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Sperm Ultrastructure in the Cirripedia and its Phylogenetic Significance

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ABSTRACT. Sperm morphology was examined in 42 species of Thoracica, from 32 genera in 11 families, and in one species of Acrothoracica. The filiform spermatozoa of Thoracica, Acrothoracica and Rhizocephala share: (1) an axoneme capped by a conical acrosome; (2) a filiform nucleus running parallel to the axoneme; (3) an elongate mitochondrion posterior to the nucleus; (4) glycogen deposits. This sperm type links the Thoracica, Acrothoracica and Rhizocephala and distinguishes them from the other Thecostraca. Structural differences in the sperm nucleus separate the three groups and are further consistent with segregation of the Rhizocephala from the Thoracica + Acrothoracica (= Cirripedia *sensu* Newman). In spermatozoa from seminal vesicles of Thoracica, an accessory droplet is usually present. This structure, produced by the Golgi complex during spermiogenesis, shows variation among species in its form and internal substructure. The Iblidae, Lepadidae and Scalpellidae have distinctive modifications of the accessory droplet. Among the scalpellids, the Pollicipedinae retain a generalised thoracican sperm morphology, while other subfamilies which have been studied (Lithotryinae, Scalpellinae, Calanticinae) display differing modifications of nuclear and droplet structure. Sperm morphology of *Verruca* is consistent with derivation of the Verrucomorpha from scalpelloids related more closely to the Pollicipedinae than to the Calanticinae, Lithotryinae or Scalpellinae. The retention of a generalised sperm morphology in *Catomerus* is further consistent with the derivation, on other grounds, of the Balanomorpha from a scalpelloid ancestry. Within the Balanomorpha, sperm data offer indications of phylogenetic patterns in the Chthamaloidea (*Chamaesipho* derived from catophragmids independently of the chthamalids), Coronuloidea (*Cylindrolepas* related to *Tubicinella*; tetraclitids monophyletic and including *Austrobalanus*) and Balanoidea (archaeobalanids polyphyletic; *Armatobalanus* related to Pyrgomatidae; *Balanus* and the megabalanines both probably complex groups of perhaps multiple origins). Present sperm data do not assist in the elucidation of relationships between balanomorph superfamilies. Basal members of each superfamily retain a generalised thoracican sperm structure.

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Among the Crustacea, only the maxillopod groups Thecostraca (*sensu* Grygier, 1987a; Newman, 1987), Branchiura and Mystacocarida retain an axoneme in their spermatozoa (Brown & Metz, 1967; Brown, 1970; Munn & Barnes, 1970a, 1970b; Wingstrand, 1972, 1978, 1988; Pochon-Masson, 1978; Kubo *et al.*, 1979; Grygier, 1981, 1982, 1987a). In other crustaceans, sperm motility is either wanting, (e.g. Decapoda) or achieved using other structures (e.g. Ostracoda, Baccetti & Afzelius, 1976; Pochon-Masson, 1978; Wingstrand, 1988). Grygier (1981, 1982, 1987a) showed that spermatozoa of the Ascothoracida (Fig. 1a,b) are readily discernible ultrastructurally from those of the Thoracica, Acrothoracica and Rhizocephala and suggested that the Ascothoracida should be separated from the Cirripedia as then defined, probably as a category of equal rank. A more complex interpretation of the higher classification of the bivalved maxillopodans has been proposed by Newman (1987) and Grygier (1987b), in which the Rhizocephala are also classified as a group of equal rank to the Ascothoracida and Cirripedia, all three being defined as subclasses of the maxillopodan class Thecostraca, which also includes the so-called Y-larvae or Facetotecta. In this classification, the subclass Cirripedia comprises only the Thoracica and Acrothoracica. The Thoracica, Acrothoracica and Rhizocephala, however, share similar larval stages and a common filiform spermatozoon (Fig. 1c-g), the basic features of which include a conical acrosome at the apex of the axoneme, a filamentous nucleus parallel to the axoneme, a single elongate mitochondrion and glycogen deposit parallel to the axoneme (Munn & Barnes, 1970a, 1970b; Pochon-Masson, 1971). The spermatozoa of thoracicans, acrothoracicans and possibly rhizocephalans also possess an accessory droplet, a basically fusiform body produced by the Golgi complex during the later stages of spermiogenesis (Kubo *et al.*, 1979; Azevedo & Corral, 1982), but then lost in the seminal vesicles, in the anterior portion of the ejaculatory duct, or soon after leaving the penis (Munn & Barnes, 1970b; Barnes *et al.*, 1971; Barnes *et al.*, 1977; Kubo *et al.*, 1979). The accessory droplet is unique to this sperm type and adds further weight to the concept that the filiform sperm of Thoracica, Acrothoracica and Rhizocephala is a synapomorphy, placing the Rhizocephala close to, if not within, the Cirripedia.

Beyond these arguments, the utilisation of sperm ultrastructure in the analysis of cirripede phylogeny has been restricted by lack of data and by the seemingly invariant ultrastructure of the sperm. Ultrastructural data are particularly limited in groups such as the Rhizocephala (*Sacculina carcini* — Pochon-Masson, 1971) and Lepadomorpha (*Pollicipes pollicipes* — Azevedo & Corral, 1982; *Lepas anatifera* — Anderson & Personne, 1970). A review of spermatozoa and spermiogenesis in the Rhizocephala is currently being prepared by Hoeg & Jespersen (J. Hoeg, personal communication). The accessory droplet of balanomorph spermatozoa is known to vary in internal structure from species to species (for example Munn & Barnes, 1970b; Kubo *et al.*, 1979), but no attempt has been made to relate this structure to phylogeny. In the present work, we offer comparative data on sperm

ultrastructure in a wide range of species, with particular emphasis on specific variation in the ultrastructure of the accessory droplet. We then review the ultrastructure of thecostracan sperm as presently known and consider the relevance of the accessory droplet as a phylogenetic character.

Abbreviations used in the figures are: a — acrosomal complex; ar — axial rod material (of acrosomal complex); av — acrosomal vesicle; ax — axoneme; c — centriole; co — collar; d — accessory droplet; drs — dense ring structure (annulus); g — glycogen deposits; l — lacunae (substructural element of droplet); m — mitochondrion; n — nucleus; r — rod (substructural element of droplet); t — tail; v — dense vesicle(s) (substructural element of droplet).

Materials and Methods

Spermatozoa were obtained from the seminal vesicles of one species of acrothoracican and 42 species of thoracican cirripedes. The seminal vesicles were taken either from live specimens or from sea water-formalin fixed material. Table 1 lists the species examined, together with relevant locality data and fixation used. For live specimens, seminal vesicles were dissected out, cut into small portions (1 to 2 mm³) and fixed in cold (0 to 4°C) 3% glutaraldehyde (in 0.2 M phosphate or cacodylate buffer) for two to three hours. Subsequently, the tissues were washed in buffer, as were tissues obtained from formalin-fixed specimens. All tissues, whether glutaraldehyde or formalin-fixed, were placed in a cold 1% osmium tetroxide solution (in 0.2 M phosphate or cacodylate buffer) for 80 minutes. After a thorough buffer rinse, tissues were dehydrated using ethanol and embedded in Spurr's epoxy resin. Ultrathin sections were cut using a LKB ultramicrotome, collected on uncoated copper grids and stained using 4% uranyl acetate and Reynold's lead citrate. Sections were examined using a Philips 300 transmission electron microscope operated at 60kV.

Results

Acrothoracica (*Berndtia fossata*)

In *Berndtia fossata*, the filiform sperm (Fig. 2a) is approximately 85 µm long. The acrosome is conical and basally invaginated (Fig. 2b). Axial rod material occupies the invagination. A collar appears to be present but is not well developed. A 9 + 2 axoneme, a filiform nucleus and an accessory droplet run parallel to each other (Fig. 2c,d). Transverse sections of the nucleus reveal a dumb-bell shaped profile, similar to that demonstrated by Tomlinson (1969) and Pochon-Masson (1971) for *Trypetesa* (Fig. 2c). This shape may be characteristic for Acrothoracica. The accessory droplet in *Berndtia* is very narrow and exhibits prominent sub-spherical spaces (Fig. 2d). Parallel layers, each composed of a double membrane, occur within the

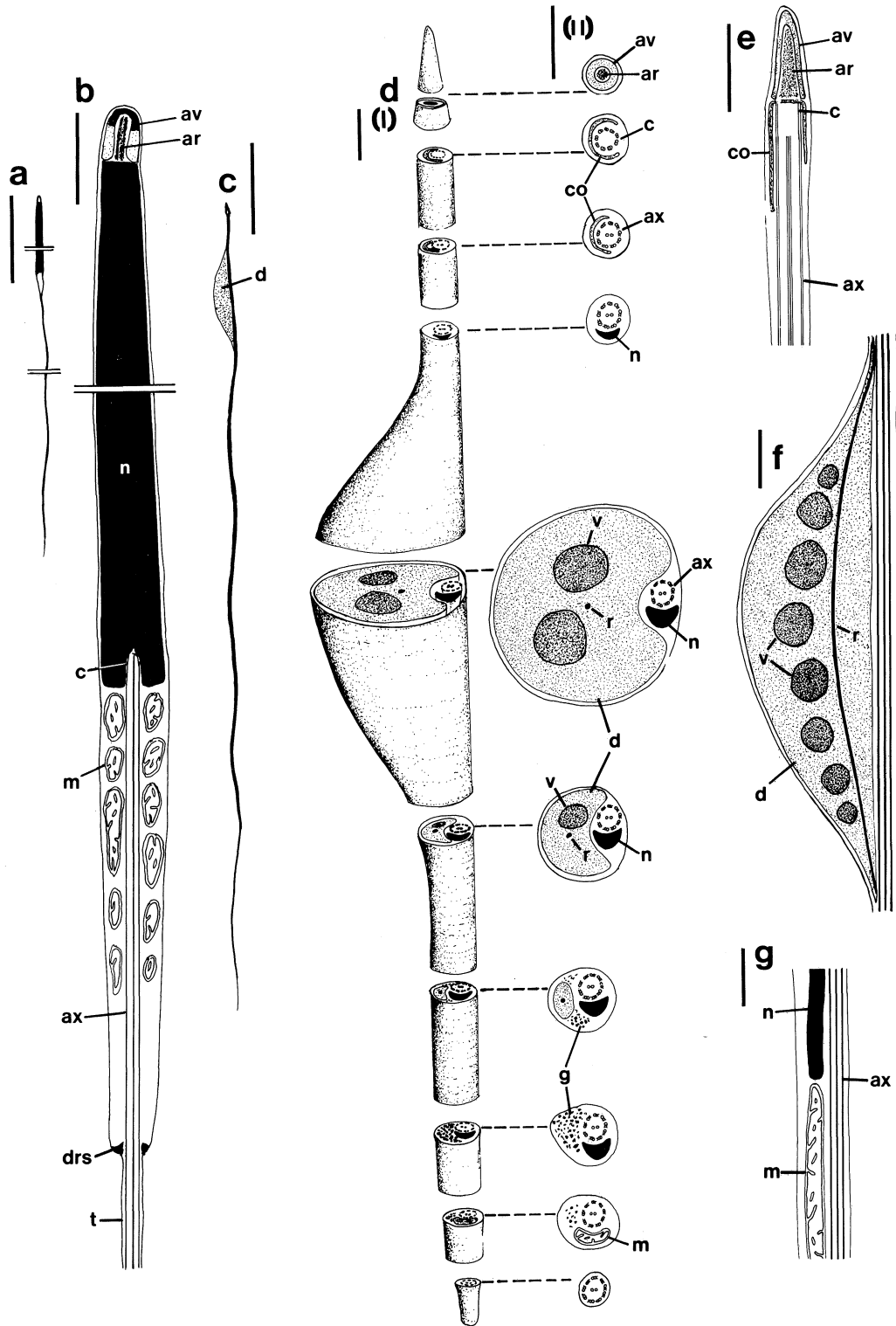


Fig.1. Sperm morphology in the Ascothoracica and Cirripedia (semi-diagrammatic). (a, b) Spermatozoon of the ascothoracican, *Ulophysema oeresundense* Brattström (based on data of Grygier, 1982). (c — g) General features of spermatozoa of the Acrothoracica, Thoracica and Rhizocephala. Elongate nucleus (n) runs parallel to axoneme (ax); centriole (c) is adjacent to base of acrosome; single mitochondrion (m) lies posterior to nucleus; accessory droplet (d) (often with internal rod (r) or dense vesicles (v), parallels axoneme/nucleus. (c) Whole spermatozoon. (dI, II) Reconstruction of main sperm regions (example *Austromegabalanus nigrescens*). (e) Longitudinal section of acrosome, centriole and collar. (f) Longitudinal section of droplet and axoneme. (g) Junction between nucleus and mitochondrion. Scale bars: a, c = 10 μ m; b = 1.0 μ m; all others = 0.5 μ m

Table 1. Material examined.

Species	Locality	Fixation	Species	Locality	Fixation
ORDER ACROTHORACICA			Suborder BALANOMORPHA (cont'd)		
<i>Berndtia fossata</i> Tomlinson	Darwin, NT	SWF	Superfamily Coronuloidea		
ORDER THORACICA			Family Coronulidae		
Suborder LEPADOMORPHA			<i>Coronula diadema</i> (Linnaeus)	unknown, Australia	SWF
Superfamily Ibloidea			<i>Chelonibia testudinaria</i> (Linnaeus)	Mon Repos, northern QLD	SWF
Family Iblidae			<i>Tubicinella cheloniae</i> Monroe	Mon Repos, northern QLD	SWF
<i>Ibla quadrivalvis</i> (Cuvier)	Balmoral, Sydney, NSW	Glut.	<i>Cylindrolepas darwiniana</i> Pilsbry	Mon Repos, northern QLD	SWF
Superfamily Scalpelloidea			<i>Platylepas coriacea</i> Monroe	Port Jackson, NSW	SWF
Family Scalpellidae			<i>Platylepas hexastylus</i> (Fabricius)	Port Jackson, NSW	SWF
<i>Scalpellum stearnsi</i> Pilsbry		SWF	Family Tetracitidae		
<i>Scalpellum</i> sp.		Os.	<i>Austrobalanus imperator</i> (Darwin)	Balmoral, Sydney, NSW	Glut.
Subfamily Lithotryinae			<i>Epopella plicata</i> (Gray)	Werrington, Otago, NZ	SWF
<i>Lithotrya valentiana</i> (Gray)	Lizard Island, northern QLD	SWF	<i>Tetracitella squamosa</i> (Bruguere)	Townsville, northern QLD	SWF
Subfamily Calanticinae			<i>Tetracitella purpurascens</i> (Wood)	Balmoral, Sydney, NSW	Glut.
<i>Calantica spinosa</i> (Quoy & Gaimard)	Werrington, Otago, NZ	SWF	<i>Tesseropora rosea</i> (Krauss)	Balmoral, Sydney, NSW	Glut.
<i>Calantica villosa</i> (Leach)	Werrington, Otago, NZ	SWF—>ETH	Superfamily Balanoidea		
<i>Smilium peronii</i> (Gray)	Perth, WA	SWF	Family Archaeobalanidae		
Superfamily Lepadoidea			<i>Hexaminus foliorum</i> Anderson, Anderson & Egan	Iron Cove, NSW	Glut.
Family Lepadidae			<i>Hexaminus popeiana</i> Foster	Iron Cove, NSW	Glut.
<i>Lepas anserifera</i> Linnaeus	Port Hacking, NSW		<i>Elminius covertus</i> Foster	Iron Cove, NSW	Glut.
<i>Lepas anatifera</i> Linnaeus	Port Hacking, NSW	Glut.	<i>Armatobalanus allium</i> (Darwin)	Darwin, NT	SWF
<i>Lepas pectinata</i> Spengler	Port Hacking, NSW	SWF	<i>Armatobalanus arcuatus</i> Hoek	Darwin, NT	SWF
<i>Conchoderma auritum</i> (Linnaeus)	Port Hacking, NSW	SWF	Family Pyrgomatidae		
<i>Conchoderma virgatum</i> (Spengler)	Port Hacking, NSW	Glut.	<i>Pyrgoma cancellata</i> Leach	Darwin, NT	SWF
Suborder VERRUCOMORPHA			<i>Creusia spinulosa</i> Leach	John Brewer Reef, Townsville, QLD	SWF
Family Verrucidae			<i>Savignium elongatum</i> (Hiro)	John Brewer Reef, Townsville, QLD	SWF
<i>Verruca ströemia</i> (O.F. Müller)	Plymouth, UK	SWF—>ETH	Family Balanidae		
Suborder BALANOMORPHA			<i>Balanus variegatus</i> Darwin	Iron Cove, NSW	Glut.
Superfamily Chthamaloidea			<i>Balanus amphitrite</i> Darwin	Iron Cove, NSW	Glut.
Family Catophragmidae			<i>Megabalanus ajax</i> (Darwin)	One Tree Island, Great Barrier Reef, QLD	SWF
<i>Catomerus polymerus</i> (Darwin)	Balmoral, Sydney, NSW	Glut.	<i>Austromegabalanus nigrescens</i> (Lamarck)	Balmoral, Sydney, NSW	Glut.
Family Chthamalidae					
<i>Chthamalus antennatus</i> Darwin	Balmoral, Sydney, NSW	Glut.			
<i>Chthamalus malayensis</i> Pilsbry	Townsville, northern QLD	SWF			
<i>Octomeris brunnea</i> Darwin	Magnetic Island, northern QLD	SWF			
<i>Chamaesipho tasmanica</i> Foster & Anderson	Balmoral, Sydney, NSW	Glut.			

Abbreviations: ETH (ethanol); Glut. (glutaraldehyde); Os. (osmium tetroxide); SWF (sea water/formalin)

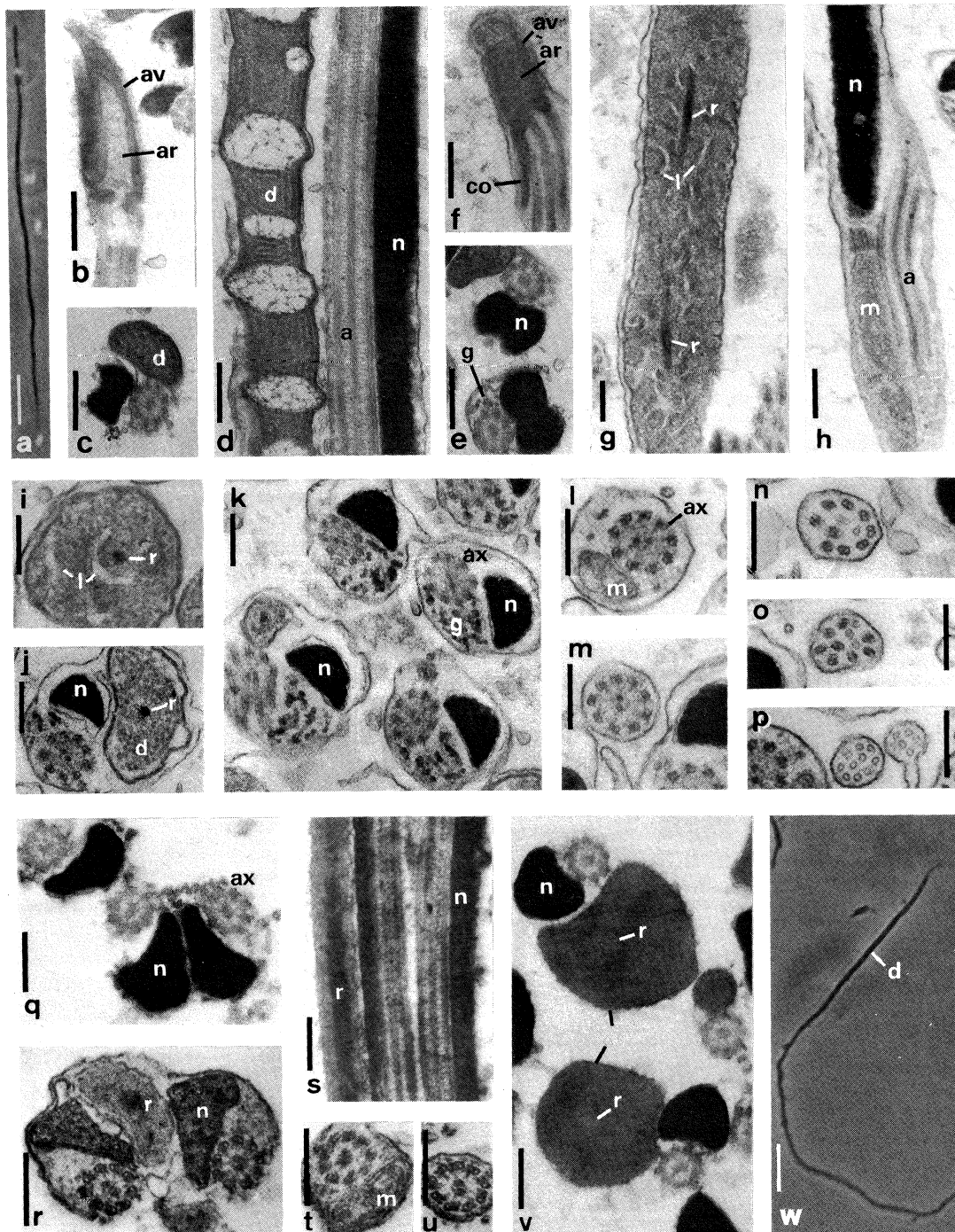


Fig. 2. (a — e) Spermatzoa of the acrothoracican *Berndtia fossata*. (a) Whole spermatozoon. (b) Longitudinal section through acrosome. (c) Transverse section through nucleus, axoneme and droplet. (d) L.S. through droplet. (e) T.S. nuclei, axonemes, glycogen deposit, droplet. (f — p) *Ibla quadrivalvis* (Thoracica, Iblidae). (f) L.S. acrosome, collar and anterior extremity of axoneme. (g) L.S. portion of droplet showing rod and lacunae. (h) Junction of nucleus and mitochondrion. (i) T.S. rod and lacunae of droplet. (j) T.S. nucleus, axoneme, droplet. (k) T.S. 'glycogen' region of sperm (right) and terminal portion of accessory droplet (left, also with glycogen). (l) T.S. mitochondrial region of sperm. (m) T.S. axoneme of free tail region. (n — p) Sequence of axoneme degeneration at posterior extremity of sperm. (q — w) Scalpellidae. (q) *Scalpellum stearnsi*, T.S. axoneme, nuclei. (r — u) *Scalpellum* sp. (original micrographs courtesy of Professor B.A. Afzelius). (r) T.S. nucleus, droplet (with thick rod) and axoneme. (s) L.S. droplet, nucleus, axoneme. (t) T.S. mitochondrial region of sperm. (u) T.S. free tail. (v, w) *Lithotrya valentiana*. (v) T.S. droplet, nucleus, axoneme. (w) Whole spermatozoon. Scale bars: a, w = 10 μm ; b–v = 0.25 μm .

droplet (Fig. 2d). These layers run axially at the periphery of the droplet and transversely at the droplet core (Fig. 2d). The length of the droplet could not be determined. Posterior to the droplet, dense deposits (probably glycogen) occur closely to the nucleus and axoneme (Fig. 2e). A single mitochondrion follows the nucleus in the posterior region of the spermatozoon.

Thoracica

Lepadomorpha

Iblidae — *Ibla quadrivalvis*

The acrosomal vesicle of *I. quadrivalvis* is 0.5 μm long, with a deep basal invagination containing axial rod material (Fig. 2f). Posterior to the acrosomal region is a collar 0.36 μm long, sheathing the centriole and the initial portion of the axoneme (Fig. 2f). Beyond the collar the axoneme becomes closely associated with the filiform nucleus, triangular in transverse section, and an elongate accessory droplet (Fig. 2g,j,k). The droplet measures 14 to 17 μm (phase-contrast observation) and contains a granular matrix, curved spaces (lacunae) and a dense rod 0.04 to 0.91 μm in diameter (Fig. 2g,i,j). Transverse sections indicate that the droplet has a maximum diameter of 0.6 to 0.7 μm (Fig. 2i) and tapers anteriorly and posteriorly (Fig. 2k). Behind the nucleus, the axoneme is accompanied by a single mitochondrion containing unmodified cristae (Fig. 2h). Dense deposits, identifiable as glycogen granules (see Anderson & Personne, 1970 — *Lepas*), form a wedge-shaped tract parallel to the axoneme and nucleus (Fig. 2k) and then to the axoneme and mitochondrion (Fig. 2l). Figures 2m to 2p illustrate degeneration of the 9 + 2 axoneme into individual microtubules at the posterior extremity of the spermatozoon. This involves firstly loss or displacement of the central pair of microtubules, then disruption of doublet positioning (Fig. 2n,o) and finally degeneration of the doublets into single microtubules (Fig. 2p). The spermatozoon of *I. quadrivalvis* is approximately 70 μm long.

Scalpellidae

Representatives of three scalpellid subfamilies were examined: the Scalpellinae (*Scalpellum stearnsi*, *Scalpellum* sp.), Lithotryinae (*Lithotrya valentiana*) and Calanticinae (*Calantica spinosa*, *Calantica villosa*, *Smilium peronii*). Acrosomes, where observed (*Smilium*, *Calantica*), consist of a conical acrosomal vesicle with a deep basal invagination occupied by axial rod material (Fig. 3b,c). The filiform nucleus and elongate accessory droplet of scalpellid spermatozoa show diversity in morphology. In *Lithotrya*, the nucleus is triangular in transverse section and the droplet is well developed, with a single, pale-staining rod (diameter 0.06 to 0.07 μm) at its core (Fig. 2v). In *Scalpellum* the nucleus is also triangular in transverse

section, but the profile has a distinct asymmetry (Fig. 2q,r). The accessory droplet of *Scalpellum* sp. is narrow and curved in cross section and contains a thick rod (diameter 0.09 to 0.1 μm) composed of a dense core and outer enveloping layer (see Fig. 2r,s). Droplets were not observed in *Scalpellum stearnsi*. Sperm nuclei of calanticines (*Calantica* spp., *Smilium peronii*) are club-shaped in transverse section (Fig. 3d,f,g,i-l). The accessory droplets of calanticine spermatozoa are long (20 to 27 μm) and up to 0.7 μm wide, with a rod and scattered dense vesicles in *Smilium* (Fig. 3d-g), but without clear evidence of substructure in *Calantica* (Fig. 3k,l). Dense glycogen granules accompany the nucleus and axoneme at and beyond the posterior extremity of the accessory droplet (Fig. 3i). In *Scalpellum*, *Smilium*, and presumably other scalpellids, the nucleus terminates abruptly and is followed by a single mitochondrion (Figs 2t, 3h,j). The mitochondrion usually appears crescentic in transverse section, and is frequently associated with glycogen granules. The posterior extremity of the spermatozoon consists solely of the axoneme and enveloping plasma membrane (Fig. 2u). Total sperm length, from phase-contrast microscopy, is 90 to 95 μm for *Calantica* and *Smilium* (Fig. 3a,m), 92 μm for *Scalpellum stearnsi* and 110 μm for *Lithotrya* (Fig. 2w).

Lepadidae and Heteralepadidae

The spermatozoa of several lepadid species were compared (*Lepas anserifera*, *Lepas anatifera*, *Lepas pectinata*, *Conchoderma auritum*, *Conchoderma virgatum*), but only limited material was available from a single species of heteralepadid (*Heteralepas japonica*).

The acrosome of *Lepas anserifera* is 0.54 to 0.6 μm long and conical, with axial rod material located within a basal invagination (Fig. 3n,p,q). Acrosomes of *Conchoderma virgatum* were swollen, possibly due to the poor condition of the live specimen collected for study. Some transverse sections were obtained which suggest that the acrosomal vesicle of *C. virgatum* is conical, as in *Lepas*. The acrosome was not identifiable in available material of *Conchoderma auritum*. A dense collar is present immediately posterior to the acrosomal region, measuring approximately 0.6 μm in *Lepas anserifera* (Fig. 3n). In transverse section the nucleus of *Lepas* and *Conchoderma* has a sharply defined, triangular profile (Fig. 3u-w). The accessory droplets of *Lepas* and *Conchoderma* are narrow (maximum width 0.4 to 0.6 μm) and contain a prominent rod composed of a dense core (diameter 0.06 to 0.07 μm in *Lepas*, 0.03 μm in *Conchoderma*) surrounded by a narrow space, then a less electron-dense outer layer, 0.05 to 0.06 μm thick in *Lepas* (Fig. 3r,s,v) and 0.07 to 0.08 μm thick in *Conchoderma* (Fig. 3w). Phase-contrast microscopy revealed a droplet length of 28 to 35 μm in *Lepas* and *Conchoderma* (Figs 3o, 4a). From available material of *Heteralepas japonica*, it was possible to determine only that the sperm nucleus is triangular in cross section, and that a rod is probably present in the droplet. In *Lepas* and *Conchoderma*, glycogen granules occur before and after termination of

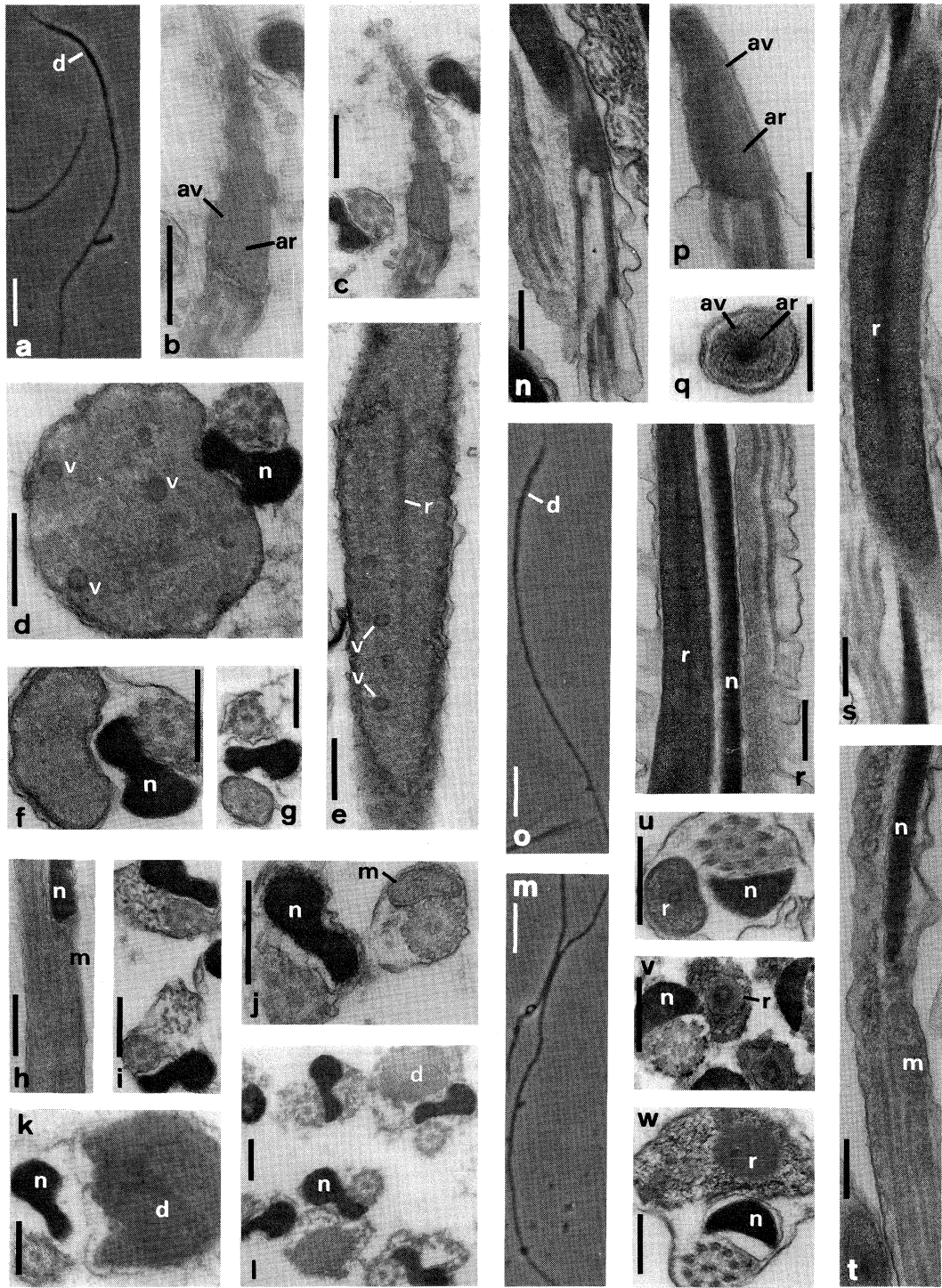


Fig.3. (a — n) Scalpellidae (continued). (a — j) *Smilium peronii*. (a) Whole spermatozoon. (b, c) L.S. acrosome. (d) T.S. droplet, nucleus and axoneme. (e) L.S. portion of droplet showing rod and scattered vesicles. (f) T.S. posterior region of droplet. (g) T.S. terminal region of droplet, with associated axoneme, nucleus. (h) L.S. junction of nucleus and mitochondrion. (i) T.S. glycogen region of sperm. (j) T.S. mitochondrial region of sperm. (k) *Calantica spinosa*. L.S. droplet, anome and nucleus. (l) *Calantica villosa*. T.S. axoneme, nucleus and droplet. (m) Whole spermatozoon of *Calantica spinosa*. (n — w) Lepadidae. (n — u) *Lepas anserifera*, (n) L.S. acrosome, collar, axoneme. (o) Whole spermatozoon. (p) L.S. acrosome. (q) T.S. acrosome. (r, s) L.S. droplet with thick rod, nucleus, axoneme. (t) Junction of nucleus and mitochondrion (note glycogen deposits). (u) T.S. droplet, nucleus, axoneme. (v) *Lepas anatifera*. T.S. droplet, nucleus, axoneme. (w) *Conchoderma virgatum*. T.S. droplet (note thick rod), nucleus, axoneme. Scale bars: a,o,m = 10 μ m; p,q = 0.2 μ m; all others = 0.25 μ m.

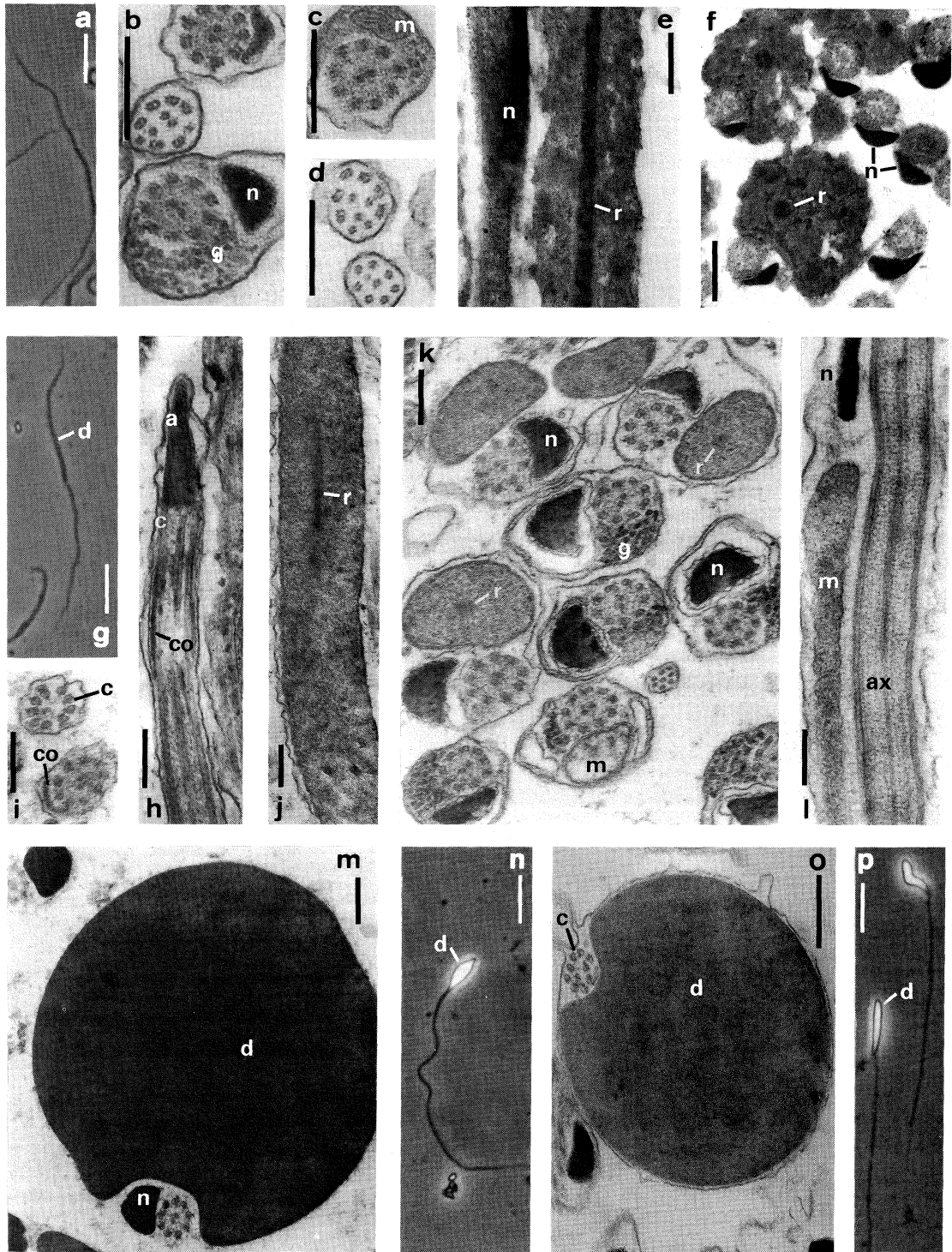


Fig.4. (a — d) Lepadidae. (a) *Conchoderma virgatum*, whole spermatozoon. (b — d) *Lepas anserifera*. (b) T.S. anterior region of axoneme and nucleus (top of figure); glycogen region of sperm (bottom), posterior extremity of sperm (middle). (c) T.S. mitochondrial region. (d) Transition of 9 + 2 axoneme into single microtubules at posterior extremity of sperm. (e, f) *Verruca ströemia*. (e) L.S. droplet, with thick rod. (f) T.S. droplet, axoneme and nucleus. (g — p) Balanomorpha. (g — l) *Catomerus polymerus* (Catophragmidae). (g) Whole spermatozoon. (h) L.S. acrosome, collar, centriolar zone, axoneme. (i) T.S. centriole, collar/axoneme. (j) L.S. portion of droplet showing rod. (k) T.S. axoneme/nucleus with droplet, axoneme/nucleus with glycogen granules, axoneme with mitochondrion. (l) Junction of nucleus and mitochondrion, with accompanying axoneme. (m — p) Chthamalidae. (m, n) *Octomeris brunnea*. (m) T.S. droplet, axoneme, nucleus. (n) Whole spermatozoon. (o, p) *Chthamalus antennatus*. (o) T.S. droplet, centriole, nucleus. (p) Whole spermatozoa. Scale bars: a, g, n, p = 10 µm; all others = 0.25 µm.

the accessory droplet (Figs 3t, 4b). These granules are also associated with the single mitochondrion (Figs 3t, 4c) which replaces the nucleus posteriorly. The 9 + 2 axoneme degenerates, first into nine disordered doublets with persistence of the central pair of microtubules, then into single microtubules (see Fig. 4b,d). Spermatozoa of *Lepas* spp. measure 90 to 95 μm , (Fig. 3o) while those of *Conchoderma* spp. (Fig. 4a) are approximately 80 to 85 μm long.

Verrucomorpha

Verrucidae (*Verruca ströemia*)

Available material of *Verruca ströemia* was useful only for determining the shape of the nucleus and some features of the accessory droplet. In transverse section, the nucleus has a sharply-defined triangular profile, and the droplet (maximum observed width 0.6 μm) contains a thick rod (Fig. 4f). Longitudinal sections (Fig. 4e) through the droplet suggest the the rod may be composed of two elements, a dense core with a diameter of 0.07 μm and a more electron-lucent enveloping layer about 0.01 μm thick. These and other details of the spermatozoa of *Verruca* will be fully resolved only through a study of better-fixed tissues. Barnes *et al.* (1971) give a length of 6 μm for the droplet of *V. ströemia*, but their photographs suggest that the length may be up to 12 μm .

Balanomorpha Chthamaloidea

Catophragmidae (*Catomerus polymerus*)

The acrosomal vesicle of *Catomerus polymerus* is 0.05 μm long, with a basal invagination containing axial rod material (Fig. 4h). A collar 0.7 μm long extends posteriorly from the centriole, sheathing the initial portion of the axoneme (Fig. 4h,i). The centriole is composed of nine doublets (Fig. 4i). Posterior to the collar, the axoneme becomes associated with the nucleus, triangular in transverse section (Fig. 4k), and a long, narrow accessory droplet (length 22 to 25 μm , maximum width 0.7 to 0.8 μm , Fig. 4g,j,k). A single rod lies within the granular matrix of the droplet (Fig. 4j,k). A tract of glycogen granules lies close to the axoneme and nucleus, particularly behind the accessory droplet (Fig. 4k). The nucleus is succeeded by a single mitochondrion possessing typical cristae (Fig. 4k,l). Degeneration of the axoneme into microtubules follows the pattern outlined for *Ibla*.

Chthamalidae (*Chthamalus antennatus*, *C. malayensis*, *Octomeris brunnea*, *Chamaesipho tasmanica*)

Spermatozoa of chthamalids differ from those of

Catomerus principally in an increased diameter of the accessory droplet and, in *Chthamalus* and *Octomeris*, a reduced droplet length. Acrosomal morphology was traced only for *Chamaesipho tasmanica*, where the vesicle is conical, 0.5 to 0.6 μm long, and has axial rod material lodged within its invaginated base (Fig. 5a). In this species, the accessory droplet is 17 to 18 μm long (Fig. 5d,e) and up to 1.0 μm wide, and contains a single rod together with electron-lucent areas within the matrix (Fig. 5b,c). The shorter, wider droplets of *Chthamalus* and *Octomeris*, 8 to 11 μm long and up to 2 μm wide, have no rod or other substructural elements (Fig. 4m-p). Sperm nuclei of *Chthamalus*, *Octomeris* and *Chamaesipho* are triangular in transverse profile. Phase-contrast microscopy gave the following lengths for chthamalid spermatozoa: *C. antennatus*, 70 μm ; *C. malayensis*, 100 μm ; *O. brunnea*, 68 to 70 μm ; *C. tasmanica*, 70 to 75 μm , (Figs 4n,p, 5d,e).

Balanomorpha Coronuloidea

Coronulidae (*Coronula diadema*, *Chelonibia testudinaria*, *Cylindrolepas darwiniana*, *Tubicinella cheloniae*, *Platylepas coriacea*, *Platylepas hexastylus*)

The acrosomal vesicles of these species are conical with an invaginated base, and range in length from 0.6 to 0.7 μm (*Chelonibia*, *Tubicinella*) to 0.38 μm (*Platylepas*) (Fig. 5g). A collar appears to be present in all species, but in no case could its length be determined. The accessory droplets of *Chelonibia testudinaria* (Fig. 5h-j), *Cylindrolepas darwiniana* (Fig. 5m,n), *Coronula diadema* (Fig. 5o-q) and *Tubicinella cheloniae* (Fig. 5f,k,l) are moderately long (14 to 20 μm) and narrow (markedly so in *Tubicinella* and *Cylindrolepas*). In *Platylepas* spp., the droplet is only 6 to 7 μm long and much wider than in other coronulids (Fig. 5r-t). A rod is present in the droplets of *Chelonibia*, *Tubicinella* and *Cylindrolepas* (Fig. 5h,j,k-m), but lacking in *Coronula* and *Platylepas* (Fig. 5o,p,t). In *Coronula*, *Tubicinella* and probably *Cylindrolepas*, the axial core of the droplet is appreciably less electron-dense than the surrounding area (Fig. 5l,o,p). The nucleus in all coronulid spermatozoa is triangular in cross section, and is succeeded posteriorly by a single mitochondrion. Phase-contrast microscopy gave the following lengths for whole spermatozoa: *Chelonibia*, 48 to 50 μm (Fig. 5i); *Coronula*, 58 to 60 μm (Fig. 5q); *Tubicinella*, 50 μm (Fig. 5f); *Cylindrolepas*, 56 μm ; *Platylepas*, 40 μm (Fig. 5r,s).

Tetraclitidae (*Austrobalanus imperator*, *Epopella plicata*, *Tesseropora rosea*, *Tetraclita squamosa*, *Tetraclitella purpurascens*)

Among the tetraclitids there is variation in the length of the accessory droplet and in its position along the axoneme, nuclear complex, as well as some variation in acrosoma

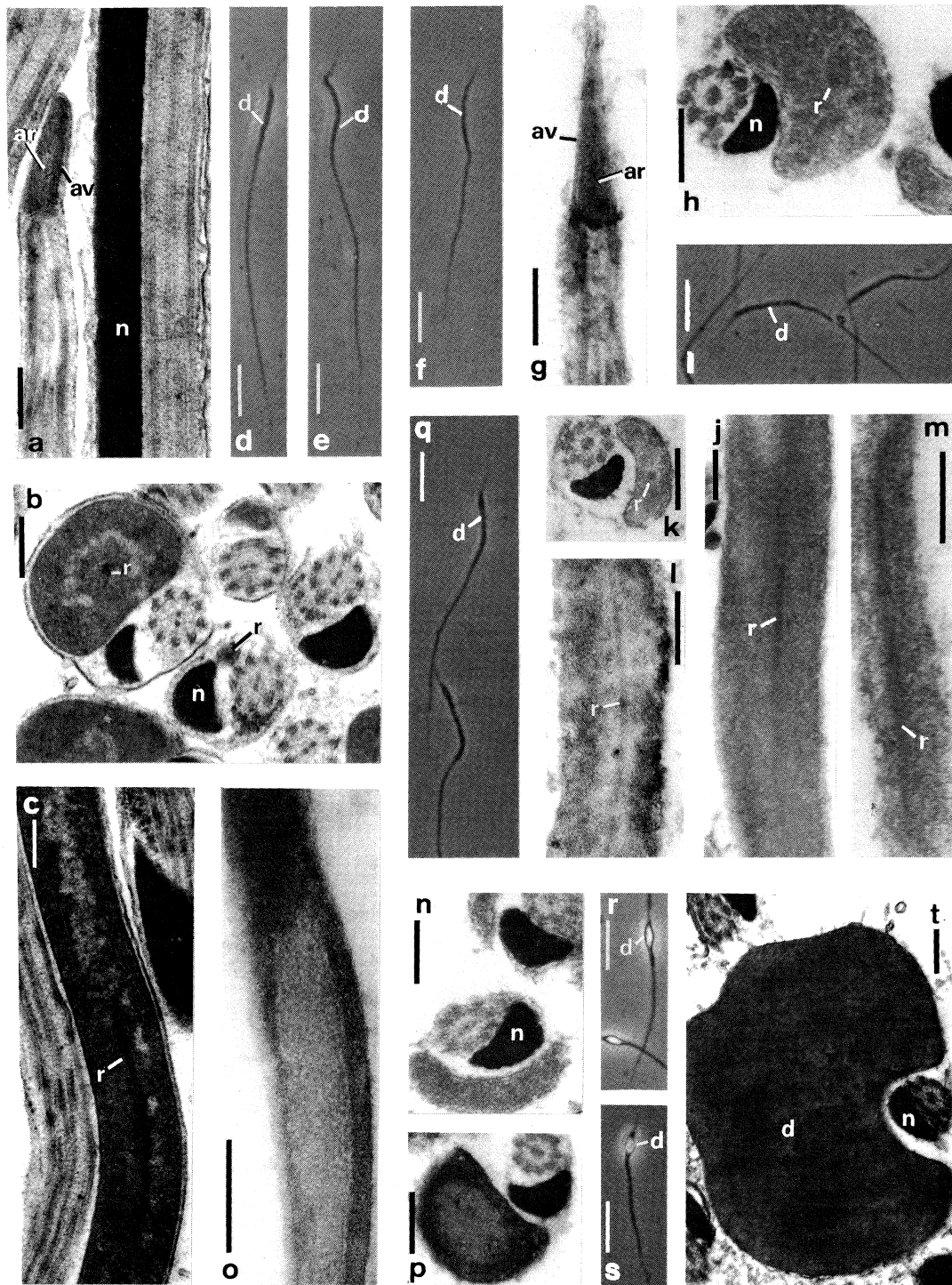


Fig.5. Balanomorpha. (a — e) Chthamalidae, *Chamaesipho tasmanica*. (a) L.S. acrosome, collar, axoneme, nucleus. (b) T.S. axoneme/nucleus with accessory droplet and with glycogen (right). (c) L.S. droplet and axoneme. (d, e) Whole spermatozoa. (f — t) Coronulidae. (f) Whole spermatozoon of *Tubicinella cheloniae* (g — j) *Chelonibia testudinaria*. (g) acrosome. (h) T.S. droplet, axoneme, nucleus. (i) Whole spermatozoon. (j) L.S. portion of droplet showing rod. (k, l) *Tubicinella cheloniae*. (k) T.S. droplet, axoneme, nucleus. (l) L.S. portion of droplet showing rod. (m, n) *Cylindrolepas darwiniana*. (m) L.S. portion of droplet with rod. (n) T.S. slightly oblique through droplet, nucleus, axoneme. (o — q) *Coronula diadema*. (o) L.S. portion of droplet with differentiated central zone. (p) T.S. droplet. (q) Whole spermatozoon. (r — t) *Platylepas*. (r) Whole spermatozoon of *Platylepas coriacea*. (s) Whole spermatozoon of *Platylepas hexastylus*. (t) T.S. droplet, axoneme, nucleus of *Platylepas coriacea*. Scale bars: d-f,i,q-s = 10 μm ; all others = 0.25 μm .

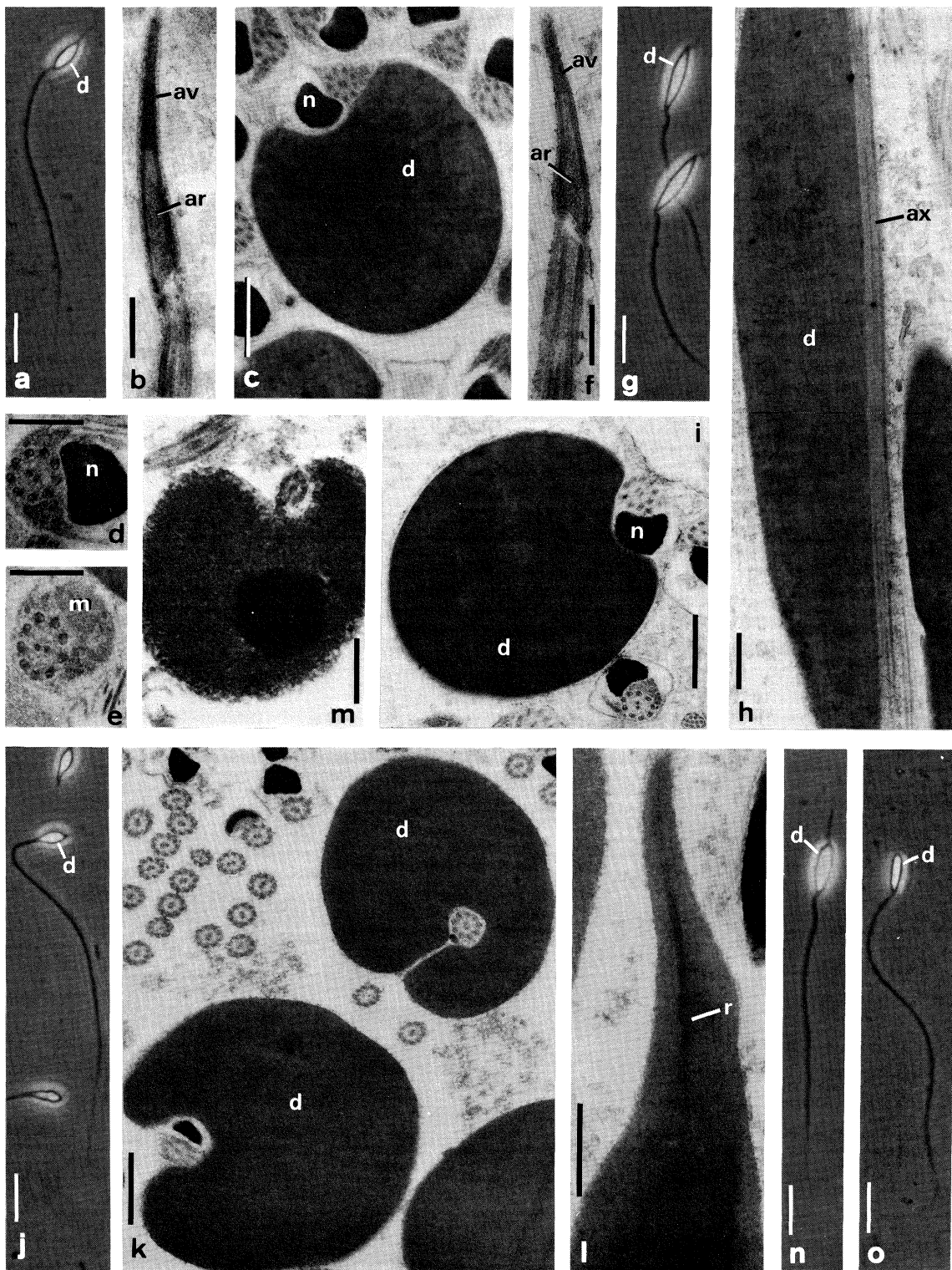


Fig.6. Balanomorpha, Tetracitidae. (a — e) *Tesseropora rosea*. (a) Whole spermatozoon. (b) L.S. acrosome. (c) T.S. droplet, axoneme, nucleus. (d) L.S. glycogen region. (e) T.S. mitochondrial region. (f — i) *Austrobalanus imperator*. (f) L.S. acrosome. (g) Whole spermatozoa. (h) L.S. droplet and axoneme. (i) T.S. droplet, axoneme, nucleus. (j — m) *Epopella plicatus*. (j) Whole spermatozoon. (k) T.S. showing deep enclosure of axoneme/nucleus by droplet. (l) L.S. tapered extremity of droplet. (m) T.S. of droplet. Vesicle-like structure is probably an artefact of droplet degeneration in this spermatozoon. (n) Whole spermatozoon of *Tetraclita squamosa*. (o) Whole spermatozoon of *Tetraclitella purpurascens*. Scale bars: a,g,j,n,o = 10 μ m; b,d-f = 0.25 μ m; all others = 0.5 μ m.

length. In *Austrobalanus imperator* and *Tesseropora rosea* the acrosomal vesicle is long in comparison with other thoracicans, and measures up to 1 μm (Fig. 6b,f). In *Tetraclitella purpurascens* the acrosomal vesicle is about 0.7 μm long (Fig. 7b). Acrosomes were not traced in *Epopella plicata* or *Tetraclita squamosa*. In all species, the accessory droplet is wide, with a groove containing the axoneme and nucleus (Fig. 6c,i,k). Nuclei are triangular in transverse section. In *Epopella*, the axoneme and nucleus may be almost totally enclosed by the droplet at some levels (see Fig. 6k). No evidence of a rod could be found in the droplets of *Tesseropora*, *Austrobalanus*, *Tetraclita* or *Tetraclitella* (Figs 6c,h,i, 7a) but a rod was observed in the droplet of *Epopella*, most clearly at the tapered extremities (Fig. 6l). Kubo *et al.* (1979) mentioned that the droplet of *Tetraclita squamosa* was of 'dual texture', exhibiting spherical, differentiated zones, but we could find no clear evidence of this in our material (sea water/formalin-fixed). Droplet lengths range from 6.5 μm in *Epopella* (Fig. 6j), to 7.5 to 8 μm in *Tesseropora* and *Tetraclitella* (Fig. 6a,o), 10 μm in *Tetraclita* (Fig. 6n) and 14 μm in *Austrobalanus* (Fig. 6g). Whole sperm lengths range from 65 μm in *Austrobalanus* to 75 to 82 μm in the other genera (Fig. 6a,g,j,n,o).

Balanomorpha Balanoidea

Archaeobalanidae (*Hexaminus foliorum*, *H. popeiana*; *Elminius covertus*; *Armatobalanus allium*, *A. arcuatus*; *Acasta spongites*)

The spermatozoa of the archaeobalanids examined in the present study showed substantial variation in the length and substructure of the accessory droplet. Spermatozoa of *Hexaminus* and *Elminius* are similar to those of the generalised chthamaloid *Catomerus polymerus*. The acrosomal vesicle measures 0.4 to 0.5 μm and is basally invaginated (Fig. 7d,e,m). A collar 0.7 to 0.9 μm long extends posteriorly from the base of the acrosome, where it then sheaths the centriole and the initial portion of the axoneme (Fig. 7e,j). The accessory droplet is long (17 to 20 μm) and narrow (maximum width 0.6 μm), with a single rod (diameter 0.02 to 0.04 μm) (Fig. 7c,f,i,l,n,o). Nuclei have a triangular transverse profile (Fig. 7h,i,l,o). The axoneme/nucleus complex lies along the weak indentation in the droplet. A single mitochondrion, measuring 2.4 μm in *H. foliorum*, replaces the nucleus posteriorly (Fig. 7g). Glycogen deposits are associated with the axoneme/nucleus, particularly behind the level of the accessory droplet, and the axoneme/mitochondrion (Fig. 7f-h,o). Sperm length is approximately 67 μm in *Hexaminus* and *Elminius* (Fig. 7c,n).

In *Armatobalanus* spp., the droplet is 7 to 8 μm long and 0.7 to 1.0 μm wide, and contains a variable number (8 to 10) of dense spheres, which at the light microscopic level give the droplet a beaded appearance (Fig. 8a,c-g). These spherical vesicles are linked by a single rod (Fig. 8g). The

acrosomal vesicle of *Armatobalanus* is short (approximately 0.25 μm) and conical, with its basal invagination filled by axial rod material (Fig. 8b). A collar is present, but is not well developed. Nuclei have a triangular transverse profile (Fig. 8c). A single mitochondrion replaces the nucleus posteriorly (Fig. 8d). Whole spermatozoa of *Armatobalanus* are 52 to 54 μm long (Fig. 8a,e — phase-contrast microscopy).

Spermatozoa of *Acasta spongites* have a droplet which is the same length as in *Armatobalanus* but is much wider, and lacks either a rod or any vesicle substructure (Fig. 8i). The length of the whole spermatozoon is 65 to 67 μm (Fig. 8h).

Pyrgomatidae (*Pyrgoma cancellata*; *Creusia spinulosa*; *Savignium elongatum*)

Light microscopy clearly shows that the accessory droplets of pyrgomatid spermatozoa are beaded (Fig. 8k,n,o), like those of *Armatobalanus* (Fig. 8a,e). Droplet length varies from 8.5 to 10 μm in *Pyrgoma* and *Creusia* to 16 to 18 μm in *Savignium*. Internally the droplet contains spherical, dense vesicles 0.3 μm in diameter linked by a rod (Fig. 8j,l,m,p). Nuclei remain triangular in transverse section. The lengths of the spermatozoa are as follows: 55 to 57 μm (*P. cancellata*); 75 to 77 μm (*S. elongatum*); 85 μm (*C. spinulosa*).

Balanidae (*Balanus variegatus*, *B. amphitrite*; *Austromegabalanus nigrescens*; *Megabalanus ajax*)

Considerable variation also exists in the morphology of the accessory droplet and, to some extent, the acrosome of balanid spermatozoa. The acrosome is short in *Balanus variegatus* (0.3 μm , Fig. 9a,b), but longer in *B. amphitrite* (0.56 μm , Fig. 9i-k), *Austromegabalanus nigrescens* (0.54 μm , Fig. 10b) and *Megabalanus ajax* (0.56 μm , Fig. 10m). In all species the acrosome is conical, with axial rod material located within a deep basal invagination. The accessory droplet is narrow and slightly elongate in *B. variegatus* (0.45 x 20 μm , Fig. 9c-e,g) and *B. amphitrite* (0.7 x 17 to 20 μm , Fig. 9h,l,m), with a row of widely separated spherical to oblong vesicles connected by a rod. In *Austromegabalanus nigrescens*, the droplet also shows spherical to oblong vesicles (Fig. 10c-e), very dense in comparison to the surrounding matrix, and a rod (Fig. 10c-d). However the droplet of *A. nigrescens* is much wider (1.8 μm) and shorter (5 to 6.5 μm) than those of *B. variegatus* or *B. amphitrite*, and frequently shows more than one row of vesicles (Fig. 10e). The droplet in *Megabalanus ajax* is very wide (maximum diameter 2.26 to 2.3 μm) and short (6.5 to 7.5 μm). It appears to lack either spherical vesicles or a rod, and has a deep groove which contains the nucleus and axoneme (Fig. 10i-k). Transverse sections show that the profile of the accessory droplet in *M. ajax* is crescentic towards the tapered extremities

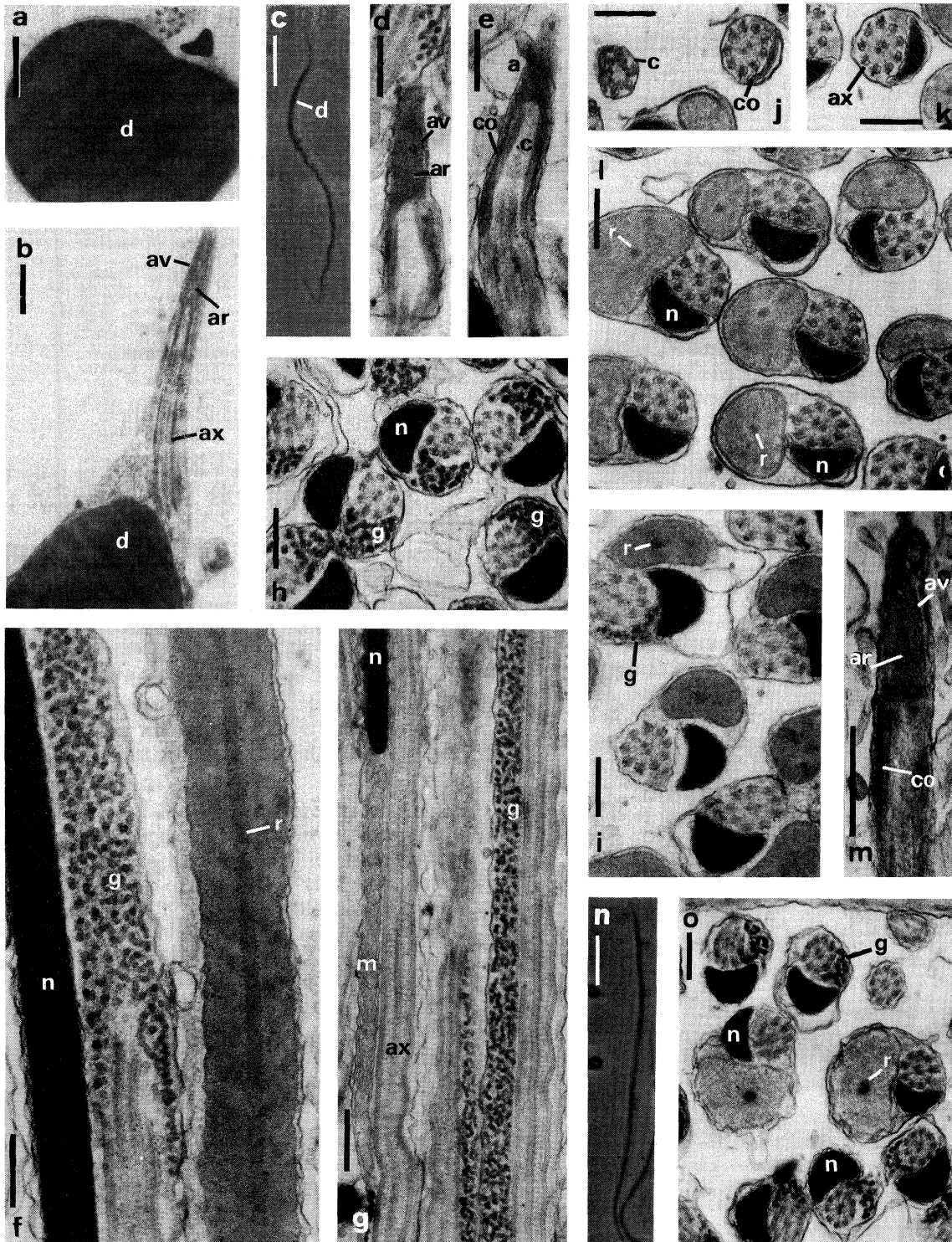


Fig.7. Balanomorpha. (a, b) *Tetraclitella purpurascens* (Tetraclitidae). (a) T.S. droplet, axoneme, nucleus. (b) L.S. acrosome, axoneme and anterior extremity of droplet. (c — o) Archaeobalanidae. (c — i) *Hexaminus foliorum* (c) Whole spermatozoon. (d, e) L.S. acrosome, collar, centriole, proximal region of axoneme. (f) L.S. droplet (with rod), nucleus and glycogen granules. (g) L.S. junction of nucleus and mitochondrion (left), glycogen and axoneme (right). (h) T.S. glycogen region. (i) T.S. droplet (with rod), axoneme, nucleus. (j — l) *Hexaminus popeiana*. (j) T.S. centriole (left), axoneme/collar (right). (k) T.S. axoneme and anterior region of nucleus. (l) T.S. droplet, axoneme and nucleus. (m — o) *Elminius covertus*. (m) L.S. acrosome, collar, proximal portion of axoneme. (n) Whole spermatozoon. (o) T.S. droplet and glycogen regions of spermatozoon. Scale bars: c,n = 10 μ m; a,b = 0.5 μ m; all others = 0.25 μ m.

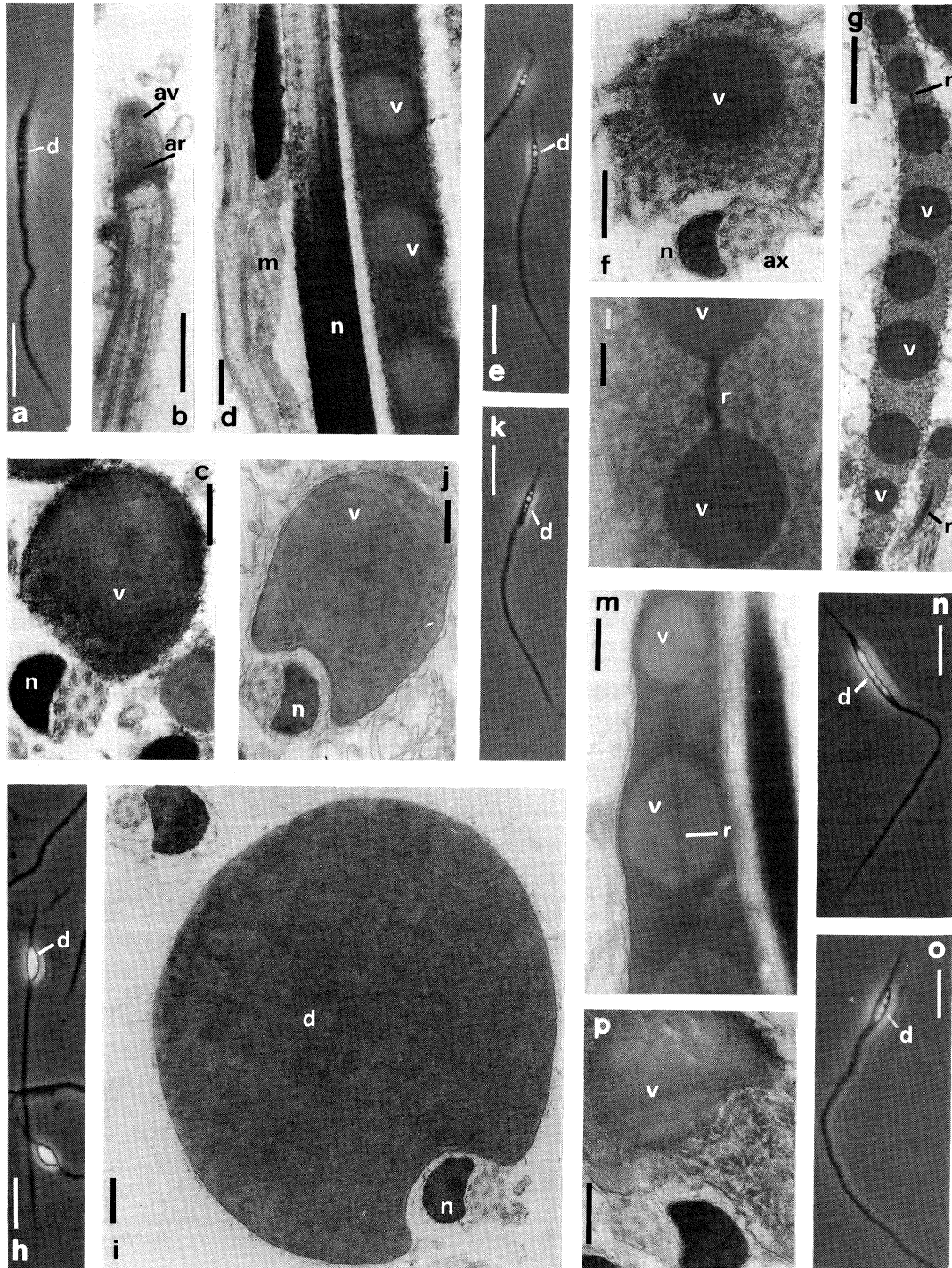


Fig.8. Balanomorpha. (a — i) Archaeobalanidae. (a — d) *Armatobalanus allium*. (a) Whole spermatozoon — internal vesicles of accessory droplet visible as beads. (b) L.S. acrosome, collar, proximal portion of axoneme. (c) T.S. droplet (note large internal vesicle). (d) L.S. droplet and nucleus — mitochondrion junction. (e — g) *Armatobalanus arcuatus*. (e) Whole spermatozoon, beading visible in droplet. (f) T.S. droplet with dense vesicle, axoneme, nucleus. (g) L.S. droplet showing vesicles and rod. (h, i) *Acasta spongites*. (h) Whole spermatozoon, absence of beading in droplet. (i) T.S. droplet (no vesicle present), nucleus, axoneme. (j — p) Pyrgomatidae. (j — m) *Pyrgoma cancellata*. (j) T.S. droplet, nucleus, axoneme. (k) Whole spermatozoon, beading visible in droplet. (l) Vesicles of droplet connected and penetrated by rod. (m) Vesicles and rod in droplet. (n, o, p) *Creusia spinulosa*. (o) Whole spermatozoon, with beading in droplet. (p) T.S. droplet with vesicle, nucleus, axoneme. Scale bars: a = 15 μm ; e, h, k, n, o = 10 μm ; all others = 0.25 μm .

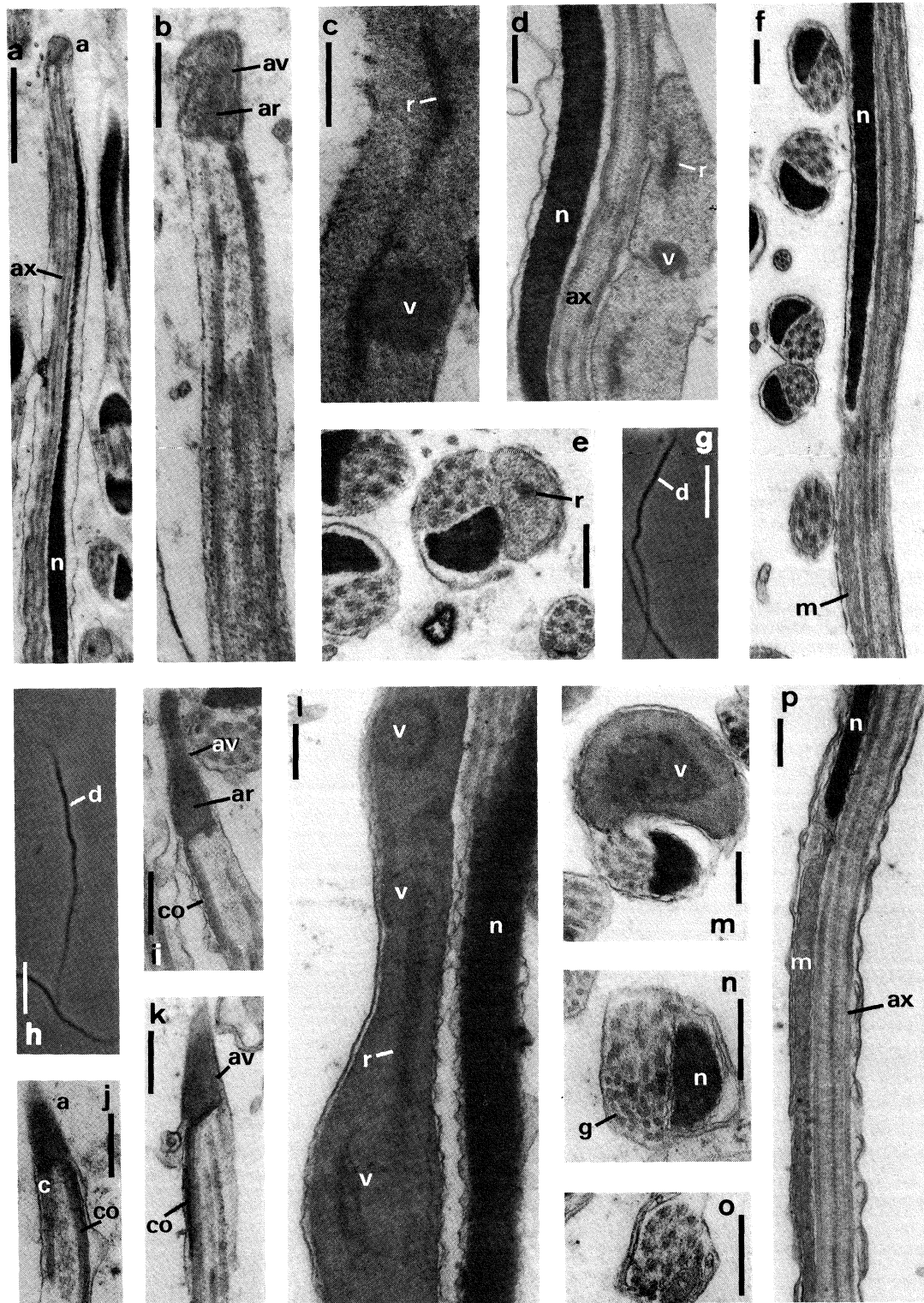


Fig.9. Balanomorpha, Balanidae. (a — g) *Balanus variegatus*. (a) L.S. acrosome, axoneme, nucleus. (b) L.S. acrosome, centriole/axoneme/collar region of sperm. (c) L.S. portion of droplet, with rod and dense vesicle. (d) L.S. portion of nucleus, axoneme and droplet, with rod and vesicle. (e) T.S. droplet, axoneme, nucleus. (f) L.S. nucleus — mitochondrion junction. (g) Whole spermatozoon. (h — p) *Balanus amphitrite*. (h) Whole spermatozoon. (i — k) L.S. acrosome, centriole, collar, proximal portion of axoneme. (l) L.S. nucleus, droplet (with rod and vesicles). (m) T.S. droplet, axoneme, nucleus. (n) T.S. glycogen region. (o) T.S. mitochondrial region. (p) L.S. nucleus-mitochondrion junction. Scale bars: g,h = 10 μm ; a = 1.0 μm ; all others = 0.25 μm .

(Fig. 10l). Balanid sperm nuclei are triangular in transverse section. The lengths of balanid spermatozoa (phase-contrast microscopy) are: *B. variegatus*, 52 to 54 μm ; *B. amphitrite*, 55 μm ; *Austromegabalanus nigrescens*, 50 to 52 μm ; *Megabalanus ajax*, 51 to 53 μm (Figs 9g,h, 10a,j).

Figure 11 summarises the variations in shape, position and internal morphology of the accessory droplet among major lepadomorph and balanomorph taxa.

Discussion

Thecostracan Spermatozoa

This study, in conjunction with other work on sperm ultrastructure in the Thecostraca (Bocquet-Vedrine & Pochon-Masson, 1969; Turquier & Pochon-Masson, 1969; Brown, 1970; Pochon-Masson *et al.*, 1970; Pochon-Masson, 1971; Munn & Barnes, 1970a, 1970b; Kubo *et al.*, 1979; Azevedo & Corral, 1982; Grygier, 1981, 1982; also Anderson & Personne, 1970; Klepal, 1985), confirms that the Thoracica, Acrothoracica and Rhizocephala share a distinctive type of filiform spermatozoon (Fig. 1c-g), the chief characteristics of which are: (1) a simple, conical acrosome capping the axoneme; (2) a filiform nucleus running parallel to the axoneme; (3) a single, rod shaped mitochondrion posterior to the nucleus; (4) substantial glycogen deposits associated with the axoneme in the posterior half of the spermatozoon. Thoracican and acrothoracican sperm also share a fusiform accessory droplet, often with internal substructural elements, running parallel to the nucleus and axoneme. An accessory droplet has not been documented for rhizocephalan sperm.

This type of sperm differs considerably from the more generalised spermatozoa of acrothoracidans (Grygier, 1981, 1982) and also from the distinctive flagellate sperm of mystacocaridans (Brown & Metz, 1967) and branchiurans (Brown, 1970; Wingstrand, 1972). The typical 'cirripede' spermatozoon presumably originated from an ascothoracidan-like spermatozoon, although spermiogenesis in thoracicans (Bocquet-Vedrine & Pochon-Masson, 1969; Kubo *et al.*, 1979) does not recapitulate an 'ascothoracidan' stage. In other crustaceans such as Decapoda (Pochon-Masson, 1969), Ostracoda (Gupta, 1968; Reger, 1970; Lopez-Camps *et al.*, 1979a; Wingstrand, 1988), Branchiopoda (Wingstrand, 1978) and Copepoda (Brown, 1970; Lopez-Camps *et al.*, 1979b), the spermatozoa lack an axoneme, often have unusual shapes (e.g. the stellate sperm of many decapods and discoidal sperm of some copepods) and are commonly immotile. There does not appear to be a homologue of the accessory droplet of cirripede sperm in any other group of crustaceans, save perhaps for the pseudoacrosome of *Argulus* (Brown, 1970; Wingstrand, 1972). As emphasised by Munn & Barnes (1970b), Barnes *et al.* (1971), Bocquet-Vedrine & Pochon-Masson (1969) and Kubo *et al.* (1979), the accessory droplet of cirripedes is not homologous with the cytoplasmic droplet of immature mammalian sperm. It is, in fact, a distinct Golgi product formed at an advanced stage

of spermiogenesis (Kubo *et al.*, 1979). According to Kubo *et al.* (1979) the accessory droplet reacts negatively for lipid, glycolipid, polysaccharide and acid phosphatase, as well as to digestion tests using DNA-ase and RNA-ase. It does, however, appear to contain lipoprotein (Bocquet-Vedrine & Pochon-Masson, 1969). Motility is increased when the droplet is lost, although Barnes *et al.* (1971) and Barnes *et al.* (1977) considered that sperm motility in cirripedes is more dependent on physiological maturity than on structural features. Passage through the pores of the oviducal sac may be difficult or impossible for sperm which retain the droplet (see Walker, 1980 for discussion of the structure of the pores of the oviducal sac in balanomorphs). Probably the accessory droplet serves a nutritional and/or capacitative function.

The present study, together with those of Bocquet-Vedrine & Pochon-Masson (1969), Munn & Barnes (1970b), Kubo *et al.* (1979), Azevedo & Corral (1982) and Klepal (1985), shows that the accessory droplet of cirripede sperm often exhibits internal substructures such as a simple or structured rod, spherical, differentiated zones (sometimes connected to the rod) and more rarely, lacunae, parallel arrays of membranes, coiled tubules (corpuscle) and crystalloid bodies (Fig. 11). These substructural elements, when considered in conjunction with the shape and size of the droplet, may be of phylogenetic importance, as will be discussed below.

It is well known that the droplet in testicular sperm of cirripedes is frequently ovoid compared with the more streamlined droplet of sperm taken from the seminal vesicle (Barnes *et al.*, 1971). Barnes *et al.* (1971) also found that in some balanomorphs such as *Chthamalus stellatus*, the form of the accessory droplet was variable and that shape changes or final loss of the droplet took place abruptly. We have observed that most sperm within the seminal vesicle possess droplets of a characteristic size and shape for each species.

Cirripede Spermatozoa and Phylogeny

One of the principal aims of this study has been to attempt to evaluate the relevance of sperm morphology to cirripede phylogeny. As indicated earlier and by previous authors (for example Pochon-Masson, 1971; Baccetti, 1979; Grygier, 1981, 1982), the Thoracica, Acrothoracica and Rhizocephala share a similar modification of sperm architecture and are collectively separable from a sister group Ascothoracida, which retain a more generalised sperm (Grygier, 1981, 1982, 1983). Grygier (1987b, 1987c) and Newman (1982, 1987) also list other grounds for recognition of the Ascothoracida as a sister-group.

Newman (1987), in a review of recent investigations bearing on the phylogenetic relationships of the Rhizocephala, has argued for an origin of the Rhizocephala from a mobile, biting stock, closely related to the ancestors of the Cirripedia (= Thoracica and Acrothoracica) within the Thecostraca. Although the shared filiform sperm is evidence of a close relationship

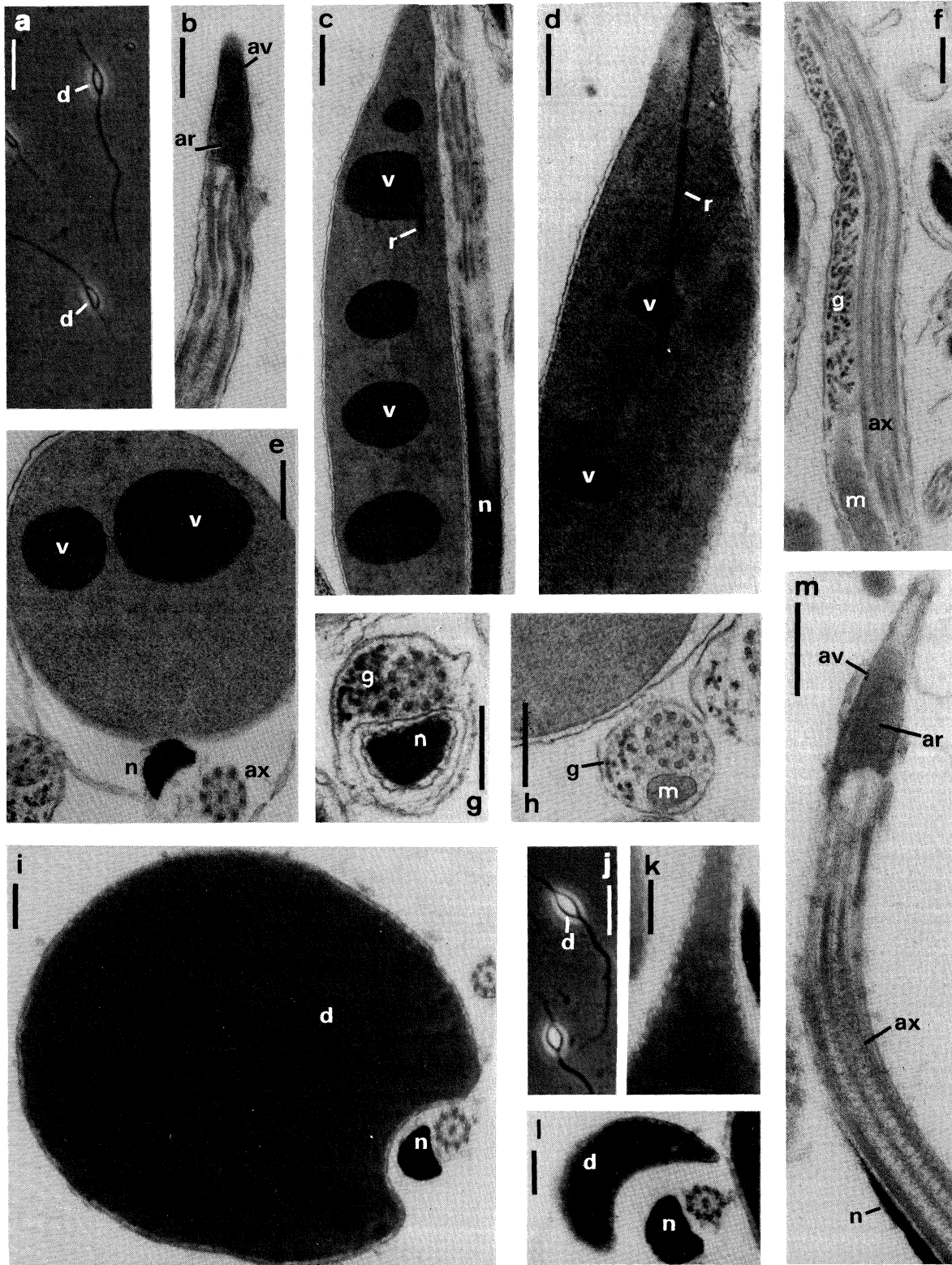


Fig.10. Balanomorpha, Balanidae. (a — h) *Austromegabalanus nigrescens*. (a) Whole spermatozoa. (b) L.S. acrosome, collar, centriole, axoneme. (c) L.S. portion of droplet, nucleus, axoneme. (d) L.S. portion of droplet showing rod and vesicle. (e) T.S. droplet, nucleus, axoneme. (f) L.S. glycogen region, axoneme, mitochondrion. (g) T.S. glycogen region. (h) T.S. axoneme, glycogen, mitochondrion. (i — m) *Megabalanus ajax*. (i) T.S. droplet, axoneme, nucleus. (j) Whole spermatozoa. (k) L.S. tapered extremity of droplet. (l) T.S. posterior region of droplet, with axoneme and nucleus. (m) L.S. acrosome, axoneme, nucleus. Scale bars: a,j = 10 μ m; c,d = 0.5 μ m; all others = 0.25 μ m.

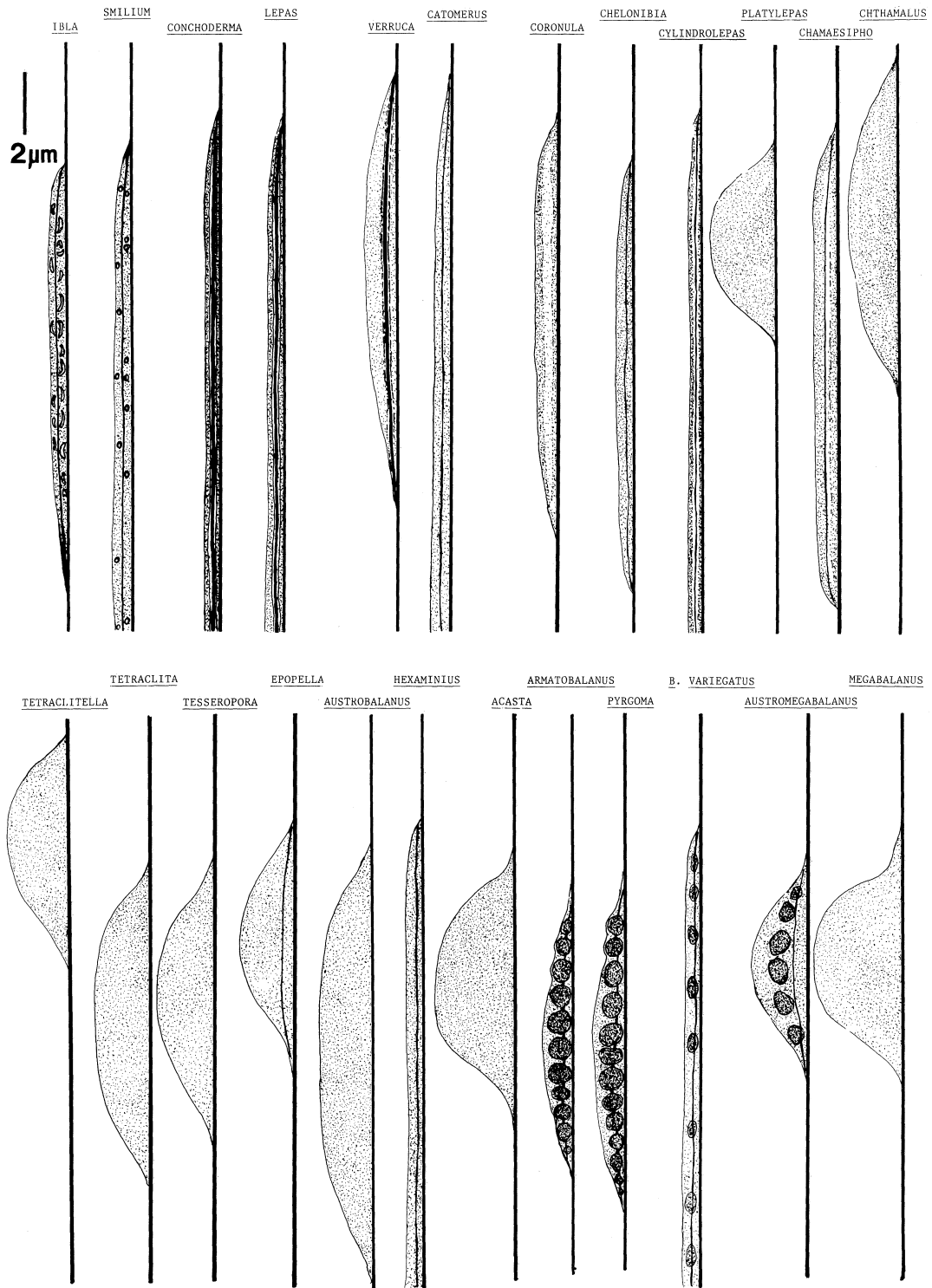


Fig.11. Shape, position and substructure of accessory droplet in thoracican spermatozoa relative to anterior portion of spermatozoon (diagrammatic). Acrosomes are not shown: only anterior portions of elongate droplets are illustrated. Substructural elements of droplets include: curved lacunae (*Ibla*); dense vesicles (small — *Smilium*; large — *Armatobalanus*, *Pyrgoma*, *Balanus variegatus*, *Austromegabalanus*); rods (thick, structured — *Lepas*, *Conchoderma*, *Verruca*; thin — many examples). Note: narrow droplets (with rod) in lepadomorphs, and some balanomorphs (*Catomerus*, some coronuloids, the archaeobalanids *Hexaminus* and *Elminius*); wide, shorter droplets, lacking any substructural elements, in other coronuloids and some balanoids; similarity between droplets of *Armatobalanus* and pyrgomatids.

between the Thoracica, Acrothoracica and Rhizocephala, the nuclear transverse profile of the sperm is distinctive in each group. That of thoracicans is triangular (Fig. 12), that of acrothoracicans, dumb-bell shaped (Tomlinson, 1969; Pochon-Masson *et al.*, 1970; Pochon-Masson, 1971; this account) and that of rhizocephalans, crescentic (*Sacculina*, *Clistosaccus*; Pochon-Masson, 1971; J. Hoeg, personal communication). An early divergence of the three groups is therefore indicated. The shared basic structure of the accessory droplet of the thoracicans and acrothoracicans adds another feature to the extensive morphological, developmental and fossil evidence in favour of a divergence of Thoracica and Acrothoracica from a common, sessile, cirripede ancestry (Newman, 1987).

The club-shaped nuclear profile of calanticine sperm, while similar in some respects to the acrothoracican profile, appears to be a modification of the asymmetrical pattern found in *Scalpellum* (Fig. 12). Except for these modifications, a triangular nuclear profile is characteristic of thoracican sperm, including the Ibloidea.

Thoracican Phylogeny

The complexities of phylogenetic evolution within the Thoracica have become well recognised in recent years (Newman & Ross, 1976; Zevina, 1978, 1981, 1982; Anderson, 1981; Newman, 1982, 1987; Buckeridge, 1983; Foster & Buckeridge, 1987; Newman & Hessler, 1989). In Figs 11 to 13, we have attempted to interpret the possible significance of the patterns of ultrastructure of the accessory droplets of thoracican spermatozoa in relation to current views on thoracican phylogeny. For the most part, the data are confirmatory of these views, although some unexpected problems have arisen.

Taking account of a condition shared with Acrothoracica, we make the assumption that the basic form of the thoracican accessory droplet is that retained in *Pollicipes* (Azevedo & Corral, 1982), *Catomerus*, *Chelonibia*, *Hexaminus* and *Elminius* (this account) and *Semibalanus balanoides* (Munn & Barnes, 1970b). The droplet is long and narrow, with a crescentic transverse profile, and contains a thin, central rod within a uniform granular matrix.

Among lepadomorphs, *Ibla cumingi* and *I. quadrivalvis* are immediately distinguished from all other thoracicans in having a unique droplet substructure (Klepal, 1985; this account; Figs 12, 13). This is in accord with the view that ibloids, with their four plated capitulum, postoral adductor scutorum (Darwin, 1851; Klepal, 1985) and generalised larval musculature (Anderson, 1987) diverged early from other thoracicans (Newman, 1982, 1987).

For non-ibloid lepadomorphs other than scalpellids, data on the accessory droplet are still limited. Heteralepadids appear to retain the basic sperm structure, as might be expected if they occupy the basal status proposed by Foster (1978), but this needs to be confirmed. Lepadids, in contrast, have a modified rod, with a dense core separated by a space from a less dense peripheral sheath. A similar

rod has evolved convergently in *Scalpellum* (Scalpellidae) and in the *Verrucomorpha* (see below). The scalpellids provide useful evidence that variations in droplet structure are of phylogenetic value, in that consistent differences are recognisable between major subfamilies (classification of Zevina, 1981), supported by differences in nuclear transverse profile (Fig. 12). While the Pollicipedinae retain the basic condition, there are distinctive and different modifications of droplet and/or nuclear structure in the Lithotryinae (*Lithotrya*), Scalpellinae (*Scalpellum*) and Calanticae (*Calantica*, *Smilium*). On present evidence, these modifications offer no indications of relationships between the subfamilies. Darwin (1854), on morphological grounds, favoured the Pollicipedinae as close relatives of the scalpellid ancestors of the Balanomorpha. Newman (1987) has pointed out the difficulties of a direct derivation of balanomorphs from pollicipedine scalpelloids and has demonstrated an evolutionary route from a core scalpellid stock via the Brachylepadomorpha. At the same time, Newman continues to recognise the closeness of *Pollicipes* to the core stock of scalpelloids, and thus to the balanomorphs, a view previously supported by Anderson (1983) from functional morphology and more recently by Egan & Anderson (1989) from larval comparisons.

The Verrucomorpha are another group with origins among the Brachylepadomorpha, independently of the Balanomorpha (Newman, 1987; Newman & Hessler, 1989). The limited data presented here on sperm ultrastructure in *Verruca* are in accord with the distinctiveness of the Verrucomorpha (Fig. 12). The basic triangular nuclear profile is retained but the accessory droplet is modified through shortening and the development of a complex rod with a thick, dense core, closely covered by a less dense sheath. A somewhat similar rod is found in the elongate droplet of *Scalpellum* sperm, but the sperm nucleus of *Scalpellum* is modified in transverse profile. Sperm morphology does not support earlier suggestions of a calanticine (Newman *et al.*, 1969; Newman, 1982; Buckeridge, 1983) or lithotryine-calanticine (Newman, 1982) origin for the Verrucomorpha.

The broad outlines of the phylogenetic evolution of the Balanomorpha were elucidated by Newman & Ross (1976) and have been refined in certain details in subsequent work (Anderson, 1981, 1987; Anderson & Buckle, 1983; Buckeridge, 1983; Anderson & Anderson, 1985; Foster & Buckeridge, 1987; Newman, 1982, 1987; Egan & Anderson, 1988, 1989) but various problems remain. The relationships of the basic balanomorph stocks Chionelasmatoidea, Pachylasmatoidea and catophragmid Chthamaloidea to each other and to the ancestry of the Balanomorpha are not fully resolved. The origin of the Coronuloidea and the later routes of divergent evolution of the tetraclids and, independently, the Balanoidea from the coronuloid stock are also in need of further elucidation. Within the Balanoidea, there are also indications, particularly from functional morphology (Anderson & Southward, 1987), that the Archaeobalanidae and Balanidae as presently defined may be polyphyletic groupings.

The sperm data of the present work (Fig. 13) are largely in accord with current interpretations of balanomorph

evolution. They also offer some pointers towards the solution of remaining problems. These will be mentioned as we review the summary presented in Figures 11 and 13.

Among early balanomorphs, no sperm data have yet been obtained for *Chionelasmus* or *Pachylasma*. We predict the retention of the generalised structure seen in *Catomerus* and *Chelonibia* and thus a typical symplesiomorphic situation of no elucidatory value. Within the Chthamaloidea, however, the sperm data are more revealing, in separating *Chamaesipho*, in which an elongate droplet is retained but has a distinctive substructure, from the *Octomeris* — *Chthamalus* line of evolution, with a short, swollen droplet lacking a rod. Other evidence that *Chamaesipho* has evolved from a catophragmid ancestry independently of the other chthamalids is available from larval studies (Egan & Anderson, 1989) and adult morphology (Foster & Newman, 1987; D.T. Anderson, in preparation).

For the Coronuloidea, the lack of sperm data on the Bathylasmatidae presents a serious problem (see below), but interesting trends emerge in the Coronulidae and Tetracitidae. In the former, several divergent modifications can be traced from the basic thoracican sperm type retained in *Chelonibia*. *Platylepas* presents another example of the shortened, swollen droplet, lacking a rod, previously encountered in the chthamalids. This form of droplet has evolved independently several times (see Fig. 13) and is not, of itself, a useful indication of relationships at higher phylogenetic levels. Within the Coronulidae, however, it serves to separate *Platylepas* from the other genera for which we have sperm data. *Coronula* also lacks a rod, but retains a relatively long, narrow droplet, with an electron-lucent core. Droplets with an even narrower, crescentic transverse profile, retaining a rod, unexpectedly link the *Tubicinella* of turtles with *Cylindrolepas*. On morphological grounds, Monroe (1981) derived *Cylindrolepas* from *Platylepas* and *Platylepas* from *Coronula*, all within the Coronulinae, and placed *Tubicinella* in a separately derived subfamily, Xenobalaninae. The sperm data suggest that phylogenetic evolution within the Coronulidae may be in need of further study.

The Tetracitidae, on the other hand, share a derived pattern of sperm ultrastructure in which the droplet is short, swollen and, except in *Epopella*, rodless. In the absence of suitable data on the Bathylasmatidae, we are in doubt about the origin of the tetracitid sperm pattern. The arrows in Figure 13 tentatively reflect relationships based on functional morphology (Anderson & Anderson, 1985) and, except for a different view of tetracitellan relationships, those of Foster & Buckeridge (1987) derived from palaeontology. Synapomorphies of sperm ultrastructure (this account) and larval development (Egan & Anderson, 1989) fully support the proposal by Newman & Ross (1976) that *Austrobalanus imperator* is a 'six-plated tetracitid'.

The concept of an origin of the Balanoidea from bathylasmatid coronuloids (Newman & Ross, 1976) has also found further support in recent studies on larval development (Egan & Anderson, 1989). At present we have no sperm data on the bathylasmatids, but can

recognise that a number of archaeobalanid genera (*Hexaminius* [Fig. 13], *Elminius*, *Semibalanus*) retain the generalised thoracican sperm. Within the Archaeobalanidae, we are much in need of data on sperm ultrastructure in *Solidobalanus*, *Chirona*, *Membranobalanus* and *Conopea*, but can point to some striking divergences. *Acasta*, for example, is an archaeobalanid in which the accessory droplet displays a short swollen form lacking internal substructure. Since *Acasta* is now known (D.T. Anderson, unpublished) to retain a mode of cirral extension feeding of a very generalised kind, involving prolonged cirral extension together with particle capture by individual rami, the evolutionary origin and phylogenetic relationships of this "archaeobalanid" genus become matters of great interest. The coral-associated genus *Armatobalanus*, on the other hand, is distinctive among archaeobalanids in the possession of prominent vesicles within the sperm accessory droplet, giving it a beaded appearance at the light microscope level (Figs 11, 13). The same beaded modification characterises the coral-inhabiting Pyrgomatidae (e.g. *Creusia*, *Pyrgoma*, *Savignium*), supporting the proposal of Ross & Newman (1973) that *Armatobalanus* might be closely related to the ancestors of the pyrgomatids. A similar dense vesicle substructure, although not obvious as beads under light microscopy, occurs in the accessory droplet of the sperm of certain species of *Balanus* (*B. amphitrite*, *B. variegatus*, this account; *B. trigonus*, Kubo *et al.*, 1979), and may be indicative of a relationship of these *Balanus* "groups" (*sensu* Newman & Ross, 1976) to *Armatobalanus*. The group of *Balanus balanus*, in contrast, lacks this beaded arrangement and has a somewhat swollen accessory droplet in which the dense central rod is surrounded by a less dense sheath (Munn & Barnes, 1970b). The accessory droplet of *B. perforatus* is also wide and contains a rod, but is distinguished by a 'crystalloid corpuscle', a spherical body comprised of tubules (Bocquet-Vedrine & Pochon-Masson, 1969; Munn & Barnes, 1970b). These patterns, distinct from each other and from the configuration of the droplet shared between *B. trigonus*, *B. amphitrite* and *B. variegatus*, raise in another form the question posed by Anderson (1981) on the basis of differences in patterns of cirral activity: is the presently recognised genus *Balanus* (*sensu stricto*, Newman 1979) an assemblage of groups with more than one origin among the archaeobalanids?

Newman (1979) recognised the subfamily Megabalaninae, comprising warm water (*Megabalanus*) and austral (*Austromegabalanus*, *Notomegabalanus*) subgroups, but did not comment on possible affinities with other balanids. Newman & Ross, (1976) had previously hinted at a derivation of *Megabalanus* (*sensu lato*; now the megabalanines) from the group of *Balanus perforatus*. Some features of cirral structure and activity also suggest this relationship (Anderson & Southward, 1987). Comparative sperm ultrastructure (Figs 11, 13) reveals a short, wide, featureless accessory droplet in *Megabalanus* (*M. ajax*, *M. tintinnabulum*; this account, Kubo *et al.*, 1979), convergently like that of the archaeobalanid *Acasta*; and a less swollen droplet in *Austromegabalanus nigrescens*,

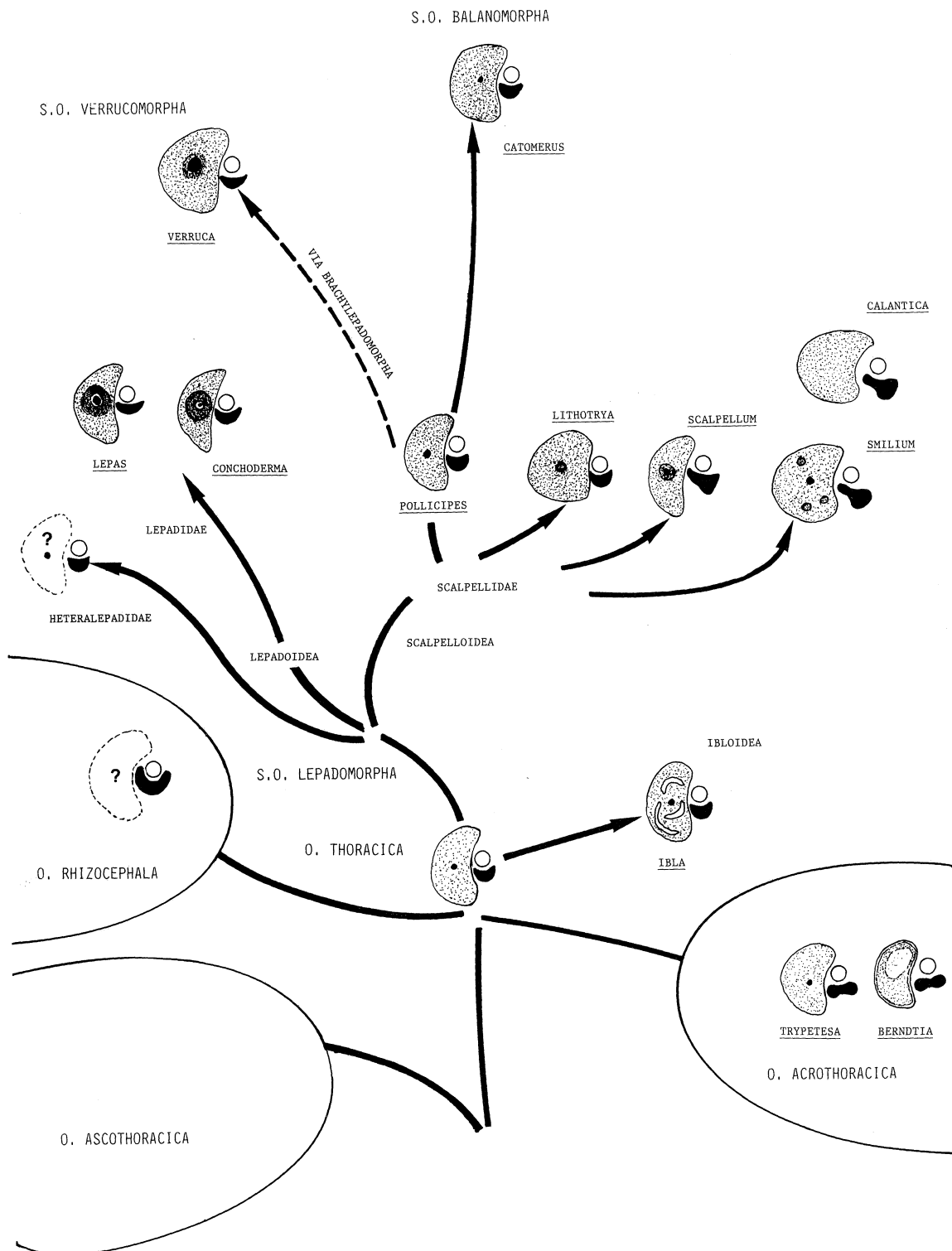


Fig.12. Phylogeny of cirripede sperm. Part 1 — Ascothoracida, Rhizocephala, Acrothoracica and Thoracica (Lepadomorpha). Spermatozoa of the Ascothoracida (see Fig. 1a,b) are relatively unmodified in comparison to the filiform spermatozoa of the Rhizocephala, Acrothoracica and Thoracica. Presence or absence of an accessory droplet in Rhizocephala has yet to be established. Nuclear profiles in Rhizocephala (crescentic) and Acrothoracica (dumb-bell shaped) differ from those of thoracicans (triangular). Among lepadomorpha, lepadids are distinct from scalpellids — the latter showing variation in nuclear profile and droplet substructure in different subfamilies. Verrucomorpha were probably derived via the Brachylepadomorpha from a scalpelloid source.

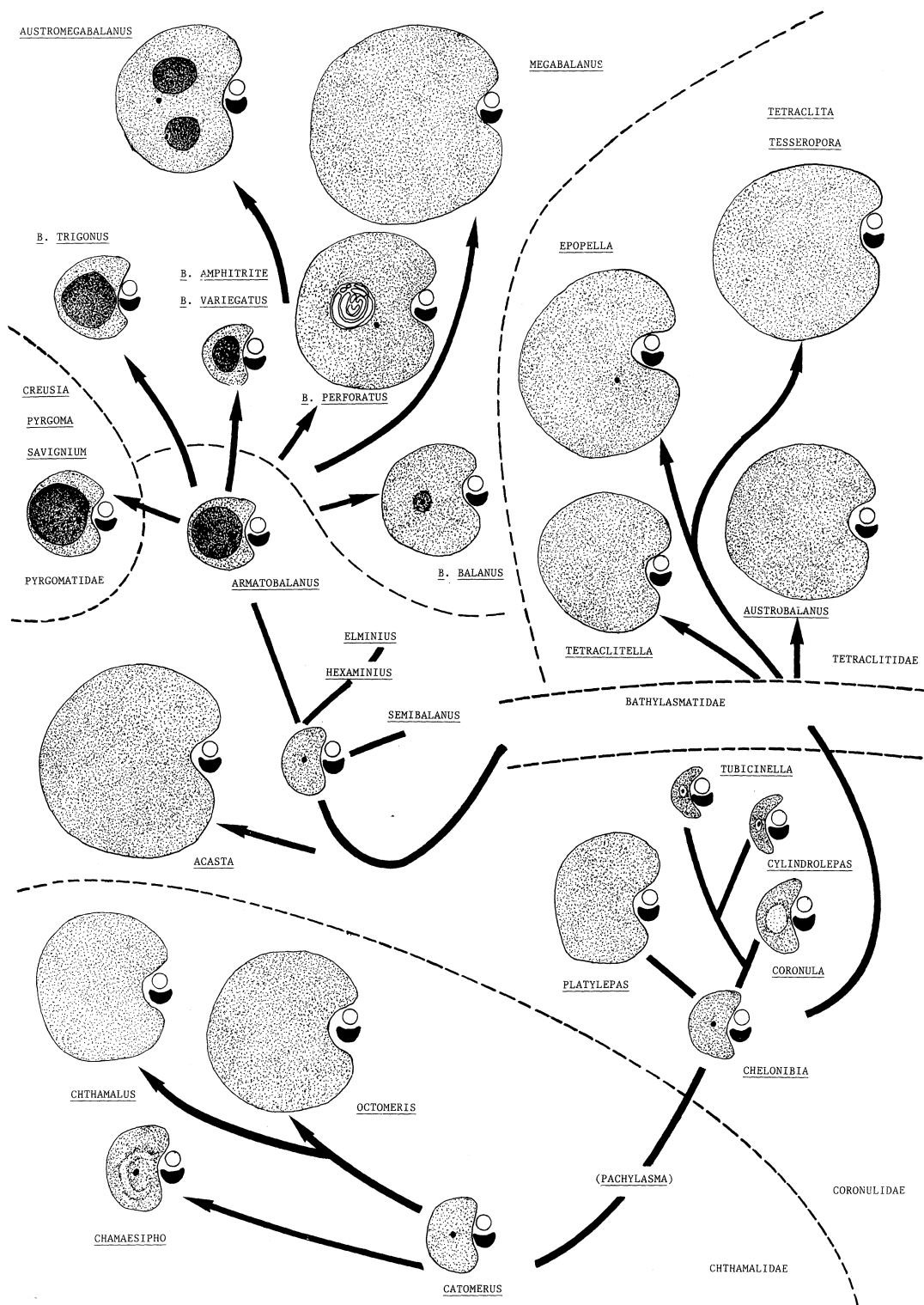


Fig.13. Phylogeny of cirripede sperm. Part 2 — Thoracica (Balanomorpha). The sperm of *Catomerus* represents basic balanomorph sperm structure. Repeated trends toward loss of the rod and increase in diameter of the droplet are evident through the Balanomorpha. The basic sperm type is retained in some coronuloids (e.g. *Chelonia*) and some archaeobalanids (e.g. *Hexaminus*, *Elminius*, *Semibalanus*). Other archaeobalanids show marked divergences in sperm structure, raising the question of whether this is a heterogeneous assemblage. Close links are evident between *Armatobalanus* and the Pyrgomatidae, and with certain balanid taxa. Differences between *Megabalanus* and *Austromegabalanus* are pronounced, suggesting that they may not be closely related to each other.

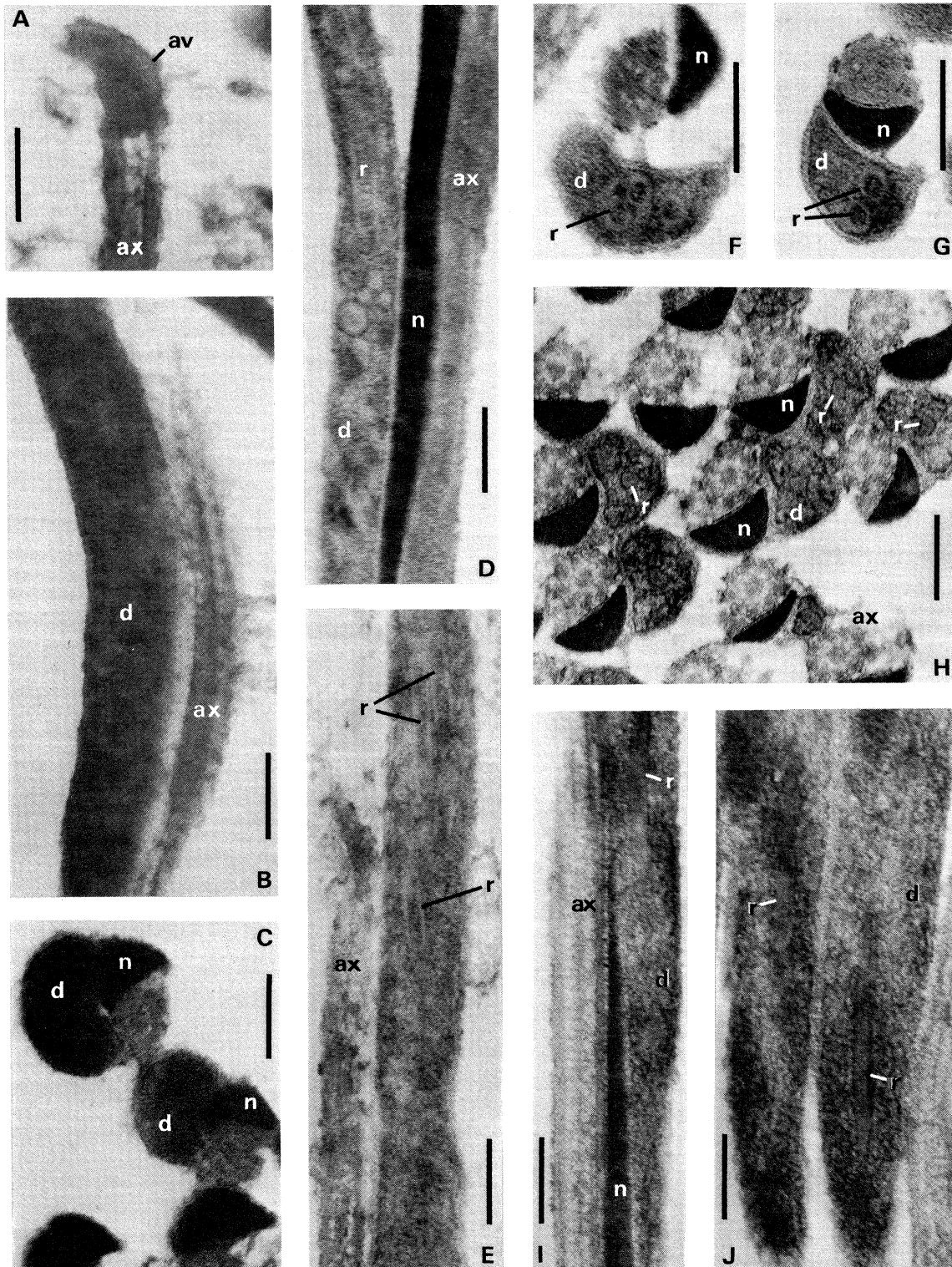


Fig.14. (a — c) *Neolepas zeviniae*. (a) L.S. acrosome and anterior extremity of axoneme. (b) L.S. accessory droplet and axoneme. (c) T.S. showing droplet, axoneme and nucleus of two spermatozoa. (d — g) *Neoverruca brachylepadoformis*. (d) L.S. showing nucleus, axoneme and droplet. (e) L.S. droplet. Two rods and electron-lucent spaces are visible. (f) T.S. droplet, showing five rods, nucleus and axoneme. (g) T.S. droplet with two rods, nucleus and axoneme. (h — j) *Eochionelasmus* sp. (h) T.S. spermatozoa showing droplet (one rod and a fibrous reticulum), nucleus and axoneme. (i) L.S. droplet, nucleus and axoneme. (j) L.S. droplets of two spermatozoa. Scale bars: 0.25 μ m.

with a complex internal substructure of one or two rows of dense vesicles and a separate rod. While a relationship of *Megabalanus* to *B. perforatus* is conceivable on this basis, it seems likely that the austral megabalanines are distinct and perhaps separately derived. Much more needs to be known of all aspects of comparative balanoid biology before the complex phylogenetic evolution of the Balanoidea is fully elucidated.

Addendum

Since submitting this paper for review, we have had the opportunity to examine spermatozoa from the seminal vesicles of three important thoracicans from the fauna associated with hydrothermal vents, *Neolepas zeviniae* Newman, *Neoverruca brachylepadoformis* Newman & Hessler, and *Eochionelasmus* sp. The specimens were kindly provided by Professor W.A. Newman of the Scripps Institution of Oceanography, California, U.S.A. and had been fixed in formalin before transfer to 70% ethanol. The results are illustrated in Fig. 14.

The spermatozoon of *Neolepas zeviniae* has a triangular nuclear transverse profile and an elongate accessory droplet about 0.43 μm wide, containing finely granular, homogenous material (Fig. 14a–c). No rod could be detected in the droplet.

The spermatozoon of *Neoverruca brachylepadoformis* (Fig. 14d–g) also retains a triangular transverse nuclear profile and an elongate droplet about 0.40 μm wide, but the droplet contains several rods, each composed of a dense core and an electron-lucent outer layer.

The spermatozoon of *Eochionelasmus* (Fig. 14h–j) has a similar nuclear profile and elongate droplet, but with only a single, simple rod in the centre of the droplet.

All three species thus exhibit the generalised thoracican nuclear profile. *Eochionelasmus* also retains a generalised droplet substructure, but the droplets in *Neolepas* and *Neoverruca* are interestingly distinctive. If the absence of a rod is not a result of poor fixation, *Neolepas* is divergent in this respect from the generalised condition retained in *Pollicipes* and *Lithotrya*. The multiple, distinctive rods of *Neoverruca* have not been observed in any modern scalpellid, but resemble the specialised single rod in the droplet of *Verruca*.

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available copies of micrographs or rhiizocephalan sperm and spermatids. The Electron Microscope Unit of the University of Sydney provided facilities for the work. We also thank Mrs J.T. Anderson for assistance in collecting and sorting our material. Miss S.F. Warren and Mrs J.T. Anderson typed the manuscript.

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