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Olfactory and Vomeronasal Systems of Caecilians (Amphibia:Gymnophiona)

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ABSTRACT The morphology of both the main nasal cavity and the vomeronasal organ differs among species representing six families of caecilians. The main nasal cavity is either divided or undivided. The vomeronasal organ differs in position (mediolateral, lateral), size (large vomeronasal organ in the aquatic species), and shape (mediolateral extension, vomeronasal organ with a lateral rostral projection). The great amount of respiratory epithelium of the main nasal cavity, the large vomeronasal organ, and its extensive innervation in typhlonectids may reflect both phylogeny and habitat adaptation, for these taxa are secondarily aquatic or semi-aquatic and have several concomitant morphological and physiological modifications. The vomeronasal organ is associated with the caecilian tentacle as the tentacular ducts open into it. This association is further evidence for the involvement of the caecilian tentacle in vomeronasal chemoperception and may represent the mechanism by which these animals smell though the main nasal cavity is closed during burrowing or swimming. Labelings of primary olfactory and vomeronasal projections by means of horseradish peroxidase reaction reveal that the pattern of vomeronasal projections is similar in *Ichthyophis kohtaoensis, Dermophis mexicanus,* and *Typhlonectes natans,* even though *T. natans* possess stronger vomeronasal projections relative to olfactory projections than *I. kohtaoensis* and *D. mexicanus.* However, there are differences with respect to the patterns of olfactory projections. The olfactory projection of *I. kohtaoensis* is characterized by many displaced glomeruli. *T. natans* has the smallest olfactory projection. The nervus terminalis is associated with the olfactory system as shown by selective labelings of olfactory projections.

Six characters potentially useful for phylogenetic analysis emerge from this study of comparative morphology. The characters were subjected to analysis using PAUP to see 1) if any resolution occurred and 2) if any groups were distinguished, whether they corresponded to phylogenetic arrangements based on other morphological characters. The characters are too few to produce nested dichotomous sets for all cases, but they do support the two typhlonectid genera examined and *Dermophis* and *Gymnopis* as sister taxa discrete from other groups, and they show that species within genera cluster together.

Comparative studies of the olfactory and vomeronasal systems provide useful data with which to consider the question of a monophyletic or polyphyletic origin of terrestrial vertebrates. Neuroanatomical studies on phylogenetic aspects of the olfactory and vomeronasal systems of amphibians thus far have dealt largely with the morphology of the nasal cavities (e.g., Parsons, '67; Jürgens, '71) and olfactory and vomeronasal projections in salamanders (Schmidt et al., '88). The vomeronasal organ has been discussed either as an adaptation to terrestrial life (Bertmar, '81) or as an originally aquatic olfactory organ resembling the olfactory mucosa of fishes (Broman, '20). The vomeronasal organs of members of the three extant amphibian orders differ with regard to size and location.

In the gymnophione amphibians, the caecilians, the olfactory and vomeronasal systems are nearly completely separated. There is only a small connection between the primary olfactory cavity and the vomeronasal organ. Chemorecep-

tion in the vomeronasal organ is considered to be facilitated by the caecilian tentacle (summarized in Badenhorst, '78; Billo and Wake, '87).

The present study is directed to the question of whether the anatomy of the olfactory and vomeronasal systems, e.g., the anatomy of the nasal cavities and primary olfactory and vomeronasal projections, might provide information on phylogenetic relationships and/or correlate with different habitats of caecilians. Our results offer the basis for a comparison of the olfactory and vomeronasal systems in the three amphibian orders. We also further investigate the relationship of the caecilian tentacle to chemoreception by the vomeronasal system.

MATERIALS AND METHODS

Species examined

The morphology of the nasal cavities was studied in representatives of all six families included in the Order Gymnophiona (see Duellman and Trueb, '86). Species studied include Family Rhinatrematidae (northern South America), *Epicrionops petersi;* Ichthyophiidae (southeast Asia), *Ichthyophis glutinosus* and *I kohtaoensis;* Uraeotyphlidae (India), *Uraeotyphlus narayani;* Caeciliaidae, (Central and South America, Africa, Seychelles, India), subfamily Caeciliainae, *Caecilia occidentalis* (Peru) and *Oscaecilia ochrocephala* (Panama), subfamily Dermophiinae, *Dermophis mexicanus* (Central America), *Gymnopis multiplicata* (Central America), *Hypogeophis rostratus* (Seychelles), *Geotrypetes seraphini* (Ghana), *Sylvacaecilia grandisonae* (Ethiopia), *Afrocaecilia taitana* (Kenya), *Boulengerula boulengeri* (Kenya), and *Idiocranium russeli* (Cameroon); Scolecomorphidae (East and West Africa), *Scolecomorphus kirkii* and *Scolecomorphus uluguruensis;* Typhlonectidae, *Typhlonectes natans* and *Chthonerpeton indistinctum.*

Primary olfactory and vomeronasal projections were examined in an ichthyophiid, *I. kohtaoensis* (five animals), a caeciliaid, *D. mexicanus* (five animals), and a typhlonectid, *T. natans* (one animal).

METHODS

The morphology of nasal cavities was examined in heads of adults, which were serially sectioned (transversely, sagittally, or frontally) at 7–10 μm. Every third slide was stained with picroponceau, hematoxylin-eosin, or Mallory's azan. Sections were drawn with the aid of a camera lucida. Measurements of circumferences of the main nasal cavity, the vomeronasal organ, and their sensory and respiratory epithelia were digitized from drawings of five sections that included the greatest extent of each component to provide comparative data. These measurements, however, do not reflect the volumes of the structures because all extensions could not be included.

The primary projections of the olfactory and vomeronasal mucosae were demonstrated using the horseradish peroxidase (HRP) method. The animals were anesthetized in a 1:150 (w/v) solution of tricaine methanesulfonate (MS 222). A solution of 30% (w/v) HRP (HRP Boehringer, grade I) in 0.8% (w/v) NaCl, 5% (v/v) Nonidet (P 40), and 2% (v/v) dimethylsulfoxide was injected by inserting a syringe medially and laterally through the naris into the main nasal cavity. A selective staining of olfactory projections was achieved by injecting HRP exclusively into the medial olfactory sac. In order to stain vomeronasal projections selectively, we removed the skull and tissues covering the vomeronasal organ and injected HRP precisely into the vomeronasal organ. We reacted the nasal cavities as whole mounts in order to ascertain that HRP was injected only into the vomeronasal organ. We also injected HRP into the tentacle sac to investigate the connection between the tentacle and the vomeronasal organ.

The animals were sacrificed after 48 to 72 hours by overdose of anesthetic. They were transcardially perfused with a 0.8% (w/v) solution of NaCl followed by a solution of 2% (v/v) glutaraldehyde and 1% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) and 2.5% sucrose for fixation. The brain was removed, postfixed for 30 minutes, and rinsed for at least 2 hours in 0.1 M cacodylate buffer (pH 7.2). The brains were reacted as whole mounts in 0.2% (w/v) diaminobenzidine (Sigma) in 0.1 M cacodylate buffer (pH 5.45) and 0.015% hydrogen peroxide for 60 minutes. The reactions were performed according to the protocols of Fritzsch ('80, '81). Whole mounts were embedded in Histosec or Epon and cut into 30 μm sections.

RESULTS

Morphology of nasal cavities

The morphology of nasal cavities differs in size, shape, and epithelial distribution in the caecilians examined. The differences concern both the main nasal (primary olfactory) cavity, the "Hauptnase" (e.g., Wiedersheim, 1879; Sarasin and Sarasin, 1887–1890), as well as the vomeronasal organ, the "Nebennase." (Throughout this work, we will refer to these paired

structures in the singular for simplicity of expression.) Badenhorst ('78) reviewed the literature on these structures. He resolved some of the inconsistencies of terminology and description found in several papers that described the morphology of the heads, including olfactory components, of various species. We follow his usage.

Two types of main nasal cavities are distinguished (types A [undivided] and B [divided]; Table 1, Fig. 1) In *T. natans, C. indistinctum, B. boulengeri, S. grandisonae, H. rostratus, C. occidentalis, U. narayani, I. russeli,* and *O. ochrocephala* (the latter ascertained with difficulty due to preservation artifact), the main nasal cavity is uniform and undivided (type A; Table 1, Fig. 1). Respiratory epithelium covers the lateral part of the caudal cavity. The medial cavity and the lateral rostral cavity are lined with sensory epithelium. The only exceptions are *T. natans* and *C. indistinctum.* In these species sensory epithelium is found only in the rostral fourth of the cavity (Fig. 1). Most of the main nasal cavity is covered by respiratory epithelium. In contrast to all other species examined, the longitudinal axis of the main nasal cavity in *B. boulengeri* is not parallel to the longitudinal axis of the head. The axes form an angle of about 60°.

In *S. kirkii, S. uluguruensis, I. glutinosus, I. kohtaoensis, A. taitana, D. mexicanus, Gym. multiplicata, Geo. seraphini,* and *E. petersi* the ventral main nasal cavity is divided into medial and lateral cavities (type B; Fig. 1, Table 1). In these species the lateral cavity is lined by respiratory epithelium caudally and sensory epithelium rostrally. The medial cavity is coated with sensory epithelium. In *Epicrionops* the division of the main nasal cavity is not complete, i.e., the lateral and medial cavity join at its caudal part (Fig. 1). Most of the lateral cavity is covered by respiratory epithelium. *Afrocaecilia* has a slightly divided nasal cavity.

The connection between the main nasal cavity and the vomeronasal organ is very small in caecilians. The size and position of the vomeronasal organ differ in the species examined. In accord with earlier studies in amphibians (Parsons, '67; Badenhorst, '78, Seydel, 1895), the size and the position of the vomeronasal organ are described relative to the main nasal cavity. These relations do not reflect the position relative to the axis of the head, because the species examined differ with regard to head shape and to the rostrocaudal extension of nasal cavities. *S. kirkii, S. uluguruensis, I. glutinosus, I. kohtaoensis,* and *A. taitana* possess a vomeronasal organ that is situ-

TABLE 1. Morphology of the main nasal cavity and the vomeronasal organ in gymnophiones[1]

Family, genus, species	Vomeronasal organ	Main nasal cavity
Rhinatrematidae		
Epicrionops petersi	Lateral, extends caudad	B
Ichthyophidae		
Ichthyophis kohtaoensis	Mediolateral I	B
Ichthyophis glutinosus	Mediolateral I	B
Scolecomorphidae		
Scolecomorphus uluguruensis	Mediolateral I	B
Scolecomorphus kirkii	Mediolateral I	B
Caeciliaidae		
Subfamily Dermophiinae		
Afrocaecilia taitana	Mediolateral I	B
Boulengerula boulengeri	Mediolateral II	A
Sylvacaecilia grandisonae	Mediolateral II	A
Gymnopis multiplicata	Lateral	B
Dermophis mexicanus	Lateral	B
Geotrypetes seraphini	Mediolateral with lateral rostral projection	B
Hypogeophis rostratus	Mediolateral with lateral rostral projection	A
Idiocranium russeli	Mediolateral, probably with lateral rostral projection	A
Subfamily Caeciliainae		
Caecilia occidentalis	Mediolateral with lateral rostral projection	A
Oscaecilia ochrocephala	Mediolateral with lateral rostral projection	A(?)
Uraeotyphlidae		
Uraeotyphlus narayani	Mediolateral with lateral rostral projection	A
Typhlonectidae		
Typhlonectes natans	Mediolateral II	A
Chthonerpeton indistinctum	Mediolateral II	A

[1]A, uniform cavity; B, divided cavity; mediolateral I, vomeronasal organ lies between the medial and lateral cavity of the main nasal cavity; mediolateral II, vomeronasal organ lies beneath the uniform main nasal cavity.

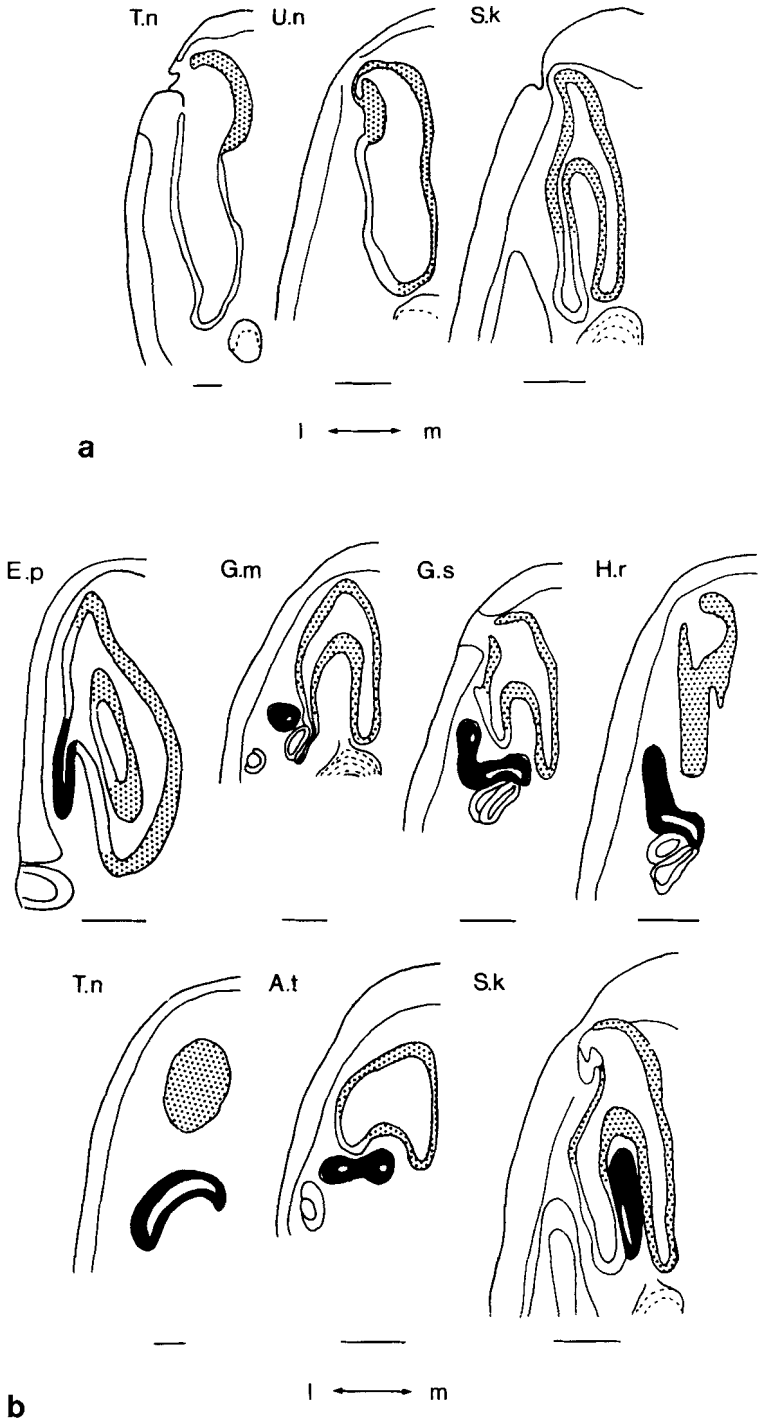

Figure 1

ated between the medial and the lateral cavities of the main nasal cavity (Fig. 1) and extends mediolaterally ventral to the lateral cavity of the main nasal cavity in *I. glutinosus, I. kohtaoensis,* and *A. taitana.* In *S. ulugurensis* the vomeronasal organ extends rostrocaudally between the lateral and medial cavities of the main nasal cavity and shows only a slight mediolateral extension. In *T. natans, C. indistinctum, B. boulengeri,* and *S. grandisonae* the vomeronasal organ extends mediolaterally ventral to the undivided main nasal cavity (Fig. 1). *D. mexicanus* and *Gym. multiplicata* possess the smallest vomeronasal organs; they lie ventral to the lateral cavity of the main nasal cavity and extend laterally (Fig. 1). In *Epicrionops* the entire vomeronasal organ extends caudad, lateral to the main nasal cavity. The vomeronasal organ joins the main nasal cavity at the lateral wall of the lateral cavity (Fig. 1). A caudal extension of the vomeronasal organ, as occurs in *Epicrionops,* has not been observed in any other taxa. *Geo. seraphini, H. rostratus, C. occidentalis, O. ochrocephala, U. narayani,* and *I. russeli* also possess a vomeronasal organ that extends mediolaterally ventral to the main nasal cavity and bend laterally to form a rostral extension (Figs. 1, 2; Table 1). *T. natans* and *C. indistinctum* are characterized by a very large vomeronasal organ, which is correlated with a very extensive innervation. Whole-mount preparations of the nasal cavities indicate a patent connection between the tentacular ducts and the vomeronasal organ (Fig. 3), as also shown by other workers (see summaries in Badenhorst ['78] and Billo and Wake ['87]).

Fig. 1. Line drawings of the morphology of **a:** main nasal cavities (sensory epithelium is shaded; respiratory epithelium is unshaded) in T.n, *Typhlonectes natans;* U.n, *Ureaotyphlus narayani;* S.k, *Scolecomorphus kirkii;* and **b:** vomeronasal organs (black) in E.p, *Epicrionops petersi;* G.m, *Gymnopis multiplicata;* G.s, *Geotrypetes seraphini;* H.r, *Hypogeophis rostratus;* T.n, *Typhlonectes natans;* A.t, *Afrocaecilia taitana;* S.k, *Scolecomorphus kirkii* all at the level of greatest extensions. Lateral is to the left and medial to the right. Two types of main nasal cavities can be distinguished: a uniform cavity (T.n and U.n) and a divided cavity (S.k). The main nasal cavity of T.n is characterized by a great amount of respiratory epithelium. The vomeronasal organ is characterized with respect to extension and position relative to the main nasal cavity: a lateral vomeronasal organ extending caudally in E.p, a small lateral vomeronasal organ with a slight mediolateral extension in G.m, a vomeronasal organ that extends mediolaterally and bends laterally to form a lateral rostral projection in G.s and H.r, a mediolateral vomeronasal organ in T.n and A.t extending mediolaterally beneath the undivided main nasal cavity in T.n and extending mediolaterally ventral to the lateral cavity of the main nasal cavity in A.t, and a vomeronasal organ extending mainly rostrocaudally between the lateral and the medial main nasal cavity in S.k. l, lateral; m, medial. Bars = 1 mm.

Olfactory and vomeronasal projections

Horseradish peroxidase experiments show that in *I. kohtaoensis* and *D. mexicanus,* paired dorsal and ventral nervi olfactorii originate in the olfactory mucosa and enter the bulbus olfactorius dorsally and ventrally, respectively (Figs. 5, 6). *T. natans* does not have a clear separation of the dorsal and ventral nervi olfactorii where they enter the bulbus olfactorius. The bulbus olfactorius constitutes the rostral telencephalon and is concentrically laminated (Fig. 6b). Fibers of the dorsal nervus olfactorius originate exclusively in the olfactory mucosa. The ventral nervus olfactorius consists of fibers which innervate both the olfactory mucosa and the vomeronasal mucosa. Ventral fibers of the ventral nervus olfactorius come from the olfactory mucosa while dorsal fibers of the ventral nervus olfactorius originate in the vomeronasal mucosa. Vomeronasal fibers cross the bulbus olfactorius caudally to enter the bulbus olfactorius accessorius (Figs. 4–6). The bulbus olfactorius accessorius lies caudal to the bulbus olfactorius and forms only a part of the lateral telencephalic wall (Fig. 6).

Olfactory projections

In *D. mexicanus* and *I. kohtaoensis* olfactory projections cover the bulbus olfactorius entirely (Fig. 4). The extensions of the olfactory projections are similar in the two species. The area of termination of olfactory fibers is smaller in *Typhlonectes* than in *Dermophis* and *Ichthyophis.* In *Typhlonectes* olfactory projections cover only the rostral bulbus olfactorius (Fig. 4). In *Ichthyophis* we find many glomeruli outside the main olfactory and vomeronasal projection areas (Figs. 4, 5a). These displaced glomeruli surround the olfactory and vomeronasal projection area at their caudal extent and consist exclusively of olfactory fibers, as demonstrated by selective stainings of olfactory projections (Fig. 5b). The *T. natans* examined has only one displaced glomerulus in the dorsal bulbus olfactorius, and *D. mexicanus* does not possess any displaced glomeruli.

Vomeronasal projections

In all species vomeronasal fibers cross the olfactory projection area dorsolaterally and terminate caudal to the bulbus olfactorius within the bulbus olfactorius accessorius, which is well separated from the bulbus olfactorius in *I. kohtaoensis* and *D. mexicanus* and less in *T. natans.* All species examined have only one vomeronasal projection field (Fig. 4). Injections of HRP into

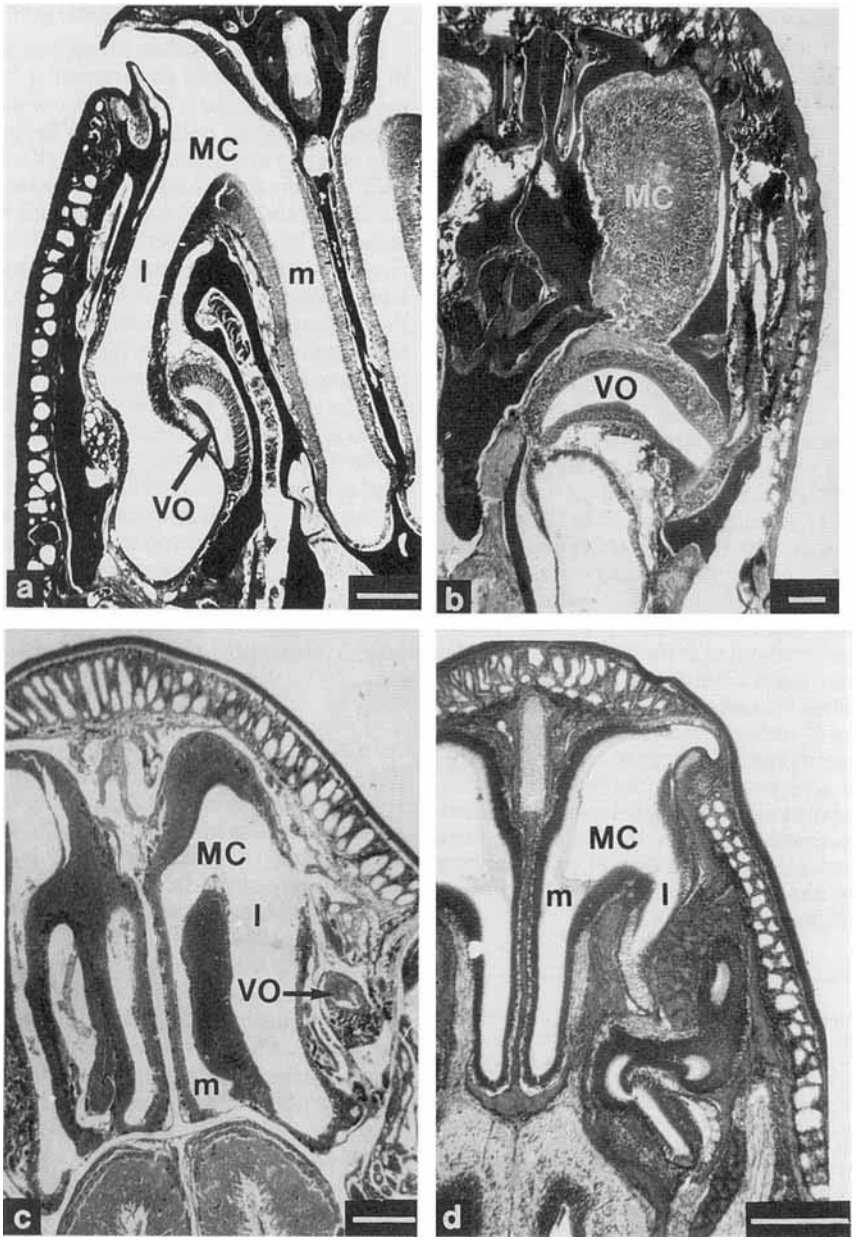

Fig. 2. Horizontal sections of nasal cavities in **a:** *Ichthyophis kohtaoensis* (divided main nasal cavity, vomeronasal organ between the medial and lateral main nasal cavity); **b:** *Typhlonectes natans* (uniform main nasal cavity, large vomeronasal organ); **c:** *Dermophis mexicanus* (divided main nasal cavity; lateral, small vomeronasal organ); and **d:** *Geotrypetes seraphini* (divided main nasal cavity, mediolateral vomeronasal organ with a lateral rostral projection). l, lateral cavity; m, medial cavity; MC, main nasal cavity; VO, vomeronasal organ. Bars = 1 mm.

the tentacular sac produce selective stainings of vomeronasal projections.

Nervus terminalis

Selective stainings of fibers that originate in the olfactory epithelium in *I. kohtaoensis* reveal fibers that branch off the displaced glomeruli (Figs. 4, 5b). These fibers run caudad to the anterior commissure and extend to the hypothalamus. *T. natans* has fibers which leave the olfactory projection area medially and run caudad to the septum. The course of all these fibers and

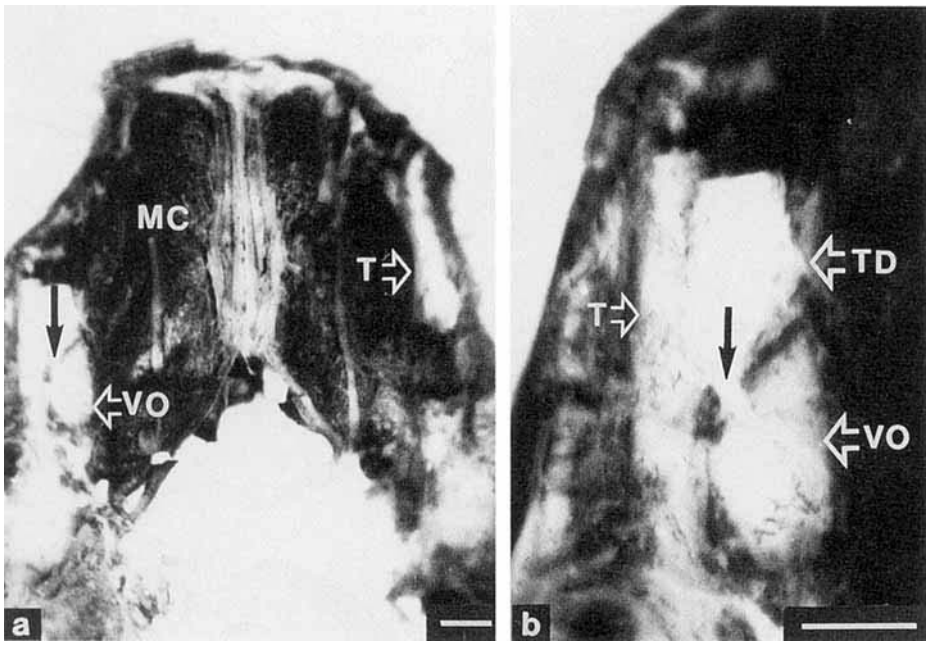

Fig. 3. Whole-mount preparation of the nasal cavities in *Typhlonectes natans* to show the connections between the tentacle ducts and the vomeronasal organ (arrows). The tentacle and the tentacle duct are cut at the left side. **b** shows a higher magnification of the connection at the left side in **a**. MC, main cavity; T, tentacle; TD, tentacle duct; VO, vomeronasal organ. Bars = 1 mm.

their terminations within the septum and the hypothalamus suggest that they are fibers of the nervus terminalis, as described for many vertebrates (Herrick, '09; Johnston, '13; Crapon de Caprona and Fritzsch, '83; Demski and Northcutt, '83; Münz et al., '82; von Bartheld and Meyer, '86; Schmidt et al., '88). Primary olfactory and vomeronasal projections terminate exclusively within the olfactory bulb and the accessory olfactory bulb, respectively. Fibers of the nervus terminalis are not labeled by selective stainings of vomeronasal projections.

DISCUSSION

Morphology of nasal cavities

Gymnophione species exhibit differences in the morphology of nasal cavities with regard to both the main nasal cavity and the vomeronasal organ. We distinguish two morphological types of main nasal cavity: an undivided cavity and a cavity that is divided into medial and lateral components. The vomeronasal organ differs with regard to shape, position, and size. We recognize a mediolateral position of the vomeronasal organ in *I. glutinosus, I. kohtaoensis, A. taitana, B. boulengeri, S. grandisonae, S. kirkii, S. uluguruensis, T. natans,* and *C. indistinctum;* a lateral position in *G. multiplicata, D. mexicanus,* and *E. petersi;* and one mediolateral with a lateral rostral extension in *U. narayani, H. rostratus, C. occidentalis, O. ochrocephala,* and *G. seraphini.*

E. petersi is the only species examined that has a vomeronasal organ which lies completely lateral to the main nasal cavity and which enters the main nasal cavity laterally. This position resembles that found in urodeles, although in urodeles the vomeronasal organ forms a lateral outpocketing of the main nasal cavity with a continuous connection between the main cavity and the vomeronasal organ. In *Epicrionops* the main nasal cavity and the vomeronasal organ are almost completely separated. In most anurans the vomeronasal organ lies ventromedially, though *Pipa* has a lateral vomeronasal organ (Parsons, '67). Jürgens ('71) hypothesized that in anurans a shifting of the vomeronasal organ from anterolateral to anteromedial has taken place during phylogeny. The phylogenetic implications of these morphological patterns are discussed below.

The main nasal cavities of *T. natans* and *C. indistinctum* have less sensory epithelium than respiratory epithelium. In other species the main nasal cavity mostly is covered by sensory epithelium. These results likely reflect the phylogenetic history, and particularly adaptation to the

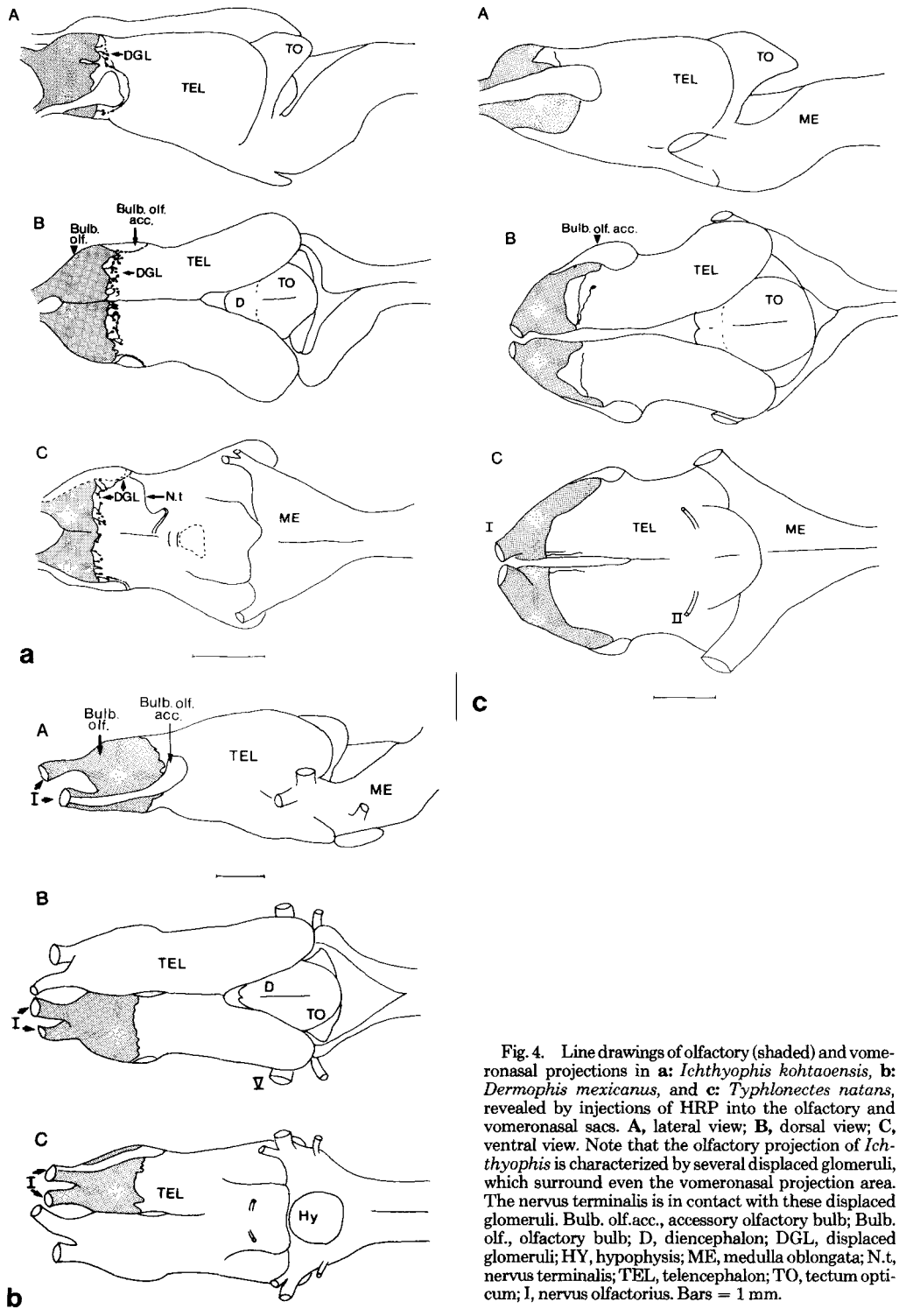

Fig. 4. Line drawings of olfactory (shaded) and vomeronasal projections in **a:** *Ichthyophis kohtaoensis*, **b:** *Dermophis mexicanus*, and **c:** *Typhlonectes natans*, revealed by injections of HRP into the olfactory and vomeronasal sacs. **A,** lateral view; **B,** dorsal view; **C,** ventral view. Note that the olfactory projection of *Ichthyophis* is characterized by several displaced glomeruli, which surround even the vomeronasal projection area. The nervus terminalis is in contact with these displaced glomeruli. Bulb. olf.acc., accessory olfactory bulb; Bulb. olf., olfactory bulb; D, diencephalon; DGL, displaced glomeruli; HY, hypophysis; ME, medulla oblongata; N.t, nervus terminalis; TEL, telencephalon; TO, tectum opticum; I, nervus olfactorius. Bars = 1 mm.

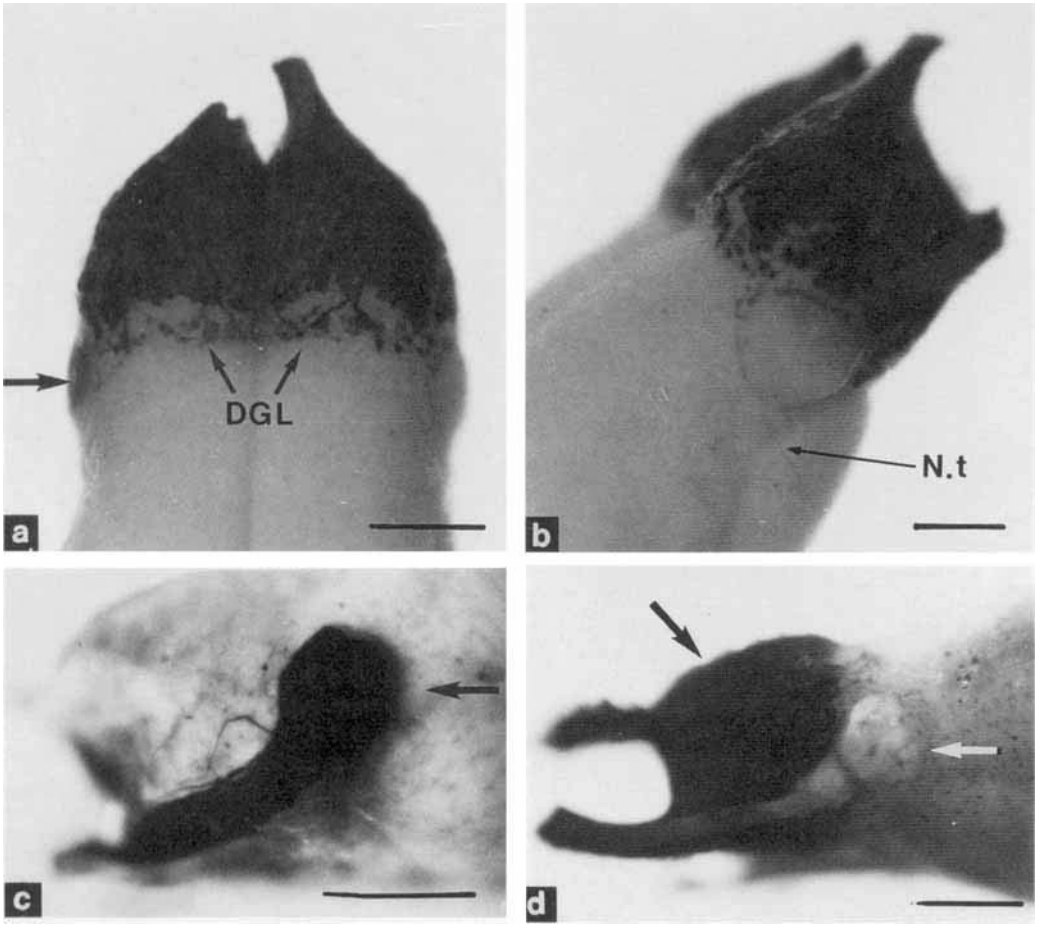

Fig. 5. Dorsal (**a**) and lateral (**b**) views of the rostral telencephalon in *Ichthyophis kohtaoensis*. In a, olfactory projections are stained at both olfactory bulbs while vomeronasal projections are stained only at the left side (arrow). In b, the nervus terminalis joins fibers that run around the spared vomeronasal projection field at the right side. Lateral views (**c,d**) of the rostral telencephalon in *Dermophis mexicanus*. Selective staining of vomeronasal projections by application of HRP to the vomeronasal mucosa is shown in c; selective staining of olfactory projections (black arrow) in *Dermophis mexicanus* by application of HRP to the olfactory mucosa is shown in d. Vomeronasal projections are unstained (white arrow). DGL, displaced glomeruli; N.t, nervus terminalis. Bars = 500 μm.

aquatic to semiaquatic habitat, of these animals. It is assumed that the typhlonectid ancestor was terrestrial and that extant species are secondarily aquatic. Several morphological features unique among caecilians are thought to be associated with aquatic life. For example, typhlonectids are the only caecilians that have extensively bilateral lung development, though the length of the second lung varies among taxa. Species of all other families characteristically have only the right lung well developed, though some have small "tracheal" lungs as well. The great amount of respiratory epithelium in typhlonectids may be related functionally to the existence of bilateral lungs and to a requirement for extensive gaseous exchange epithelium in low oxygen tension aquatic habitats. Typhlonectids come to the surface to breathe and stay submerged for long periods of time.

There are different opinions of the evolutionary history of the vomeronasal organ. Broman ('20) and Parsons ('67) hypothesize that the vomeronasal epithelium corresponds to the olfactory epithelium of fishes. Bertmar ('81) and Jürgens ('71) regard the vomeronasal organ as an adaptation to terrestrial life. This was indicated by Anton ('12) and Bruner ('14), who found a small vomeronasal organ in aquatic urodeles,

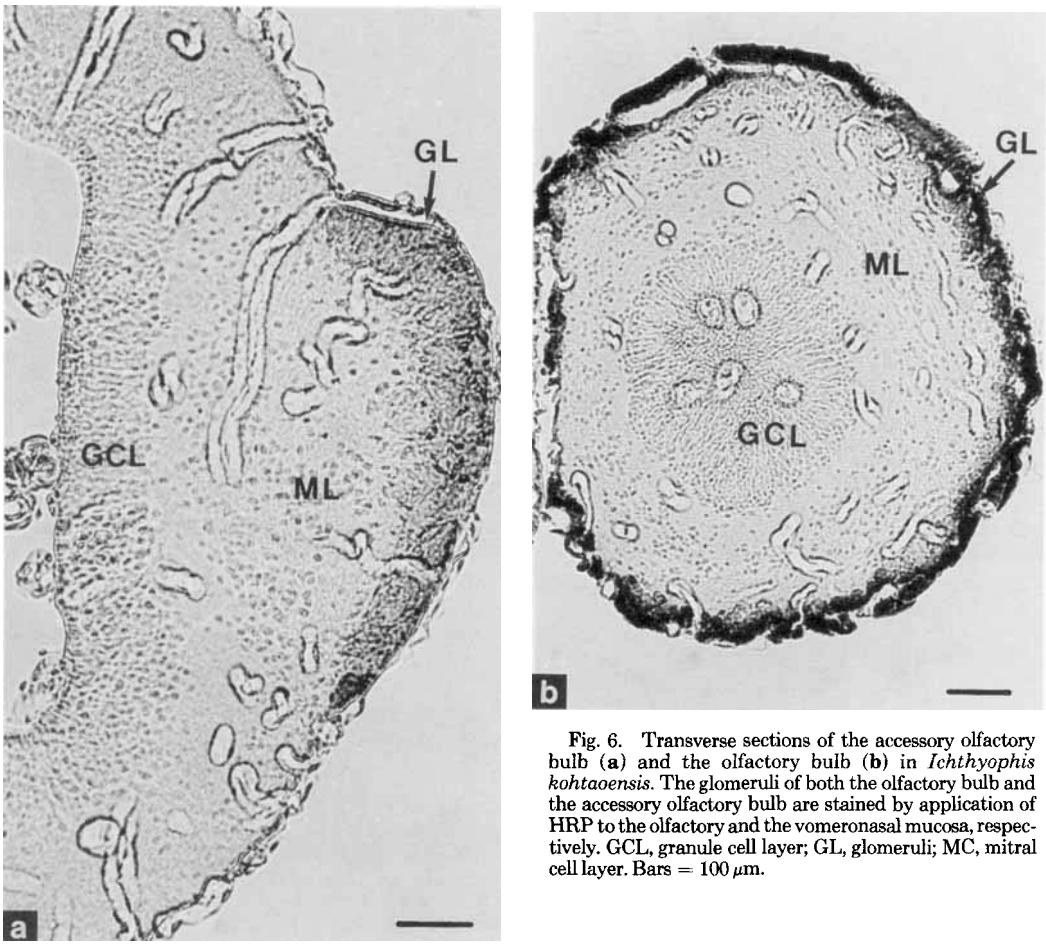

Fig. 6. Transverse sections of the accessory olfactory bulb (**a**) and the olfactory bulb (**b**) in *Ichthyophis kohtaoensis*. The glomeruli of both the olfactory bulb and the accessory olfactory bulb are stained by application of HRP to the olfactory and the vomeronasal mucosa, respectively. GCL, granule cell layer; GL, glomeruli; MC, mitral cell layer. Bars = 100 μm.

and by Medvedeva ('67), who described a reduction of the vomeronasal organ in animals that returned to water secondarily. However, this is not the case in *T. natans* and *C. indistinctum*. Our studies show that the typhlonectids examined have a very large vomeronasal organ. We believe that Medvedeva's conclusions regarding a loss of functional demands for the vomeronasal organ in animals which return to water secondarily may not be appropriate. We question that there is a loss of functional demand because the vomeronasal system may well facilitate the perception of water soluble odorants.

Olfactory and vomeronasal projections

Though the vomeronasal projections are similar in the species examined via HRP stainings, there are differences in olfactory projections. *T. natans* has the smallest olfactory projection area, corresponding to its small amount of olfactory epithelium. The olfactory projection of *I. kohtaoensis* is characterized by many displaced glomeruli which surrounded the olfactory and vomeronasal projection area at their caudal extent. It remains unresolved whether these displaced glomeruli represent a functional specialization or whether they are the beginning of an extended projection. Displaced glomeruli, although fewer in number, have been found in urodeles as well (Schmidt et al., '88). In urodeles the displaced glomeruli appeared to be in contact with the nervus terminalis. Our experiments show that this is also the case in caecilians.

Staining of the nervus terminalis was achieved by selective stainings of olfactory projections. HRP labelings of vomeronasal projections did not label fibers of the nervus terminalis. The nervus terminalis is thought to be involved in the perception of pheromones (see Demski and Northcutt, '83, for summary). The terminations

of the nervus terminalis within the septum, the preoptic region, and the hypothalamus, which have been shown in many vertebrates (Herrick, '09; McKibben, '11; Johnston, '13; Crapon de Caprona and Fritzsch, '83; Demski and Northcutt, '83; Münz et al., '82; von Bartheld and Meyer, '86; Schmidt et al., '88) and evidence for gonadotropin-releasing factor immunoreactivity in neurons of the nervus terminalis (summarized in Muske and Moore, '88) lend credence to the hypothesis of pheromone perception. In this context, it is surprising that fibers of the nervus terminalis stained in our experiments originated exclusively from the olfactory projection.

Olfactory projections in caecilians are different from those of urodeles and anurans. In urodeles primary olfactory projections form an oval shaped lateral termination area (Schmidt et al., '88). In anurans primary olfactory projections enter the olfactory bulb ventrally (Northcutt and Kicliter, '80).

The vomeronasal projections that we have shown for three caecilian species bear resemblance to vomeronasal projections found in anurans, forming one vomeronasal projection field (Weiss, personal communication) and to those found in urodelan species that are considered to be derived. Even though many urodeles show several vomeronasal projection areas, other urodeles such as the strictly terrestrial Plethodontini and Bolitoglossini (highly specialized plethodontids: Wake, '66) have vomeronasal projection fields that are reduced in number and extent (Schmidt et al., '88). The reduction of vomeronasal projection fields in Plethodontini and Bolitoglossini is due to a reduction of the entire vomeronasal system of these animals (unpublished results) and may be correlated with the terrestrial life of these groups. In caecilians the number of vomeronasal projections relative to the amount of olfactory projections differed. *T. natans* has the strongest vomeronasal projection of the species examined. This projection size is correlated with the large vomeronasal organs we found in typhlonectid species. Even though *T. natans* has only one vomeronasal projection field, it was very enlarged compared to those of *I. kohtaoensis* and *D. mexicanus*.

Role of the caecilian tentacle in vomeronasal perception

Opinions differ on the functional role of the caecilian tentacle. Some authors have considered it tactile (Sarasin and Sarasin, 1887–1890; Engelhardt, '24; Laubmann, '27; Marcus, '30; Fox, '85). The investigations of Badenhorst ('78), Billo ('86), and Billo and Wake ('87) indicate that the tentacle is chemosensory as well as tactile. Billo ('86) found Merkel cells, which are thought to be tactile, in the tip of the tentacular fold, though he did not state which of the species (*I. kohtaoensis* and *T. compressicauda*) he examined. Fox ('85) stated that Merkel cells are absent in *I. kohtaoensis* and *I. orthoplicatus*. The studies of development and adult structure of the tentacle and the vomeronasal organ by Marcus ('30) on *Hypogeophis*, by Badenhorst ('78) on *Ichthyophis*, and by Billo and Wake ('87) on *Dermophis* and *Gymnopis* clearly show that there is a connection between the tentacle and the vomeronasal organ. Billo and Wake ('87) demonstrate that patent ducts from the tentacular sac join the vomeronasal organ in fetuses that are near birth size (approximately 105 mm total length [TL] in *Dermophis;* 84 mm TL in *Gymnopis*).

Our experiments verify that there is a patent connection between the tentacular ducts and the vomeronasal organ in adults of all species examined. Injections of HRP into the tentacular sac led to selective stainings of vomeronasal projections. This is further evidence that the tentacle is functionally related to the vomeronasal organ. Odorants entering the tentacular lumen may be led via the tentacular ducts to the vomeronasal organ. Therefore animals would be able to smell even if the main nasal cavity is closed by constriction of the nostrils during burrowing or swimming.

Phylogenetic relationships

We believe that when surveys of morphology across taxa are conducted, the data should be examined to see if characters of systematic significance emerge. Our data on olfactory and vomeronasal organ morphology contribute new characters potentially useful for assessment of phylogenetic relationships.

Six characters emerged from analysis of olfactory and vomeronasal morphology (the character states and the data matrix are presented in Appendix A). Characters were polarized according to the condition in the outgroup to caecilians (urodeles) and to the ontogeny of the character (if known). Characters include 1) shape of the main nasal cavity; 2) orientation of the main nasal cavity to the axis of the head; 3) extent of respiratory epithelium lining; 4) vomeronasal organ position; 5) vomeronasal with or without a caudal extension; and 6) size of vomeronasal organ.

The six characters were subjected to a PAUP computer analysis (Swofford, '84) to see whether character states of the olfactory/vomeronasal system showed correlations with each other and with the current associations of genera in fami-

lies based on other characters. We recognize that six characters for 18 species do not have real resolution power, especially since some derived states are shared broadly (see Table 1 and Appendix A). Characters 1 and 4 (and to some degree 5) seemed in conflict in terms of numbers of reversals or convergences. Running the program with characters 1 and 4 (see above) unordered and unpolarized did not produce further resolution. When the analysis is run with character 4 polarized in accord with the outgroup state, *Gymnopis* and *Dermophis* are sister groups with *Epicrionops* their sister taxon, and the group is nested high in the tree; the polychotomy of *Idiocranium, Hypogeophis, Uraeotyphlus, Oscaecilia,* and *Caecilia* is lowest in the tree. When the analysis is run with characters 1, 4, and 6 unordered, or with all characters unordered, *Epicrionops* is the most primitive taxon and *Gymnopis–Dermophis* its sister group. This grouping is in better accord with the phylogenetic hypothesis based on other morphological criteria (cf. Duellman and Trueb, '86). Depending on how the characters were ordered, three to six equally parsimonious trees were produced.

Character 2, the angled main nasal cavity, is an autapomorphy unique to *Boulengerula,* hence uninformative. Characters 3 and 6 are autapomorphies for *Typhlonectes* and *Chthonerpeton,* the two members of the family Typhlonectidae examined (see below). The state of character 5 is shared by all taxa except *Epicrionops,* which is primitive on other grounds. Similarities among the PAUP trees and that from a compatibility analysis indicate that *Dermophis* and *Gymnopis* are closely allied; one large, poorly resolved block of taxa includes *Ichthyophis, Scolecomorphus,* and *Afrocaecilia,* with *Boulengerula* and *Sylvacaecilia* less closely allied; the other large, poorly resolved block consistently includes *Uraeotyphlus, Geotrypetes, Idiocranium, Hypogeophis, Caecilia,* and *Oscaecilia,* with the typhlonectids their sister group.

The postulated relationships show some variations of interest. For example, the Old World ichthyophiids and scolecomorphids, and the "caeciliaid" *Afrocaecilia* cluster together, with *Boulengerula,* sometimes postulated to be related to *Afrocaecilia,* less closely associated. Ichthyophiids are considered primitive in a number of characters of osteology and reproductive biology, while *Afrocaecilia* and scolecomorphids are derived. Uraeotyphlids are not allied in any way to ichthyophiids by the morphology of the olfactory system, though many other kinds of morphological characters suggest a relationship. Further, *Sylvacaecilia* and *Geotrypetes,* until recently congeneric (Wake, '87), do not have similar olfactory morphology at all. However, we suggest that typhlonectid morphology—the large size of the vomeronasal organ, its extensive innervation, its limited olfactory projections, and the reduced sensory epithelium but increased respiratory epithelium of the main nasal cavities—is in contrast to the condition in all other species examined and likely is correlated with their secondarily aquatic mode of life, including effective function of the vomeronasal organ.

The analysis does show, however, that species in the same genus, at least in the cases we tested (*Ichthyophis* and *Scolecomorphus*), share the same morphology, as do the genera *Dermophis* and *Gymnopis,* terrestrial viviparous Central American dermophiines considered closely related based on other characters (Case and Wake, '77). Indications that closely related taxa share the same morphology suggest that changes in the olfactory and vomeronasal organs are relatively old. Further, since genera assigned to a diversity of families based on other data share certain characters of the olfactory system in a mosaic fashion, we infer that change is relatively constrained and that many features are in common.

We recognize that while the position of the vomeronasal organ suggests that caecilians and urodeles are sister taxa, the anatomy of the olfactory bulb and the olfactory projections in the three taxa that we examined (*Ichthyophis, Dermophis,* and *Typhlonectes*) is different from the urodelan condition.

We are accumulating information that provides new characters from morphological "systems" not previously examined comparatively. Neuroanatomical data are not often used in phylogenetic analysis. While data from any one "system" may not be conclusive, an examination of overall neuroanatomical patterns is likely to be useful in understanding both phylogeny and adaptive radiation. Our data on the morphology of the olfactory and vomeronasal system of selected caecilian species facilitate testable hypotheses of function and life history and of some phylogenetic correlates.

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LITERATURE CITED

Anton, W. (1912) Die Nasenhöle der Perennibranchiaten (Ein Beitrag zur Phylogenese des Jacobson schen Organs). Morphol. Jb. *44*:179–199.

Badenhorst, A. (1978) The development and the phylogeny of the organ of Jacobson and the tentacular apparatus of *Ichthyophis glutinosus*. Ann. Univ. Stellenbosch Ser. A2 (Sölogie) *1*:1–26.

Bertmar, G. (1981) Evolution of vomeronasal organs in vertebrates. Evolution *35*:359–366.

Billo, R. (1986) Der Tentakel der Gymnophionen (Amphibia, Gymnophiona) Dissertation, Universität Basel.

Billo, R., and M.H. Wake (1987) Tentacle development in *Dermophis mexicanus* (Amphibia, Gymnophiona) with an hypothesis of tentacle origin. J. Morphol. *192*:101–111.

Broman, I. (1920) Das Organon vomero-nasale Jacobsoni—ein Wassergeruchsorgan! Anat. Hefte *58*:143–191.

Bruner, H.L. (1914) Jacobson's organ and the respiratory mechanism of amphibians. Morphol. Jb. *48*:157–165.

Case, S.M., and M.H. Wake (1977) Immunological comparisons of caecilian albumins (Amphibia: Gymnophiona). Herpetologica *33*:94–98.

Crapon de Caprona, M.-D., and B. Fritzsch (1983) The development of the retinopetal nucleus olfacto-retinalis of two cichlid fishes as revealed by horseradish peroxidase. Dev. Brain Res. *11*:281–301.

Demski, L.S., and R.G. Northcutt (1983) The terminal nerve: a new chemosensory system in vertebrates? Science *220*: 435–437.

Duellman, W.E., and L. Trueb (1986) Biology of Amphibia. New York: McGraw Hill, Inc.

Engelhardt, G. (1924) Tentakelapparat und Auge von *Ichthyophis*. Jen. Zeitschr. Naturw. *60*:241–305.

Fox, H. (1985) The tentacles of *Ichthyophis* (Amphibia: Caecilia) with special reference to the skin. J. Zool. Lond. [A] *205*:223–234.

Fritzsch, B. (1980) Retinal projections in European Salamandridae. Cell Tissue Res. *213*:325–341.

Fritzsch, B. (1981) Efferent neurons to the labyrinth of *Salamandra salamandra* as revealed by retrograde transport of horseradish peroxidase. Neurosci. Lett. *26*:13–17.

Herrick, C.J. (1909) The nervus terminalis (nerve of Pinkus) in the frog. J. Comp. Neurol. *37(3)*:175–190.

Johnston, J.B. (1913) Nervus terminalis in reptiles and mammals. J. Comp. Neurol. *23*:150–156.

Jürgens, J.D. (1971) The morphology of the nasal region of Amphibia and its bearing on the phylogeny of the group. Ann. Univ. Stellenbosch Ser. A, No. 2, *46*:1–146.

Laubmann, W. (1927) Uber die Morphogenese vom Gehirn und Geruchsorgan der Gymnophionen (Beitrag zur Kenntnis der Gymnophionen Nr. 10). Z. Anat. Entw. *84*:597–637.

Marcus, H. (1930) Uber die Bildung von Geruchsorgan, Tentakel und Choanen bei *Hyphogeophis*, nebst Vergleich mit Dipnoern und Polypterus (Beitrag zur Kenntnis der Gymnophionen Nr. XIII). Z. Ges. Anat. 1: Z. Anat. Entw. Gesch. *91*:657–691.

McKibben, P.S. (1911) The nervus terminalis in urodele amphibia. J. Comp. Neurol. *21*:261–309.

Medvedeva, I.M. (1967) Die Homologie des Jacobsonschen Organs bei Anura und Urodela. In T. Orvig (ed.): Current Problems of Lower Vertebrate Phylogeny. Stockholm: Almquist & Wiksell, pp. 331–341.

Münz, H., B. Claas, W.E. Stumpf, and L. Jennes (1982) Centrifugal innervation of the retina by luteinizing hormone releasing hormone (LHRH): Immunoreactive telencephalic neurons in teleostean fishes. Cell Tissue Res. *222*: 313–323.

Muske, L.E., and F.L. Moore (1988) The nervus terminalis in amphibians: Anatomy, chemistry and relationship with the hypothalamic gonadotropin-releasing hormone system. Brain Behav. Evol. *32*:141–150.

Northcutt, G., and E. Kicliter (1980) Organization of the amphibian telencephalon. In S.O.E. Ebbesson (ed.): Comparative Neurology of the Telencephalon. New York: Plenum Press, pp. 203–255.

Parsons, T.S. (1967) Evolution of the nasal structure in the lower tetrapods. Am. Zool. *7*:397–413.

Sarasin, P., and F. Sarasin (1887–1890) Zur Entwicklungsgeschichte und Anatomie der ceylonesischen Blindwühle *Ichthyophis glutinosus*. 1. bis 4. Teil. Wiesbaden: C.W. Kreidel's Verlag.

Schmidt, A., C. Naujoks-Manteuffel, and G. Roth (1988) Olfactory and vomeronasal projections and the pathway of the nervus terminalis in ten species of salamanders: A whole-mount study employing the horseradish-peroxidase technique. Cell Tissue Res. *251*:45–50.

Seydel, C. (1895) Uber die Nasenhohle und das Jacobson'sche Organ der Amphibien. Morphol. Jb. *23*:453–543.

Swofford, D.L. (1984) Phylogenetic analysis using parsimony (PAUP), version 2.3. Illinois Natural History Survey.

von Bartheld, C.S., and D.L. Meyer (1986) Tracing of single fibers of the nervus terminalis in goldfish brain. Cell Tissue Res. *245*:143–158.

Wake, D.B. (1966) Comparative osteology and evolution of the lungless salamanders, Family Plethodontidae. Mem. South. Calif. Acad. Sci. *4*:1–111.

Wake, M.H. (1987) A new genus of African caecilian (Amphibia: Gymnophiona). J. Herpetol. *21*:6–15.

Wiedeisheim, R. (1879) Die Anatomie der Gymnophionen. Jena: Gustav Fischer Verlag, pp. 101.

Appendix appears on following page.

Appendix A. Characters and character code matrix

Character 1: Main nasal cavity undivided (0), slightly divided (1a), divided (1b)
Character 2: Longitudinal axis of main nasal cavity parallel to longitudinal axis of the head (0), longitudinal axis of main nasal cavity at 60° angle to longitudinal axis of the head (1)
Character 3: Respiratory epithelium concentrated in lateral part of caudal region of main nasal cavity (0), respiratory epithelium covering most of main nasal cavity (1)
Character 4: Vomeronasal organ mediolateral with lateral projection (0), mediolateral (1a), lateral (1b)
Character 5: Vomeronasal organ with caudal extension (0), without caudal extension (1)
Character 6: Vomeronasal organ moderate in size (0), vomeronasal organ large (1)

	Character					
Species	1	2	3	4	5	6
Epicrionops petersi	1a	0	0	1b	0	0
Ichthyophis glutinosus	1b	0	0	1a	1	0
Ichthyophis kohtaoensis	1b	0	0	1a	1	0
Uraeotyphlus narayani	0	0	0	0	1	0
Caecilia occidentalis	0	0	0	0	1	0
Oscaecilia ochrocephala	0	0	0	0	1	0
Afrocaecilia taitana	1b	0	0	1a	1	0
Boulengerula boulengeri	0	1	0	1a	1	0
Hypogeophis rostratus	0	0	0	0	1	0
Sylvacaecilia grandisonae	0	0	0	1a	1	0
Geotrypetes seraphini	1b	0	0	0	1	0
Idiocranium russeli	0	0	0	0	1	0
Gymnopis multiplicata	1b	0	0	1b	1	0
Dermophis mexicanus	1b	0	0	1b	1	0
Scolecomorphus kirkii	1b	0	0	1a	1	0
Scolecomorphus uluguruensis	1b	0	0	1a	1	0
Typhlonectes natans	0	0	1	1a	1	1
Chthonerpeton indistinctum	0	0	1	1a	1	1