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Spermatozoan morphology of 19 species of prosobranch limpets (Patellogastropoda) with a discussion of patellid relationships

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SUMMARY

The spermatozoon morphology of 19 species of Indo-Pacific, East Atlantic and Mediterranean patellid limpets was examined by transmission electron microscopy. All nine species of *Patella* and *Helcion* from the South Atlantic (southern Africa) and the Indo-Pacific, as well as three species from the North Atlantic, have sperm which correspond to one of the sperm groups I, II or III, previously described for patellacean limpets from southern Africa (Hodgson & Bernard 1988). With the exception of *P. safiana* and *P. canescens*, all 7 *Patella* species from the N.E. Atlantic/Mediterranean have sperm morphologies which closely resemble one another but are distinctly different from the sperm types of species in the southern hemisphere. These N.E. Atlantic/Mediterraean limpets have been assigned to a new sperm group, group VI. On the basis of sperm structure it is suggested that patellid limpets had three main centres of radiation: a N.E. Atlantic/Mediterranean centre; an East Atlantic centre with its focal point on the S.W. coast of southern Africa; an Indo-Pacific centre with its focal point on the S.E. coast of South Africa.

Despite similarities between the species within groups, each has a unique sperm. An examination of spermatozoa of one patellid from Namibia and one from S. Angola (both initially identified as *Patella miniata*), has revealed that their sperm are distinctly different, as well being different from that of *P. miniata* from South Africa. Subsequently, the species from Namibia was identified as *P. adansonii*. Furthermore, *P.* cf. *miniata* from S. Angola had a sperm which is remarkably similar to that of *P. safiana* from N.W. Africa and the two may be conspecific.

1. INTRODUCTION

Patellid limpets are some of the commonest inhabitants of intertidal and subtidal hard substrates and these animals have been the subject of numerous ecological, physiological and behavioural investigations (for reviews, see Branch 1981, 1985*a*, *b*). Within the Patellidae, species of *Patella* and *Helcion* have a broad global distribution, being found throughout the eastern Atlantic, Mediterranean and parts of the Indo-Pacific. They reach their greatest diversity in southern Africa (Powell 1973; Lindberg 1988). Despite numerous studies on the biology of *Patella*, there have been very few on *Helcion*. Moreover, there are only a few investigations which have attempted to unravel the relationships within and between these two genera. In reviews of the genus *Patella* (Christiaens 1973; Powell 1973) species were placed into a number of subgenera based on shell and radula characters. More recently the cyto- and population genetics of the North-East Atlantic and Mediterranean species of *Patella* have been investigated using karyology and electrophoresis of allozymes (Bacci & Sella 1970; Sella & Bacci 1971; Wilkins 1977; Badino & Sella 1980; Sella & Badino 1982; Lavie *et al.* 1987; Cervella *et al.* 1988; Ramella *et al.* 1988; Côrte-Real 1992; Sella *et al.* 1993; Cretella *et al.* 1994), and relationships of southern African species (including *Helcion*) were examined using sperm morphology (Hodgson & Bernard 1988; Jamieson *et al.* 1991). Clearly if the relationships of patellids are to be fully understood, it will be essential to combine information from ecological and morphological studies, cytogenetics and biochemical genetics including electrophoresis of allozymes and more recent molecular techniques.

Numerous studies have shown that an examination of sperm morphology can provide useful insights into taxonomic and phylogenetic relationships within and between invertebrate taxa (e.g. Chia *et al.* 1975; Franzén 1977; Jamieson 1984, 1985; Hodgson & Bernard 1986; Eckelbarger & Grassle 1987; Healy 1988; Hodgson & Foster 1992; Hodgson & Chia 1993). A spermatological study of 15 species of prosobranch limpets from the Patellidae of the South African coast (Hodgson & Bernard 1988) was the first to shed some light on possible relationships within and between species of *Patella* and *Helcion*. Although Hodgson & Bernard (1988) were able to examine the

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Table 1. *Dimensions* (μm) *of spermatozoa of 19 species of patellid limpet*

(All head + mid-piece dimensions (except *P. safiana*, *P. canescens* and *P. ferruginea*) are measured from 20 mid-longitudinal sections from which a mean ± s.d. has been calculated. Maximum dimensions only are given for the above three species. All nuclear and acrosome dimensions are maximum measurements recorded from mid-longitudinal sections. Geographic locality refers to the region from which specimens were collected. L:B refers to length to breadth ratio.)

species	geographic locality	morphological sperm type	head + mid-piece length	nucleus			acrosome		acrosomal differentiation
				length	breadth	L:B	length	breadth	
P. chapmani Tenison Woods, 1875	S. Australia	I	3.2 ± 0.2	2.0	1.2	1.7:1	0.5	0.7	no
P. laticostata Blainville, 1825	S. Australia	I	3.6 ± 0.3	2.4	1.0	2.4:1	1.0	0.6	no
P. pica Reeve, 1854	South Africa – Zululand	I	3.3 ± 0.3	2.0	1.1	2.0:1	1.0	0.7	no
P. flexuosa Q & G, 1834	Japan	I	2.9 ± 0.1	1.7	1.2	1.4:1	0.8	0.8	no
P. peronii Blainville, 1825	S.W. Australia	II	12 ± 1.1	9.0	0.3	30:1	2.6	0.4	no
Patella miliaris Philippi, 1848	S. Angola	II	7 ± 0.9	5.0	0.4	12:1	1.6	0.3	yes
Patella adansonii Dunker, 1853	S. Namibia	III	3.4 ± 0.1	2.0	0.9	2.2:1	1.1	0.9	yes
Patella cf. *miniata*	S. Angola	III	3.2 ± 0.2	1.6	1.0	1.6:1	1.7	0.9	yes
P. safiana Lamarck, 1819	N.W. Africa	III	3.4	1.5	0.9	1.8:1	1.8	0.8	yes
P. canescens Gmelin, 1791	St Helena	III	3.0	1.4	0.9	1.6:1	1.4	0.9	yes
P. rustica Linneus, 1758	N.E. Atlantic	VI	7.6 ± 0.4	5.6	0.5	11:1	1.4	0.3	no
P. vulgata Linneus, 1758	N.E. Atlantic	VI	5.7 ± 0.3	4.0	0.6	6.6:1	1.9	0.5	no
P. depressa Pennant, 1777	N.E. Atlantic	VI	4.7 ± 0.1	3.5	0.7	5.0:1	0.9	0.4	no
P. aspera Röding, 1798	N.E. Atlantic	VI	5.1 ± 0.2	3.7	0.6	6:1	1.0	0.3	no
P. candei d'Orbigny, 1840	N.E. Atlantic	VI	5.4 ± 0.2	3.8	0.7	5.4:1	1.1	0.6	no
P. ferruginea Gmelin, 1791	Mediterranean	VI	4.0	2.5	0.6	4.2:1	1.0	0.4	no
P. caerulea Linneus, 1758	Mediterranean	VI	4.6 ± 0.3	3.4	0.8	4.0:1	0.7	0.4	no
H. dunkeri Krauss, 1848	South Africa	III	3.4 ± 0.1	1.9	1.0	1.9:1	1.3	0.9	yes
H. pellucidum Linneus, 1758	N.E. Atlantic	III?	3.1 ± 0.1	1.9	0.9	2:1	0.6	0.6	yes

spermatozoa of the majority of species within these two genera from southern Africa, several species were missing from their study. In addition the study did not include the North Atlantic, Mediterranean and other Indo-Pacific species. Of the North Atlantic/Mediterranean species the spermatozoa of only *Helcion pellucidum* (Sousa & Oliviera 1994), *Patella rustica* (= *P. lusitanica*) (Azevedo 1981), *P. caerulea* (Kohnert & Storch 1983) and *P. vulgata* (Smaldon & Duffus 1985) have been described, the latter two species of *Patella* only briefly. As part of an ongoing investigation into a study on the systematics and phylogeny of prosobranch limpets we describe here the spermatozoa of a further 19 species of Patellidae: six species from southern Africa, nine species from the North Atlantic and Mediterranean (including re-descriptions of *P. rustica*, *P. vulgata*, *P. caerulea* and *H. pellucidum*), three from Australia and one from Japan. The results are compared with those of Hodgson & Bernard (1988) and the potential relationships between and within *Patella* and *Helcion* are discussed.

2. MATERIALS AND METHODS

Animals were collected either by the authors or colleagues (see acknowledgements) from a number of localities (table 1). All specimens were then forwarded to either the Department of Zoology & Entomology at Rhodes University or the Department of Zoology at the British Museum, where tissues were subsequently processed for transmission electron microscopy.

Portions of the testis were fixed for variable lengths of time either in 2.5% glutaraldehyde in filtered sea water (osmolarity of 1200 mOsm) or in 2.5%

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glutaraldehyde in 0.1 M sodium cacodylate buffer and sea water (pH 7.4 and isosmotic with sea water; after Hackney *et al.* 1983). The fixed samples were washed for at least 30 mins in 0.1 M sodium cacodylate buffer and postfixed for 90 minutes in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer and sea water (after Hackney *et al.* 1983), dehydrated in a graded ethanol series and embedded in an Araldite/Taab resin mixture (Cross 1989) via propylene oxide. Thin sections (silver/gold interface) were cut, stained in 5% aqueous uranyl acetate (30 mins) and lead citrate (3 mins) and examined with a Jeol JSM 100CX II electron microscope.

Two of the patellids collected from the west coast of southern Africa (one from S. Namibia and one from S. Angola) were originally identified as *Patella miniata*. These species have also been termed *P. safiana* (Penrith & Kensley 1970), but it was not clear if they corresponded to *P. safiana* of N.W. Africa. Therefore this resulted in some doubts as to their taxonomic status. A more detailed examination of specimens from S. Namibia identified this limpet as *P. adansonii* Dunker 1853 *sensu* Powell (1973). As the taxonomy of the limpets from S. Angola has still to be clarified this species is referred to as *P.* cf. *miniata* (S. Angola) throughout the paper.

3. RESULTS

The spermatozoa of all 19 species are of the primitive (after Franzén 1956) or ect-aquasperm type (after Rouse & Jamieson 1987). The sperm consist of a head of variable length, which is composed of a nucleus and acrosome, a simple mitochondrial midpiece and a flagellum with a 9+2 arrangement of microtubules. The mid-piece of all species examined is similar and consists of a ring of four to five spherical mitochondria (about 0.3–0.5 μM diameter) with well developed cristae (figure 1*b*). In the centre of the mitochondrial ring are the proximal and distal centrioles, the proximal lying in a small posterior nuclear fossa and the distal giving rise to the axoneme. A small cytoplasmic collar surrounds the flagellum as it emerges from the sperm mid-piece (figure 1*b*) although this collar can only be seen in sections cut in the mid-longitudinal plane. Small electron-dense particles presumed to be glycogen are present in the residual cytoplasm.

Although there is little variation in the morphology of the midpiece between species, there are considerable differences in head morphology. In a previous study of the spermatozoa of 13 species of *Patella* and two species of *Helcion*, Hodgson & Bernard (1988) suggested that based on head morphology, species could be assigned to one of four types of spermatozoa (termed Types I–IV). Twelve of the species examined in this study could be placed into one of three of these four sperm types; the remaining seven species had to be assigned to a separate category. To avoid any conflict with the sperm types described by Hodgson & Bernard (1988), this additional category has been termed Type VI (Type V sperm having been previously assigned to *Cellana capensis* by Hodgson & Bernard 1988).

(*a*) *Type I: Spermatozoa with short cylindrical nuclei ('bullet-shaped') and small undifferentiated acrosomes*

Four species examined in the genus *Patella* (*P. chapmani*, *P. laticostata*, *P. pica*, *P. flexuosa*) have this type of sperm. The electron-dense nucleus is slightly

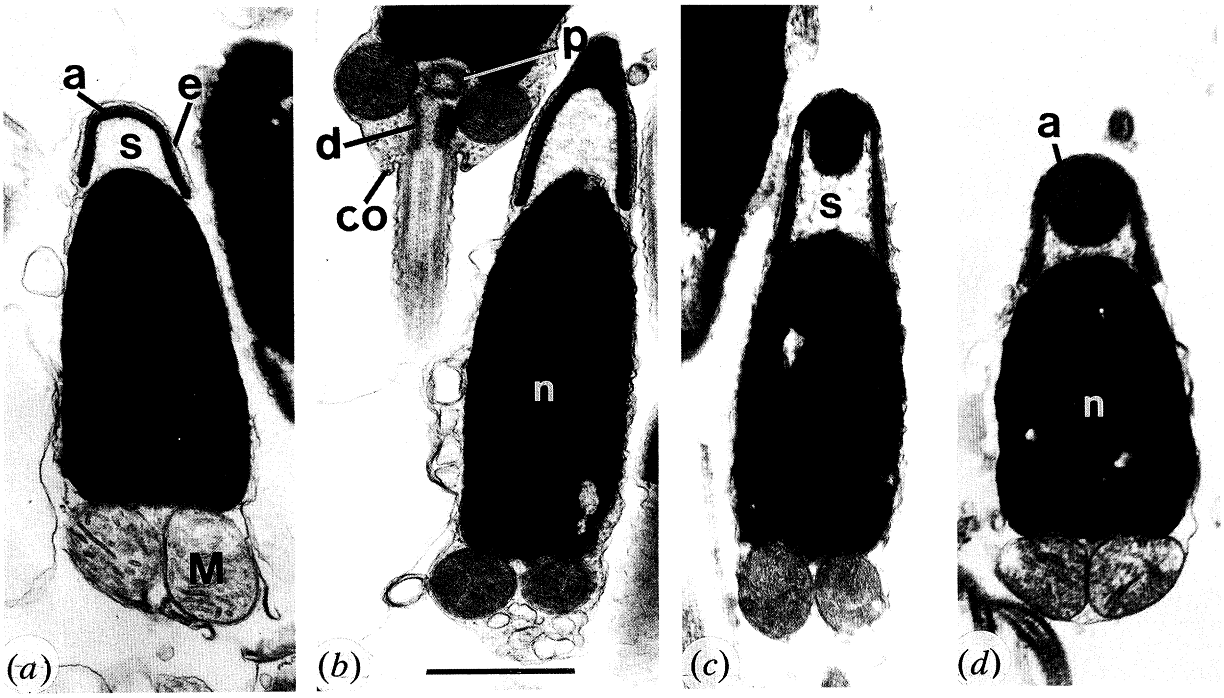

Figure 1. Type I sperm. Mid-longitudinal sections through the spermatozoa of: (*a*) *Patella chapmani*; (*b*) *P. laticostata*; (*c*) *P. pica*; and (*d*) *P. flexuosa*. a, acrosome; co, cytoplasmic collar; d, distal centriole; e, extra-acrosomal layer; M. mitochondrion; n, nucleus; p, proximal centriole; s, subacrosomal space. Scale bar = 1 μM.

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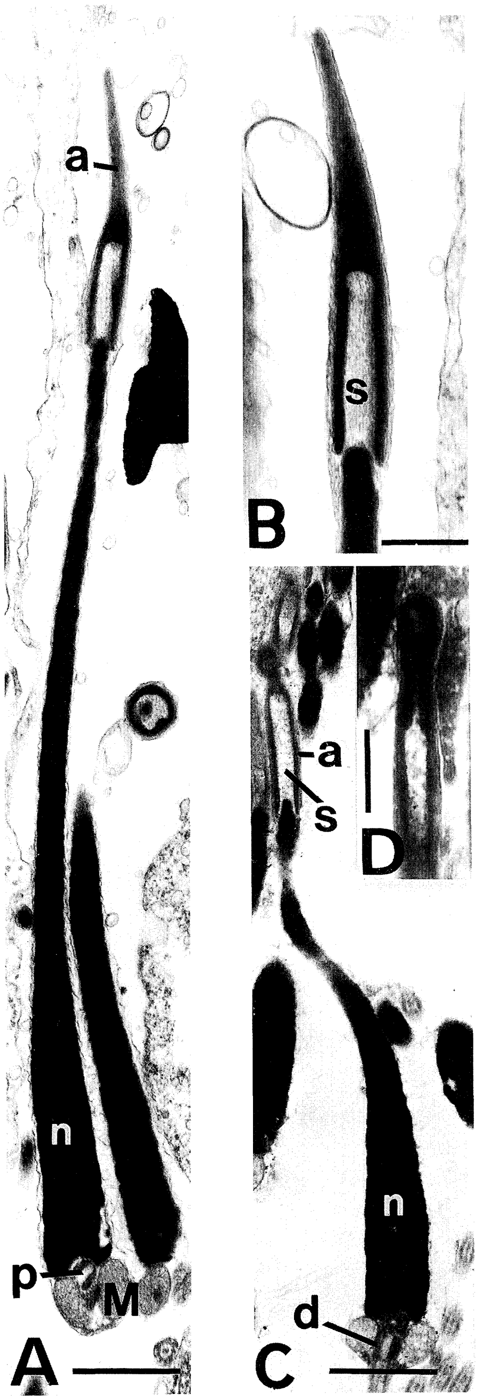

Figure 2. Type II sperm. Mid-longitudinal sections through the spermatozoa and acrosomes of: (*a*, *b*) *P. peronii*; and (*c*, *d*) *Patella miliaris*. a, acrosome; d, distal centriole; M, mitochondrion; n, nucleus; p, proximal centriole; s, subacrosomal space. (*a*, *b*) scale bars = 1 μm; (*c*, *d*) scale bars 0.5 μm.

elongate (L:B < 3:1) and rounded at the anterior end. In *P. chapmani* and *P. pica* the nucleus is about 2.0 μm long × 1.1 μm wide whereas in *P. laticostata* the nucleus is slightly longer and narrower (about 2.4 μm long × 1.0 μm wide) (table 1). *P. flexuosa* has a smaller nucleus (about 1.7 μm × 1.2 μm). In both species the nucleus is capped by a small membrane-bound acrosome (< 32% of total head length), the contents of which are uniformally electron-dense (i.e. undifferentiated) (see table 1 for dimensions). The acrosome is in the shape of a hollow cone, which is rounded anteriorly in *P. chapmani*, *P. pica* and *P. flexuosa* and more pointed in *P. laticostata*. In *P. chapmani* the acrosomal vesicle is of uniform thickness (about 55 nm) (figure 1*a*) whereas in *P. laticostata* the acrosome has a small anterior extension (figure 1*b*). The acrosome of both *P. pica* and *P. flexuosa* have a rounded posterior lobe which projects into the subacrosomal space (figure 1*c*, *d*). In all species, a thin but distinct extra-acrosomal layer is sandwiched between the cell membrane and the outer acrosomal membrane. In the hollow of the acrosome lies subacrosomal material which has a granular appearance.

(*b*) *Type II: Spermatozoa with very elongate, tapering 'flask-shaped' nuclei and acrosomes with long anterior extensions*

Two species, *P. peronii* and *Patella miliaris*, have this type of spermatozoon. In both species the nucleus is very elongate (about 9 μm in *P. peronii* and 5 μm in *P. miliaris*) so that the nuclear length: breadth ratio is greater than 12:1. In both species the nucleus has a basal width of about 0.8 μm, and a midwidth of 0.3–0.4 μm which narrows to 0.2 μm anteriorly. The pointed anterior of the nucleus projects into the subacrosomal space. The nucleus is capped by an elongate hollow cone-shaped acrosome (see table 1 for dimensions) which in *P. miliaris* is differentiated internally. The wall of the posterior section is about 85–90 nm thick. The anterior extension of the acrosome is solid (and about 0.2 μm diameter).

(*c*) *Type III: Spermatozoa with nuclei which intrude into the subacrosomal space (often 'bottle-shaped') and complex acrosomes*

Four species of *Patella* and two species of *Helcion* were assigned to this sperm type i.e. *P.* cf. *miniata* (S. Angola) *P. adansonii*, *P. safiana*, *P. canescens*, *H. pellucidum* and *H. dunkeri* (table 1, figure 3). However it should be noted that the placement of *H. pellucidum* in this group is tentative as the sperm does not possess all the features of Type III. In common with the Type III species, it shows a differentiated and complex acrosome. However its nucleus is not bottle-shaped and does not intrude into the subacrosomal space, and in these resembles sperm of Type I species.

In all these species the heads of the sperm are small, with a diameter of about 1.0 μm and a length ranging from 3.0 to 3.5 μm. The nuclei are small with a L:B < 2.5:1 (table 1), and either rounded anteriorly or narrow at the point they intrude into the acrosome (the latter form being termed 'bottle-shaped' by Hodgson & Bernard 1988); in both cases the anterior portion intrudes into the subacrosomal space beneath the acrosome. The anterior extension of the nucleus is particularly well developed in *Patella* cf. *miniata* (S. Angola), *P. adansonii*, *P. safiana* and *P. canescens* (figure 3*a*–*g*).

The acrosome is complex and constitutes a large proportion (40–50%) of the head length of the sperm in all these species except *H. pellucidum* (figure 3*j*, *k*) (see table 1 for species dimensions). The acrosome is in

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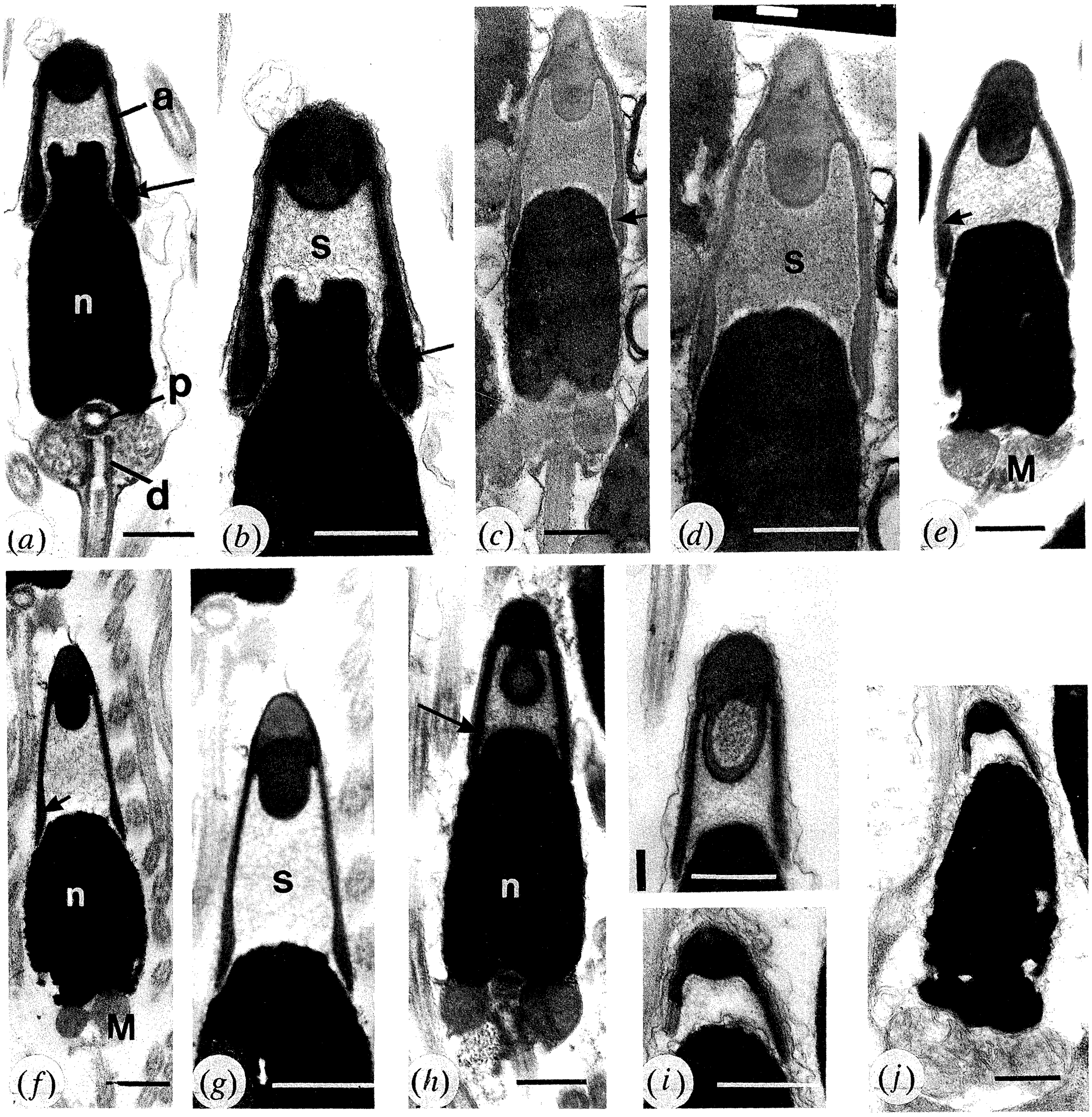

Figure 3. Type III sperm. Mid-longitudinal sections through the spermatozoa and acrosomes of: (*a*, *b*) *Patella adansonii*; (*c*, *d*) *P. safiana*; (*e*) *P.* cf. *miniata* (Angola); (*f*, *g*) *P. canescens*; (*h*, *i*) *H. dunkeri* (*j*, *k*) *H. pellucidum*. a, acrosome; d, distal centriole; M, mitochondrion; n, nucleus; p, proximal centriole; s, subacrosomal space. Note the slightly bulbous postior region of the acrosomes (arrowed). Scale bars = 0.5 μm.

the form of a hollow cone, which is seated over the anterior section of the nucleus. Posteriorly the wall of the acrosomal vesicle is slightly bulbous (except in *H. pellucidum*) (figure 3) so that the inner acrosomal membrane lies close to the nuclear envelope. The anterior section of the acrosome is solid with a posterior lobe projecting into the subacrosomal space which contains a granular material. The acrosomal contents are differentiated into electron-dense and electron-lucent regions (figure 3). This last feature is the major reason *H. pellucidum* has been provisionally assigned to Type III sperm: no representatives of Type I sperm have differentiated acrosomes.

Two of the species examined, *P. safiana* and *Patella* cf. *miniata* (Angola) have spermatozoa which were extremely similar in size and in the morphology of the nucleus and acrosome (compare figure 3*c*, *d* and *e*). This extreme similarity suggests that they are very closely related and may be the same species. Indeed *P.* cf. *miniata* (from both Angola and Namibia) has been previously identified as *P. safiana* (Penrith & Kensley 1970).

(*d*) *Type VI: Spermatozoa with elongate cylindrical nuclei and conical acrosomes*

Seven of the North Atlantic and Mediterranean species could be assigned to this category i.e. *P. rustica*, *P. vulgata*, *P. depressa*, *P. aspera*, *P. candei*, *P. ferruginea* and *P. caerulea*. Their spermatozoa are characterized by an elongate nucleus (L:B ranging from 5:1 to 11:1) which is cylindrical (not tapering), and rounded anteriorly. The acrosome is distinctly conical in shape and sharply pointed in all species except *P. caerulea*

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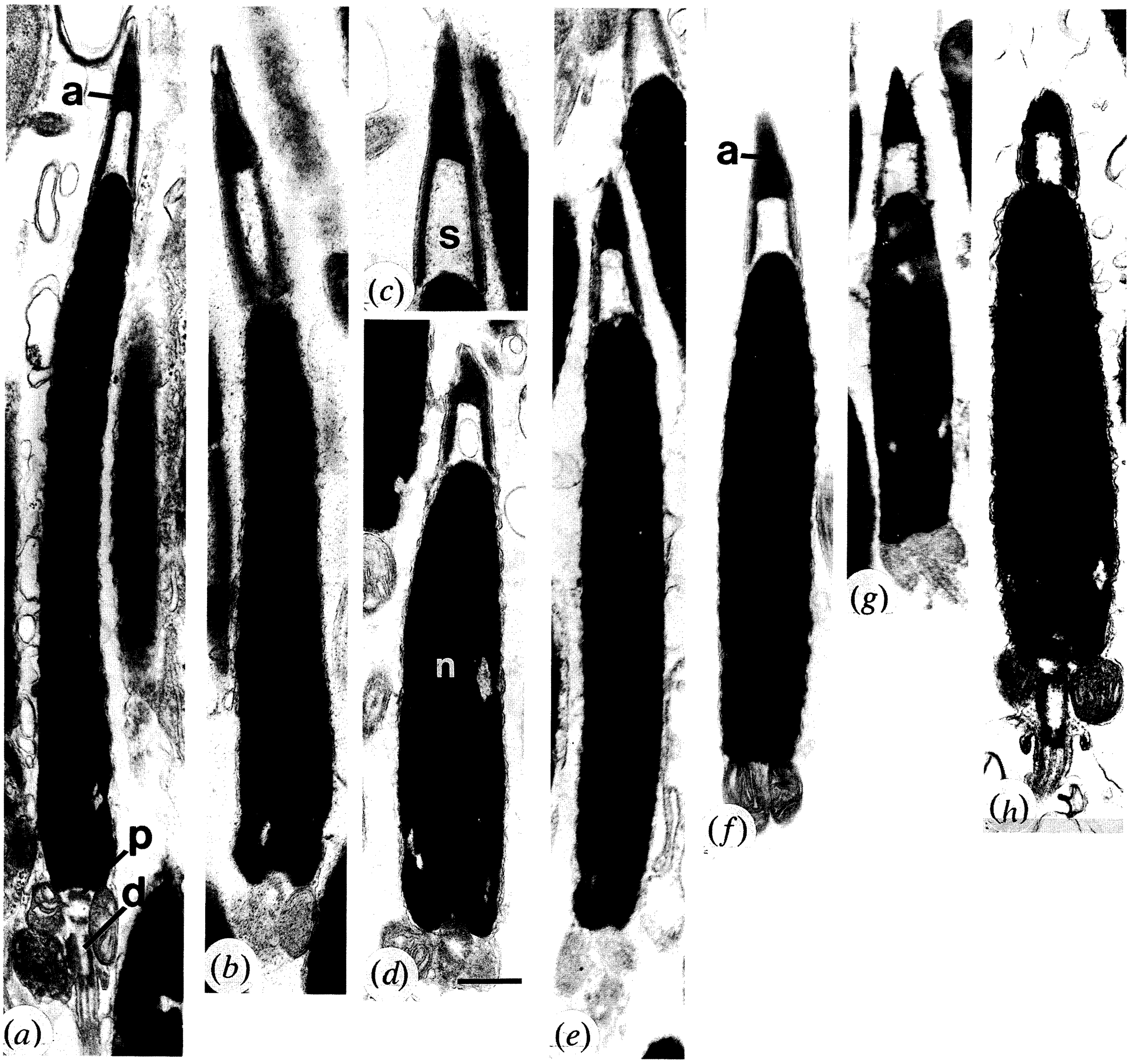

Figure 4. Mid-longitudinal sections through the spermatozoa and acrosomes of: (*a*) *Patella rustica*; (*b*, *c*) *P. vulgata*; (*d*) *P. depressa*; (*e*) *P. aspera*; (*f*) *P. candei*; (*g*) *P. ferruginea*; (h) P. caerulea. a, acrosome; d, distal centriole; n, nucleus; p, proximal centriole. Scale bar = 0.5 μm.

(figure 4*h*), which is more rounded anteriorly. Posteriorly all acrosomes are invaginated, the invagination extending for about 50 % of the length of the acrosome. The anterior section of the acrosome is solid, almost triangular in longitudinal section, and the acrosomal contents are undifferentiated.

4. DISCUSSION

Combining the results of this study with those of previous workers (Azevedo 1981; Kohnert & Storch 1983; Smaldon & Duffus 1985; Hodgson & Bernard 1988; Sousa & Oliviera 1994), the spermatozoa of the majority of *Patella* species and all *Helcion* species are now described. Known outstanding species are *P. mexicana* from the Pacific coast of central America, *P. kermadecensis* from the Kermadec islands in the southern Pacific, *P. depsta* from Amsterdam Island in the Indian Ocean and *P. lugubris* from the Cape Verde Islands in the North Atlantic. Examination of alcohol preserved *P. mexicana* has revealed that this species most probably has Type I or II sperm. Although tissue preservation was poor and no acrosomal detail could be ascertained, the nucleus was found to be similar in shape to that of *P. granularis* and at least 8 μm long (A. N. Hodgson, unpublished data). Similarly a study of alcohol preserved *P. lugubris* suggested that this species has Type VI sperm, similar to other North Atlantic species (Ridgway 1994).

Hodgson & Chia (1993) suggested that each family within the Patellogastropoda has spermatozoa with characteristic features. It is perhaps not surprising therefore that the spermatozoa of all species examined in this study conform to a morphology which has previously been described as typifying the Patellidae (Hodgson 1995). Features include an acrosome which is deeply invaginated posteriorly and constitutes < 50 % of the head length, the absence of an axial rod (or its equivalent) and a small cytoplasmic collar.

In their examination of the spermatozoa of 13 species of *Patella* Hodgson & Bernard (1988) suggested that within this genus there are three morphological

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types of sperm (grouped as Types I–III). The results from this study have revealed that all of the species of *Patella* from southern Africa and the Indo-Pacific (8 species), as well as two species from the North Atlantic (*P. safiana* and *P. canescens*), have one of these three types of sperm. Among them are species which are spermatologically closely related even though they are separated by several thousand kilometers. Table 2 and figure 5 summarize the morphology of the sperm in the five types and the grouping of sister species within these types. It is interesting to note that in their study, Hodgson & Bernard (1988) were only able to find one species, *P. granularis*, with Type II spermatozoa. Two further spermatological sister species have now been found, *P. peronii* from Australia and *Patella miliaris* from S. Angola. All three species have very long sperm nuclei. No functional significance can be attached to sperm head length, but it is known that head length is not correlated with egg size in the Patellidae (Hodgson & Bernard 1988) contrary to the correlation that exists in bivalves (Franzén 1983).

Table 2. *Summary of species of* Patella *and* Helcion *grouped as spermatologically similar species*

(These may or may not represent groupings of true sister-species. Sperm Type V is restricted to Cellanidae.)

species with Type I spermatozoa
P. tabularis, *P. aphanes*, *P. obtecta*, *P. barbara*, *P. argenvillei*, *P. cochlear*, *P. longicosta*, *P. chapmani*, *P. laticostata*, *P. pica*, *P. flexuosa*, *P. mexicana*?, *P. kermadecensis* (predicted only)
species with Type II spermatozoa
P. granularis, *P. peronii*, *Patella miliaris*, *P. mexicana*?
species with Type III spermatozoa
P. oculus, *P. granatina*, *P. concolor*, *P. miniata*, *P. compressa*, *P. safiana*, *Patella adansonii*, *Patella* cf. *miniata* (Angola), *P. canescens*, *Helcion dunkeri*, *H. pellucidum*?
species with Type IV spermatozoa
H. pruinosus, *H. pectunculus*
species with Type VI spermatozoa
P. rustica, *P. vulgata*, *P. depressa*, *P. aspera*, *P. candei*, *P. ferruginea*, *P. caerulea*, *P. lugubris*

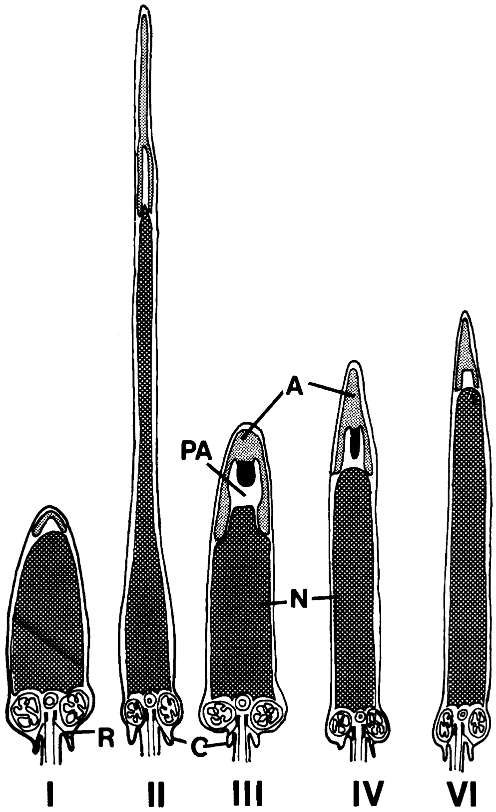

Figure 5. Semi-diagrammatic longitudinal sections through representatives of the five morphological types of sperm of patellid limpets. For names of the species within each type refer to table 2. Type V is not illustrated as this type was assigned to cellanid limpets (Hodgson & Bernard 1988). A, acrosome; C, collar; N, nucleus; PA, posterior acrosomal invagination; R, radial arm.

Within the three sperm morphology groups some species have spermatozoa which are more similar to one another than to others. Thus within Type I, *P. chapmani* more closely resembles *P. tabularis*, *P. barbara* and *P. argenvillei* than other members of this group (listed in table 2). Similarly within Type III the sperm of *Patella adansonii* is very similar to that of *P. compressa* and *P. miniata*, whereas *P. safiana*, *P. canescens* and *P.* cf. *miniata* (S. Angola) resembles *P. oculus* and *P. granatina*.

The similarities in sperm morphology between species with such a large geographic spread invites speculation on the evolutionary history of this genus. Because the greatest species diversity of *Patella* is found in southern Africa it might be tempting to suggest that this is a result of regional radiation. An alternative plausible hypothesis is that southern Africa is the meeting point of at least two separate radiations, one from the Atlantic and the other from the Indo-Pacific. Thus there is maximum species diversity where the two overlap.

Except for *P. safiana* and *P. canescens*, the *Patella* species from the North Atlantic/Mediterranean have sperm which closely resemble one another but are distinctly different to the sperm types from species in the southern hemisphere. Lindberg (1988) has suggested that the northeastern patellid fauna is a result of a single endemic radiation and that this limpet fauna is linked to a patellid clade of southern Africa. Electrophoretic studies of many of the N.E. Atlantic species have shown that they are closely related (Côrte-Real 1992; Cretella *et al.* 1994; L. I. Weber & S. J. Hawkins, personal communication). The great similarities in sperm morphology of the North Atlantic limpets would support the idea of a single endemic radiation possibly a result of allopatric events caused by glaciation, the opening and closing of the Mediterranean and island speciation. It is not clear to which southern African clade the N.E. Atlantic patellids are linked although a recent cladistic analysis of the group indicates that the Northern Atlantic spp. with Type VI sperm may be closely related to those species from South Africa with Type I or II sperm (Ridgway 1994).

Hodgson & Bernard (1988) determined that two species of *Helcion* (*H. pruinosus* and *H. pectunculus*) have a spermatozoon morphology which is distinctly different to that of *Patella* species. They therefore placed *Helcion* in a separate sperm group Type IV (figure 5), characterized by sperm with cylindrical nuclei and complex acrosomes with a linear posterior lobe containing central microfilaments. The sperm of *H.*

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dunkeri bears a closer resemblence to those species of *Patella* with Type III sperm. This could suggest that *H. dunkeri* is more closely related to those species of *Patella* with Type III spermatozoa which in turn has interesting taxonomic implications. The sperm of *H. pellucidum* does not fit conveniently into any of the current sperm types. It was tentatively assigned to Type III on the basis of the differentiated acrosomal contents. *Helcion pectunculus* and *H. pruinosus* were assigned a separate sperm group (Type IV) largely because of their more elongate cylindrical nucleus and more complex acrosome. It is possible that the sperm of these two species are a more apomorphic form of Type III spermatozoa and should also be grouped within this category. However cladistic analysis based on sperm, radula, shell and soft part morphology has shown *Helcion* to be a monophyletic group with *H. pectunculus* and *H. pruinosus* being the most derived species and *H. pellucidum* the basal species (Ridgway 1994), which supports the hypothesis that Type IV sperm may be apomorphic. Enzyme electrophoresis has also shown that within the genus *Helcion*, *H. pectunculus*, *H. pruinosus* and *H. dunkeri* are most closely related and *H. pellicidum* more distant (L. I. Weber, D. R. Gray, A. N. Hodgson and S. J. Hawkins, unpublished data).

Two species collected from southern Africa (*P. adansonii* from S. Namibia and *P.* cf. *miniata* from S. Angola) were initially thought to be *P. miniata*. Although both have Type III spermatozoa, their differing sperm morphologies clearly show that they are not the same species and in addition their sperm are different from that of *P. miniata* (described by Hodgson & Bernard 1988). The taxonomy of *P.* cf *miniata* still needs clarification. It is however interesting to note that the sperm of *P. safiana* from North Africa is remarkably similar to that of *P.* cf. *miniata* from S. Angola, and we believe that a more detailed examination will reveal that they are the same species.

In summary we would suggest that of the five morphological sperm groups of patellids, three of these i.e. I, III and VI (see table 2 for species), represent clearly identifiable groups within the Patellidae. In a preliminary spermiocladistic study of southern African limpets Jamieson *et al.* (1991) suggested that there might be four monophyletic groups. Group III of the present study corresponds to the '*concolor*' group of Jamieson *et al.* (1991), thus adding further support to this clade. Group I is however a combination of the '*argenvillei*' and '*aphanes*' clades of Jamieson *et al.* (1991), two clades which were separated on only one sperm character. The affinities of group II are questionable and group IV (*H. pectunculus* and *H. pruinosus*) may be part of group III. We therefore propose that there were three main centres of patellid radiation. Group VI radiated in the North East Atlantic/Mediterranean region; Group III comprises an East Atlantic (West Africa) grouping extending around to the South East coast of South Africa; Group I is predominantly Indo-Pacific with a focal point on the South East coast of South Africa extending around on to the West coast of South Africa. We would predict therefore that *P. kermadecensis* will fall within this group.

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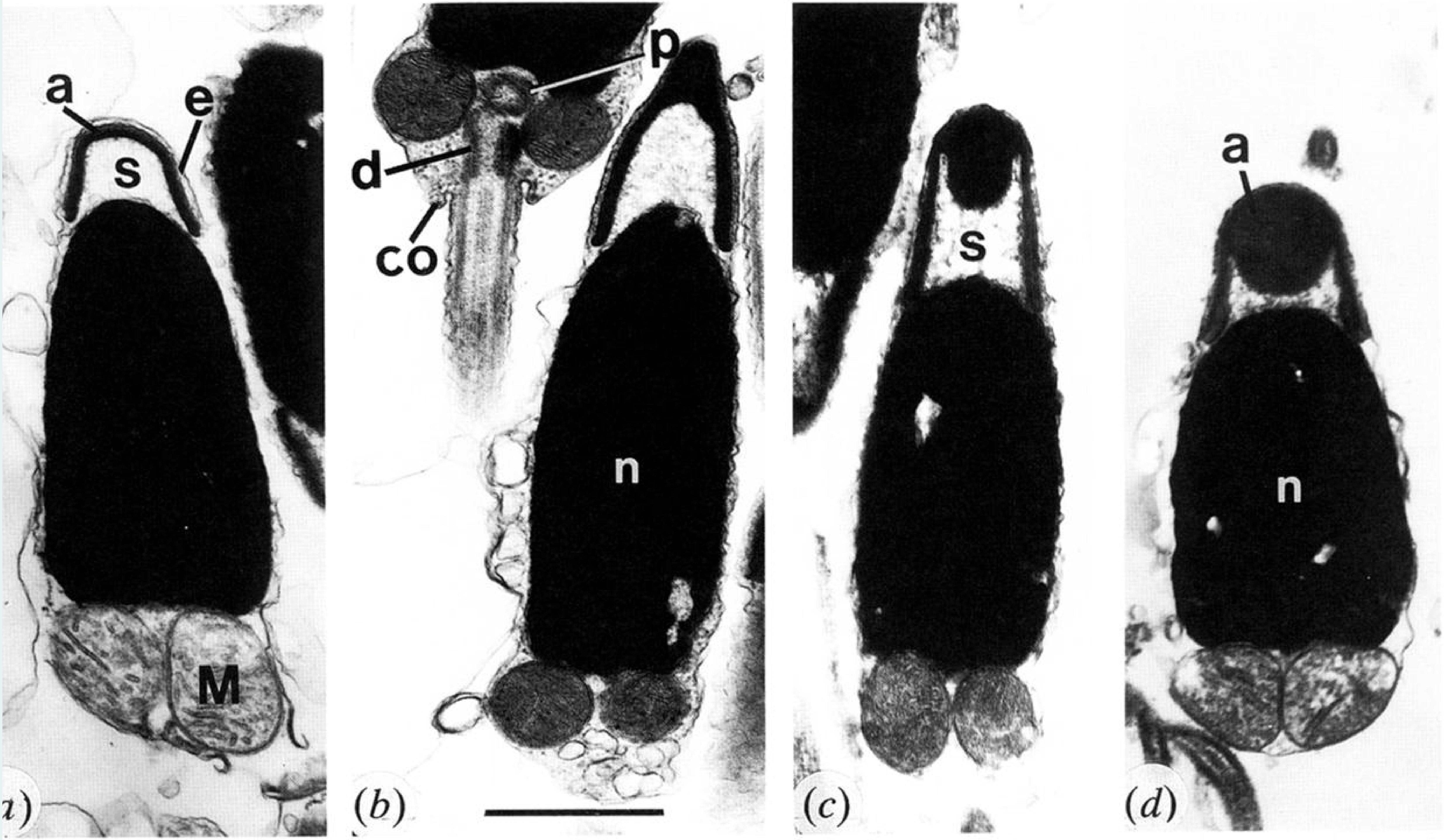
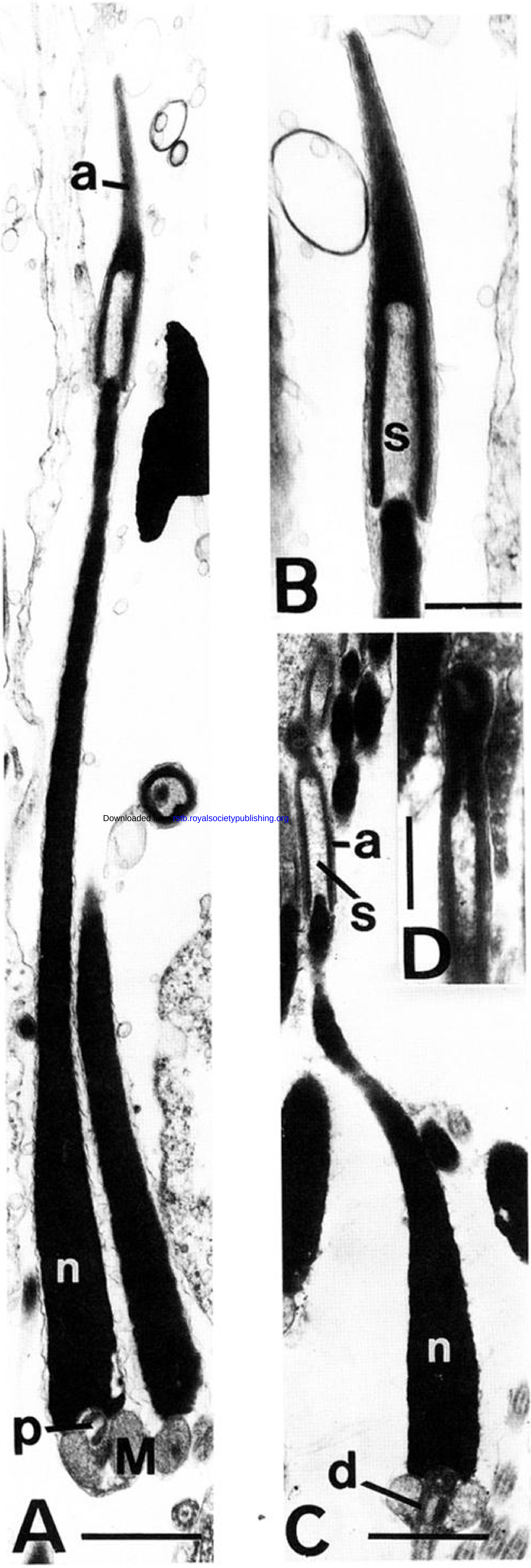


Figure 1. Type I sperm. Mid-longitudinal sections through the spermatozoa of: (*a*) *Patella chapmani*; (*b*) *P. laticostata*; (*c*) *P. pica*; and (*d*) *P. flexuosa*. a, acrosome; co, cytoplasmic collar; d, distal centriole; e, extra-acrosomal layer; M. mitochondrion; n, nucleus; p, proximal centriole; s, subacrosomal space. Scale bar = 1 μm.



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gure 2. Type II sperm. Mid-longitudinal sections through e spermatozoa and acrosomes of: (*a*, *b*) *P. peronii*; and (*c*, *Patella miliaris*. a, acrosome; d, distal centriole; M, itochondrion; n, nucleus; p, proximal centriole; s, subacrosomal space. (*a*, *b*) scale bars = 1 μm; (*c*, *d*) scale bars 0.5 μm.

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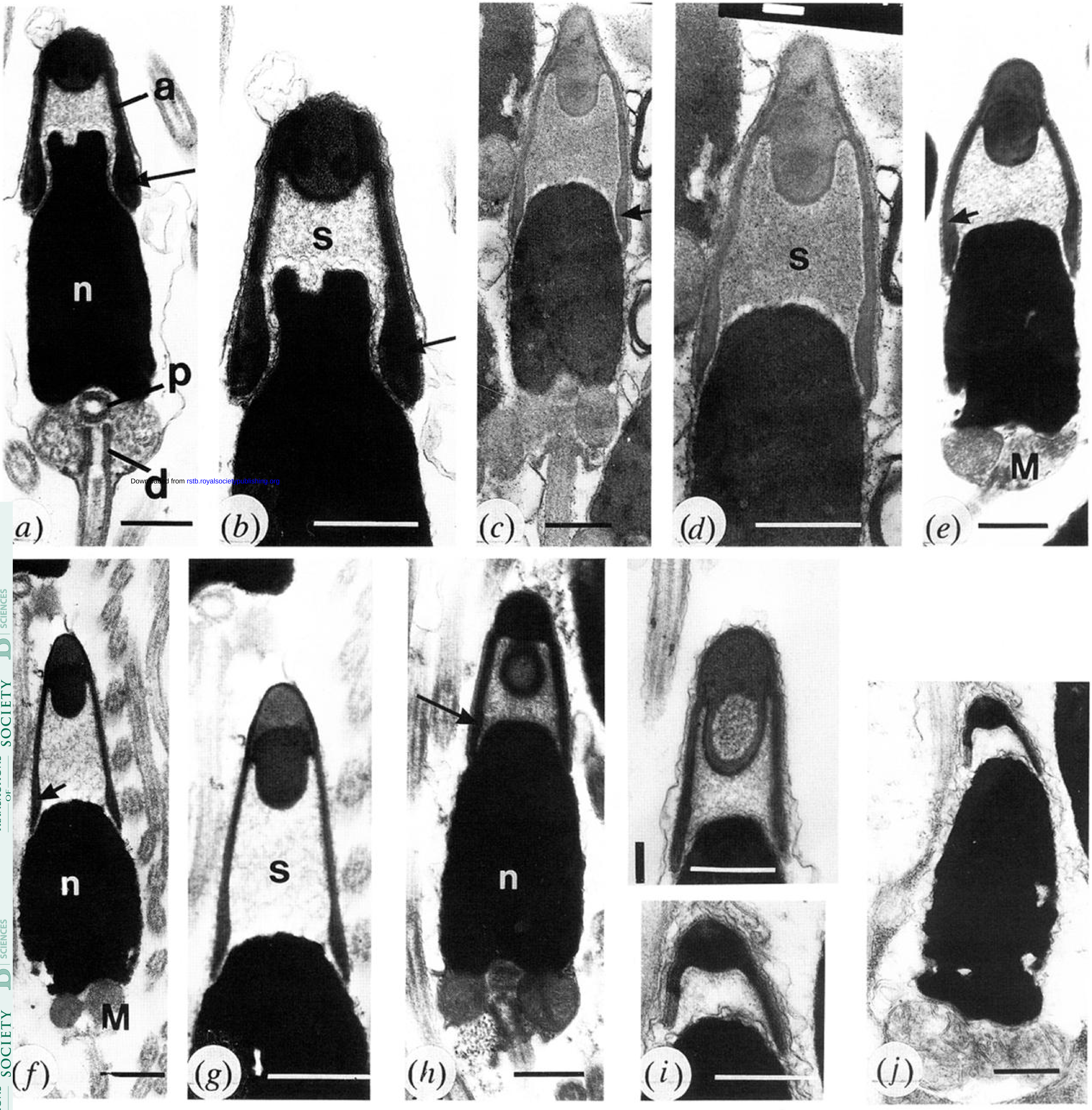

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Figure 3. Type III sperm. Mid-longitudinal sections through the spermatozoa and acrosomes of: (*a*, *b*) *Patella adansonii*; (*c*, *d*) *P. safiana*; (*e*) *P.* cf. *miniata* (Angola); (*f*, *g*) *P. canescens*; (*h*, *i*) *H. dunkeri* (*j*, *k*) *H. pellucidum*. a, acrosome; d, distal centriole; M, mitochondrion; n, nucleus; p, proximal centriole; s, subacrosomal space. Note the slightly bulbous postior region of the acrosomes (arrowed). Scale bars = 0.5 μm.

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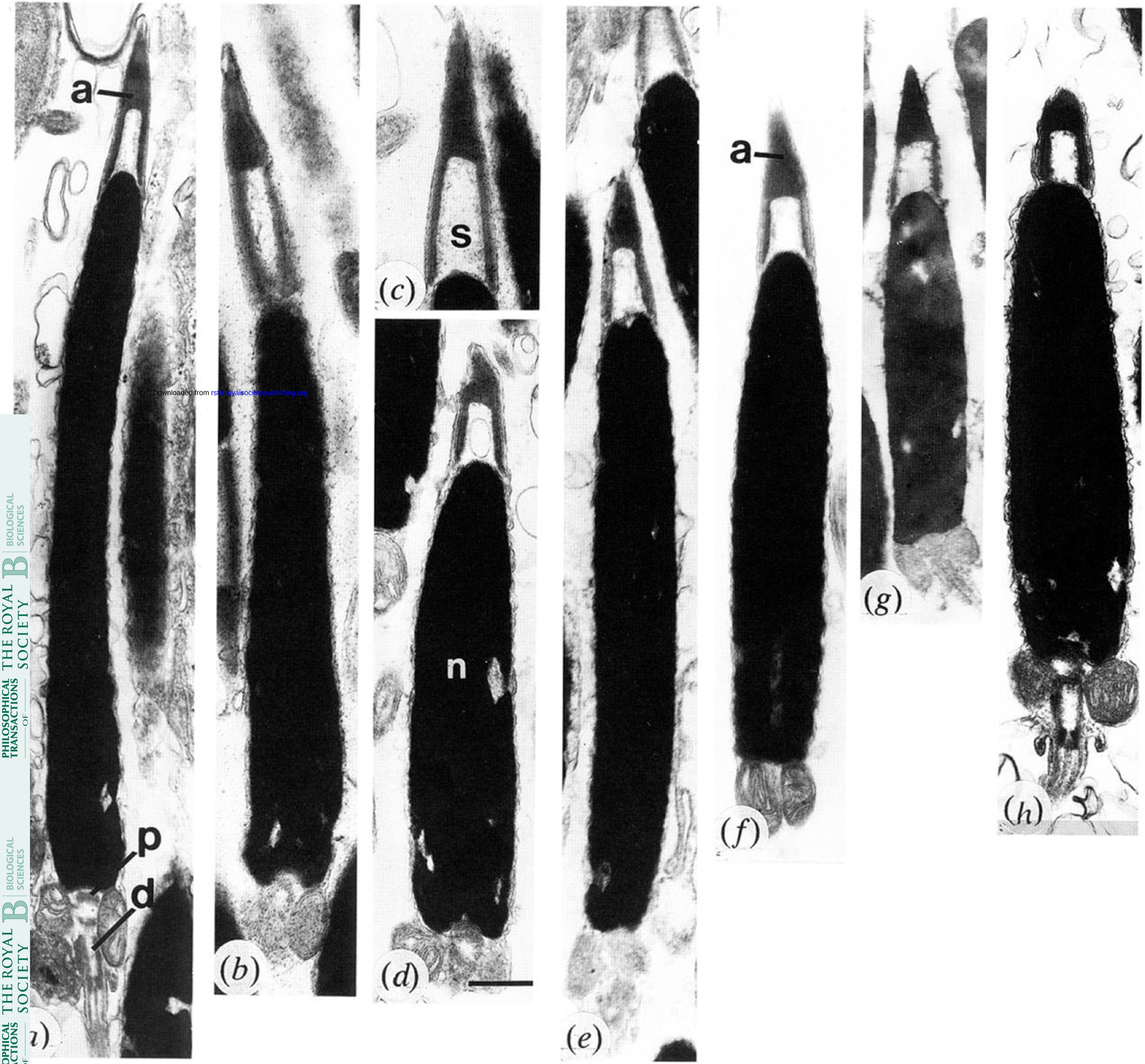

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Figure 4. Mid-longitudinal sections through the spermatozoa and acrosomes of: (*a*) *Patella rustica*; (*b*, *c*) *P. vulgata*; (*d*) *P. depressa*; (*e*) *P. aspera*; (*f*) *P. candei*; (*g*) *P. ferruginea*; (h) P. caerulea. a, acrosome; d, distal centriole; n, nucleus; p, proximal centriole. Scale bar = 0.5 μm.