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Ultrastructure of the Mature Spermatozoa of Caecilians (Amphibia: Gymnophiona)

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ABSTRACT The spermatozoa of Gymnophiona show the following autapomorphies: 1) penetration of the distal centriole by the axial fiber; 2) presence of an acrosomal baseplate; 3) presence of an acrosome seat (flattened apical end of nucleus); and 4) absence of juxta-axonemal fibers. The wide separation of the plasma membrane bounding the undulating membrane is here also considered to be apomorphic. Three plesiomorphic spermatozoal characters are recognized that are not seen in other Amphibia but occur in basal amniotes: 1) presence of mitochondria with a delicate array of concentric cristae (concentric cristae of salamander spermatozoa differ in lacking the delicate array); 2) presence of peripheral dense fibers associated with the triplets of the distal centriole; and 3) presence of a simple annulus (a highly modified, elongate annulus is present in salamander sperm). The presence of an endonuclear canal containing a perforatorium is a plesiomorphic feature of caecilian spermatozoa that is shared with urodeles, some basal anurans, sarcopterygian fish, and some amniotes. Spermatozoal synapomorphies are identified for 1) the Uraeotyphlidae and Ichthyophiidae, and 2) the Caeciliidae and Typhlonectidae, suggesting that the members of each pair of families are more closely related to each other than to other caecilians. Although caecilian spermatozoa exhibit the clear amphibian synapomorphy of the unilateral location of the undulating membrane and its axial fiber, they have no apomorphic characters that suggest a closer relationship to either the Urodela or Anura. J. Morphol. 258:179–192, 2003.
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KEY WORDS: *Gegeneophis*; *Ichthyophis*; *Typhlonectes*; *Uraeotyphlus*; spermatozoa; phylogeny

Caecilians (Gymnophiona) are one of the three orders of extant Amphibia. They are relatively poorly known snake or worm-like limbless amphibians. Approximately 160 species, 32 genera, and six families of these distinctive amphibians are currently recognized, although the lower-level taxonomy of the group is not stable (Nussbaum and Wilkinson, 1989). Given that the group has a primarily tropical distribution and the majority of species are fossorial, it is perhaps not surprising that knowledge of many aspects of caecilian biology lag behind that of frogs (Anura) and salamanders (Urodela). The single biggest obstacle to advances in caecilian biology has been the paucity of material for study. However, some caecilian species, such as the aquatic *Typhlonectes natans*, are often available commercially through the pet trade, and at least some terrestrial species are known to be locally abundant, offering improved opportunities for ecological as well as morphological study (e.g., Oommen et al., 2000; Measey et al., 2001).

The only caecilian spermatozoon that has been examined ultrastructurally is that of *Typhlonectes natans* (Typhlonectidae) (van der Horst and van der Merwe, 1991; van der Horst et al., 1991). Until recently, light microscope observations had been reported for just five other taxa, *Ichthyophis glutinosus* (Ichthyophiidae), *Uraeotyphlus narayani* (Uraeotyphlidae), *Siphonops annulatus* (Caeciliidae), and *Gegeneophis carnosus* (Caeciliidae) by Seshachar (1939, 1940, 1943, 1945), and *Chthonerpeton indistinctum* (Typhlonectidae) by de Sa and Berois (1986). Owing to changes in caecilian taxonomy and an absence of known voucher specimens, the specific, although not the generic, identity of the material referred to by Seshachar as *I. glutinosus, G. carnosus*, and perhaps also *U. narayani* must be considered uncertain. Recently, Wake (1994) has greatly augmented the number of caecilians (22 genera, 29 species, representing all families) examined for spermatozoal morphology using light microscopy. Unfortunately, due to the age and fixation of some of the samples used by Wake (1994), major features of spermatozoal morphology, such as the presence or absence of an undulating membrane, were often uncertain. Furthermore, important features such as the structure of the acrosome or the

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occurrence of axial and/or juxta-axonemal fibers were not determinable by the techniques employed by Wake (1994).

The present account is the first ultrastructural description of mature spermatozoa of representatives of the caecilian families Ichthyophiidae (*Ichthyophis beddomei* and *I. tricolor*), Uraeotyphlidae (three forms, probably distinct species, of *Uraeotyphlus*), and Caeciliidae (*Gegeneophis ramaswamii)*. The spermatozoa of *Typhlonectes natans* (Typhlonectidae) is reexamined in order to obtain more detail of its structure. Description of spermatozoal ultrastructure in four of the six currently recognized caecilian families allows a preliminary discussion of caecilian interrelationships based on spermatozoal characters.

MATERIALS AND METHODS

Caecilians used in this study were collected from synanthropic cultivated habitats in the Western Ghats regions of Kerala, Southern India, with the exception of a single specimen of the South American *Typhlonectes natans* (Fischer, 1880 "1879") which was obtained commercially and is believed to have come from Colombia. Voucher specimens of all Indian caecilians are deposited in the collection of the Department of Zoology, University of Kerala, as follows.

Gegeneophis ramaswamii Taylor, 1964: MW 79 collected at Bonaccord, Thiruvananthapuram, Kerala (08°40′46.3″N, 77°10′08.6″E, 593m asl) on 14 October 1999; MW 180-182 collected either at Bonaccord or Thekkada, Thiruvananthapuram, Kerala (08°37′43.1″N, 76°57′38.1″E, 60m asl) in either 1998 or 1999.

Ichthyophis beddomei Peters, 1880 "1879": MW 283, collected at Thalapuzha, Wayanad, Kerala (11°49′59.4″N, 75°57′53.6″E, 700m asl) on 21 October 1999. *Ichthyophis beddomei* is a poorly circumscribed species. The type specimen may be lost (Taylor, 1968) and it is unclear that all reports of this species actually pertain to the taxon described by Peters (1879). The type locality, the Nilghiris Hills, is ~60 km from Thalapuzha and of similar altitude. MW 283 fits the current but probably poorly refined concepts of this species (Taylor, 1968; Pillai and Ravichandran, 1999) reasonably well. However, the assignment of MW 283 to *I. beddomei* is with the caveat that it might be affected by any future revision of *I. beddomei. Ichthyophis tricolor* Annandale, 1909: MW 127, collected at Kollamom, Thiruvananthapuram, Kerala (08°32′21.8″N, 77°06′37.9″E, 139m asl) on 14 October 1999; MW 183-4, collected at Thekkada, Thiruvananthapuram, Kerala (08°37′43.1″N, 76°57′38.1″E, 60m asl) on 18 December 1998.

Uraeotyphlus A: MW 202, collected at Kattayad, Wayanad, Kerala (11°40′00″N, 75°16′53.36″E, 906m asl) in either October 1998 or on 06 October 1999. *Uraeotyphlus* B: MW 309, collected at Chittadi Estate, Kottayam, Kerala (09°33′18.3″N, 76°51′42.5″E ~50m asl) on 24 October 1999. *Uraeotyphlus* C: MW 177-179, collected at Muvattupuzha, Ernakulam, Kerala (09°58′00″N, 76°34′36″E, 27m asl) on 20 June 1999.

There are five nominate species in the genus *Uraeotyphlus*. Although we are confident that the *Uraeotyphlus* described here are not *U. malabaricus*, the lower-level taxonomy of the group is not well understood and precludes us from assigning the *Uraeotyphlus* material to any of the four other species at this time. We suspect, but are not certain, that the three forms referred to here as A, B, and C are distinct species and this is under further investigation.

The testes were removed and fixed for TEM in 3% glutaraldehyde in 0.1 M sodium phosphate buffer (PB; pH 7.2) at 4°C for at least 2 h before being transported at ambient temperature to Brisbane for processing and sectioning. The material was then rinsed in 0.1 M PB, postfixed for 80 min in similarly buffered 1% osmium tetroxide, rinsed in buffer, dehydrated through an ascending ethanol series (20–100%), and infiltrated and embedded in Spurr's epoxy resin (Spurr, 1969).

Sections were cut with diamond knives on an LKB 2128 UM IV microtome. Thin sections, 50–80 nm thick, were collected on carbon stabilized, colloidin-coated, 200 μm mesh copper grids, stained for 30 sec in Reynolds' lead citrate (Reynolds, 1963), rinsed in distilled water, then placed in 6% aqueous uranyl acetate for 4 min, rinsed in distilled water, and stained for a further 2 min in lead citrate before final rinsing (Daddow, 1986). Electron micrographs were taken on a Hitachi 300 electron microscope at 75 kV. Light microscopic observations and photographs of spermatozoa, from glutaraldehyde-fixed tissue squashes, were made using an Olympus BH2 microscope with Nomarski interference contrast and an attached OM-2 camera.

RESULTS

Spermatozoa of *Ichthyophis* and *Uraeotyphlus*

Examination of the spermatozoa of all three species of *Uraeotyphlus*, and the two species of *Ichthyophis*, revealed no difference in ultrastructure within a genus; however, differences in dimensions (Table 1) of the spermatozoa were detected between species and give some support to the proposition that at least *Uraeotyphlus* A and B are distinct species. Due to the small number of mature spermatozoa present in the testis material of *Uraeotyphlus* C, no measurements were obtained for this species.

The general structure of the spermatozoa of *Ichthyophis tricolor, I. beddomei*, and *Uraeotyphlus* species A, B, and C is sufficiently similar to be described together, while noting the few observed differences between the two genera. The spermatozoa are filiform and under light microscopy the acrosome and nucleus appear as distinct structures, with a rounded acrosome tip (Fig. 1A,E). Distinction can be made between the differing midpieces of the two genera at the light microscope level. The midpiece of *Ichthyophis* appears homogeneous, whereas that of *Uraeotyphlus* has spherical mitochondria which resemble a cluster of grapes (racemose arrangement). Dimensions of the spermatozoa are provided in Table 1. The spermatozoon of *Ichthyophis* is represented semidiagrammatically in Figure 2, and should be referred to throughout.

Acrosome complex. The acrosome complex is composed of an acrosome vesicle surrounding an electron-dense acrosome rod, here termed the perforatorium (Figs. 3A,N, 4A). The acrosome vesicle is cylindrical and consists of three distinct regions: apically, a moderately electron-dense homogeneous zone, a granular zone, and basally an electron-dense homogeneous zone (Figs. 3A,K,N, 4A,N,O). The basal homogeneous zone of *Ichthyophis* is larger than that of *Uraeotyphlus*. The opposite is true for the granular zone, which is larger in *Uraeotyphlus*, whereas the apical homogeneous zone is of a similar size in both genera. The base of the acrosome conforms in shape to the flattened anterior tip of the

TABLE 1. Dimensions of spermatozoa taken from light and transmission electron microscopy

Species	Total length	Head length	Acrosome vesicle length	Nucleus length	Nucleus width	Midpiece length	Tail length	Endonuclear canal length	Perforatorium length
Gegeneophis carnosus[4]	100	27.5	7.5	20	1.2	7	65.5	—	—
Gegeneophis ramaswamii	99.4 (27, 3.5)	27.2 (10, 0.78)	—	—	0.69 (4, 0.06) ant. 0.97 (5, 0.08) base	5.66 (7, 0.33)	68.9 (23, 2.7)	0.25 (1, 0)	>3.5
Siphonops annulatus[2]	—	17	4	13	—	6	—	—	—
Chthonerpeton indistinctun[1]	approx. 70	17.34* ± 0.85	—	—	—	—	approx. 52	—	—
Typhlonectes natans	— 105.3 (9, 2.0)	approx. 18[5] 26.4 (12, 0.6)	6.0[5†] 8.48 (6, 0.38)	11.7[5†] 17.9 (3, 0.3)	1.2[5†] 1.13 (1, 0) ant. 1.6 (1, 0) base	5.8[5†] 7.47 (4, 1.11)	— 71.1 (2, 0)	— 1.4 (1, 0)	— approx. 10
Ichthyophis beddomei	98.8 (6, 3.3)	13.2 (9, 2.0)	4.75 (7, 0.75)	8.99 (5, 1.71)	1.36 (5, 0.27) ant. 1.26 (6, 0.04) base	6.13 (3, 0.54)	81.0 (6, 3.3)	1.19 (4, 0.05)	5.06 (3, 0.24)
Ichthyophis glutinosus[3]	110	13	5	8	2	5.5	91.5	—	—
Ichthyophis tricolor	85.4 (5, 3.5)	14.2 (6, 1.5)	4.68 (1, 0)	9.35 (2, 0.21)	1.49 (6, 0.15) ant. 1.11 (8, 0.08) base	4.1 (1, 0)	67.7 (5, 3.8)	1.53 (4, 0.14)	—
Uraeotyphlus A	95.3 (11, 2.4)	18.7 (5, 0.2)	4.86 (5, 0.16)	13.8 (5, 0.2)	1.5 (1, 0) ant. 1.35 (3, 0.11) base	4.56 (6, 0.45)	71.4 (11, 2.1)	1.75 (1, 0)	—
Uraeotyphlus B	97.6 (13, 1.6)	20.1 (4, 0.15)	5.06 (5, 0.21)	15.0 (4, 0.3)	1.23 (3, 0.11) ant. 1.14 (2, 0.19) base	5.57 (2, 0.42)	72.7 (11, 1.53)	1.95 (1, 0)	4.8 (1, 0)
Uraeotyphlus narayani[4]	120	16.6	5.5	11.1	1.4	5	98.4	—	—

[1]de Sa and Berois (1986).
*This value may also include the midpiece.
[2]Seshachar (1940), calculated from drawing (Fig. 3)—note that the whole spermatozoon was not drawn.
[3]Seshachar (1943).
[4]Seshachar (1945).
[5]van der Horst et al. (1991).
†calculated from Figure 2.
Values (means) given are in μm (n, SD).

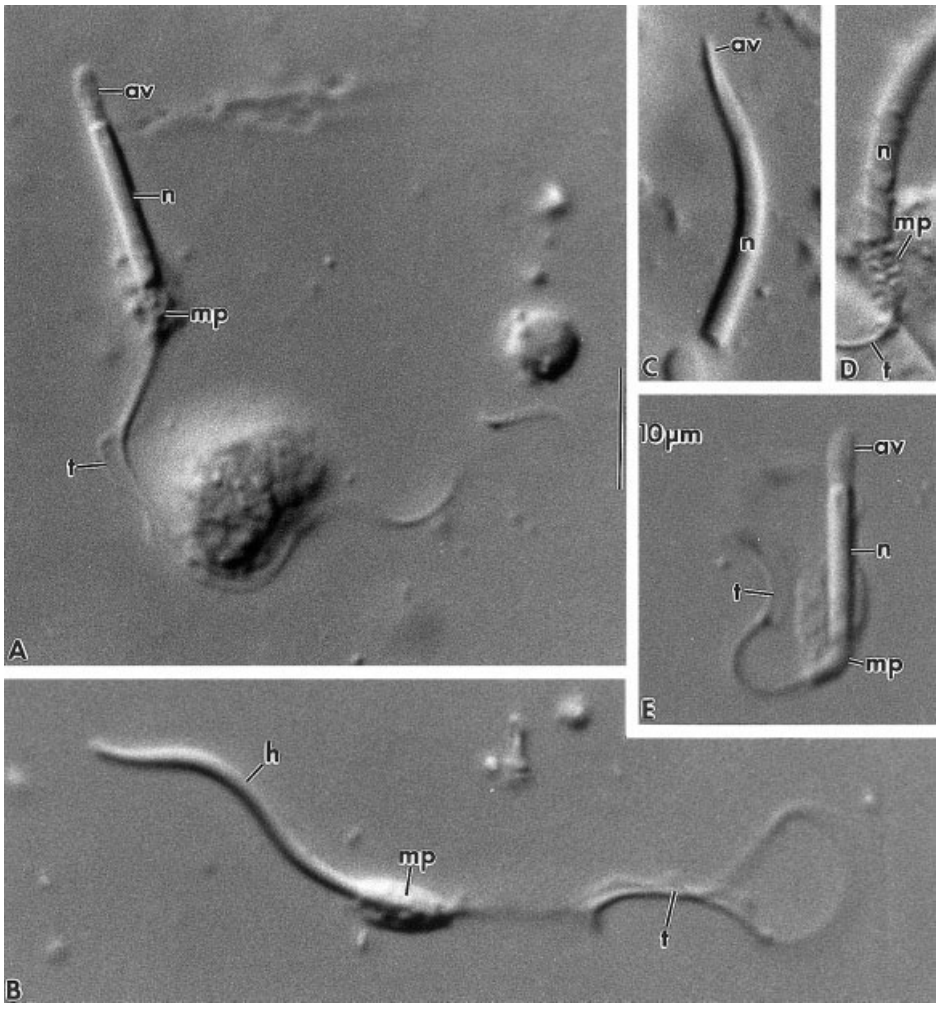

Fig. 1. Light micrographs of spermatozoa. **A:** *Uraeotyphlus* sp. A (whole spermatozoon). **B:** *Gegeneophis ramaswamii* (whole spermatozoon). **C:** *Typhlonectes natans* (head). **D:** *Typhlonectes natans* (midpiece). **E:** *Ichthyophis tricolor* (whole spermatozoon, note tail broken off). All to the same scale as indicated. av, acrosome vesicle; h, head (acrosome and nucleus); mp, midpiece; n, nucleus; t, tail.

nucleus but is separated from it by a thin disc of granular material, the acrosomal baseplate (Figs. 3A,L,N, 4A,H,O). The acrosome is circular in transverse section throughout most of its length (Figs. 3E–G, 4D–H), becoming slightly irregular at its apex (Figs. 3D, 4C).

The perforatorium extends from the apical homogeneous zone of the acrosome to within the endonuclear canal (Figs. 3A,H,L, 4A,I). It does not extend to the apical tip of the acrosome vesicle. Anteriorly, the perforatorium becomes closely associated with the acrosome vesicle's "inner" membrane, from which distinct lateral projections, which appear barb-like in longitudinal section, are observed (Figs. 3A,K, 4A,N). This barb-like extension occurs between the apical homogeneous and granular zones of the acrosome vesicle.

Basally, within the subacrosomal space, between the perforatorium and the acrosome vesicle, is a ring of granular material of a texture similar to the acrosomal baseplate, but separate from it (Figs. 3A,L,N, 4A,O). In *Ichthyophis* the granular subacrosomal material forms a continuous ring around the perforatorium (Fig. 3G), whereas in *Uraeotyphlus* it forms a discontinuous ring of distinct parts (Fig. 4G).

Nucleus. The nucleus has the form of a relatively short cylinder of constant diameter, circular in cross section, with strongly condensed chromatin (Figs. 3N, 4K). The anterior tip of the nucleus is flat and indented medially for a short distance as an anterior nuclear fossa, which is here regarded as an endonuclear canal (Figs. 3H,L,N, 4A,I,J,O). The endonuclear canal is wide and contains the base of the perforatorium for most of its length. Posteriorly, the base of the canal is rounded in *Ichthyophis* (Fig. 3L), which differs from that seen in *Uraeotyphlus*, where a narrow extension of the canal exists (Fig. 4J,O). Basally, the nucleus ends with an asymmetrical nuclear fossa (Fig. 3B,N).

Midpiece. The midpiece consists of the centrioles, anterior part of the tail, and the mitochondria. The proximal centriole is close to the base of the nucleus and is surrounded by pericentriolar material that connects it to the nuclear fossa and the distal centriole (Fig. 3B). It lies at a right angle to the long axis of the nucleus, whereas the distal centriole is in same axis and forms the basal body of the axoneme.

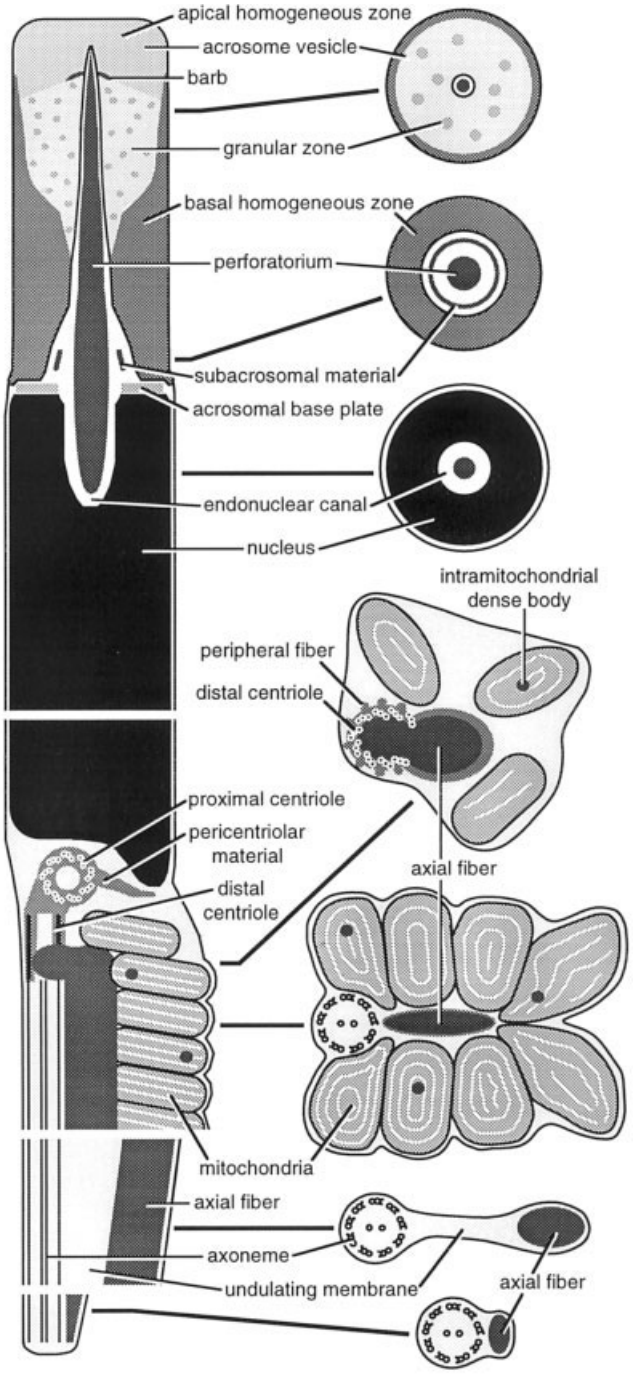

Fig. 2. Highly diagrammatic representation of the spermatozoon of the Ichthyophiidae as exemplified by *Ichthyophis beddomei* and *I. tricolor*.

Basally, the distal centriole is penetrated by the anterior portion of the axial fiber (Figs. 3B,I,4L,P). A short peripheral fiber is associated with, questionably, each of the nine triplets of the distal centriole (Figs. 3I, 4L). Eight, or occasionally only seven, peripheral fibers can be seen in transverse section through the distal centriole; however, it is not possible to determine if a "ninth" fiber exists due to the presence of the axial fiber, or whether the axial fiber is actually a greatly enlarged "ninth" fiber. The peripheral fibers of *Uraeotyphlus* are well developed compared to those of *Ichthyophis*. In both genera the peripheral fibers do not continue beyond the length of the centriole.

The centrioles, axial fiber, and the anterior part of the axoneme are surrounded by mitochondria. The structure of the mitochondria differs between the two genera. Those of *Uraeotyphlus* are spherical, have an extensive array of delicate concentric cristae, number ~35 per spermatozoon, and occur in a racemose arrangement (Fig. 4B,L,M,P). In transverse section the number of mitochondria seen alternates between four and five per layer. In contrast, the mitochondria of *Ichthyophis*, when viewed in longitudinal section, appear flattened and to spiral around the tail (Fig. 3C), and although the cristae are concentric, they do not form the delicate array seen in *Uraeotyphlus* (Fig. 3C,I,J,M). In transverse section a maximum of eight mitochondria is seen in *Ichthyophis*. The mitochondria of both genera contain dense bodies (Figs. 3I,M, 4B), never completely surround the axoneme (Figs. 3J, 4M), and clearly define the length of the midpiece (Figs. 3M, 4B). A thin, but distinct, annulus is present at the base of the midpiece and defines the beginning of the tail (Figs. 3M, 4B).

Tail. The tail consists of the 9+2 axoneme and axial fiber, enclosed by a plasma membrane, typical of amphibian spermatozoa (Figs. 3O, 4Q). Anteriorly, within the midpiece, the axoneme and elongate axial fiber run closely adjacent to each other (Fig. 4M). More posteriorly, within the tail, the axoneme is separated from the round/oval-shaped axial fiber by an undulating membrane (Figs. 3O, 4Q). For much of the length of the tail the plasma membrane of the two faces of the undulating membrane is not closely apposed but is widely separated by cytoplasm. Immediately beneath the plasma membrane there is a dense layer, which is little more in appearance than a thickening of the membrane (Figs. 3O, 4Q). The width of the axial fiber varies from between one to two times that of the axoneme for most of the length of the tail. Towards the posterior end of the tail the axial fiber decreases in size and again becomes closely associated with the axoneme. Although no juxta-axonemal fibers are present in the tail of the mature spermatozoon, what appears to be a small juxta-axonemal fiber associated with doublet 3 is occasionally seen in the tail of spermatids (Fig. 3P).

Spermatozoa of *Gegeneophis ramaswamii* and *Typhlonectes natans*

The spermatozoon of *Typhlonectes natans* has been previously examined by van der Horst et al. (1991) and does not require detailed description here. Important structures will be shown in order to

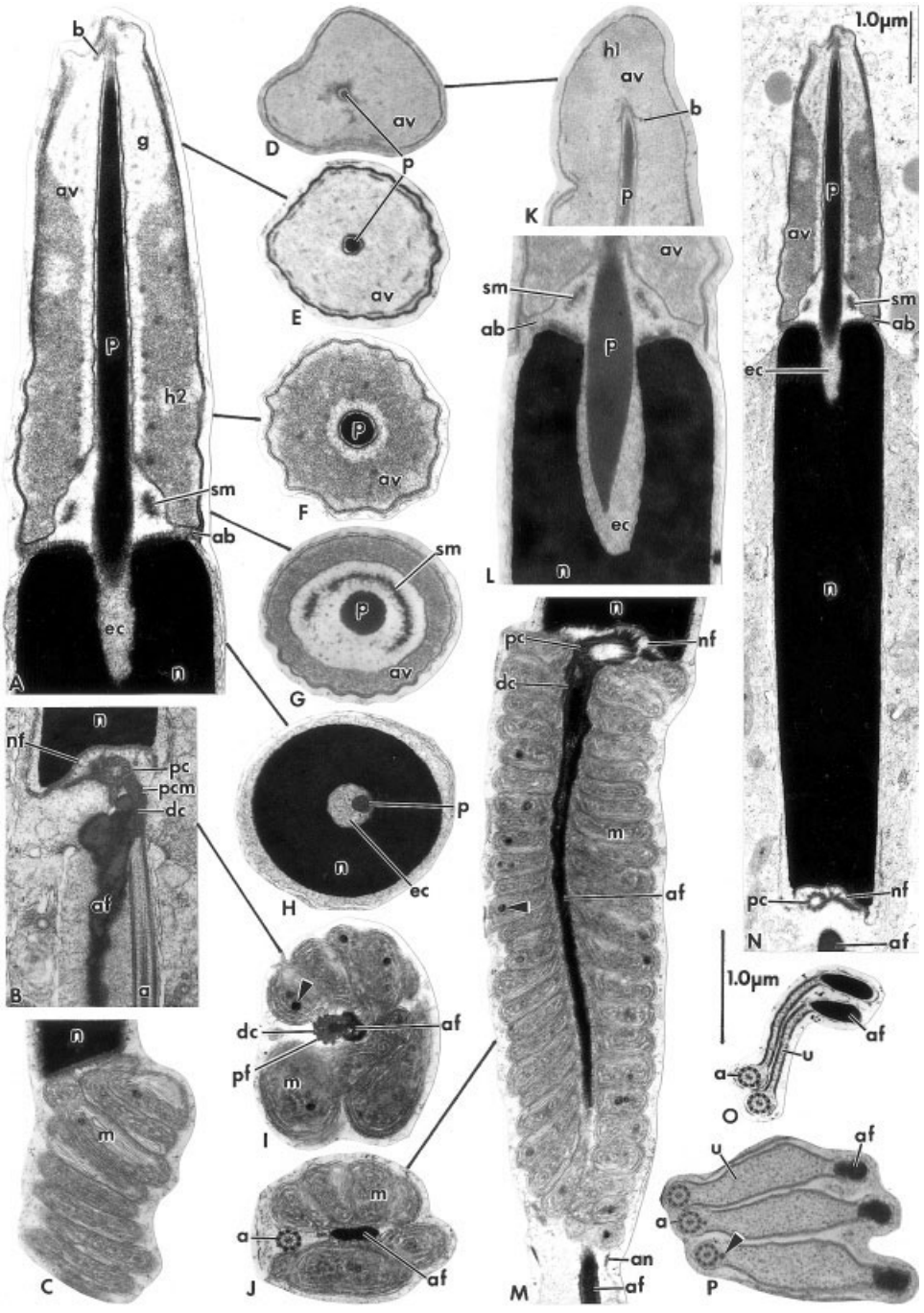

Fig. 3. *Ichthyophis beddomei* (**A,C–G,I–K,M–O**) and *I. tricolor* (**B,H,L,P**) spermatozoa. TEM. **A:** Longitudinal section (L.S.) of the acrosome complex. **B:** L.S. through the centriolar (neck) region of a late spermatid showing the axial fiber extending into the distal centriole (also see **I**). **C:** Oblique L.S. of the midpiece showing the spiral pattern of the mitochondria. **D–J:** Successive transverse sections (T.Ss) through the spermatozoon as indicated: **D–G**, through the acrosome complex, **H**, through the anterior region of the nucleus showing the endonuclear canal, **I**, through the distal centriole (arrow indicates intramitochondrial dense body), **J**, through the midpiece. **K:** L.S. through the apical region of the acrosome vesicle. **L:** L.S. through the anterior region of the nucleus showing the endonuclear canal. **M:** L.S. through the entire midpiece (arrow indicates intramitochondrial dense body). **N:** L.S. through the entire head. **O:** T.S. of the tail of a mature spermatozoon (note the absence of juxta-axonemal fibers). **P:** T.S. through the tail of a late spermatid (note the wide undulating membrane and rudimentary juxta-axonemal fiber (arrow) associated with doublet 3 of the axoneme). **A–M, O–P** to the same scale as indicated, **N** scale as indicated. a, axoneme; ab, acrosomal baseplate; af, axial fiber; an, annulus; av, acrosome vesicle; b, "internal" barb of acrosome vesicle; dc, distal centriole; ec, endonuclear canal; g, acrosome vesicle – granular zone; h1, acrosome vesicle – moderately electron-dense apical homogeneous zone; h2, acrosome vesicle – electron-dense basal homogeneous zone; m, mitochondrion; n, nucleus; nf, nuclear fossa; p, perforatorium; pc, proximal centriole; pcm, pericentriolar material; pf, peripheral fiber; sm, subacrosomal material; u, undulating membrane.

provide comparisons with the caecilians described in this study and to complement previous work.

The spermatozoa of *Gegeneophis ramaswamii* and *Typhlonectes natans* are filiform and under light microscopy the head (acrosome and nucleus) is elongate and pointed anteriorly (Fig. 1B,C). Spherical mitochondria can clearly be seen in the midpiece in a racemose arrangement (Fig. 1D). Dimensions of the spermatozoa are provided in Table 1. The spermatozoon of *G. ramaswamii* is represented semidiagrammatically in Figure 5 and should be referred to throughout.

Acrosome Complex. The acrosome complex is composed of an acrosome vesicle surrounding an electron-dense perforatorium (Fig. 6A). The acrosome vesicle is sharply attenuated apically and is initially circular in transverse section (Fig. 6A,C). Posteriorly, the acrosome becomes laterally flattened, then spatulate before again appearing circular (Fig. 6D–F). It is composed of moderately

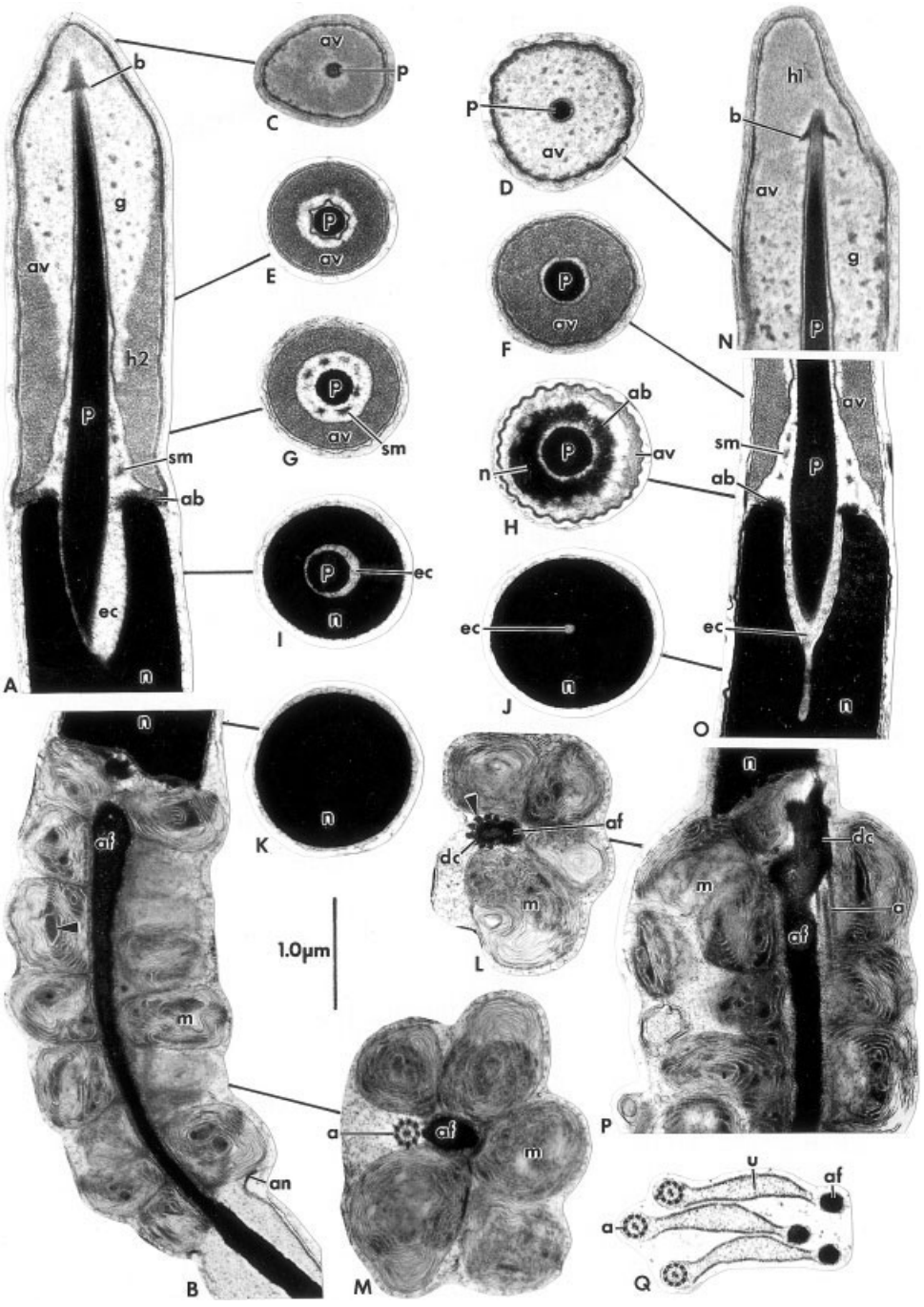

Fig. 4. *Uraeotyphlus* sp. A (**B,C,F,H–L,N**) and *U.* sp. B (**A,D–E,G,M,O–Q**) spermatozoa. TEM. **A:** Longitudinal section (L.S.) of the acrosome complex. **B:** L.S. through the entire midpiece (arrow indicates intramitochondrial dense body). **C–M:** Successive transverse sections (T.Ss) through the spermatozoon as indicated: **C–G**, through the acrosome complex, **H**, slightly oblique section through the acrosomal baseplate, **I,J**, through the endonuclear canal, **K**, through the nucleus, **L**, through the distal centriole, showing the axial fiber extending into the distal centriole (arrow indicates peripheral fibers associated with each of the nine triplets of the centriole), **M**, through the midpiece. **N:** L.S. through the apical region of the acrosome vesicle. **O:** L.S. through the anterior region of the nucleus showing the endonuclear canal. **P:** L.S. through the anterior region of the midpiece. **Q:** T.S. of tails. All to the same scale as indicated. a, axoneme; ab, acrosomal baseplate; af, axial fiber; an, annulus; av, acrosome vesicle; b, "internal" barb of acrosome vesicle; dc, distal centriole; ec, endonuclear canal; g, acrosome vesicle – granular zone; h1, acrosome vesicle – moderately electron-dense apical homogeneous zone; h2, acrosome vesicle – electron-dense basal homogeneous zone; m, mitochondrion; n, nucleus; p, perforatorium; sm, subacrosomal material; u, undulating membrane.

electron-dense material throughout its length. For an indeterminate length a second rod-shaped fiber, the acrosome vesicle fiber, lies longitudinally within the acrosome parallel to the perforatorium (Fig. 6E,F,K). A thin veneer of electron-lucent material occurs just within the outer limits of the acrosome vesicle. When observed in transverse section this thin veneer does not form a complete circle (Fig. 6D). The base of the acrosome conforms in shape to the anterior tip of the nucleus but is separated from it by a thin disc of granular material, the acrosomal baseplate (Fig. 6A,K,L).

The perforatorium extends from the apical tip of the acrosome to within the endonuclear canal. The acrosome vesicle of *Typhlonectes natans* differs in being composed basally of granular material of differing electron densities and anteriorly of homogeneous material and in lacking both an acrosome vesicle fiber and the thin electron-lucent veneer (Fig. 7A,C–H).

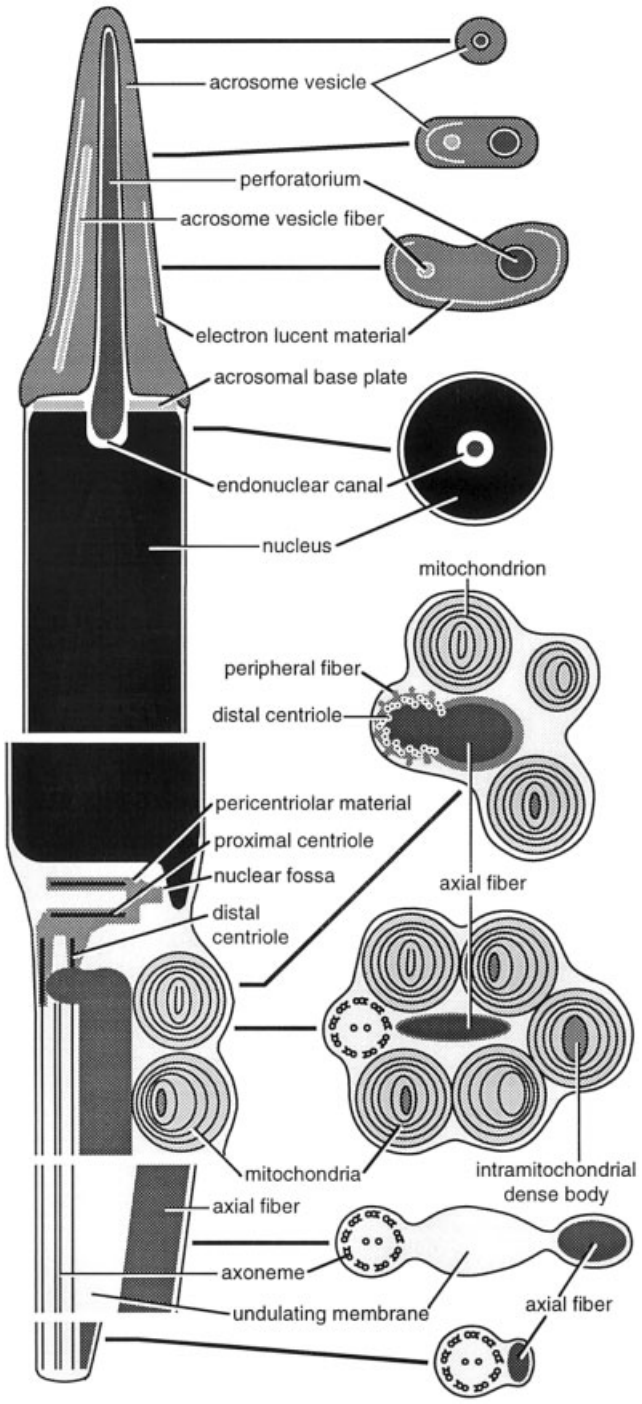

Fig. 5. Highly diagrammatic representation of the spermatozoon of the Caeciliidae as exemplified by *Gegeneophis ramaswamii*.

Nucleus. The nucleus is in the form of an elongate cylinder that is slightly narrower anteriorly, circular in cross section, and composed of strongly condensed chromatin (Fig. 6G,K,M). The anterior tip of the nucleus is flat and indented axially for a short distance as an endonuclear canal (Fig. 6L). The endonuclear canal is relatively short and contains the base of the perforatorium. Posteriorly, the base of the canal is rounded. Basally, the nucleus ends with an asymmetrical nuclear fossa (Fig. 6N). The endonuclear canal of *Typhlonectes natans* (Fig. 7A) is longer than that of *Gegeneophis ramaswamii* (Fig. 6L).

Midpiece. The midpiece consists of the centrioles, anterior part of the tail, and the mitochondria. The proximal centriole is close to the base of the nucleus and is surrounded by pericentriolar material that connects it to the distal centriole (Fig. 6M). It lies at a right angle to the long axis of the nucleus, whereas the distal centriole is in the same axis and forms the basal body of the axoneme (Fig. 6H,M,N). Basally, the distal centriole is penetrated by the anterior portion of the axial fiber (Fig. 6B,I,M,N). Short peripheral fibers are associated with the triplets of the distal centriole (Fig. 6I). The fibers appear as little more than indistinct swellings of the pericentriolar material in *Gegeneophis ramaswamii*. For reasons described previously, the exact number of fibers could not be determined. The presence or absence of peripheral fibers in *Typhlonectes natans* was not determined.

The centrioles, axial fiber, and the anterior part of the axoneme are surrounded by mitochondria (Fig. 6B). The mitochondria are spherical and have an extensive array of delicate concentric cristae, number ~35 per spermatozoon, and occur in a racemose arrangement (Fig. 6B,I,J). In transverse section the number of mitochondria seen alternates between four and five per layer (Fig. 6J). The mitochondria never completely surround the axoneme and clearly define the length of the midpiece (Fig. 6J). Dense bodies are present within the mitochondrion (Fig. 6I). Although the presence of an annulus was not determined for the mature spermatozoa, it is clearly present in late spermatids (Fig. 6M). The midpiece of *Typhlonectes natans* (Fig. 7B,I) is of a similar structure to that described for *Gegeneophis ramaswamii* above.

Tail. The tail consists of a typical 9+2 axoneme and an axial fiber enclosed by a plasma membrane. Anteriorly, within the midpiece, the axoneme and elongate axial fiber run closely adjacent to each other (Fig. 6J). More posteriorly, as the tail, the axoneme is separated from the round/oval-shaped axial fiber by an undulating membrane (Fig. 6O). For much of the length of the tail, the plasma membrane of the two faces of the undulating membrane is not closely apposed but is widely separated by cytoplasm (Fig. 6O). Immediately beneath the plasma membrane there is a dense layer, which appears as little more than a thickening of the membrane. The width of the axial fiber varies from between one and two times that of the axoneme for most of the length of the tail. Towards the posterior end of the tail the axial fiber decreases in size and again becomes closely associated with the axoneme (Fig. 6P,Q). No juxta-axonemal fibers are present in

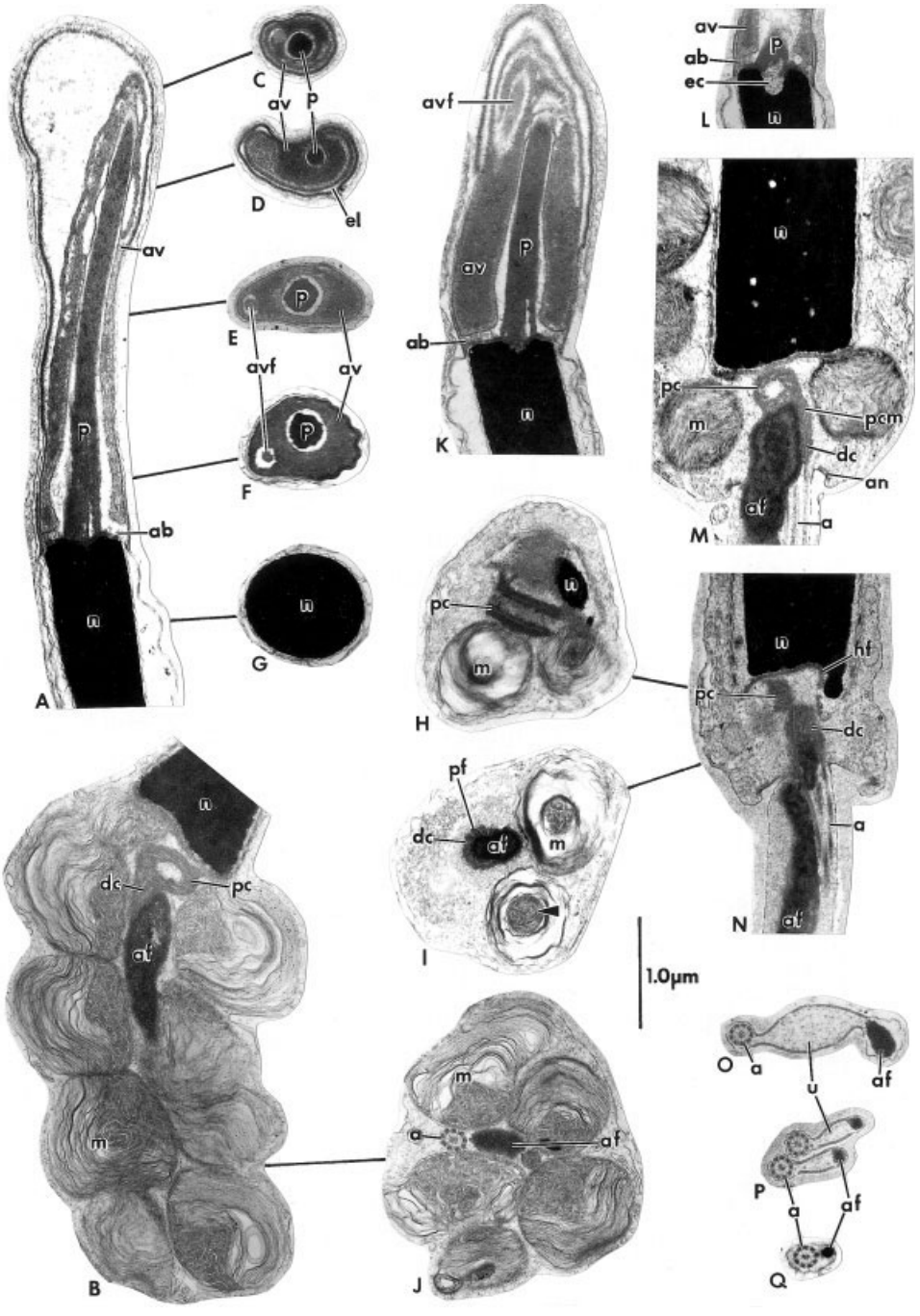

Fig. 6. *Gegeneophis ramaswamii* spermatozoa. TEM. **A:** Longitudinal section (L.S.) of the acrosome complex. **B:** Oblique L.S. through the anterior region of the midpiece. **C-J:** Successive transverse sections (T.Ss) through the spermatozoon as indicated: **C–F**, through the acrosome complex, **G**, through the nucleus, **H**, through the proximal centriole, **I**, through the distal centriole, showing the axial fiber extending into the distal centriole (arrow indicates intramitochondrial dense body), **J**, through the midpiece. **K:** L.S. through the basal region of the acrosome vesicle. **L:** L.S. through the anterior region of the nucleus showing the short endonuclear canal. **M,N:** L.Ss through the centriolar region of a spermatid. **O–Q:** Successive T.Ss through the tail. All to the same scale as indicated. a, axoneme; ab, acrosomal baseplate; af, axial fiber; an, annulus; av, acrosome vesicle; avf, acrosome vesicle fiber; dc, distal centriole; ec, endonuclear canal; el, veneer of electron-lucent material; m, mitochondrion; n, nucleus; nf, nuclear fossa; p, perforatorium; pc, proximal centriole; pcm, pericentriolar material; pf, peripheral fiber; u, undulating membrane.

the mature spermatozoon (Fig. 6O,P). The tail of the spermatozoon of *Typhlonectes natans* (Fig. 7J) is of a similar structure.

DISCUSSION

Our principal aim in surveying the ultrastructure of the spermatozoa of a small but phylogenetically disparate set of caecilian taxa is to explore the potential phylogenetic significance of commonalties and differences in the ultrastructure of caecilian spermatozoa. The interrelationships of the three groups of extant Amphibia (frogs, salamanders, and caecilians) have, as yet, not been compellingly resolved by molecular and/or morphological data. Similarly, interrelationships among caecilians are far from fully understood. Thus, there is potential for careful interpretation of comparative spermatozoal data to provide useful evidence of relationships at a variety of levels.

The spermatozoa of Gymnophiona share the following unique autapomorphies: 1) penetration of the

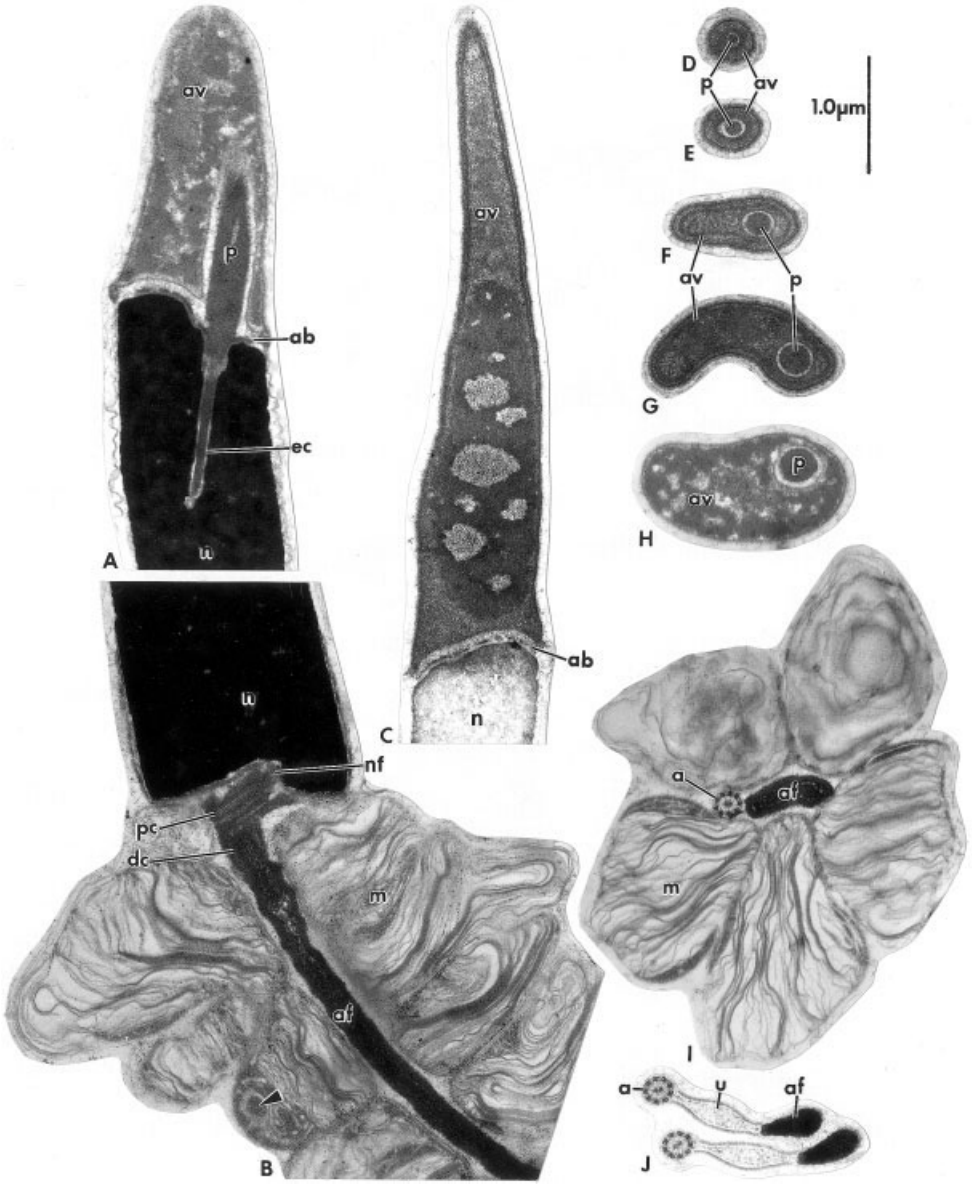

Fig. 7. *Typhlonectes natans* spermatozoa. TEM. **A:** Longitudinal section (L.S.) through the anterior nucleus showing the endonuclear canal containing the perforatorium. **B:** L.S. through the anterior midpiece (arrow indicates intramitochondrial dense body). **C:** L.S. through the acrosome vesicle showing the apical homogeneous zone and the basal granular zone of differing electron densities. **D–J:** Successive transverse sections through the spermatozoon: **D–H**, through the acrosome complex, **I**, through the midpiece, **J**, through the tail. All to the same scale as indicated. a, axoneme; ab, acrosomal baseplate; af, axial fiber; av, acrosome vesicle; dc, distal centriole; ec, endonuclear canal; m, mitochondrion; n, nucleus; nf, nuclear fossa; p, perforatorium; pc, proximal centriole; u, undulating membrane.

distal centriole by the axial fiber; 2) the presence of an acrosomal baseplate; 3) "acrosome seat," i.e., junction between acrosome complex and the flattened anterior end of the nucleus, although the perforatorium and endonuclear canal are themselves symplesiomorphies; and 4) juxta-axonemal fibers absent throughout the length of the axoneme of mature spermatozoa.

Penetration of the distal centriole by the axial fiber and the presence of an acrosomal baseplate are not observed in the spermatozoa of any other vertebrate. The acrosome seat is considered apomorphic because the acrosome complex of those fish possessing one (Jamieson, 1991), urodeles (Picheral, 1979; Selmi et al., 1997), anurans (Pugin-Rios, 1980; Kwon and Lee, 1995), and amniotes (Jamieson, 1995) caps the pointed nucleus. Juxta-axonemal fibers are absent from the flagellum of all caecilians so far examined. In contrast, a juxta-axonemal fiber is associated with doublet 8 in urodeles (Picheral, 1979; Lee and Jamieson, 1993; Selmi et al., 1997) and doublet 3 in Anura (Pugin-Rios, 1980; Lee and Jamieson, 1992, 1993; Kwon and Lee, 1995) with the exception of *Leiopelma* (Scheltinga et al., 2001), and occasional *Bufo marinus* (Swan et al., 1980) spermatozoa, which have juxta-axonemal fibers at both 3 and 8. Juxta-axonemal fibers are also prefigured in Dipnoi (Jamieson, 1999).

Another potential apomorphy of the Gymnophiona, as tentatively suggested by Jamieson (1999), is the wide separation of the plasma membrane of the two faces of the undulating membrane by a considerable amount of cytoplasm. In the Urodela, Dipnoi, and most Anura the plasma mem-

brane is closely apposed for most of the length of the flagellum. In those Anura where the two faces of the undulating membrane are widely separated, they are separated by a paraxonemal rod (Jamieson et al., 1993; Meyer et al., 1997; Jamieson, 1999) and not cytoplasm, as in caecilian sperm. Thus, the separation of the two faces of the undulating membrane in caecilians and some frogs does not show detailed similarity and both the anuran and caecilian conditions are considered most likely to be independent apomorphies.

The presence of an endonuclear canal containing a perforatorium is a plesiomorphic feature of caecilian spermatozoa that is shared with urodeles (Fawcett, 1970; Picheral, 1979; Selmi et al., 1997), and some basal Anura (Sandoz, 1970; Furieri, 1975; Jamieson et al., 1993). It is also seen in some amniotes (turtles, crocodiles, and tuatara; Healy and Jamieson, 1992, 1994; Jamieson, 1995) and in sarcopterygian fish (see Jamieson, 1991). However, the endonuclear canal of amphibians differs from those observed in the sarcopterygian fish and amniotes in being singular and containing only one perforatorium. With the exception of some plethodontid salamanders (pers. obs.), the endonuclear canal in caecilians is also distinctive in penetrating only the extreme tip of the nucleus. It was first described in caecilians, for *Typhlonectes natans*, by van der Horst et al. (1991:445) as "an indentation at the anterior wall of the nucleus" that is "identical to the cup-shaped depression" described for other caecilians by Seshachar (1945). Thus, the precise form of the endonuclear canal in caecilians, rather than its presence, appears apomorphic with a degree of convergence with some plethodontids.

The presence of mitochondria with concentric cristae occurs in the spermatozoa of the Gymnophiona and Urodela (van der Horst et al., 1991; Selmi et al., 1997; pers. obs.), although the mitochondria of urodeles differ in being smaller and containing fewer cristae that are not in the form of an extensive array. Concentric cristae are interpreted as plesiomorphic in salamanders and caecilians because they also occur in turtles, crocodiles, tuatara (Jamieson and Healy, 1992), and some marsupials, i.e., opossums (Fawcett, 1970; Phillips, 1970; Temple-Smith and Bedford, 1980) and the macropod *Lagorchestes hirsutus* (Jamieson, 1999), and therefore appear to be an autapomorphy of the Tetrapoda, although multiple homoplastic origin of the concentric condition cannot categorically be dismissed. Similarly, intramitochondrial dense bodies are considered symplesiomorphic because they occur in caecilians (van der Horst et al., 1991; present study), salamanders (Selmi et al., 1997), turtles, crocodiles, and the tuatara (Jamieson and Healy, 1992). The short peripheral dense fibers associated with the distal centriole observed in *Ichthyophis, Uraeotyphlus*, and *Gegeneophis ramaswamii* spermatozoa are not seen in any other amphibian but similar structures occur in some fish (see Jamieson, 1991), some molluscs (see Healy, 1996), and amniote spermatozoa (see Jamieson, 1995). That they are strictly homologous is debatable but the combination of mitochondria with concentric cristae, intramitochondrial dense bodies, annulus, and peripheral fibers is not inconsistent with homology of these characters with those of lower amniotes. This suggests that, of the extant Amphibia, caecilian spermatozoa are the most similar (albeit greatly modified) in these and perhaps other respects to those of the common ancestor of Amphibia and Amniota. However, it has previously been suggested that the presence of bilateral fibers associated with the axoneme of dipnoan spermatozoa indicates that bilateral fibers are plesiomorphic for amphibian spermatozoa (Jamieson, 1999). That there are nine fibers associated with the axoneme in the spermatozoa of lampreys and cephalopods reveals that this pattern has been acquired repeatedly in animal spermatozoa. Thus, caution is required in interpreting the peripheral fibers in caecilians as homologous with those of amniotes.

In many metazoan spermatozoa there is a postmitochondrial dense ring that is called an annulus, although its homology wherever it occurs is doubtful. The presence of an annulus may be a tetrapod apomorphy (or an apomorphic reacquisition) as it is not seen in any fish but is seen in caecilians, salamanders, and amniotes. However, a "ring body" (Jespersen, 1971) or "retronuclear body" (Jamieson, 1999) is present in the lungfish *Neoceratodus,* where it has the form of a postmitochondrial ring and is here tentatively considered to be the precursor to the tetrapod annulus. An annulus (or annulus-like structure) is not seen in any other Dipnoi. The retronuclear body observed in the spermatozoa of the lungfish *Protopterus* appears to be the homolog of the "neck" or "connecting piece" of urodele spermatozoa (Jamieson, 1999). An annulus is absent from anuran spermatozoa and highly modified in some salamander spermatozoa (Picheral, 1979). The annulus seen in caecilians appears more similar to those occurring in basal amniotes than it does to the elongate structure of higher urodeles. We tentatively suggest that the absence of the annulus in anurans and its modification in salamanders are independent apomorphies, while acknowledging that evolutionary history and homologies of the annulus are unclear.

The unilateral location of the mitochondria relative to the axoneme, the unilateral undulating membrane, and axial fiber occur in all three amphibian orders, and as suggested by Jamieson et al. (1993) and Jamieson (1999), appear to be amphibian autapomorphies. There are no apomorphic characters seen in caecilian spermatozoa that suggest a closer relationship to either the Urodela or Anura.

The spermatozoa of Uraeotyphlidae and Ichthyophiidae share the following unique character

states: 1) barbed (lateral extensions) acrosome membrane associated with the perforatorium tip; 2) presence of subacrosomal granular material: a continuous ring in *Ichthyophis*; a "dis-continuous" ring in *Uraeotyphlus*; 3) acrosomal vesicle divided into three distinct zones; 4) wide, blunt-ended endonuclear canal: round-ended in *Ichthyophis*; round-ended but with a narrow, axial extension in *Uraeotyphlus*; and 5) cylindrical and apically blunt-ended acrosome vesicle.

The polarity of characters one, two, and five only can be readily determined. These characters are unique to the Uraeotyphlidae and Ichthyophiidae within the Gymnophiona, and as they are not found in any outgroup they are here considered to be synapomorphies. The polarities of characters three and four are uncertain as a shortening of the endonuclear canal and subdivision of the acrosome vesicle into different zones occurs, although with notable differences, in all caecilians. For example, at present it is not possible to determine whether division of the acrosome vesicle into two (as in Typhlonectidae) or three (as in Uraeotyphlidae and Ichthyophiidae) zones is the plesiomorphic condition. However, the presence of an acrosome vesicle fiber in *Gegeneophis* appears to be apomorphic, as it does not occur in any outgroup. The acrosomes of several salamanders have been described as being barbed (Picheral, 1979; Wortham et al., 1982; Selmi et al., 1997), but these differ from the condition observed here in that the barb of salamanders is an "external" outgrowth along one side of the acrosome vesicle, whereas that observed in *Ichthyophis* and *Uraeotyphlus* is a lateral extension of the "internal" acrosome vesicle membrane associated with perforatorium tip. Van der Horst et al. (1991) considered the curved tip of the acrosome in *Typhlonectes natans* to be comparable with the barbed condition in higher urodeles. However, a distinct "external" barb structure was not observed here and we consider homology between the caecilian and salamander conditions doubtful.

Although the mitochondria of all caecilian spermatozoa examined to date possess concentric cristae, mitochondria of Ichthyophiidae differ from those of the Uraeotyphlidae, Caeciliidae, and Typhlonectidae in several respects. The mitochondria of Ichthyophiidae appear flattened and to spiral around the tail, do not possess a delicate array of cristae, and are not in a racemose arrangement. The flattened shape appears to be apomorphic because spherical mitochondria are widespread in many distant and more proximate outgroups. The absence of the delicate array of concentric cristae is more difficult to interpret given that this is present in some amniotes (turtles and tuataras) but absent in salamanders and frogs.

The spermatozoa of the Caeciliidae and Typhlonectidae display the synapomorphic condition of having a spatulate/flattened acrosome vesicle. The acrosomes of Dipnoi, those fish possessing them, urodeles, anurans, and basal amniotes are round in transverse section (Picheral, 1979; Pugin-Rios, 1980; Jamieson, 1991, 1999; Healy and Jamieson, 1992, 1994). The acrosomes of *Ichthyophis glutinosus, Uraeotyphlus narayani*, and *Siphonops annulatus* have been described as spatulate by Seshachar (1940) from light microscopy. However, this is highly doubtful for *I. glutinosus* and *U. narayani,* as it is clearly shown as circular here for other species of *Ichthyophis* and *Uraeotyphlus*. The presence of the acrosome vesicle fiber and a veneer of electron-lucent material within the acrosome vesicle are unique to *Gegeneophis ramaswamii* spermatozoa and presumably derived within caecilians. Their presence in other Caeciliidae requires examination.

Wake's (1994) light microscopic examination of the morphology of caecilian spermatozoa found that the caecilians could be divided into two groups on the shape and size of the head. One group contained long heads with a pointed acrosome, the other having wider short heads with a blunt-ended acrosome. Using the criteria of Wake, spermatozoa of all species examined here have mid-length acrosomes (5–8 μm), short (<8 μm) midpieces, and short (<100 μm) tails. As observed here (see Table 1) and noted by Wake (1994), tail and midpiece length are variable within genera (and above) and do not correlate with higher systematic relationships. The acrosome length and shape as well as the head size and shape are more consistent within genera and appear to be more phylogenetically informative. The acrosomes of Ichthyophiidae and Uraeotyphlidae examined here are short (4.86–5.06 μm) and blunt-ended, whereas those of Caeciliidae and Typhlonectidae (8.48 μm) are long and pointed. The head is short in Ichthyophiidae (13.2–14.2 μm), moderately long in Uraeotyphlidae (18.7–20.1 μm), and long in the Caeciliidae (27.2 μm) and Typhlonectidae (26.4 μm) examined here.

As shown above, spermatozoa of caecilians show four (possibly five) autapomorphies that strongly support their monophyly. From spermatozoal ultrastructure the Ichthyophiidae appear more closely related to the Uraeotyphlidae than to caeciliids or typhlonectids, a view strongly supported by other morphological data and molecular evidence (Wilkinson and Nussbaum, 1996; Wilkinson, 1997; Wilkinson et al., 2002). Ichthyophiidae and Uraeotyphlidae share at least three synapomorphic characters, whereas Caeciliidae and Typhlonectidae share only one synapomorphy. Apart from the amphibian autapomorphies, the Uraeotyphlidae, Caeciliidae, and Typhlonectidae share only the plesiomorphic racemose arrangement of spherical mitochondria with an extensive array of delicate concentric cristae.

Examination of the spermatozoa of *Uraeotyphlus* A, B, and C, and of the two species of *Ichthyophis*, revealed no discernible difference in ultrastructure within a genus, indicating that the characters de-

rived from spermatozoal ultrastructure are relatively constant at generic levels within the Gymnophiona. In contrast, considerable differences in the dimensions of spermatozoa occur between the species of *Ichthyophis*, and differences between *Uraeotyphlus* A and B may lend some support to the hypothesis that they are distinct species (unfortunately, because of the paucity of mature spermatozoa present in the testis material of *Uraeotyphlus* C, no measurements were obtained for this taxon). Substantial differences exist between the measurements of spermatozoa reported from light microscopic investigations by Seshachar (1943, 1945) for *Ichthyophis glutinosus* and *Uraeotyphlus narayani* and the members of these genera studied here. There is some uncertainty regarding the specific identity of the material studied by Seshachar, particularly in the case of *I. glutinosus*. This species is considered to be restricted to Sri Lanka (Nussbaum and Gans, 1980), whereas Seshachar's "*I. glutinosus*" were from India. We doubt that the differences in the dimensions of spermatozoa of *Ichthyophis* and *Uraeotyphlus* observed by Seshachar (1943, 1945) and those determined here for these genera are attributable to different microscopic techniques employed, and interpret these differences as indicating that Seshachar's material is not conspecific with any of the taxa reported here.

At this time spermatozoal characters do not appear to provide much phylogenetically informative data for resolving relationships between the Gymnophiona, Urodela, and Anura. However, it does appear that comparative spermatozoal ultrastructure can provide characters of potential use for elucidating evolutionary relationships within the Gymnophiona and that a more comprehensive survey of the ultrastructure of caecilian spermatozoa would be worthwhile. Lower-level caecilian taxonomy is difficult and unstable because of a paucity of external characters and a lack of understanding of their variation (Nussbaum and Wilkinson, 1989). Variation in the proportions of caecilian spermatozoa may be of considerable assistance in distinguishing caecilian taxa at low taxonomic levels, more especially in the context of testing hypotheses that particular populations represent distinct species, than in the context of practical identification.

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LITERATURE CITED

Daddow LYM. 1986. An abbreviated method of the double lead stain technique. J Submicr Cytol 18:221–224.

de Sa R, Berois N. 1986. Spermatogenesis and histology of the testes of the caecilian, *Chthonerpeton indistinctum*. J Herpetol 20:510–514.

Fawcett DW. 1970. A comparative view of sperm ultrastructure. Biol Repro Suppl 2:90–127.

Furieri P. 1975. The peculiar morphology of the spermatozoon of *Bombina variegata* (L.). Mon Zool Ital 9:185–201.

Healy JM. 1996. Molluscan sperm ultrastructure: correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia. In: Taylor JD, editor. Origin and evolutionary radiation of the Mollusca. Oxford: Oxford University Press. p 99–113.

Healy JM, Jamieson BGM. 1992. Ultrastructure of the spermatozoon of the tuatara (*Sphenodon punctatus*) and its relevance to the relationships of the Sphenodontida. Philos Trans R Soc Lond B 335:193–205.

Healy JM, Jamieson BGM. 1994. The ultrastructure of spermatogenesis and epididymal spermatozoa of the tuatara *Sphenodon punctatus* (Sphenodontida, Amniota). Philos Trans R Soc Lond B344:187–199.

Jamieson BGM. 1991. Fish evolution and systematics: evidence from spermatozoa. Cambridge, UK: Cambridge University Press.

Jamieson BGM. 1995. Evolution of tetrapod spermatozoa with particular reference to amniotes. In: Jamieson BGM, Ausio J, Justine J-L, editors. Advances in spermatozoal phylogeny and taxonomy. Paris: Mémoires du Muséum national d'Histoire naturelle. vol. 166. p 343–358.

Jamieson BGM. 1999. Spermatozoal phylogeny of the vertebrata. In: Gagnon C, editor. the male gamete: from basic science to clinical applications. St. Louis, MO: Cache River Press. p 303–331.

Jamieson BGM, Healy JM. 1992. The phylogenetic position of the tuatara, *Sphenodon* (Sphenodontida, Amniota), as indicated by cladistic analysis of the ultrastructure of spermatozoa. Philos Trans R Soc Lond B 335:207–219.

Jamieson BGM, Lee MSY, Long K. 1993. Ultrastructure of the spermatozoon of the internally fertilizing frog *Ascaphus truei* (Ascaphidae: Anura: Amphibia) with phylogenetic considerations. Herpetologica 49:52–65.

Jespersen Å. 1971. Fine structure of the spermatozoon of the Australian lungfish *Neoceratodus forsteri* (Krefft). J Ultrastruct Res 37:178–185.

Kwon AS, Lee YH. 1995. Comparative spermatology of anurans with special references to phylogeny. In: Jamieson BGM, Ausio J, Justine J-L, editors. Advances in spermatozoal phylogeny and taxonomy. Paris: Mémoires du Muséum national d'Histoire naturelle. vol. 166. p 321–332.

Lee MSY, Jamieson BGM. 1992. The ultrastructure of the spermatozoa of three species of myobatrachid frogs (Anura, Amphibia) with phylogenetic considerations. Acta Zool (Stockh) 73:213–222.

Lee MSY, Jamieson BGM. 1993. The ultrastructure of the spermatozoa of bufonid and hylid frogs (Anura, Amphibia): implications for phylogeny and fertilization biology. Zool Scr 22:309–323.

Measey GJ, Gower D J, Oommen OV, Wilkinson M. 2001. Permanent marking of a fossorial caecilian, *Gegeneophis ramaswamii* (Amphibia: Gymnophiona: Caeciliidae). Sth Asian J Nat Hist 5:109–115.

Meyer E, Jamieson BGM, Scheltinga DM. 1997. Sperm ultrastructure of six Australian hylid frogs from two genera (*Litoria* and *Cyclorana*): phylogenetic implications. J Submicr Cytol Path 29:443–451.

Nussbaum RA, Gans C. 1980. On the *Ichthyophis* (Amphibia: Gymnophiona) of Sri Lanka. Spolia Zeylanica 35:137–154.

Nussbaum RA, Wilkinson M. 1989. On the classification and phylogeny of caecilians (Amphibia: Gymnophiona), a critical review. Herpetol Monogr 3:1–42.

Oommen OV, Measey GJ, Gower DJ, Wilkinson M. 2000. Distribution and abundance of the caecilian *Gegeneophis ramaswamii* (Amphibia: Gymnophiona) in southern Kerala. Curr Sci 79:1386–1389.

Peters WCH. 1879. Uber dei eintheilung der caecilien und insbesondere uber die gattungen *Rhinatrema* und *Gymnopis*. Monatsbericht Konigl Preuss Akad Wiss Berlin 1879:924–943.

Phillips DM. 1970. Ultrastructure of spermatozoa of the woolly opossum *Caluromys philander*. J Ultrastruct Res 33:381–397.

Picheral B. 1979. Structural, comparative, and functional aspects of spermatozoa in urodeles. In: Fawcett DW, Bedford JM, editors. The spermatozoon. Baltimore: Urban and Schwarzenberg. p 267–287.

Pillai RS, Ravichandran MS. 1999. Gymnophiona (Amphibia) of India: a taxonomic study. Rec Zool Survey India, Occ Pap 172: 1–117.

Pugin-Rios E. 1980. Étude Comparative sur la Structure du Spermatozoïde des Amphibiens Anoures. Comportement des Gamètes lors de la Fécondation. Ph.D. Thèse, L'Université de Rennes.

Reynolds ES. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J Cell Biol 17: 208–212.

Sandoz D. 1970. Étude ultrastructurale et cytochimique de la formation de l'acrosome du discoglosse (Amphibien Anoure). In: Baccetti B, editor. Comparative spermatology. Rome: Accademia Nazionale dei Lincei. p 93–113.

Scheltinga DM, Jamieson BGM, Eggers KE, Green DM. 2001. Ultrastructure of the spermatozoon of *Leiopelma hochstetteri* (Leiopelmatidae, Anura, Amphibia). Zoosystema 23:157–171.

Selmi MG, Brizzi R, Bigliardi E. 1997. Sperm morphology of salamandrids (Amphibia, Urodela): implications for phylogeny and fertilization biology. Tissue Cell 29:651–664.

Seshachar BR. 1939. The spermatogenesis of *Uraeotyphlus narayani* Seshachar. Cell 48:63–76.

Seshachar BR. 1940. The apodan sperm. Curr Sci, Bangalore 10:464–465.

Seshachar BR. 1943. The spermatogenesis of *Ichthyophis glutinosus* Linn. 3. Spermateleosis. Proc Natl Inst Sci India 9:271–285.

Seshachar BR. 1945. Spermateleosis in *Uraeotyphlus narayani* Seshachar and *Gegeneophis carnosus* Beddome (Apoda). Proc Natl Inst Sci India 11:336–340.

Spurr AR. 1969. A low viscosity epoxy-resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43.

Swan MA, Linck RW, Ito S, Fawcett DW. 1980. Structure and function of the undulating membrane in spermatozoan propulsion in the toad *Bufo marinus*. J Cell Biol 85:866–880.

Taylor EH. 1968. The caecilians of the world. Lawrence: University of Kansas Press.

Temple-Smith PD, Bedford JM. 1980. Sperm maturation and the formation of sperm pairs in the epididymis of the opossum, *Didelphis virginiana*. J Exp Zool 214:161–171.

van der Horst G, van der Merwe L. 1991. Late spermatid-sperm/sertoli cell association in the caecilian, *Typhlonectes natans* (Amphibia: Gymnophiona). Elect Microsc Soc Sthn Afr 21:247–248.

van der Horst G, Visser J, van der Merwe L. 1991. The ultrastructure of the spermatozoon of *Typhlonectes natans* (Gymnophiona: Typhlonectidae). J Herpetol 25:441–447.

Wake MH. 1994. Comparative morphology of caecilian sperm (Amphibia: Gymnophiona). J Morphol 221:261–276.

Wilkinson M. 1997. Characters, congruence and quality: a study of neuroanatomical and traditional data in caecilian phylogeny. Biol Rev 72:423–470.

Wilkinson M, Nussbaum RA. 1996. On the phylogenetic position of the Uraeotyphlidae (Amphibia: Gymnophiona). Copeia 1996: 550–562.

Wilkinson M, Richardson MK, Gower DJ, Oommen OV. 2002. Extended embryo retention, caecilian oviparity and amniote origins. J Nat Hist 36:2185–2198.

Wortham JWE Jr, Murphy JA, Martan J, Brandon RA. 1982. Scanning electron microscopy of some salamander spermatozoa. Copeia 1982:52–60.