The evolution of venom-delivery systems in snakes

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The Colubroidea represents approximately 2300 of the 2700 species of living snakes and includes all venomous taxa. Although many morphological studies of colubroid snakes have been carried over the last hundred years, the phylogenetic relationships within this group are poorly known. In this study, components of the venom-delivery system (VDS) were examined within the context of two conflicting phylogenetic hypotheses proposed in 1988 by Cadle and in 1998 by Kraus & Braun. The results suggest that several major morphological changes occurred early in colubroid evolution: a Duvernoy's gland evolved, the posterior maxillary teeth became specialized relative to the anterior maxillary teeth, and the attachment of the pterygoideus muscle moved forward to a position associated with the posterior maxillary teeth. These innovations may have allowed the great radiation of colubroid snakes that led to the Colubroidea representing such a large percentage of living snakes. More recently, three separate lineages of colubroids have independently evolved highly specialized front-fanged VDSs with large and complex venom glands, venom gland compressor muscles, and tubular fangs.   © 2003 The Linnean Society of London, *Zoological Journal of the Linnean Society,* 2003, **137**, 337–354.

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INTRODUCTION

Venom-delivery systems (VDSs) have evolved independently several times among vertebrates. The earliest known example is a hollow fang from a Triassic 'reptile' of uncertain taxonomic affiliation (Sues, 1991) Among living vertebrates, VDSs are found in mammals and squamates. Mammalian VDSs include mildly toxic salivary secretions associated with grooved teeth in some insectivores (Vaughan, 1986) and the bizarre crural VDS of the platypus (Grant, 1989). Among squamates, helodermatid lizards have mildly toxic salivary secretions and grooved teeth (Kochva, 1978). Snakes, however, are the masters of VDSs; no other group approaches them in terms of sophistication, efficiency, and diversity.

The present study aims to integrate existing morphological data on the VDS accumulated by numerous authors over the last 100 years with my own observations. The combined data are examined within the context of two recent conflicting phylogenetic hypotheses of colubroid relationships, in an attempt to find

out whether they can reveal the same story about the evolution of characters of the VDS. Morphological components include: (1) the venom and Duvernoy's glands, (2) the muscles associated with the VDS, and (3) the fangs and maxillary dentition. The two recent studies are Cadle's (1988) hypothesis of colubroid relationships and Kraus & Brown's (1998) transversion parsimony tree. The rationale behind their selection is explained below. Recognizing that no consensus on the phylogeny of colubroid snakes yet exists, every effort has been made to present results in such a way as to allow reanalysis of the data as new phylogenies become available in the future.

The ophidian VDS has been the subject of study by comparative anatomists since the late 1500s (reviewed by Russell, 1983). More recently, evolutionary biologists have studied the question of how the VDS might have evolved (Bogert, 1943; Anthony, 1955; Haas, 1962; Kardong, 1980; Kochva, 1987), and of what selective forces might have been involved (Kardong, 1996; Savitzky, 1980). In addition, there has been considerable interest in the mechanisms of action of snake venoms, the study of which has made important contributions to medical research (Tu, 1991). The question of how the VDSs of differ-

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ent groups of snakes evolved, however, remains unanswered.

Of the c. 2700 recognized species of living snakes, c. 450 are considered venomous. All venomous taxa occur within the large (2300 species) and morphologically and ecologically diverse clade Colubroidea ('advanced snakes'; Fig. 1), in which venomousness is thought to have evolved independently several times. Just how many times, and under what circumstances, are the subject of this study.

Traditionally, three taxa were recognized within Colubroidea: (1) Viperidae, venomous snakes with a moveable maxilla and tubular front fang unit; (2) Elapidae (taken to include Hydrophiidae, since Hydrophiidae and Elapidae together form a clade; McCarthy, 1985), venomous snakes with fixed, tubular front fangs; and (3) Colubridae (not to be confused with Colubroidea), a huge group (approximately 1800 species) of non- and semi-venomous snakes which may have enlarged or grooved posterior fangs. Following the work of Bourgeois (1961, 1968), a fourth group has been recognized, Atractaspididae, venomous snakes with moveable, tubular front fangs. The most important change, however, has been the recognition that although Viperidae, Elapidae, and Atractaspididae probably represent monophyletic groups, Colubridae certainly does not (see below). Because of the probable non-monophyly of Colubridae it is important to sample diverse colubrid subfamilies (e.g. Aparallactinae, Boodontinae, Colubrinae, Homalopsinae, Natricinae, Pareatinae, Xenoderminae, Xenodontinae), some of which probably represent monophyletic groups, in order to understand the evolution of the VDS.

The following terms are used to describe different types of dentition in colubroid snakes: (1) solenoglyphous (viperids and atractaspidids), in which the short-ened maxilla rotates so that the fang is effectively moveable; (2) proteroglyphous (elapids) in which the fang is fixed and at the front of the maxilla; (3) opisthoglyphous (some colubrids) in which there is a posterior fang which may be enlarged or grooved; (4) aglyphous (other colubrids) in which the posterior fang is unspecialized or nonexistent. (The term 'fang' will be used here to indicate any enlarged, grooved, or tubular tooth associated with a Duvernoy's gland or venom gland.) As these terms are used with slightly different meanings by different authors they are avoided here, except within the context of a historical review, in favour of brief, less ambiguous descriptions of the dentition.

COLUBROID PHYLOGENY

Knowledge of the phylogenetic relationships among the Colubroidea is essential for reconstructing the evolution of the VDS. Currently there is considerable uncertainty, although much progress has been made over the years.

Boulenger (1893) regarded elapid and viperid snakes as representing independent derivations of the front-fanged VDS: "The elapoids, long regarded as forming a passage from the Colubroids to the vipers, stand apart, the author considering the Viperine maxillary as derived from the opisthoglyphous type." Can we then assume that he thought the elapid fang was derived from an anterior tooth? It would be some years before anyone again thought of elapid snakes as anything other than an intermediate step between colubrids and viperids.

Cope (1900) considered the aglyphous colubroids to represent the most primitive colubroid condition. From this ancestral stock, he derived two separate lineages: the opisthoglyphs and the proteroglyphs. He derived the solenoglyphs from the latter.

On the basis of dentition and the structure of the fangs, Bogert (1943) concluded that venomousness had evolved only once in snakes, that all venomous colubroids were derived from a nonvenomous colubrid ancestor, that the first venomous snakes resembled elapids, and that these in turn gave rise to the more specialized dentition of the viperids.

Anthony (1955) used a series of measurements of the maxilla and morphological observations of the teeth and fangs of a large number of colubroid snakes to establish an elaborate reconstruction of the evolution of the venom system. His scheme placed living taxa in a morphocline, and his conclusion was that the venom systems of Elapidae and Viperidae had evolved independently. The elapids, he believed, were derived from a colubrid ancestor with enlarged anterior maxillary teeth which evolved into tubular fangs. The viperids, on the other hand, came from a colubrid ancestor with
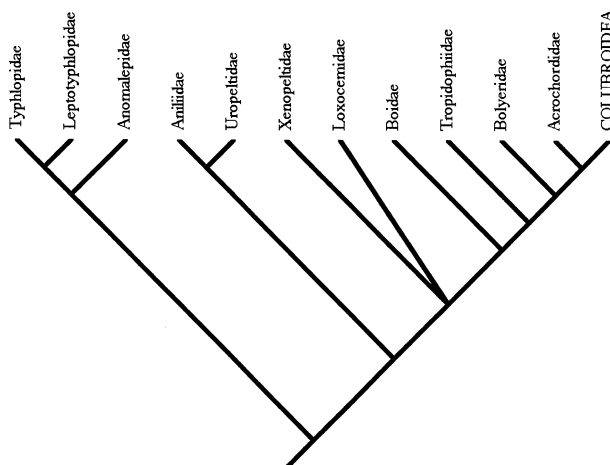

Figure 1. Phylogenetic relationships among living snakes (Pough *et al.*, 2001).

posterior fangs which migrated anteriorly along the maxilla, and evolved from grooved to tubular.

Bourgeois (1961, 1968) studied the morphology of the skull in a variety of African colubroids. She described the relationships among the Colubroidea as '*touffu*' ('bushy'), but recognized Elapidae and Viperidae as separate venomous lineages. She also recognized, on the basis of skull morphology, that *Atractaspis*, previously considered a viperid, represented an independent derivation of the solenoglyph venom system (a point discussed in detail below).

Underwood (1967) produced a classification of all snakes based on several morphological characters, including hard and soft tissues. Some were related to the VDS (e.g. maxillary teeth, adductor externus superficialis muscle) but most were not (e.g. vertebral hypapophyses, hemipenes, renal arteries, visual cells). In his analysis of the evolution of Caenophidia (= Colubroidea + Acrochordidae), he concluded that venom was acquired early in caenophidian history and was later lost in several aglyphous and opisthoglyphous lineages.

McDowell (1968) argued, on the basis of several morphological characters, that *Homoroselaps* (formerly *Elaps*) was not an elapid as had been thought previously, but rather an aparallactine. This would suggest another independent derivation of a front-fanged VDS. However, a later study showed that the characters that allied *Homoroselaps* with aparallactines are more likely derived characters associated with fossoriality (Kochva & Wollberg, 1970), and that *Homoroselaps* belongs with the elapids.

Marx & Rabb (1972; Rabb & Marx, 1973) undertook a quantitative phylogenetic analysis of colubroid snakes, using 50 morphological characters. The characters were those traditionally employed in herpetology such as scale counts and skull and dentitional characters. Surprisingly, they used Colubridae as their outgroup for determining the polarity of characters. They concluded that the elapid-hydrophiid line and the rear-fanged colubrid-viperid line represent parallel, i.e. separate, derivations of the front-fanged VDS.

Savitzky (1978) proposed on the basis of a number of morphological characters that the New World coral snakes, *Micrurus* and *Micruroides*, represent a derivation of the proteroglyph VDS separate from other elapids. However, this hypothesis has not been supported by subsequent immunological studies (Cadle, 1981, 1982).

Kardong (1980, 1982) argued, based on functional morphological studies of the maxilla of colubroid snakes and consideration of the postorbital position of the venom gland in front-fanged snakes, that the elapid and viperid front fang both evolved from an enlarged posterior fang in a colubrid ancestor.

McDowell (1986) argued, based on morphological features associated with the VDS, that elapids represent a basal branch of Colubroidea. For example, in elapids the anterior attachment of the pterygoideus muscle is on the ectopterygoid, similar to that of basal snakes. In contrast, in most other colubroids, the attachment is on the posterior end of the maxilla (McDowell, 1986).

Over the past two decades, molecular techniques have been used in an attempt to solve the key problem of resolving relationships among the colubroids. Cadle (1982) used immunological distance methods to determine the affinities of *Atractaspis* and to assess which colubroid group it is most closely related to. His results, while tentative, indicate that *Atractaspis* is not closely allied to Viperidae. It could possibly be a basal branch of Elapidae, but until further data are available Atractaspididae is considered a separate colubroid clade, forming a trichotomy with Elapidae and Colubridae.

In a larger study, Cadle (1988) (Fig. 2) used immunological comparisons to address three further questions about colubroid relationships. The first concerned whether Colubroidea is monophyletic. Using *Boa* as an outgroup, Cadle concluded that it is. The second question was to determine relationships among the major colubroid lineages. Here, viperids were found to be basal relative to the other colubroids included in the study (elapids, atractaspidids, colubrines, and xenodontines). Beyond that, however, the results were less certain, and Elapidae, Colubridae, and *Atractaspis* formed an unresolved trichotomy. The third question addressed the relationships among colubrid lineages, an issue discussed below.

Knight & Mindell (1994) used DNA sequence data to determine relationships among Viperidae, Elapidae, and Colubridae. Using *Boa* as an outgroup, and including only a single representative of each group in the study, they reached the same conclusion as Cadle (1988), with Elapidae and Colubridae (represented by the colubrine, *Coluber*) grouping together, and Viperidae as their sister group.

Dessauer *et al*. (1987) compared proteins to determine relationships among ophidian higher taxa. Their results indicated that Colubroidea is monophyletic, and that Viperidae is the sister group to the others (Elapidae, Colubridae, and *Atractaspis*).

One limitation of all these studies is that they rely on a small number of colubrid taxa. If, as seems likely, Colubridae is not a monophyletic group relative to Elapidae, Viperidae, and *Atractaspis*, but rather made up of several lineages, then a very different conclusion may be reached concerning the relationships among the colubroid groups, depending on which taxa are used as 'representatives' of the Colubridae. For example, Cadle (1982) used four colubrids, all of which were
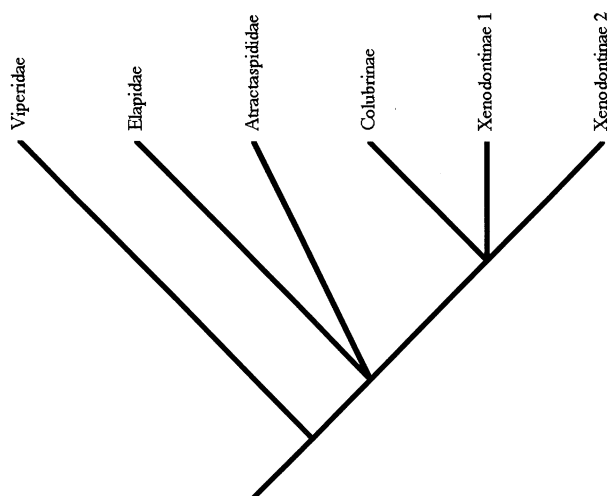
Figure 2. Relationships among colubroid snakes proposed by Cadle (1988).

xenodontines. In his 1988 study, he expanded the number of taxa; in addition to elapids, viperids and *Atractaspis*, several colubrid taxa were included. These fell into three lineages: South American xenodontines, Central American xenodontines, and colubrines; together, these groups were monophyletic relative to other colubroids. Knight & Mindell (1994) used only one specimen of one species for each of the three families that they examined. Their colubrid was the colubrine, *Coluber*. Thus, although their results may correctly indicate the position of *Coluber* relative to elapids and viperids, it is unlikely that it would hold true for all members of the probably paraphyletic Colubridae.

Building on these results, more recent studies have recognized the importance of determining the relationships among colubrid lineages. Cadle (1994) again used immunological distance methods to examine the relationships among African colubrids. A large number of taxa were included in this study. However, Viperidae was used as an outgroup, based on the fact that previous studies had shown the family to be an outgroup to all colubroids previously examined, though not all those included in the study. The result was a large polytomy comprised of Elapidae, *Atractaspis*, and five colubrid lineages.

Heise *et al.* (1995) used sequence data to determine the relationships among major groups of snakes, including Caenophidia. Their results in many ways confirmed those of previous molecular studies. For example, Colubroidea was found to be monophyletic; Viperidae and Elapidae were each found to be monophyletic, with viperids basal to elapids and colubrids,

and colubrids were found to be probably paraphyletic. Of the colubrids included in the study, Colubrinae was found to be monophyletic, as were the xenodontines, although the latter were found to comprise at least two lineages. Interestingly, the one homalopsine included in the study ended up in a trichotomy with the viperids and the clade made up of elapids and other colubrids, suggesting that homalopsines may belong in a basal position relative to other colubrids. However, the methodology of this study was criticized by Kraus & Brown (1998).

Kraus & Brown (1998) (Fig. 3) used DNA sequence data from a large number of taxa to determine which higher-level colubroid taxa are monophyletic and establish the relationships among the groups. Several monophyletic colubroid groups were identified, including Viperidae, Elapidae, Thamnophiinae, Pareatinae, Homalopsinae, and Xenoderminae. Other groups that were probably also monophyletic, but less strongly supported, were Xenodontinae, Colubrinae, and Boodontinae. Establishing the relationships among the groups however, proved to be inconclusive. Two methods of analysis produced quite different trees; the authors favoured the one produced using transversion parsimony. Their most interesting findings were that *Atractaspis* grouped with aparallactines, and that Xenoderminae was found to occupy a basal position within Colubroidea, or possibly to belong to Acrochordidae, outside Colubroidea.

Vidal *et al.* (2000) used DNA sequence data to study the relationships among xenodontines. While not directly relevant to determining relationships among colubroid higher taxa, their results confirm the probable monophyly, proposed by other authors, of the Colubrinae and Xenodontinae.

Gravlund (2001) compared mitochondrial sequence data from 43 species of colubroid snakes to identify monophyletic groups and determine the relationships among them. The result was a trichotomy. The first clade was Psammophiinae, while the second included Elapidae, *Atractaspis*, and some xenodontines (an unusual grouping, only weakly supported). The third clade included Viperidae, *Enhydris*, a homalopsine, and all other Colubridae. Perhaps the most surprising result from this study is that Viperidae was not found to be a basal branch of Colubroidea (i.e. the sister-group to the Elapidae and Colubridae).

Most recently, Slowinski & Lawson (2002) produced a phylogeny of snakes using nuclear and mitochondrial genes. They included several colubroid taxa in their study, and confirmed the results of other recent studies, finding that acrochordids are the sister group to colubroids, that viperids are basal among colubroids, and that the Natricinae are paraphyletic. They included one aparallactine in their study but unfortunately no atractaspidids.
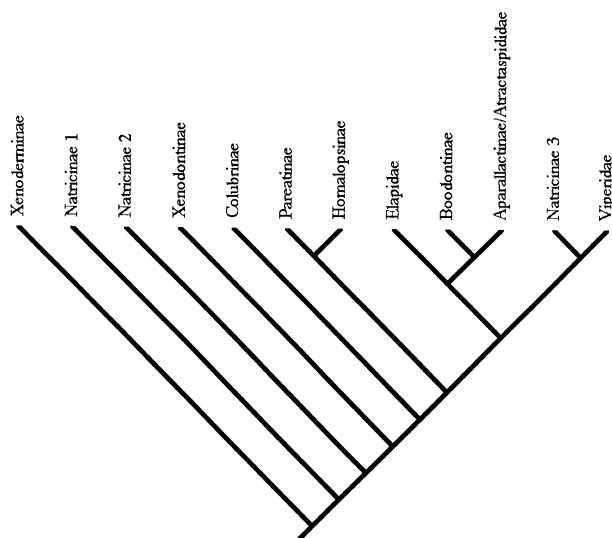
Figure 3. Relationships among colubroid snakes proposed by Kraus & Brown (1998).

METHODS

Morphological data relating to components of the VDS were considered within the context of two phylogenetic hypotheses of colubroid relationships, those of Cadle (1988) and Kraus & Brown (1998), selected because they included a large number of colubroid taxa and were the most methodologically sound of all the phylogenies considered. Character evolution was traced using MacClade version 3.05. Morphological information about venom glands, Duvernoy's glands, and about the muscles associated with the VDS was obtained from the literature. Observations of fangs were taken from Jackson & Fritts (1995) and Jackson (2002), and new observations were made of the teeth of dried skeletonized museum specimens, using light microscopy.

GLANDS

A large number and wide variety of glands are present in the heads of reptiles (see reviews by Gabe & Saint Girons, 1969 and Kochva, 1978; those of snakes specifically are reviewed by Taub, 1966). The ophidian cephalic glands of greatest relevance to the VDS are the venom glands of viperid, elapid, and atractaspidid snakes, and the Duvernoy's glands of other colubroids. These glands are present only in colubroid snakes, but not all colubroid snakes possess them (Taub, 1966).

One of the earliest accurate accounts of the venom gland was included in Fontana's (1781) description of that of the viper. Duvernoy (1832) described a 'less perfect' venom gland in colubrid snakes. The term 'Duvernoy's gland' was coined by Taub (1966) to refer

to the venom gland of colubrid snakes, in order to avoid the confusion generated by earlier terms. The currently favoured terminology is 'venom gland' for the venom glands of viperids, elapids, and atractaspidids, and 'Duvernoy's gland' for the venom glands of all other colubroids. One weakness of this terminology is that it implies homology of all venom glands. Another is its implicit suggestion about their function and medical importance, as some colubrids, such as *Dispholidus* and *Thelotornis*, have 'Duvernoy's glands' but are capable of inflicting lethal bites on humans.

Both Duvernoy's and venom glands are innervated by the maxillary branch of the trigeminal nerve (V2) with contributions from the facial nerve (VII) (Kochva, 1965; Taub, 1966), and their vascular supply is from branches of the internal carotid artery (Phisalix, 1922; Kochva, 1965). There is strong developmental evidence that both originate from dental glands, which are present in most squamates (Kochva, 1978). Glands extend in a line along the colubroid upper jaw. The supralabial glands extend along the entire length of the upper jaw, whereas the venom or Duvernoy's glands lie further posterior. Reshef (1994) showed in the natricine, *Natrix tessellata*, that the maxillary dental lamina in the embryo extends along the entire length of the upper jaw, and has two parts. The anterior teeth arise from the anterior portion of the maxillary dental lamina, and the Duvernoy's gland and the posterior fang arise from the posterior portion. Earlier studies had already demonstrated that in both viperids (Kochva, 1963; Wollberg & Kochva, 1967) and in rear-fanged colubrids with grooved or ungrooved fangs, respectively (Kochva, 1965), the fang and the venom or Duvernoy's gland develop from a common primordium at the posterior end of the dental lamina (although in viperids the dental lamina is so short that is difficult to distinguish between an anterior and a posterior end). It is especially noteworthy that the venom gland and fang develop from a common primordium even for species in which the adult fang ends up at the anterior end of the mouth, with the gland posterior to the eye.

McDowell (1986) proposed that the venom gland of viperid, elapid, and atractaspidid snakes (as well as those of two colubrids) is not the homologue of the colubrid Duvernoy's gland, but rather is derived from the rictal gland present at the corner of the mouth of non-colubroid squamates. However, this hypothesis is refuted by the embryological evidence that both venom and Duvernoy's glands are derived from dental glands, and by the presence of a rictal gland, in addition to the venom gland, in several viperid, elapid, and atractaspidid snakes (Wollberg *et al.*, 1998).

The Duvernoy's gland of colubrid snakes was extensively surveyed, both grossly and histologically, by Taub (1967), whose study investigated 180 species

from 120 genera, including representatives of most colubrid subfamilies. In contrast to the more anteriorly positioned supralabial glands, which are made up entirely of mucous cells, Duvernoy's gland is positioned posterior to the eye, is encased in a usually thin cover of connective tissue, and consists mostly of serous cells. A single, short duct extends anteromedially from the lumen of the gland to the base of the posterior fangs. Beyond this general state, however, considerable variation was observed across taxa. Duvernoy's gland was absent in all xenodermines examined, and in some of the colubrines. It was also absent in one specimen of the xenodontine, *Farancia abacura*, but since it was found to be present in another specimen of the same species, it seems likely that the former represents an artifact, possibly resulting from poorly preserved museum material. Taub (1967) also found that it differed in overall size, size of lumen, thickness of capsule, and relative abundance of mucous vs. serous cells. Not surprisingly, the venomous colubrines *Dispholidus* and *Thelotornis* were found to have large, highly developed Duvernoy's glands, with thick capsules and some muscle fibre attachment.

Taub (1967) rejected the hypothesis that Duvernoy's gland had evolved only once and had been subsequently lost in some taxa, suggesting that it had evolved independently at least twice and possibly as many as four times. The hypothesis of a single derivation of Duvernoy's gland, he argued, would not adequately explain the condition seen in Xenoderminae, in which it is absent and the supralabial gland is made up of a mixture of mucous and serous cells (in contrast to other supralabial glands which are composed entirely of mucous cells).

Although they are all referred to as 'venom glands', and are all encased in a fibrous sheath of connective tissue allowing muscle attachment, the venom glands of viperids, elapids, and atractaspidids differ greatly in their structure. The viperid venom gland is large and generally triangular in shape, with the longest side of the triangle along the upper lip, the rounded apex directed dorsally. The main gland has a complex tubular structure and is divided into several lobules by infoldings of the outer sheath. The lumen is capacious and a large quantity of venom is stored in it. Anteriorly, the triangle comes to a point, as the lumen becomes the primary duct. This duct passes through the mainly mucous accessory gland (the main gland is serous), leaving it as the secondary duct which extends to the sheath of the fangs (Kochva & Gans, 1965).

The elapid venom gland, in contrast, is oval in shape. Like the viperid venom gland, it consists of a main (serous) gland and a mucous accessory gland. The main gland is made up of many simple or branching tubules. The lumen is narrow, and most of the venom is stored within the cells rather than in the lumen. The elongate mucous accessory gland surrounds the entire duct, so that the narrow lumen continues through it (Rosenberg, 1967).

The atractaspidid venom gland also has a distinctive pattern, although this was only discovered after Bourgeois' (1961) suggestion that *Atractaspis* did not belong among the viperids. It is cylindrical and in some taxa is highly elongate, extending posteriorly along the body well beyond the head (elongate venom glands are found in some viperids and elapids as well). A central lumen extends along the length of the gland, with a characteristic pattern of unbranched tubules radiating outward, as can be seen in transverse section. There is no distinct accessory gland, but mucous cells line the lumen along most of its length (Kochva *et al.*, 1967; Underwood & Kochva, 1993).

Tracing the evolution of morphological characters on phylogenetic trees depends heavily on the assumptions one makes about the homologies of the structures being studied when ordering the characters. In the case of ophidian cephalic glands, there is strong embryological, neural, and vascular evidence for the homology of the Duvernoy's glands of colubrid snakes with the venom glands of viperid, elapid, and atractaspidid snakes. However, the great structural differences among the latter suggest that although they probably all evolved from a Duvernoy's gland, they did so independently of one another. It is noteworthy that no snake has yet been discovered that possesses both a Duvernoy's gland and a venom gland.

If one considers the evolution of Duvernoy's and venom glands within the context of the alternate phylogenetic hypotheses proposed by Cadle (1988) and Kraus & Brown (1998), one finds results which are in some ways similar. If we order the characters X (no Duvernoy's gland or venom gland present), D (Duvernoy's gland present), V_V (venom gland of the viperid type present), V_E (venom gland of the elapid type present), and V_A (venom gland of the atractaspidid type present), in the following manner:

$$X \longleftrightarrow D \begin{array}{c} \nearrow V_V \\ \longleftrightarrow V_E \\ \searrow V_A \end{array}$$
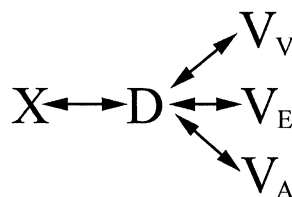
and consider this in the context of Cadle's (1988) phylogeny (Fig. 4A), it is most parsimonious to conclude that Duvernoy's gland evolved only once, at the base of the colubroid tree. In the viperid, elapid and atractaspidid lineages, it evolved into a venom gland of the viperid, elapid and atractaspidid types respectively. It was lost in some of the colubrines and retained in the
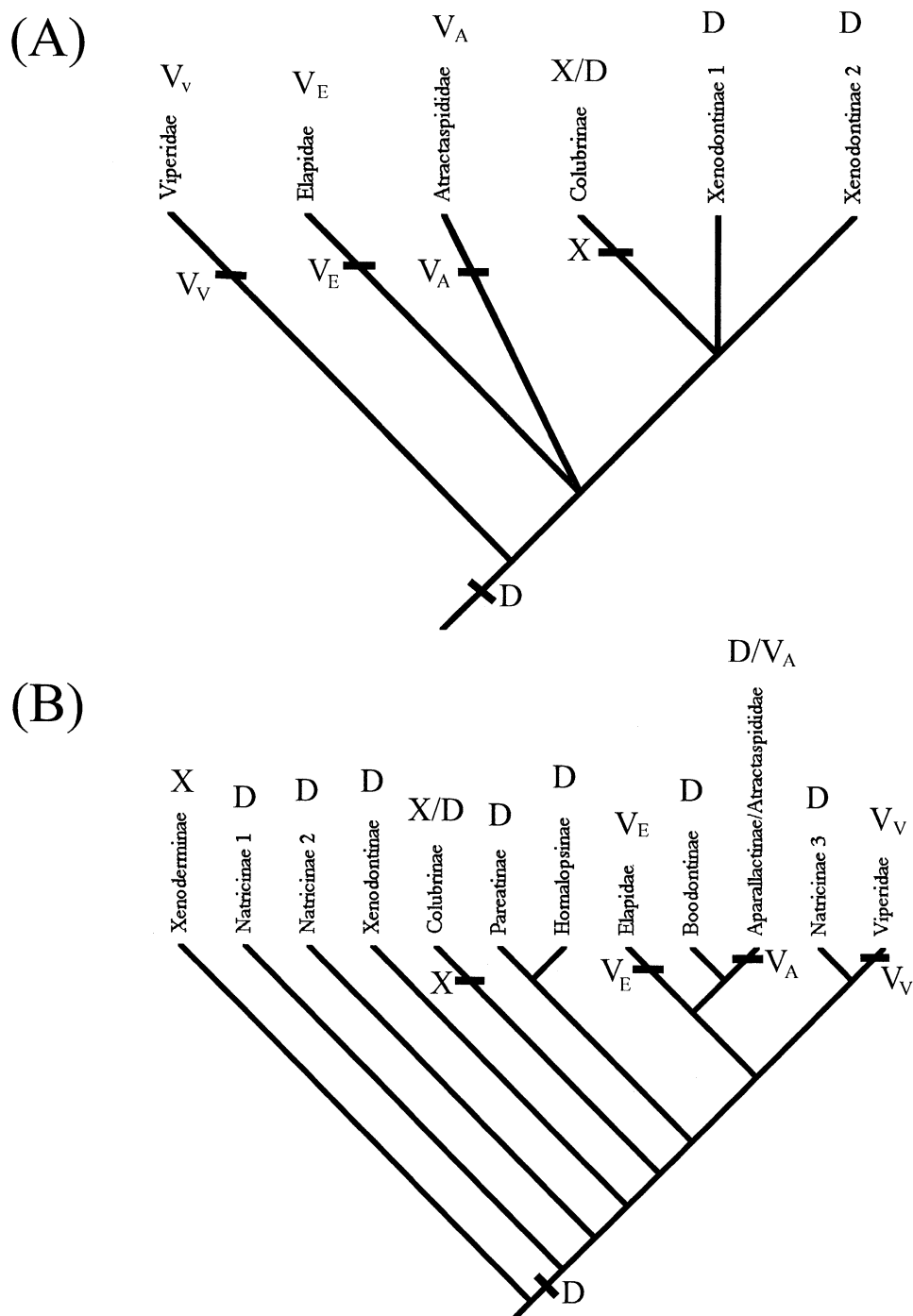
Figure 4. Most parsimonious reconstruction of the evolution of venom glands and Duvernoy's glands in the context of (A) Cadle's (1988) and (B) Kraus & Brown's (1998) phylogeny. X, Duvernoy's gland and venom gland absent; D, Duvernoy's gland present; V_V, venom gland of viperid type present; V_E, venom gland of elapid type present; V_A, venom gland of atractaspidid type present.

xenodontines. The lack of representation of other colubrid subfamilies from this phylogeny makes it impossible to draw further conclusions. If the analysis is repeated with the characters unordered, the only difference in the most parsimonious reconstruction is that Duvernoy's gland evolves after the branching off of the viperid, atractaspidid, and elapid lineages.

In Kraus & Brown's (1998) phylogeny (Fig. 4B) the basal position of the Xenoderminae provides a possible explanation of Taub's (1967) observation that xenodermines lack a Duvernoy's gland but have an unusual supralabial gland. Using this phylogeny, the most parsimonious reconstruction, using ordered characters, is that Duvernoy's gland evolved only once, near the base of the colubroid tree, but after the branching off of the Xenoderminae, and was lost in some colubrines. In the elapid and viperid lineages, it evolved into a venom gland of the elapid and viperid types, while in the aparallactine-atractaspidid lineage it was retained in the former and evolved into a venom gland of the atractaspidid type in the latter. What seems to hold true for both trees is that Duvernoy's gland evolved early in colubroid evolution and that 'true' venom glands have evolved independently three times in viperids, elapids, and atractaspidids. Repeating the analysis with the characters unordered results in the same single most parsimonious reconstruction.

MUSCLES

In elapids, viperids, and atractaspidids, the venom gland is associated with muscles that compress the gland during biting. This squeezes venom out of the lumen of the gland through the duct and into the canal of the tubular fangs. Assessing the homologies of the venom gland compressor muscles in different groups of snakes has the potential to help resolve the question of what the sequence of acquisition of VDS characters was.

The cranial muscles of snakes, associated with the VDS, may be divided into four major functional groups (Table 1): jaw adductors and abductors, and palatal protractors and retractors. Of these four groups, the jaw adductors and the palatal protractors are most involved in the VDS. The jaw adductors compress the venom gland (additionally, the pterygoideus, a retractor, contributes to venom gland compression in *Crotalus* (Young *et al*. 2000) and possibly in other snakes). The palatal protractors erect the moveable fang of viperids and atractaspidids. The palatal protractors and retractors are functionally highly conserved in colubroid snakes (Cundall, 1983), though some structural variability is observed. and I therefore initially focus on the venom gland compressor muscles.

VENOM GLAND COMPRESSOR MUSCLES

The venom gland is compressed by the adductor externus, which is divided into three or four parts depending on which author is naming them (Table 2). For the purposes of this study, the three parts of the adductor externus are referred to as adductor externus superficialis (AES), adductor externus medialis (AEM), and

Table 1. Snake cephalic muscles associated with the VDS, grouped by function

Muscle group	Muscles	Innervation	Function
Jaw adductors	adductor externus (including levator anguli oris, AES, AEM, AEP) adductor posterior	CN V$_3$	adduction of jaws compression of venom gland
Jaw abductors	depressor mandibulae costo-mandibularis retractor quadrati	CN VII	abduction of jaws
Palatal protractors	levator pterygoidei protractor pterygoidei	CN V$_3$	erection of solenoglyph fang pterygo-palatine walk'
Palatal retractors	retractor pterygoidei pterygoideus pterygoideus accessorius	CN V$_3$	'pterygo-palatine walk'

Table 2. Terminology: jaw adductor muscles of snakes

Phisalix (1922)	temporal antérieur	temporal moyen	temporal postérieur
Haas (1934)	adductor externus superficialis, part 1b	adductor externus profundus	adductor externus medialis
Kochva (1962)	adductor externus superficialis, + levator anguli oris	adductor externus medialis	adductor externus profundus
Haas (1962, 1973)	adductor externus superficialis, part 1b	adductor externus medialis	adductor externus profundus
Cundall (1983)	adductor externus superficialis	adductor externus medialis	adductor externus profundus
McDowell (1986)	levator anguli oris (1a + 1b)	adductor externus medialis	adductor externus superficialis
Present study	adductor externus superficialis	adductor externus medialis	adductor externus profundus

Table 3. Position of the attachment of the pterygoideus in different groups of snakes. Anterior: near to the ectopterygoid-maxilla attachment, close to the posterior-most maxillary teeth. Posterior: near the middle of the ectopterygoid

Taxon	Position of attachment
Colubroidea	
Aparallactinae	anterior or posterior
Atractaspididae	anterior[1]
Elapidae	posterior
Viperidae	anterior
other Colubroidea	anterior
Acrochordidae	anterior
Tropidophiidae	posterior
Bolyeridae	posterior[2]
Aniliidae	Posterior
Boidae	Posterior

[1]Data from Kochva (1962).
[2]Data from Cundall & Irish (1989).
All other data from McDowell (1986).

adductor externus profundus (AEP) (Fig. 5A). The AES is innervated by an anterior branch of V_3 and the AEP by a posterior branch of V_3. The innervation of the AEM is variable and it appears to receive nerves from both branches (Kochva, 1962). The blood supply to the AES is from one branch of the facial artery, while another supplies the AEM and AEP (Kochva, 1962).

The substantial literature that exists on the comparative anatomy of the ophidian adductor externus is greatly complicated by variations in the terminology used by different authors (Table 2), and, more seriously, by disagreements over the homologies of the external adductor muscles. Fortunately, these disagreements concern muscle homologies between snakes and lizards (the terminology used for snake cranial muscles is taken from Lakjer's (1926) work on lizards), and have no bearing on the question of external adductor homologies among colubroid snakes, except in contributing to the problem of inconsistent terminologies from author to author.

The term 'compressor glandulae' (CG) is often used to describe the muscle that compresses the venom gland in venomous snakes. However, the CG is derived from different portions of the adductor externus in different groups of venomous snakes.

The most complicated pattern of venom gland musculature is seen in Viperidae (Fig. 5B). Here the CG is derived from the AEP. The homology of these two muscles is supported by comparative studies of muscle innervation in a variety of viperid taxa (Kochva, 1962), and by embryological studies of *Vipera palaes-*

tinae (Kochva, 1963) and of the basal viperid *Causus rhombeatus* (Wollberg & Kochva, 1967). These studies show that in both of these cases the CG originates embryologically as part of the AEP. Fibres of the CG originate along the lower jaw and extend dorsal and medial to the AES; after passing deep to the AES, they turn dorsally and anteriorly, wrapping around it, ultimately inserting on the venom gland (Kochva, 1958).

In elapids, by contrast, the AES has become the CG (Fig. 5C) (Kochva, 1962; McDowell, 1968). This is supported by comparative studies of head muscle innervation across a variety of elapid taxa (Kochva, 1962). In elapids the CG (= AES) is usually divided by the venom gland into dorsal and ventral portions. The dorsal portion (larger than the ventral portion), extends from the postorbital bone to the dorsal and caudal surfaces of the venom gland. The ventral portion extends from the lower jaw to the ventral surface of the venom gland.

The AES is absent in *Atractaspis* (Fig. 5D). The venom gland is compressed by a long arc of muscle fibres that extends from the parietal bone and sheath of the Harderian gland (posterior to the eye) caudally along the length of the venom gland. At the caudal end of the gland (which is elongate in some taxa) the arc turns ventrally, wraps around the gland, and proceeds anteriorly along its ventral surface to the angle of the mouth and the lower jaw (McDowell, 1968). Along the way, many fibres insert on the surface. The homologies of the atractaspidid CG are not certain. Kochva (1962) noted that the fibres are innervated by the posterior branch of V_3; at that time *Atractaspis* was still thought to be a viperid, so there would have been no reason to suspect that the CG was derived from anything other than the AEP. However, McDowell (1968) interpreted the position of the dorsal attachment of the CG as evidence that the AEM may be involved, and Kochva's (1962) evidence of innervation by the posterior branch of V_3 does not rule this out. Thus Figure 5D shows the atractaspidid CG as derived from the AEM, although this conclusion could change with further research. McDowell (1968) stated that the atractaspidid CG is derived from the AES, but provided no additional explanation. He altered his terminology in the 1986 paper (Table 2) where he refers to the AES as the levator anguli oris.

Finally, colubrids generally lack compressor muscles associated with their VDSs. However, there are some colubrids in which some muscle fibres are associated with Duvernoy's gland. Examples include the colubrine *Dispholidus* and the boodontine *Mehelya*, where fibres from the AES and pterygoideus, respectively, are associated with the gland (Kochva & Wollberg, 1970; McDowell, 1987).

Neural, vascular, and embryological evidence suggests that the function of venom gland compression
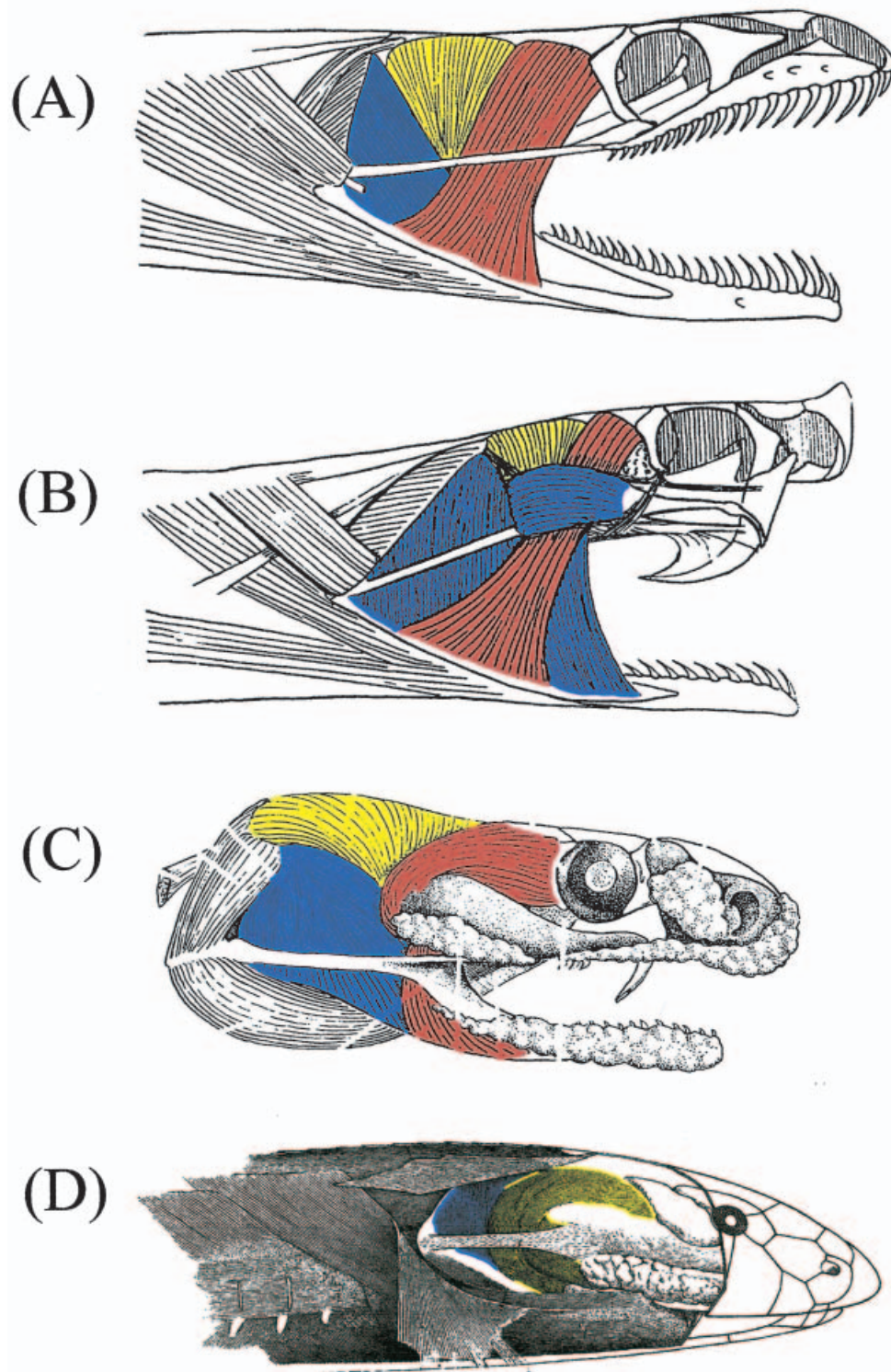
Figure 5. Head muscles of (A) *Python regius* (modified from Phisalix, 1922), showing an unspecialized pattern of external adductor muscles; (B) the viperid, *Vipera aspis* (modified from Phisalix, 1922); (C) the elapid, *Elapsoidea sundevalli* (modified from McDowell, 1968), and (D) the atractaspidid, *Atractaspis dahomeyensis* (modified from Underwood & Kochva, 1993). The adductor externus muscles are shown in colour: red, AES and derived fibres; yellow, AEM and derived fibres; blue, AEP and derived fibres. Snakes not drawn to the same scale.

must have evolved independently at least three times in snakes. The viperid CG is the AEP, the elapid CG is the AES, and the atractaspidid CG is probably the AEM. This holds true when either Cadle's (1988) phylogeny or Kraus & Brown's (1998) transversion parsimony tree are considered. Cadle's phylogeny places the Viperidae (in which the CG is derived from the AEP) as the basal colubroid group. The Elapidae (in which the AES is the CG), the Atractaspididae (in which the AEM forms the CG) form a trichotomy with the colubrids included in the phylogeny.

In Kraus & Brown (1998), the taxa are arranged very differently compared to Cadle. However, the most parsimonious reconstruction of the evolution of the compressor muscles is the same. The AES became the CG in the Elapidae, a CG derived from the AEP evolved in the Viperidae, and a CG probably derived from the AEM evolved in the Atractaspididae. Thus, the morphological and phylogenetic evidence indicates that the CG must have evolved three times in colubroid snakes.

ANTERIOR ATTACHMENT OF THE PTERYGOIDEUS

The palatal protractors and retractors are conservative in colubroid snakes (Cundall, 1983) and do not display the diversity of the external adductor muscles. The thick end of the pterygoideus originates on the ventral side of the angular process of the mandible. It extends anteriorly, with its thin end inserting on either the shaft of the ectopterygoid or the maxilla, depending on the species. Some fibres may insert on the ventral surface of the venom gland as the pterygoideus passes beneath it (Kochva, 1958).

In viperids the anterior attachment of the pterygoideus is continuous with the sheath of the fang, on the maxilla (Kochva, 1958). The condition is similar in atractaspidids, which also have an erectable fang. In *Atractaspis heterochilus* and *A. engaddensis* the attachment is on the maxilla and the sheath of the fang (Kochva, 1962); in elapids, in which the fang is not moveable, it is much further back, on the shaft of the ectopterygoid (McDowell, 1986).

McDowell (1986) places great importance on the position of the attachment. He notes that in 'stab and inject' envenomators (fixed front-fanged colubroids [= elapids]) it is far back on the ectopterygoid, whereas in 'slash and swab' envenomators (rear-fanged colubroids) the pterygoideus attaches further anterior, near the ectopterygoid-maxilla articulation and the posterior fangs. McDowell argues that an anterior (near the fangs) attachment is an essential part of the rear-fanged VDS, allowing the precise manipulation of the fangs. This, he believes, represents the derived condition, a specialization of rear-fanged colubroid snakes. As evidence, he points out that several out-

groups to Colubroidea (representatives of Boidae, Aniliidae, Tropidophiidae, and Bolyeridae) have a posterior attachment (near the middle of the ectopterygoid). Elapids also have a posterior attachment, and McDowell interprets this as evidence that they must be basal within Colubroidea. However, *Acrochordus*, the immediate outgroup to Colubroidea, has an anterior attachment. Considering the phylogenetic relationships among the groups involved, and the position of the anterior attachment, it is difficult to determine whether it is more likely that (a) elapids retain a primitive condition of the anterior attachment, or (b) they have secondarily reacquired an anterior attachment that is located posteriorly on the ectopterygoid.

The problem of the evolution of the attachment of the pterygoideus in elapids is evaluated within the context of Cadle (1988) and Kraus & Brown (1998). The phylogeny from Pough *et al.* (2001) is used for the basal snakes considered here as outgroups to Colubroidea. The position of the anterior attachment in different groups of snakes is shown in Table 4.

Cadle's (1988) phylogeny does not include most colubroid subfamilies, making it difficult to draw firm conclusions. However, the most parsimonious scenario to explain the evolution of anterior attachment positions is as follows: a posterior position was the plesiomorphic condition for snakes. Before the branching off of Acrochordidae, it moved anteriorly. Then the

Table 4. Condition of the fang in colubroid snakes

	Ungrooved	Grooved	Tubular
Aparallactinae[1]		X	
Atractaspididae[1]			X
Boodontinae[2,3]	X	X	
Colubrinae[1]	X	X	
Elapidae[1]			X
Homalopsinae[1,2]		X	
Natricinae[1,3]	X		
Pareatinae[1,3]	X		
Viperidae[1]			X
Xenoderminae[3,4]	X	X	
Xenodontinae[1,3]	X	X	

[1]Personal observation: as reported in Jackson & Fritts (1995), in addition to *Aparallactus* sp. (MCZ 131453), *Amblyodipsas polylepis hildebrandti* (MCZ 54834), *Chrysopelea o. ornata* (MCZ 3113), *Dispholidus typus* (MCZ 54621), *Enhydris polylepis* (MCZ 137282), *Fordonia leucobalia* (MCZ 129944), *Myron richardsoni* (MCZ 134738), *Elaphe obsoleta* (MCZ 46047), *Opheodrys vernalis* (MCZ 32225), *Dendrelaphis caudolineatus* (MCZ 9030), *Nerodia fasciata* (MCZ 176470), *N. n. natrix* (MCZ 32226).
[2]McDowell (1987).
[3]Boulenger (1893).
[4]McDowell (1986).

position of attachment secondarily became posterior in elapids and in some aparallactines (Fig. 6A).

Kraus & Brown's (1998) transversion parsimony tree includes a larger sample of colubrid subfamilies. For the purposes of this character the most significant difference between this tree and Cadle's is that the aparallactines and atractaspidids are grouped together. The result is still the same. The most parsimonious scenario is that a posterior position of attachment is the plesiomorphic condition. Before the branching off of Acrochordidae, it moved anteriorly. Then it secondarily became posterior in Elapidae and in some aparallactines. Thus, both these phylogenies support the view that the posterior position in elapids and in some aparallactines represents a secondary move, and that an anterior position is the plesiomorphic condition for Caenophidia (Fig. 6B).

FANGS

Colubroid snakes have teeth on the maxillary, palatine, pterygoid, and dentary bones. In all venomous taxa, the fang is a modified maxillary tooth, and for this reason the maxillary dentition has been studied extensively and is an important taxonomic character. Specialization of the maxillary teeth for the conduction of oral secretions or venom is almost certainly a colubroid synapomorphy. Among the Colubridae the posterior maxillary teeth are usually specialized. They may be enlarged relative to the anterior maxillary teeth and have a groove extending along the labial or anterior surfaces. Often they are separated from the rest by a diastema. Even in cases where the posterior teeth are not grooved or notably enlarged relative to other teeth, they are distinguishable by shape from the anterior teeth: the former have ridges on their anterior and posterior surfaces, while the latter have ridges along their labial and lingual surfaces (Jackson & Fritts, 1995). Only a few colubrids lack specialized posterior teeth.

Fangs may be grooved or tubular. Grooved fangs are modified teeth with a groove extending along the labial, anterolabial, or anterior surface of the tooth, from base to tip. The groove may be shallow or deep, and grooved fangs are usually, though not always, at the posterior end of the maxilla, with unspecialized anterior maxillary teeth in front of them. Tubular fangs have a completely enclosed venom canal for the conduction of venom into a bite wound. This canal probably evolved as a result of selective pressures acting on a grooved fang to deepen the groove and draw its edges closer together until they met. Tubular fangs are always positioned at the anterior end of the maxilla, which is shortened relative to that of most rearfanged snakes. In elapids the fang is positioned at the anterior end of the maxilla, and in viperids and atrac-taspidids the maxilla is greatly reduced and there are no maxillary teeth other than the fangs. The enclosed canal lies in close approximation with the venom duct via the fang sheath.

The condition of the maxillary fang in colubroid snakes is summarized in Table 4. In colubrines, boodontines, xenodermines, and xenodontines, the posterior maxillary teeth may be grooved or ungrooved. In natricines they are ungrooved but in some cases greatly enlarged. In homalopsines the posterior fang is grooved, and in pareatines it is ungrooved. In aparallactines it is deeply grooved and in some cases almost tubular, and the maxilla is short relative to that of other colubrids. In most aparallactines ungrooved anterior maxillary teeth are present anterior to the fangs, but in some the latter are positioned on the anterior end of the maxilla. In elapids the fang is tubular and positioned on the anterior end of the maxilla. In some taxa there are additional maxillary teeth posterior to the fang. In viperids and atractaspidids the fang is tubular and elongate. The maxilla is greatly shortened, forming a small base to which the fang is attached. There are no other maxillary teeth besides the fangs. To accommodate the elongate fang when the mouth is closed, the maxilla with the attached fang rotates, so that the fang is erected into a vertical position when the mouth is open, and lies with the tip pointing caudally when not in use. Bourgeois (1961, 1968) noted that although atractaspidids and viperids both have an erectable fang, they differ in the mechanism by which the fang is erected. In both groups, the point of rotation is between the maxilla and the prefrontal. However, in atractaspidids the maxilla articulates with a socket in the prefrontal, whereas in viperids it does not.

There has been much speculation about the homologies of colubroid fangs, with the preponderance of evidence favouring the view that all tubular anterior fangs are homologous to grooved posterior fangs, and that the fangs of different tubular-fanged taxa are homologous to one another. Kardong (1980, 1982) argued that the tubular fangs of viperids and elapids are both derived from grooved posterior fangs, citing as evidence, among other things, the postorbital position of the venom gland and Duvernoy's gland. Another important point is that tubular front fangs and grooved posterior fangs develop the same way, from a common primordium with the venom gland or the Duvernoy's gland (Kochva, 1963, 1965). Early authors who believed that all venomous snakes formed a clade (e.g. Cope, 1900; Bogert, 1943) presumably thought the tubular fangs of different venomous snakes were homologous with one another. Indeed, it was the great morphological similarity of all tubular fangs that led them to this view of the relationships among colubroid snakes in the first place. More
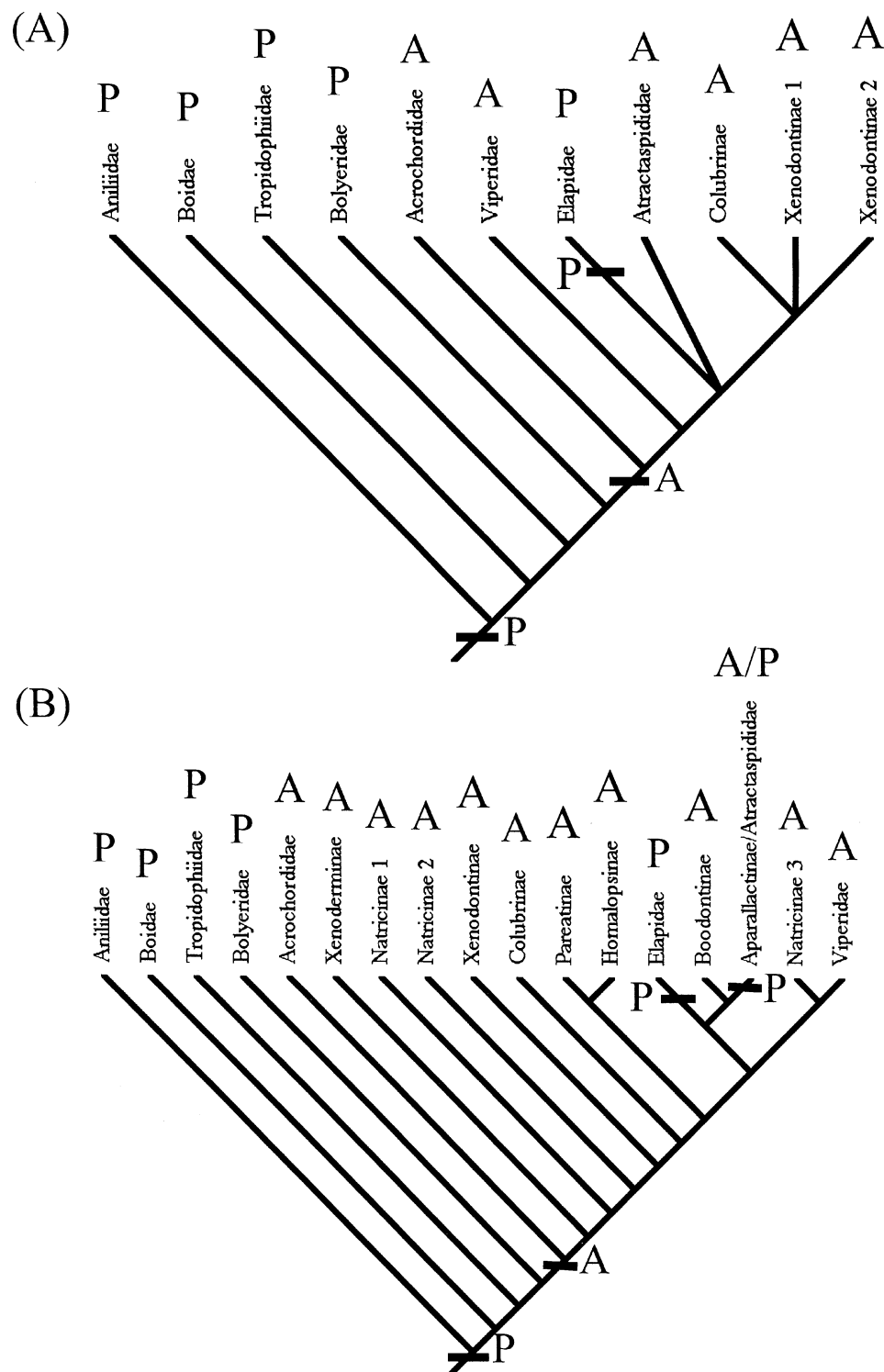
Figure 6. Most parsimonious recontruction of the evolution of the position of attachment of the pterygoideus within the context of the hypotheses of (A) Cadle (1988) and (B) Kraus & Brown (1998). A, anterior attachment near the posterior maxillary teeth, on the posterior end of the maxilla; P, anterior attachment on the ectopterygoid (i.e. posterior).

recently, a detailed study of the morphology and development of tubular fangs in viperid and elapid snakes confirmed the close similarity of these fangs, and found no evidence of homoplasy (Jackson, 2002).

Parsimony analysis of character evolution of fang condition in colubroid snakes within the context of Cadle (1988) and Kraus & Brown (1998) yields inconclusive results. The first decision to be made is whether to order the characters 'ungrooved', 'grooved', and 'tubular'. Doing so might seem reasonable, since it makes biological sense that a grooved fang might evolve from an ungrooved fang as a result of selective pressures, and that selective pressures acting on a grooved fang might deepen the groove leading to the evolution of a tubular fang. Similarly, if there are costs associated with having tubular or grooved fangs and advantages in some cases in not having them, then it might seem reasonable to suppose that venom canals and grooves might be secondarily lost. A transition from ungrooved to tubular with no intermediate is harder to imagine. The problem with ordering the characters 'ungrooved', 'grooved', and 'tubular', is that doing so assumes that the fangs being considered are homologous with one another. This may be a fair assumption considering the evidence discussed above, but I ran my analysis with the characters both ordered and unordered, for both phylogenetic hypotheses.

Using Cadle's (1988) phylogenetic hypothesis with the characters ordered or unordered, the most parsimonious reconstruction finds that a tubular fang is the plesiomorphic condition for Colubroidea. This is probably an artifact of the lack of representation in the tree of so many colubroid subfamilies with grooved and ungrooved fangs. The only difference between the trees obtained with ordered vs. unordered characters is that when the characters are ordered the character state evolves from tubular to grooved after the branching off of Atractaspididae, and then from grooved to ungrooved in some of the colubrines and xenodontines (Fig. 7A). With the unordered characters, the character state after the branching off of Atractaspididae could be either grooved or ungrooved.

Analysis of fang character evolution using Kraus & Brown's (1998) tree produces more complicated results, owing to the larger number of taxa represented in the tree. Ordering the characters produces seven equally parsimonious trees (one example is shown in Fig. 7B), while repeating the analysis with the characters unordered produces 12. Interestingly, all 19 trees have some features in common: they all find that the ungrooved fang represents the plesiomorphic condition for Colubroidea, and they all derive tubular fangs independently three times in viperids, elapids, and atractaspidids.

One conclusion that does become apparent is that tubular fangs have probably evolved several times, while grooved fangs have probably been acquired and lost several times in colubroid history. How is this reconcilable with the morphological evidence that all colubroid fangs are homologous? The answer may lie in the definition of homology used. By a strict phylogenetic definition, the tubular fangs of different groups of venomous snakes can only be considered homologous if the different groups arise from a shared common ancestor with tubular fangs. On the other hand, following the biological concept (Wagner, 1989), a shared developmental pathway could be considered the appropriate criterion. Tubular fangs of viperids, elapids, and atractaspidids may best be considered latent homologues (*sensu* Wake, 1999), suggesting that the morphogenetic system for forming tubular fangs may have been present in the shared common ancestor of these taxa, even though the fangs themselves were not.

CONCLUSIONS

Savitzky (1980) postulates that with the advent of open habitats during the Miocene, the heavy-bodied henophidians, which were the ancestors of colubroids and survived as sit-and-wait predators relying on constriction to kill their prey, gave way to smaller, more agile snakes. Having given up the large bodies typical of henophidian constrictors, these smaller snakes had to find another way to subdue their prey, and perhaps this way was the VDS.

It becomes clear from the present study that several major morphological changes associated with components of the VDS occurred near the base of the colubroid tree. For the first time, dental glands became modified into Duvernoy's glands, posterior maxillary teeth became morphologically specialized relative to anterior maxillary teeth, and the anterior attachment of the pterygoideus muscle moved anteriorly, placing it in close association with the posterior maxillary teeth or fangs. It is also possible, as discussed in the section on fangs, that the developmental pathway for forming tubular venom-conducting fangs was laid down near the base of the colubroid tree. Could these morphological changes indicate the evolution of the first snake VDS?

Later in colubroid history, three lineages of colubroids (not one as believed by early authors, and not five as believed by some researchers in recent decades) independently evolved front-fanged VDSs. In each case (viperids, elapids, and atractaspidids) these changes consisted of large and highly complex venom glands derived from Duvernoy's glands, specialized external adductor muscles to compress them, and
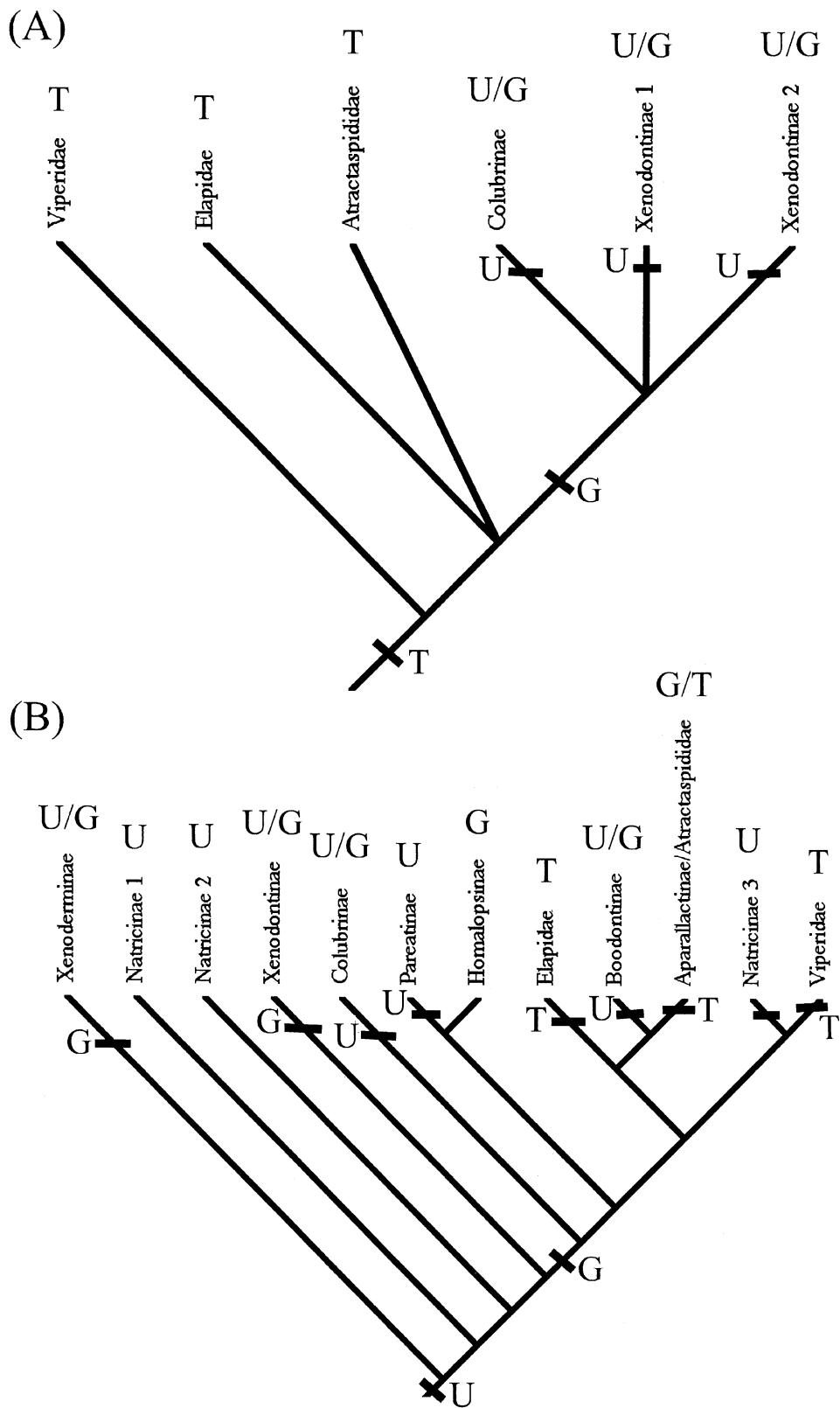
Figure 7. Most parsimonious reconstructions of the evolution of fang character states, with characters ordered, in the context of the phylogenies of (A) Cadle (1988) and (B) Kraus & Brown (1998). The Kraus & Brown tree is an example of just one of seven equally parsimonious reconstructions. U, ungrooved; G, grooved; and T, tubular.

tubular fangs for the more efficient introduction of venom into prey.

Comparative biologists often avoid attempts to reconstruct character evolution when the phylogeny of the taxonomic group to be studied is not well-resolved. One interesting result of the present study was that conclusions about the evolution of most morphological characters were consistent even when analyses were repeated for two very different phylogenetic hypotheses.

However there are still many aspects of the evolution of VDSs in snakes about which we know little. First of all, it is abundantly clear that a reliable and complete phylogeny of colubroid taxa is needed before many questions can be settled. Second, this study is concerned only with the gross morphological aspects of the VDS. Research on the venoms themselves, in a phylogenetic context, has the potential to open a whole new field and provide new information about the biology and evolution of colubroid snakes. At present, most studies of venoms have focused on snakes that pose a medical threat to humans, and on the effect of venoms and Duvernoy's secretions on mammals. As a result, little is known about the venoms of nonmedically important colubroid snakes, and about the effects on nonmammalian prey, even though these factors may be more significant to the biology of snakes. Finally, ecological studies of snakes have the potential to shed new light on how venoms and VDSs are actually used by snakes in real biological situations, and perhaps even to elucidate what selective pressures drive the evolution of VDSs of different types.

ACKNOWLEDGEMENTS

REFERENCES

Anthony J. 1955. Essai sur l'évolution anatomique de l'appareil venimeux des ophidiens. *Annales des Sciences Naturelles, Zoologie* **11:** 7–53.

Bogert CM. 1943. Dentitional phenomena in cobras and other elapids with notes on adaptive modifications of the fangs. *Bulletin of the American Museum of Natural History* **131:** 285–360.

Boulenger GA. 1893. *Catalogue of snakes in the British Museum (Natural History)*, Vol. 1. London: Taylor & Francis.

Bourgeois M. 1961. *Atractaspis* – a misfit among the Viperidae. *News Bulletin of the Zoological Society of South Africa* **3:** 29.

Bourgeois M. 1968. *Contribution à la morphologie comparée du crâne des Ophidiens de l'Afrique Centrale*, Vol. XVIII. Publications de l'Université Officielle du Congo à Lubumbashi.

Cadle JE. 1981. An immunological assessment of the phylogenetic position of New World coral snakes. *Journal of Zoology, London* **195:** 157–167.

Cadle JE. 1982. Problems and approaches in the interpretation of the evolutionary history of venomous snakes. *Memoirs of the Institute of Butantan* **46:** 255–274.

Cadle JE. 1988. Phylogenetic relationships among advanced snakes: a molecular perspective. *University of California Publications in Zoology* **119:** 1–77.

Cadle JE. 1994. The colubrid radiation in Africa (Serpentes: Colubridae): phylogenetic relationships and evolutionary patterns based on immunological data. *Zoological Journal of the Linnean Society* **110:** 103–140.

Cope ED. 1900. The crocodilians, lizards, and snakes of North America. *Reports of the US National Museum* 153–1270.

Cundall D. 1983. Activity of the head muscles during feeding by snakes: a comparative study. *American Zoology* **23:** 383–396.

Cundall D, Irish FJ. 1989. The function of the intramaxillary joint in the Round Island boa, *Casarea dussumieri*. *Journal of Zoology, London* **217:** 569–598.

Dessauer HC, Cadle JE, Lawson R. 1987. Patterns of snake evolution suggested by their proteins. *Fieldiana (Zoology) NS* **34:** 1–34.

Duvernoy DM. 1832. Mémoire sur les caractères tirés de l'anatomie pour distinguer les serpents venimeux des serpents non venimeux. *Annales des Sciences Naturelles* **26:** 113–160.

Fontana F. 1781. *Sur les Poisons et sur le Corp Animal. Traité sur le Venin de la Vipère.* Paris.

Gabe M, Saint Girons H. 1969. Données histologiques sur les glandes salivaires des lépidosauriens. *Mémoires du Musée de l'Histoire Naturelle SérieA, Zoologie* **58:** 1–112.

Grant TR. 1989. Ornithorhynchidae. In: Walton DW, Richardson BJ, eds. *Mammalia*, Vol. 1B. Canberra: Australian Government Publishing Service, 436–450.

Gravlund P. 2001. Radiation within the advanced snakes (Caenophidia) with special emphasis on African opisthoglyph colubrids, based on mitochondrial sequence data. *Biological Journal of the Linnean Society* **72:** 99–114.

Haas G. 1934. Beitrag zur Frage der Homologisierung der Kiefermuskulatur der Ophidia und Sauria. *Biol. Gen.* **10:** 211–226.

Haas G. 1962. Remarques concernant les relations phylogéniques des diverses familles d'ophidiens fondées sur la differenciation de la musculature mandibulaire. In: Lehman J-P, ed. *Problèmes actuels de paléontologie (evolution des vertèbres)*, Vol. 104. Paris: Centre National de la Recherche Scientifique, 215–241.

Haas G. 1973. Muscles of the jaws and associated structures in the Rhychocephalia and Squamata. In: Gans CT, Parsons T, eds. *Biology of the Reptilia, morphology D*, Vol. 4. London, New York: Academic Press, 285–490.

Heise PJ, Maxson LR, Dowling HG, Hedges SB. 1995. Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. *Molecular Biology and Evolution* **12:** 259–265.

Jackson K. 2002. How tubular venom-conducting fangs are formed. *Journal of Morphology* **252:** 291–297.

Jackson K, Fritts TH. 1995. Evidence from tooth surface morphology for a posterior maxillary origin of the proteroglyph fang. *Amphibia-Reptilia* **16:** 273–288.

Kardong KV. 1980. Evolutionary patterns in advanced snakes. *American Zoology* **20:** 269–282.

Kardong KV. 1982. The evolution of the venom apparatus in snakes from colubrids to viperids and elapids. *Memoirs of the Institute of Butanan* **46:** 105–118.

Kardong KV. 1996. Snake toxins and venoms: an evolutionary perspective. *Herpetologica* **52:** 36–46.

Knight A, Mindell DP. 1994. On the phylogenetic relationship of Colubrinae, Elapidae, and Viperidae and the evolution of front-fanged venom systems in snakes. *Copeia* **1994:** 1–9.

Kochva E. 1958. The head muscles of *Vipera palaestinae* and their relation to the venom gland. *Journal of Morphology* **102:** 23–54.

Kochva E. 1962. On the lateral jaw musculature of the Solenoglypha with remarks on some other snakes. *Journal of Morphology* **110:** 227–284.

Kochva E. 1963. Development of the venom gland and trigeminal muscles in *Vipera palaestinae*. *Acta Anatomica* **52:** 49–89.

Kochva E. 1965. The development of the venom gland in the opisthoglyph snake *Telescopus fallax* with remarks on *Thamnophis sirtalis* (Colubridae, Reptilia). *Copeia* **1965:** 147–154.

Kochva E. 1978. Oral glands of the Reptilia. In: Gans CK, Gans A, eds. *Physiology B*, Vol. 8. London, New York: Academic Press, 43–162.

Kochva E. 1987. The origin of snakes and the evolution of the venom apparatus. *Toxicon* **25:** 65–106.

Kochva E, Gans C. 1965. The venom gland of *Vipera palaestinae* with comments on the glands of some other viperines. *Acta Anatomica* **62:** 365–401.

Kochva E, Wollberg M. 1970. The salivary glands of Aparallactinae (Colubridae) and the venom glands of *Elaps* (Elapidae) in relation to the taxonomic status of this genus. *Zoological Journal of the Linnean Society* **49:** 217–224.

Kochva E, Wollberg M, Sobol R. 1967. The special pattern of the venom gland in *Atractaspis* and its bearing on the taxonomic status of the genus. *Copeia* **1967:** 763–772.

Kraus F, Brown WM. 1998. Phylogenetic relationships of colubroid snakes based on mitochondrial DNA sequences. *Zoological Journal of the Linnean Society* **122:** 455–487.

Lakjer T. 1926. *Studien über die Trigeminus-Versorgte Kaummuskulatur der Sauropsiden*. Copenhagen: C. A. Reitzel.

Marx H, Rabb GB. 1972. Phyletic analysis of fifty characters of advanced snakes. *Fieldiana (Zoology)* **63:** 1–321.

McCarthy CJ. 1985. Monophyly of the elapid snakes (Serpentes: Reptilia): An assessment of the evidence. *Zoological Journal of the Linnean Society* **83:** 79–93.

McDowell SB. 1968. Affinities of the snakes usually called *Elaps lacteus* and *E. dorsalis*. *Journal of the Linnean Society (Zoology)* **47:** 561–578.

McDowell SB. 1986. The architecture of the corner of the mouth of colubroid snakes. *Journal of Morphology* **20:** 353–407.

McDowell SB. 1987. Systematics. In: Seigel RA, Collins JT, Novak SS, eds. *Snakes: ecology and evolutionary biology*. New York: Macmillan, 3–50.

Phisalix M. 1922. *Animaux Venimeux et Venins*, Vol. II. Paris: Masson.

Pough FH, Andrews RA, Cadle JE, Crump M, Savitzky AH, Wells KD. 2001. *Herpetology*, 2nd edn. New York: Prentice Hall.

Rabb GB, Marx H. 1973. Major ecological and geographical patterns in the evolution of colubroid snakes. *Evolution* **27:** 69–83.

Reshef R. 1994. Ontogenetic mechanisms of teeth and oral gland development in snakes –a possible contribution to the understanding of evolutionary processes in reptiles (English summary only). PhD Dissertation, Tel-Aviv University.

Rosenberg HI. 1967. Histology, histochemistry, and emptying mechanism of the glands of some elapid snakes. *Journal of Morphology* **123:** 133–156.

Russell FE. 1983. *Snake Venom Poisoning*. New York: Scolum International, Inc.

Savitzky AH. 1978. The origin of the New World proteroglyphous snakes and its bearing on the study of venom delivery systems in snakes. PhD Dissertation, University of Kansas, Lawrence.

Savitzky AH. 1980. The role of venom-delivery strategies in snake evolution. *Evolution* **34:** 1194–1204.

Slowinski JB, Lawson R. 2002. Snake phylogeny: evidence from nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution* **24:** 194–202.

Sues HD. 1991. Venom-conducting teeth in a Triassic reptile. *Nature* **351:** 141–143.

Taub AM. 1966. Ophidian cephalic glands. *Journal of Morphology* **118:** 529–542.

Taub AM. 1967. Comparative histological studies of Duvernoy's gland of colubrid snakes. *Bulletin of the American Museum of Natural History* **138:** 1–50.

Tu AT. 1991. Reptile venoms and toxins. In: Tu, AT, ed. *Handbook of natural toxins*, Vol. 5. New York: Marcel Dekker, Inc.

Underwood G. 1967. *A contribution to the classification of snakes*. London: British Museum (Natural History).

Underwood G, Kochva E. 1993. On the affinities of the burrowing asps *Atractaspis* (Serpentes: Atractaspididae). *Zoological Journal of the Linnean Society* **107:** 3–64.

Vaughan TA. 1986. *Mammalogy*. Philadelphia: Saunders College Publishing.

Vidal NV, Kindl SG, Wong A, Hedges SB. 2000. Phylogenetic relationships of xenodontine snakes inferred from 12S and 16S ribosomal RNA sequences. *Molecular Phylogenetics and Evolution* **14:** 389–402.

Wagner GP. 1989. The biological homology concept. *Annual Review of Ecological Systematics* **20:** 51–69.

Wake DB. 1999. Homoplasy, homology, and the problem of 'sameness' in biology. In: Bock GR, Cardew G, eds. *Homology*. Novartis Foundation Symposium 222. Chichester: Wiley, 24–46.

Wollberg M, Kochva E. 1967. Embryonic development of the venom apparatus in *Causus rhombeatus* (Viperidae, Ophidia). *Herpetologica* **23:** 249–259.

Wollberg M, Kochva E, Underwood G. 1998. On the rictal glands of some atractaspid snakes. *Herpetological Journal* **8:** 137–143.

Young BA, Zahn K, Blair M, Lalor J. 2000. Functional subdivision of the venom gland musculature and the regulation of venom expulsion in rattlesnakes. *Journal of Morphology* **246:** 249–259.