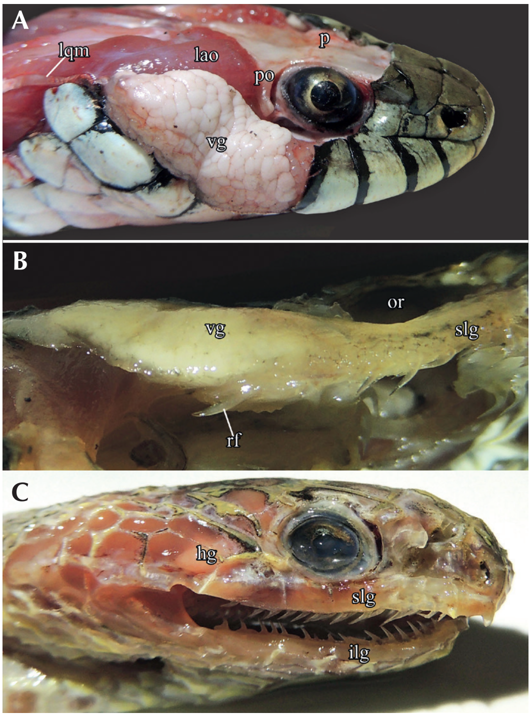

Figure 2. Adult specimens of *Natrix helvetica* and *Zamenis longissimus* from Abruzzo, Italy. (**A**) *Natrix helvetica* dissected head in right lateral view. (**B**) *Natrix helvetica* dissected head in ventrolateral view. (**C**) *Zamenis longissimus* dissected head in right lateral view. Abbreviations: hg, Harderian gland; ilg infralabial salivary gland; lao, levator anguli oris; lqm, ligamentum quadrato-maxillare; or, orbit; p, parietal bone; po, postorbital bone; rf, rear fang; slg, supralabial salivary gland; vg, venom gland.

Palatomaxillary Arches

Of the three species discussed, the palatomaxillary arches were examined in both dead and in vivo specimens, with the aim of comparing them with other species of European and non-European aglyphous and rear-fanged colubroids (Figure 3). In the material examined, in the palatomaxillary arch of *H. viridiflavus* (Figure 3A) the cuffs/sheaths that medially extend from the venom glands and cover the posterior maxillary teeth are easily visible and distinguishable (Figure 3B). This trait is also observable in other inspected opistoglyphous species, such as the European whip snakes *Platyceps najadum, Hemorrhois hippocrepis, Hemorrhois ravergieri,* and *Dolichophis caspius* (Figure 3E–H), and the extra-European opistoglyphous *Boiga dendrophila, Philodryas baroni,* and the rear-fanged *Heterodon nasicus* (Figure I–K). Cuffs/sheaths covering the enlarged maxillary posterior teeth are present in *N. helvetica* (Figure 3C), but less visible than the ones found in whip snakes (Figure 3D) and in the American natricid *Thamnophis marcianus,* where instead these are more distinguishable (Figure 3L). Consequently, the palatomaxillary arches of this European natricid appear similar, at first sight, to those of the sympatric aglyphous colubrids such as *Elaphe quatuorlineata, Z. longissimus,* and *Zamenis scalaris* (Figure 3M–O), as no color variations highlight the cuffs/sheaths. The substantial difference is that in the natricids the lateromedial space between the walls of the supralabials and the maxillary bones is filled by the venom gland (Figure 3), while in ratsnakes this is hollow, generating a large longitudinal canal that occurs between the maxilla and the posterior supralabial scales (Figure 3M–O). Furthermore, in aglyphous colubrids, the posterior maxillary teeth are uncovered and visible, lacking the structures present in the other analyzed groups (Figure 3 M–O).

In the analysis of the palatal arches of another European colubrid, *Coronella austriaca,* the presence of enlarged posterior maxillary teeth covered by cuffs/sheaths that morphologically resemble those previously described is observable. Analogous to *N. helvetica,* these structures show the same color as the surrounding mucosa, and the openings from which the posterior maxillary teeth unsheathe are observable (Figure 3P).

As control tests, other colubrids from the Old World, including *Elaphe anomala, Elaphe*

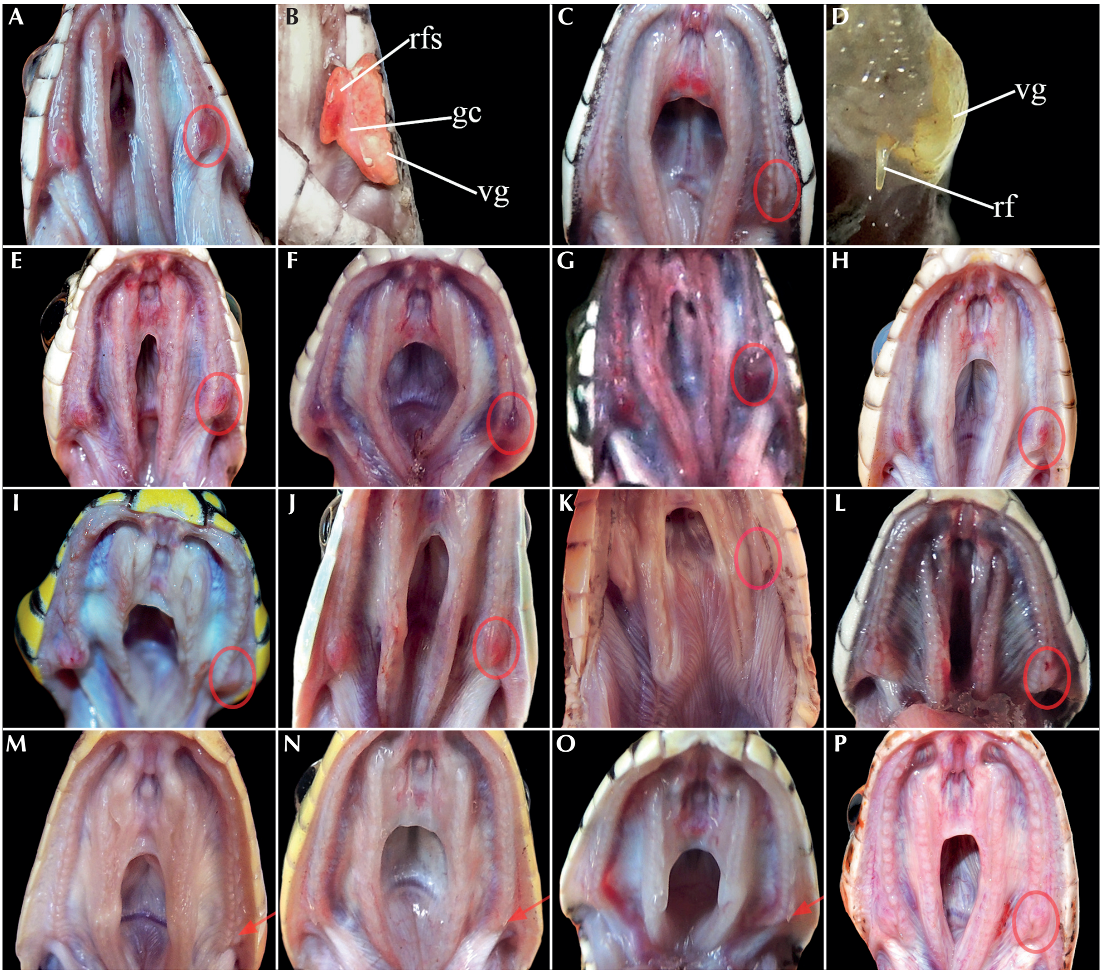

Figure 3. Analogies and differences among the palatomaxillary arches of aglyphous, opisthoglyphous, and blade-fanged colubroids. From the top, first horizontal row: (**A**) *Hierophis viridiflavus carbonarius*, (**B**) *Hierophis viridiflavus carbonarius*, dissected right maxilla in ventral view, (**C**) *Natrix helvetica*, (**D**) *Natrix helvetica*, dissected right maxilla in ventral view. Second row, European whipsnakes: (**E**) *Platyceps najadum*, (**F**) *Hemorrhois hippocrepis*, (**G**) *Hemorrhois ravergieri*, (**H**) *Dolichophis cascpius*. Third row, extra-European rear-fanged snakes: (**I**) *Boiga dendrophila divergens*, (**J**) *Philodryas baroni*, (**K**), *Heterodon nasicus,* (**L**) *Thamnophis marcianus*. Fourth row, ratsnakes and the smooth snake: (**M**) *Elaphe quatuorlineata*, (**N**) *Zamenis longissimus*, (**O**) *Zamenis scalaris*, (**P**) *Coronella austriaca*. Red circles delimitate the sheaths of rear fangs, red arrows indicate the last (naked) maxillary tooth. Abbreviations: gc, connective tissue capsule of the gland; rf, rear fang; rfs, sheath of rear fangs; vg, venom gland.

bimaculata, Elaphe carinata, Elaphe dione, Orthriophis taeniurus friesi, Zamenis persicus, and from the New World, including *Pantherophis guttatus* and *Pituophis catenifer sayi,* were examined. In the palatomaxillary arches of the Old World species, observed in *E. quatuorlineata, Z. longissimus,* and *Z. scalaris,* there are no cuffs/sheaths in relation to the posterior maxillary teeth, much less the lateromedial thickening of the supralabial walls, while in the two New World species small cuffs/sheaths are present in the posterior margins of the maxillae, although less well defined than the whip snakes, grass snakes, and the other rear-fanged snakes analyzed.

Discussion

In addition to the presence of Duvernoy's glands/venom glands, several traits may be completely or partially shared in opisthoglyphous species. These are: 1) enlarged posterior maxillary teeth; 2) grooved posterior maxillary teeth; 3) blade-shaped posterior maxillary teeth; 4) alveolar diastema separating the posterior maxillary teeth from the anterior ones; 5) mediolateral or/and dorsoventral deviation of the maxillary bone corresponding to the alveolar diastema; and 6) presence of cuffs/sheaths covering the posterior maxillary teeth in the palatomaxillary arch (Table 1). A seventh trait, which does not affect European colubrids, is the extreme reduction of the jaw in which the number of teeth is considerably reduced, as in the case of *Dispholidus* (Kardong 1982). Deviations of the maxillae and diastemas that separate the posterior teeth from the anterior ones, as in the case of *Hierophis viridiflavus* and *Dolichophis* (Paterna 2023, Paterna and Grano 2024), are traits shared between opistoglyphous species (Weinstein *et al*. 2011) and not present in the aglyphous colubroid species, or in those in which only enlarged posterior maxillary teeth are present. Such a condition is documented in *Elaphe xiphodonta* (Qi *et al*. 2021) and in other species of Asian colubrids (Cundall and Irish 2008, Qi *et al*. 2021), many of which predominantly prey on anurans (Knox and Jackson 2010). The separation of the posterior maxillary teeth from the anterior ones is instead present in opisthoglyphous colubrids as in the case of *Boiga irregularis, Thrasops flavigularis, Thrasops jacksoni,* and *Philodryas baroni* (Broadley and Wallach 2002, Weinstein *et al*. 2011). The presence of grooves on the enlarged maxillary posterior teeth further justifies the role they have in the conveyance of the secretions of the venom glands. This trait does not seem to be a constant within the ophidians currently considered opisthoglyphous, where it may even be absent, as in the case of *Heterodon platirhinos, Rhabdophis subminiatus, Rhabdophis tigrinus, Helicops modestus,* and *Alsophis cantherigerus* (Mittleman and Goris 1974, Jackson 2003, Weinstein *et al*. 2011, Oliveira *et al*. 2016), where the term "rear-fanged" better fits these species. A further trait that does not represent a constant in opisthoglyphous snakes is the deviation of the maxillary bone, which repositions the enlarged and/or modified posterior teeth on a different plane than the anterior ones. This is the case of *Hydrodynastes gigas, Liophis breviceps, Helicops leopardinius,* and *Homalopsis buccata* (Knox and Jackson 2010). The occurrence of visible cuffs/sheaths in the posterior ends of the maxillae, as observed in the European whip snake/racers (*Hierophis, Hemorrhois, Platyceps,* and *Dolichophis*), it is a trait present in many species of opisthoglyphous colubroids such as the sympatric *Malpolon monspessulanus* and the extra-European species analyzed here: *Boiga dendrophila divergens, Philodryas baroni,* and *Heterodon nasicus* (Figure 3). The presence of this trait, obvious in whip snakes, and the absence of this trait in purely constrictor colubrids (Figure 3) represents yet another marked feature that firmly places these animals within the group of opisthoglyphous snakes. Together with the presence of these cuffs/sheaths, the presence of the venom gland/ Duvernoy's gland is notable, which fills the space occurring between these and the supralabial

Table 1. Variation of the presence of the opisthoglyphous traits within the three European colubroids examined and three allopatric opisthoglyphous snakes.

Species	Duvernoy's glands	Enlarged rear teeth	Grooved rear teeth	Blade rear teeth	Alveolar diastema	Deviation of the maxilla	Rear fangs' cuffs/sheats
Hierophis viridiflavus	•	•	•		•	•	•
Natrix helvetica	•	•		•			•
Zamenis longissimus							
Boiga dendrophila	•	•	•		•		•
Heterodon nasicus	•	•			•	•	•
Philodryas baroni	•	•	•		•	•	•

scales (Figure 3). In *Natrix helvetica* and *Coronella austriaca* the cuffs/sheaths are different and less evident from those of the whip snakes, in which the color is homogeneous with the surrounding mucosa, even if the bulging at the posterior margin of the maxillary bone and the bulking of the venom gland are distinguishable (Figure 3C, D, P).

No less worthy of the same consideration are the traits observed in *N. helvetica.* Unlike *H. viridiflavus* and the Western Palearctic clade of whip snake/racers, this species possesses enlarged posterior maxillary teeth, but these are characterized by large distal and a smaller mesial carinae, which confer a blade-like shape to the enlarged rear fangs (Paterna 2023). Although the maxillae of *N. helvetica* do not have many of the features typical of the opisthoglyphous colubroids (Table 1), such as alveolar diastemas and deviations (Paterna 2023), the large venom glands and the blade-like teeth still play a role in predation and the inoculation of mixed oral secretions (Jackson *et al*. 2016). In the past, some authors argued that in the species having a specialized diet of anurans, these teeth functioned to pierce the tegument of the prey to neutralize the anti-predatory mechanism by which many amphibians inflate to resist swallowing (Pope 1947, Edgren 1955, McAlister 1963). Today we know instead that these function to lacerate the prey's tegument and to introduce buccal secretions (Weinstein and Kardong 1994, Averill-Murray 2006). In defense of this last thesis, many natricids are today considered "mid-venomous" or "low toxic" (Weinstein *et al*. 2011). Some are also highly venomous to humans, as in the case of the genus *Rhabdophis,* in which the bite can have serious and even fatal consequences in humans (Nakayama *et al*. 1973, Ogawa and Sawai 1986, Kikuchi *et al*. 1987). In our case, analogous to the Old World whip snakes/racers (Paterna and Grano 2024), there are reports in the literature in which the clinical consequences following the bite of *Natrix natrix* (*sensu lato*) in humans (Gardner-Thorpe 1967, Satora 2004, Gläßer-Trobisch and Trobisch 2008) and in anurans are described (Delisle 1981). Similar cases are reported in the related American natricids of the genus *Thamnophis* (Vest 1981, Hayes and Hayes 1985, Gomez *et al*. 1994, Grenard 1994). Perry *et al*. (2018) discussed how the genes that encode protein composition of the venom of *Thamnophis sirtalis* "find their evolutionary origins in a rear-fanged venomous colubrid species," while other studies have been conducted on the mechanical action involving the emptying of the glands of Duvernoy present in this genus of snakes (Jansen and Foehring 1983, Kardong and Luchtel 1986).

Regarding the presence of Duvernoy's glands/venom glands in both *N. helvetica* and *H. viridiflavus,* information is provided in the previous literature. Taub (1967) analyzed 180 species from 120 genera of colubroids, which he divided into groups. One group consisted of colubrids that lack Duvernoy's gland, generally in the genera *Boaedon, Elaphe* (*sensu lato*), and *Lampropeltis*. The Aesculapian Snake *Z. longissimus* is included in this group. A second group contains colubroids that possess a Duvernoy's gland composed of mucous cells intermingled with serous cells. This group include the natricids *Thamnophis* and *Nerodia* (genera related to *Natrix*), and *Rhabdophis*. A third group consists of colubroids that have a purely serous Duvernoy's gland. Among the species examined are the European *Hemorrhois ravergieri* (Whip Snake of the same clade as *H. viridiflavus*), *Malpolon monspessulanus,* and *Telescopus fallax,* together with other non-European opisthoglyphous species of the genera *Boiga, Spalerosophis, Philodryas,* and *Psammophis* (more species belonging to this last group have been chosen to be part of the actual research). Together with the "officially considered" opisthoglyphous species, the smooth snake, *Coronella austriaca,* is included in this group (Taub 1967). The inclusion of the European species examined by Taub in this last group confirms the morphological and anatomical analogies observed in this study (Figure 1, 3) and the consideration of the opisthoglyphous model in whip snakes discussed in previous studies (Paterna 2023, Paterna and Grano 2024). Subsequently, many studies have analyzed these types of glands and their secretions in different species (Hill and Mackessy 2000, Mackessy 2002). Some of these studies provide photographs, for example Jackson *et al*. (2016), in which it is noted that the large serous-secreting venom gland of *M. monspessulanus* is morphologically similar to that observed in *H. viridiflavus* (Figure 1). The Montpellier snake *M. monspessulanus* has been considered the most venomous colubroid among European opisthoglyphous species. In addition, the cuffs/sheaths covering the posterior maxillary teeth in this species are similar to those found in *H. viridiflavus*. The morphology of the supralabial and infralabial glands of *H. viridiflavus* is described (Baccari *et al*. 2002), but no further references are made to other glands.

Regarding the genus *Natrix,* many authors state that the anatomy of these ophidians is unclear; in fact, Phisalix (1922) described the presence of Duvernoy's glands in *N. natrix,* while Smith and Bellairs (1947) reported the opposite. Akat *et al*. (2011) reconfirmed Taub's description, stating that the venom glands of *Natrix tessellata* consists of many small lobules mainly composed of mucous and seromucous acini. The authors also noted that Duvernoy's gland secretions of *Natrix tessellata* have toxic effects on prey. The glands were composed mainly of seromucous cells. and the authors compare them to those of known venomous ophidians, suggesting that further research on the serum secreted by these glands could reveal medical and pharmacological properties (Akat *et al*. 2011).

The smooth snake, *Coronella austriaca,* possesses venom glands of the same type as the opisthoglyphous snakes (Taub 1967, Kochva, 1978), and has enlarged posterior maxillary teeth covered by cuffs/sheaths visible in its palatomaxillary arch. This snake, together with the congeneric *C. girondica,* requires further anatomical investigation to establish whether both share this condition, and in which sub-group of the rear-fanged snakes these species belong. The genus *Coronella* separated from the macro-group of Old World "aglyphous" rat snakes (Taub 1967, Nagy *et al*. 2004, Figueroa *et al*. 2016), followed by New World rat snakes, of which *Pantehrophis guttatus* and *Pituophis catenifer sayi* have small cuffs/sheaths at the posterior end of the maxillae. Jackson *et al*. (2016) reported the presence of a small venom gland in *P. guttatus.* Although Old World rat snakes appear to lack modified fangs and venom glands, they have residual traits that can be traced back to these adaptations. The four-lined rat snake, *Elaphe quatuorlineata,* features a longitudinal groove and depressions in the posterior maxillary teeth (Paterna 2023), and *Z. longissimus* appears to have a small residual gland that may differ from the supralabial salivary gland. These features are unusual in a fully aglyphous model because they represent relics of a previous opisthoglyphous model. This would explain the different levels of adaptation phylogenetically related to colubrine snakes, in which the rear-fanged model is more conserved in *Coronella,* scarcely conserved in *Pantherophis* and *Pituophis,* and completely lost in *Elaphe* and *Zamenis.* Another case of loss of the opisthoglyphous model within European colubrids is that of the genus *Eirenis,* a group of dwarf snakes descended from whip snakes, and which, following miniaturization and a specialized diet, developed a type of aglyphous dentition suitable for the predation of arthropods (Çiçek and Mermer 2007, Rajabizadeh 2019, Paterna and Grano 2024). The "loss" of the opistglyphous model across entire genera within rear-fanged colubrids is not limited to the two cases just discussed. Examining the phylogenetic trees of this family (Figueroa *et al*. 2016) another striking case is that of the genus *Dasypeltis.* This genus of snakes is equipped with small rudimentary teeth, an adaptation to their specialized diet consisting of bird eggs, which derived from the opisthoglyphous snakes of the genera *Toxicodryas* and *Boiga.* The latter are highly developed rear-fanged snakes whose bite can cause clinical consequences in humans.

Conclusions

From the data and evidence obtained, it is clear how European whipsnake/racers came to share traits of the opisthoglyphous model. In some cases, these species even have more traits than other snakes currently considered to be opisthoglyphous. From anatomical analyses of the species examined, it is possible to generate guidelines for identification of the opisthoglyphous state of certain ophidians, even when this has not yet been verified or is currently poorly identified. The most explicit features are observable using dissection, bone preparation, and microscopy, but a preliminary evaluation can also be obtained without resorting to these practices. An initial identification of a possible opisthoglyphous model can be obtained by inspecting the head and jaws of a particular colubroid. Confirmed by examination of the palatomaxillary arches, the presence of cuffs/sheaths at the posterior margin of the maxillae is a trait that indicates the opistoglyphus model. The color and morphology of the mucosa covering the posterior maxillary teeth are easily distinguishable from the surrounding mucosa. A second feature that can be observed in the open jaws of opisthoglyphous colubroids is the thickness that occurs lateromedially between the posterior portion of the maxilla and the supralabial scales. In many opisthoglyphous species, the presence of the venom gland indicates a larger or more filled space than in the aglyphous species, where this transverse space is generally hollow (Figure 3). A third factor is not diagnostic on its own, but in some species could represent further confirmation following the observation of the first two traits, is the shape of the ventral margin of the supralabials. As observed in European whipsnakes/racers and non-European opisthoglyphous species, a distinctly wavy silhouette in the posterior supralabials (e.g., *Hemorrhois, Spalerosophis*) could correspond to the dorsoventral deviation of the maxillary bones, the presence of enlarged posterior teeth, and the venom gland. A fourth consideration, and not at all obvious, is whether cases of envenomation or clinical reactions in humans or animals occur following a snakebite.

Particular attention should be paid to colubroids such as *Natrix helvetica,* which presents fewer opistoglyphous traits than whip snakes/racers, but which nevertheless may have structures for the production and inoculation of toxins. Both by definition and for anatomical reasons, it would be wrong to consider these snakes opisthoglyphous, but because they are capable of inflicting toxic bites, they can be grouped into a different category of colubroids that could be called "blade-fanged." The distinction of this new category from the opisthoglyphous group might seem counterintuitive because the term "opisthoglyphous" is rarely used. Snakes in which these dentition models are present are generally referred to as "non-front-fanged" snakes (Weinstein *et al*. 2011, Jackson *et al*. 2016, Weinstein 2017). Furthermore, the use of the name "Duvernoy's glands" is not recommended because these structures are homologous to the venom glands of elapids and viperids (Kochva and Gans 1970, Weinstein and Kardong 1994, Fry *et al*. 2008). Because the toxic oral secretions produced by rear-fanged snakes are intended to subdue prey, these secretions are considered "venom" in all respects (Jackson *et al*. 2016), and "it is most incorrect to view colubrid venoms as imperfect venoms" (Mackessy 2002). The use of the terms "opisthoglyphous" for whip snakes/racers, and "blade-fanged" for natricids is, however, necessary in this work to distinguish the different morphology and biology of the taxa taken into consideration. Within the "non-front-fanged" snakes several variations of the "rear-fanged" model occur, including some cases in which the enlarged teeth are not positioned at the posterior end of the maxillae and the "aglyphous" snakes.

In light of this study and in the two previous works (Paterna 2023, Paterna and Grano 2024), we can group European colubroids into three main categories corresponding to the different dentiferous models: "opisthoglyphous, blade-fanged, and aglyphous." Contrary to the past, current estimates indicate that the group comprising the largest number of European species is that of the opisthoglypha, consisting of taxa of Colubridae and Lamprophiidae, followed by the "aglypha" composed exclusively of colubrids, and finally the blade-fanged group, composed of natricids (Table 2). It is interesting and at the same time an inevitable consequence to see how within the aglypha, the medium- to large-sized colubrids all belong to *Elaphe* and *Zamenis,* are all powerful constrictors, and are all phylogenetically related. In the past, all were placed within the former genus *Elaphe* (Fitzinger) (Schulz 1996). The term "aglyphous" has almost always been reported in quotation marks because

Table 2. Dentition models within European colubroids. Within "rear-fanged" are indicated those genera that need further investigation to determine in which sub-group these should be placed.

Group	Genus	Type	Past consideration
Whip snakes	*Hierophis*	opisthoglyphous	aglyphous
	Hemorrhois	opisthoglyphous	aglyphous
	Platyceps	opisthoglyphous	aglyphous
	Dolichophis	opisthoglyphous	aglyphous
Dwarf snakes	*Eirenis*	aglyphous	aglyphous
Smooth snakes	*Coronella*	rear-fanged	aglyphous
False smooth snakes	*Macroprotodon*	rear-fanged	opisthoglyphous
Catsnakes	*Telescopus*	opisthoglyphous	opisthoglyphous
Ratsnakes	*Elaphe*	aglyphous	aglyphous
	Zamenis	aglyphous	aglyphous
Grass/water snakes	*Natrix*	blade-fanged	aglyphous
Lamprophiids	*Malpolon*	opisthoglyphous	opisthoglyphous

following what has been discussed, its application to colubrids would become technically incorrect, since this word describes the dentiferous model of ophidians of other families (e.g., Boidae and Pythonidae), and no longer refers to European colubrid genera such as *Eirenis, Elaphe,* and *Zamenis,* which were part of an earlier opisthoglyphous model. Obviously, as happened for the ophidians analyzed in this study, in-depth analyses are necessary to verify certain conditions in species that are still unsuspected or little studied to date. Discovery of such conditions could lead to a further reclassification of these, and determine if a certain species is in the process of development or suppression of a rear-fanged model. Additionally, the evolution of venom glands is not parallel with the evolution of certain types of posterior maxillary fangs, and of the number and arrangement of these (Taub 1967). Within the species analyzed in this study, the "adaptation" to the different types of rear-fanged models present profound variations even within the same group or genus (e.g., Whipsnakes).

A final consideration is that being opisthoglyphous, blade-fanged, or rear-fanged (to indicate other intermediate groups) does not mean being toxic or harmful to humans. An opistoglyphous species does not necessarily have to cause effects in humans to be considered as such, but it must present certain anatomical characteristics. The structures seen in these reptiles have developed in colubroids as adaptations and specializations derived from the diet and the ecology of the latter (Modahl *et al.* 2016) in relation to predation and digestion of prey, as well as in proteroglyphous and solenoglyphous snakes. The case of European colubroids confirms the thesis that the rear fangs (venom fangs) did not evolve independently multiple times but find their origin in common ancestors (Palci *et al*. 2021), and loss of fangs is a recurring trend within this superfamily (Westeen *et al*. 2020). Fangs and venom glands developed prior to the appearance of colubroids (Fry *et al*. 2009, Figuero *et al*. 2016, Streicher and Ruane 2018, Zaher *et al*. 2019), whose most basal species alive today are opisthoglyphous, which has subsequently differentiated within the several groups that have preserved, developed, or lost various traits. The key model used in this study can be applied to reclassify colubroids in other regions of the world that have a similar ophidiofauna.

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