29 *Spermatozoal Phylogeny of the Vertebrata*

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The ultrastructure of the spermatozoa of fish, from agnathans to Dipnoi, and its phylogenetic significance has been reviewed by Jamieson (1991) and Mattei (1991) and the evolution of tetrapod sperm, with particular reference to amniotes, was discussed by Jamieson (1995a). In this chapter, spermatozoal ultrastructure and phylogeny from the Sarcopterygii (lobed-finned fish and their descendants) will be examined and an attempt will be made to deduce the spermatozoal synapomorphies which distinguish the major constituent groups.

Spermatozoa of Sarcopterygii

Extant sarcopterygians consist of the Actinistia (containing a single species, the coelacanth, *Latimeria chalumnae*), the Dipnoi (containing the three lungfish genera *Neoceratodus*, *Protopterus* and *Lepidosiren*), and the Tetrapoda (containing the Lissamphibia and the Amniota). Alternative classifications and phylogenies of these three major groups are discussed by Jamieson (1991). Despite some equivocal results, recent molecular studies appear to confirm the finding (for example, Meyer, Wilson 1990; Meyer, Dolven 1992) that lungfishes are the sister group of the Tetrapoda though the closest relatives of *Latimeria* (Yokobori et al. 1994; Zardoya, Meyer 1996, 1997a, b). All endorse the sarcopterygian status of *Latimeria* demonstrated by Hillis et al. (1991).

The spermatozoa of the sarcopterygian fish have been described ultrastructurally for the actinistian *Latimeria chalumnae* (Mattei et al. 1988); for the Australian lungfish, *Neoceratodus forsteri* (Jamieson 1995a; Jespersen 1971) and in this account (Figs. 1A-R); for *Protopterus* (Boisson 1963; Boisson et al. 1967; Purkerson et al. 1974); and for *Lepidosiren* (Matos, Azevedo 1989); tetrapod sperm literature is briefly summarized in Jamieson (1995a) and in this chapter.

Symplesiomorphies of Sarcopterygian Sperm

As deduced from the sperm of sarcopterygian fish (*Latimeria* and the Dipnoi) and a survey of tetrapod sperm, the following features appear plesiomorphic for the spermatozoa of Sarcopterygii.

Sarcopterygian fish sperm have a very long, slenderly conical acrosome vesicle (Figs. 1A-E) but lack the subacrosomal cone that is basic to tetrapods. There is only one endonuclear canal in the coelacanth, *Latimeria chalumnae*, but this contains two or three perforatoria (Jamieson 1991, 1995a; Mattei et al. 1988). In *Neoceratodus* there are two or three (Figs. 1C-F, H, I) or sometimes four perforatoria initially in one canal but more posteriorly (Fig. 1H) in as many canals as there are perforatoria (Jamieson 1995a; Jespersen 1971). *Neoceratodus* is exceptional in sarcopterygians

in that the perforatoria re-emerge from the nucleus at their posterior ends (Fig. 1I). The number of endonuclear canals and of enclosed perforatoria is one in basal Lissamphibia, in the caiman (though poorly substantiated by micrographs), tinamou, rhea and non-passerines (for example, galliforms), but in the Chelonia and *Crocodylus johnstoni* there are two or three canals and in *Sphenodon* there are two. There are three endonuclear canals in the sperm of the sturgeon, *Acipenser sturio*, in the Actinoperygii (ray-finned fish). It is therefore probable that the presumed common ancestor of Lissamphibia and amniotes possessed more than one perforatorium and possibly more than one endonuclear canal. A single canal occurs in the Lissamphibia, except where lost in more advanced Anura (Jamieson et al. 1993), and appears basic to all amniotes above turtles and *Sphenodon*; that is, in birds, squamates and mammals (Jamieson, Healy 1992). In *Acipenser* the canals are spiralled around each other as they are in turtles, *Sphenodon* and *Crocodylus johnstoni*. The spiral arrangement, or at least the presence of one or more endonuclear canals, may well be a synapomorphy for the Osteichythes, a monophyletic clade including the Actinopterygii, Sarcopterygii and, within the latter, the Tetrapoda. The canals are absent (presumed lost) in the highly simplified sperm of holosteans (a paraphyletic group) and Neopterygii (Jamieson 1991).

The nucleus is long in the sarcopterygian fish (*Neoceratodus*, Fig. 1A). This may be a plesiomorphic retention from osteichthyan fish, as it is also long in *Acipenser*, a basal actinopterygian, and in Chondricthyes (Jamieson 1991, 1995a). Further elongation in sarcopterygian fish appears apomorphic.

A simple midpiece, as in *Neoceratodus* (Figs. 1G, J-L), and in *Acipenser*, with some of the mitochondria in a cytoplasmic collar, is presumably plesiomorphic for the Sarcopterygii. The location of a putatively mitochondrial sleeve, usually incomplete, lateral to the nucleus in *Latimeria* is clearly apomorphic.

A 9+2 axoneme is plesiomorphic for the Sarcopterygii. Whether the lateral fins (Figs. 1M-O) are a plesiomorphy held over from osteichthyan ancestors, and basal to Actinopterygii, or are a new, homoplasic development is debatable.

Spermatozoal Synapomorphies of the Sarcopterygii

If one accepts the validity of the Sarcopterygii as defined above, at least four synapomorphies for the group can be proposed on the basis of sperm ultrastructure. These pertain to the perforatoria, the nucleus, the retronuclear body and the structure of the flagellum.

The great length of the nucleus in Actinistia (acrosome and nucleus 25-26 μm long) and Dipnoi may be

an initial synapomorphy of the Sarcopterygii. The nucleus reaches a length of 70 μm in *Neoceratodus forsteri* (Jespersen 1971), the longest recorded in fish sperm (Jamieson 1991).

The extension, anterior to the nucleus, of rod-like structures, the perforatoria, is a new development in the Sarcopterygii and thus constitutes a synapomorphy, and an autapomorphy, for the group. The portions of these within the nucleus lie in one or more endonuclear canals. Perforatoria, or at least endonuclear canals indicating the existence of these, are present in lampreys, in which an acrosomal filament is extruded on reaction (Afzelius et al. 1957); the cladistian *Polypterus senegalus*, in which there is an axial endonuclear canal but a perforatorium remains to be identified (Mattei 1970); the chondrostean *Acipenser stellatus*, in which an acrosome reaction involving subacrosomal material has been demonstrated, although the role of the material in the three endonuclear canals is uncertain (Cherr, Clark 1984; Detlaf, Ginzburg 1963); the dipnoan *Neoceratodus forsteri* (Jamieson 1995a; Jespersen 1971); urodele amphibians; primitive frogs, including *Ascaphus* (Jamieson 1995a; Jamieson et al. 1993); and amniotes, including "reptiles" and non-passerine birds, of which the most basal are the Chelonia (Furieri 1970; Healy, Jamieson 1992; Hess et al. 1991; Jamieson 1995a; Jamieson, Healy 1992) and the sphenodontidan *Sphenodon punctatus* (Healy, Jamieson 1992, 1994; Jamieson, Healy 1992). With the exception of lampreys, *Acipenser* and *Polypterus*, therefore in the sarcopterygians, the perforatorial rods extend anterior to the nucleus (Figs. 1C-F, for *Neoceratodus*). It is probable, in view of the presence of rods and endonuclear canals in *Latimeria* and the ceratodontiform *Neoceratodus*, that their absence in the lepidosireniforms *Protopterus* (Boisson 1963; Mattei 1970) and *Lepidosiren* (Matos, Azevedo 1989) is secondary.

A large dense body, between the nucleus and centrioles and termed the retronuclear body, has been described for *Protopterus annectens* by Boisson (1963; Fig. 2) and *P. aethiopicus* by Purkerson et al. (1974). It has been homologized with a smaller structure which, though postmitochondrial, originates behind the nucleus, in *Neoceratodus* by Jespersen (1971) and Jamieson (1991; Fig. 1G); and a postnuclear structure, termed by Mattei et al. (1988) the "paracentriolar body," in *Latimeria chalumnae* by Jamieson (1991). Its cross striation in *P. aethiopicus* has led to its being compared with the striated columns of mammalian sperm (Purkerson et al. 1974). It is tentatively considered homologous with the neck region of urodele and anuran sperm (Jamieson 1991, 1995a), being, in urodeles, most strongly developed in ambystomatoids, plethodontids (Fig. 6C) and salamandroids (Figs. 6D, E) (Baker 1962, 1963, 1966; Furieri 1962; Jamieson 1995a; Picheral

1967, 1979; Picheral et al. 1966; Werner et al. 1972) and weakly developed in cryptobranchs (Baker 1963). The retronuclear body is here considered to be a synapomorphy, and autapomorphy, of the Sarcopterygii.

In *Neoceratodus*, lateral fin-like prolongations of the sperm flagellum are present at doublets 3 and 8 (Jamieson 1995a; Jespersen 1971), (Figs. 1L-O, Q), except at the endpiece (Fig. 1R). Shortly behind the distal centriole, within the mitochondrial collar (Fig. 1L), and behind this (Fig. 1M), for a short distance, each fin is supported by a large dense juxta-axonemal rod and by a smaller lateral rod within its free extremity (Jamieson 1995a). The fin becomes more extensive behind this short anterior region but the rods are reduced in size (Figs. 1N, O) and soon only the lateral fiber persists (Figs. 1O, Q). Such lateral prolongations, though questionably with supporting rods, in *Latimeria* were appropriately termed undulating membranes by Tuzet and Millet (1959). Lateral fins (also at doublets 3 and 8) in many actinopterygian fish (Jamieson 1991; Mattei 1988) could conceivably have been precursory to actinistian and dipnoan undulating membranes but homoplasy cannot be ruled out as lateral axonemal fins occur also in some echinoderms and protostomes (Jamieson 1995a). Two bilateral elements which also occur at doublets 3 and 8 in Chondrichthyes were presumably convergently acquired.

It is here accepted, as proposed by Jamieson (1995a), that dipnoan axonemal fins are homologous with the undulating membrane of lissamphibian sperm. It is thus proposed that presence of two undulating membranes is a sarcopterygian synapomorphy. As supporting rods are not reported for *Protopterus* (Boisson 1963; Purkerson et al. 1974) or *Lepidosiren* (Matos, Azevedo 1989), nor for *Latimeria* (Mattei et al. 1988), it is possible that presence of such rods is a ceratodontiform-amphibian synapomorphy.

Even if lateral axonemal fins are sarcopterygian symplesiomorphies carried over from an osteichthyan ancestor, their elaboration in dipnoans and amphibians is considered synapomorphic.

Spermatozoal Synapomorphies of the Tetrapoda

A generalized tetrapod spermatozoon manifesting the shared features of basal lissamphibians (for example, *Ascaphus*) and basal amniotes (Chelonia, *Sphenodon*) is illustrated in Figure 3. As it shares features of basal, extant tetrapods, delineation of this hypothetical ancestral tetrapod sperm is not unduly speculative. It should be borne in mind that ancestral tetrapods, in being non-amniote, are classifiable as Amphibia. Their descendants are the Lissamphibia and the Amniota.
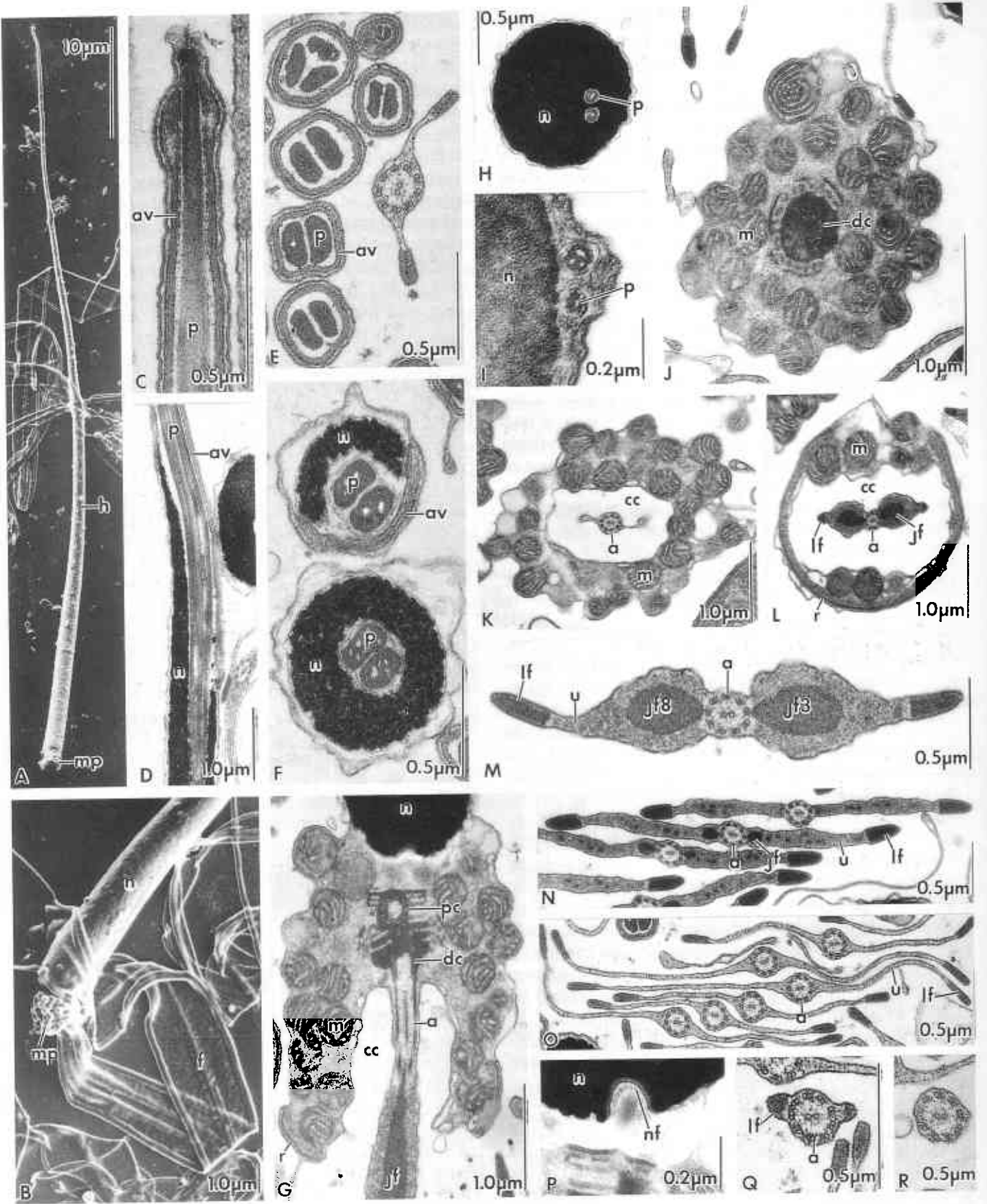
The tetrapod sperm is derived relative to that of sarcopterygian fish (Actinistia and Dipnoi) in a remodeling of the acrosome complex and nucleus. This involves development of a subacrosomal cone and, presumably to house this, correlated reshaping of the proximal end of the nucleus.

A cone of subacrosomal material, not seen in sarcopterygian fish, is developed, in addition to the basal sarcopterygian perforatorial rods, in the Lissamphibia in *Ascaphus* (Fig. 4) and discoglossoids, and in the amniotes in Chelonia, *Sphenodon* (Fig. 11), crocodiles (Fig. 12), squamates (Fig. 15A) and monotremes. Its function is not known but it may be perforatorial, as suggested by the fact that it is present in the absence of perforatorial rods in monotremes. Birds have lost the subacrosomal cone but plesiomorphically retain a perforatorial rod.

A second synapomorphy of the tetrapod spermatozoon relative to sarcopterygian fish is the development of an abrupt shoulder-like transition from the anterior tapered portion of the nucleus (constituting the nuclear rostrum) within the acrosome complex to the long cylindrical portion of the nucleus. The nuclear shoulders are illustrated for *Ascaphus* (Fig. 4), *Sphenodon* (Fig. 11), *Crocodylus johnstoni* (Fig. 12) and the squamate *Carlia rubrigularis* (Fig. 15A). The shoulders and associated narrowing of the proximal end of the nucleus as the nuclear rostrum, within the acrosome complex, presumably were an adaptation allowing the newly evolving acrosome cone to be housed between the nucleus and the acrosome vesicle.

Spermatozoa of the Lissamphibia

The extant Amphibia comprise the subclass Lissamphibia. These consist of the Urodela (newts,
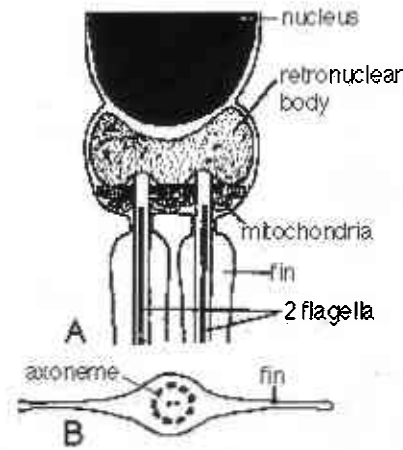

Figure 2. *Protopterus annectens.* Spermatozoon, showing retronuclear body. (From Jamieson 1991, after Boisson 1963.)

salamanders and sirenians), the Anura (frogs and toads) and the Gymnophiona (caecilians). It is argued (Jamieson 1995a; Lee, Jamieson 1993) that internal fertilization is basic (plesiomorphic) in the Lissamphibia. It occurs in the great majority of urodeles and in all gymnophionans, but in only primitive frogs.

A survey of the ultrastructure of the spermatozoa of the three orders of Lissamphibia permits delineation of a generalized lissamphibian spermatozoon (Jamieson 1995a; Fig. 5). Many features of this spermatozoon are deduced to be plesiomorphies carried over from the presumed tetrapod ancestral spermatozoon as they are also found in basal amniotes or even in Dipnoi and Actinistia.

Spermatozoal Symplesiomorphies in the Lissamphibia

Features of the ascaphid (Fig. 4) and hypothetical ancestral lissamphibian spermatozoon (Fig. 5) that are

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Abbreviations used in figures: a, axoneme; af, axial fiber; an, annulus; av, acrosome vesicle; b, barb; cc, cytoplasmic canal; cd, cytoplasmic droplet; cy, cytoplasm; d3, density (juxta-axonemal fiber?) at 3; db, dense, intermitochondrial body; dc, distal centriole; el, electron lucent space; ec, endonuclear canal; f, flagellum; fs, fibrous sheath; h, head; hmt, helical microtubules; jf, juxta-axonemal fiber; jf3, juxta-axonemal fiber at 3; jf8, juxta-axonemal fiber at 8; lc, longitudinal column; lf, lateral fiber; m, mitochondrion; mp, midpiece; mts, sheath of microtubules; n, nucleus; nf, basal nuclear fossa; nk, neck; ni, infolding of nucleus into neckpiece (retronuclear body); nr, nuclear rostrum; nri, nuclear ridge; p, perforatorium; pa, paraxonemal rod; pc, proximal centriole; pf, peripheral fiber; r, retronuclear body; sc, subacrosomal cone; sdb, small dense body; stc, striated (segmented) column; su, subacrosomal material; u, undulating membrane.

Figure 1. *Neoceratodus forsteri*, the Australian lungfish. Ultrastructure of the spermatozoon. A: Scanning electron micrograph (SEM) of the head and midpiece. B: SEM of the base of the nucleus and midpiece and the flagellum with its lateral fins or undulating membranes. C, D: Longitudinal sections (LS) of the perforatoria, showing their extension anterior to the nucleus and, in D, in an endonuclear canal. E: Transverse sections (TS) of the perforatoria within the acrosome. F: TS of perforatoria entering and within the nucleus. G: LS of midpiece, showing mitochondrial collar. H: TS of two perforatoria, each in a separate endonuclear canal. I: TS of two perforatoria posteriorly emergent from the nucleus. J: TS of midpiece through the distal centriole. K: TS of far anterior region of axoneme, within the cytoplasmic canal. L: Same further distally, showing beginning of lateral and juxta-axonemal fibers on each side. M: Same shortly behind the midpiece. N: Further distally, showing more slender undulating membranes still with lateral and greatly reduced juxta-axonemal fibers. O: Still further distally, the slender undulating membranes now lacking the juxta-axonemal fibers. P: LS of basal nuclear fossa. Q: Axoneme far distally, shortly before the endpiece, with greatly reduced undulating membranes. R: TS of endpiece. (Original.)
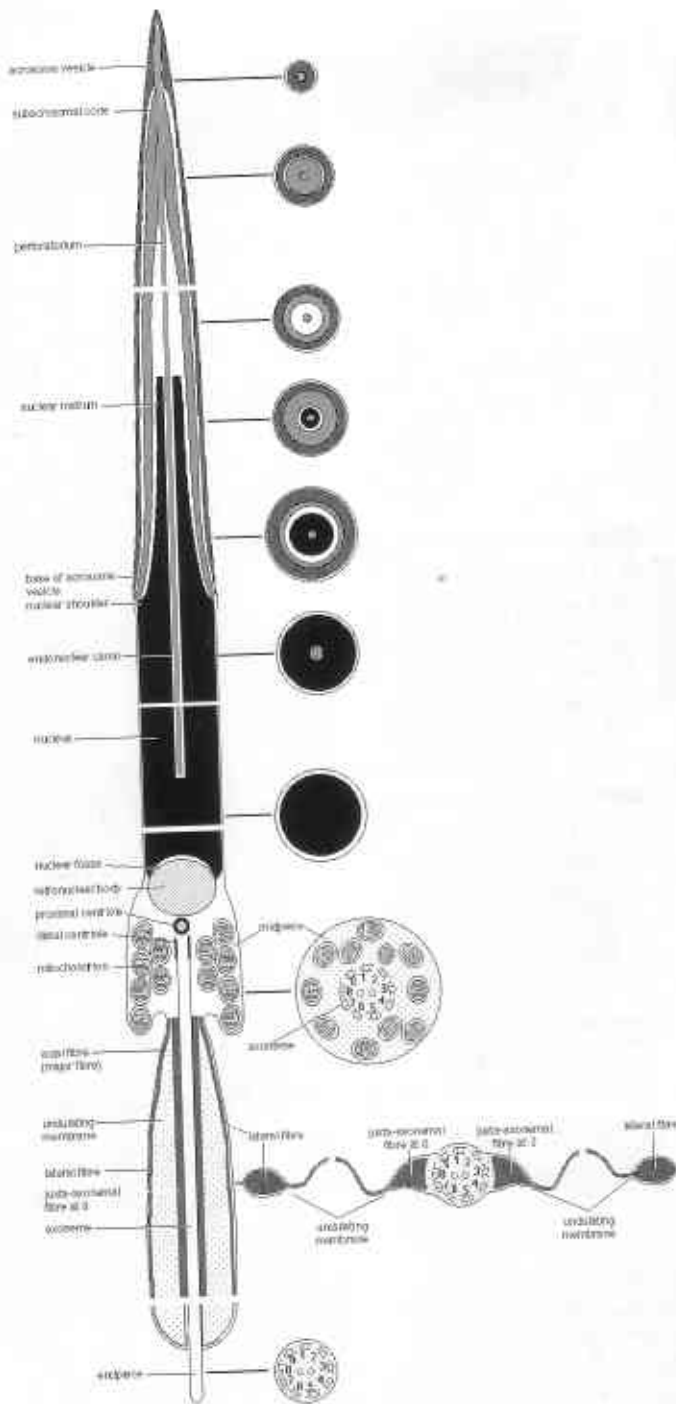
Figure 3. Generalized or hypothetical ancestral tetrapod spermatozoon.

Figure 4. *Ascaphus truei*. Diagrams of spermatozoal ultrastructure as seen in longitudinal section and transverse sections by transmission electron microscopy. (From Jamieson et al. 1993.)

lissamphibian symplesiomorphies are as follows. An anterior acrosomal vesicle forms a hollow cone (sarcopterygian symplesiomorphy) which overlies a cone of subacrosomal material; the subacrosomal cone embraces the tapered anterior end of the nucleus (lissamphibian symplesiomorphy and tetrapod synapomor-
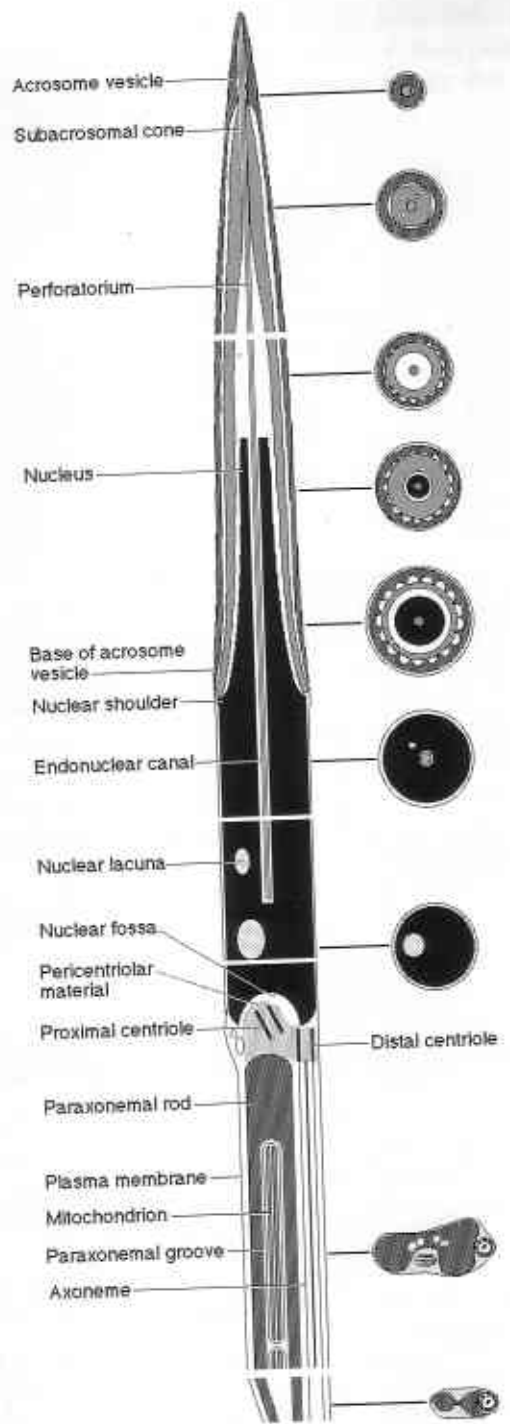
phy) which is elongate (lissamphibian symplesiomorphy and sarcopterygian synapomorphy). At the posterior end of the acrosome (vesicle and subacrosomal cone), the nucleus forms characteristic "shoulders" (lissamphibian symplesiomorphy and tetrapod synapomorphy)

posterior to which its form is cylindrical. Axially, within the acrosome and therefore extending anterior to the nucleus (lissamphibian symplesiomorphy and sarcopterygian synapomorphy), there is a rod, the putative perforatorium, which deeply penetrates the nucleus within an endonuclear canal (lissamphibian, tetrapod and sarcopterygian symplesiomorphy). The basal sarcopterygian feature of a prenuclear, and endonuclear, axial rod (perforatorium), is present not only in *Ascaphus* but also in other primitive frogs, *Discoglossus* and the bombinids *Bombina* and *Alytes*. It is also present in gymnophionids though there the perforatorium lodges posteriorly in a much shorter endonuclear canal.

The base of the nucleus is indented as a basal nuclear fossa (possibly a tetrapod symplesiomorphy as also seen in *Neoceratodus*, Fig. 1P) which, unlike the dipnoan, contains the proximal centriole. Behind this is the distal centriole, which forms the basal body of the axoneme.

Spermatozoal Synapomorphies in the Lissamphibia

Synapomorphies of the lissamphibian sperm relative to an ancestral tetrapod may now be considered.

The presence of an undulating membrane within the flagellar complex has long been thought distinctive of the Lissamphibia but it is proposed here, as in Jamieson (1995a), that it is the loss of the undulating membrane adjacent to doublet 8 which is distinctive and synapomorphic of the Lissamphibia, with retention of that at doublet 3. Evidence for the former existence of an undulating membrane at doublet 8, as in Actinistia and Dipnoi, is the persistence of the juxta-axonemal fiber at doublet 8, in the absence of a membrane on that side, in urodeles (Figs. 6N, O) and exceptionally in Anura.

Concomitant with the loss of one undulating membrane has been the development of a condition in which the axoneme undulates around the remaining lateral fiber which has, therefore, long been termed the axial fiber. This condition is particularly well demonstrated by the Urodela, particularly in the non-cryptobranchs (Figs. 6N, O) as these have a stiffened axial fiber. An axial fiber with undulating membrane is present in caecilians (Fig. 6I), as basically in anurans (Figs. 5, 6P).

Spermatozoal Synapomorphies in the Urodela

Ultrastructural aspects of urodele spermatozoon are illustrated for the salamandrid *Taricha granulosa* (Figs. 6D, E, O) and the plethodontids *Stereochilus marginatus* (Fig. 6C) and *Eurycea quadridigitata* (Figs. 6M, N).

The structure of urodele sperm is uniform relative to the great diversity in anurans though the ground plan has many similarities with that of anuran and caecilian sperm. The more striking, synapomorphic, urodele
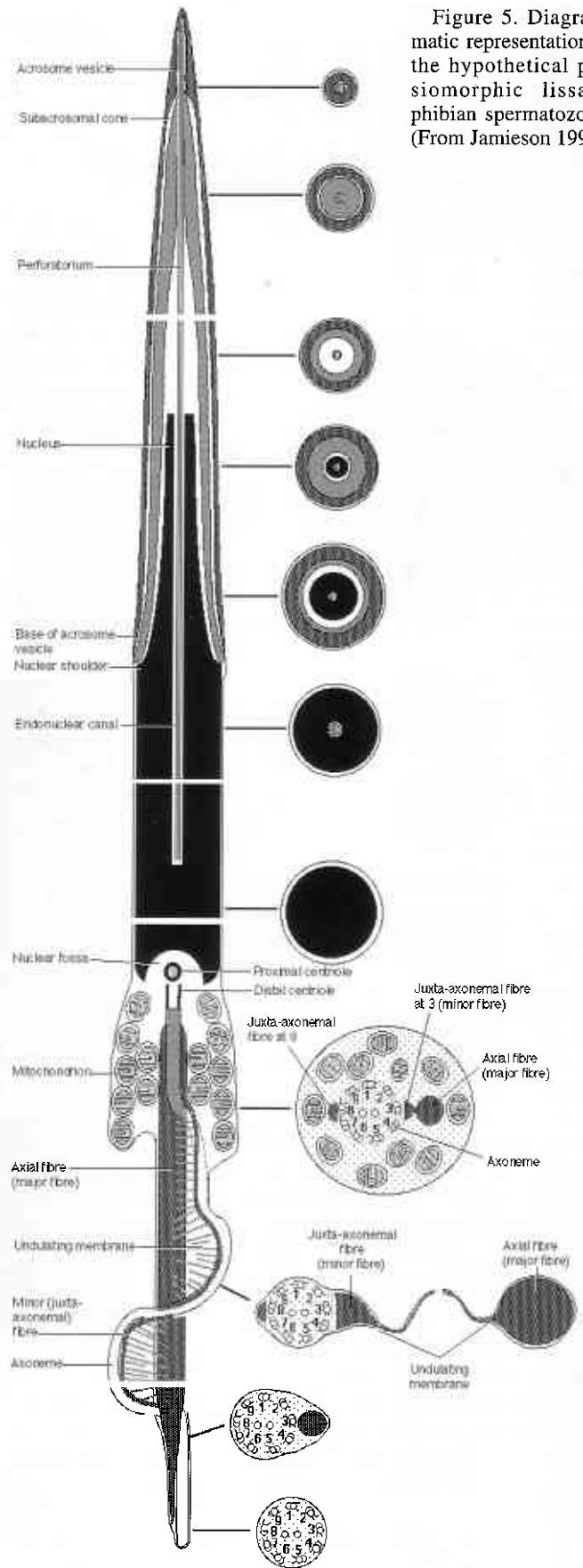

Figure 5. Diagrammatic representation of the hypothetical plesiomorphic lissamphibian spermatozoon. (From Jamieson 1995.)

sperm features will now be discussed, general lissamphibian features having been outlined above.

The acrosome in salamandrids, plethodontids (*Eurycea quadridigitata*, Fig. 6A) and ambystomatids typically ends subapically in a distinct barb (Retzius 1906; Wortham et al. 1982). It is uncertain that a barb is absent in cryptobranchs, amphiumids and sirenids. However, the transverse section of the acrosomal cap (vesicle) in the cryptobranch *Hynobius nebulosus* is strongly trifoliate in a transmission electron micrograph (Picheral 1967, 1979), a condition which is compatible with presence of a barb or at least some unilateral modification of the tip of the vesicle. The presence of a terminal barb to the acrosome of *Amphiuma* claimed by McGregor (1899) was denied by later authors (Baker 1962; Barker, Biesele 1967) and a barb was not described in an optical study of *Pseudobranchus striatus* (Austin, Baker 1964). It is possible that the barb is an internal and not basal synapomorphy of the Urodela.

The barb has no equivalent in anurans. Modification of the tip of the spermatozoon in *Neoceratodus forsteri* (Fig. 1C) is possibly precursory to the urodele condition.

At least the salamandrids and plethodontids (*Eurycea quadridigitata*, Fig. 6A) are distinguished by a stronger development of the subacrosomal cone than that occurring in anurans and gymnophionans. The cone is so large that it projects distally beyond the acrosome vesicle. The condition is uncertain in cryptobranchs and hypermophosis may be a synapomorphy of higher urodeles only.

A nuclear ridge (as in the salamandrid *Taricha granulosa*, Fig. 6M), composed of closely adpressed microtubules internal to the nuclear envelope, is restricted to urodele sperm. It is known for cryptobranchs and salamandrids (Picheral 1967, 1979; Picheral et al. 1966), in addition to plethodontids (Fig. 6M), and can thus be considered a basal synapomorphy and, because unique, an autapomorphy of urodeles.

Behind the nucleus, urodele sperm have a neck region characterized by a structure, considered to be the homolog of the basal sarcopterygian retronuclear body (Jamieson 1991). It is not seen in gymnophionans and is questionably present in anurans. In salamandrids, the neck (also termed the connecting piece) is a long cylinder which fits into the deep fossa at the basal end of the nucleus in such a way that the neck is surrounded by a thin sheath of chromatin (nuclear collar) limited by two nuclear envelopes (Picheral 1967, 1979; Picheral et al. 1966; this study, Fig. 6E). It may present deep indentations into the neckpiece (as in the salamandrid *Taricha granulosa*, Fig. 6D). The length of the neck varies from one-quarter to about one-sixth of the length of the nucleus.

In the ambystomatoids, plethodontids (*Stereochilus marginatus*, Fig. 6C) show a constant and conspicuous difference in the structure of the neckpiece as compared with that of salamandroids. There is no basal nuclear fossa. The posterior end of the nucleus abuts on the neckpiece, which equals the base of the nucleus in width, at a straight transverse border and there is no continuation of nuclear material around the neckpiece (Fig. 6C). The densified nuclear envelope intervenes between nucleus and neckpiece. The form of the neckpiece in Ambystomatidae is unknown.

The sperm of the cryptobranchs *Hynobius* and *Cryptobranchus* have a very short neckpiece, which may be no more than an enlargement of the anterior end of the axial fiber, and resembles the connecting piece of mammalian sperm (Baker 1963). It also resembles the nuclear-centriolar junction of anuran sperm, to which an additional similarity is the presence and subcircular cross section of the major fiber.

The great development of the neck in salamandrids and plethodontids is possibly a synapomorphy of higher urodeles but, alternatively, the modest development in cryptobranchs might be a reduction relative to the intermediate size seen in at least some Dipnoi (*Protopterus*). Although usually considered primitive, cryptobranchs are apomorphic in having reduced spermatophoral glands and therefore external fertilization, which is deduced to be a secondary condition relative to basic internal fertilization of lissamphibians (Lee, Jamieson 1993).

It is possible that the material of the neck in urodeles is homologous with the pericentriolar material of anurans, which is continuous with the major (axial) fiber of the tail.

Figure 6 (right). Spermatozoal ultrastructure in Lissamphibia. A: The plethodontid urodele *Eurycea quadridigitata*. LS of acrosome, showing terminal barb. B: The myobatrachid frog *Limnodynastes peronii*. LS of acrosome and tip of nucleus. C: The plethodontid urodele *Stereochilus marginatus*. LS of anterior region of neck (retronuclear body) and transverse junction with nucleus. D: The salamandrid urodele *Taricha granulosa*. LS of posterior region of neck (retronuclear body) showing that it is ensheathed by the posterior region of the nucleus which intrudes into it. E: Same, showing basal nuclear fossa; F: The microhylid *Cophixalus ornatus*. TS of sperm flagella showing absence of undulating membranes and fibers. G: The rhacophorid frog *Chiromantis xerampelina*. LS (top and left) of five spiral sperm nuclei below which are many cross sections of the paired terminal flagella. H: Same, a TS of the paired flagella embedded in a sheath of microtubules in pseudocrystalline array. I: The gymnophionan *Typhlonectes natans*. TS of axoneme and undulating membrane. J: The hylid frog *Cyclorana alboguttata*. TS of axoneme and thick undulating membrane. K: The hylid frog *Litoria rheocola*. LS of eubufonoid mitochondrial collar. L: The myobatrachid, limnodynastine frog *Limnodynastes convexiusculus*. LS of basal nuclear fossa penetrated by axial fiber. M: *Taricha granulosa*. TS of nucleus showing microtubules of nuclear ridge. N: *E. quadridigitata*. TS of two sperm flagella, showing crescent of mitochondria. O: *Taricha granulosa*. TS of sperm flagella, showing crescent of mitochondria and Y-shaped axial fiber. P: The hylid frog *Litoria eucnemis*. TS of flagella, showing hypermorphosed juxta-axonemal fiber at 3. (Original.)
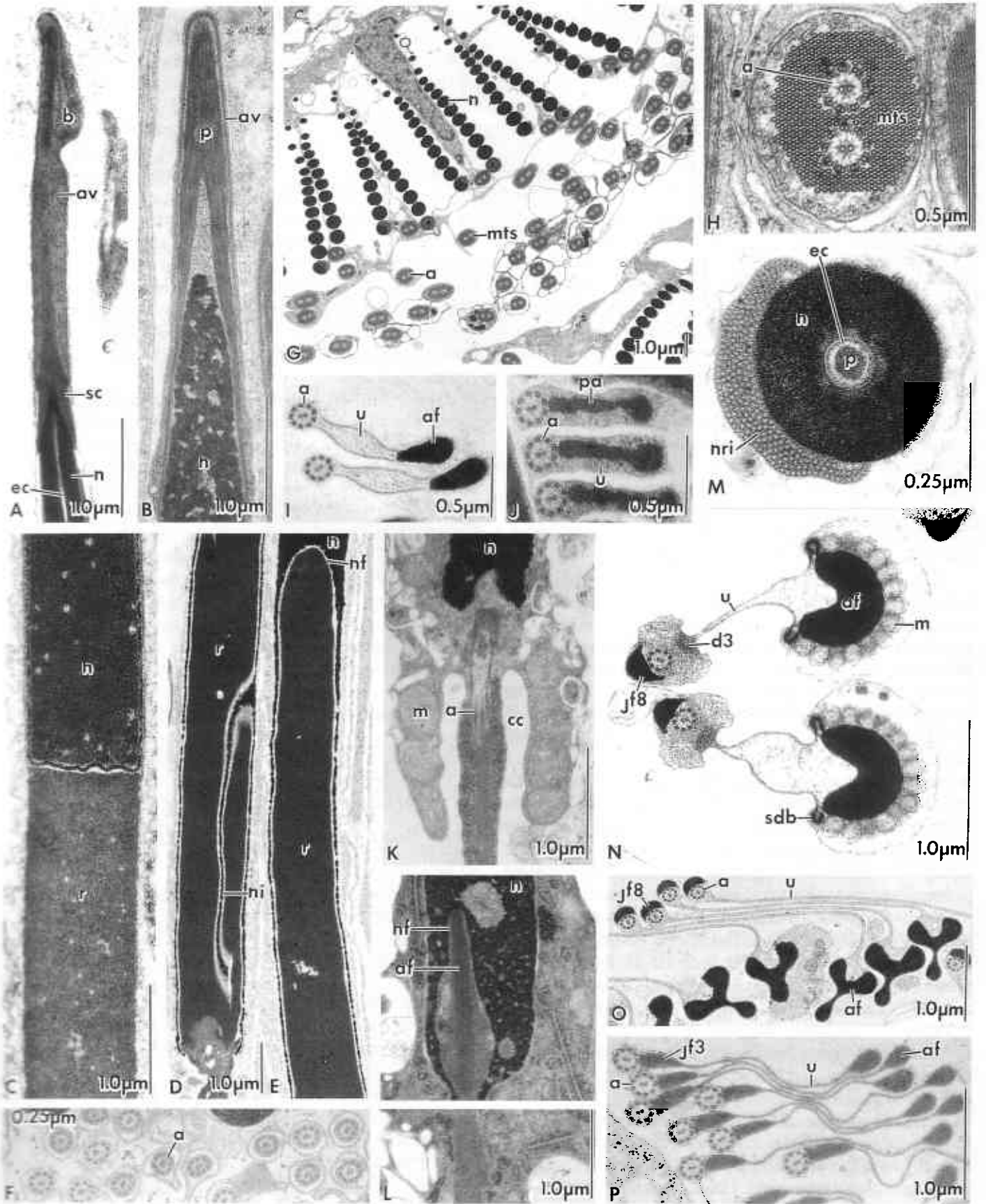
At least some urodele sperm have a dense ring-like structure questionably identified with an annulus (Picheral 1979). The inner wall of a cytoplasmic collar, equivalent to that in many fish sperm (Jamieson 1991), is occupied by two large dense structures: the ring, forming a lining to much of the canal, and opposite the ring, and in *Amphiuma* contiguous with it (Barker, Biesele 1967), the axial fiber. It is uncertain whether the strong development of the ring is a general urodele synapomorphy or is restricted to higher urodeles.

In urodele sperm, the axial fiber, at doublet 3, is connected to the axoneme by the undulating membrane but, typically (*Taricha granulosa*, Fig. 6O), there is no intervening juxta-axonemal fiber. This may represent a synapomorphic loss from a basic lissamphibian condition with juxta-axonemal fibers at 3 and 8. However, the condition in cryptobranchs requires investigation. The plethodontoid ambystomatoids (for example, *Plethodon albagula*, Fig. 7 and *Eurycea quadridigitata*, Fig. 6N) are exceptional in having a density on the adaxonemal end of the undulating membrane but it is questionable, because of its connection to dense bodies near the major fiber rather than to the fiber (Figs. 6N, 7), that this is homologous with the anuran juxta-axonemal fiber (Jamieson 1995a).

The axial fiber has the presumed plesiomorphic subcircular cross section in the cryptobranch *Hynobius nebulosus* (Picheral 1979), as in the Gymnophiona (Fig. 6I) and Anura (Fig. 6P), though in cryptobranchs differing from the typical condition in the latter two orders in also having the smaller fiber on the opposite side of the axoneme at doublet 8.

In the higher urodeles, as an internal synapomorphy, the axial fiber acquires, for much of its length, a Y-shaped or trifoliate cross section (*Taricha granulosa*, Fig. 6O). This sectional profile corresponds with that used in human tools and engineering structures to confer strength and rigidity and is deduced to stiffen the fiber against bending. The trifoliate cross section, and the accompaniment of a fiber on the opposite side of the axoneme, has been demonstrated ultrastructurally in salamandrids (Baker 1966; Furieri 1960, 1962; Picheral 1967, 1972, 1979; *Taricha granulosa*, Fig. 6M); in amphiumids (Baker 1962); in ambystomatids (Russell et al. 1981) and the plethodontids *Eurycea* sp., *Eurycea bislineata bislineata*, *E. quadridigitata*, *E. wilderae*, *Gyrinophilus porphyriticus*, *Plethodon albagula*, *Stereochilus marginatus*, and *Typhlomolge rathbuni* (all in this study).

Presence of an additional juxta-axonemal fiber in many anurans at doublet 3, connected with axial fiber by a thin lamina within the undulating membrane, may well be plesiomorphic, and attributable to the generalized lissamphibian sperm.

In some ambystomatid salamanders (*Ambystoma*

and *Rhyacosiredon*) a short tail membrane, absent in the ambystomatid *Rhyacotriton* and unknown in other lissamphibians, has been observed by light microscopy on the opposite side of the major fiber from the undulating membrane at the posterior end of, and extending longitudinally for a fraction of the length of, the membrane (Brandon et al. 1974; Martan, Wortham 1972; Wortham et al. 1982). It does not appear to represent pairing of the undulating membrane, on opposite sides of the axoneme, which is here hypothesized as an ancestral, possibly pre-lissamphibian stage.

Sperm of the sirens, exemplified by *Siren intermedia*, *S. lacertina* and *Pseudobranchus striatus axanthus*, are biflagellate and have two interconnected undulating membranes (Austin, Baker 1964). Such secondary duplication of the axoneme also occurs in some Anura.

The condition of the mitochondria in *Neoceratodus forsteri* in which the mitochondria are located in a short midpiece and in a collar-like posterior extension of this around the base of the axoneme (Figs. 1G, K, L) may represent the plesiomorphic condition for lissamphibia. In the cryptobranch urodeles *Cryptobranchus alleganiensis bishopi* (Baker 1963) and *Hynobius nebulosus* (Picheral 1979), the mitochondria seem to be located in a protoplasmic bead around the nucleus, even in the mature sperm. This possibly represents a reduction from the plesiomorphic condition. The cryptobranch sperm tail lacks mitochondria and there is no remnant of the ring (putative annulus) around the major fiber.

In salamandrids (Fig. 6O), ambystomatids, plethodontids (Figs. 6N, 7) and amphiumids, small, ovoid mitochondria are present in cytoplasm around a long anterior region of the axial (major) fiber where, in cross section of the sperm, they form an arc, the whole constituting the intermediate piece or midpiece (Baker 1966; Fawcett 1970; Grassé 1986; Jamieson et al. 1993; Picheral 1967, 1979; Wortham et al. 1977). This appears to be a derived condition.

This type of midpiece has been said not to occur in anurans but, clearly homoplasically, in *Limnodynastes peronii* an incomplete ring of mitochondria surrounds the major fiber (Fig. 8) much as in salamandrids (Lee et al. 1992); a somewhat similar arrangement is seen in *Bombina variegata* (Folliot 1979; Furieri 1975b; Pugin-Rios 1980) and *Neobatrachus pelobatoides* (Lee, Jamieson 1992) in which the mitochondria lie on each side of the axial fiber.

A major difference of salamandrids, ambystomatids and plethodontids from anurans is the great length of the mitochondrial region, occupying a considerable proportion of the length of the tail. This appears to be an apomorphic elongation as the midpiece is short in dipnoans (Figs. 1B, G) as it is in basal actinopterygians such as *Acipenser* (Jamieson 1991).