# Comparative Spermatology of the Crustacea Entomostraca

## 2. Subclass Ostracoda

*By* KARL GEORG WINGSTRAND

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*Abstract*

The structure of the spermatozoa was studied in 72 species of ostracods. This material is regarded as representative for it includes most of the major taxa and 24 of the 46 families usually accepted in modern systems (Table 1).

Ostracod spermatozoa are often said to be well known, with reference to the excellent papers of Gupta (1964, 1968), Zissler (1966-1970), and Reger et al. (1969, 1970). However, these authors describe only a single complicated cell type which is restricted to the superfamily Cypridacea. This type is not representative of the subclass, for other ostracods have completely different spermatozoa.

Actually there are eight very different types of spermatozoa in ostracods. Since these types are easily recognizable and are restricted to well defined taxa in the current system, they can be used as diagnostic features in taxonomy and as arguments at ordinal and family levels in phylogeny. Reliable synapomorphic sperm characters thus support the monophyly of the superfamilies Cypridacea and Cytheracea, and the complex Cypridinidae + Sarsiellidae + Rutidermatidae.

Other sperm types characterize the families Cylindroleberididae, Polycopidae, Bairdiidae and Cytherellidae, but in these cases the phylogenetical conclusions are somewhat weakened by the small number of species examined in each taxon.

Some morphological features show striking variation also at the generic and specific levels, particularly in the Cypridacea and Cytheracea, and can clearly be used for taxonomical work at these levels.

An analysis of the spectacular variation revealed several unknown and unique morphological features, e.g.: 1) in the Halocyprididae the numerous normal mitochondria are all inside the nuclear envelope of mature sperm; 2) in the Cylindroleberididae the small round spermatozoa are all enclosed in "private" vacuoles in unique, syncytial "cytophores"; and 3) unique external "spermatophores" are formed in the furrow of the genital lobes in the females of Cypridinidae, Sarsiellidae and Rutidermatidae, when plastic secretions, deposited by the male during copulation, harden to form a capsule around the sperm mass.

It is very difficult to find homologous characters common to all ostracods, and it is also difficult to reconstruct a morphotype showing the probable sperm type of ancestral ostracods. Spermatology does thus not contribute significantly to discussions on the ancestry of ostracods among the other crustacean groups.

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# Contents

# A. Introduction

The present account of ostracod spermatozoa is the second of a series of papers originally planned to give a survey of the spermatology of the Entomostraca. The first, on the subclass Branchiopoda, was published in 1978 (Wingstrand 1978), and a third paper on the Copepoda has been under preparation for several years, but the material is still far from sufficient for monographic treatment.

After the appearance of the excellent papers by Gupta (1964, 1968), Zissler (1966-1970) and Reger et al. (1969, 1970), the spermatozoa of ostracods are often supposed to be well known. However, the papers deal exclusively with a complicated and unique sperm type which occurs in freshwater Cypridacea but is unknown in other ostracods. This type can therefore not be regarded as representative of the subclass Ostracoda.

As will be shown below, there are eight fundamentally different types of spermatozoa within the Ostracoda, each type being restricted to well defined taxa in the acknowledged system (Figs. 3 and 4). In fact they are so different that homologous features are hard to find and an overall comparison of the types becomes uncertain. I have therefore chosen to treat each of the eight types separately, describing the mature sperm, its spermatogenesis and occurrence in the system, and discussing the phylogenetical and functional aspects when this is possible.

As in my previous monograph on branchiopods, this paper is primarily focussed on the structure and variation of the spermatozoa and the usefulness of the spermatozoa in phylogeny and taxonomy. This approach seemed promising, for ostracod spermatozoa are even more complicated than those of branchiopods (Wingstrand 1978), making convergent evolution less probable. Moreover, the difference from one taxon to another is often striking, making the spermatozoa useful as distinctive characters at some levels in the system.

The idea that ostracod spermatozoa might be useful in systematical work is far from new. Zenker (1854) used spermatozoan structure and dimensions when defining species and when discussing which males and females belong to the same species. He even considered a subdivision of his large genus *Cythere* into two genera on the basis of spermatozoan structure (Zenker 1854:61). Later G. W. Müller used the bulb-shaped swelling on the spermatozoa of *Propontocypris pirifera* to characterize this species and was clearly successful (G. W. Müller 1889:678, 1894:249; compare with chapter 8.3.2 of the present work). And Retzius (1909:14) concluded his chapter on ostracod spermatozoa with a suggestion that their structure could be useful in phylogenetical work.

The functional understanding of ostracod spermatozoa is hampered by the fact that most of them have never been seen moving under the light microscope, even when living material has been used. The spermatozoa of Cypridacea do move but only after they have been transferred to the female during copulation. This unique chance was utilized by myself and others for studies of locomotory organelles, and a summary of the results obtained is included in the chapter on cypridacean spermatozoa (Chapter 8.5).

Slight movements have also been seen in spermatozoa extracted from a few female cytherids, but up to now these movements have not revealed much about the function of the sperm in this family.

In case no movements are known, the function of the organelles can sometimes be deduced from the structural picture and from knowledge of the process of fertilization. This usually requires some information on the genital apparatus. I have therefore

added to each chapter a short summary of what is known about the genital organs, but details are often lacking or are poorly described in the available literature, and my own material is not satisfactory for a systematic account in all groups.

These remarks on the genitalia are partly added as an aid for future work, for I hope that when the present investigation is published, it will stimulate some scientists to study the very variable strategies of fertilization which evidently have developed in different ostracod groups.

## *Literature on ostracod spermatozoa*

### 1. *Historical review*

Ramdohr (1808) was probably the first to see ostracod spermatozoa, for when dissecting "*Cypris incongruens*", now called *Heterocypris incongruens* (Ramdohr), he observed some hairlike structures come out of the sperm duct and straighten themselves out in the water. Ramdohr did not interpret these long filaments as spermatozoa but called them "Härchen" or "Blutgefässe". That these filaments were spermatozoa was first recognized by Wagner (1836), who also made some remarks about their exceptional size.

The fascinating morphology and great size of the spermatozoa in candonids and cypridids made them a favourite object for light microscopists during a hundred years (1850-1950) and also inspired several electron microscopists after 1955. Zissler (1969a) published a very competent review of the older literature, so I refer to him for particulars and restrict myself to a list of references and to comments on some important points:

*Light microscopy:* Ramdohr (1808), Wagner (1836), Zenker (1850, 1854), Lilljeborg (1853), Plateau (1867), Metschnikoff (1868), G. W. Müller (1880, 1884, 1889, 1894, 1927), Stuhlmann (1886), G. O. Sars (1889, 1928), Retzius (1909), Schmalz (1912), Lowndes (1935), Bauer (1940), Hartmann (1955-1968).

*Electron microscopy*: Bradfield (1955), Gupta (1964, 1968), Zissler (1966, 1969a,b, 1970), Tětart (1967), Reger & Florendo (1969a, b), Reger (1970), Baccetti & Afzelius (1976), López-Camps et al. (1979).

Most light microscopical studies and all electron microscopical studies have been made on freshwater Cypridacea, particularly of the genera *Candona, Notodromas, Cypris, Heterocypris, Cypridopsis* and *Cypricercus,* which all have the same type of spermatozoa. In contrast there are only scattered remarks or no information at all on the very different spermatozoa in other families.

### 2. *Literature on the cypridacean families Candonidae, Cyprididae and Cypridopsidae*

Zenker (1854), Stuhlmann (1886), G. W. Müller (1889, 1894) and Retzius (1909) described cypridacean spermatozoa in such detail as could be resolved in the light microscope. They were astonished to find that the spermatozoa were sometimes longer than the animals themselves, usually c. 1 mm long, but sometimes several millimeters. Each spermatozoon was found to consist of a thicker and a thinner piece of about the same length (Fig. 8). The thinner part of some species is shaped like a corkscrew. In the thick part they could find up to five longitudinal filaments coiled around each other.

Opinions differed about the orientation of this spermatozoon. Müller regarded the thin end as the "tail" whereas Stuhlmann thought it was the head end, and Retzius (1909) realized that the arguments were insufficient and left the question open. The problem was still regarded as unsettled in 1976 (Baccetti & Afzelius), although Lowndes (1935) had shown that the spermatozoa move with the slender end first. Of course this criterion is purely functional and could be misleading, for movements can be backwards.

In 1968 Gupta found that an acrosomal vesicle develops at the apex of the thin segment which, accordingly, must be the homologue of the the anterior end of other spermatozoa.

Movements of cypridacean spermatozoa were first seen by Zenker (1854), who described undula-

tions of the "spiral plates" which extend as two parallel helices over the thick part of cypridacean spermatozoa. These undulations were called "ripple movement" by Lowndes (1935), who studied several candonid and cypridid species.

Gupta (1968) tried to correlate these movements with ultrastructure and was of the opinion that the "ripple movement" is generated by wavelike bending of the "contractile organelles" which underlie the undulating surfaces of the spermatozoon. He was supported by Zissler (1969a,b), who used the term "Flügelorgane" for the "contractile organelles". On the other hand, Reger & Florendo (1969) and Reger (1970) believed the pair of long coiled mitrochondria to be the motor producing the ripple movement.

Spermatogenesis of candonid and cyprid spermatozoa was repeatedly studied by the light microscopists (Zenker 1854, Stuhlmann 1886, G. W. Müller 1889, Schmalz 1912), who could register the main sequence of developmental stages. But a final identification of nucleus and mitochondria among the five coiled filaments in the spermatid did not come until the electron microscope was used (Gupta 1964, 1968; Zissler 1966, 1969a,b, 1970; Reger & Florendo 1969a,b). It was shown that the "central filament" of classical authors is the elongated nucleus, containing the perforatorium, and that it extends throughout the spermatozoon.

The formation of two gigantic "Nebenkerne" by fusion of the mitochondria in early spermatids, and the elongation of these "Nebenkerne" into the coiled mitochondrial filaments were confirmed.

Opinions still differ with regard to the "contractile organelles". Gupta and Zissler describe the development of these structures from the endoplasmic reticulum, whereas Reger & Florendo regard them as a modified acrosomal apparatus, because the Golgi complex seems to be involved in their development.

3. *Literature on other families*

G. W. Müller (1894:134) noticed that spermatozoa of the cypridaceans *Macrocypris* (Macrocyprididae) and *Pontocypris* (Pontocyprididae) differ from those of cypridids and candonids with respect to coiling and behaviour of the contractile organelles. The present investigation has shown that he was right.

Spermatozoa of *Bairdia* (superfam. Bairdiacea) were drawn as thin filaments, without detail, by G. W. Müller (1894:pl. 38:3).

Spermatozoa of the superfamily Cytheracea have previously only been studied with the light microscope, e.g., by Zenker (1854), who published drawings and brief comments. However, I doubt that he had found mature spermatozoa in "*Cythere gibba*" (= *Cytherura gibba* (O. F. Müller), for his drawing of the sperm has little in common with the spermatozoa of this species, which are long and extremely thin filaments in my photographs (unpublished).

Retzius (1909) made some beautiful drawings of cytheracean spermatozoa, showing that they are filamentous and consist of two regions. His picture (1909:pl. 3) of the sperm of *Loxoconcha impressa* fits well with scanning pictures taken during the present investigation (Fig. 5; Pl. 18:114). His figure of the spermatozoa of "*Cytheridea dentata*" (now *Cyprideis sorbeyana* (Jones)) is certainly also good but shows that he had confused some species in his material. Actually the spermatozoon drawn is that of *Xestoleberis aurantia* (Baird), which has very characteristic spermatozoa (Fig. 5). This also fits with the fact that Retzius had taken his material in the Gullmar Fjord on the Swedish west coast, where *Xestoleberis aurantia* is common but *Cyprideis sorbeyana* never found (see Elofson 1941). The two species are superficially similar but certainly are not closely related.

Very little is known of the spermatozoa of the myodocopid ostracods. G. W. Müller (1894:129) states that *Cylindroleberis* (Cylindroleberididae) has simple round spermatozoa, wheres those of *Cypridina* (Cypridinidae) are said to be round with a long tail (actually the acrosome). Also Sars (1928) observed that the spermatozoa of *Cypridina* (now *Vargula*) are small nucleated cells with a hairlike appendage.

TABLE 1

*Systematic position of ostracods investigated*

System according to Hartmann & Puri (1974)

Genera and species are listed in each chapter

| | Genera examined | Species examined |
|---|---|---|
| *Order Myodocopida* | | |
| *Suborder Myodocopa* (4 fam.) | | |
| Fam. Cypridinidae | 4 | 5 |
| Fam. Cylindroleberididae | 2 | 3 |
| Fam. Sarsiellidae | 1 | 3 |
| Fam. Rutidermatidae | 1 | 1 |
| *Suborder Halocypriformes* (4 fam.) | | |
| Fam. Halocyprididae | 1 | 2 |
| *Suborder Cladocopa* (1 fam.) | | |
| Fam. Polycopidae | 1 | 1 |
| *Order Podocopida* | | |
| *Suborder Platycopa* (1 fam.) | | |
| Fam. Cytherellidae | 1 | 1 |
| *Suborder Metacopa* (1 fam.) | – | – |
| *Suborder Podocopa* | | |
| *Superfam. Bairdiacea* (2 fam.) | | |
| Fam. Bairdiidae | 1 | 1 |
| *Superfam. Cytheracea* (25 fam.) | | |
| Fam. Leptocytheridae | 1 | 3 |
| Fam. Limnocytheridae | 2 | 2 |
| Fam. Cytherideidae | 1 | 1 |
| Fam. Krithidae | 1 | 1 |
| Fam. Trachyleberididae | 1 | 5 |
| Fam. Hemicytheridae | 1 | 2 |
| Fam. Loxoconchidae | 3 | 4 |
| Fam. Cytheruridae | 2 | 7 |
| Fam. Xestoleberididae | 1 | 5 |
| Fam. Paradoxostomatidae | 2 | 7 |
| *Superfam. Terrestricytheracea* (1 fam.) | – | – |
| *Superfam. Darwinulacea* (1 fam.) | – | – |
| *Superfam. Cypridacea* (6 fam.) | | |
| Fam. Macrocyprididae | 2 | 2 |
| Fam. Ilyocyprididae | 1 | 1 |
| Fam. Pontocyprididae | 4 | 4 |
| Fam. Candonidae | 4 | 5 |
| Fam. Cyprididae | 4 | 4 |
| Fam. Cypridopsidae | 2 | 2 |
| Total | 44 | 72 |

Numbers in parentheses indicate the number of recent families described in each suborder or superfamily

G. W. Müller (1894) figured spermatozoa of *Chonchoecia* (Halocyprididae) as long filaments, and Hartmann (1955, 1968) described the sperm of *Polycope* (Polycopidae) as 15-45 µm long rods, but both these descriptions, based on light microscopy, lack details.

## *Material and methods*

The choice of material was partly dictated by the availability of the species, but in general I tried to get a fair representation of all the major taxa. Table 1 indicates the systematic positions of the 72 species examined for sperm structure. The system used is that of Hartmann & Puri (1974).

Actually more than twice as many species were caught, fixed, identified and examined, but many had to be discarded for different reasons: some were immature, parthenogenetic, poorly fixed or were damaged when I determined the genus and species. The list shows only the species which were identified with reasonable certainly to the genus and species level and which gave acceptable electron micrographs.

Of the six recent suborders, only the Metacopa (genus *Saipanetta*) is lacking (Table 1). These minute ostracods are only known from samples of mud from the SW Pacific (McKenzie 1967a,b; Hartmann & Puri 1974). The lack of material of the superfamily Darwinulacea is also regrettable, but up to now male specimens of *Darwinula* have only been mentioned by Brady & Robertson (1870) and have not to my knowledge been found later. My abundant Danish material consists only of parthenogenetic females.

The fixatives used for most material were:

1 % $OsO_4$, with veronal acetate (Palade 1952), in some cases with bicarbonate or 0.1M cacodylate buffer, pH 7.4.

2 % $OsO_4$, with 0.1M cacodylate buffer, pH 7.4.

3-A – Trialdehyde fixative according to Kalt & Tandler (1971). For marine animals the 3-A fixative was made up on a mixture of sea water and distilled water (2:1).

Fixation was at 4-10 °C for 1-2 hours. When osmium fixatives were used the animals were dissected as soon as they stopped moving in the fixative. 3-A fixative worked excellently on intact specimens, probably because of the rapid penetration, but also because this fixative causes relaxation of the muscles so the valves tend to open. All aldehyde material was postfixed in 2 % $OsO_4$ with 0.1M cacodylate for 1-2 hours.

Before osmification and embedding, the valves were more or less completely removed, except in species in which the testicles are situated in the mantle fold under the valves (Cyprididae, Candonidae). However, removal of the valves was not always necessary, for prolonged stay in several changes of buffer (0.1M cacodylate with 0.1M sucrose, pH 7.4) decalcifies also some heavily mineralized valves.

Embedding in Epon, ultramicrotomy, contrasting the grids with uranyl acetate and lead citrate, and microscopy with a Zeiss EM 9S or Zeiss 109 followed standard procedures. 1-2 µm epon sections stained with toluidine blue were used for light microscopical control.

In many cases plankton samples or mud samples with animals were fixed directly in the 3-A mixture and preserved for several weeks in buffer. Good results were still obtained in many cases even if the sample could not be kept at 4 °C all the time. This was particularly useful when material was collected by other people at distant localities. In such cases, to avoid deterioration of the 3-A fixative before use, premeasured portions of the components were mixed in the field immediately prior to fixation.

Preparation of spermatozoa for SEM was difficult, for the few spermatozoa obtained each time are often lost if centrifugation procedures are tried when media are changed. For this reason a few drops of water with living extended spermatozoa were pipetted into a 2-3 cm piece of dialysis tubing, which was clamped with plastic clips at

both ends. The tube was fixed for some hours in 3-A, washed for 2 days in several changes of buffer, osmified in 2 % $OsO_4$, and washed in buffer for 2 days again. The plastic clips were then exchanged with metal clips resistant to benzene, and the tube was transferred through graded alcohols to some changes of pure analytical quality benzene. The benzene-soaked tube was then frozen at −25 °C and dried at −10 °C in vacuum (method originally from Nørrevang & Wingstrand 1970). The dry tube with adhering spermatozoa was then cut into pieces which were mounted on SEM stubs, coated with carbon and gold and examined in a Cambridge scanning electron microscope.

The procedure was useful for the present purpose but the dialysis tube was a somewhat unstable support and tended to break when the electron beam was too intense.

# B. Results

## TYPE 1. CYPRIDINIDAE, SARSIELLIDAE AND RUTIDERMATIDAE

### 1.1. *Material*

Fam. Cypridinidae

Subfam. Cypridininae

*Vargula norvegica* (Baird). – Korsfjorden, Espegrend, Norway, May 1975, ♂♂ + ♀♀, fix. Karnowsky (1965), coll. A. Andersson, Lund. – Korsfjorden, Espegrend, Norway, 400 m, 30.IX.1975, ♂♂ + ♀♀, 3-A, 2 % $OsO_4$.

*Gigantocypris agassizii* Müller. – Eastern Pacific Ocean, Oct. 1973, 1 ♂, 1 ♀, Osmium fixation, coll. N. Holland, Scripps Institution, La Jolla, California.

Subfam. Philomedinae.

*Philomedes globosus* (Lilljeborg). – Kattegat, N. of Zealand, 2.XI.1972, ♀♀, 1 % $OsO_4$, coll. H. Lemche, Copenhagen.

*P. lilljeborgi* (Sars). – Skagerrak, 220 m, 21.III.1973, 1 ♀, 1 % $OsO_4$, coll. R/V Dana, stat. 16206.

*P. paucichelata* Kornicker. – Outside Bellair's Research Inst., Barbados, 36 m, 26.III.1976, 4 ♂♂ + 5 ♀♀, 3-A, coll. J. Just.

Fam. Sarsiellidae

*Sarsiella multispinosa* (Poulsen, 1965). – Thailand. 22.II.1982, ♀♀, 3-A, coll. J. Just.

*Sarsiella* sp. 2. – Outside Bellair's Research Inst., Barbados, 15-20 m, 27.III.1976, ♂♂ + ♀♀, 3-A, coll. J. Just. The species is similar to *S. sculpta* Brady (see Kornicker 1958) and *S. costata* Kornicker, but I had to give up final identification because of some differences.

*Sarsiella* sp. 3. – Outside Bellair's Research Inst., Barbados, 36 m, 26.III.1976, 1 ♂, 3-A, coll. J. Just. This specimen is similar to *S. carinata* Scott (see Kornicker 1958) but the endopodite of the ♂ 2nd antenna is somewhat different.

Fam. Rutidermatidae

*Rutiderma dinochelata* Kornicker. – Barbados 1978, ♀♀, 3-A, coll. J. Just. Only ♀♀ were caught, but some of them had well-preserved spermatophores attached to the genital lobes.

These three families are similar with regard to sperm structure and mechanism of sperm transfer. It is therefore practical to treat them together.

### 1.2. *Genital organs*

*Male.* The testicles are simple sacs lying on each side of the posterior intestine (Sars 1928:pl. 3). The two vasa deferentia fuse into one sperm duct before opening on an unpaired penis papilla behind the 7th pair of legs. The penis papilla is surrounded from each side by limblike copulatory appendages. These look like the penes of podocopid ostracods but are not penetrated by the sperm duct. The homologies of these external genital parts are still uncertain (G. W. Müller 1894:129, Skogsberg 1920:58, Weygoldt 1960:429, Hartmann 1968:415).

*Cypridina stellifera* (Claus) is said to be different, with testicles situated far anteriorly and below the heart (Claus 1873:212). The paired copulatory appendages are also variable, especially in *Sarsiella*, where they may be strongly asymmetrical and may fuse to a single rod as in *S. capsula* (G. W. Müller 1894:130, pl. 4; Hartmann 1968:268).

In the males examined (of *Vargula norvegica, Sarsiella* spp., *Gigantocypris agassizii*) the vasa deferentia are wide and function as a vesicula seminalis, for they are filled with mature spermatozoa together with some secretions which stain deeply with toluidine blue. Also the unpaired sperm duct next to the gonopore may be enlarged and contain spermatozoa and secretions (in *Spinacopia,* see Kornicker 1969:fig. 2). The secretions probably come from the wall of vas deferens, but in *Gigantocypris agassizii*

I observed a large flat gland, also observed by Lüders (1909:139), lying along the vas deferens.

In *Vargula norvegica* the spermatozoa are oriented perpendicular to the axis of the vas deferens and are parallel, with the head end in the same direction in each duct. This was not so distinct in the other species.

The compact spermatozoa and the secretions in the vas deferens become very hard during fixation so ultrathin sections of this part were difficult to cut.

*Female.* The two ovaries are flat sacs lying on each side of the intestine in the posterior part of the body above the furca. The two oviducts are narrow. In *Vargula norvegica* they may be difficult to follow because of their delicate walls, but according to Ramsch (1906:393) they remain paired to the genital openings, which are situated on each side just anterior to the anus.

An elongated "genital lobe" (Kornicker 1976) or "Genitalhöcker" (Claus 1873), often with some bristles on the medial end, extends laterally from the gonopore region on each side (Pl. 1:1). Each genital lobe is divided into two ridges by a distinct furrow (Pl. 1:1). The ridges look like potential homologues of the copulatory appendages of the male.

This homology is uncertain, but the function is clear. The genital lobes receive the spermatophores deposited by the male during copulation. Some females of all examined species had hard spermatophores attached to the genital lobes, usually one on each side. In some cases, as in a specimen of *Philomedes globosus*, the spherical, hard spermatophore was almost hidden in the furrow (Pl. 1:2). The lips of the furrow had not really fused over the spermatophore but were close together. In other specimens the spermatophore protruded from the gaping furrow like a hemisphere (Pl. 1:3, 4), and in several specimens of *Vargula norvegica*, one of *Gigantocypris agassizii* and one of *Sarsiella multispinosa* the furrow was wide open and the spermatophore was more external, attached to the female body only at the bottom of the furrow.

Some spermatophores were so hard and impermeable that embedding and sectioning were impossible. They could then be seen as small shining spheres in the epon blocks. The surrounding capsule of these spermatophores is noncellular, often very thick, irregular and droplike on one side (Pl. 1:3-4). It is not continuous with the cuticle of the female but is in contact with it, at least in the bottom of the furrow (Pl. 1:4). The capsule stains deeply with toluidine blue, like the secretions around the spermatozoa in the vas deferens of the male.

These observations support the interpretation of G. W. Müller (1927:428-429), where he accepts the opinion of Ramsch (1906) that the capsules are spermatophores, not walls of a receptaculum as was usually maintained (Hartmann 1968:456).

According to G. W. Müller, the secretions of the vas deferens together with sperm are injected as a plastic droplet into the furrow on the genital lobe of the female and may be pressed out through the more or less open cleft before the secretions harden and form the spermatophore capsule. Müller's idea fits with the fact that the capsule's shape fits perfectly in the space it occupies in the furrow on the genital lobe (Pl. 1:2-4). Müller suggests that the genital appendages of the male can be bent to form a tubular structure guiding the ejaculate and securing the injection on both sides during copulation.

Of course it must be supposed that the spermatozoa can be liberated from the spermatophore and fertilize the eggs coming out of the oviduct. I failed to find preformed openings of the spermatophores in my few continuous section series so I find it likely that the capsules can be enzymatically dissolved to let the sperm out. Kornicker (1969:fig. 2m) has drawn a spermatophore from his light microscopical sections of *Spinacopia*, which may show how the liberation takes place, but I have not completely understood the picture.

Some spermatophores in my material contain only disintegrating spermatozoa, but a number of spermatophores of the species *Philomedes globosus*, *P. lilljeborgi*, *Sarsiella* spp. and *Rutiderma dinochelata*

contain well preserved spermatozoa similar to those in the testicle of the male. In some cases, however, the hard capsule made it difficult to obtain good ultrathin sections.

It should be recalled that female *Philomedes globosus* and *P. lilljeborgi* probably preserve viable sperm for a very long time. Copulation probably takes place during a relatively short planktonic period. After that the young females lose their swimming bristles and live permanently in the mud on the bottom. The living spermatozoa in the spermatophores must be sufficient to fertilize the eggs produced by the females throughout the rest of their life, for repeated copulations seem to be excluded (Elofson 1941:402ff.).

The presence of external spermatophores deposited and hardening on the surface of the female during copulation indicates a unique and unparalleled type of sperm transfer in the families Cypridinidae, Sarsiellidae and Rutidermatidae. The evidence is good for all three families for I have seen such external spermatophores in sections of the following species: *Vargula norvegica, Gigantocypris agassizii, Philomedes globosus, P. lilljeborgi, P. pauci-chelata, Sarsiella multispinosa, Sarsiella* sp. 2, and *Rutiderma dinochelata*. More evidence comes from the drawings and SEM pictures in Kornicker's papers (1969-1976), which show such spermatophores in many sarsiellids and cypridinids:

Cypridinidae: *Vargula hamata* (1975:fig. 85), *Metavargula adinothrix* (1975:fig. 66), *Skogsbergia costai* (1974:fig. 3), *S. squamosa* (1974:fig. 2), *Doloria levinsoni* (1975:fig. 50), *D. septenaria* (1975:fig. 55), *Paradoloria dorsoserrata* (1976:fig. 17), *Siphonostra hallex* (1975:fig. 73).

Sarsiellidae: *Sarsiella capsula* (1974:fig. 12), *Spinacopia sandersi* (1969:7, fig. 2), *Cymbicopia brevicostata* (1975:fig. 398), *Adelta theta* (1975:fig. 403).

There is no evidence for the presence of such external spermatophores in other families of ostracods. A similar formation of spermatophores is supposed to occur in bairdiids (Podocopida) (G. W. Müller 1894, Hartmann 1968:480-481), but in bairdiids the spermatophore is very different and is found inside the female in a well developed receptaculum seminis, and the spermatozoa inside are completely different.

### 1.3. *Mature spermatozoa*

Mature spermatozoa were studied in the testicles and vesicula seminalis of males and in spermatophores attached to females. The hard secretions usually made ultramicrotomy problematic, but the spermatozoa of 7 species including representatives of Cypridinidae, Sarsiellidae and Rutidermatidae could be analyzed in detail. The material of *Gigantocypris agassizii* and *Sarsiella multispinosa* had to be partly given up and only some single sections were used.

All 9 species examined have spermatozoa of the same basic type, containing a round nucleus, a large spear-shaped acrosomal apparatus and normal scattered mitochondria with cristae (Fig. 1). There is no trace of a flagellum or of axonemal structures, and the mitochondria do not form Nebenkerne or other large complexes.

The total length of these spermatozoa is 10-12 μm in *Philomedes globosus* and *Sarsiella* spp., 40 μm in *Rutiderma dinochelata* and 50-60 μm in *Vargula norvegica*. The spermatozoa of *Gigantocypris agassizii* are even larger than those of *V. norvegica*, but I could not isolate single spermatozoa and can therefore not record the length with certainty.

The nucleus is rounded or oval, often with a depression on the anterior side where the acrosomal apparatus makes contact (Fig. 1). In *V. norvegica* there is instead a furrow on the surface of the nucleus for the proximal end of the perforatorium, which passes around the nucleus to its posterior side. The nuclear content is usually homogeneously granular but in *V. norvegica* there are scattered light spaces (Fig. 1:A; Pl. 3:19).

The most characteristic structure in these spermatozoa is the long acrosomal rod (perforatorium), which is the main supporting element in the acrosomal apparatus. Its proximal (posterior) end is in contact with the nucleus whereas the anterior

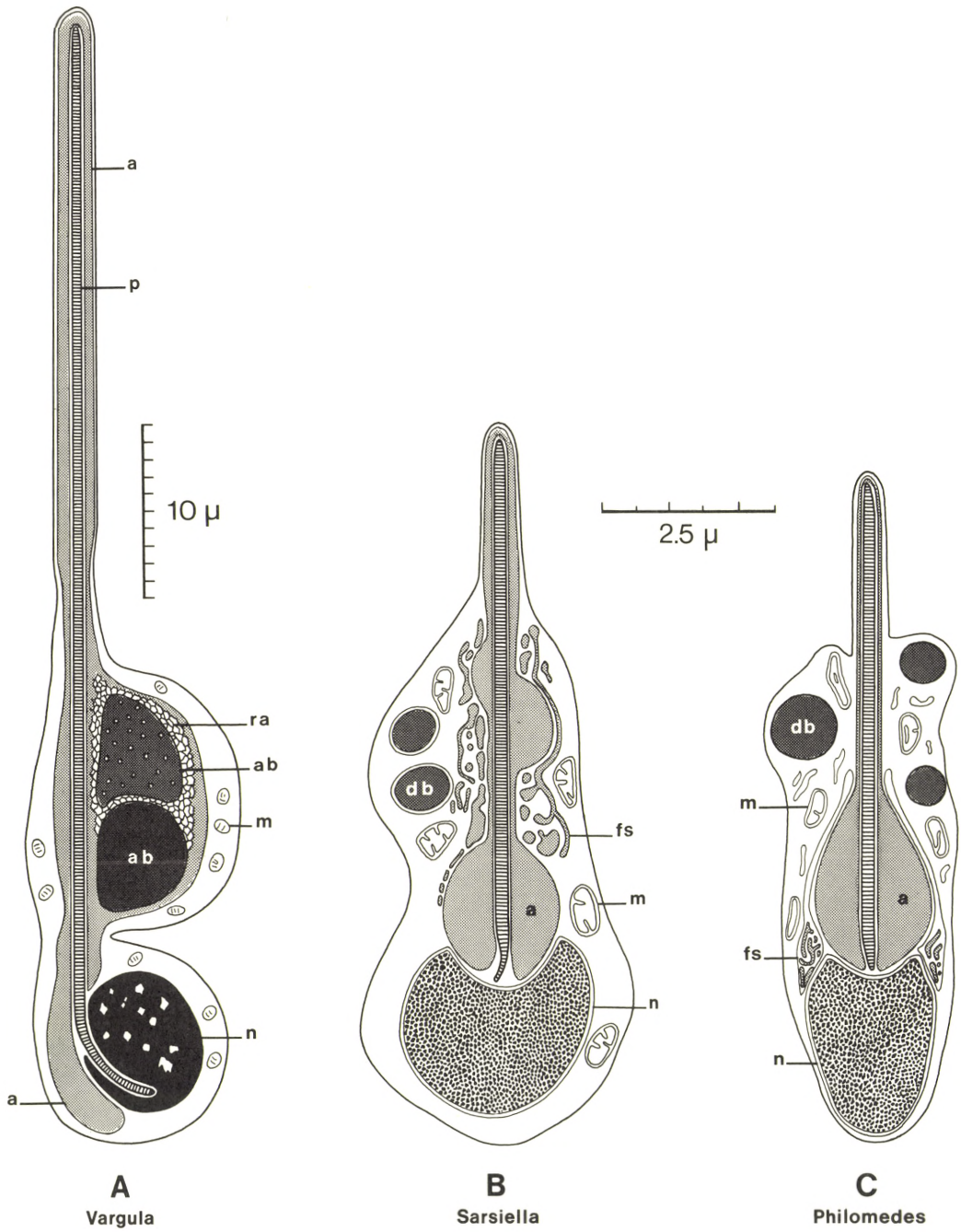



Fig. 1. Diagrams of spermatozoa. A. *Vargula norvegica*, B. *Sarsiella* sp. 2, and C. *Philomedes paucichelata*. Combined from TEM pictures of spermatozoa in testicles and vesiculae seminales. Compare with Pls. 2 and 3.

*Legends*: a = acrosomal vesicle, ab = acrosomal bodies, db = dark bodies surrounded by membrane, fs = flat irregular sacs with dark contents around acrosome, m = mitochondria, n = nucleus, p = perforatorium, ra = reticulate outer zone of acrosomal bodies.

end extends forwards as an axis in the acrosomal apparatus, throughout its length surrounded by the acrosomal vesicle. The latter appears to have been invaginated from behind and is strongly elongated, so it covers the entire perforatorium, also its anterior point, with double membranes (Fig. 1).

In all species examined, *Gigantocypris agassizii* included, the perforatorium has a crystalline structure, visible in longitudinal sections as a longitudinal or transverse striation, in cross sections as a transverse striation or square pattern (Pl. 2:8; Pl. 3:13-16). The appearance obviously depends on the angle of the plane of the section in relation to the three-dimensional lattice of the crystalline matter.

The acrosomal vesicle is simplest in *Philomedes globosus* and *Rutiderma dinochelata*: a voluminous sac with a simple unit membrane all around, containing a granular matter with light or medium contrast (Fig. 1:C; Pl. 1:6; Pl. 2:8). The vesicle is best developed, i.e., broadest, where it is in contact with the nucleus. The perforatorium obviously develops in the space between the nucleus and the acrosomal vesicle, invaginating the latter and forming a cap-shaped covering on its distal end. When the perforatorial rod grows further anteriorly the acrosomal cap remains on its top and elongates correspondingly, so the rod is covered all the way by the two walls of the acrosomal vesicle (Fig. 1). The narrow anterior extension of the acrosomal vesicle is surrounded by a diminutive amount of plasm and by the plasma membrane of the cell surface.

In *Sarsiella* spp. the acrosomal vesicle is more irregular, with dilatations also from the distal part. In *Vargula* and *Gigantocypris* it forms very large dilatations in front of the nucleus (Fig. 1:A). The dilated parts contain one or two large and dense bodies of condensed intraacrosomal matter, called "acrosomal bodies". These are not limited by membranes but often have a superficial zone with loose reticulate structure (Pl. 2:11).

Many normal-looking mitochondria with distinct cristae are scattered in the cytoplasm around the nucleus and the basal part of the acrosome (Fig. 1; Pl. 2:7, 12). In *Philomedes globosus* and *P. lilljeborgi* some mitochondria are packed together in a deep pocket in the nucleus, formed by invagination of the nuclear membrane (Pl. 2:12). Other plasmatic organelles observed in *Philomedes paucichelata* and *Sarsiella* sp. 2 are membrane-bound globules with dark contents and some branching and anastomosing sacs, also with dark contents (Fig. 1:B and C; Pl. 2:7, 8). Such irregular dark sacs sometimes fill out the furrow between the acrosomal vesicle and the nucleus (Fig. 1:C; Pl. 2:8).

### 1.4. *Spermatogenesis*

The material of *Vargula norvegica* is large enough to give a general picture of the spermatogenesis, but the other species give only scattered pieces of information.

In *Vargula norvegica* the spermatogenesis from the early spermatid stage to nearly mature sperm is dominated by the acrosomal apparatus, the components of which fill out the cells and tend to obscure the picture (Pl. 3:17, 18).

Development of the acrosomal vesicle differs from that reported for most animals. Late spermatocytes and early spermatids contain numerous small Golgi stacks scattered in the large cell body, which in early spermatids of *Vargula norvegica* measures about 30 μm in diameter. The Golgi stacks pinch off small vesicles with dark contents, and such "proacrosomal vesicles" coalesce to form larger vesicles in which the contents differentiate to form a central dark body and a light rim, sharply separated from each other (Pl. 3:17). These characteristic vesicles with a dark central body dominate throughout much of the spermatogenesis and slowly increase in size. Finally all the vesicles coalesce, forming a single large acrosomal vesicle with a diameter of about 10 μm. This vesicle becomes attached to the nucleus.

The perforatorium was first seen as a dark granular body in the cleft between the nucleus and the acrosomal vesicle. In *Vargula norvegica* the primordial body grows out to form a rod but does not

immediately invaginate the acrosomal vesicle. Instead it develops along the surface of the nucleus to the prospective posterior side, throughout its length covered by a diverticulum of the acrosomal vesicle (Pl. 3:19). Finally the opposite end of the rod grows anteriorly to form the long spear, invaginating the large acrosomal vesicle and transforming it to the cap seen in Fig. 1:A.

The cytodifferentiation of the acrosome begins quite early, before the 2nd meiotic division is completed. Many spermatids in which the chromosomes are still contracted (the beginning of anaphase) contain numerous Golgi stacks and proacrosomal vesicles, all with a dark central body (Pl. 3:18). Such vesicles are also abundant at the time when the new nuclear membrane is formed after the nuclear division.

The origin of the acrosomal vesicle from the Golgi stacks is an argument for the homologization with the typical acrosomal vesicle in other animals, and its attachment to the nucleus and relation to the perforatorium make this homology obvious. But the origin of the vesicle from numerous Golgi stacks in *Vargula norvegica* is different from that in most other animals, in which it is formed on the concave side of a single Golgi apparatus. The same development as in *Vargula norvegica* with many Golgi stacks and long-lived and numerous proacrosomal vesicles was described in the lamellibranch *Spisula solidissima* by Longo & Anderson (1969).

It can be added that the large and numerous proacrosomal vesicles were observed also in spermatids of *Gigantocypris agassizii*, *Philomedes paucichelata* and *Sarsiella* spp., but the material was not sufficient for a complete description of the entire spermatogenesis.

The mitochondria in all species examined remain unchanged during the development.

## 1.5. *Comments*

The spermatozoa of Cypridinidae, Sarsiellidae, and Rutidermatidae are all of the same general type and can in practice be distinguished morphologically from those of other ostracods. The long, crystalline perforatorium and the exceptionally enlarged and extended acrosomal vesicle are the most striking morphological features. These organelles are usually present also in other ostracods. The characteristics evolved in cypridinids, sarsiellids and rutidermatids are mainly quantitative and are hardly complicated enough to support a strong case for synapomorphy.

The simple round nucleus and the scattered normal mitochondria are not informative, for both occur in other ostracods and other crustaceans.

The particular development of the acrosomal vesicle from numerous Golgi stacks and proacrosomal vesicles seems to be characteristic of the three ostracod families but has also been observed outside the ostracods in the bivalve *Spisula*.

The most convincing synapomorphic feature of cypridinids-sarsiellids-rutidermatids is the remarkable "spermatophores" which harden and get their shape on the surface of the female, in the furrow on the genital lobe. The original sperm droplet ejaculated by the male is obviously plastic and contains some secretions which harden and form the rigid walls after transfer to the females. This is not found in other groups and I suggest that the combination of this type of sperm transfer with the particular morphological type of the spermatozoon is a reasonable argument for a monophyletic origin of the three families within the ostracods.

Among most other ostracods the sperm is transferred in the usual way while suspended in a fluid. In bairdiids some spermatophorelike baskets are believed to be formed in a similar way to that described above, but they are formed inside the female in a regular receptaculum seminis and contain spermatozoa of a completely different type. In cylindroleberidids the reduced spermatozoa become included in soft "cytophores" which are transferred to the female receptaculum, but these "cytophores" are unique structures formed as syncytia of the plasm bodies of the spermatids and are not comparable to the "spermatophores" in cypridinids, sarsiellids and rutidermatids.

## TYPE 2. CYLINDROLEBERIDIDAE

### 2.1. *Material*

Subfam. Cylindroleberidinae

*Parasterope muelleri* Skogsberg. – Outside Bellair's Research Station, Barbados, 36 m, 26.III.1976, ♂♂ + ♀♀, 3-A, coll. J. Just.

*P. corrugata* Poulsen. – Same locality, 26.III.1976, 1 ♂, 1 ♀, 3-A, coll. J. Just.

Subfam. Cycloleberidinae

*Asteropella mortenseni* Poulsen. – Same locality, 26.III.1976, ♂♂ + ♀♀, 3-A, coll. J. Just.

### 2.2 *Genital organs*

*Male.* The male genital organs of cylindroleberidids are not well known. In my sections I found the paired compact testicles on each side behind the middle of the body, between the posterior intestine and the body wall. They are actually difficult to identify in non-serial sections, for spermatozoa and spermatids are not very characteristic and look like ganglion cells or glandular cells in the light microscope (Pl. 5:26). Much material was destroyed because I identified the testicles too late to shift to ultramicrotomy. But, of course, the presence of meiotic chromosome plates makes it possible to identify the testicles with certainty also in case the other features are atypical (Pl. 4:21).

G. W. Müller (1894:121 and Pl. 5:41) found that both vasa deferentia are dilated to form vesiculae seminales before they fuse and form the unpaired sperm duct. These observations were made on *Cylindroleberis oblonga* Grube. Müller also depicts fairly independent copulatory appendages flanking the male genital opening in this species, whereas Sars (1928:16) talks of "almost wholly confluent copulatory appendages" in *Asterope mariae* (Baird), a species which seems to be identical with *Cylindroleberis oblonga* Grube (see Skogsberg 1920:518 and Kornicker 1974:40-41).

*Female.* The female genital organs differ from those of cypridinids, sarsiellids and rutidermatids in important respects. Cylindroleberidids have a paired subcutaneous receptaculum seminis on each side. This was described by G. W. Müller for *Cylindroleberis oblonga* and I can confirm his description for *Parasterope muelleri* and *Asteropella mortenseni*, of which I have abundant material. The receptacula are situated deep in the tissue on each side of the anus and are simple sacs, lined on the luminal side by a thick cuticle (Pl. 4:22-25; Pl. 5:31). Müller's (1927:418) idea that the receptaculum is derived from an invaginated furrow of the type found on the genital lobe of *Vargula* is thus possible or even reasonable.

Each receptaculum is connected with the outside by two ducts, a somewhat wider one and a narrow one. According to G. W. Müller the wide duct serves the entrance of the spermatozoa during copulation, whereas the narrow duct is a fertilization canal, opening in or near the oviduct aperture. In my sections both ducts are lined by a thick cuticle (Pl. 4:22-25).

No indications of hard spermatophores were seen in my fairly numerous section series of *Parasterope* spp. and *Asteropella mortenseni*. Round dark spermatozoa enclosed in vacuoles in the syncytial plasm of "cytophores" were common in the vesiculae seminales of males and in the receptaculum of females (Pl. 4:22-24; Pl. 5:31). In *Parasterope muelleri* the plasm of the cytophore is probably stripped off during the stay in the receptaculum, for it seems to be disintegrating, and in the fertilization duct of this species I have seen only naked spermatozoa which lack the cytoplasmic encasing (Pl. 4:25).

### 2.3. *Mature spermatozoa*

It was mentioned by G. W. Müller (1894:129) that *Cylindroleberis* has simple round spermatozoa. This could be verified for *Parasterope* spp. and *Asteropella mortenseni* in my material, but the structural background is more complicated than indicated by Müller.

*Parasterope muelleri* and *P. corrugata*. The spermatozoa as seen in the vesiculae seminales of males and the receptaculum seminis of females are round or oval, 2-3 μm in diameter. They consist almost exclusively of a strongly condensed nucleus with dark, homogeneous contents (Fig. 2:B; Pl. 4), covered on the surface by the normal double nuclear envelope and an extra plasma membrane which has formed secondarily (see spermatogenesis). Some cytoplasm would be expected between the nuclear envelope and the plasma membrane but little or none remains in mature sperm. When sometimes present in young spermatozoa, this cytoplasm is restricted to small patches on the nuclear surface. Two typical centrioles are sometimes preserved in this cytoplasm in the spermatids.

Both in males and females these spermatozoa occur in groups of four, each group being enclosed in a "cytophore", a roundish body of syncytial cytoplasm formed by fusion of the four plasmatic bodies of the spermatid quartet during spermatogenesis (Pl. 4:20, 22).

In *Parasterope* spp. these cytophores contain large open vacuoles in which the spermatozoa are floating freely (Pl. 2:22). The four vacuoles in each cytophore are often irregular and sometimes communicate with each other. The plasm of the cytophores contains normal mitochondria with cristae (Pl. 4:24) and is crowded with smaller vesicles, but the outer surface of the cytophore is well defined by a plasma membrane.

The syncytial cytophores still surround the spermatozoa in the female receptaculum, although some disintegration of the plasm is seen in the female duct system. In the receptaculum some spermatozoa may even be in direct contact with the cuticular lining, showing that they are no longer completely surrounded by the cytophore (Pl. 4:24). Completely naked spermatozoa were seen in the narrow "fertilization duct", in which no intact cytophores were found. Therefore it is concluded that cytophores with sperm quartets are transferred to the female during copulation, that the cytophore disintegrates and is stripped off in the receptaculum, and that the spermatozoa are naked when they pass out of the fertilization duct and fertilize the eggs (Pl. 4).

*Asteropella mortenseni*. Spermatozoa of *A. mortenseni* show some differences but the general morphology is the same as in *Parasterope* spp. The spermatozoa of *A. mortenseni* are larger, 5-6 μm, more lobulated and more closely packed within each cytophore (Pl. 5:28, 31). There are no open vacuoles in which the spermatozoa float. In fact the flat vacuoles, which in *Parasterope* spp. surround the sperm nuclei, isolating them from the surrounding plasm, are present also in *A. mortenseni*, but here their walls remain closely attached, covering the nuclear envelope from the outside. The space between the two walls, which in *Parasterope* widens and forms the large vacuole, remains narrow and is filled by a thin lamella of dark matter (Pl. 5:29, 31). In *A. mortenseni* the normal nuclear envelope is thus surrounded by two secondary membranes with some dark matter between them (Pl. 5:29, 31). As in *Parasterope* spp. there is practically no plasm between the nuclear envelope and the double external cover, but two centrioles have been seen in this space in some young spermatids (Pl. 5:29, inset).

The cytophore is also different from that of *Parasterope*. In *A. mortenseni* the plasm of the cytophore is more homogeneous, containing some large mitochondria with cristae and scattered small vesicles (Pl. 5:28, 30). A system of tubular endoplasmic reticulum extends into the peripheral plasm of the cytophore and communicates with the surface. In the receptaculum of the female the picture of the cytophore changes: all small vacuoles, mitochondria and other organelles are packed around the four spermatozoa forming a darker body of "endoplasm". In contrast, the "exoplasm" remaining peripherally under the distinct plasma membrane is light and is also well defined (Pl. 5:31).

No naked spermatozoa were seen in females of *A. mortenseni* so it is not known whether the cytophore is cast off in this species before the spermatozoa enter the fertilization duct and fertilize the eggs.

## 2.4. *Spermatogenesis*

Spermatogenesis could be followed in the fairly abundant material of *Parasterope muelleri* and *Asteropella mortenseni*.

In both genera the testicles appear compact, filled with germ cells and with a germ zone with meiotic figures at one end. Meiosis and development up to the early spermatid stage look quite ordinary and were not studied in detail.

Early spermatids are simple cells with a large vesicular nucleus, normal mitochondria and some ribosomes. They are more or less distinctly grouped together in fours, each quartet probably being the result of two meiotic divisions of a single primary spermatocyte (Pl. 4:20; Pl. 5:26, 27).

Spermatogenesis is characterized by three simultaneous processes:

1) the coalescence of the plasm of the four spermatids of each quartet to form the cytophore;
2) the formation of flat membranous sacs which spread around each nucleus and isolate it from the surrounding plasm;
3) strong successive condensation of the nucleus.

In *Parasterope muelleri* the spermatid cytoplasm is strongly vesicular with very complicated vacuoles having light contents. Some of these vesicles spread around the nucleus. In somewhat more advanced spermatids these vesicles have fused around the nucleus, which becomes isolated in a large vacuole to which several different vesicles contribute.

The complicated course of vacuoles and membranes makes this process difficult to follow in detail, but it is obvious that the vesicles, when fusing around the nucleus, contribute to the new cell membrane forming outside the nuclear envelope.

The fusion of the plasm of the four spermatids in each quartet must imply fusion and partial reduction of the cell membranes separating them, but this process could not really be followed. One can see that early spermatids in a quartet with large vesicular nuclei are separated by clear cell limits, although sometimes interdigitating in a confusing way. In contrast, more advanced quartets with contracted nuclei lie in a common plasmatic body with an uninterrupted surface membrane and a separate vacuole for each of the four nuclei (Pl. 4:22).

In the vesicula seminalis of *Parasterope* the cytophores with spermatozoa inside float in a fluid which contains numerous dark globules of different size.

*Asteropella mortenseni* is easier to analyze than *P. muelleri* as the spermatids and the cytophores are larger and not so vesicular.

The fusion of the plasm of the four spermatids could thus be followed clearly. Early spermatids with large vesicular nuclei are separated by distinct and smooth cell walls (Pl. 5:26, 27). More advanced spermatids with condensed nuclei have fused and formed cytophores without any cell separation (Pl. 5:28).

The development of the extra walls outside the nuclear envelope could be followed step by step. First a number of flat sacs are seen in the plasm around the nucleus (Pl. 5:27). Unlike conditions in *Parasterope muelleri*, these sacs have no open lumen. Their opposite walls are close together, separated by a lamella of dark amorphous matter (Pl. 5:29). The sacs are intimately related to the Golgi stacks, which are numerous in these spermatids (Pl. 5:29). The sacs become attached to the nuclear envelope and fuse, forming a double investment outside it (Pl. 5:30).

## 2.5. *Comments*

The spermatozoa of the three species of cylindroleberidids described are maximally simplified, consisting only of the haploid nucleus derived from the spermatid, a nuclear envelope and one or two "secondary plasma membranes". All other organelles usually found in spermatozoa are lacking (acrosome, perforatorium, axonema, mitochondria, etc.). The designation of these bodies as spermatozoa is justified by the fact that they are transferred to the female and must be supposed to fertilize the eggs.

The term "cytophore" is used in the present text for the syncytial body containing the spermatozoa. It is realized that it is not homologous with typical cytophores in other animals. In cylindroleberidids the spermatozoa are contained in vacuoles in the plasm of the "cytophore", not attached to the outside as in typical cytophores in annelids and molluscs.

Sperm transfer by injection of such "cytophores" with spermatozoa in their vacuoles is unique among the ostracods, and so is the extremely reduced state of the spermatozoa. The "cytophore" obviously serves as a vehicle for spermatozoa during copulation and disintegrates in the receptaculum of *Parasterope muelleri*, so the spermatozoa become free before they fertilize the eggs. Whether such disintegration of the "cytophore" takes place also in *Asteropella mortenseni* is not known.

With only three species investigated it may well be optimistic to claim that their shared features characterize the family Cylindroleberididae as a whole. But the examined genera *Parasterope* and *Asteropella* represent both subfamilies accepted within the family, thus suggesting that the specialized spermatozoa and sperm transfer may be useful arguments for keeping the cylindroleberidids together. This would support the classical synapomorphy of the group: The presence of a paired series of multiple abdominal gills.

Both the extreme reduction of the spermatozoa and the sperm transfer by means of "cytophores" appear to be typical examples of "substitution" (Wirth 1984), i.e., a phylogenetical process by which completely new features are introduced without any indication of the original (plesiomorphic) state.

It is usually believed that cylindroleberidids are related to cypridinids, sarsiellids and rutidermatids. The spermatological features neither support nor weaken these ideas about relationships to other ostracods. The strong reduction of the spermatozoa and the specialized "cytophore" may have evolved from spermatological states in most ostracod groups. The absence of mitochondrial Nebenkerne in these families is a poor piece of evidence, since nothing indicates that this is an apomorphic state.

## TYPE 3. HALOCYPRIDIDAE

### 3.1. *Material*

*Conchoecia elegans* Sars. – Koster, Bohuslän, Sweden, 230 m, 3.VI.1975, 2 ♂♂, many ♀♀, 2 % $OsO_4$. – Raunafjord, S. of Bergen, Norway, 240 m, 31.V.1976, 1 ♂, 3-A. – Skagerrak, 300-600 m, Jan. 1978, many ♂♂ + ♀♀, 3-A, Coll. J. Just.

*C. borealis* Sars. – Korsfjorden, S. of Bergen, Norway, 670 m, 29.IX.1975, 2 ♂♂, many ♀♀, 3-A.

### 3.2. *Genital organs*

*Male.* The male genital organs of halocyprids were described by Claus (1890, 1894), G. W. Müller (1894, 1927), Sars (1928) and Hartmann (1968:416).

The paired testicles are situated in the posterior end of the male, dorsal to the intestine in the recurved abdomen. Each testicle is connected with a vas deferens which is expanded and forms a vesicula seminalis. The vasa deferentia meet and form an unpaired sperm duct which enters the penis.

The penis consists of two components: a thinner rod containing the sperm duct and a thicker flattened lobe which partly encloses the former (G. W. Müller 1894:figs. 19, 20). Although Claus

and G. W. Müller agree that the penis is asymmetrical, developed only on one side, they place it on opposite sides of the midline (Claus 1890:32, left side; G. W. Müller 1894:130, right side; Sars 1928, left side; see also Skogsberg 1920:573).

The testicle is largely compact, with the lumen restricted to the end where the vas deferens is emitted. Each testicle is filled with densely packed lobules of germinal cells arranged so that the early spermatogenic lobules are in the periphery, and the mature spermatozoa are released from the mature lobules near the lumen. All cells in one lobule are at the same developmental stage, and this greatly facilitates the analysis of spermatogenesis. There is no distinct barrier or wall between the lobules, each of which is defined by having all germ cells in the same developmental stage.

*Female*. Female halocypridids have a single receptaculum seminis in front of the furca, near the ventral side of the body. It is a simple or two-lobed sac, buried below the body wall and connected with the outside by two ducts, one on each side. The right duct is believed to serve as a vagina during copulation; the left one opens into the terminal part of the oviduct and is probably used when the spermatozoa approach the eggs for fertilization (G. W. Müller 1894:152, Hartmann 1968:457).

The receptaculum is lined by a cuticle. The filamentous spermatozoa are found free in the lumina of the vesicula seminalis and receptaculum seminis. Spermatophores or cytophores were not seen.

### 3.3. *Mature spermatozoa*

Mature spermatozoa from the vesicula seminalis of both species are filiform; those of *Conchoecia borealis* (65 µm long) are a little shorter than those of *C. elegans* (80 µm).

Two distinct regions of the spermatozoa can be distinguished: an anterior thinner region (0.35 µm thick), containing complicated acrosomal structures, and a posterior region, about 1 µm thick, dominated by the bandlike nucleus which contains intranuclear mitochondria (Fig. 2; Pl. 6:34-36).

The anterior region is only 9 µm long. It begins with a "claw" which has a slight dorsal bend and is approximately 1 µm long (Fig. 2; Pl. 6:32).

"Dorsal" and "ventral" as used for orientation in cross sections of the spermatozoa are purely descriptive terms and may perhaps be used in different ways for spermatozoa in other ostracods, for the directions they indicate are not clearly homologous. In the *Conchoecia* sperm, "dorsal" is the side marked by the concavity of the claw and "ventral" is the side where the nucleus approaches the plasma membrane.

*The anterior region* begins with the claw, which contains the remnants of the acrosomal vesicle. Originally, in spermatids, the acrosomal vesicle is supposed to be an open bladder, but at an early stage it is invaginated from behind by the perforatorium and forms a double-walled cup. In mature spermatozoa the two membranes forming the walls of this cup are so close together that the original lumen of the acrosomal vesicle is hardly visible, and the double wall is in close contact with the plasma membrane. Actually the acrosomal cup is wrapped around the point of the perforatorium from the dorsal side and the fusion of its lateral walls in the ventral midline is incomplete, except near the point proper (Fig. 2; Pl. 7:38).

The *claw* is the acrosomal region in the strict sense, containing the remnants of the acrosomal vesicle. The region behind it to the level of the anterior end of the nucleus could be called the "postacrosomal region", for it contains some dark amorphous rods which begin in the acrosomal vesicle or behind it and extend backwards to the anterior end of the nucles (Fig. 2). These dark rods therefore appear to be the homologue of the perforatorium in other animals.

The most characteristic structures developed in the postacrosomal region are:

1) *A central rod* with a light cylindrical core and thick dark walls. It begins in the tip of the claw and extends backwards to the oblique anterior end of the nucleus, where it tapers and ends (Fig. 2; Pl. 6:32, 33).

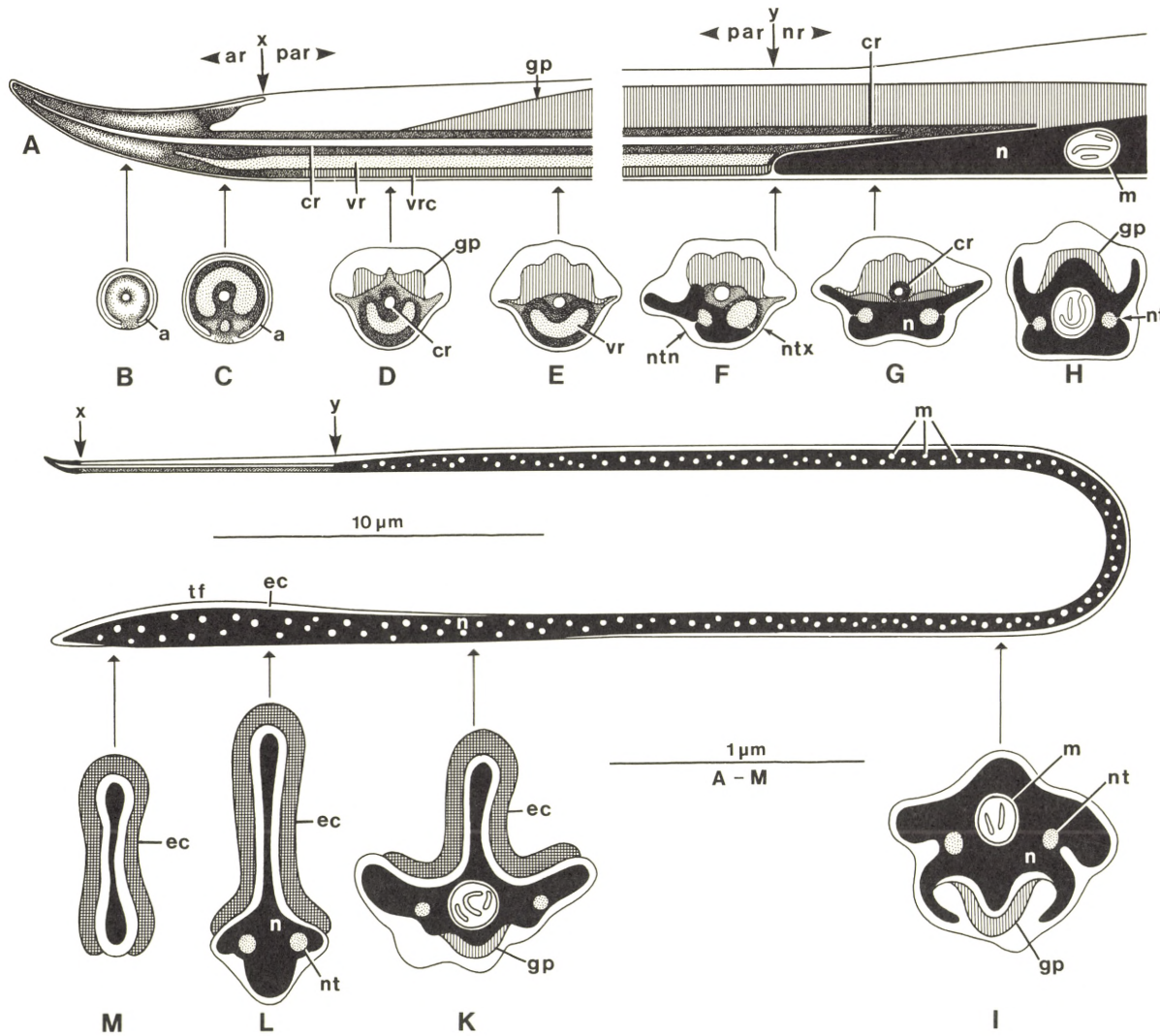



Fig. 2. Diagrams of spermatozoon of *Conchoecia borealis*. Combined from light microscopical pictures of whole spermatozoa and TEM pictures of cross and longitudinal sections. Compare with Pls. 6 and 7. For further details see text.

Scale different for whole sperm (10 µm) and for figures A-M (1 µm).

*Legends*: a = acrosomal vesicle, ar = acrosomal region ("claw"), cr = central rod, ec = extracellular coat, gp = dorsal granular plate, m = mitochondria, n = nucleus, nr = nuclear region, nt = intranuclear tubules, ntn = light core of intranuclear tubule, surrounded by nuclear matter, ntx = corresponding light tubule on the opposite side, surrounded by postacrosomal matter, both derived from core of ventral rod, par = postacrosomal region, tf = tail fin, vr = ventral rod, vrc = ventral crystalline wall of ventral rod, X = limit between acrosomal region (claw) and postacrosomal region, Y = limit between postacrosomal region and nuclear region.

2) *A ventral, U-shaped rod,* situated ventral to the central rod and partly surrounding it. This has a flattened U-shaped light core and dark walls, which are partly confluent with those of the central rod. Its walls have a crystalline structure, seen as a cross-striation with 200 Å periodicity (Pl. 6:32, inset). The ventral rod begins in the posterior part of the claw. At the level of the anterior end of the nucleus its light core is divided into two rods which are wedged into the nucleus and can be followed as the light cores of the "intranuclear tubules" to near the posterior end of the spermatozoon (Fig. 2; Pl. 6).
3) *A plate of granular matter,* covering the dorsal side of the central rod in the postacrosomal region and continuing on the dorsal side of the nucleus in the posterior region. This plate has a paired or triple origin just behind the claw and is coarsely granular. It has an even and marked dorsal contour but real unit membranes are not involved (Fig. 2; Pl. 6, 7). Similar matter forms several dorsal and lateral ridges from the rod complex in the postacrosomal region of immature spermatids (Pl. 7:41).

*The posterior region* is dominated by the long, band-like nucleus which can be followed into the extreme posterior end of the spermatozoon (Fig. 2; Pl. 6). In spermatids the nuclear envelope is typical, consisting of two membranes with a perinuclear space between. In mature spermatozoa the perinuclear space is difficult to see except where it is in contact with the granular plate. Here the perinuclear space is enlarged and filled by dark amorphous matter (Pl. 6:34, 36).

The chromatin is moderately condensed, appearing as granules or filaments.

Three features make this nucleus unique:
1) It contains hundreds of scattered normal mitrochondria.
2) It contains a symmetrical pair of "intranuclear tubules" which appear to be part of the postacrosomal apparatus.
3) The "dorsal granular plate" of the postacrosomal region extends also in the dorsal midline above the nucleus and, more remarkably, the perinuclear space in contact with the granular plate is enlarged and contains a bandlike lamella of very dark amorphous matter (Pl. 6:34, 36).

The *mitochondria* are spherical, 1.3-1.7 μm in diameter, with a normal double membrane and typical cristae (Pl. 6:36). There are hundreds of them, but the precise number has not been counted. They are evenly distributed along the entire nucleus. It should be noted that the mitochondria are truely inside the nuclear envelope and are surrounded only by the two mitochondrial membranes, the inner one forming the cristae. If the mitochondria had been folded into the nucleus from the plasm outside the nuclear envelope one would expect some extra membranes around them. As described in the following, the mitochondria invade the nucleus during the 2nd meiotic division, when the nuclear envelope is absent.

The two *intranuclear tubules* are symmetrically placed and extend as straight cylindrical tubes just under the nuclear envelope throughout the nucleus of the mature spermatozoon (Fig. 2; Pl. 6:34). The wall of the tubules is a double membrane like the nuclear envelope, so it appears possible that the light core of the tubules has sunk into a longitudinal fold of the nuclear surface from an original extranuclear position. This interpretation is actually supported by late stages of spermatogenesis, where the two light rods are seen lying in more or less open furrows on the nuclear surface (Pl. 7:40). In later stages the nuclear envelope fuses over the furrow, forming a closed tubule in which the light core lies. However, the outer membrane of the tubules remains in contact with the inner nuclear membrane also in mature spermatozoa (Pl. 6:34, 35; Pl. 7:40).

It can thus be concluded that the light core of the tubules has no wall of its own; the double wall is supplied by the nuclear envelope. The light cores themselves are to be regarded as posterior continu-

ations of the U-shaped rod seen in the postacrosomal region. The light flattened core of this rod bifurcates when the tip of the nucleus is approached and each resulting light cylinder penetrates into the nucleus and continues backwards in the center of an intranuclear tubule. The bifurcation of the light core can be seen in somewhat oblique sections through the anterior end of the nucleus. In Pl. 6:33 the U-shaped light rod is divided into two; one is still surrounded by postacrosomal matter (ntx) whereas the other is inside the nucleus and is surrounded by the dark and distinct nuclear membranes (ntn). See also Fig. 2:E.

The *dorsal granular plate* is a thick band of coarsely granular matter of medium contrast covering the dorsal aspect of the central postacrosomal rod and the nucleus (Fig. 2). The plate begins just behind the "claw" and is continuous to a point about 10 μm from the posterior end of the spermatozoon. The plate is not separated from the surrounding plasm by any regular trilaminar unit membrane but its contour is nevertheless quite distinct (Pl. 6:33-36; Pl. 7:41; Fig. 2).

The nuclear envelope in contact with the granular plate is specialized in a characteristic way. The two membranes of the envelope part and the cleft between them is filled by a lamella of very dark, amorphous matter. That this lamella is situated in the perinuclear space is sometimes diffucult to see in mature spermatozoa, but it can be seen in some cases when the resolution of the membranes is particularly good (Pl. 6:36) and in spermatids (Pl. 7:40).

The *extracellular coat* is restricted to the finlike posterior end of the spermatozoon, where it covers the plasma membrane in the ventral part of the cross section (Fig. 2; Pl. 6:35). Near the posterior end where the nucleus has the shape of a vertical plate, the coat extends also dorsally in cross sections and covers the spermatozoon all around. The coat is about 0.08 μm thick and has an amorphous appearance. No such coat was found in sections of sperm from the receptaculum of the female.

### 3.4. *Spermatogenesis*

The study of spermatogenesis in *Conchoecia* spp. was focussed on some events which are important for the interpretation of the remarkable structures in the mature sperm.

*Mitochondria.* Secondary spermatocytes are large cells with a round nucleus and numerous small, rod-shaped mitochondria scattered in the cytoplasm. When these cells enter 2nd meiotic division and the nuclear envelope disappears, all mitochondria are dislocated to the center of the cells and are found attaching to the contracted chromosomes (Pl. 7:42, 43; Pl. 8:44). They remain attached to the chromosomes through ana- and telophase and are thus incorporated in the nucleus when the new nuclear membrane is formed (Pl. 8:45). This envelope is first seen as flattened sacs of endoplasmic reticulum, which spread around the prospective nucleus and fuse to form a continuous double wall (Pl. 7:43). In spermatids the mitochondria are thus located in the nucleoplasm, inside the nuclear envelope (Pl. 8:45).

During spermatogenesis the shape of the mitochondria changes from long and rod-shaped in spermatocytes and early spermatids to round or slightly oval in late spermatids and mature spermatozoa (cf. Pl. 7:43 and Pl. 6:34, 36). The mitochondria remain in the nucleus and become evenly scattered when the nucleus extends as a long rod or band during subsequent spermatogenesis. No mitochondria were ever seen outside the nuclear envelope after the 2nd meiotic division.

*Acrosomal complex.* The acrosomal vesicle and the postacrosomal matter are first seen in early spermatids after the new nuclear membrane has been formed. A large Golgi complex is always present nearby, but it is variable and has no constant spatial relation to the nucleus and the acrosomal vesicle. This makes identification of the early acrosomal vesicle problematic.

It is possible that the primordial acrosomal vesicle is round, as in most other animals. Such vesicles were seen but could not be recognized with certain-

ty because the spatial relation to the Golgi apparatus is so variable. The earliest acrosomal vesicles identified with certainty are therefore fairly advanced, about 0.4 μm in diameter, and each is invaginated as a double-walled cup around the top of a perforatorium (Pl. 7:37, 38). The earliest acrosomal vesicles lie near the nuclear membrane, and the perforatorium inside is short and poorly defined, protruding from the opening of the cup to the surface of the nuclear envelope. The acrosomal vesicle is actually wrapped around the top of the perforatorium from the dorsal side and does not cover it completely in the ventral midline (Pl. 7:38, 41).

At this stage the nucleus becomes drop-shaped, its anterior pointed end being parallel with the short perforatorium. Later the perforatorium grows out rapidly in front of the nucleus, with the small acrosomal vesicle on its end, pushing the vesicle into the extreme anterior end of the elongating sperm, away from the nucleus (stage shown in Pl. 7:37). Here the vesicle makes contact with the plasma membrane all around, elongates and bends slightly dorsally (Pl. 7:39).

The space between the "claw" thus formed and the anterior end of the nucleus is the prospective postacrosomal region, which contains a thick string of dark amorphous matter. This thick string appears to be formed by addition of amorphous material to the originally formed perforatorium.

The postacrosomal organelles (central rod, ventral U-shaped rod, dorsal granular plate) develop in the thick string of amorphous postacrosomal material. The organelles seem to form by simple condensation of material, and the process provides little information about their phylogenetic origin.

In addition to the rods and plates already mentioned the postacrosomal matter forms two pairs of very distinct longitudinal ridges, which are quite constant in intermediate stages of spermatids (Pl. 7:41). These ridges disappear before final maturation (Pl. 6:33).

*The posterior region.* The elongation of the nucleus begins when it attains drop-shape, with a slender anterior process parallel with the postacrosomal rod. The anterior process of the nucleus does not keep pace with the growth of the perforatorium, so a postacrosomal region develops between the "claw" and the anterior end of the nucleus (Pl. 7:37). The bulk of the nucleus is then drawn out and remodelled to form the complicated cross sections shown in the mature sperm (Fig. 2; Pl. 6:34, 35).

Some amorphous postacrosomal matter extends behind the nuclear tip and spreads along the dorsal side of the nucleus (Pl. 7:40), condensing as the dorsal granular plate. When it appears this plate is stratified (Pl. 7:40 gp). The membranes of the nuclear envelope under this plate are separated, and a dark lamella of amorphous matter fills the enlarged perinuclear space (Pl. 7:40; Pl. 6:34, 36).

The development of the intranuclear tubules could be followed step by step. First a pair of dark strings can be seen symmetrically attached to the anterior end of the nucleus. These strings can be followed forwards to the postacrosomal region and are continuous with the dark matter there. In cross sections of early stages these strings can be seen as amorphous tufts attached to the outside of the nuclear envelope (Pl. 7:40, inset). Later the nuclear envelope is folded inwards under each string, forming a furrow into which the string sinks down (Pl. 7:40). Each furrow becomes deeper and is expanded as a regular cylindrical tubule which at first communicates with the extranuclear plasm through a narrow slit. Finally the outer nuclear membrane fuses over the slit, forming a closed tubule, whereas the inner nuclear membrane, although fusing in a similar way, tends to remain in contact with the (outer) membrane of the tubule (Pl. 7:40; Pl. 6:34, 35).

The contents of each tubule, originally a dark string, become light during further development. The continuity of the core of the tubules with the U-shaped ventral rod in the postacrosomal region could only be clearly seen in mature stages (Pl. 6:33).

### 3.5. *Comments*

The filiform spermatozoa of *Conchoecia* spp. differ from those of all other ostracods in the presence of numerous normal mitochondria in the nucleoplasm, i.e., inside the normal nuclear envelope. No doubt this is a unique feature which can be used as an autapomorphy of the genus *Conchoecia*. If the character is present also in other halocypridids and in *Thaumatocypris* it is a good synapomorphy of the order Halocypriformes, but this is unknown so far.

The paired symmetrical intranuclear tubules of *Conchoecia* seem to be another good apomorphy, but similar dark strings emitted from the postacrosomal or acrosomal region in spermatids in some Podocopa (Cyprididae) could be homologous, although their fate is different.

The "claw" with the acrosomal vesicle and the long postacrosomal region and perforatorium bears some resemblance to the structure of *Cytherella* and some Podocopa, but in most features *Conchoecia* is unique.

The *Conchoecia* sperm does not seem to share significant features with the sperm of other Myodocopa except for the absence of Nebenkerne and other mitochondrial complexes, but for reasons mentioned in Chapter C this character is doubtful as a phylogenetic argument.

The filiform spermatozoa of *Conchoecia* are probably transferred to the female in the usual way, suspended in a fluid. There are no spermatophores or cytophores, and the sperm when transferred to the female has the same structure as it had in the male, with the possible exception that the extracellular coat on the posterior end has disappeared. No movements of the spermatozoa were seen although I examined live specimens of both sexes.

## TYPE 4. POLYCOPIDAE

### 4.1. *Material*

*Polycope orbicularis* G. O. Sars. – Koster, Bohuslän, Sweden, 80-120 m, 2.VI.1975, ♂♂ and ♀♀, 2 % $OsO_4$.

### 4.2. *Genital organs*

*Male.* The male genital organs have been described in different species of *Polycope* by G. W. Müller (1894:233-239), Sars (1928), Klie (1938) and Hartmann (1955, 1968). The latter also describes the inner organs. The testicle is an unpaired sac with paired, anteriorly directed "horns", lying above and on the sides of the anterior intestine, above the furca. The germinal layer is mainly present in the "horns" of the testicle and is compact (Hartmann 1968:418). Areas with more advanced spermatogenesis have a follicular structure with the spermatids forming an epithelium along one side of the follicular lumina (Pl. 9:51). The follicles are separated by delicate walls of flattened cells (Pl. 8:46). Mature follicles open into larger lumina which communicate with the unpaired vas deferens. This is enlarged as a vesicula seminalis and opens on the unpaired penis, which is on the left side and is supported by specializations of the left furcal ramus.

*Female.* As described by Hartmann (1968:448-456), the ovary and the oviduct are unpaired, developed only on the left side. Also the large receptaculum is unpaired. It is connected with the outside by a vagina situated on the right side. A fertilization duct leading to the terminal part of the oviduct is said to be present in some species.

The spermatozoa are free in the receptaculum and no spermatophores have ever been seen.

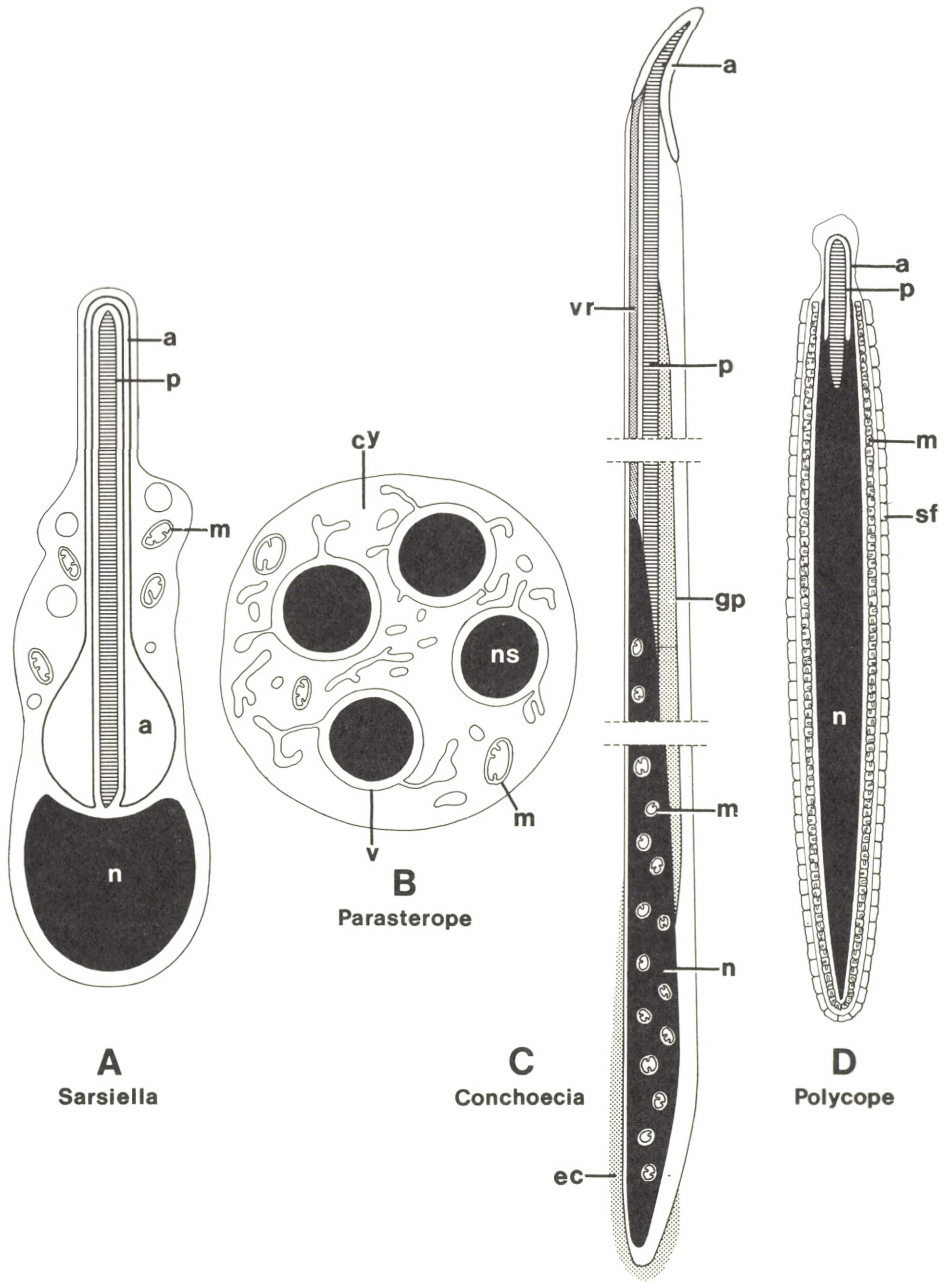



Fig. 3. Simplified diagrams of myodocopid spermatozoa. A. *Sarsiella* sp. 2, B. *Parasterope* sp., C. *Conchoecia borealis*, D. *Polycope orbicularis*. Proportions and relative size partly arbitrary.

*Legends*: a = acrosomal vesicle, cy = cytophore, ec = extracellular coat, gp = dorsal granular plate, m = mitochondria, n = nucleus, ns = spermatozoon, i.e., nucleus with a few membranes (in B), p = perforatorium (in C = central rod), sf = superficial folds of plasma membrane (in D), v = vacuoles in the cytophore, in which the spermatozoa float (in B), vr = ventral rod.

### 4.3. *Mature spermatozoa*

Hartmann (1955, 1968) remarks that the spermatozoa of the species he examined in the light microscope are small rods, measuring between 45 µm (*Polycope dispar*) and 15 µm (*P. cancellata*).

In my material of *P. orbicularis* the spermatozoa are needle-shaped and somewhat flattened. Cross sections measure about 0.4×1.3 µm. The posterior end is flattened out to form a finlike tail, about 0.2 µm thick (Pl. 8:50). The anterior end is a short acrosomal region, about 0.8 µm long, containing the acrosomal vesicle (Fig. 3; Pl. 8).

This slightly flattened needle-shape is characteristic of the spermatozoa both in the vesiculae seminales of males and in the receptaculum of females (Fig. 3; Pl. 8:46-50). No twisting of the spermatozoa or coiling of contained structures was ever seen. The most obvious differences seen in spermatozoa transferred to the female receptaculum are increased condensation of the nucleus and progressive disintegration of the original spermatid cell body, which attaches to the acrosomal end.

The core of the band-shaped body of the sperm is the long and flattened nucleus, the contents of which condense to longitudinal filaments (Pl. 8:46-50). In mature spermatozoa the filaments are so densely packed that they are difficult to resolve, and after transfer to the females the nucleus looks almost compact and black (Pl. 8:49).

Along each margin of the nucleus there is a single row of densely packed, roughly cubical mitochondria. These have distinct cristae and look normal in late spermatids, but their margins are more irregular and partly disorganized after transfer of the sperm to the females (Pl. 8:48, 49).

The surface of the spermatozoon is complicated by a strictly arranged pattern of folds from the superficial plasma membrane (Pl. 8:46-50). Each fold is about 4800 Å long, 400 Å broad and 500 Å or more high. Such folds are arranged parallel to each other in transverse belts which cover the entire surface of the spermatozoon with the exception of the minute acrosomal region (Fig. 3; Pl. 8:46). Where two belts are in contact (Pl. 8:47), the folds of one belt alternate with the folds of the neighbouring belts. The number of folds in one belt (cross sections) is 30-39 in the middle of the body but decreases in the flat posterior end of the sperm.

The usual height of the folds is 600-700 Å, but some folds, often every second one, are higher than the others (Pl. 8:50). Such exceptionally high folds may reach 2000 Å or more into the surrounding sperm fluid and are sometimes vesicular; they are circular in cross sections.

Although the folds start as simple wrinkles of the plasma membrane in spermatids, in mature sperm they are often delimited at the base by an "amorphous membrane". This sometimes looks like a floor to which the folds attach (Pl. 8:49, 50).

The posterior end of the spermatozoon has the same structure as the main part, except for the strong flattening and the absence of mitochondria (Pl. 8:50).

*The acrosomal region.* The acrosomal vesicle is preserved in the mature spermatozoon, also after transfer to the female. It is partly buried in the anterior end of the nucleus (Fig. 3; Pl. 9:56-57). The very end with the acrosomal vesicle thus can be called the acrosomal region, but this definition is not very clear for it includes also parts of the nucleus, and the region is very short, only about 1 µm long.

The anterior end of the nucleus is strongly flattened with sharp lateral edges (Pl. 8:46; Pl. 9:57). The anterior point of the nucleus produces two symmetrical lobes which envelope the acrosomal vesicle from the sides (Pl. 9:57).

The acrosomal vesicle, originally spherical, is at an early stage invaginated by the perforatorium, forming a cup which opens posteriorly, i.e., towards the nuclear membrane. Later the acrosomal cup elongates, forms a tube, and sinks partly into the end of the nucleus with its open (posterior) end first. The blind end of the tube remains outside but is in contact with the lateral lobes of the nucleus

(Pl. 9:56, 57; Pl. 8:46). The walls of the acrosomal cup remain double, still containing a cleftlike lumen originating from that of the acrosomal vesicle.

The perforatorium is first seen in the invagination of the acrosomal vesicle. Later it grows also in the posterior direction, into the nucleus, surrounded by the nuclear envelope (Pl. 9:55). But the intranuclear part of the perforatorium remains small and does not grow very much.

In mature spermatozoa the acrosomal vesicle is a tube with double walls, about 0.8 μm long and 0.17 μm thick. It contains the perforatorium in its center. The intranuclear posterior end of the perforatorium is short and irregular and appears to disintegrate in the mature stage. The posterior end of the acrosomal vesicle is buried in the end of the nucleus, and the free part of it is surrounded by the anterior nuclear lobes (Pl. 9:56, 57; Pl. 8:46).

No crystalline structure could be found in the perforatorium proper, but some dark matter lying inside the acrosomal vesicle and surrounding the perforatorial tube has a hexagonal pattern.

### 4.4. *Spermatogenesis*

Early spermatids are 4-5 μm large, regular cells lying in clumps of 15-20. Intercellular bridges between these cells are common in this and later stages. All cells in such a clump are at the same stage of development.

When differentiation begins the spermatids are drawn apart, creating a small lumen in each clump. This follicular lumen becomes larger and the spermatids are arranged as an epithelium around it, usually covering only one side of the circumference (Pl. 9:51).

The chromatin of the spermatid nuclei is at an early stage arranged as a thick layer in contact with the nuclear envelope, whereas the center of the nucleus stands out as a large and light vesicle (Pl. 9:52-54). Normal mitochondria are scattered in the plasm. No centrioles were ever seen in spermatids of this species.

In a following stage the nucleus becomes attached to the part of the plasma membrane facing the lumen of the follicle. This end of the cell is the prospective posterior pole. At an early stage this part of the plasma membrane, i.e., that in contact with the nucleus, forms several blebs, which are the forerunners of the regular membrane folds of later stages (Pl. 9:52-54).

A large Golgi apparatus appears near the opposite (anterior) pole of the nucleus, with the concave side facing the nuclear membrane. A spherical acrosomal vesicle is produced in a very schematical way in contact with the nucleus, between the nuclear membrane and the Golgi apparatus (Pl. 9:52).

The adnuclear wall of the acrosomal vesicle invaginates, forming a cup-shaped structure, and a dark perforatorium immediately becomes visible in the invaginated, tubulelike space (Pl. 9:53-55). After the establishment of the acrosomal primordia, the posterior pole of the nucleus, covered by the plasma membrane, grows out as a long rod extending into the follicle (Pl. 9:51, 54). This rod develops into the main part of the maturing spermatozoon, remaining attached to the original spermatid cell in the wall of the follicle. Later this cell, which contains the acrosomal apparatus, shrinks and is detached, freeing the spermatozoon (see below), but remnants of its plasm still surround the acrosomal apparatus of some cells after they have been transferred to the female receptaculum.

The mitochondria, with normal cristae, are originally scattered in the spermatid plasm (Pl. 9:52, 54). When the nucleus grows out to form the long rod, they become crowded at its base. They are still inside the main plasm of the spermatid (Pl. 9:56, 57) and none are seen in the nuclear process as long as its cross section is circular (Pl. 10:58). When the nuclear process begins to flatten, the mitochondria are concentrated in two dense aggregations. They do not fuse but are massed together in a kind of "ready position" at the base of the nuclear margins and are still inside the cell proper (Pl. 10:59). From here they apparently slip out in one row along each

margin of the nucleus, for they suddenly appear here, strictly arranged, when the nucleus is distinctly flat at a fairly late stage.

The perforatorium is first seen in the invagination of the acrosomal vesicle but grows posteriorly, out of the opening of the cup, and extends into the nucleus, invaginating the nuclear envelope (Pl. 9:55). The acrosomal vesicle elongates and becomes a long tube with the perforatorium in its axis, and sinks into the anterior end of the nucleus with the open end first (Pl. 9:55-57). The perforatorium does not extend far into the nucleus and seems to partly disintegrate in advanced spermatozoa.

The two anterior lobules from the nucleus are seen in some very early spermatids (Pl. 9:52) and regularly surround the acrosomal vesicle from the sides in more advanced stages (Pl. 9:56, 57).

Some condensations of dark matter in the nucleus sometimes have the shape of an irregular oval contour (Pl. 10:58). In the anterior lobules lateral to the acrosome, such matter appears to form regular double intranuclear lamellae (Pl. 9:57), which attach to the acrosomal vesicle. I believe these membranelike structures are amorphous chromatin condensations, not true membranes.

The folds of the superficial plasma membrane are seen as soon as the nucleus makes contact with the cell surface. When the nuclear process grows out the folds multiply, so the entire surface of the process is covered during the growth (Pl. 9:52-54; Pl. 10:58). At first these folds are open and somewhat irregular vesicles but they are later arranged in regular transverse belts (Pl. 8:47-50; Pl. 10:58).

When the spermatid after considerable elongation of the nucleus, condensation of the chromatin, reallocation of the mitochondria, etc., has become mature, the follicle opens into the testicular lumen. The spermatozoa become free, still with remnants of the original cell plasm attached to the acrosomal end. The plasm is sometimes partly separated from the spermatozoa by a deep constriction and is perhaps pinched off in this way. In the receptaculum of females similar plasm bodies are sometimes attached to the spermatozoa whereas others seem to be free. Some spermatozoa in the receptaculum have little or no plasm at the acrosomal end, but the acrosome itself is always preserved.

### 4.5. *Comments*

As in other Myodocopida the mitochondria of *Polycope orbicularis* remain free and do not form mitochondrial complexes (Nebenkerne). Such Nebenkerne are typical of all the Podocopida. Instead the mitochondria of *Polycope orbicularis* are arranged into two characteristic rows, one along each lateral margin of the nucleus, but they do not fuse.

Another very characteristic feature of *Polycope orbicularis* is the presence of regular surface folds of the plasma membrane, arranged in transverse belts.

Spermatophores and cytophores have not been observed. The spermatozoa are probably suspended in a liquid when they are transferred to the female during copulation.

Movements of the spermatozoa of *Polycope* spp. are assumed to exist, but are not really reported although some formulations in Hartmann (1968:419) could be misunderstood to mean this. I have not had living material of both sexes under suitable conditions and am therefore unable to shed more light on this question.

## TYPE 5. CYTHERELLIDAE

### 5.1. *Material*

*Cytherella abyssorum* G. O. Sars. – Skagerrak, 60 km NW of Skagen, 400 m, R/V Dana, Stat. 17084, 4.IX.1976, 1 ♂, 10 ♀♀, 3-A. – Korsfjorden, S. of Bergen, Norway, 684 m, 2.VI.1976, 1 ♂, more than 50 ♀♀, 3-A.

Remarks: ♂♂ of this species appear to be rare in some localities where I have been collecting. I caught more than 100 ♀♀ from Korsfjorden during three visits to the Espegrend station (30.IX.1975, 2.VI.1976 and 16.X.1984), but got only one male.

### 5.2. *Genital organs*

The *male* has paired testicles lying in the posterior part of the body. They are subdivided into large lobules which appear compact, filled with spermatids or spermatozoa. Spermatogenesis is largely simultaneous in each lobule. No walls or vegetative tissue is seen inside the lobules. The mature spermatozoa are emptied into a large vesicula seminalis on each side. The large paired penis is figured by Sars (1928:19) and G. W. Müller (1894:32). No spermatophores were seen in my sections of males or females.

*Female*. A receptaculum seminis (possibly paired) was seen in my sections of a few females. It was so hard because of its content of densely packed spermatozoa that ultrastructural techniques failed almost completely.

### 5.3. *Mature spermatozoa*

Spermatozoa in the vesicula seminalis of the males of *Cytherella* are filamentous, very thin (0.5-1 µm) and probably very long. They are more than 50 µm, but I failed to isolate single spermatozoa so the total length could not be recorded.

The anterior end is a dorsoventrally flattened, dorsally bent "claw" about 1.7 µm long. It contains the remnants of the acrosomal vesicle and the anterior end of the perforatorium (Pl. 10:60-62).

The following regions, called postacrosomal and nuclear regions, respectively, are long and filamentous and constitute the main part of the spermatozoon (Fig. 4:A). The frequency of the cross sections of these two regions indicates that they have a similar length.

I have used the descriptive designations "dorsal" and "ventral" for orientation in cross sections, calling the nuclear side for dorsal as in the podocopid ostracods, but I am not certain that these terms cover real homologies.

The "*claw*" or *acrosomal region* is surrounded by some indifferent plasm and is delimited by an irregularly wrinkled cell membrane (Pl. 10:60-62). The dark structures inside are the vestigial acrosomal vesicle, the anterior end of the perforatorium and the anterior extremities of the endoplasmic vesicles which are characteristic of the following, postacrosomal region.

The perforatorium has a nearly central position in the "claw" and reaches into its very tip. It has a central light core, continuous with that of the perforatorium further back in the postacrosomal region (Pl. 10:60-62).

The end region of the perforatorium is covered by two dark membranes which are indistinctly separated by a light space. I believe that these two membranes represent the partly obliterated cup-shaped acrosomal vesicle which covers the end of the perforatorium in early spermatids (Pl. 11:68). If this is true the originally open, cup-shaped vesicle must have been drawn out to a tube over the point of the perforatorium, and its lumen must have almost become obliterated (Pl. 10:61, 62).

The acrosomal vesicle is fairly distinctly delimited posteriorly, where the membranes of the vesicle seem to end or double back (Pl. 10:61). The entire region is dorsoventrally compressed with sharp

margins and is slightly bent, with the concave side dorsal (Pl. 10:61, 62).

The *postacrosomal region* is somewhat flattened from the sides, about 0.6 µm high and 0.35 µm broad. Its cross section is the same throughout the region: the dorsal half is occupied by the perforatorium and attached structures, whereas the ventral half contains four rows of longitudinal flat sacs of endoplasmic origin (Pl. 10:64).

Also the external contour is characteristic: on each side of a blunt dorsal keel is a longitudinal fold with a more or less double edge. The fold on each side is not straight but regularly undulating and is believed to perform undulating movements in the living sperm (see below).

The axis of the postacrosomal region is the perforatorium, which is a straight rod with triangular cross section (Pl. 10:64). One angle of the triangle is

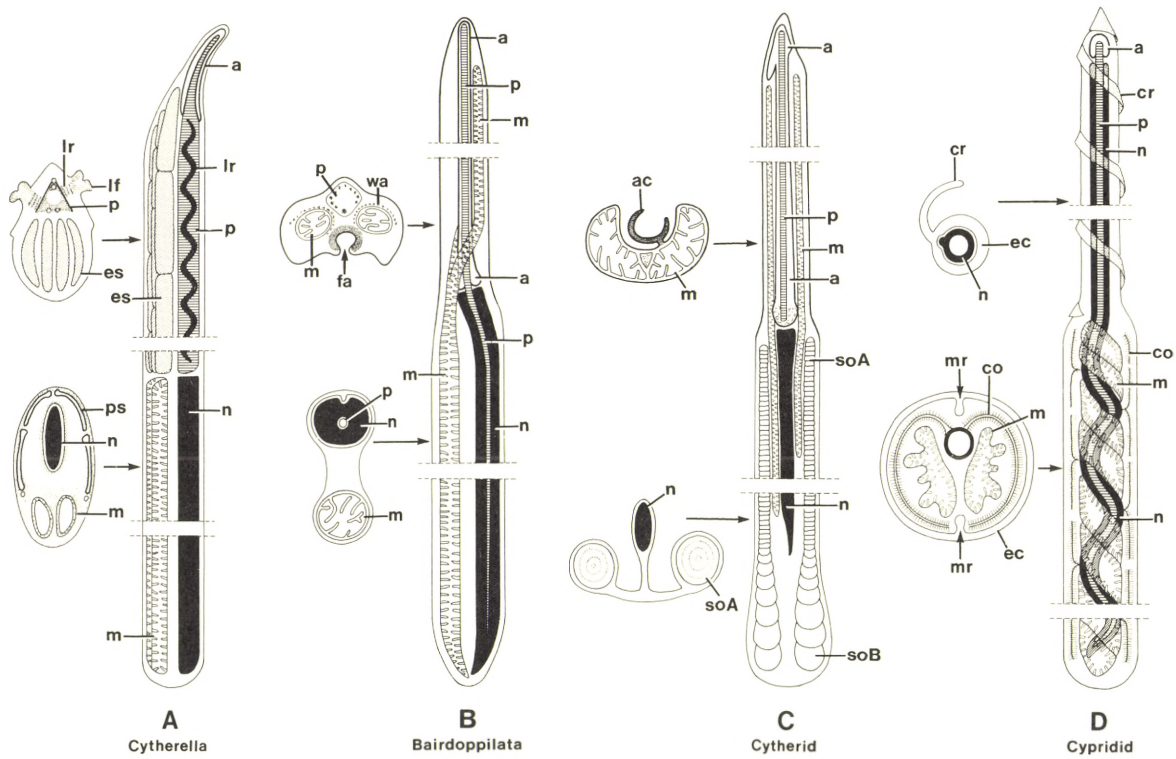



Fig. 4. Strongly simplified comparison of podocopid spermatozoa. A. *Cytherella abyssorum*, B. *Bairdoppilata cushmani*, C. Cytherid, D. Cypridid. Proportions and relative dimensions are partly arbitrary. See Pls. 10-34 and Figs. 5-8 for more details.

Figs. A and B are seen from the side, C and D are seen from the dorsal side. In B-D the extension of the acrosomal vesicle and perforatorium is drawn as in spermatids, although these structures change in mature spermatozoa.

*Legends to all figures*: a = acrosomal vesicle, ac = acrosomal crest, co = contractile organelles, cr = coiling ridge on anterior region of cypridid (D), ec = extracellular coat, es = endoplasmic sacs (in A), fa = longitudinal furrow on acrosomal region (B), lf = longitudinal undulating fold on acrosomal region (A), lr = lamellate rods (A), m = mitochondria, mr = monorails, dorsal and ventral (D), n = nucleus, p = perforatorium, ps = platelike endoplasmic sacs around nucleus (A), soA = segmented organelles A, soB = segmented organelles B.

middorsal and the symmetrical sides are sharply delimited and even. The surface of each side is superficially delimited by a sharp light lamella, covered by a dark "membrane", about 20 Å thick. It seems probable that together with the light core, the lamellae on these surfaces maintain the constant shape and rigidity of the perforatorium.

In the top region of the triangle there are two small light tubules, one above the other, in the median plane (Pl. 11:65). A pair of similar tubules are in contact with the basal surface of the triangle, one on each side of the median plane (Pl. 10:64; Pl. 11:65).

*Lamellate rods.* Each lateral surface of the perforatorium is in contact with a "lamellate rod", which has a mainly longitudinal course. The two rods are about 500×1000 Å in cross sections, and each of them is continuous throughout the postacrosomal region.

The rods appear stratified in cross sections, consisting of three or four dark lamellae alternating with light ones (Pl. 10:64; Pl. 11:67; Fig. 4:A). Hence the name "lamellate rods". The outer lamellae are less distinct and are connected with the ipsilateral fold on the outer surface by some amorphous matter which spreads in the fold.

The lamellate rods have an undulating course with regular bends in a plane parallel with the sides of the triangular perforatorium. Different cross sections therefore show the lamellate rods in contact with the plane surfaces of the perforatorium at different levels (Pl. 10:64; Pl. 11:65-67). Similar undulations are found in the plasma fold on the external surface, to which the lamellate rod is connected. When the lamellate rod is high up on the surface of the triangular perforatorium, the external plasma fold is high up. In other cross sections where the rod is in a low position the external fold is also low (Pl. 11:65, 66).

Longitudinal sections confirm that the lamellate rods and the external fold have an undulating course whereas the triangular perforatorium is strictly straight (Pl. 10:60, 63; Pl. 11:67). It can also be seen that the rod and the fold on the same side have the same period and are correlated (Pl. 10:63; Pl. 11:67). But the undulation of the right lamellate rod is often out of phase with that of the left one (Pl. 11:65, 66).

This is the static situation seen in fixed material. However, it seems likely that the undulations recorded reflect a dynamic situation in the living spermatozoon, which has been "frozen" by fixation. It is quite possible that the two folds on the surface of living spermatozoa do undulate in such a way. If this is true, the force probably comes from sliding up and down of the lamellate rods on the sides of the perforatorium.

This may be the mechanism by which these spermatozoa move, but it must be admitted that no such movements have been seen in the microscope, although fresh living material was looked at. The negative result of these efforts is not significant, for the lamellate rods and the superficial folds are too small to be resolved in the light microscope (less than 0.3 μm).

*The four rows of endoplasmic sacs* in the ventral part of cross sections of the postacrosomal region develop in continuity with the irregular endoplasmic reticulum of spermatids. The sacs have a single unit membrane in the wall and cristae are not present. The contents of the sacs are light, with a few strands and flakes (Pl. 10:64; Pl. 11:71).

In the mature speramtozoon, each of the four rows consists of regular, laterally compressed sacs, about 1.7 μm long. In each row the sacs are arranged end to end in a very regular way (Pl. 10:60; Pl. 11:71).

*The nuclear region.* The nucleus is a long band, somewhat compressed from the sides. It occupies the dorsal part of the cross section in the nuclear region, whereas the two filamentous mitochondria are symmetrically located in the ventral part (Pl. 11:73, 74).

The chromatin forms longitudinal filaments, visible as black dots in the cross sections. The nuclear envelope is thick, often very dark, but could not be resolved in detail. Young spermatids have a typical double nuclear envelope (Pl. 11:69). Outside the

nuclear envelope is a zone of light, empty-looking plasm (Pl. 11:74).

Flat, platelike vesicles are fitted together as an almost continuous layer inside the plasma membrane in the dorsal part of cross sections through the nuclear region (Fig. 4; Pl. 11:74). Each plate is 0.3-0.4 μm broad but thin, with only about 230 Å between the two outer surfaces. Development shows that the plates are strongly flattened sacs; they appear to be pinched off from the Golgi complexes in the early spermatids. In mature spermatozoa the sacs are so strongly compressed that their lumen may be difficult to see.

*The mitochondria* are a pair of long, bandlike bodies which pass uninterrupted through the nuclear region. They are in contact with each other in the ventral midline but do not fuse. In spermatids the double walls and the cristae are distinct (Pl. 11:73), but in mature spermatozoa they are less typical: the cristae disappear and the two membranes of the wall become connected by numerous regularly spaced cross bridges (Pl. 11:74).

## 5.4. *Spermatogenesis*

Since the large testicular lobes are few and contain only a single developmental stage each, the available material from two males gives a somewhat discontinuous picture of the spermatogenesis. Nevertheless some important facts could be derived from the stages present.

*Early spermatids* were seen in many lobes. They have a round nucleus and many scattered mitochondria with cristae. The Golgi complexes are large and very complicated, but no acrosomal vesicle could be detected.

*Early acrosomal apparatus.* In the next stage studied the nucleus has elongated, the mitochondria have fused into two elongate rods extending along the nucleus, and there is a typical acrosomal vesicle at the prospective anterior pole of the cell (Pl. 11:68). The vesicle is invaginated from its posterior (adnuclear) end and is cup-shaped, containing a well developed perforatorium. The rodlike perforatorium has also invaginated the anterior pole of the nucleus, forming an intranuclear canal about 0.8 μm long.

The acrosomal pole of the nucleus, surrounded by some plasm and the plasma membrane, extends as a narrow process, about 0.5 μm thick, in front of the main plasma body of the spermatid, which is 5-6 μm in diameter.

Unfortunately no intermediate stages were present to show whether the mitochondria fuse and form two spherical Nebenkerne before elongating, or if they coalesce in another way.

*Postacrosomal region, perforatorium.* After the stage described above there is again a gap in the series of stages. In the next stage seen the perforatorium has grown out and formed a long rod in front of the nucleus, and an indistinct "claw" with remnants of the acrosomal vesicle is present on its top.

In younger spermatids (Pl. 11:68) the perforatorium penetrates deeply into the anterior end of the nucleus, a fact checked in more than 20 spermatids. But in the present stage it must have retracted again, for the anterior nucleus has no invagination in this and subsequent stages. Instead the anterior end of the nucleus is squarely cut off and is in contact with a thick plate of dark matter which is continuous with the hind end of the perforatorium (Pl. 11:69).

When the perforatorium grows longer, a large postacrosomal region develops between the end of the nucleus and the small acrosomal vesicle (the "claw"). During the first phases of this growth the pair of bandlike mitochondria extends ventral to both nucleus and perforatorium (Pl. 11:72). Later the mitochondria seem to slide backwards, and disappear at an early stage from the anterior end of the postacrosomal region. Finally they disappear completely from cross sections containing the perforatorium and become restricted to the nuclear region, as in mature spermatozoa.

The postacrosomal region is originally surrounded by plentiful cytoplasm. This cytoplasmic body is elongate and is slightly compressed dorsoventrally, with the perforatorium in its center. Deep

longitudinal furrows cut into the plasm from each side to the neighbourhood of the perforatorium (Pl. 11:72). These furrows separate two longitudinal plasma folds on each side. When the plasm is reduced these folds are reduced to low ridges, but the separating furrow is probably the same as in earlier stages (Pl. 11:72, 70). The two ridges on each side tend to fuse and are connected with the lamellate rods, which appear on each side of the perforatorium.

When the mitochondria retract from the postacrosomal region, their place becomes occupied by many irregular sacs of endoplasmic reticulum. Towards the end of spermatogenesis some of these sacs elongate and become rearranged into the four rows of flat endoplasmic vesicles characteristic of the mature postacrosomal region.

The perforatorium originally looks amorphous in cross sections (Pl. 11:70). The different structures such as the triangular shape, the light core, the two pairs of light tubules and the lamellate rods arise in the amorphous matter in a way that provides little information as to their phylogenetical origin.

### 5.5 *Comments*

The most characteristic feature of *Cytherella abyssorum* is the morphology of the postacrosomal region: the strictly triangular perforatorium with plane lateral surfaces, connected with "lamellate rods" which have an undulating course.

It is suggested above that the lamellate rods undulate in living spermatozoa, and that the undulations are transferred to the longitudinal superficial folds which have a corresponding undulating course in fixed material. If this is true the locomotory mechanism of *Cytherella abyssorum* is unique and is so complicated that it must be regarded as an apomorphy of the genus.

The mitochondria of the mature spermatozoa of *C. abyssorum* are fused, forming two long bands in the nuclear region. This looks like a synapomorphy with the other Podocopida, supporting an origin of the Cytherellidae within this group. But it is not known how the fusion of the mitochondria takes place in *Cytherella abyssorum*, e.g., if a typical Nebenkern is formed as in the other podocopids. Moreover, we do not know if the fusion of the mitochondria into two complexes is an apomorphic (new) character in Podocopida or if it is plesiomorphic, derived from ancestors. This weakens the conclusions.

The small, clawlike acrosomal region of *Cytherella abyssorum* is superficially like that of of the Halocyprididae but I am uncertain of the significance of this simple feature.

The long postacrosomal region with its long perforatorium looks like structures in Halocyprididae, Cypridídae and particularly Cytheridae and Bairdiidae (Fig. 4), but there are also differences in detail between these families. I am uncertain about the significance of these features.

## TYPE 6. BAIRDIIDAE

### 6.1. *Material*

*Bairdoppilata cushmani* (Tressler, 1949). – Barbados, Bellair's Research Inst., on Barrier Reef, 36 m, 27.III.1976, ♂♂ and ♀♀, 3-A, coll. J. Just.

### 6.2. *Genital organs*

The *male* genital organs of bairdiids are described by G. W. Müller (1894:147) and Hartmann (1968:420). The testicles are paired, each consisting of four lobes of which one is vestigial. Sper-

matogenesis is simultaneous in each lobe, but spermatocytes and early spermatids may form small clusters along the walls, from which subsequent generations can start. The lobes are compact, filled with spermatids and spermatozoa, and no vegetative tissue is present.

Mature spermatozoa from the three lobes on each side empty into an unpaired lumen in the middle of the animal. Paired vasa deferentia come from this seminal vesicle and are coiled up to a large ball-like structure on either side before the duct passes down to the penis. Here the vas deferens enters a long, flagellumlike copulatory tube which is partly independent of the "penis proper". The latter is a large structure consisting of several movable parts as in other Podocopida.

*Female.* According to G. W. Müller (1894:153) the receptaculum is a large sac lined by a cuticle. It is connected with a wide vagina through which sperm is believed to enter and a long, coiled fertilization duct leading to the terminal part of the oviduct.

The receptaculum of bairdiids often contains complicated spermatophores of considerable size. G. W. Müller (1894:153) has developed the idea that these spermatophores are formed within the receptaculum. They are far too large to pass the sperm ducts in the male, and their shape fits with the lumen available in the receptaculum and the first part of the fertilization duct. The secretions forming the spermatophore are believed to come from the vas deferens of the male and to be injected together with the sperm through the copulatory tube directly into the female receptaculum. I can confirm Müller's statement that parts of the coiled duct in the male are strongly glandular but have no direct observations on spermatophores in this species. Müller's interpretation is, however, quite convincing.

## 6.3. *Mature spermatozoa*

As indicated in G. W. Müller's figures, the mature spermatozoa of bairdiids are long and filamentous (1894:pl. 38). In my sections of *Bairdoppilata cushmani* they are less than 1 μm in diamater and probably more than 100 μm long. Isolated spermatozoa could not be obtained from the fixed material available so the exact length is unknown.

The spermatozoa consist of two main regions: an *acrosomal region* and a *nuclear region.* The acrosomal region can be subdivided into an anterior, nonmitochondrial part and a posterior mitochondrial part (Fig. 4:B; Pl. 12:75, 76).

The *acrosomal region* is originally established in spermatid stages by the large acrosomal vesicle and the perforatorium, but is later modified so the original pattern is difficult to recognize. Its posterior end contains two mitochondrial rods which have invaded it from the following nuclear region (Fig. 4:B).

The anterior, nonmitochondrial part has a characteristic cross section with a pair of lateral "horns", which are separated by a T-shaped invagination (Pl. 12:75). The cross section is not perfectly symmetrical. One of the horns is larger, corresponding to the side where the plasm contains a dark longitudinal rod, which is absent on the opposite side. The T-shaped furrow is supported by a well-defined mantle of dark matter which has a crystalline structure, seen as a cross striation in longitudinal sections (Pl. 12:76, inset).

The anterior end of the spermatozoon is simple and pointed. The two lips of the T-shaped furrow meet and fuse, and the crystalline matter of the mantle spreads and seems to cover the very point.

The posterior part of the acrosomal region differs mainly in the presence of the mitochondria, which are symmetrically located and in close contact with each other (Pl. 12:76). Between them is a narrow unpaired rod.

The cross section changes from dorsoventrally flattened in the nonmitochondrial region to fairly high in the mitochondrial region, and the longitudinal furrow becomes deeper (Pl. 12:76). The mitochondrion extends further anteriorly on the side where the above-mentioned longitudinal plasma rod is present than it does on the opposite side.

The *nuclear region* has a handle-shaped cross sec-

tion with the cylindrical nucleus situated in the dorsal dilatation and the single mitochondrial rod filling the ventral bulge (Pl. 12:77). The nucleus has the normal double envelope, which is evaginated as a low fold in the dorsal midline. The chromatin is moderately condensed, forming dark longitudinal filaments. A small central tubule is the last remnant of the perforatorium and its investment, which are better preserved in early spermatids (Pl. 12:77; Pl. 13:84-87).

The single mitochondrion is cylindrical with a double envelope and typical cristae (Pl. 12:77). Longitudinal sections through the transition between acrosomal and nuclear regions show that the single mitochondrion of the nuclear region bifurcates in this zone and enters the acrosomal region as two symmetrical rods (Fig. 4:B). The space between the nucleus and the mitochondrion is filled by dark granular cytoplasm, but a somewhat irregular lamella is seen to connect these two rods in some cross sections (Pl. 12:77). Sections of submature spermatozoa show that this lamella is a longitudinal sac, strongly flattened from the sides (Pl. 13:86).

Both nucleus and mitochondrion extend to near the posterior end of the spermatozoon.

## 6.4. *Spermatogenesis*

Early spermatids are isodiametrical cells, about 10 μm in diameter, with a large (5 μm) nucleus and numerous free mitochondria with cristae. In somewhat older spermatids the mitochondria are assembled into a dense cluster on one side of the nucleus, whereas a large Golgi apparatus (4 μm across) occupies the opposite side of the cell.

In the following stages the mitochondria coalesce as two spherical Nebenkerne. These are in contact with each other with flattened surfaces between which there is some medium-contrast matter (Pl. 12:78, 79).

A very large acrosomal vesicle is formed on the concave side of the Golgi apparatus. It attains the same size as the nucleus, to which it is attached. The vesicle grows out and elongates, attaining contact with the plasma membrane in the prospective anterior end of the spermatozoon (Pl. 12:78).

The three major organelles have a somewhat irregular arrangement in very early spermatids but soon become lined up in the way shown in Pl. 12:78, with acrosomal vesicle, nucleus and the two mitochondria in contact with each other.

At this stage the nuclear envelope in contact with the acrosomal vesicle invaginates, forming a blind-ending perforatorial tube into the nucleus. The postacrosomal matter in this tube condenses and forms one or two longitudinal rods which are an obvious homologue of the perforatorium in other animals (Pl. 12:78). Somewhat later a similar invagination is formed into the acrosomal vesicle, and the two invaginations straighten up and elongate, forming a continuous perforatorium through the acrosomal vesicle and nucleus (Pl. 12:78).

The very large acrosomal vesicle, covered by the plasma membrane, elongates and becomes roughly triangular in cross sections (Pl. 12:80). The sharp edge on one side of the triangle corresponds to the nuclear side further back, so it is here called dorsal. The perforatorium is located near this ridge in cross sections, i.e., dorsally. The opposite, ventral side of the triangular acrosomal vesicle has a broader, flattened surface in contact with the plasma membrane (Pl. 12:80).

*The nucleus* with the perforatorium inside elongates and develops a dorsoventral asymmetry. Two dark rods, which are thickenings of the plasma membrane, extend from the acrosomal region into the anterior end of the nuclear region. The dorsal rod is clearly the direct continuation of the sharp dorsal ridge of the acrosomal vesicle (Pl. 13:81, 84). The ventral rod is less marked. It is continuous with the broad ventral base of the acrosomal vesicle and does not extend so far into the nuclear region.

The nuclear envelope develops a dorsal and a ventral "nuclear band" under each of the mentioned rods. There is no contact between the rods from the acrosomal region and the nuclear membrane, but it is possible that the rods induce the

nuclear membrane to form the bands, which are very distinct (Pl. 13:81, 84, 85). The rods soon disappear but the bands on the nucleus extend to its posterior end, where the bands meet and fuse.

The mitochondria, still two large spherules in contact with each other, are in contact with the nuclear bands and fuse at the posterior end of the nucleus, where the bands meet (Pl. 12:78).

*Final development of the mitochondria.* The two globular mitochondria, attached to the posterior end of the nucleus, are strongly modified when the sperm elongates. The ventral mitochondrion, attached to the ventral nuclear band, extends forwards as a tongue in contact with the band (Pl. 13:85). The dorsal mitochondrion was seen to emit a similar tongue along the dorsal band in some early spermatids, but is probably abortive, for no dorsal mitochondrion was ever seen along the nucleus in submature or mature spermatozoa.

The ventral mitochondrion grows forwards to the acrosomal region, where it bifurcates into two rods which lie symmetrically, one on each side of the perforatorium (Pl. 13:82, 83). The ventral mitochondrion is directly in contact with the ventral band when it grows forwards (Pl. 13:85). Later it is withdrawn from the nucleus but remains connected with it by a laterally flattened sac (Pl. 13:86, 87). The sac is sometimes preserved as an irregular membrane between nucleus and mitochondrion in mature spermatozoa (Pl. 12:77) but often disappears completely, like the dorsal and ventral nuclear bands.

The fate of the dorsal mitochondrion could not be seen with certainty. Perhaps it fuses with its ventral partner and is used up when the single ventral mitochondrion grows forwards.

*Final development of the nuclear region.* Final maturation includes additional elongation of the long nucleus. The chromatin in the nucleus is condensed into longitudinal lamellae, which form a labyrinth-like pattern in cross section (Pl. 13:86, 87). Finally the lamellae are again scattered and transformed into filaments.

*Final development of the acrosomal region.* The acrosomal vesicle, originally several μm thick, becomes longer and filiform, and the diameter is reduced to about 0.5 μm. The mitochondrial rods invade the acrosomal region from behind and extend symmetrically on each side of the midline, at first ventral to the perforatorium (Pl. 13:82, 83). Later the two rods have a more dorsal location and approach each other in the dorsal midline (Pl. 12:76) and are separated by an unpaired rod which is believed to contain the remnants of the perforatorium. The latter becomes smaller during maturation.

The acrosomal vesicle changes and shrinks during further development and can no longer be clearly recognized in mature spermatozoa.

The ventral midline of the acrosomal region is folded in as a longitudinal furrow, which becomes surrounded by a U-shaped dark mantle. This seems to be a derivative of the acrosomal vesicle (Pl. 13:82, 83).

It is also probable that matter from the acrosomal vesicle contributes to the septum between the mitochondria and to the lateral "wings" covering the dorsal aspect of the mitochondria in intermediate spermatids (Pl. 13:82, 83). These wings are later reduced.

## 6.5. *Comments*

The hard-walled spermatophores of bairdiid females indicate a type of sperm transfer during copulation similar to that in cypridinids, sarsiellids and rutidermatids. In bairdiids, ejaculated fluid from the male obviously hardens to a spermatophore inside the receptaculum, whereas this takes place externally, in the furrow on the female genital lobe, in the other three families. However, this does not prevent homology, for the receptaculum may have evolved from the furrow on the genital lobe (G. W. Müller 1927:418) and the mechanism, involving specializations of morphology and secretions, appears similar.

Such spermatophores formed on or in the female are unknown in other ostracods, and could be an

argument for a close phylogenetic association between bairdiids and the three myodocope families. However, we cannot eliminate the possibility that the mechanism, including the spermatophores, was developed early in ostracod ancestors and was preserved in cypridinids, sarsiellids and rutidermatids, and in bairdiids in a somewhat modified form. If so, it does not show that the bairdiids are more related to the myodocope families than to other ostracods and the character is plesiomorphic at this level.

With regard to the development of the typical, double Nebenkern, *Bairdoppilata cushmani* resembles the podocopids, while myodocopids have numerous free mitochondria. This fits with the idea that bairdiids belong to the podocopid line, an idea supported by several other characters such as the reduction of the exopodite of the 2nd antenna and the morphology of the furca (see Hartmann & Puri 1974). However, the Nebenkern is not very strong as an argument, for its state as an apomorphic feature in podocopids and bairdiids is purely hypothetical (see chapter C).

The very large acrosomal vesicle in spermatids resembles that of cytherids and the invasion of the acrosomal region by the mitochondria and the presence on the acrosomal region of a longitudinal furrow supported by a dark mantle both in *Bairdoppilata* and some cytherids points in the same direction. It would strengthen the homology if the acrosomal furrow is ventral in both cases, but this is a difficult question because of the complicated symmetry conditions in cytherids.

On the other hand, the spermatozoa of *Bairdoppilata cushmani* differ clearly in some respects from cytherid spermatozoa. The "segmented organelles" so characteristic of cytherids are lacking in *B. cushmani*, and the perforatorium invades the nucleus in *B. cushmani* but not in cytherids (Fig. 4).

The bifurcation and behaviour of the mitochondria is a very striking feature of *B. cushmani*, the only bairdiid species investigated, but little can be said about its phylogenetical significance.

## TYPE 7. SUPERFAMILY CYTHERACEA

### 7.1. *Material*

Fam. Leptocytheridae

*Leptocythere castanea* G. O. Sars. – Kristineberg, Bohuslän, Sweden, 5.X.1972, ♂♂ + ♀♀, $OsO_4$-bicarbonate buffer.

*L. lacertosa* (Hirschmann). – Nivå Bay (Øresund), Denmark, 26.I.1973, from laboratory culture by B. Theisen, ♂♂ + ♀♀, Palade's $OsO_4$. – Nivå Bay, Denmark, 15.III.1973 and 30.IV.1976. ♂♂ + ♀♀, 3-A. – Espegrend, Norway, brackish bay, 27.IX.1975, 30.V.1976 and 14.X.1984, ♂♂ + ♀♀, 3-A.

*L. pellucida* (Baird). – Espegrend, Norway, 28.IX.1975, ♂♂ + ♀♀, 3-A.

Fam. Limnocytheridae

*Limnocythere reticulata* Sharpe. – Silver Lake, N. of Baker, California, culture of dry mud, 17.XII.1977, coll. Å. Jespersen and J. Lützen, ♂♂ + ♀♀, 3-A.

*Metacypris cordata* Brady & Robertsons. – Løg Sø, Zealand, Denmark, 16.VIII.1974, ♂♂ + ♀♀, 2% $OsO_4$-cacodylate, 3-A.

Fam. Cytherideidae

*Cyprideis litoralis* (Brady) (not distinguished from *C. torosa* Jones). – Nivå Bay, Denmark, 21 and 26.I.1973, cultured by B. Theisen, ♂♂ + ♀♀, Palade's $OsO_4$. – Nivå Bay, Denmark, 27.VIII.1973, ♂♂ + ♀♀, 3-A. – Espegrend, Norway, 14.X.1984, ♂♂ + ♀♀, 3-A.

Fam. Krithidae

*Krithe bartonensis* (Jones). – Skagerrak, R/V Dana, stat. 16206, 200 m, 21.III.1973, ♀♀, Palade's $OsO_4$. – Espegrend, Norway (Fanafjord), 26.IX.1975 and 15.X.1984, ♂♂ + ♀♀, 3-A.

Fam. Trachyleberididae

*Cythereis albomaculata* (Baird). – Tromsø, Norway, VIII.1973, 1 ♂, Palade's $OsO_4$, coll. Å. Jespersen.

*C. jonesi* (Baird). – Kristineberg, Sweden, 6.X.1972, 2 ♂♂, $OsO_4$-bicarbonate. – Tjärnö, Koster, Sweden, 2.VI.1975, ♂♂ + ♀♀, 2 % $OsO_4$-cacodylate.

*C. dunelmensis* Norman. – Øresund off Helsingør, Denmark, 10.IV.1973, ♂♂ + ♀♀, Palade's $OsO_4$. – Espegrend, Norway, 31.V.1976, ♂♂ + ♀♀, 3-A.

*C. tuberculata* G. O. Sars. – Tjärnö, Koster, Sweden, 3.VI.1975, ♀♀, 2 % $OsO_4$-cacodylate.

*C. echinata* G. O. Sars. – Espegrend, Norway (Korsfjorden), 26.IX.1975, 31.V.1976 and 16.X.1984, ♂♂ + ♀♀, 3-A.

Fam. Hemicytheridae

*Hemicythere villosa* (G. O. Sars). – Espegrend, Norway, off the harbour, 30.IX.1975, ♀♀, 3-A.

*H. oblonga* (Brady). – Øresund off Ellekilde Hage, Zealand, Denmark, 30.V.1975, ♂♂ + ♀♀, 3-A, coll. R. M. Kristensen.

Fam. Loxoconchidae

*Loxoconcha elliptica* Brady. – Nivå Bay, Denmark, 26.I.1973 and 15.III.1973, ♂♂ + ♀♀, Palade's $OsO_4$. – Nivå Bay, XI.1973 and 30.VI.1976, ♂♂ + ♀♀, 3-A.

*L. impressa* (Baird). – Kristineberg, Sweden, 5.X.1972, ♂♂ + ♀♀, $OsO_4$-bicarbonate. – Espegrend, Bergen, Norway, 27.IX.1975 and 15.X.1984, ♂♂ + ♀♀, 3-A. – Drøbak, Norway, 1.VI.1976, ♂♂ + ♀♀, 3-A, coll. J. Lützen.

*Elofsonia baltica* (Hirschmann). – Nivå Bay, Denmark, 30.VI.1976, ♂♂ + ♀♀, 3-A.

*Hirschmannia viridis* (O. F. Müller). – Espegrend, Bergen, Norway, 29.V.1976, ♂♂ + ♀♀, 3-A.

Fam. Cytheruridae

*Cytherura gibba* (O. F. Müller). – Nivå Bay, Denmark, 27.VIII and 28.VIII.1973, ♂♂ + ♀♀, Palade's $OsO_4$. – Espegrend, Bergen, Norway, brackish bay, 27-29.IX.1975, ♂♂ + ♀♀, 3-A.

*C. sella* G. O. Sars. – Espegrend, Bergen, Norway, 30.IX.1975 and 30.V.1976, ♂♂ + ♀♀, 3-A.

*C. acuticostata* G. O. Sars. – Espegrend, Bergen, Norway, 1.VI.1976, ♂♂ + ♀♀, 3-A.

*C. nigrescens* (Baird). – Tromsø, Norway, VIII.1973, ♂♂ + ♀♀, Palade's $OsO_4$, coll. Å. Jespersen. – Espegrend, Bergen, Norway, 30.V. and 1.VI.1976, ♂♂ + ♀♀, 3-A.

*Cytheropteron alatum* G. O. Sars. – Espegrend, Bergen, Norway (Fanafjord), 26.IX.1975, ♂♂ + ♀♀, 3-A. – Tjärnö, Koster, Sweden, VI.1975, ♂♂ + ♀♀, 2 % $OsO_4$-cacodylate.

*C. latissimum* (Norman). – Kristineberg, Sweden, 6.X.1972, ♂♂ + ♀♀, $OsO_4$-bicarbonate. – Tjärnö, Koster, Sweden, 2.VI.1975, ♂♂ + ♀♀, 2 % $OsO_4$-cacodylate.

*C. ? pyramidale* Brady (the few males are not convincingly separate from preceding species). – Tjärnö, Koster, Sweden, 3.VI.1975, ♂♂, 3-A and 2 % $OsO_4$-cacodylate.

Fam. Xestoleberididae

*Xestoleberis aurantia* (Baird). – Kristineberg, Sweden, 6.X.1972, ♂♂ + ♀♀, Palade's $OsO_4$ and $OsO_4$-bicarbonate. – Same place, 30.IX.1976, ♂♂ + ♀♀, 3-A. – Tjärnö, Koster, Sweden, 3.VI.1975, ♂♂ + ♀♀, $OsO_4$-cacodylate.

*X. depressa* G. O. Sars. – Espegrend, Bergen, Norway, 1.VI.1976, ♂♂ + ♀♀, 3-A.

*Xestoleberis* sp. 1. – Shore of Barbados, 8.III.1978, ♂♂ + ♀♀, 3-A, coll. J. Just.

*Xestoleberis* sp. 2. – Shore of Mauritius, XII.1978, ♂♂ + ♀♀, 3-A, coll J. Dyck.

*Xestoleberis* sp. 3. – Espegrend, Bergen, Norway, small brackish pond on small island W. of harbour, 30.V.1976, ♂♂ + ♀♀, 3-A.

Fam. Paradoxostomatidae

*Paradoxostoma variabile* Baird. – Espegrend, Bergen, Norway, 1.VI.1976, ♂♂ + ♀♀, 3-A.

*P. ensiforme* Brady. – Espegrend, Bergen, Norway, 27.IX.1975, ♂♂ + ♀♀, 3-A.

*Paradoxostoma* sp. 1. – Shore of Barbados, 8.III.1978, ♂♂, 3-A, coll. J. Just.

*Cytherois fischeri* G. O. Sars. – Nivå Bay, Zealand, Denmark, XI.1973, ♂♂ + ♀♀, 3-A. – Espegrend, Bergen, Norway, 14.X.1984, ♂♂ + ♀♀, 3-A.

*C. pusilla* G. O. Sars. – Kristineberg, Sweden, 6.X.1972, ♂♂, Palade's $OsO_4$.

*C. vitrea* G. O. Sars. – Espegrend, Bergen, Norway, 1.VI.1976, ♂♂, 3-A.

*C. arenicola* Klie. – Nivå Bay, Zealand, Denmark, 30.VI.1976, only ♀♀, length of spermatozoa recorded.

## 7.2. *Genital organs*

The genital organs of some cytherids are briefly described by G. W. Müller (1894), Hirschmann (1912) and Hartmann (1968), but these texts deal mainly with the external genital appendages. A comprehensive survey of the internal organs is therefore difficult to give and becomes uncertain because of great variation within the superfamily.

*Male.* The testicle is said to consist of four vesicles on each side, situated behind the valve adductor above and lateral to the intestine. That of *Cyprideis torosa litoralis* is described in some detail by Hartmann (1968:423). The efferent ducts from the four vesicles on each side fuse and form a vas deferens which passes down to the ipsilateral copulatory appendage. The vasa deferentia are paired but right and left vas deferens are connected by a transverse duct just behind the testicles. Complicated ejaculatory ducts are sometimes evolved where the vas deferens enters the copulatory limb. Widened parts of the ducts are often filled with mature sperm and function as vesiculae seminales.

*Female.* The oviducts are said to be paired in some Cytheracea, fused in other species (G. W. Müller 1894). Although fusions may occur, Hartmann (1968:453) believes that the terminal parts, including vagina and receptaculum, are paired in all cytherids. I have found paired receptacula with mature sperm in most species in my material.

The function of the copulatory appendages, e.g., how the sperm is transferred to the female during copulation, has been discussed by Elofson (1941) and Hartmann (1968). It appears obvious that the sperm is transferred while suspended in a fluid, and spermatophores have not been observed.

## 7.3. *Mature spermatozoa*

Cytheracean spermatozoa show an almost bewildering variation (Figs. 5, 6, 9), but significant differences between specimens of the same species have not been recorded. Material from males and females of the same species was compared many times in considerable detail but no obvious differences were found.

Cytheracean spermatozoa are long and filamentous, between 30 μm and 480 μm in length (Table 2, page 41), but are in general shorter than those of the Cypridacea, which often reach 1000 μm or more.

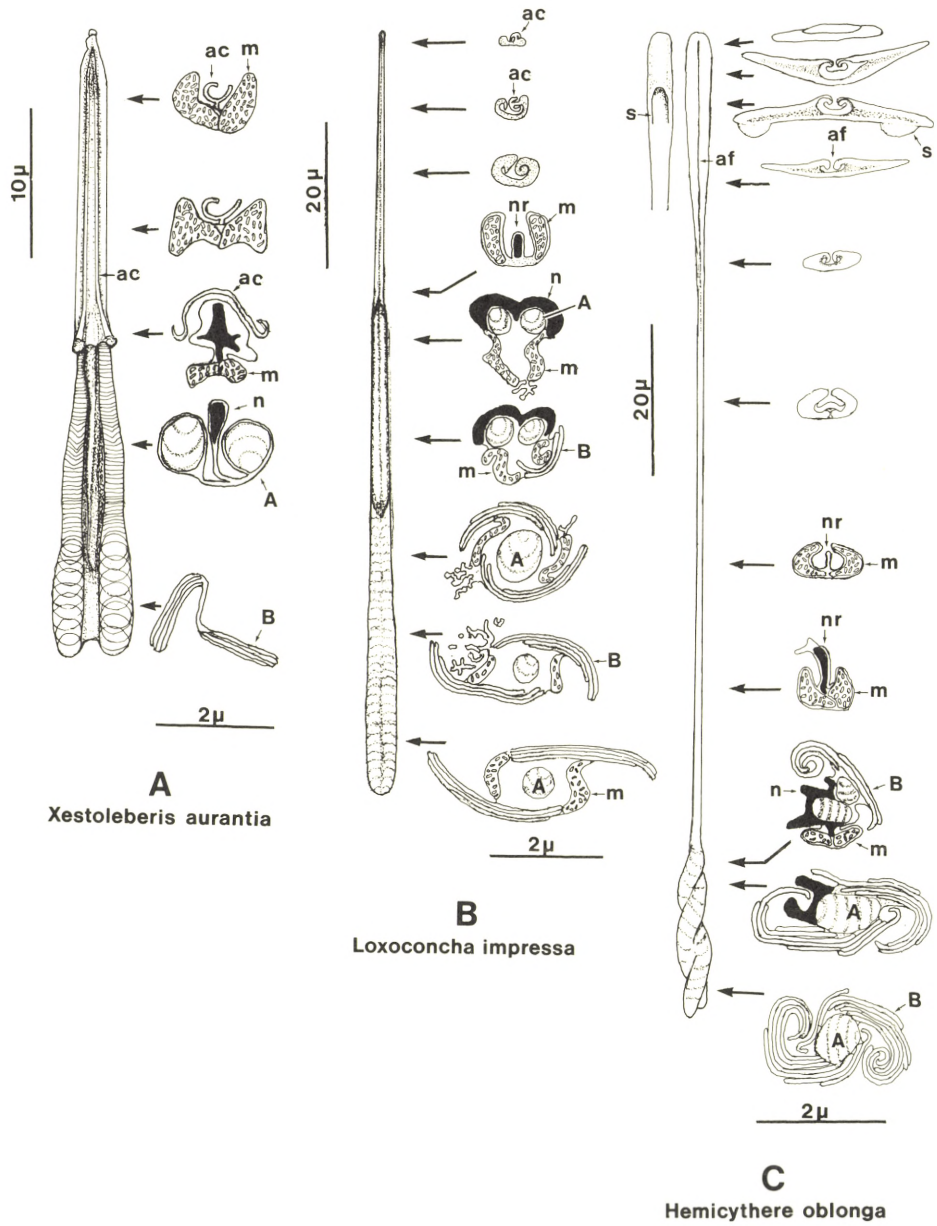
Cytheracean spermatozoa have no "contractile organelles" of the type so characteristic of the Cypridacea, and do not even have probable homologues of such organelles.

Spiral coiling, when present, is usually restricted to the "tail fin", but in *Xestoleberis depressa* and *Elofsonia baltica* the entire sperm is coiled (Fig. 7; Pl. 19:121). It is doubtful whether this coiling has anything to do with the coiling in cypridaceans, for the organelles involved are different.

TABLE 2
*Lengths of cytheracean spermatozoa (μm)*

| | Length, μm | Condition |
|---|---|---|
| Leptocytheridae | | |
| *Leptocythere lacertosa* | 100-117 | Fixed |
| Limnocytheridae | | |
| *Limnocythere reticulata* | 43 | Living |
| *Metacypris cordata* | 80-100 | Living |
| Cytherideidae | | |
| *Cyprideis litoralis* | 200-215 | Living |
| Krithidae | | |
| *Krithe bartonensis* | 400-480 | Living |
| Trachyleberididae | | |
| *Cythereis dunelmensis* | 205-220 | SEM |
| *C. echinata* | 100 | SEM |
| *C. echinata* | 90-100 | Living |
| Hemicytheridae | | |
| *Hemicythere villosa* | 80 | Fixed |
| *H. oblonga* | 120-140 | SEM |
| Loxoconchidae | | |
| *Loxoconcha elliptica* | 82-86 | Living |
| *L. impressa* | 95-107 | Fixed |
| *Elofsonia baltica* | 30-40 | SEM |
| *Hirschmannia viridis* | 70 | Living |
| Cytheruridae | | |
| *Cytherura gibba* | 380 | Living |
| *C. nigrescens* | 66-70 | Living |
| *C. sella* | 70-71 | Living |
| Xestoleberididae | | |
| *Xestoleberis aurantia* | 70 | Fixed |
| *X. depressa* | 25-31 | Living |
| *Xestoleberis* sp. 1 | 32 | Fixed |
| *Xestoleberis* sp. 3 | 49-51 | Living |
| Paradoxostomatidae | | |
| *Cytherois fischeri* | 30-35 | Living |
| *C. arenicola* | 40 | Living |

Although the shape and the proportions are variable, all cytheracean spermatozoa share a number




Figs. 5-7. The structure of spermatozoa of cytherids, drawn as outlines of mature spermatozoa from light microscopic and SEM pictures (to the left) and from TEM cross sections (right). The approximate level of each cross section is indicated. Magnification is indicated by separate scales for complete spermatozoa and for cross sections, but all cross sections of Fig. 6: D, E, and F have the same magnification (scale below Fig. 6: F).

*Legends to all figures*: A = segmented organelles A, B = segmented organelles B, ac = acrosomal crest, af = longitudinal furrow on dorsal side of acrosomal region, dr = dark rod in acrosomal crest, m = mitochondria, n = nucleus, nr = nuclear ridge in transitional zone between acrosomal and nuclear regions, s = slipperlike excavation on ventral side of acrosomal end of *Hemicythere oblonga*.

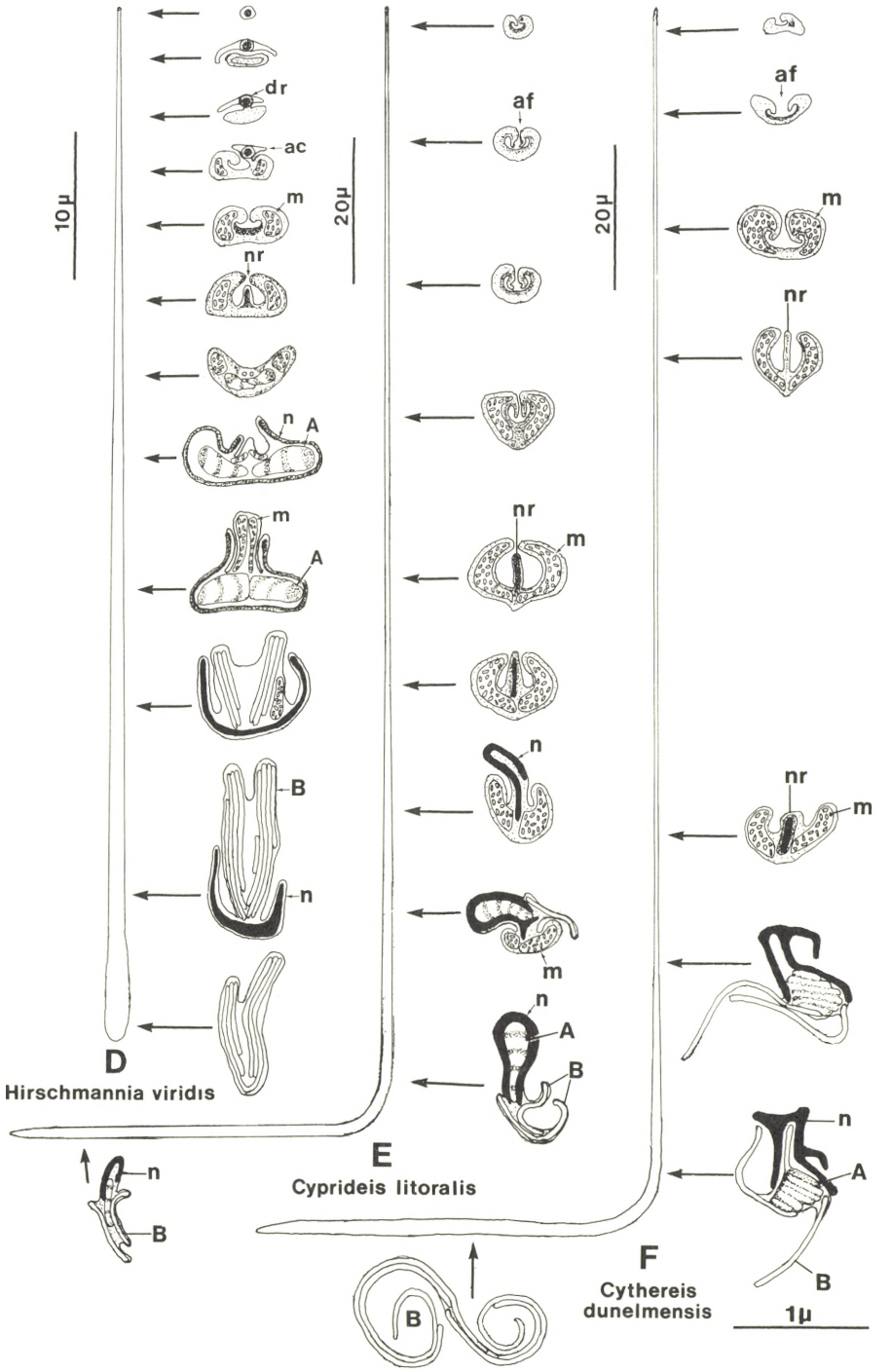



Fig. 6. The structure of spermatozoa of cytherids, continued. For legends see Fig. 5.

All cross sections of 6:D, E and F have the same magnification (See scale below F).

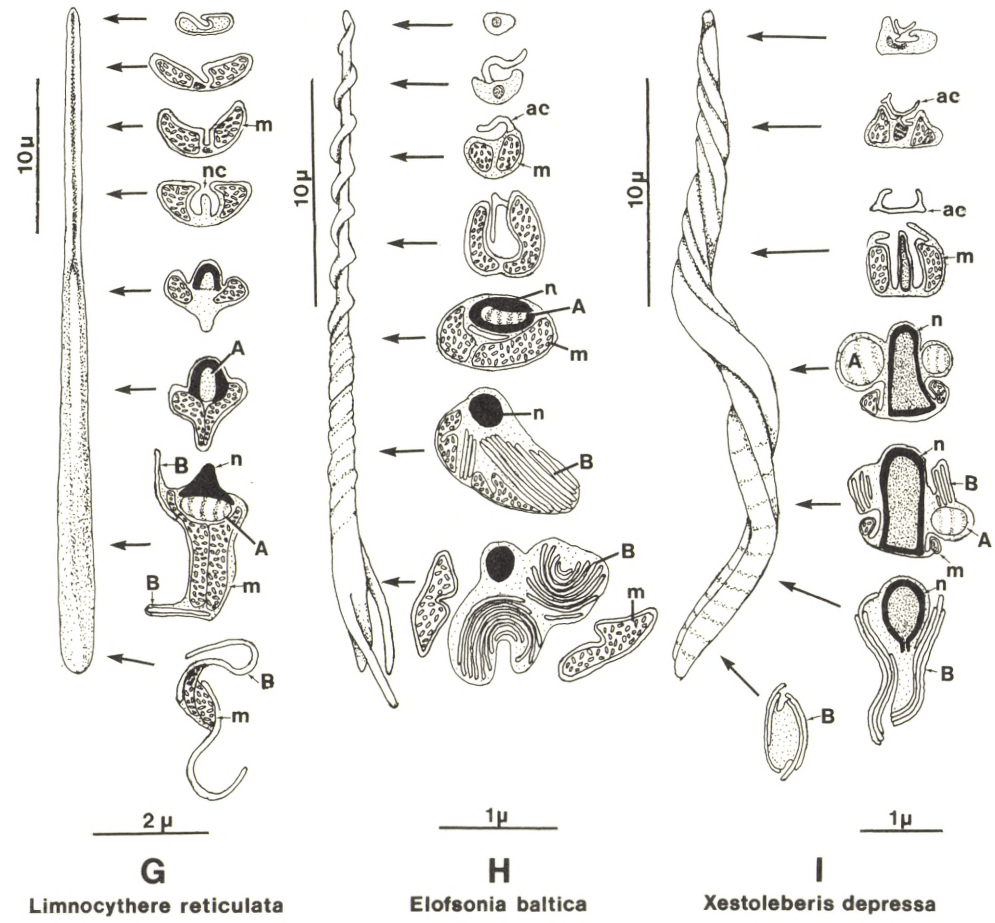



Fig. 7. The structure of the spermatozoa of cytherids, continued. For legends see Fig. 5.

of features which can be summarized as a "cytheracean spermatozoan pattern". In mature spermatozoa this pattern involves a regional differentiation into three parts (Figs. 4-7):

1) *An acrosomal region*, containing an acrosomal vesicle, a perforatorium and the anterior parts of the paired mitochondria, with all three structures strongly modified. With rare exceptions a longitudinal "acrosomal crest" with a complicated, species-specific cross section is present (Pls. 14, 15).
2) *A nuclear region*, dominated by the bandlike nucleus. This region usually contains the hind parts of the two mitochondria and of some structures called "segmented organelles A".
3) *A flattened tail fin*, sometimes coiled, and always supported by some structures called "segmented organelles B".

As seen in Figs. 5-7, this pattern is modified in several ways. Overlapping of organelles, particularly of mitochondria and segmented organelles, from

one region to another is sometimes seen. But the cytheracean pattern can always be recognized. This common basic structure of cytheracean spermatozoa is underlined by spermatogenesis, which is very similar in early stages of all examined species.

Cytheracean spermatozoa are very often strongly asymmetrical in cross sections, particularly in the spermatid stages. This often makes it difficult to follow the organelles from one section to another through long spermatozoa.

I have tried to maintain the terms "ventral" and "dorsal" for descriptive purposes, calling the nuclear side in cross sections for dorsal. However, I am far from sure that these terms cover homologous orientation in other ostracods, in which "dorsal" and "ventral" are used in an analogous way.

The main organelles and variations of the cytheracean spermatozoa are described below, but I have given up trying to describe the structure of each species separately. Instead, the reader is referred to the diagrammatic figures (Figs. 5-7) and to the SEM and TEM photographs (Pls. 14-22) showing the spermatozoa of some species.

*The acrosomal region* is long and straight, between 0.4 μm and 1.9 μm thick in most species. The spoonlike anterior end of the sperm of *Hemicythere oblonga* is 3 μm broad and is exceptional (Fig. 5). The acrosomal region occupies about half of the length of the sperm or a little less. Only *Elofsonia baltica* and *Xestoleberis depressa* among the examined species show distinct spiral coiling of the acrosomal region (Fig. 7).

In many species a longitudinal furrow cuts into the acrosomal region from the surface and partly separates the two mitochondria inside. When this furrow is present, the acrosomal crest is often attached inside it, often in a strongly asymmetrical way (Pl. 14:90; Pl. 15:94-99; Pl. 22:140).

In some species the furrow is supported on the plasmatic side by a dark mantle (Pl. 15:97; Pl. 22:140). It is tempting to homologize this furrow (with its mantle) with the furrow on the acrosomal region of *Bairdoppilata cushmani*, but the cytheracean furrow seems to be dorsal whereas that of *Bairdoppilata* is ventral. If the furrows are homologous it is probable that the definition of dorsal and ventral is incorrect in one of these ostracods.

Whatever the case, the furrow is not a simple mechanical consequence of the presence of the two mitochondria inside the acrosomal region, for the furrow sometimes extends beyond the anterior tips of the mitochondria, as in *Hemicythere* (Fig. 5), and *Cytheropteron* has no furrow but distinct, paired mitochondria are present (Pl. 14:88, 89).

*The acrosomal crest* begins at the anterior tip of the spermatozoon, which it actually forms, and passes posteriorly for a longer or shorter distance, attached inside the longitudinal furrow if such is developed (Pls. 14, 15). In *Cytheropteron* there is no furrow and the crest is attached to the convex side of the acrosomal region (Pl. 14:88, 89).

In cross section the acrosomal crest is often very complicated (Pls. 14, 15). It is usually double, consisting of two unequal lamellae, which in some species are rolled up (Pl. 14:90, 91) or supplied with secondary lamellae (Pl. 14:92).

The variations are often surprisingly great, even between species of the same genus (Pl. 14:88, 89; Pl. 15:98, 99). The appearance of the crest can therefore probably be used as a diagnostic feature at the species level, for different specimens within the same species appear identical.

The crest becomes simpler and lower and the furrow, if present, disappears when the anterior end is approached. This end is a simple conical point which seems to be formed by the crest.

Posteriorly the crest may end before reaching the end of the acrosomal region, but in the species of *Xestoleberis* and *Cytheropteron* it extends over the transitional zone to the nuclear region. Here it becomes a broad plate which loses contact with the spermatozoon, extending as a free apron over the anterior end of the nucleus (Fig. 5; Fig. 7; Pl. 16:101).

The core of the crest is usually filled by medium-constrast amorphous matter, but in some species it contains a dark, longitudinal rodlike condensation (Pl. 14:90; Pl. 15:94-95).

The surface of the acrosomal region is covered by a thickened plasma membrane, often supplied with extracellular coats of different kinds. A characteristic hairlike coat is seen in the species of *Cytherois* (Pl. 14:93), and the coats of species of *Cythereis* and *Paradoxostoma* contain small regularly spaced longitudinal rods which are darker than the normal coat substance (Pl. 14:90; Pl. 16:105).

The numerous mitochondria of early spermatids fuse to form two giant mitochondria, closely attached to each other as a Nebenkern. This is originally located at the posterior end of the nucleus, but the two mitochondria later elongate and grow forwards along the nucleus, invading the acrosomal region of the mature sperm (Figs. 5-7; Pls. 14-15).

In mature sperm of *Xestoleberis aurantia* the mitochondria are restricted to the acrosomal region, having retracted from the regions further back (Fig. 5), but in most species they are present throughout acrosomal and nuclear regions. In *Limnocythere reticulata* and *Loxoconcha impressa* they even extend into the tail fin (Figs. 5, 7). In spermatids the mitochondria have a double envelope and typical cristae. This pattern is sometimes preserved in mature sperm (Pl. 14; Pl. 17:109), but in some species it becomes strongly modified (Pl. 15:96, 98, 99; Pl. 16:104).

The *nucleus* is always long, bandlike or rod-shaped, but its cross section varies considerably from species to species (Pl. 17:106-112; Figs. 5-7).

The anterior end of the nucleus is usually lodged in a median ridge in the furrow between the mitochondria in the transitional region between acrosomal region and nuclear region. This ridge could be a backward extension of the acrosomal crest, but the two ridges are clearly separated from each other, and I have no evidence for these ridges being identical (see *Xestoleberis* and *Loxoconcha,* Fig. 5).

In *Xestoleberis aurantia* the nuclear ridge continues backwards, containing the nucleus in its free margin, whereas the basal part of the ridge is reduced to a mesentery-like thin lamella (Fig. 5; Pl. 16:100). In species of *Loxoconcha, Cyprideis, Cythereis* and in *Hemicythere oblonga* the anterior part of the nucleus is also inside such a nuclear ridge, but the posterior part expands and forms a plate covering the dorsal aspect of the spermatozoon or shows more complicated configurations in cross sections (Figs. 5-7; Pl. 17:109-112).

In *Hirschmannia viridis* the nucleus seems to start in a typical nuclear ridge but further back probably shifts in some way to the opposite side of the sperm where it forms a superficial plate (Fig. 6; Pl. 17:106). I have not been able to analyze this very long and thin spermatozoon completely.

A flat but spirally coiled nucleus was found in cross sections of the tail fin of *Paradoxostoma variabile* but this species was also difficult to analyze completely (Pl. 17:107).

*Segmented organelles A and B* are previously undescribed, very characteristic structures of cytherid spermatozoa. They consist of flattened sacs with simple walls, piled up in columns and more or less densely packed. The two kinds, organelles A and B, are both variable and show only relative differences, so they are difficult to keep apart and define critically. In *Xestoleberis aurantia* even a continuous change from organelles A to organelles B was seen when the segemnted organelles were followed in a posterior direction (Fig. 5).

However, I have maintained organelles A and B as subclasses of segmented organelles, for most spermatozoa clearly have two (one pair) of each kind, and organelles A and B have different and probably constant localization during spermatogenesis (Pl. 16:102-103; Pl. 22:138, 139, 141).

*Segmented organelles A,* when typical, are two symmetrical columns of vesicles in the nuclear region. The vesicles are flattened but have a distinct lumen, often filled with dark, amorphous matter so that the organelles may be very dark in sections (*Xestoleberis* and *Loxoconcha,* Pl. 16:103; Pl. 22:139, 141). The vesicles in such an A-column are more or less packed together, in contact with their flattened sides.

In early spermatids there are two independent A-organelles, and they often remain independent in mature spermatozoa, e.g., in species of *Xestoleberis*

(Fig. 5; Pl. 16:100, 103). In species of *Loxoconcha* the two original rods fuse into one in the posterior, finlike tail (Fig. 5). In other species they may be modified in different ways, e.g., being encircled by the nucleus as in *Elofsonia baltica* (Fig. 7) and *Cyprideis litoralis* (Fig. 6).

*Segmented organelles B* consist of more compressed sacs in which the lumen is reduced to a narrow cleft with light contents (Pl. 16:102; Pl. 18:113-118; Pl. 22:138, 139, 141). The successive flat sacs in a row imbricate (Pl. 16:102). This arrangement can be seen also in SEM pictures (Pl. 18:113, 114).

The two symmetrical rows of segmented organelles B form the flat lateral parts of the tail fin in all examined species except *Elofsonia baltica*, in which the organelles B are present in the tail but are embedded in a conical, massive portion of plasm (Fig. 7; Pl. 19:121). The two plates of the tail fin form a single plane surface in *Xestoleberis aurantia*, *Loxoconcha impressa* and *L. elliptica*, but are usually bent around the axis as a spiral configuration. The spiral arrangement is distinct in species of *Cytheropteron* and *Cytherura*, in *Hemicythere oblonga* and in species of *Cythereis* (Figs. 5-7; Pl. 18:113-118).

*Surface microvilli.* A dense growth of long, microvilli-like, branching plasma processes is present along one margin of the posterior spermatozoon in some species, e.g., *Krithe bartonensis* and species of *Loxoconcha* and *Cythereis* (Pl. 19:119, 120). The microvilli have a plasma membrane on the surface. They are present also on spermatozoa taken from the receptaculum of females, so they are not a temporary, developmental phenomenon.

## 7.4. *Spermatogenesis*

Stages of spermatogenesis were seen in most species of which males have been sectioned for TEM. They indicate that early spermatogenesis is very similar in all cytherids examined.

The lobes of the testicle contain nests of cells in which spermatogenesis is simultaneous. The early spermatids are large polygonal cells with an oval nucleus and scattered normal mitochondria.

Differentiation begins when the mitochondria concentrate to a Nebenkern on one side of the nucleus. The Nebenkern consists of two closely attached giant mitochondria (Pl. 19:122, 123). A very large acrosomal vesicle is formed on the opposite side of the nucleus, while a large Golgi apparatus is situated nearby (Pl. 19:123, 124).

In some species the acrosomal vesicle is at an early stage filled with dark, amorphous matter (Pl. 19:123; Pl. 20:125). The adnuclear part of its lumen becomes complicated by a 3-dimensional net of tubules, which develop from the surface of the vesicle (Pl. 19:124; Pl. 20:125; Pl. 21:129-130). The acrosomal vesicle attains contact with the plasma membrane with a plane or somewhat convex surface (Pl. 20:125).

The next thing to happen is that a perforatorium develops in the space between acrosomal vesicle and nucleus. It does not invade the nucleus but extends into the acrosomal vesicle, invaginating its wall and forming a tube into which the perforatorium grows (Pl. 21:130).

The perforatorium grows further in the anterior direction, obviously causing the acrosomal vesicle to extend and form a long anterior spear (Pl. 21:133). This spear is surrounded by some plasm and a plasma membrane which becomes thick and distinct. When the acrosomal spear with the perforatorium inside has grown out in front of the nucleus, the prospective acrosomal region is defined (Pl. 21:132, 133).

Each of the two large mitochondria in the Nebenkern, originally situated behind the nucleus, sends out a narrow tongue in an anterior direction. These mitochondrial tongues follow the nucleus symmetrically and grow further into the acrosomal region on either side of the perforatorium (Pl. 22:135-141).

Great and poorly understood changes take place in the acrosomal region during further development. The perforatorium, in earlier stages distinct from the end of the nucleus to the top of the acrosomal spear, becomes irregular and shrinks (Pl. 21:131-133; Pl. 20:128). The acrosomal vesicle, originally surrounding the perforatorium, also

shrinks. In intermediate stages it is reduced to a double-walled tube around the perforatorium (Pl. 21:131; Pl. 22:135), and its top proliferates in several species and forms asymmetrical sacs, some with dark contents, others seemingly empty (Pl. 20:126-127). It can be supposed that some of these vesicles, like the perforatorium, contribute to the acrosomal region and the acrosomal crest, but details of this development could not be followed in the present material, partly because of the strong asymmetries.

The remnants of the perforatorium remain for some time in the axis of the acrosomal spear (Pl. 22:136) but finally disappear. The 3-dimensional net of tubules in the base of the acrosomal vesicle remains intact and in contact with the nucleus to fairly late stages (Pl. 20:128; Pl. 22:134, see also Pl. 19:124; Pl. 21:129), but is not preserved in mature stages.

*The paired "vanes"*, not previously mentioned, are temporary structures which are prominent on the acrosomal region of late spermatids of all species examined. In cross sections they are seen as a pair of slender symmetrical "legs", usually attached in the furrow between the mitochondria (Pl. 22:135-137). A dark and distinct rod is always seen at the base of each "leg" and can perhaps be the structure inducing the development of the vane. These basal rods are formed early in the contact zone between plasma membrane and acrosomal vesicle and extend longitudinally below the surface of the acrosomal region (Pl. 22:134). The dark rod at the base of each vane may perhaps also serve as a guide for the mitochondria when these enter the acrosomal region, for the mitochondria are always attached to the rods when they appear at this level.

Asymmetrical development of the vanes was seen in a few species, particularly in *Elofsonia baltica* (Pl. 22:137). The vanes disappear during final maturation, and no clear observations support a theory that they should contribute to the acrosomal crest or the nuclear ridge, which become visible in later stages in similar locations.

*Nucleus*. When the nucleus elongates it becomes a long band. In *Loxoconcha elliptica* it is flattened, forming a thin plate which is S-shaped in cross sections (Pl. 22:141). Its shape is modified in very different ways in other species.

The mitochondria, the segmented organelles A and B, and the nucleus form a characteristic pattern in spermatids of many species (Pl. 22:138, 139, 141). Following the segmented organelles during their development proved difficult because of the confusing asymmetries of the nuclear region. This can be described as a rotational symmetry, like the symmetry of an S, and terms such as dorsal, ventral, lateral, etc., cannot be defined (see Pl. 22:141). Organelles A and B are recognizable as such from an early stage, at least in *Loxoconcha*, for the sacs of organelles A are filled with dark matter and therefore appear very dark both in cross- and longitudinal sections.

In the more advanced stages organelles A remain in the nuclear region in contact with the nucleus, whereas organelles B, which have a more superficial position, slide backwards and finally form the skeleton of the "tail fin" (compare with Pl. 18; Fig. 5).

### 7.5. *Movements of cytheracean spermatozoa*

When living females of *Loxoconcha impressa* and *L. elliptica* were dissected or crushed in sea water under the microscope, the spermatozoa could be seen moving. They bent intermittently and rapidly and then straightened out again, much like small nematodes. This was confirmed several times with material from different localities, but the movements were never seen to result in real locomotion.

No such movements were ever seen in other cytheraceans although living females of many species were examined under similar conditions. It should be particularly remarked that males and females of *Elofsonia baltica*, a species earlier referred to *Loxoconcha* (as *Loxoconcha baltica* Hirschmann), were examined with negative results. The spermatozoa of this species differ strikingly from those of *L. elliptica* and *L. impressa* (Pl. 19:121; Figs. 5 and 7).

Retzius (1909:6) observed movements of the sperm in *Loxoconcha rhomboidea* Fischer (= *L. elliptica* Brady) and even talks of "lebhafte Bewegungen", but this would be an overstatement for my observations.

Unfortunately the movements of *Loxoconcha* sperm are not very informative, for they do not result in regular swimming, and I was not able to correlate the bending with the structure of the organelles.

## 7.6. *Comments*

Cytherid spermatozoa can always be recognized because of the presence of "segmented organelles", a kind of structure always present in Cytheracea and restricted to this superfamily.

The mitochondria of cytherid spermatids form a large double Nebenkern as in other podocopid ostracods. This is in agreement with the general belief that cytherids belong to the podocopid line, but as there is uncertainty about the apomorphic state of the Nebenkern, the conclusion is not very strong.

A perforatorium is formed as in the majority of ostracods, and invades the acrosomal vesicle, but it does not extend into the nucleus as in spermatids of bairdiids and cypridids.

Although variation is surprisingly great between genera and species of Cytheracea, some general features can be compared with those of Bairdiacea, supporting the relationship accepted by most recent taxonomists.

The acrosomal vesicle in both superfamilies is very large and looks similar in early spermatids, and it finally disintegrates in both superfamilies, probably contributing, together with the perforatorium, to the long and narrow acrosomal region. The longitudinal furrow on the surface of this region, distinct in *Bairdoppilata cushmani* and many cytheraceans, may also be a homologue, but the comparison is made uncertain by the confusing symmetry conditions in cytheraceans.

# TYPE 8. SUPERFAMILY CYPRIDACEA

## 8.1. *Material*

Fam. Macrocyprididae

*Macrocypris minna* (Baird). – Säcken, Koster, Sweden, 2.VI.1975, 1 ♂, 3-A.

*Macrocypria angusta* G. O. Sars. – Säcken, Koster, Sweden, 120 m, 1.VI.1975, ♂♂, 2 % $OsO_4$. – Fanafjord, Espegrend, Norway, 150-160 m, ♂♂ + ♀♀, 26.IX.1976, 3-A. – Korsfjorden, Espegrend, Norway, 640 m, 31.V.1976, ♂♂ + ♀♀, 3-A.

Fam. Ilyocyprididae

*Ilyocypris* sp. – Culture of mud from dry pond, 20 km S. of Casablanca, Morocco, 10.IV.1977, ♂♂ + ♀♀, 3-A, coll. Å. Jespersen and J. Lützen.

Fam. Pontocyprididae

*Pontocypris trigonellus* G. O. Sars. – Espegrend, Norway, in the harbour, 27.IX.1975, ♂♂ + ♀♀, 3-A.

*Erythrocypris mytiloides* (Norman). – Espegrend, Norway, 1.VI.1976, ♂♂ + ♀♀, 3-A.

*Argilloecia cylindrica* G. O. Sars. – Säcken, Koster, Sweden, 160 m, 2.VI.1975, 1 ♀, 2 % $OsO_4$.

*Propontocypris litoricola* Maddocks, 1969. – Mauritius, near shore, 2.I.1978, 1 ♂ + ♀♀, 3-A, coll. J. Dyck.

Fam. Candonidae

*Aglaiocypris* sp. – Cultured from mud collected on Barbados, 1976, ♂♂ + ♀♀ 3-A, coll. J. Just.

*Candona suchi* Hartwig, 1901. – Zoological Central Institute, Copenhagen, in artificial pond. This rare and little known species has been living here since 1970 and has been collected several times in the 1970's, ♂♂ + ♀♀, 3-A.

*C. sarsi* Hartwig. – Løg Sø, Zealand, Denmark, 13.VIII.1974, ♂♂ + ♀♀, 3-A.

*Cyclocypris ovum* (Jurine). – Copenhagen, Denmark, small pond. Living specimens, ♂♂ + ♀♀, for studies of sperm dimensions.

*Cypria ophthalmica* (Jurine). – Zoological Central Institute, Copenhagen, small artificial pond, 28.V.1974, ♂♂ + ♀♀, 3-A.

Fam. Cyprididae

*Notodromas monacha* (O. F. Müller). – Løg Sø, Zealand, Denmark, 8.VIII.1974, ♂♂ + ♀♀, 2 % $OsO_4$.

*Heterocypris incongruens* (Ramdohr). – Culture of dry mud from Ramle, Sinai, Israel, 21.II.1973, ♂♂ + ♀♀, 2 % $OsO_4$, coll. C. Dimentman.

*Eucypris* sp. (*?cisternina* Furtos). – Culture of dry mud from Silver Lake, California, 17.XII.1977, ♂♂ + ♀♀, 3-A, coll. Å. Jespersen and J. Lützen.

*Chlamydotheca* sp. (? *flexilis* (Brady)). – Culture of mud from Barbados, 1976, ♂♂ + ♀♀, 3-A, coll. T. Wolff and J. Just.

Fam. Cypridopsidae

*Cypridopsis* sp. – Culture of dry mud from Barbados, 1976, ♂♂ + ♀♀, 3-A, coll. T. Wolff and J. Just.

*Potamocypris* sp. – Culture of dry mud from Barbados, 1976, ♂♂ + ♀♀, 3-A, coll. T. Wolff and J. Just.

## 8.2. *Genital organs*

Cypridacean genital organs are paired, with one complete set on each side. The gonads are generally dislocated into the mantle folds under the valves, a unique situation in ostracods. The only exception in Cypridacea from this rule is the Macrocyprididae, in which the gonads lie inside the posterior body as in noncypridacean ostracods (G. W. Müller 1894, 1927; G. O. Sars 1928:7).

The *male* organs of *Candona suburbana* are well described by Kesling (1957), and the following additional works indicate that his description has a fairly general validity within the Cypridacea: Lilljeborg (1853), Weismann (1880), Nordquist (1885), Stuhlmann (1886) and G. W. Müller (1894, 1927). The older papers, particularly those of Zenker (1850, 1854) and the early works of G. W. Müller (1880, 1889) are somewhat confusing because of misinterpretation of the ejaculatory duct.

The testicles of the macrocyprids are four pairs of rounded sacs which fill part of the posterior body region. The other cypridaceans have instead four tubelike testicles lying in the mantle fold under the posterior part of each valve. The following description follows Kesling's account of *Candona suburbana*.

After a somewhat variable course the four testicular tubes on each side fuse and form a vas deferens which is a long duct with loops both in the mantle fold and inside the body proper. In pontocyprids it forms a prominent spiral whorl under the anterior half of each valve.

The vas deferens finally enters the seminal vesicle, which is a saclike expansion of the duct inside the anterior part of the body and serves as a store for mature spermatozoa. The vesicle on each side continues as a strongly muscular ductus ejaculatorius. This has a cuticular lining from which cuticular spines extend into the surrounding muscle sheath of the wall. The ductus ejaculatorius is often called "Zenker's organ" and has been extensively studied by taxonomists, who use the number of whorls of cuticular spines as an important taxonomic character (see Sars 1889, G. W. Müller 1894:142-146). From the ductus ejaculatorius the sperm duct leads down to the inner lobe of the ipsilateral penis.

G. W. Müller (1894, 1927) has described the complicated mechanism by which the variable orientation of the spermatozoa is readjusted so that all pass the ejaculatory duct with the same end first.

The *female genital system* is only briefly mentioned in most current texts, but details are given by G. W. Müller (1894, 1927). The paired ovaries are situated in the mantle fold under the posterior end of the valves, except in the Macrocyprididae, which have ovaries inside the posterior body like noncypridacean ostracods. The paired oviducts are sometimes difficult to follow, but their terminal parts form the so-called vagina, which is distinct and opens in front of the furca behind the last pair of limbs.

There is a paired recptaculum seminis in the female, also in species which are known to be parthenogenetic (!) (Weismann 1880, Lowndes 1935). The receptaculum is connected with the vagina by

a single very thin and coiled duct, which may have a considerable length when straightened out. The spermatozoa must obviously pass upwards from the vagina to the receptaculum during copulation, and they must use the same duct downwards to the vagina in order to fertilize the eggs during oviposition.

According to G. W. Müller (1927:418), *Macrocypris* has two receptaculum ducts, presumably homologous with the two canals which connect the receptaculum with the outside in the Bairdiidae, Cytheridae, and some Myodocopida.

What really happens during copulation when sperm is transferred from the male penis to the receptaculum is not known, although some ideas have been expressed (e.g., Klie 1938:13). This may perhaps be a kind of passive transport, caused by muscular contractions of the ducts in the male and the female. But the downward travel to the vagina during oviposition is probably active swimming performed by the spermatozoa which rotate around their axis when they move. Such swimming through the receptaculum duct was observed directly by Lowndes (1935:40).

The rotatory movements and the drill-like anterior end of the spermatozoa in most species also suggest that the actual syngamy occurs by active boring of the sperm into the egg (López-Camps et al. 1979). This has not been directly observed but Lowndes (1935:407) saw a spermatozoon of *Candona compressa* bore into an egg, but incompletely, stopping short very soon. Bauer's (1940:630) observation on *Heterocypris incongruens* makes it almost necessary to assume that the spermatozoon bores into the egg in this way. After fertilization Bauer found the long (1.3 mm) spermatozoon wound up in 3.5 parallel coils under the membrane of the much smaller eggs.

## 8.3. *Mature spermatozoa*

*Size*

Cypridacean spermatozoa are filiform and have long been known as some of the largest in the animal kingdom. They are usually between 0.2 and 1.5 mm long (Table 3), but larger spermatozoa are found, e.g., in *Cypricercus dentifera* (4 mm), *Chlamydotheca* sp. (4.74 mm) and *Propontocypris monstrosa* (5.0-7.0 mm). The latter has a body length of only 0.6 mm, so the spermatozoa are about 10 times as long as the animal (G. W. Müller 1927:416). The maximum recorded in ostracods appears to be 10 mm, found by Bauer (1940:630) in an Australian species, "*Cypris Sydnea*" (should probably be *Cypris sydneia* King, 1855).

This record is, however, beaten by the absolute lengths of the spermatozoa of some hemipteran insects: *Notonecta undulata,* 16 mm (Tandler & Moriber 1974) and *N. glauca,* 12mm (Pantel & Sinety 1906). But the relative size of the spermatozoa of these bugs is not so impressive, for the animals themselves are about 13 mm long.

*General structure, polarization*

If allowed to straighten out, cypridacean spermatozoa are long filaments, consisting of two regions with different thickness (Fig. 8). In the Macrocyprididae such regions are not distinct. In other Cypridacea the thinner part makes up ½ to ⅕ of the entire length of the sperm and usually has the appearance of a drill or a corkscrew. The thicker part has a characteristic coiled surface pattern like that of a rope (Fig. 8; Pl. 28).

Older works are uncertain which part of the sperm should be called anterior from a morphological point of view. Lowndes (1935) found that the spermatozoa of several cypridid and candonid ostracods, while remaining straight and stiff, rotate around their axis and proceed through the water with the thin end first. He therefore called this end the anterior end. Movements were only seen in spermatozoa from the receptaculum of females.

This movement was later studied by Gupta (1964, 1968), who confirmed Lowndes' results. Gupta also found that spermatids of *Notodromas monacha* develop an acrosomal vesicle and a perforatorium at the prospective anterior end; this

TABLE 3
*Dimensions of the spermatozoa of the Cypridacea*

| | Anterior region, mm | Posterior region, mm | Total length, mm |
|---|---|---|---|
| Macrocyprididae | | | |
| *Macrocypris minna* | | | 1.4-1.5 |
| *Macrocypria angusta* | | | 1.4-1.6 |
| Ilyocyprididae | | | |
| *Ilyocypris* sp. | 0.3 | 0.8 | 1.1 |
| Pontocyprididae | | | |
| *Propontocypris litoricola* | 0.09 | 0.26 | 0.35 |
| *Erythrocypris mytiloides* | 0.31 | 0.81 | 1.12 |
| Candonidae | | | |
| *Aglaiocypris* sp. | | | 1.0 |
| *Candona suchi* | 0.23 | 0.27 | 0.50 |
| *Cyclocypris ovum* | 0.28 | 0.70 | 0.98 |
| *Cypria ophthalmica* | 0.18 | 0.72 | 0.90 |
| Cyprididae | | | |
| *Notodromas monacha* | 0.40 | 0.51 | 0.91 |
| *Heterocypris incongruens* | | | 1.3 |
| *Eucypris ?cisternina* | | | 1.6 |
| *Chlamydotheca ?flexilis* | 2.24 | 2.50 | 4.74 |
| Cypridopsidae | | | |
| *Potamocypris* sp. | 0.53 | 0.80 | 1.33 |
| According to G. W. Müller (1894:134) | | | |
| *Pontocypris setosa* | 0.088 | 0.579 | 0.667 |
| *P. mediterranea* | 0.78 | 2.49 | 3.27 |
| *P. monstrosa* | | | 5.0-7.0 |
| *P. succinea* | | | 0.26 |
| *P. subfusca* | 0.06 | 0.28 | 0.34 |
| *Pontocypria spinosa* | 0.07 | 0.16 | 0.23 |
| According to Gupta (1968:118) | | | |
| *Notodromas monacha* | | | 1.0 |
| *Cypria ophthalmica* | | | 1.0 |
| *Cyclocypris* sp. | | | 1.0 |
| *Cypricercus dentifera* | | | 4.0 |
| *Candona punctata* | | | 0.6 |
| *C. truncata* | | | 0.6 |

appears to definitely settle the old problem about anterior and posterior, for an acrosome certainly marks the anterior end of animal spermatozoa.

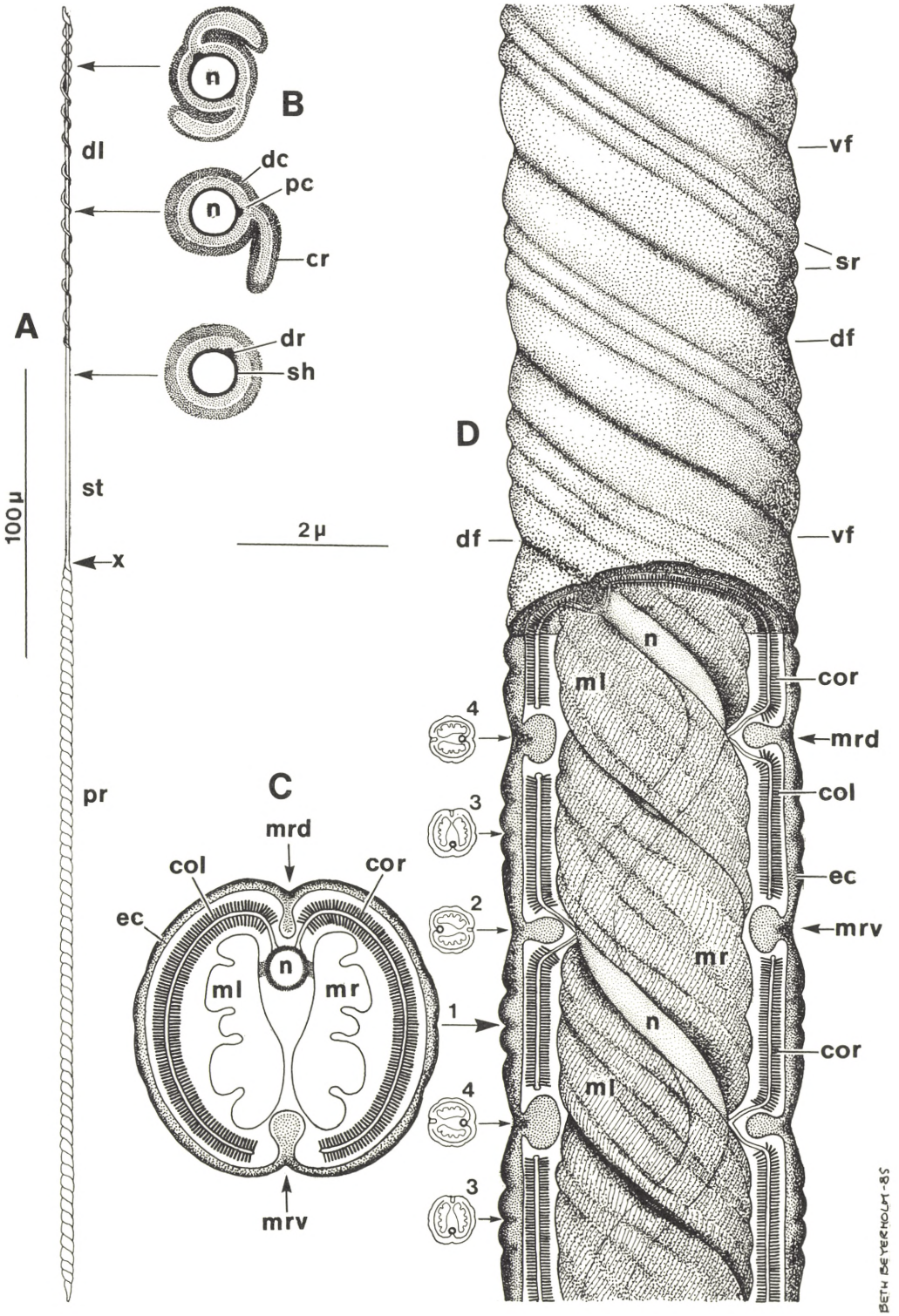
As described in the following, I have confirmed these observations on movements and acrosomal development. I consequently call the slender, often corkscrew-shaped, end anterior and the thicker, ropelike part posterior. This is in agreement with Lowndes (1935), Gupta (1964, 1968), Zissler (1969) and López-Camps et al. (1979).

The posterior end tapers and forms a kind of "end piece" which contains the posterior extremities of some of the organelles. It is about 25 µm long in *Heterocypris incongruens* (López-Camps et al. 1979) but is much less conspicuous in most species (Fig. 8). Since it contains no specific organelles I prefer the neutral term "end piece" and think it is impractical to give it a higher rank and call it "posterior region" as suggested by López-

Fig. 8. *Candona suchi*. A is a drawing of an entire spermatozoon, based on SEM. B shows three cross sections of the anterior region of a spermatozoon from the vesicula seminalis (with deciduous coat intact), based on TEM. C is a cross section of the posterior region showing the positions of internal structures at different levels, based on TEM. D is a three-dimensional reconstruction of the coiled posterior region showing the internal organelles, based on TEM cross sections of mature sperm. The drawings numbered 1 to 4 show levels of the counter-clockwise spiral (when seen from the posterior end of the sperm), separated by rotations of 90°.

All drawings in B, C and D, except the miniatures, have the same magnification (the 2 µm scale bar).

*Legends to all figures*: col = left contractile organelle, cor = right contractile organelle, cr = coiling ridge of anterior region, dc = deciduous coat, df = dorsal furrow of coat, dl = drill (or corkscrew) of anterior region, dr = dark ridge in nucleo-perforatorial sheath, ec = extracellular coat, ml = left mitochondrion, mr = right mitochondrion, mrd = dorsal monorail of coat, mrv = ventral monorail of coat, n = nucleus (nucleo-perforatorium), pc = permanent coat, pr = posterior region, sh = nucleo-perforatorial sheath, st = smooth shaft of anterior region, sr = superficial coat ridges, vf = ventral furrow of coat, x = limit between anterior and posterior regions of sperm.


A
B
C
D
n
dl
dc
pc
cr
dr
sh
st
pr
x
100 µ
2 µ
mrd
col
cor
ec
ml
mr
mrv
df
vf
sr
1
2
3
4
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Camps et al. (1979). This would interfere with the chief subdivisions of the spermatozoon, for which the terms anterior and posterior regions have been well established.

There are three principle types of cypridacean spermatozoa, all of which are variants of the same basic pattern, characterized by the contractile organelles:

*The standard type* is found – with small variations – in the families Candonidae, Cyprididae, Ilyocyprididae and Cypridopsidae. It is the type described by most recent authors and is characterized by a clear distinction between anterior and posterior regions and by dense, ropelike coiling of the posterior region (Fig. 8; Pl. 28). In the latter the contractile organelles extend just under the cell surface, covering the mitochondria (Fig. 8; Pl. 24).

*The pontocyprid type*, found in the Pontocyprididae exclusively, has a more variable and not always sharply delimited anterior region. The coiling of the posterior region is more open (less dense) and the contractile organelles are situated deeper, partly inside the mitochondria (Pl. 29).

*The macrocypridid type* has no distinct regions and little or no coiling of the posterior region. The situation of the contractile organelles differs from that of the other two types (Pl. 31).

These differences make it practical to keep the three subtypes apart in the descriptions and to begin with the best known standard type.

### 8.3.1. The standard type (Candonidae, Cyprididae, Ilyocyprididae, Cypridopsidae)

*The anterior region*

The anterior region makes up between ⅕ and ½ of the entire length of most standard spermatozoa (Table 3). In *Cypricercus dentifera* it is said to be very short, about 1/10 of the entire length (Gupta 1968).

The anterior region is a straight cylindrical rod, but the surface is usually elevated as one or two longitudinal ridges which have a coiled course (Figs. 4, 8; Pl. 23:142, 144, 146, 147). This anterior region can be bent and straightened out passively but has no inherent motility in living sperm.

The anterior end is different in different species. Usually one or both ridges increase in height towards the very point and form what looks like a drill or a corkscrew (Fig. 8; Pl. 23:142, 144). *Cypria ophthalmica* and *Notodromas monacha* lack such a terminal drill and the spermatozoon ends with a simple knob (Pl. 23:143).

In *Candona suchi* the top of the drill is formed by two parallel ridges. One of them disappears after 3-4 coils, the other continues further backwards (Fig. 8). As far as could be seen in SEM pictures, in most species the drill is formed by one ridge only. The ridges become lower and disappear posteriorly, so most species have a smooth shaft behind the drill, extending to the point where the transition to the posterior region begins. In *Heterocypris incongruens* the single ridge can be followed all the way until it merges with the coiling structures of the posterior region (López-Camps et al. 1979, and my own material).

The axis of the anterior region is usually straight, but slight indications of coiling of the axis were seen in *Cypria ophthalmica* (Pl. 23:143).

The internal structure of the anterior region looks simple, with few organelles: 1) a dominating *axial rod* or "nucleo-perforatorium", 2) a dark composite *nucleo-perforatorial sheath* around this axis, and 3) several layers of *extracellular coat substance*. In addition the cross section shows some dark bands which may be derivates of the acrosomal vesicle (Fig. 8; Pl. 23:145-147).

The axial rod is continuous with that of the posterior region and thus extends through the entire spermatozoon. It was called "nucleo-perforatorial rod" by Gupta (1964, 1968), because it is formed in spermatids from the strongly elongated nucleus and its contained perforatorium. In early spermatids the perforatorium is surrounded by an intranuclear tube formed by invagination of the anterior end of the nucleus (Pl. 33:200). In mature spermatozoa the invaginated nuclear membrane disappears and there is no clear separation between nuclear and perforatorial material (Pls. 23, 24).

In mature spermatozoa of the standard type the

axial rod usually looks homogeneous (Pls. 23, 24), although a distinct perforatorium is present in spermatids (Pl. 33:194-200). In macrocypridids there is a central core probably representing the perforatorium, but there is no membrane around it (Pl. 31). Also some pontocypridids have a similar core, which differs from the surrounding matter with regard to structure and contrast, but it is more indistinct (Pl. 29:175, 175). The perforatorium preserves its invaginated nuclear membrane to fairly late stages in spermatids, particularly in macrocypridids and pontocypridids, and this supports the above interpretation (Pl. 32).

The *nucleo-perforatorial sheath* is a thick and dense membrane surrounding the axial rod. It is clearly composite, formed by fusion of the plasma membrane with the nuclear envelope (Pls. 32-34). In the early stages the nuclear envelope is still separate from the plasma membrane outside (Pl. 34:204, 206), but in the anterior region of advanced spermatids and spermatozoa the two membranes have always fused (Pl. 23:145-147).

That the sheath is composite can sometimes be seen at the base of the ridges, where the two membranes part and give place for a dark longitudinal band (Pl. 23:146, 147). These bands, one or two in each cross section, mark the space between the nuclear envelope and the plasma membrane, where they are situated in younger spermatid stages (Pl. 34:203-206).

I do not believe that the nucleo-perforatorial sheath contains remnants of the original perforatorial tube, which is invaginated from the anterior nuclear membrane in early spermatids (Pls. 33, 34). This tube is distinct in early spermatid stages but seems to disintegrate and disappear completely.

*Extracellular coat structures* are added on the entire surface of the spermatozoon when it lies in the vas deferens and begins to spiralize. The coat material is believed to be secreted by the wall cells of the duct which surround and envelope the spermatozoa at this stage (Pl. 23:147). The coat is deposited in several (up to five) strata with slightly different structure and contrast.

In standard spermatozoa the coat consists of two functionally different main layers: an inner *permanent coat* and an outer *deciduous coat*. The latter is shed or disintegrates when the sperm is transferred to the female receptaculum. In the anterior region of the spermatozoon the permanent coat forms the pattern of ridges which coil over the surface and is the main constituent of the drill and the anterior point (Pl. 23:146, 147). The deciduous coat covers all external furrows and clefts as a uniform layer, making the superficial contour more even.

That some structures, including the drill, are formed by the permanent coat alone is shown by some species (*Ilyocypris* sp., *Chlamydotheca* sp.) in which the coat has an unmistakable ridge structure on the surface. This pattern is seen on the outer surface of spermatozoa from the receptaculum but is covered by the deciduous coat on spermatozoa from the vas deferens of the male (Pl. 24:148, 149; and Pl. 26:162-164). The coat and its main subdivisions extend over the entire sperm, also over the posterior region.

*Remnants of the acrosomal vesicle.* Early spermatids have a typical acrosomal vesicle at the anterior end of the nucleus (Pl. 33:196-199). This vesicle is at an early stage filled with dark matter (Pl. 33:198, 199). Later, when the corkscrew pattern develops in this region there is only some ill-defined dark matter left, in which an acrosomal vesicle is difficult to identify.

It is also possible that the dark bands, which follow a coiling course under the ridges of the anterior region, have grown out from the acrosomal vesicle (Pl. 33:196). This is discussed on the background of spermatogenesis in chapter 8.4.

*Posterior region*

I can confirm most of the accounts of the structure of the posterior region published by Gupta (1964, 1968), Zissler (1969a, b, 1970), Reger & Florendo (1969a, b) and López-Camps et al. (1979). The text will therefore be a kind of summary, with addition of some new facts and remarks on variation of structures.

The posterior region is dominated by the spiral pattern of all structures (Fig. 8). The characteristic organelles are all long, coiled filaments which extend throughout this region and conform to this general spiral arrangement:

1) *The nucleo-perforatorial rod,* which is unpaired.
2) *Paired mitochondrial derivatives,* situated on each side of the nucleo-perforatorial rod.
3) *Paired contractile organelles* (Müller 1889, Gupta 1968). These are unique motor structures, attached to the nuclear membrane and arching over the other organelles in close contact with the plasma membrane (Fig. 8).
4) *The extracellular coat,* continuous with that of the anterior region.

For orientation in cross sections I use the terms "ventral" for the mitochondrial side and "dorsal" for the nuclear side, following Zissler (1969a, b). I emphasize, however, that the coiling of most structures has the result that ventral and dorsal sides, defined in this way, coil around the sperm (Fig. 8).

The *nucleo-perforatorial rod* extends throughout the spermatozoon. As mentioned above, it is straight and cylindrical in the anterior region. In the posterior region of standard-type spermatozoa it is regularly coiled and its cross section often changes to triangular, 8-shaped or with lateral horns in different species (Fig. 8; Pls. 24, 27). It is surrounded by a dense membrane, which seems to be the original nuclear envelope. The plasma membrane remains on the surface of the cell and is not fused with the nuclear membrane as in the anterior region (Pl. 25:154).

In standard-type spermatozoa the nucleo-perforatorial rod is regularly coiled, at a constant depth under the surface of the sperm, as seen in Fig. 8. It has a more central position than the other organelles.

Spermatogenesis shows that the rod contains both nuclear and perforatorial material. The localization of DNA under these circumstances is problematic. Feulgen reaction on light microscopical sections proved difficult because of the small dimensions and the birefringence of the structures. In TEM cross sections there is often some high-contrast material, sometimes located in the lateral "horns" (Pl. 24:152), but critical localization of DNA was not successful in my preparations. Gupta (1964, 1968) found that the chromatin is concentrated in the posterior part of the long nucleus or (in *Candona*) in its middle part. He based his conclusion on the Feulgen reaction and on the presence of birefringence (Gupta 1968:120).

The mitochondrial derivatives, formed by elongation of the two gigant mitochondria of the Nebenkern in early spermatids, extend as two flattened coiled bands throughout the posterior region. In cross sections they are symmetrically disposed in relation to the nucleo-perforatorial rod and follow its coiling throughout (Fig. 8; Pls. 24, 27). They are attached to the ventrolateral aspect of the nuclear envelope by some dark matter. In standard-type spermatozoa they are completely inside (covered by) the contractile organelles (Fig. 8; Pls. 24, 27).

The outer surface of the mitochondrial bands is often lobulated in cross sections. The lobules correspond to parallel ridges which follow the general coiling of the mitochondrion (Fig. 8).

The mitochondria have regularly spaced lamellar cristae which stand at right angles to the coiling mitochondrial axis. They are therefore best seen in longitudinal sections and in spermatids in which coiling is incomplete (Pl. 26:161).

Reger & Florendo (1969b) described dark "filaments" in the center of the cristae of *Cypridopsis* sp. and suggested that these filaments may play a role in the contractions (movements) of the cell. In my material the cristae contain what appears to be a dark lamella in several species, particularly in *Chlamydotheca* sp. (Pl. 26:161). It is about 70-80 Å in diameter and obviously corresponds to the "filament" seen by Reger & Florendo in *Cypridopsis*. But I must remark that the dark matter has the shape of a flat lamella or plate. If it had the shape of a filament it would not be regularly cut as in my Pl. 26:161 or in Reger & Florendo's fig. 13 (1969b). It must also be added that the cristae are regularly

lamellar in cypridacean spermatozoa, not tubular as indicated by Reger & Florendo.

*The contractile organelles* are the most remarkable structures of cypridacean spermatozoa. They were called "contractiles Band" by G. W. Müller (1889), "contractile bands" by Gupta (1964, 1968), "couche tubulaire" by Tétart (1967), "Flügelstrukturen" by Zissler (1969a, b), "membranaceous organellae" by Reger & Florendo (1969a, b), and "feather-like organellae" by López-Camps et al. (1979).

These organelles are a pair of long flat plates with membranous walls, covered on both sides by densely set "pins"; the organelles attach symmetrically to the dorsolateral aspect of the nuclear envelope and follow the coiling of the nucleo-perforatorium throughout the posterior region (Fig. 8; Pls. 24, 25, 27).

Cross sections show that the contractile organelles attach to the nuclear envelope dorsal to the mitochondria (Pls. 24, 25:154). From here they extend symmetrically just under the plasma membrane, arching over the other organelles in a wing-like fashion (Fig. 8). They do not meet ventrally but leave an interspace which in standard-type spermatozoa is filled by an internal ridge of the permanent coat, called the "ventral monorail" by Gupta. The ventral margins of the two contractile organelles attach from each side to this "monorail" (Pl. 25:156; Fig. 8).

The supporting structure of each contractile organelle is a flat "core-sac" (Gupta 1968) with a narrow, cleftlike lumen, delimited on each side by a thick wall, each originally consisting of two unit memebranes. Basally each of the walls of the core-sac is continuous with the outer nuclear envelope, as described by Gupta (1968:fig. 2). Each wall of the core-sac can thus be regarded as a fold of the outer nuclear membrane and would be expected to consist of two trilaminar membranes with an extension of the perinuclear space between them. In mature spermatozoa this space is obliterated and the trilaminar structure of the two membranes is no longer distinct (Pl. 25:154, 158, 159). The lumen enclosed between the two thick walls of the core-sac is an enclosed extranuclear space (Pl. 25). The development of the core-sac supports this description, but the endoplasmic reticulum also contributes some elements (Chapter 8.4).

The "pins" form a uniform and continuous cover on both sides of the core-sac. In standard-type spermatozoa the pins are slightly conical rods, about 1000 Å long and 200-300 Å in diameter. In mature spermatozoa they look compact or indistinctly cross-striated. The base is usually somewhat enlarged and bulb-shaped, forming a slightly convex foot plate (Pl. 25:159). This is attached to a slight depression of the external membrane of the core-sac wall, but in some sections no such depression is visible (Pl. 25:158).

Sections passing tangential to the surface of the core-sac show that the pins are attached in a hexagonal pattern, in which the distance between adjacent pins is about the same as the diameter of the single pins. In some spermatozoa the surface of the pins shows projections and blebs (probably artifacts) but regular bridges between nearby pins were also seen in some cases (Pl. 25:157, 159). Such bridges were also observed by Gupta (1968:fig. 2). It is possible that these bridges are contractile and are involved in bending the contractile organelles when the spermatozoon moves.

Some additional structures which may be significant for the movement will be mentioned, although they are not really understood. Gupta (1968:121) described an almost continuous layer of long fine filaments on the luminal side of the walls of the core-sac. The filaments are parallel, with the same direction as the axis of the contractile organelles, and Gupta thinks that they follow the the coiling throughout the posterior region (Pl. 25:155, 156).

These coiling filaments in the lumen of the core-sac are visible in several standard-type spermatozoa in my collection, e.g., in *Heterocypris incongruens* (Pl. 25:156), but they are very high and distinct in the pontocypridid *Propontocypris litoricola* (Pl. 25:155).

Another structure which may be relevant for movements was only seen clearly in *Aglaiocypris* sp. (Pl. 26:160). It is a distinct zone, cross striated with 130 Å periodicity, which covers the top of the pins on the mitochrondrial side of the contractile organelles. It is perhaps present in other species as well but was not so clearly preserved.

*The extracellular coat* is continuous with that of the anterior region. It is added to the surface of the spermatozoon in the first part of the vas deferens, probably by secretion of the wall cells. In standard-type spermatozoa it consists of an inner permanent coat and an outer deciduous one. Both are present on spermatozoa in the vesicula seminalis of males, but the deciduous coat has disappeared from all spermatozoa in the female receptaculum.

As mentioned above, the permanent coat forms the coiling ridges on the surface of the anterior region and also the corkscrew at the anterior end.

In the posterior region the permanent coat has deep furrows which correspond to similar furrows of the plasma membrane and reflect the coiling pattern of internal organelles. In addition the permanent coat can develop secondary patterns of ridges, furrows and valves, and can also develop coiling ridges from the inner side, called "monorails" (Fig. 8).

The existence of a deciduous coat in the posterior region is best illustrated in genera such as *Ilyocypris*, *Chlamydotheca*, *Candona*, *Aglaiocypris* and *Potamocypris*, in which the permanent coat has a recognizable and characteristic surface pattern (Pls. 26-28). When both coat strata have been formed in the male, this pattern is seen (in sections) on the surface of the inner, permanent layer, for instance in *Ilyocypris* (Pl. 27:167; Pl. 26:162). Spermatozoa transferred to the female show this pattern on the external surface for the deciduous coat has disappeared (Pl. 26:164). This pattern can also be seen on the surface of empty coats, which remain in the receptaculum a long time after the contents of the spermatozoa have disintegrated (Pl. 26:164).

When still present the deciduous coat fills the furrows on the surface of the permanent coat. Sperm from the seminal vesicle of the male therefore appear more smooth and with an indistinct surface pattern when examined in SEM. Sperm from the female receptaculum are, on the contrary, devoid of the deciduous coat and show furrows, ridges, etc. distinctly (Pl. 28:170-172).

The coat has been discussed since it was discovered by Stuhlmann (1886), who regarded the spermatozoon with its coat as a kind of spermatophore containing one spermium. The same author was one of the first to observe that the receptaculum of cypridacean females often contains great numbers of empty coats in addition to living sperm. This is characteristic of all cypridaceans with standard-type spermatozoa: Candonidae, Cyprididae, Ilyocyprididae and Cypridopsidae (Pl. 26:164).

Stuhlmann suggested that the spermatozoa lose the coat when they arrive in the receptaculum, and that this "molt" is the reason why they suddenly can move, whereas spermatozoa from the male can not. Stuhlmann's idea appeared reasonable and was widely accepted, but spermatozoa in act of losing the coat still have not been critically observed.

Gupta (1964, 1968) also observed the empty coats in the receptaculum of many females. He suggested that they were "exhausted" spermatozoa from earlier copulations, and that the internal parts had disintegrated.

Since spermatozoa with partly disintegrated contents are far from rare in the receptacula of some females, I am inclined to believe that Gupta is right. At least it is certain that the actively moving spermatozoa in the receptaculum have preserved the permanent coat, and that this coat looks identical to the empty coats present in the same organ (Pl. 26:164-166). Only the deciduous coat has disappeared from the spermatozoa in the receptaculum.

The good preservation of the empty coats in the receptaculum suggests that they consist of resistant material. Gupta (1964) said that they contain a polysaccharide, presumably chitin, but admitted that his tests for chitin were not conclusive.

The ridges, furrows and structural patterns on

the outer surface are briefly mentioned below (Pls. 26, 28). The distinct ridges from the inner side of the coat called "monorails" by Gupta (1968) obviously anchor the inner structures to the coat (Fig. 8). Typically, as in the investigated species of *Notodromas, Chlamydotheca, Aglaiocypris, Cypridopsis, Eucypris* and *Ilyocypris,* there is a single ventral monorail, which fits between the ventral margins of the contractile organelles and follows the coiling over the entire length of the posterior region (Pl. 24:150-153; Pl. 25:156). In *Candona suchi* there is in addition a dorsal, smaller monorail, between the dorsal margins of the contractile organelles, in close contact with the nucleus (Fig. 8; Pl. 26:166). The monorails may be simple crests from the inner side of the permanent coat (Pl. 26:166) but are sometimes more complicated in cross sections, with lateral horns or other processes (Pl. 26:163-165). It is believed that the monorails propagate the forces from the contractile organelles to the coat.

*The relation of the surface pattern to the inner organelles*
The outer surface of the posterior region in standard-type spermatozoa is dominated by coiling superficial bands, which are slightly elevated zones deliminated by deep furrows (Fig. 8; Pl. 28). In the simplest case, as in *Candona suchi, Aglaiocypris* sp. and *Cypridopsis* sp., there are two parallel coiling bands, which correspond to right and left contractile organelles. They are separated by a middorsal and a midventral furrow (Fig. 8). In *Candona suchi* both furrows are matched on the inner side by a dorsal and ventral monorail (Pl.326:166). In *Aglaiocypris* sp. and *Cypridopsis* sp. there is only one monorail, which lies under the ventral furrow.

López-Camps et al. (1979) demonstrated by SEM technique that the relation of the surface pattern to internal organelles differs between the species. In most species each of the primary bands, which match the right and left wings of the contractile organelles, is subdivided into two secondary bands. There are thus in all four secondary bands which coil over the surface (Pl. 27:167, 169; Pl. 28:170, 171). The bands are separated by four furrows, of which one is dorsal, one ventral and two lateral. But there is still only one pair of contractile organelles. Each lateral wing of the contractile organelles shows only a slight fold at the level of the lateral furrow on the coat in species with secondary subdivision of the primary bands (Pl. 27:167, 169). This four-banded arrangement is found in *Notodromas monacha, Heterocypris incongruens, Cypria ophthalmica, Chlamydotheca* sp., *Potamocypris* sp. and *Ilyocypris* sp.

The interrelations of these structures are best seen in longitudinal sections of the posterior region, but SEM pictures and sections of empty coats may be quite helpful (Pls. 26-28).

The superficial bands and furrows, either primary or secondary, are present in all standard-type spermatozoa, but the pattern on the surface is further complicated by ridges and furrows formed by the permanent coat alone. In *Candona suchi* there are two low parallel ridges following the midline of each primary band (Fig. 9; Pl. 26:166; Pl. 28:172). *Aglaiocypris* sp. has five low ridges on each primary band (Pl. 27:168). *Ilyocypris* sp. has up to eight ridges on each secondary band, but the number varies in different regions (Pl. 27:127). *Heterocypris incongruens* and *Eucypris* sp. have a valvelike ridge along the posterior margin of the secondary bands (Pl. 24:152; Pl. 28:171). *Chlamydotheca* sp. has such valves along all four furrows, and the bottom of each furrow is covered by 4-5 microridges (Pl. 26:163, 165).

8.3.2. Special features of the Pontocyprididae
G. W. Müller (1894:134) remarked that the pontocypridid spermatozoa have a less dense coiling than those of the cyprids. This difference could be confirmed for the four pontocyprids I have examined and is in fact correlated with a very different basic structure, which made a separate description necessary (Pl. 29).

The main subdivisions of the spermatozoon, a thin anterior region and a thicker posterior region, are similar to those of other Cypridacea. But the transition from one region to the other is more

gradual and may be difficult to see in the light microscope. *Propontocypris litoricola* is a specialized exception which will be mentioned separately below. For the dimensions of the spermatozoa see Table 3.

The anterior region is a thin smooth cylinder in *Erythrocypris mytiloides*, at some levels with a low coiling ridge on the surface. The ridge is more pronounced but still low in *Pontocypris trigonellus* (Pl. 29:177, inset) and very high and lamellar in *Argilloecia cylindrica* (Pl. 29:177). The corkscrew on the anterior end is always present.

Cross- and longitudinal sections of the thick posterior region show that the unique pontocypridid features are present in all examined species, *Propontocypris litoricola* included (Pls. 29, 30).

The nucleo-perforatorium is straight in the anterior region but has a distinctly coiling course in the posterior region and becomes angular in cross sections. It has a core without membranous investment. The core has a crystalline or tubular structure, particularly distinct in *Argilloecia cylindrica* (Pl. 29:174, inset).

The mitochondria of *Erythrocypris mytiloides* and *Pontocypris trigonellus* are two long coiled bands, which meet in the ventral midline below the nucleo-perforatorial rod (Pl. 29:176). In *Argilloecia cylindrica* and *Propontocypris litoricola* they are intimately connected and form an unpaired ventral plate below the nucleo-perforatorium (Pl. 29:174, 175). From their ventral contact with the nuclear envelope they extend dorsally along the surface of the sperm, partly covering the more deeply situated contractile organelles. In the two latter species the dorsal margin of the mitochondria is flattened out to a thin plate (Pl. 29:174, 175). The cristae are not regularly perpendicular to the longitudinal axis but have a more irregular, radiating course in the cross sections (Pls. 29, 30).

The contractile organelles are similar to those of standard-type spermatozoa but have a strikingly different position. From the attachment to the nuclear envelope each core-sac bends dorsally along the side wall of the nucleo-perforatorium, inside the mitochondria (Pl. 29:174-176). Only the dorsal margins of the contractile organelles are in contact with the sperm surface; the basal parts are covered by the mitochondria.

It should also be noted that the lumen of the core-sac is more open than in standard-type spermatozoa. The "pins" are exceptionally long in *Propontocypris litoricola* (2800 Å) and *Argilloecia cylindrica* (1600 Å), whereas standard-type pins are only about 1000 Å. The longitudinal filaments inside the core-sac are strongly developed and almost lamellar in *Propontocypris litoricola* (Pl. 25:155).

The striking difference between standard-type spermatozoa and the pontocypridid type is immediately seen when Fig. 8 and Pl. 24 are compared with Pl. 29. In the four pontocypridids the contractile organelles develop mainly in a dorsal direction and are in part covered by the more superficially situated mitochondria (Pl. 29). In the standard-type spermatozoa the contractile organelles soon turn ventrally and follow the external surface, investing the mitochondria from the outside (Fig. 8; Pl. 24). This can be seen directly by comparing the cross sections, for they are all orientated in the same way with the ventral side downwards.

This orientation might seem to be misinterpreted, but Pl. 32 shows that early spermatids of cypridaceans, also of pontocypridids and macrocypridids, are identical with regard to relative position of organelles. Also the attachment mechanisms of mitochondria and contractile organelles are established at the stage shown. The ventral side of the spermatids, defined by the relative positions of mitochondria, nucleus and contractile organelles, is clearly homologous and can be regarded as irreversibly fixed. The differences found in the location of the organelles in mature stages are thus real and do not depend on misinterpretation of dorsal and ventral.

The partial retraction of the contractile organelles from the surface in pontocypridids has to be considered when the movements of cypridacean spermatozoa are discussed (Chapter 8.5).

The extracellular coat of pontocypridid spermatozoa is a most problematic structure and even if

parts of it are present, it is not directly comparable with the coat of standard-type spermatozoa. There is no typical continuous coat covering the pontocypridid spermatozoa in the vas deferens and the vesicula seminalis. But some specialized structures such as the corkscrew at the anterior end and the remarkable bulb in *Propontocypris litoricola* may be homologous to parts of the standard-type coat. No "monorails" are present.

In the vas deferens and vesicula seminalis of all four pontocypridids the posterior region of the spermatozoa is only surrounded by a very thin membrane, which by its dimensions could be a plasma membrane (Pl. 29:174-178; Pl. 30:183). Spermatozoa from the female receptaculum may preserve this membrane but it is often wrinkled and detached from the cell surface, and it is sometimes completely absent (Pl. 29:174). I hesitate to homologize this membrane with a coat.

The corkscrew at the anterior end is particularly well developed in *Argilloecia cylindrica* (Pl. 29:177). It is preserved also on spermatozoa from the female receptaculum and would thus be homologous to that part of the permanent coat which forms the corkscrew in the standard-type spermatozoa (Pl. 23:144, 147; Pl. 24:148).

Although disintegrating spermatozoa were seen in the receptaculum of pontocypridid females, I have never seen the empty coats which are so characteristic of the receptaculum of cypridids, candonids, ilyocypridids and cypridopsids (Pl. 26:164-166). These empty ghosts are easily seen when present. They are formed by the permanent coat when the internal structure of the spermatozoa disintegrates.

The absence of such empty ghosts in pontocypridids thus confirms my opinion that no continuous permanent coat covers the spermatozoa in this family.

As mentioned below, the remarkable bulbs of *Propontocypris litoricola* are preserved and fairly common in the receptaculum of the female but they are not connected with remnants of coat structures from other parts of the spermatozoon.

It should also be mentioned that degenerating spermatozoa in the receptaculum of pontocypridids and macrocypridids were usually found in bundles, packed together in secretions that stained darkly with toluidine blue.

The surface pattern of pontocypridid sperm has not been investigated by SEM technique, but is shown well by longitudinal sections of the posterior region (Pl. 29:178; Pl. 30:183). The two contractile organelles coil as in standard-type spermatozoa but are partly covered by the more or less double mitochondrial plate. It is probable that this caused G. W. Müller (1894:134) to conclude that the coiling of pontocypridid spermatozoa is less dense than that of cypridids and candonids. This is correct, for the successive coils of the contractile organelles are separated by distinct interspaces and their margins do not meet as they do in standard-type spermatozoa (cf. Pl. 27).

*Propontocypris litoricola*

Upon examining several well-fixed specimens of this species from Mauritius I was astonished to find that the spermatozoa have a thick collarlike swelling between the anterior region and the thick posterior region (Pl. 30:179). G. W. Müller (1889:pl. 33; and 1894: pl. 38) described a similar bulb on spermatozoa of *Propontocypris pirifera* G. W. Müller, a species which according to Maddocks (1969) should be referred to the same subgenus (*Ekpontocypris*) to which *P. litoricola* belongs. The presence in both species of the remarkable bulb on the spermatozoa certainly supports a close relationship between them. It should be noted that Maddocks based his grouping on nonspermatological features.

As described by Müller, the bulb is covered by a very thick layer of hyaline material (Pl. 30). The bulb is funnel-shaped with the spout of the funnel backwards, continuous with the thick posterior region of the spermatozoon (Pl. 30:181). Anteriorly this funnel ends with a transverse or somewhat concave surface, in the middle of which the slender anterior region emerges (Pl. 30:180, 181). The an-

terior region has a low spiral ridge which continues to the fore end.

The inner structure of the bulb is dominated by the enormously enlarged mitochondria, which coil around a central axis consisting of a slightly coiled nucleo-perforatorial rod and the strongly coiled contractile organelles (Pl. 30:181, 182). All these organelles are continuous with the organelles of the posterior region of the spermatozoon, which looks like that of other pontocypridids (Pl. 29:175).

The hyaline coat is restricted to the bulb, whereas the parts in front of and behind the bulb are covered by a thin irregular membrane (? cell membrane) as in other pontocypridids (Pl. 29:175; Pl. 30:183).

G. W. Müller observed that remnants of the bulb remain in the receptaculum of the female and believed that the bulbs are stripped off here in the same way as the coat was supposed to be. He described these discarded bulbs as thick plates with a central perforation.

I have seen numerous such empty bulbs, each perforated by a central hole, in the receptaculum of *Propontocypris* females. The bulbs are not associated with other remnants of the coat. The isolated bulbs consist of hyaline matter only and there are no organelles in them. They occur together with intact spermatozoa with a well-preserved bulb, whereas degenerating spermatozoa, also with a bulb, are more rare. I therefore reject Müller's stripping theory and suppose that the bulbs are what remains after the spermatozoa from earlier copulations have disintegrated.

It is probable that the hyaline substance of the bulb can be interpreted as a localized specialization of an extracellular coat. G. W. Müller's account of the spermatogenesis in *Propontocypris* gives some support to this idea. As the hyaline bulbs are preserved in the receptaculum they would, by definition, be part of the permanent coat as seen in standardtype spermatozoa. If so, the bulbs are only a partial homologue of the permanent coat, for most of the surface of the pontocypridid sperm has no cover of this kind.

8.3.3. Special features of the Macrocyprididae

*Macrocypris minna*

A male specimen with the vesicula seminalis filled with mature spermatozoa gave plentiful material of this species, and the testicles contained many stages of spermatogenesis.

In the light microscope hundreds of mature spermatozoa were seen, all about 1.5 mm long and 3 µm broad. They have no coiling and are slightly flattened throughout, and there is no clear distinction between anterior and posterior regions as in standard-type spermatozoa (Pl. 31:185).

In the light microscope one can see a dense straight rod in the center of the sperm and more tansparent borders on each side. The lateral margins of these borders are wrinkled in the fixed material, often in a regularly wave-like way (Pl. 31:185). In the presumed anterior end the dense rod extends 4-5 µm beyond the lateral borders. The opposite end is blunt without a protruding rod.

A thin tapering filament could be seen projecting from the presumed fore end of several spermatozoa. The filament is less than 1 µm thick at the base and about 25 µm long (Pl. 31:185). I first guessed that the filament is a reacted perforatorium, and this idea got some support when I found a typical acrosomal apparatus and a perforatorium in sections of spermatids (Pl. 33:194, 195). But I later learned that similar filaments develop in immature spermatids of cypridids (*Notodromas, Chlamydotheca*) without having any relation to a perforatorium. The original idea may therefore be wrong, and it has not been confirmed ultrastructurally.

Thousands of cross sections through different levels of mature spermatozoa have been examined and are uniform throughout (Pl. 31:184). The straight cylindrical axis is the nucleo-perforatorium, which extends throughout the spermatozoon without coiling (Pl. 31:187). The dark core in the center of the axis is probably the perforatorium proper, as indicated by the spermatogenesis (Pl. 33:201, 202). But there are no membranes separating the perforatorium from the light granular matter of the nucleus in mature sper-

matozoa. The invaginated nuclear envelope which surrounds the perforatorium in early spermatids must obviously have disappeared (Pl. 33:194, 195, 201, 202).

The mitochondria are slightly flattened, simple rods, attached to the ventral side of the nuclear envelope (Pl. 31:184). They have regularly spaced transverse cristae like the mitochondria of standard-type spermatozoa.

The contractile organelles attach to the nuclear envelope and extend straight laterally into the winglike borders seen in the light microscope (Pl. 31:184). They have the same structure as those of standard-type spermatozoa, but their orientation is of course completely different (cf. Pl. 24).

There is no extracellular coat of the type found in standard-type spermatozoa. A dark membrane, less than 100 Å thick, covers the outer surface. It looks like a regular cell membrane.

The spermatozoa of *Macrocypris minna* are thus remarkable in several respects: no regional differentiation, complete absence of coiling in all structures, no extracellular coat, and laterally extended contractile organelles. In most respects they look similar to very early bandlike spermatids of other cypridaceans, and it could be suggested that I have only seen immature spermatids of *Macrocypris minna*. But early bandlike spermatids of other cypridaceans have abundant plasm and appear immature in many organelles. The spermatozoa of *Macrocypris minna* I have studied are from the seminal vesicle or sperm ducts and appear cytologically mature. In fact they are cytologically very similar to mature sperm of of *Macrocypria angusta* (Pl. 31:186), for which the mature state is certain because I have seen material from the female. The spermatozoa of *Macrocypria angusta* differ from those of *Macrocypris minna* in being slightly coiled and in having a different orientation of the contractile organelles (see below).

*Macrocypria angusta*

The vesicula seminalis of many males of this species was packed with very slender spermatozoa, 0.6 µm in diameter and about 1.4 mm long. No regional differentiation can be seen in SEM or light microscopy. The presumed anterior end tapers a little, but this is restricted to a few microns and there is no clear demarcation from the predominant part of the sperm behind the fore end. SEM shows a slowly coiling pattern of indistinct ridges separated by broad interspaces. The cross section is roughly circular (Pl. 31:186).

Longitudinal sections of parts of the sperm confirm that the nucleo-perforatorial rod is straight without any trace of coiling (Pl. 31:188). The rod is somewhat angular in cross sections. It has a central core, probably derived from the perforatorium. It is surrounded by darker granular material, probably derived from the chromatin blocks in early spermatids (Pl. 32).

The contractile organelles bend straight dorsally from their attachment to the nuclear envelope. They have the usual ultrastructure (Pl. 31:186). The mitochondria are a pair of slightly flattened rods, attached to the ventral surface of the nucleus. They do not meet in the midline.

Contractile organelles and mitochondria coil in loose parallel spirals around the straight nucleo-perforatorial axis (Pl. 31:188), forming what could be called (double) contractile bands and (double) mitochondrial bands (compare Pl. 31:186, 188). These bands are low ridges which are seen coiling over the surface in SEM pictures. The coiling of these bands is not very tight, for the successive coils are separated by large interspaces (Pl. 31:188).

No coat is present. Spermatozoa from males are covered by a plasma membrane as in *Macrocypris minna* and the pontocypridids. No empty coats were ever seen in the ducts or the receptaculum of the numerous females. No "monorails" are present.

In the genital ducts and the receptaculum of females the spermatozoa occur in bundles or isolated. The bundles of sperm are often surrounded by a common cover of mucus-like matter, probably secreted by the female ducts. The cell membrane of the spermatozoa is often irregularly wrinkled as in pontocypridids under similar conditions, and some

spermatozoa show some disintegration of internal organelles. Well preserved spermatozoa in the female have the same internal structure as those in the male vesicula seminalis.

## 8.4. *Spermatogenesis in the Cypridacea*

The development of cypridacean spermatozoa has been dealt with by competent electron microscopists, so the general picture is well known for *Notodromas monacha* (Gupta 1964, 1968; Zissler 1969a, b, 1970) and *Cypridopsis* sp. (Reger & Florendo 1969).

Older light microscopical work completes this picture: Stuhlmann (1886), G. W. Müller (1889) and Schmaltz (1912), all working with candonids and cypridids, G. W. Müller also with pontocypridids.

I have regularly seen stages of spermatogenesis in EM sections of the males in my material, and I am satisfied that the early stages up to bandlike spermatids are very similar in all Cypridacea (Pl. 32). I therefore refer the reader to the papers mentioned and restrict myself to a summary and some comments.

*General remarks*

The early formation of the nucleus after the 2nd meiotic division is remarkable (see Zissler 1969a). After telophase each chromosome is surrounded by a separate nuclear envelope so 8 separate subnuclei (caryomeres) are formed. The caryomeres soon coalesce and form the single definitive nucleus.

The originally polygonal spermatids, lying in the blind end of each testicular tubule, change through oval and spindle-shaped to long, tubelike or band-shaped. Inside the spermatid the nucleus elongates and a large mitochondrial Nebenkern becomes visible. The bandlike spermatids move into the following parts of the vas deferens, become spiralized and get an extracellular coat (Zissler 1970).

*Acrosome, perforatorium*

Until Gupta (1964, 1968) reported his finding of an acrosomal vesicle attached to the prospective anterior end of the nucleus of *Notodromas*, the polarity of cypridacean sperm was uncertain and much of the interpretation of spermatogenesis was problematic. In 1968, Gupta (like Lowndes 1935) referred to the knoblike anterior end of the mature spermatozoon of *Notodromas* as an acrosomal vesicle, but this was not convincing as long as no acrosomal vesicle had been documented inside the knob. Gupta's main arguments were published in a typewritten thesis (1964), and this documentation is therefore not really available.

I can confirm Gupta's account on several points. I found a distinct membrane-bound perforatorium inside the nucleus of several cypridacean spermatids, but the acrosomal vesicle must be very short-lived for it proved difficult to find and identify with full certainty. But in *Macrocypris minna* and *Chlamydotheca* sp. I finally found many spermatids in which the perforatorium begins in the concavity of a typical but invaginated acrosomal vesicle (Pl. 33:194-199). Such cases leave no doubt about the interpretation.

These acrosomal vesicles are found in contact with the anterior end of the nucleus in early spermatids of both species. The end of the nucleus is invaginated to form a tube housing the perforatorium like in many other animals.

Still earlier stages with an open spherical acrosomal vesicle were searched for without result. Instead I found some strongly flattened vesicles of the type seen in Pl. 33:196 attached to the nuclear envelope in some early spermatids of *Chlamydotheca* sp. These "vesicles" are in fact only dark double plates with a hardly visible lumen between the two walls. They have not developed a perforatorium but sometimes have a clear relation to one of the frequent Golgi stacks. They were found in the furrow between the Nebenkern and the nucleus, fairly far back on the surface of the nucleus.

Gupta (1964) found such almost compact plates in early stages of *Notodromas* and believes that they are acrosomal vesicles. This would mean that the acrosomal vesicles start as plates with very little lumen, like those of scorpions (Jespersen & Hart-

wick 1973). It also implies that the primordial vesicles move up to the anterior end of the nucleus before they develop a perforatorium. This is certainly not impossible.

In my sections of early spermatids I found such flat primordial acrosomal vesicles attached to two or more subnuclei in the same cell, whereas there is only one acrosomal vesicle in later stages. It is thus necessary to assume that the several dark plates fuse and form one acrosomal vesicle when the single nucleus is formed by fusion of the subnuclei. This is certainly not impossible either, but these rather complicated interpretations make me somewhat uncertain about the identity of the first dark plates seen.

The earliest acrosomal vesicles identified with certainty are C-shaped in longitudinal sections, and surround the end of the perforatorium (Pl. 33:194-197). The margin of the C, where the wall of the vesicle doubles back, is in contact with the nucleus. In later stages the lumen of the vesicle is filled with dark matter but is still recognizable (Pl. 33:198). Later the entire acrosomal vesicle appears to be integrated in the dark substance filling the top of the mature sperm and can no longer be recognized (Pl. 33:199).

The early *perforatorium* contains densely packed longitudinal filaments or, in some cases, microtubules (Pl. 33:196-198, 200; Pl. 34:203, 204). Longitudinal microtubules are also present outside the nuclear envelope in the plasm (Pl. 34:203-204). The perforatorium grows backwards through the elongating nucleus inside the tube formed by the invaginated nuclear envelope (Pl. 33:200).

The membrane separating the early perforatorium from the nuclear matter is preserved for some time during elongation of the nucleus but is finally lost (Pl. 32:190-193; Pl. 33:194-202; Pl. 34:203, 204, 206). In the advanced bandlike spermatids there is thus a nucleo-perforatorial axis without separation between nuclear and perforatorial material. In *Macrocypris minna* and *Macrocypria angusta* it seems obvious that the well defined axial core in the nucleus is the perforatorium although it has no wall of its own (Pl. 31). The microtubular substructure in the center of the axial rod of *Argilloecia cylindrica* (Pl. 29:174) may also be remnants of perforatorial material. In other Cypridacea it is questionable if remnants of the perforatorium can be identified in mature spermatozoa.

The "*dark strings*" are first seen as a pair of amorphous dark bands located on each side of the nuclear process in spindle-shaped spermatids (Pl. 34:203-207). They grow backwards along the nucleus and finally reach to near its posterior end. Anteriorly, just behind the acrosomal vesicle, they are often partly hidden in furrows on the nuclear surface (Pl. 33:194; Pl. 34:203-204).

When developing backwards along the nucleus each string is subdivided into two somewhat flattened narrow plates: a medioventral one associated with the mitochondrion of that side, and a laterodorsal one involved in the formation of the contractile organelle (Pl. 33:302). A very constant and thick tubule, 5-6 times as thick as common microtubules and with double walls, is seen for a period in the space between the subdivisions of the dark string on each side (Pl. 32:191-193; Pl. 33:201-202). This tube disappears in later stages and is best seen in macrocypridids and pontocypridids.

Gupta (1964), working with *Notodromas*, regarded the dark strings as derivatives of the centrioles, which are said to lie in the furrow between the Nebenkern and the nucleus, not far from the platelike acrosomal vesicle. I hesitate about this statement for I was not able to follow the centrioles to later stages and have seen very few altogether. I have seen the very early dark strings far anteriorly, near the primordial acrosomal vesicle. It is possible that the dark strings grow out from the centrioles as suggested by Gupta, but their origin from the margin of the acrosomal vesicle can certainly not be excluded (Pl. 33:194-197).

The *mitochondria*. As described by Gupta (1964, 1968) and Zissler (1969a, b), the mitochondria of early spermatids assemble near the wall of the nucleus and coalesce, forming a double Nebenkern which can be larger than the nucleus itself

(Pl. 33:201). When fully developed the Nebenkern consists of two gigantic mitochondria which are closely attached. During elongation of the sperm each mitochondrion extends as a narrow tongue, closely attached to the mitochondrial (ventromedial) subdivision of the dark string of that side. Subsequent development implies strong elongation and spiralization of the two mitochondria (Pl. 33:201, 202; Pl. 34:205-207).

The *contractile organelles* develop in close contact with the dorsolateral division of the dark string on each side of the nucleus.

As described by Gupta (1964), Zissler (1969a, b) and Reger & Florendo (1969), the contractile organelle on each side is first seen as a pair of dark, double-walled crests that embrace between them the dorsolateral dark string of that side (Pl. 33:202; Pl. 34:203, 204). Proximally the ridges are continuous with the outer nuclear membrane; distally each double crest is continuous with sacs of the endoplasmic reticulum. The two crests grow out laterally, forming the double walls of the core-sac, while their lateral margins remain in contact with the endoplasmic reticulum (Pl. 34:203, 204).

Each of the walls of the core-sac is thus to be regarded as a flat fold from the outer nuclear membrane, opening into sacs of endoplasmic reticulum. The central lumen of the core, delimited by the two double walls, is thus extranuclear space enclosed between the two folds from the nuclear envelope (Pl. 34:204).

The entire core of the contractile organelle grows out from and is probably induced by the dorsolateral dark string of that side. It is thus situated above the double-walled thick tubule, which is a good landmark as long as it is preserved (Pl. 33:202; Pl. 34:207; Pl. 32:191, 193).

As shown by Zissler (1969b), the dark strings exist also in the prospective thin shaft (anterior region) of standard-type spermatozoa but do not induce contractile organelles there. It appears that the double crests are formed also in this region but are not supplied with pins, and no typical contractile organelles are formed. Instead the two crests form a low, somewhat flattened sac with the dark string remaining between them, i.e., in the enclosed lumen (Pl. 34:206). These two symmetrical sacs disappear before maturation but are probably retained in a reduced state in the base of the ridges of the anterior region in mature spermatozoa (Pl. 23:146, 147).

The pins on the outside of the walls of the core-sac of the contractile organelles appear as soon as the core grows out. Their origin is independent of that of the core, for they are first seen as free vesicles in the surrounding plasm and are probably detached from the Golgi complexes in the neighbourhood. This was demonstrated in the figures in Reger & Florendo (1969:figs. 10 and 11). The free vesicles are somewhat elongate but become rod-shaped or conical when they are attached to the core membrane.

In *Macrocypria angusta* the pins are first spherical and retain a large lumen for some time after their attachment to the core membrane (Fig. 34:207, 208). It can be seen that new such vesicles are added at the base of the core of the organelle, for the proximal vesicles are sometimes unattached and do not fit in the pattern (Pl. 34:207). In all species the vesicles finally lose the lumen and become solid pins (Pl. 25).

## 8.5. *Movements of cypridacean spermatozoa*

Zenker (1854:54), Stuhlmann (1886) and G. W. Müller (1889) could see different kinds of movement in cypridacean spermatozoa, but the first to study these movements systematically was Lowndes (1935) in a famous paper on freshwater Cypridacea. He used stroboscopic techniques in his analysis of the high-frequency movements and was remarkably successful. Lowndes' results were later confirmed by Gupta (1964, 1968), who used microfilming for analysis of the movement, and filming was also used for the present investigation.

Only spermatozoa from the receptaculum seminis of females were seen moving by these authours

and nobody has seen distinct movements in spermatozoa from males.

In addition to exceptional kinds of movement which appear to be artificial or secondary (bending, twisting, straightening, etc.), Lowndes described a rotation of the long, stiff sperm around its longitudinal axis, often combined with displacement in the direction of the drill (forwards). This swimming was seen in some spermatozoa in the receptaculum, which moved along the walls of the vesicle or more accidentally hit the receptaculum duct and passed downwards in the direction of the oviduct.

Moving spermatozoa also display a kind of micro-movement or "ripple movement" (Lowndes 1935) in the thick posterior region. This is difficult to study in normally moving sperm and is best seen if the spermatozoa are arrested in some way, sticking to the walls or to other sperm or simply being slowed down by oxygen deficiency under the coverslip.

Ripple movement has a high frequency and is seen in the light microscope as flimmering of the organelles in the posterior region if the frequency is normal. Analysis in the stroboscope or in film shows that transverse bulges (ripples) pass in rapid succession along the contractile organelles which coil under the surface of the posterior region (Fig. 8). The ripples start anteriorly and pass backwards to the posterior end, following the coiling of the contractile bands. According to the stroboscopic studies by Lowndes, each point on the band is passed by about 2000 ripples per minute, i.e., 35 per sec. Filming with standard frequency, 24 frames per sec., does therefore not give perfect resolution.

It is obvious that this "ripple movement" can be expected to give a rotatory momentum to the sperm and also a forward push. Lowndes consequently considers the ripple movement as the motor which drives the rotation and the forward swimming of the sperm.

Pl. 28:172 shows a *Candona* sperm from a female, fixed in $OsO_4$ and probably "frozen" in a state of moving, with the successive ripples preserved. It gives an idea of the appearance of the ripple apparatus. Of course artifacts can hardly be excluded, and the ripples may have been enlarged during fixation and subsequent preparation for scanning, but even if this has happened the picture is didactically useful as a diagram showing the principle.

Filming of candonid sperm with maximum optical resolution by Gupta (1968) and myself shows clearly that the single spermatozoon moves as described by Lowndes, in the direction of the drill, and that it rotates during this locomotion. The direction of the rotation is such that the drill is screwed forwards. The passage of the ripples over the surface of the contractile bands can also be followed, particularly if the frequency is somewhat slowed down by restricted oxygen supply.

Analysis of successive frames of the films (Pl. 28:123) reveals some details of ripple movement. The ripples are obviously formed by local bulging of the contractile organelles and the attached extracellular coat. Mitochondria and nucleus inside move very little if they move at all.

To me it appears most probable that the ripples are produced by attraction between the pins on the inner side of the core of the contractile organelles, whereas an inward bulge would be produced by the attraction of pins outside the core. Rapid spreading of impulses provoking such ripples along the core can explain the ripple movement as observed: outward bulges followed by inward bending, passing in rapid succession caudally over the sperm. The underlying mechanism of these impulses and their propagation can only be subject to guesswork.

I prefer this interpretation to Gupta's hypothesis, in which transverse contractions of the core, starting from the nucleus and spreading laterally to the ventral monorail, are supposed to cause foldings and thus produce the ripples. It is interesting in this connection that thin bridges are seen between the pins in some ultrastructural pictures (Pl. 25:157, 159). If contractile, these bridges can be responsible for the presumed attraction between the pins.

It is tempting to suppest that the nucleus or the double nuclear envelope is the structure conducting

the impulses for the ripple movement. This is not probable, however, for Lowndes, during his stroboscopic studies, showed that the two contractile organelles of one pair, both attached to the same nucleus, can show different frequency. One of the organelles can be "stopped" at one frequency of the stroboscope, and the other organelle at another frequency in several cases.

Lowndes, like Gupta and myself, is convinced that the ripple movement is oxygen dependent. It does not continue for more than a few minutes when the spermatozoa are locked up without air under the coverslip. The movements soon become slower and finally stop. They can sometimes be restarted again if fresh aerated water is added or if air bubbles are admitted.

A particular problem is presented by the pontocypridids and macrocypridids, in which no stable coat is present and the relation of the contractile organelles to the mitochondria and other structures is different (Pl. 29; Pl. 31).

In the pontocypridid *Pontocypris trigonella* I observed undoubted ripple movement in the thick posterior region of spermatozoa from the female receptaculum, but no locomotion or rotation was seen. Only the flimmering of the organelles could be seen in the fairly few preparations made. It is thus probable that the contractile organelles of pontocypridids work by producing ripples as in standard-type spermatozoa, and this is certainly also suggested by their very similar structure.

But in pontocypridids only the margin of the contractile organelles is free, the base being partly covered by the mitochondria, so the effect on locomotion of the cell must be supposed to be different (Pls. 29, 30).

In *Macrocypris minna* no living spermatozoa from females have been available and movements have not been seen, but the ultrastructure of the contractile organelles is like that described for standard-type spermatozoa. If the organelles work as in other Cypridacea one would expect that they produce propagating waves in the lateral brims of the spermatozoon, into which they extend (Pl. 31:184).

It is certainly suggestive that these lateral brims show wavy margins in spermatozoa fixed directly in $OsO_4$ solution; perhaps because they have been "frozen" by the fixative in a state of motion (Pl. 31:185). But in this case the material was from the male vesicula seminalis, so using this observation to support the theory makes it necessary to assume that also spermatozoa from males move in this species.

It should also be remarked that Gupta's idea about the mechanism of ripple formation does not seem reasonable for *Macrocypris minna*. Transverse contractions across the core cannot produce folds or ripples if the peripheral border of the contractile organelles is not fixed in the coat, and it is not in this species. But unilateral attraction between the pins as suggested by myself could well produce ripples. If this results in propagating waves along the lateral brims in *Macrocypris minna* the effect may well be a kind of forward swimming although not rotation around the axis.

*Macrocypria angusta* (males and females) were studied alive but no movements were observed. The structure of the contractile organelles is very similar to that of standard spermatozoa, although the relation to other organelles is different (Pl. 31:186). That no ripple movment could be observed is hardly astonishing, for the entire sperm is so small (less than 0.5 μm thick) that all details were below the resolution limit of the field equipment.

## 8.6. *Comments*

Cypridacean spermatozoa are characterized by the unique contractile organelles, which have been found in all cypridacean species examined. Since these organelles have a very complicated structure and spermatogenetic development, they are regarded as a clear synapomorphy of the cypridacean group, supporting its monophyletic origin. Some reservations must be made for the Darwinulacea, whose spermatozoa are unknown since males are absent in recent populations. But the phylogenetic conclusions have to be made on the basis of existing

material, and it is not customary to include characters from hypothetical animals, or organs which for some reason are unaccessible for examination, as arguments in a phylogenetic discussion. In any case such arguments must be of very limited value.

Another synapomorphic feature of all cypridaceans is the nucleo-perforatorium, formed by the enormously elongated intranuclear part of the perforatorium and the surrounding nucleus when the membrane around the perforatorium disintegrates.

It is of course satisfactory that the monophyly of the Cypridacea, formerly established mainly on the type of reduction of the exopodite of the 2nd antenna by some of our best ostracod specialists, can be supported by these complex spermatological synapomorphies. The contractile organelles show some variation which can be used to support the characterization of some of the subgroups:

*Macrocyprididae* are characterized spermatologically by the lack of subdivision of the sperm into anterior and posterior regions and by the remarkable orientation of the contractile organelles, which differs from that of other cypridacean ostracods (Pl. 31). The Macrocyprididae are maintained in recent systems as a presumed primitive subdivision of the Cypridacea. The main argument is the situation of the gonads inside the body proper, which is clearly primitive (plesiomorphic), for this is the normal siutuation in non-cypridacean ostracods. The spermatological features, which mean lack of specialization, may also be primitive (lack of regional differentiation, lack of coiling and lack of anterior corkscrew). For the classical view see G. W. Müller (1894), Alm (1915), Sars (1928), Hartmann (1963).

*Pontocyprididae*. The four examined species of pontocyprids all have a striking and unique localization of the contractile organelles, which are dorsally directed and partly covered by the mitochondria (Pl. 29; Pl. 30). G. W. Müller (1894) and Sars (1928) based the group on somewhat less conclusive characters, e.g., the simple structure (without regular rosettes) of the ductus ejaculatorius and the absence of a vibratory plate on the palp of the female. Sars was not completely satisfied with these arguments (Sars 1928:47). The orientation of the contractile organelles and the mitochondria may be added as a new and probably synapomorphic character.

*The standard type* (Cyprididae, Candonidae, Ilyocyprididae and Cypridopsidae) is most advanced. The spermatozoa have an advanced coat with monorails, clearly developed coiling of all organelles, distinct anterior and posterior regions, corkscrew at the anterior end, and superficial contractile organelles situated in contact with the coat outside the mitochondria. Most of these features are probably specializations for the particular kind of movement, and they are absent or poorly developed in pontocyprids and macrocyprids. It seems reasonable to regard these features as synapomorphies of the standard-type spermatozoa and the comparable states seen in macrocyprids and pontocyprids as plesiomorphic. It is thus possible to perform a kind of stepwise argumentation for spermatozoa within the Cypridacea.

# C. Spermatology in relation to taxonomy and phylogeny of ostracods

The present investigation has revealed an almost bewildering diversity of sperm structure among ostracods. Most of the sperm types and many organelles are completely unknown with regard to finer structure. Recent reports are restricted to a single sperm type, namely that of freshwater Cypridacea, whereas the spermatozoa of other ostracods have been neglected. The results of the present survey are therefore published in extenso and are believed to give a more representative picture of ostracod spermatology.

Eight clearly different main types of spermatozoa were recognized after examination of 72 species of ostracods, chosen so as to cover the ostracod system as well as possible. The main types are so different that they can be recognized without real possibilities for confusion, and each type has a well defined occurrence restricted to related taxa of the current ostracod system. This of course invites a consideration of the value of the spermatological characters for taxonomical definitions and as arguments in phylogenetical discussions of the ostracods.

Wirth (1984), in a comprehensive study of spermatozoan variation in relation to phylogeny in invertebrates, arrived at some general conclusions which in some cases are illustrated by the present material. The "comments" section under each sperm type often deals with some more special problems relevant to Wirth's theses; here some more general viewpoints will be discussed.

*Ostracod origin: "The ostracod type of spermatozoon"*

Successful use of the spermatozoa in discussions of ostracod ancestry would only be possible if the probable morphotype of the spermatozoa in ancestral ostracods could be reconstructed. However, the eight types of spermatozoa found in ostracods are so different that there are few if any common features which could be used for such a reconstruction. It is true that all ostracod spermatozoa lack a flagellum and that most – with the exception of the cylindroleberidids examined up to now – have an acrosomal vesicle and a perforatorium at some stage of development. But none of these features can be said to be apomorphic in ostracods, for they occur also in other crustaceans and in other arthropods. Mitochondria are present except in the mature cylindroleberidid spermatozoa, and are in hundreds included inside the nuclear envelope in halocyprids. They are in general very variable within ostracods.

Even the nucleus is subject to wide variation and sometimes, especially in the Cypridacea, includes a large perforatorium invaginated into a tube formed by the nuclear envelope.

This uncertainty of the spermatozoan morphotype and its organelles makes it difficult to use ostracod spermatozoa as an argument when the possible sister group of ostracods is discussed.

*Orders Myodocopida and Podocopida – the mitochondria*

The dichotomy of the ostracod system into Myodocopida and Podocopida was originally introduced by Sars (1866) and G. W. Müller (1894) and was subsequently followed up by modern authors, including Hartmann (1963), Scott (1961) and Hartmann & Puri (1974). The presence of a well developed exopodite with 8-9 joints on the 2nd antenna and lamella-shaped furcal rami in Myodocopida are some of the classical arguments, since Podocopida have a vestigial antennal exopodite and non-lamellate furcal rami. This dichotomy of the system seems to be supported by a spermatological argument: The mitochondria of spermatids form a typical Nebenkern during develop-

ment of Podocopida (stated for all examined Cypridacea, Cytheracea, Bairdiacea and Cytherellidae), whereas no Nebenkern is formed in the Myodocopida. In the latter the mitochondria remain free, although their fate during spermatogenesis is fairly special in polycopids, where unfused mitochondria are lined up in two rows along the margins of the nucleus; and in halocyprid ids, where the mitochondria remain scattered but are included in the nucleus.

The argument of the Nebenkern is weakened by the fact that we cannot say with certainty which state – with Nebenkern or without Nebenkern – is apomorphic in ostracods. Presence of a Nebenkern is a fairly common feature in Arthropoda (many pterygote insects, *Lepisma* but not *Petrobius*, Branchiura, Pentastomida) and some gastropod molluscs. Of course it can be said that the Myodocopida and Podocopida are different with regard to the mitochondria, and one of the states must be apomorphic if the Ostracoda are a monophyletic group. But this is not sufficient to decide whether the two groups form a sister-group system.

*Monophyletic taxa indicated by sperm types*

Ostracod spermatozoa fall within Zone 3 of Wirth's classification (1984:335) since they are aflagellate and correlated with internal fertilization. Such spermatozoa are often difficult to use in phylogenetical discussions, for they appear to change by "substitution" (sensu Wirth 1984), i.e., the new apomorphic type seems to appear without successive intermediate stages, replacing the plesiomorphic type of which no trace is left. This often makes it impossible to argue phylogenetically in a stepwise way by proceeding from plesiomorphic to more and more advanced apomorphic states as is usually done with other features.

I understand "substitution" as a descriptive term saying that a new feature seemingly arises without intermediate stages being preserved, so that a stepwise argumentation from one type to another is excluded. The term "substitution" certainly does not explain anything and does not refer to defined or well known phylogenetical processes; it only registers the pattern seen in available material.

The main ostracod spermatozoan types described above show a high degree of such "substitution". They are isolated, clearly different types and are so different that a stepwise argumentation for homology (Remane's Kontinuitätskriterium) is practically excluded, in all probability because intermediate stages are not preserved in recent species.

On the other hand, these spermatozoan types are often very complicated and restricted to well defined taxonomic groups. They can therefore be used as probable synapomorphic characters supporting the monophyletic origin of the groups and as parts of a diagnosis. Some examples will be presented below.

It should be kept in mind, however, that a "simple" structure may arise by reduction or complete disappearance of organelles. This may have happened, for instance, in the family Cylindroleberididae, whose simple spermatozoa can have been derived by reduction from more complicated ones. The derivation of cylindroleberidids from some myodocopids with more complicated spermatozoa is therefore not completely excluded (p. 20; p. 72). It is thus a doubtful hypothesis to regard cylindroleberidid spermatozoa as an apomorphic state without reservations.

1. *Cypridinidae, Sarsiellidae, and Rutidermatidae*

These three families have the same type of spermatozoon, with a long, spear-shaped acrosomal region and the nucleus as a round body at the posterior end (Fig. 1). The acrosomal spear, consisting of the elongated acrosomal vesicle, contains a long distinctly crystalline perforatorium. The mitochondria are normal in appearance and are scattered.

This morphology of the spermatozoa is not very complicated and could hardly be presented as a strong synapomorphy of the three families, although interspecific variation is moderate.

But it was shown in all three families that this spermatozoon is correlated with a unique type of

sperm transfer during copulation. Spermatozoa of this type are stored in a "spermatophore" which is deposited as a soft drop in a furrow on the external side of the female genital lobe. The hard wall of the spermatophore is obviously formed by hardening of secretions from the male vas deferens, ejaculated together with the spermatozoa.

These "spermatophores", which have been found in many members of the three families, indicate a fertilization strategy not found in other ostracods or in any other animals whatever (however, it is possible that males of the branchiuran *Dolops* ejaculate similar, hardening sperm droplets; see Fryer 1958, 1960).

I therefore conclude that the two spermatological characters, the sperm type and the external spermatophores deposited on the female genital lobe, are synapomorphic and can be used to support the monophyletic origin of the three families.

II. *Cylindroleberididae*

Cylindroleberididae are usually included in the Myodocopa together with the three families mentioned above and share a number of morphological features with some of them, e.g., the numerous muscle scars, the rostral incisure. But the cylindroleberidids have 7-8 pairs of leaflike gills on the sides of the abdomen, a feature unknown in other ostracods and therefore a probable apomorphy of Cylindroleberididae. Their spermatozoa are extremely reduced in the mature state; they consist only of simple nuclei surrounded by some membranes and are found in separate vacuoles in "cytophores", each of which has arisen by fusion of the plasm of four early spermatids. The cytophores, each with four spermatozoa lying in its vacuoles, are ejaculated into the female receptaculum. In *Parasterope muelleri* the nuclei (spermatozoa) are freed from the cytophore before they enter the fertilization duct to fertilize the eggs.

It is of course a problem whether this remarkable sperm character is present in all the numerous cylindroleberidids, for only three species have been examined. But these three species represent both subfamilies of the Cylindroleberididae, which increases the probability for the results being significant and representative.

The clearly apomorphic characters mentioned (abdominal gills and reduced spermatozoa – "cytophore") can of course be used as synapomorphies for cylindroleberidids, to argue for the monophyly of this family. But they are not conclusive for a separate origin in relation to the Myodocopida. The said features, although useful for identification of a cylindroleberidid, do not indicate a closer relation to groups outside the Myodocopida, for the two characters have only been found in cylindroleberidids. Thus, the cylindroleberidids may well have evolved together with other myodocopids, as indicated by some characters, and can have specialized as a separate branch with the said highly apomorphic spermatology and gill structure.

III. *The Halocyprididae*

The Halocyprididae have a spermatozoan type of their own, characterized by several features, particularly the presence of numerous (hundreds) of normal free mitochondria, with cristae, scattered in the nucleoplasm, inside the nuclear envelope. The kind of development leading to this arrangement has been studied and appears to be completely unique, not found in other ostracods or in other animals. It must be an autopomorphic feature of halocypridids and can be used to characterize this group if it can be shown to be a general feature within the family.

The two species examined belong to *Conchoecia* and there is no obvious reason to suppose that the other genera within the homogeneous family Halocyprididae have a different spermatology. It would be interesting to know if *Thaumatocypris* and allied genera, which constitute the other family (Thaumatocyprididae) of the suborder Halocypriformes, also have this remarkable mitochondrial feature. If so, it would be a much needed additional argument for the monophyletic origin of the Halocypriformes. The other characters of this taxon are more circumstantial or their classification as

apomorphies is more questionable (see Kornicker 1976).

The other characters of *Conchoecia* spermatozoa are more problematic. The striking enlargment of the postacrosomal region and its several longitudinal rods, including the true perforatorium, resembles that of cytherellids, and this similarity is underlined by the small acrosomal vesicle on the top. The distinct continuity of the ventral postacrosomal rod with the cores of the two intranuclear tubules further back invites comparison with the "dark strings" along the nucleus in cypridid spermatozoa. But in all these cases a precise homology is difficult to establish, because intermediate forms are lacking and the spermatogenesis of *Conchoecia* is incompletely known.

There is no obvious and unquestionable spermatological similarity to polycopids, which have been regarded as related to halocypridids on the basis of light microscopical characters (unpaired copulatory organ, loss of median eye).

IV. *Cladocopina: Polycopidae*

If the single species of *Polycope* examined can be regarded as representative of the group, the Cladocopina have very characteristic spermatozoa. They are comparatively short, flattened, with a bandlike nucleus and ordinary acrosomal structures, but the plasma membrane is elevated into longitudinal folds which are arranged into characteristic transverse belts across the surface of the spermatozoon. The mitochondria, although not fused, form a single row along each margin of the nucleus (Pl. 8; Fig. 3:D). These two features are unique in ostracods and could help to define the group, but it is desirable that more species are examined before real conclusions can be proposed.

V. *Platycopina: Cytherellidae*

The *Cytherella* sperm – like that of all the other Podocopida – develop a Nebenkern with two giant composite mitochondria. This might be taken as an indicium for the podocopid relations of the group, although it is not entirely conclusive (see above). Another feature pointing in this direction is the prevalence of the endopodite of the 2nd antenna over the small exopodite (see G. W. Müller 1894, Sars 1928, Skogsberg 1920, Hartmann 1963).

The other features of the *Cytherella* sperm are in part highly unique, e.g., the long, triangular perforatorium and the four rows of flattened sacs of endoplasmic reticulum ventral to this rod. The small acrosomal vesicle is slightly similar to that of *Conchoecia*.

Most interesting is the mechanism indicated in the postacrosomal region, where a pair of "lamellate rods" are believed to perform wavelike movements, sliding up and down along the flat lateral surfaces of the perforatorium (Fig. 4; Pls. 10, 11). If this is a locomotory mechanism it is certainly unique but shows some slight similarity (perhaps analogy) to the better known function of the contractile organelles of the Cypridacea. But confirmation of this theoretical speculation on living sperm is badly needed.

VI. *Bairdiacea*

The spermatozoa of *Bairdoppilata* develop a typical Nebenkern like that of other podocopid groups. Both during spermatogenesis and as mature the *Bairdoppilata* spermatozoa have some features which are found also in Cytheracea:

The acrosomal vesicle develops early to a very large sac and is invaded from behind by the perforatorium as in cytherids. The acrosomal vesicle and the perforatorium elongate strongly and finally disappear, being used up in the formation of the spear-shaped acrosomal region. As in the cytherids this region is invaded from behind by two mitochondrial rods, which grow forwards on each side of the perforatorium. The appearance of the mature acrosomal region, with a longitudinal furrow supported by a dark mantle, is not fundamentally different from that of cytherids.

But there are also differences: *Bairdoppilata* does not have the "segmented organelles" (A and B), which are characteristic of the cytheraceans. In *Bairdoppilata* the perforatorium also grows back-

wards and invaginates the nucleus, forming a continuous intranuclear axis until late stages of spermatogenesis, when it degenerates. In Cytheracea the perforatorium has never been observed to invade the nucleus (see Fig. 4).

Similarities between *Bairdoppilata* spermatozoa and those of Cypridacea are the invasion of the nucleus by the perforatorium, although the intranuclear part disappears in late *Bairdoppilata* stages and does not seem to fuse with the nuclear matter as in Cypridacea. But there are also important differences: The *Bairdoppilata* sperm completely lacks the contractile organelles which are so characteristic of all cypridaceans.

The remarkable spermatophore described by G. W. Müller as a capsule deposited in the receptaculum seminis of bairdiids, probably formed by hardening of sperm secretions, appears to be unique. It is tempting, however, to compare it with the external spermatophores attached to the outside of the females in Cypridinidae, Sarsiellidae and Rutidermatidae. As long as no intermediate states have been discovered this hypothesis remains uncertain, however, for there are differences in detail.

VII. *Cytheracea*

Segmented organelles of one or two types (A and B) are found in all cytheraceans examined. Since nothing really similar has been found in other animals, it is supposed to be an apomorphic feature of the group. Also the acrosomal crest, present in numerous variations in different cytheraceans, is probably synapomorphic for the superfamily, although it could perhaps be homologous with one of the ridges along the acrosomal region in cypridaceans.

The similarities to bairdiacean sperm were commented on above.

In spite of enormous variation at the generic and specific levels the cytheracean spermatozoa can be recognized because of their segmented organelles and acrosomal crest. It is particularly important that they can be easily distinguished from the spermatozoa of the Cypridacea, which have another constant and characteristic apomorphy: the contractile organelles. These have never been found in cytherids.

The distinction between Cytheracea and Cypridacea, previously based mainly on the morphology of the exopodite of the 2nd antenna, is thus made clear-cut by the spermatological features: each of the groups has a synapomorphic feature of its own which cannot be misinterpreted.

VIII. *Cypridacea*

Cypridacean spermatozoa are characterized by the unique contractile organelles which have been found in all cypridacean species examined but not in other ostracods or other animals whatever. Since these organelles have a complicated structure and spermatogenetic development I regard them as synapomorphic for cypridaceans and maintain that convergent development is practically excluded (Fig. 8; Pls. 24, 25). These organelles are therefore a strong argument for the monophyletic development of the Cypridacea. All subgroups examined have these organelles. A reservation must be made for the Darwinulacea, whose spermatozoa are unknown since males are unknown in recent populations.

Another synapomorphy of the Cypridacea is the nucleo-perforatorium, formed by complete fusion of the perforatorium with the nucleus when the separating membrane (invaginated nuclear envelope) disintegrates. Other ostracods have an invagination of the perforatorium into the nucleus during spermatogenesis (Polycopidae, Cytherellidae, Bairdiidae), but the intranuclear part of the perforatorium is withdrawn, degenerates or remains small. Only the Cypridacea have a complete fusion of nuclear and perforatorial material.

Cypridacean spermatozoa, like those of the other main types of ostracod sperm, seem to have arisen by "substitution", for no intermediate stages of contractile organelles or of nucleo-perforatorium are present and allow a stepwise comparison with other sperm types.

But within the Cypridacea three different sub-

types of spermatozoa are present: standard type, pontocypridid type and macrocypridid type. These subtypes show a certain stepwise variation.

*The standard type*, which was found in Cyprididae, Candonidae, Ilyocyprididae and Cypridopsidae, is probably most advanced. The anterior region is often shaped like a corkscrew, distinctly separate from the posterior region (Fig. 8). The nucleus is distinctly coiled. The extracellular coat is well developed, consisting of a permanent and a deciduous coat; the latter disappears after transfer to the female, while the former remains in the receptaculum as an empty "ghost" after the contents have disintegrated. The contractile organelles develop in contact with the surface dorsal to the mitochondria, which they cover. They are anchored to the coat by a "ventral monorail".

*The pontocypridid type* was found in all four pontocyprids examined. Compared with standard-type spermatozoa those of pontocyprids have less pronounced coiling, the regional differentiation is less sharp, the extracellular coat is not present over the whole spermatozoon. No empty coats (ghosts) remain in the receptaculum of the female. Most characteristic are the contractile organelles and their relation to the mitochondria. The contractile organelles, starting from the nucleus, develop dorsally but only their peripheral parts are in contact with the surface of the sperm. The basal parts are separated from the surface by the mitochondria. No "ventral monorail" is present (Pl. 29). The relative positions of the mitochondria and the contractile organelles make it easy to distinguish pontocypridid spermatozoa from those of the standard type (Pl. 29; Pl. 24; Fig. 8).

G. W. Müller (1894) and Sars (1928) based the Pontocyprididae on some light microscopical characters, e.g., the simple structure of the ductus ejaculatorius (without rosettes) and the absence of a vibratory plate on the palp of the female, but Sars was not entirely satisfied with these arguments (Sars 1928:47). The structure and orientation of the contractile organelles may be added as a new and very clear, probably synapomorphic feature of Pontocyprididae.

*The macrocypridid spermatozoa* are characterized by the lack of subdivision into anterior and posterior regions, by no or little coiling of all organelles, and by contractile organelles with a remarkable orientation which differs strikingly from that of other cypridacean ostracods (Pl. 31). Moreover, the contractile organelles are not covered by the simple rodlike mitochondria, there is no coat, there are no "monorails", and the nucleus is not coiled.

The Macrocyprididae are maintained in recent systems as a presumed primitive subdivision of the Cypridacea, as indicated by the presumed primitive situation of the gonads inside the body proper, and not in the mantle fold under the valve as in other Cypridacea (G. W. Müller 1894, Alm 1915, Sars 1928, Hartmann 1963). The characters of the spermatozoa may be primitive features like the localization of the gonads and perhaps are not apomorphic arguments for the monophyly of the family in the strict sense. The spermatological characters of Macrocyprididae, i.e., no or imperfect coiling, no corkscrew at the anterior end, no coat, no "monorail", no regional subdivision, seem to indicate imperfect specialization for the kind of movement so characteristic of advanced Cypridacea.

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# Plates

PLATE 1

Spermatophores.

1. *Vargula norvegica* ♀. Fix. Karnowsky. SEM picture of right genital lobe (gl) with spermatophore (sp) protruding from longitudinal furrow (f). Bristles on medial end of the lobe. Anterior direction shown by arrow.

2. *Philomedes globosus* ♀. Fix. 1 % $OsO_4$. Light microscopy of transverse epon section. Spermatophore (sp) enclosed in furrow (f) between two lips of genital lobe (gl).

3. *Rutiderma dinochelata* ♀. Fix. 3-A. Spermatophore with large plug of capsule secretions (cs) protruding from furrow on genital lobe. Numerous spermatozoa (s) in deeper part of spermatophore.

4. *Philomedes lilljeborgi* ♀. Fix. 1 % $OsO_4$. Spermatophore with irregularly hardened capsule secretions (cs), independent of cuticle of female (c), protruding from broad furrow on genital lobe.

5. *Philomedes globosus* ♀. Fix. 1 % $OsO_4$. Spermatozoa with nucleus (n), perforatorium (p) and acrosomal vesicle (a) in spermatophore attached to female.

6. *Rutiderma dinochelata* ♀. Fix. 3-A. Spermatozoa with nucleus (n), perforatorium (p) and acrosomal vesicle (a) in spermatophore attached to female.

*Legends to all figures*: a = acrosomal vesicle, an = anus, c = cuticle of female, cs = capsule secretions, f = furrow on genital lobe, gl = genital lobe, n = nucleus, p = perforatorium, s = spermatozoa, sp = spermatophore.

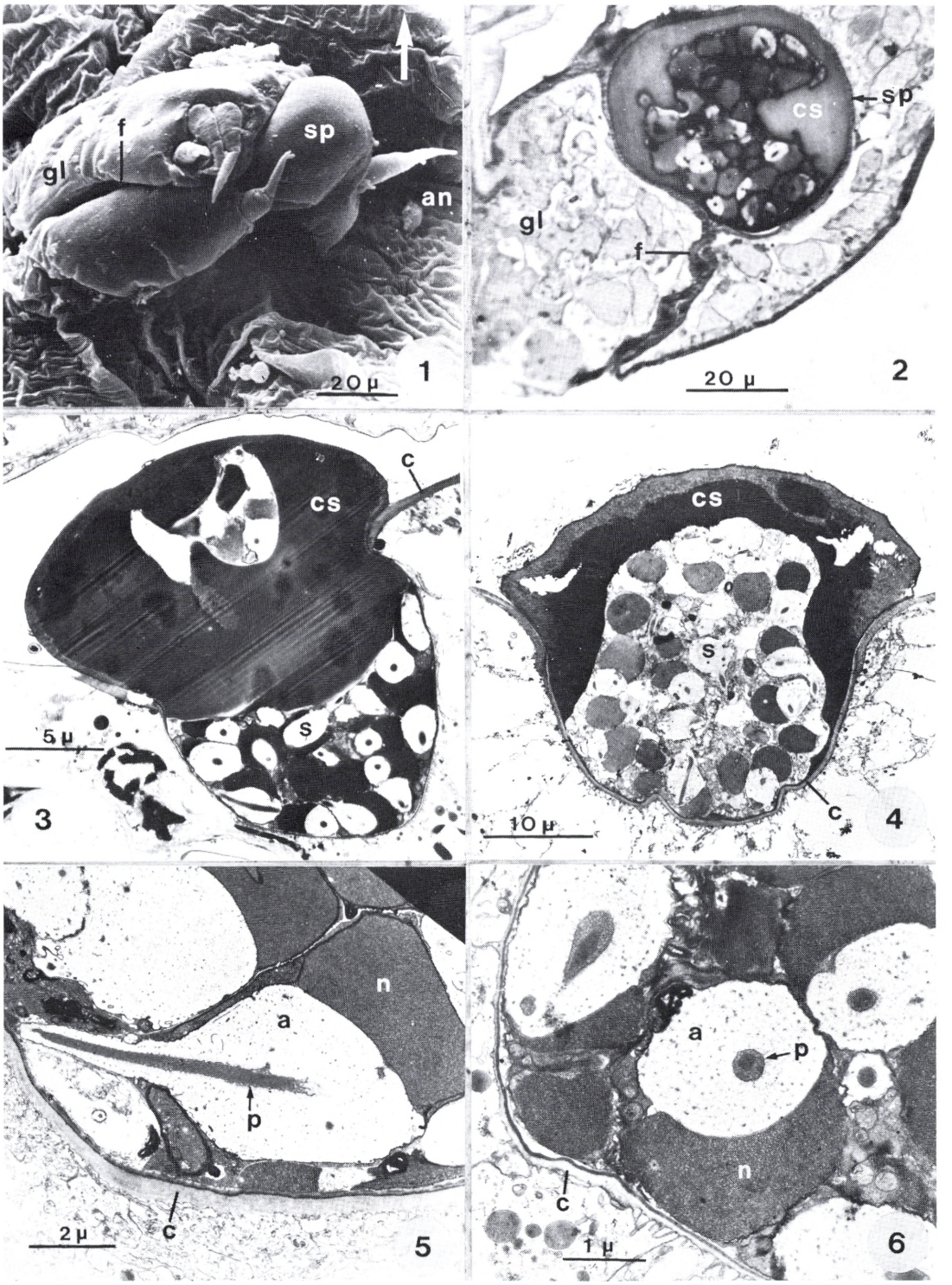
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PLATE 2

7. *Sarsiella* sp. 2 ♂. Fix. 3-A. Submedian section of almost mature sperm in testicle. Compare with Fig. 1:B.

8. *Philomedes paucichelata* ♂. Fix. 3-A. Late spermatid in testicle. Median section of transition between acrosomal vesicle (a) and perforatorium (p).

9. *Vargula norvegica* ♂. Fix. Karnowsky. Transverse section of acrosomal vesicle (a) and perforatorium (p) near base, where vesicle is voluminous (See Fig. 1:A).

10. *Vargula norvegica* ♂. Fix. Karnowsky. Transverse sections of distal parts of acrosomes of submature spermatids, still in testicle.

11. *Vargula norvegica* ♂. Fix. Karnowsky. Section of acrosomal vesicle (a) with large acrosomal bodies (ab), in testis. Note reticulate structure of surface of acrosomal bodies.

12. *Philomedes globosus* ♀. Fix. 1 % $OsO_4$. Spermatozoon in spermatophore of female. Most mitochondria (m) are scattered in the plasm but some are closely packed in a pouch of the nuclear envelope (upper left).

*Legends to all figures*: a = acrosomal vesicle, ab = acrosomal bodies, db = dense bodies surrounded by membrane, fs = flat sacs around acrosomal vesicle, m = mitochondria, n = nucleus, p = perforatorium.

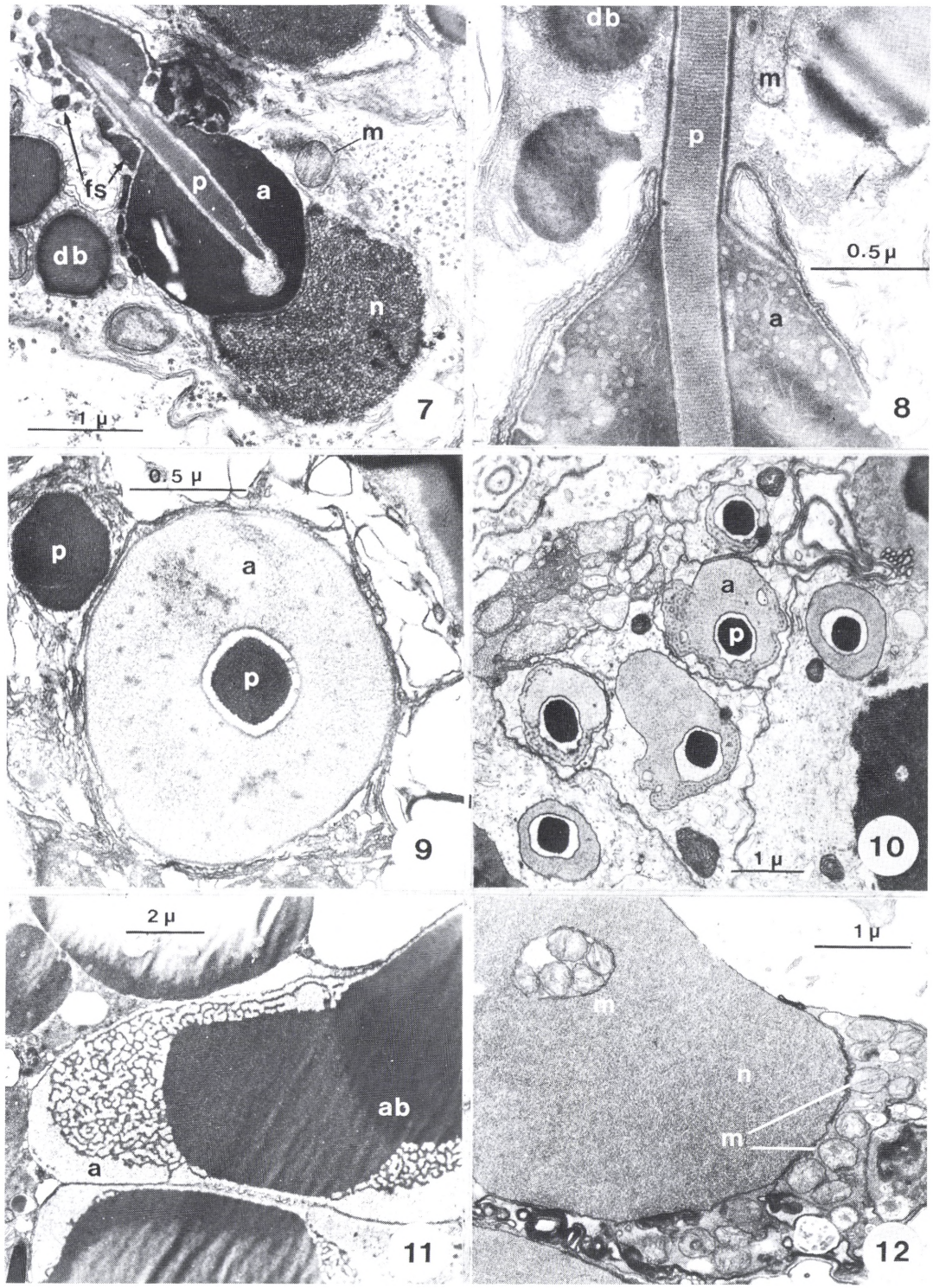
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PLATE 3

Structure of perforatorium, spermatogenesis.

13. *Sarsiella* sp. 2 ♂. Fix. 3-A. Transverse section of sperm in testicle, showing perforatorium with crystalline pattern.

14. *Philomedes lilljeborgi* ♀. Fix. 1 % $OsO_4$. Longitudinal section of sperm in spermatophore, showing crystalline pattern in perforatorium.

15. *Sarsiella* sp. 3 ♂. Fix. 3-A. Longitudinal section of spermatozoon in testicle, showing perforatorium with crystalline pattern.

16. *Rutiderma dinochelata* ♀. Fix. 3-A. Longitudinal section of perforatorium in spermatophore. Note crystalline pattern.

17. *Vargula norvegica* ♂. Fix. Karnowsky. Part of spermatid in testicle, with Golgi stacks (g) producing proacrosomal vesicles (pa) with a dark centrum and a light peripheral brim.

18. *Vargula norvegica* ♂. Fix. Karnowsky. Cell in testicle with contracted chromosomes (anaphase) and large proacrosomal vesicles (pa).

19. *Vargula norvegica* ♂. Fix. Karnowsky. Section through spermatid in testicle. Nucleus (n), acrosomal vesicle (a) and early perforatorium (p), the latter developing along the nucleus, covered by the acrosomal vesicle, before it develops anteriorly and invaginates the acrosomal vesicle.

*Legends to all figures*: a = acrosomal vesicle, ch = chromosomes, fs = flat sacs around perforatorium, g = Golgi stacks, n = nucleus, p = perforatorium, pa = proacrosomal vesicles, with dark centrum and light peripheral brim.

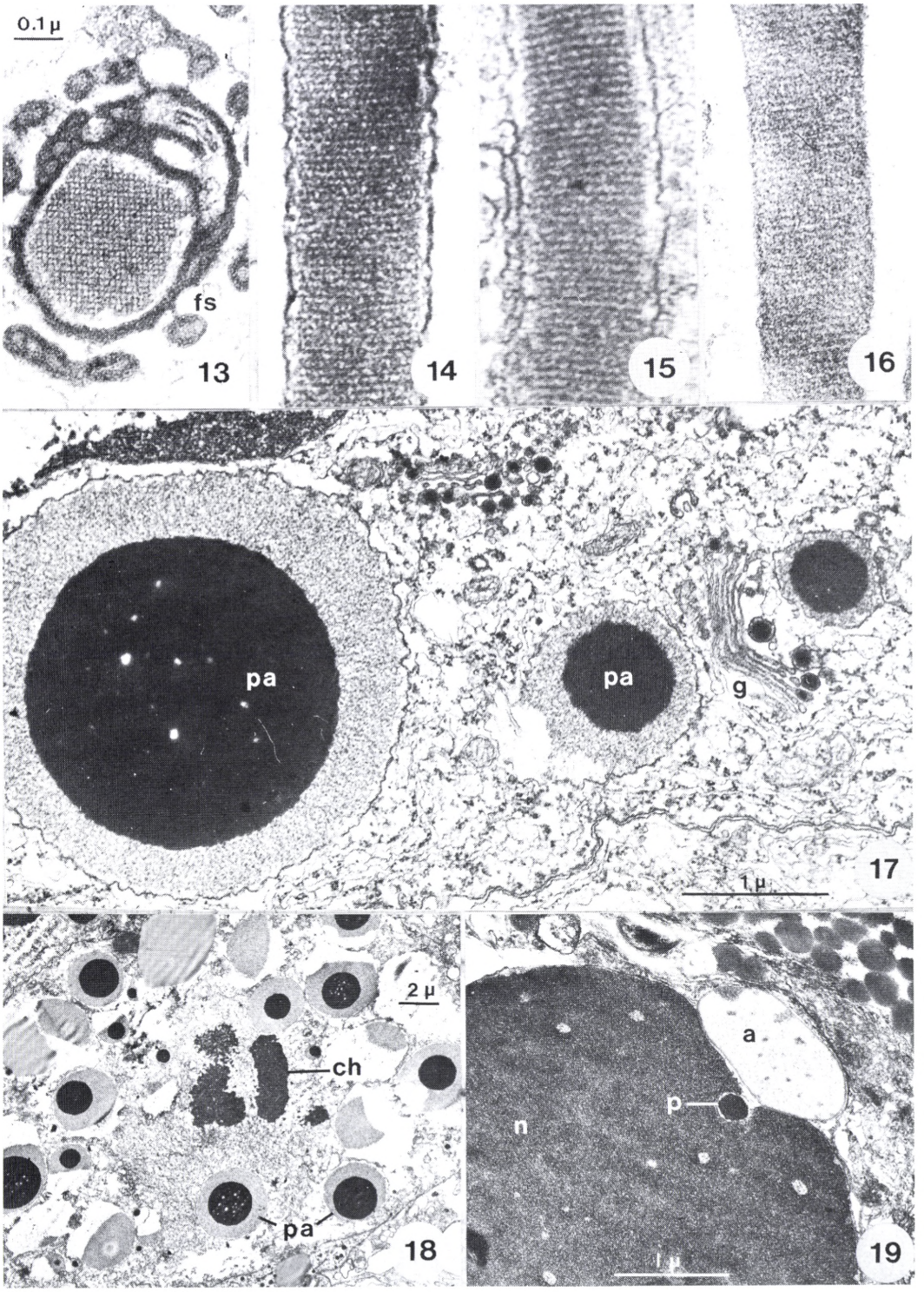
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PLATE 4

*Parasterope muelleri.*

20. *Parasterope muelleri* ♂. Fix. 3-A. Testicle with late spermatids (s) lying in groups of four (only 2-3 are in the plane of section). The plasm of each group appears confluent.

21. *P. muelleri* ♂. Fix. 3-A. part of testicle with spermatid nuclei and meiotic anaphase, showing that the organ is a testicle.

22. *P. muelleri* ♀. Fix. 3-A. Receptaculum seminis (rs) with cytophore (cy) containing four spermatozoa lying in vacuoles. One of them (sx) is out of the plane of section and only the vacuole is seen. The four original plasm bodies of the cytophore are completely fused.

23. *P. muelleri* ♀. Fix. 3-A. Cross section of receptaculum seminis and efferent duct. The external opening of the efferent duct (fertilization duct, fd) is seen; the receptaculum contains cytophores with spermatozoa.

24. *P. muelleri* ♀. Fix. 3-A. Sperm nucleus (s) and partly disintegrated cytophore (dc) in main cavity of receptaculum. Mitochondria (m) are still recognizable.

25. *P. muelleri* ♀. Fix. 3-A. Fertilization duct near its external opening, containing sperm nucleus (s) with its membranes but freed from cytophore.

*Legends to all figures*: c = cuticle of receptaculum, cy = cytophore, dc = degenerating cytophore in receptaculum, fd = fertilization duct, m = mitochondria, me = meiotic chrosmosome figure, rs = receptaculum seminis, s = spermatid and spermatozoan nuclei, sx = vacuole of fourth spermatid, the nucleus is out of the plane of section, w = wall of testicle.

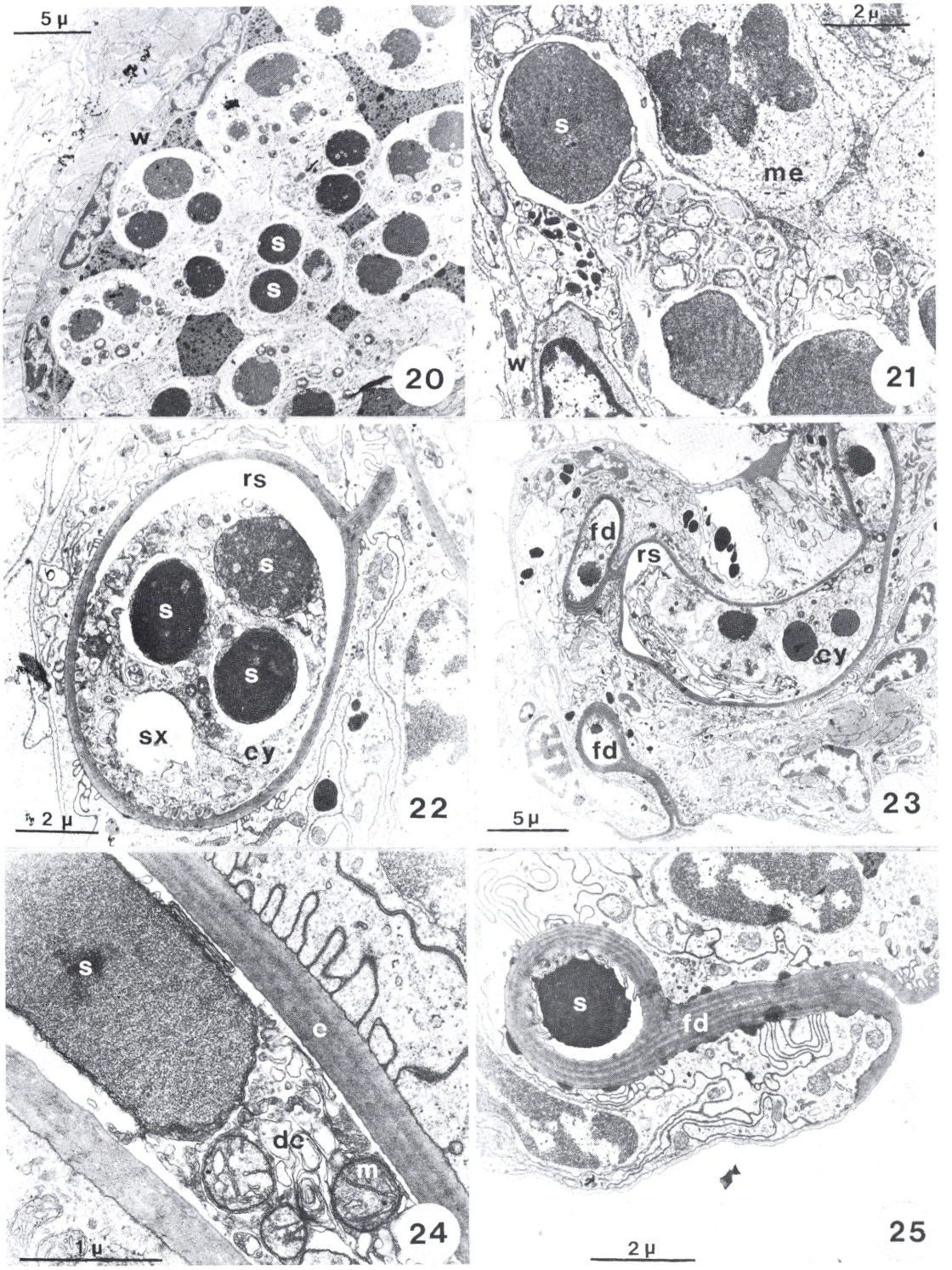
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PLATE 5

*Asteropella mortenseni.*

26. *Asteropella mortenseni* ♂. Fix. 3-A. Part of testicle with early spermatids still clearly separated from one another.

27. *A. mortenseni* ♂. Fix. 3-A. Early spermatid with normal nucleus (n) and nuclear envelope. A second wall of flat sacs (fs) has almost closed around the nucleus.

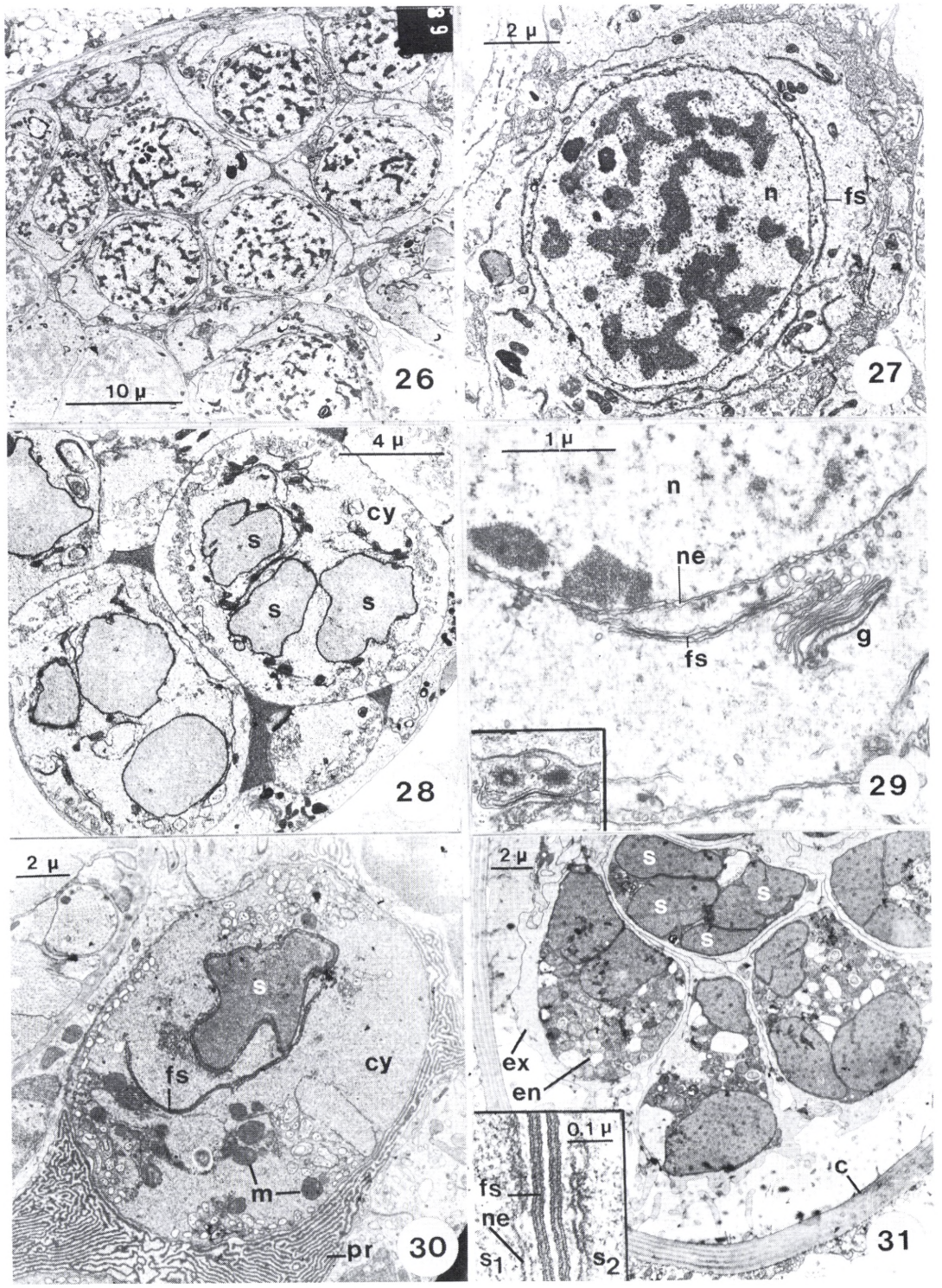
28. *A. mortenseni* ♂. Fix. 3-A. The plasm of the spermatids in each group of four (only 3 nuclei are in the plane of section) has fused and formed a cytophore (cy). Each spermatid nucleus is surrounded by external flat sacs outside the nuclear envelope.

29. *A. mortenseni* ♂. Fix. 3-A. High magnification of early spermatid, approximately same stage as in Fig. 27. Nucleus (n) surrounded by original double nuclear envelope (ne). A flat sac (fs) with a dark lamella inside approaches the nuclear envelope and seems to originate in a Golgi stack (g). Inset shows two centrioles near surface of nucleus (same scale as rest of figure).

30. *A. mortenseni* ♂. Fix. 3-A. Mature sperm (s) with flat sac around its nucleus lying in plasm of cytophore (cy) in distal part of vas deferens. One unattached piece of flat sac (fs) is still in the cytophore plasm. Note the characteristic pattern of coagulated sperm fluid (pr).

31. *A. mortenseni* ♀. Fix. 3-A. Cytophores, each with four spermatozoa, lying in receptaculum seminis of female. The contrast between endoplasm (en) and exoplasm (ex) is distinct in the cytophores. Inset shows contact between two spermatozoa. The nuclei are marked $s_1$ and $s_2$. The nuclear envelope (ne) and the secondary wall formed by a flat sac (fs) with a dark lamella inside are indicated in sperm $s_1$.

*Legends to all figures*: c = cuticle, cy = cytophore, en = endoplasm of cytophore, ex = exoplasm of cytophore, fs = flat sac with dark lamella inside, forming secondary wall of sperm, g = Golgi stacks, m = mitochondria, n = nucleus, ne = nuclear envelope, s = spermatozoon, $s_1$ and $s_2$ = the two spermatozoa making contact in Fig. 31, inset.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10 µ
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n
fs
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4 µ
cy
s
s
s
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n
ne
fs
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2 µ
s
fs
cy
m
pr
31
2 µ
s
s
s
s
ex
en
0,1 µ
fs
ne
s1
s2
c

PLATE 6

*Conchoecia*.

32. *Conchoecia borealis* ♂. Fix. 3-A. Anterior end of mature spermatozoon in vesicula seminalis. Median section, showing acrosomal region ("claw", cl), its posterior limit (pa) and part of postacrosomal region with central rod (cr) and ventral rod (vr). Inset shows longitudinal section of ventral rod, 25 % greater magnification.

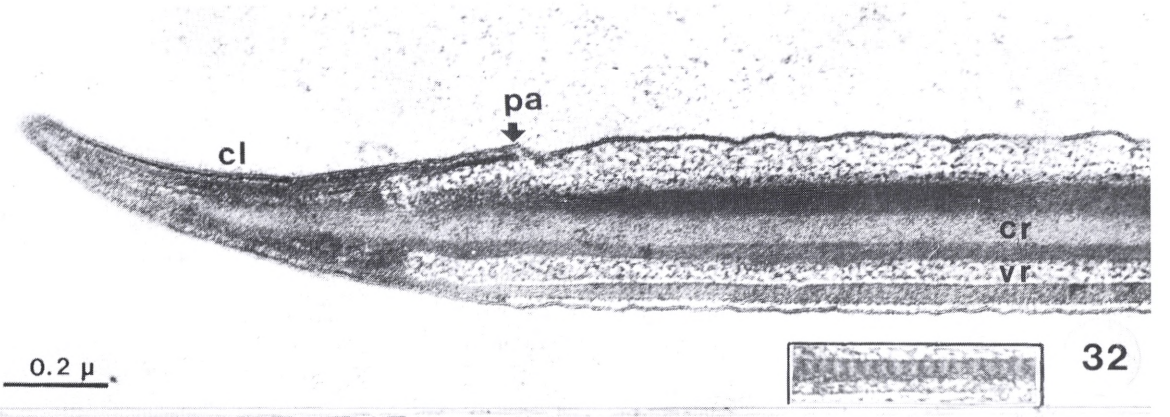
33. *C. borealis* ♂. Fix. 3-A. Cross section of postacrosomal region (right) and somewhat oblique section through transition to nuclear region (left). In the latter, the core of the ventral rod is divided into two; one rod (ntx) is still surrounded by postacrosomal material, the other (ntn) has entered the nucleus and is surrounded by the nuclear envelope.

34. *C. borealis* ♂. Fix. 3-A. Transverse section through mature sperm. One mitochondrion (m) with cristae, intranuclear tubules (nt), dorsal granular plate (gp), and dark lamella in perinuclear space (pl) are seen.

35. *C. borealis* ♂. Fix. 3-A. Cross section of posterior part of tail fin with extracellular coat (ec).

36. *C. borealis* ♂. Fix. 3-A. Median section of nuclear region with mitochondria (m), and dorsal granular plate (gp). Note enlarged perinuclear space with dark lamella (pl).

*Legends to all figures*: cl = "claw", cr = central rod, ec = extracellular coat, gp = dorsal granular plate, m = mitochondria, n = nucleus, ne = nuclear envelope, nt = intranuclear tubules, ntn = light core of intranuclear tubule, surrounded by nuclear envelope, ntx = corresponding light core on the opposite side, still in postacrosomal matter, both derived from core of ventral rod, pa = posterior limit of acrosomal vesicle, pl = dark lamella in perinuclear space, pm = plasma membrane, vr = ventral rod.


pa
cl
cr
vr
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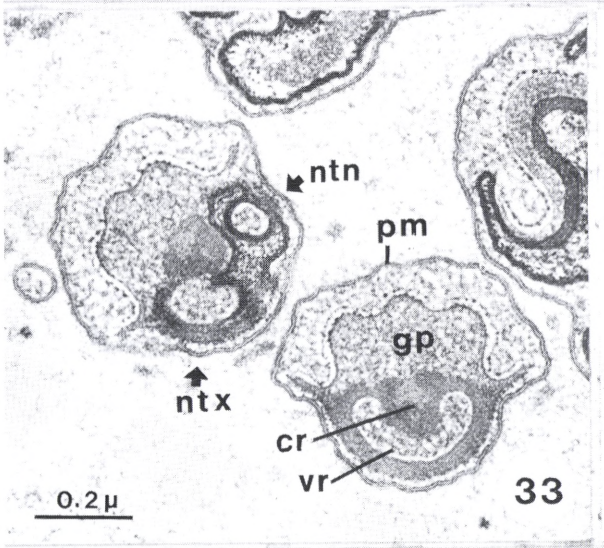
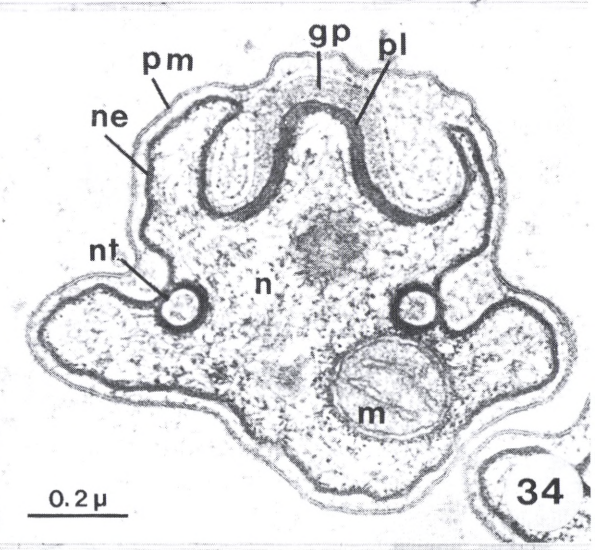
32


ntn
pm
gp
ntx
cr
vr
0.2µ
33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n
m
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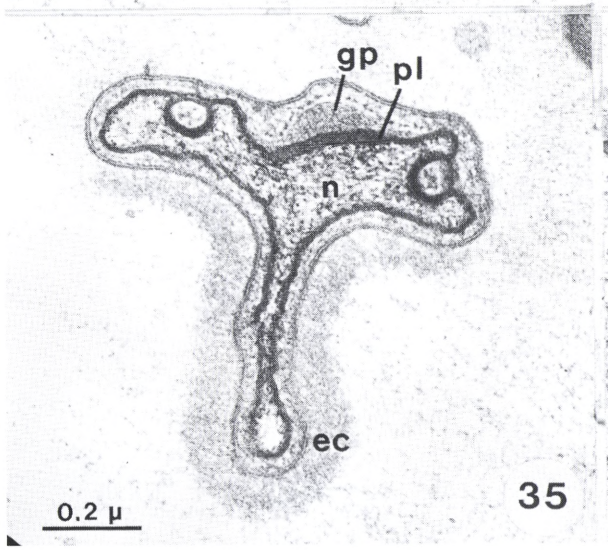
34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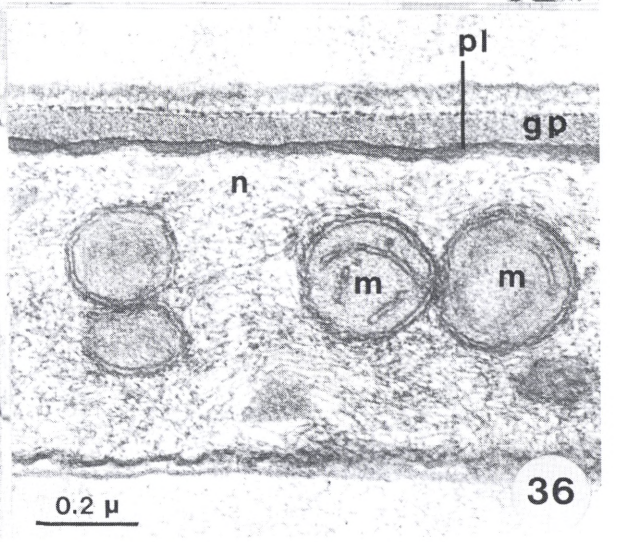

pl
gp
n
m
m
0.2 µ
36

PLATE 7

*Conchoecia.*

37. *Conchoecia elegans* ♂. Fix. 3-A. Spermatid in early stage of elongation. Postacrosomal rod or perforatorium (p) has grown out in front of nucleus (n), carrying the cap-shaped acrosomal vesicle (a) on the top.

38. *C. elegans* ♂. Fix. 2 % $OsO_4$. Close-up of median section of acrosomal vesicle (a) and perforatorium (p). Inset shows transverse section of acrosomal vesicle of *C. borealis*, fix. 3-A. Slightly greater scale than main figure.

39. *C. elegans* ♂. Fix. 2 % $OsO_4$. Median section of anterior end of not quite mature spermatid. The extension of the strongly flattened, cap-shaped acrosomal vesicle (a) is seen. Arrows mark the limits where the wall of the vesicle doubles back.

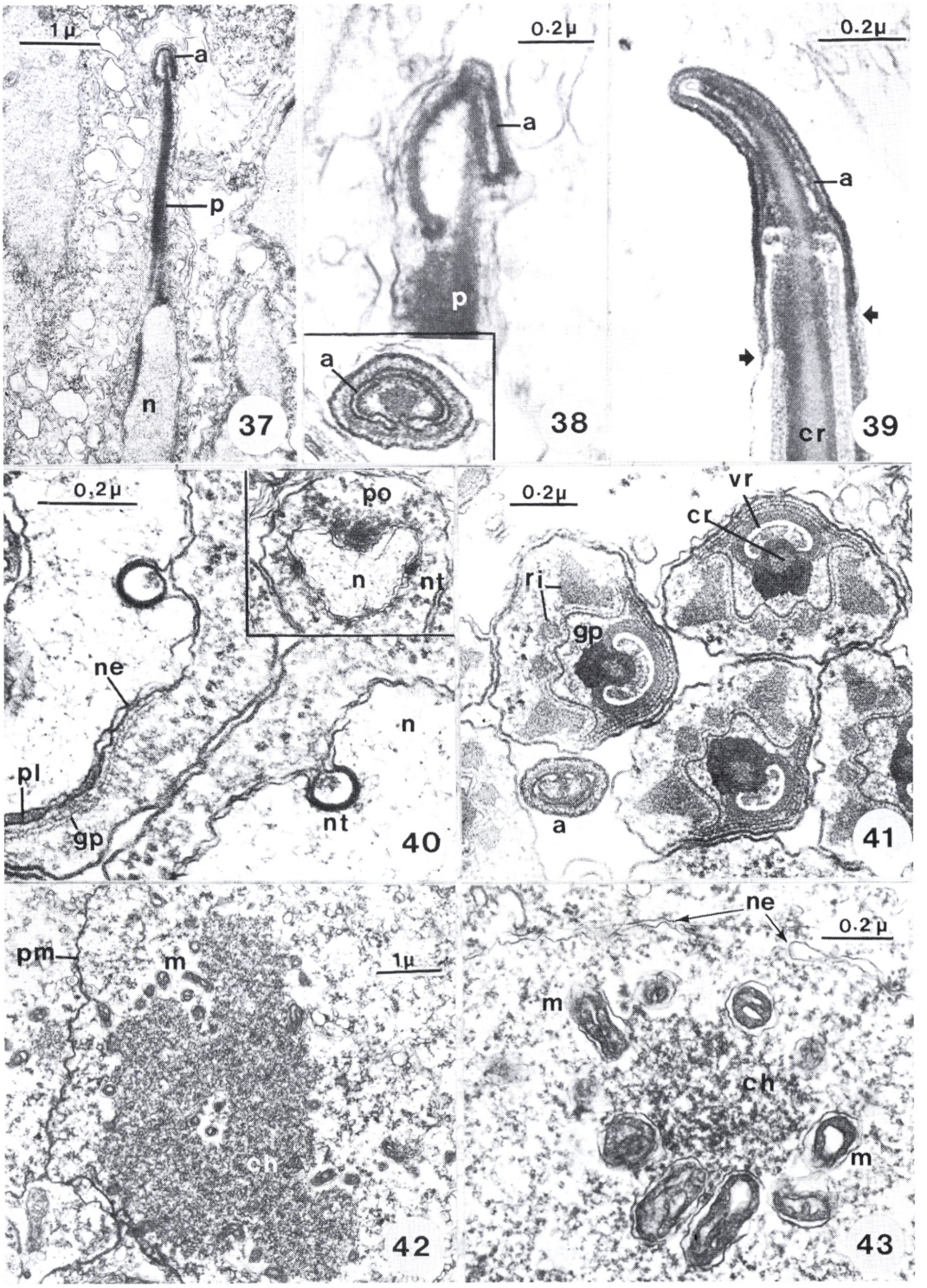
40. *C. borealis* ♂. Fix. 3-A. Cross sections of spermatids showing development of intranuclear tubules (nt) and their relation to nuclear envelope (ne). A dorsal granular plate (gp, still stratified) is forming and a dark lamella is accumulating in the perinuclear space (pl) below it.
Inset in upper right corner shows earlier stage of the nucleus with primordial dark strings (nt) attached to the surface, before the folds have arisen. The extension of postacrosomal matter (po) on the dorsal side of the nucleus is seen. Scale as main figure.

41. *C. borealis* ♂. Fix. 2 % $OsO_4$. Cross section through postacrosomal regions of elongating spermatids, with central rods (cr), U-shaped ventral rods (vr) and temporary ridges (ri).

42. *C. borealis* ♂. Fix. 3-A. Part of spermatocyte in 2nd meiotic division. Mitochondria (m) attach to the contracted chromosomes (ch). No nuclear envelope is present.

43. *C. borealis* ♂. FIx. 3-A. Somewhat more advanced stage than Fig. 42. The new nuclear envelope (ne) is forming by fusion of flat sacs of endoplasmic reticulum, locking up the mitochondria inside the nucleus.

*Legends to all figures*: a = acrosomal vesicle, ch = chromosomes, cr = central rod, gp = dorsal granular plate, m = mitochondria, n = nucleus, ne = nuclear envelope, nt = nuclear tubules, p = perforatorium, pl = dark lamella in perinuclear space, pm = plasma membrane, po = postacrosomal dark matter, ri = temporary ridges of postacrosomal matter, vr = ventral rod.


1 µ
a
p
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37
0·2µ
a
p
a
38
0·2µ
a
cr
39
0,2µ
po
n
nt
ne
n
pl
gp
nt
40
0·2µ
vr
cr
ri
gp
a
41
pm
m
1µ
ch
42
ne
0·2 µ
m
ch
m
43

PLATE 8

*Conchoecia – Polycope.*

44. *Conchoecia borealis* ♂. Fix. 3-A. Early spermatid, about the same stage as Fig. 43. End of 2nd meiotic division. New nuclear envelope (ne) is being formed, also visible in Fig. 43. Mitochondria (m) are still attached to chromosomes (ch), which are disintegrating.

45. *C. elegans* ♂. Fix. 2 % $OsO_4$. Early spermatid in which nuclear envelope (ne) has just formed. Mitochondria (m) have detached from chromosomes and are scattered in nucleus (n).

46. *Polycope orbicularis* ♂. Fix. 2 % $OsO_4$. Anterior half of nearly mature spermatid, longitudinal section. The original spermatid plasm (osp) is still attached to cyst wall. Inset shows cross section of acrosomal end of mature sperm from the receptaculum of ♀ *Polycope*, fixed in 2 % $OsO_4$.

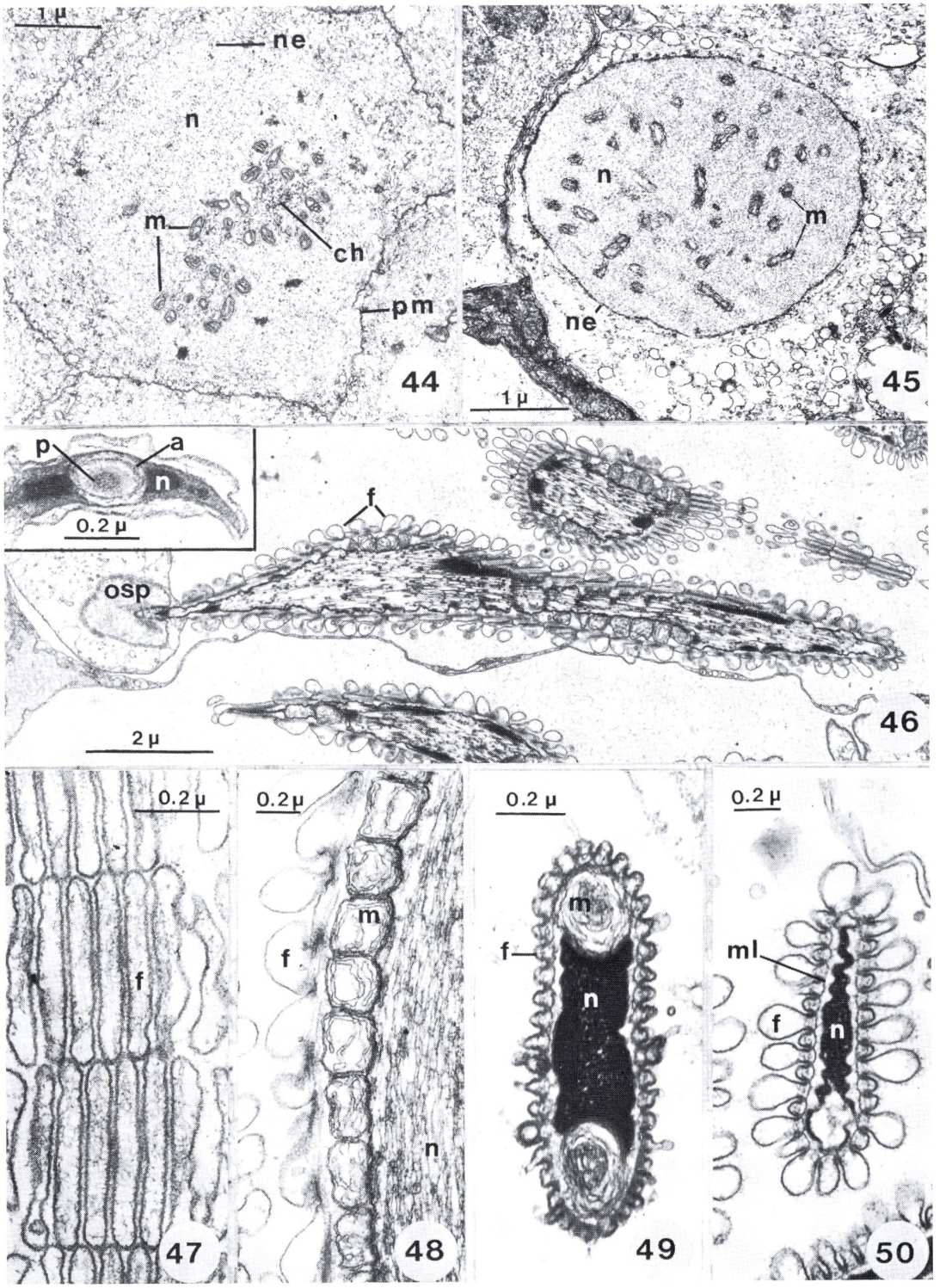
47. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Tangential section of surface of of mature spermatozoon, showing regular transverse belts of folds of the superficial plasma membrane (f).

48. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Longitudinal section through margin of mature spermatozoon, showing the single row of cubical mitochondria (m), nucleus (n) and folds of the plasma membrane (f, irregularly cut).

49. *P. orbicularis* ♀. Fix. 2 % $OsO_4$. Cross section of mature sperm from receptaculum seminis. Nucleus (n), mitochondria (m) with partly disarranged cristae, and superficial folds of the plasma membrane (f) are seen.

50. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Cross section of the tail end of mature spermatozoon. No mitochondria. Note regular arrangement of larger and smaller folds (f), and the membranelike condensation at their base (ml).

*Legends to all figures*: a = acrosomal vesicle, ch = chromosomes, f = folds of plasma membrane, m = mitochondria, ml = membranelike condensation at base of superficial folds, n = nucleus, ne = nuclear envelope, osp = original cell body of spermatid, p = perforatorium, pm = plasma membrane.


1 µ
ne
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ch
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44
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45
p
a
n
0.2 µ
f
osp
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46
0.2 µ
f
47
0.2 µ
m
f
n
48
0.2 µ
m
f
n
49
0.2 µ
ml
f
n
50

PLATE 9

*Polycope orbicularis*.

51. *Polycope orbicularis* ♂. Fix. 2 % $OsO_4$. Advanced spermatids attached to walls of follicles (sf) in the testicle.

52. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Early spermatid with acrosomal vesicle (a), Golgi apparatus (g) and first appearance of folds of the plasma membrane on the prospective nuclear process.

53. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Early spermatid. As Fig. 52 but note the dark perforatorium that has formed inside the acrosomal vesicle (See also Fig. 54).

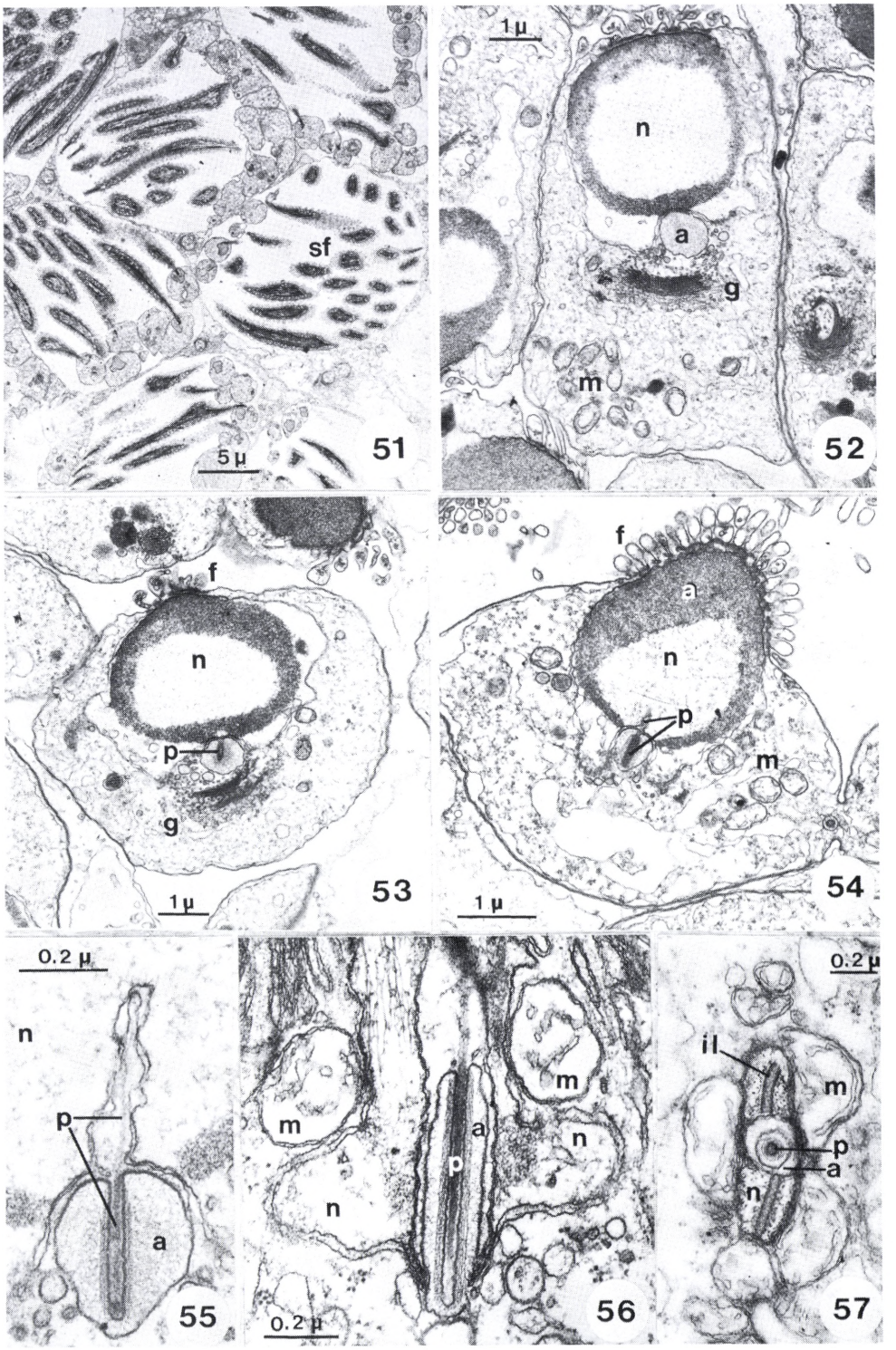
54. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Spermatid with acrosomal vesicle (a), perforatorium (p) and scattered mitochondria (m). The nucleus (n) begins to grow out, covered by folds of the plasma membrane (f).

55. *P. orbicularis* ♂. Fix. $OsO_4$. Spermatid with acrosomal vesicle (a). The perforatorium has invaginated the acrosomal vesicle (a) and the nucleus (n).

56. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Advanced spermatid with elongated acrosomal vesicle (a) containing long perforatorium (p) which extends into the nucleus. Mitochondria begin to concentrate near the base of the nucleus, which extends forwards on each side of the acrosomal vesicle.

57. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Not quite mature spermatozoon. Cross section of acrosomal vesicle (a), perforatorium (p) and anterior nuclear processes (n). The intranuclear lamellae (il) are distinct.

*Legends to all figures*: a = acrosomal vesicle, f = folds of plasma membrane on nuclear process, g = Golgi apparatus, il = intranuclear lamellae, m = mitochondria, n = nucleus, p = perforatorium, sf = spermatogenic follicle in testis.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a
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f
n
p
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f
a
n
p
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1 µ
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0.2 µ
n
p
a
55
m
m
a
p
n
n
0.2 µ
56
0.2 µ
il
m
p
a
n
57

PLATE 10

*Polycope – Cytherella.*

58. *Polycope orbicularis* ♂. Fix. 2 % $OsO_4$. Cross section of nuclear process of spermatid, before the invasion of mitochondria. Membrane folds (f) of plasma membrane are well developed, a membranelike condensation (ml) is beginning to appear at their base. Intranuclear condensation (il) is seen as an oval profile.

59. *P. orbicularis* ♂. Fix. 2 % $OsO_4$. Cross section of acrosomal end of spermatid, showing mitochondria (m) crowded in "ready position" around base of the nucleus before they invade the nuclear process.

60. *Cytherella abyssorum* ♂. Fix. 3-A. Median section of anterior end of spermatozoon in vesicula seminalis, showing one of the four rows of flattened endoplasmic sacs (es), the perforatorium (p), the "claw" (cl, acrosomal region), and wavy course of lamellate rods (lr).

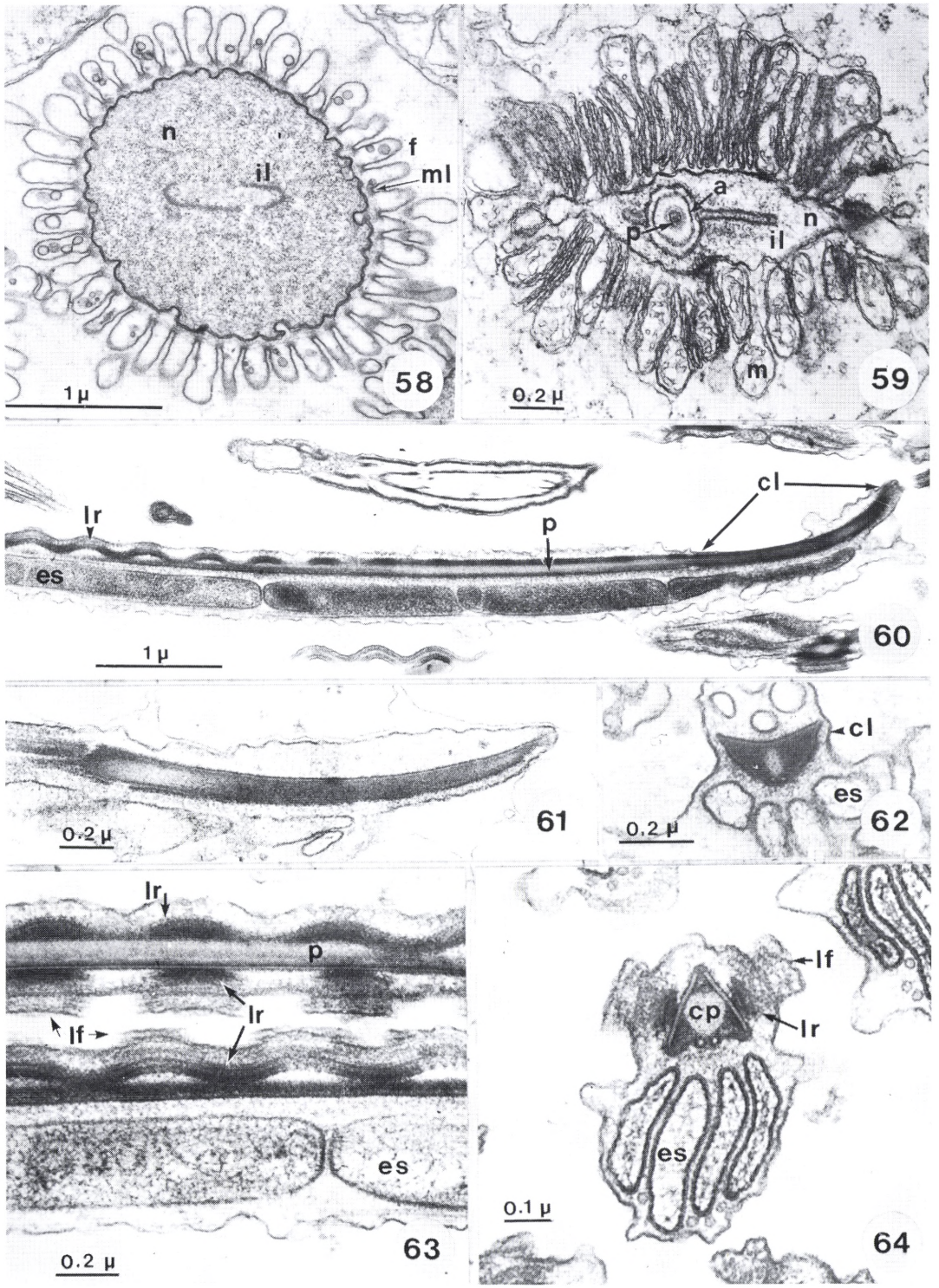
61. *C. abyssorum* ♂. Fix. 3-A. Median section of the "claw" (acrosomal vesicle) of nearly mature sperm.

62. *C. abyssorum* ♂. Fix. 3-A. Cross section of the "claw" showing its central light core. Nearly mature sperm.

63. *C. abyssorum* ♂. Fix. 3-A. Longitudinal section tangential to plane sides of triangular perforatorium, showing wavy course of lamellate rods (lr). Compare with Fig. 64.

64. *C. abyssorum* ♂. Fix. 3-A. Cross section of postacrosomal region of mature sperm, showing triangular perforatorium with lamellate rods (lr) and their connection with longitudinal folds (lf) on surface. Perforatorium with light central core (cp). A cross section of the four rows of flattened vesicles (es) is seen in the ventral part of the figure.

*Legends to all figures*: a = acrosomal vesicle, cl = the "claw" (mature acrosomal vesicle), cp = central light core of perforatorium, es = flattened endoplasmic sacs, f = folds of plasma membrane, il = intranuclear condensations, lf = longitudinal folds of surface, lr = lamellate rods, with wavy course, ml = membranelike condensations at base of folds of plasma membrane, m = mitochondria, n = nucleus, p = perforatorium.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il
f
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p
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il
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lr
p
cl
es
1 µ
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0.2 µ
61
cl
es
0.2 µ
62
lr
p
lr
lf
es
0.2 µ
63
lf
cp
lr
es
0.1 µ
64

PLATE 11

*Cytherella abyssorum.*

65 and 66. *Cytherella abyssorum* ♂. Fix. 3-A. Cross sections of postacrosomal region of mature sperm in testicle. Note the different levels of the lamellate rods (lr) and longitudinal folds (lf). Both figures to same scale.

67. *C. abyssorum* ♂. Fix. 3-A. Longitudinal section of perforatorium (p), showing lamellate structure of lamellate rods (lr).

68. *C. abyssorum* ♂. Fix. 3-A. Longitudinal section of anterior end of nucleus (n), acrosomal vesicle (a) and perforatorium (p) of young spermatid.

69. *C. abyssorum* ♂. Fix. 3-A. Older stage than Fig. 68, longitudinal section. The perforatorium has withdrawn from the nucleus (n) and a dark plate (dp) has formed at the base of the perforatorium. The mitochondria (m) are still along the perforatorium in front of this plate.

70. *C. abyssorum* ♂. Fix. 3-A. Cross sections of anterior parts of postacrosomal region with perforatorium (p), two pairs of primordial longitudinal folds (lf) and endoplasmic vesicles (es). The latter are still irregular and are not ordered in four regular rows as in Figs. 60, 64, and 71. Same stage as Fig. 69.

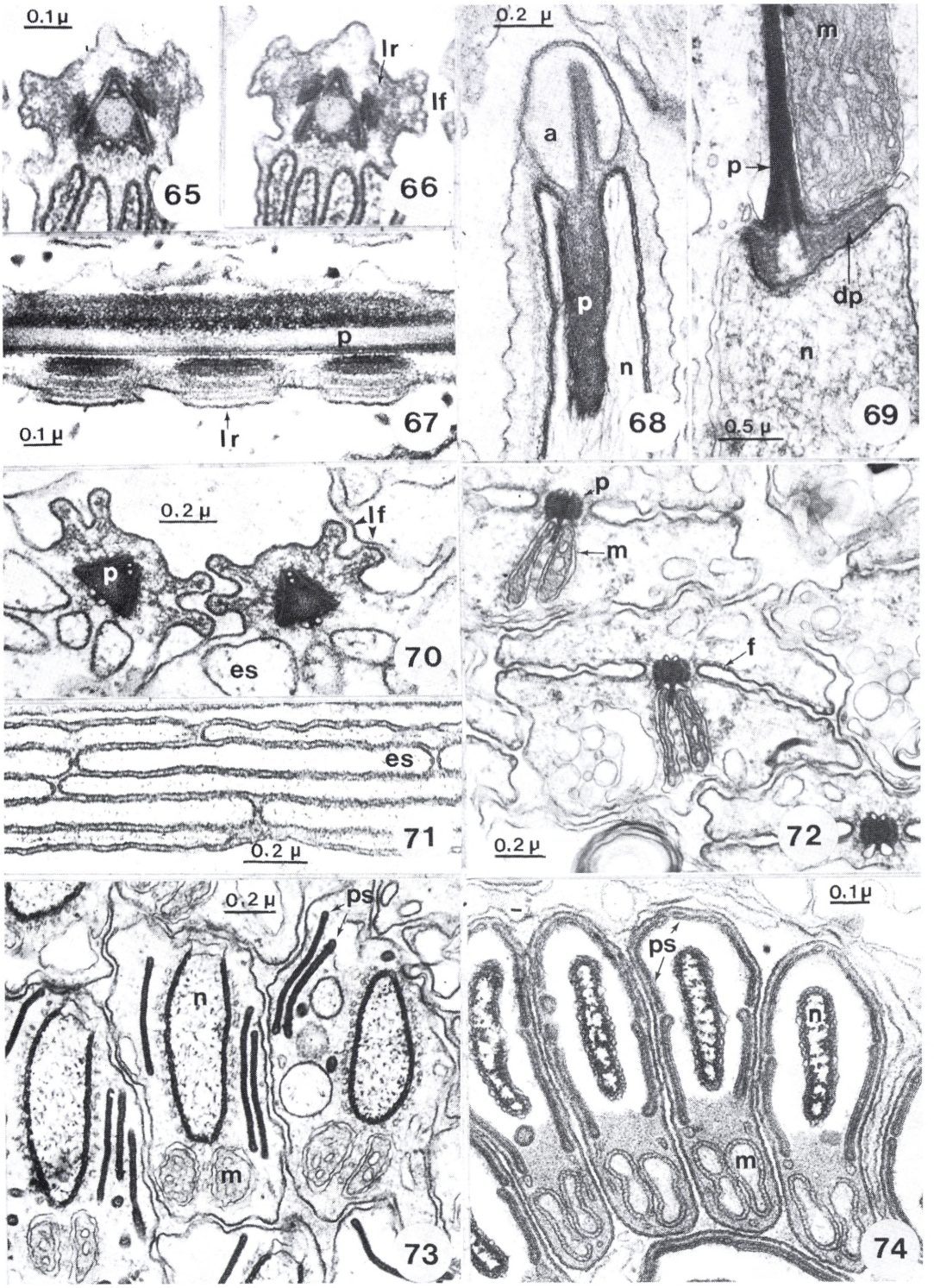
71. *C. abyssorum* ♂. Fix. 3-A. Horizontal section of postacrosomal region showing the four rows of endoplasmic sacs (es). Mature spermatozoon. Compare with Fig. 64.

72. *C. abyssorum* ♂. Fix. 3-A. Cross sections of the postacrosomal region of spermatids. The mitochondria (m) have not yet retracted from the region. Deep lateral furrows (f) divide the plasm on each side. Stage approximately as Fig. 69.

73. *C. abyssorum* ♂. Fix. 3-A. Cross sections through nuclear regions of late spermatids in which mitochondria have just appeared at this level. The dark platelike sacs (ps) of endoplasmic reticulum are still scattered.

74. *C. abyssorum* ♂. Fix. 3-A. Cross sections of nuclear regions of mature sperm. The mitochondria (m) have lost the cristae, and the platelike sacs (ps) form a continuous and regular layer under the plasma membrane.

*Legends to all figures*: a = acrosomal vesicle, dp = dense plate at base of perforatorium, es = endoplasmic sacs, irregular in Fig. 70, arranged in four rows in Fig. 71, f = lateral furrows, dividing the plasm in Fig. 72, lf = longitudinal folds in Figs 65, 67 and 70, lr = lamellate rods, m = mitochondria, n = nucleus, p = perforatorium, ps = platelike sacs.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0.5 µ
69
0.2 µ
lf
p
es
70
es
0.2 µ
71
p
m
f
0.2 µ
72
0.2 µ
ps
n
m
73
0.1 µ
ps
n
m
74

PLATE 12

*Bairdoppilata cushmani.*

75. *Bairdoppilata cushmani* ♂. Fix. 3-A. Section of testis showing one complete cross section of nuclear region with mitochondrion (m) and three cross sections of acrosomal region in front of the mitochondria, all of mature spermatozoa.

76. *B. cushmani* ♂. Fix. 3-A. Mature spermatozoon. Cross section of mitochondrial part of acrosomal region. The inset shows the longitudinal section of the dark mantle around the acrosomal furrow, with distinct cross striation (magnification 1.15 times that inmain figure).

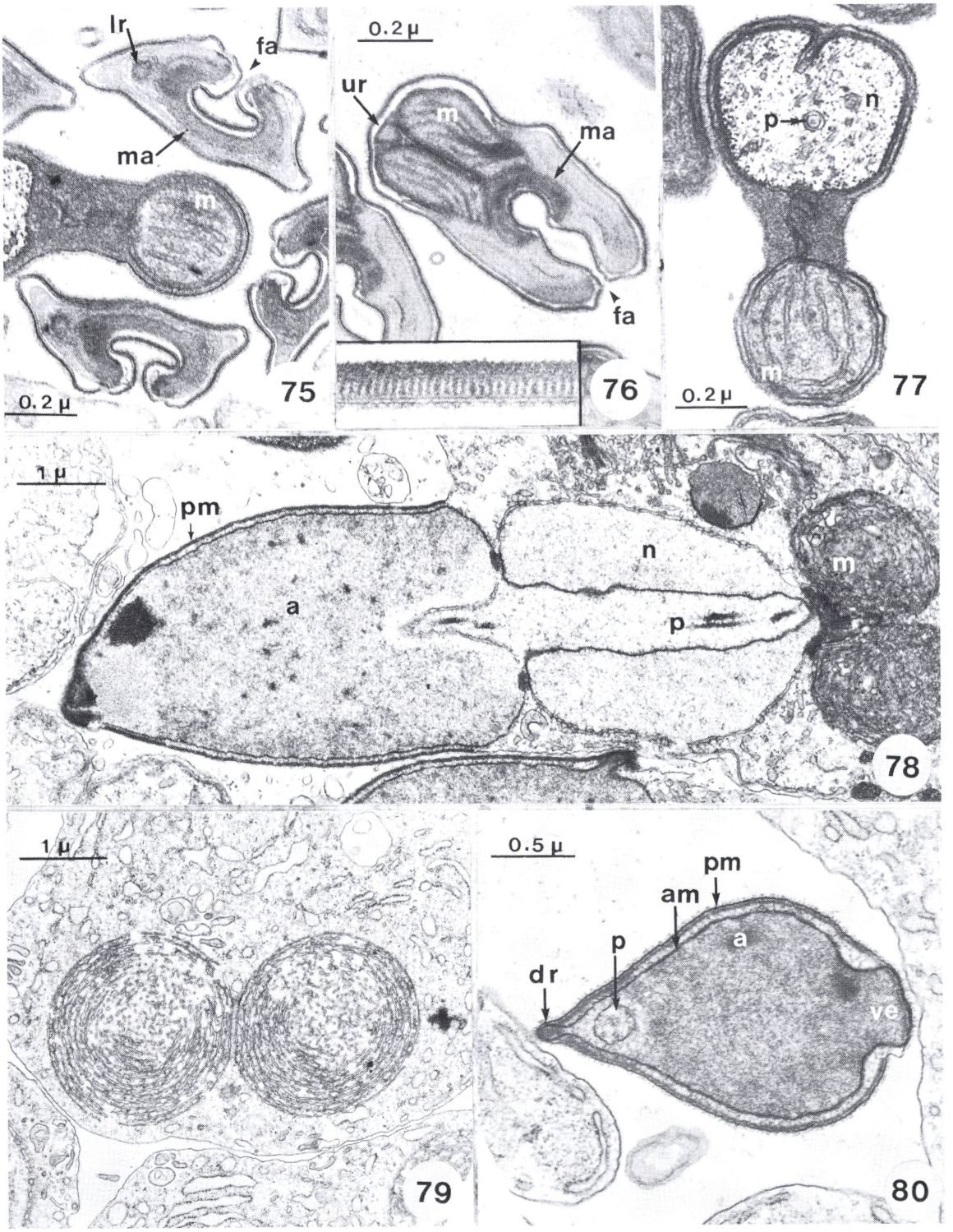
77. *B. cushmani* ♂. Fix. 3-A. Cross sectionof nuclear region of mature speramtozoon, with mitochondrion (m), nucleus (n) and vestigial perforatorium (p).

78. *B. cushmani* ♂. Fix. 3-A. Longitudinal section of spermatid with acrosomal vesicle (a), perforatorium (p), nucleus (n) and mitochondria (m, Nebenkern). The perforatorium consists of two dark rods. Thickened plasma membrane (pm) on acrosomal vesicle.

79. *B. cushmani* ♂. Fix. 3-A. Cros section of Nebenkern of young spermatid. The two large mitochondria are attached to one another by dark matter.

80. *B. cushmani* ♂. Fix. 3-A. Cross section of acrosomal vesicle of spermatid, somewhat older than Fig. 78. Dorsal rod (dr), ventral rod (ve), perforatorium (p) and thickened plasma membrane (pm) can be seen.

*Legends to all figures:* a = acrosomal vesicle, am = membrane of acrosomal vesicle, dr = dorsal rod of acrosomal vesicle, fa = longitudinal furrow on acrosome, lr = longitudinal dark rod (asymmetrical) in acrosome, m = mitochondrion, ma = dark mantle around acrosomal furrow, n = nucleus, p = perforatorium, pm = thickened palsma membrane, ur = unpaired rod between the mitochondria, ve = ventral rod or plate of acrosomal vesicle.


lr
fa
ma
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75
0.2 µ
ur
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ma
fa
76
n
p
m
0.2 µ
77
1 µ
pm
a
n
p
m
78
1 µ
79
0.5 µ
pm
am
p
a
dr
ve
80

PLATE 13

*Bairdoppilata cushmani.*

81. *Bairdoppilata cushmani* ♂. Fix. 3-A. Median section through acrosomal vesicle (a), nucleus (n) and perforatorium (p) of spermatid, more advanced than stage shown in Fig. 78.

82. *B. cushmani* ♂. Fix. 3-A. Cross section of acrosomal region of espermatid, more advanced than Figs. 78 and 80.

83. *B. cushmani* ♂. Fix. 3-A. As Fig. 82, but the section cuts the basal part of the acrosomal region. Perforatorium (p), mitochondria (m), acrosomal furrow (fa) and the dark mantle of the latter (ma) can be seen.

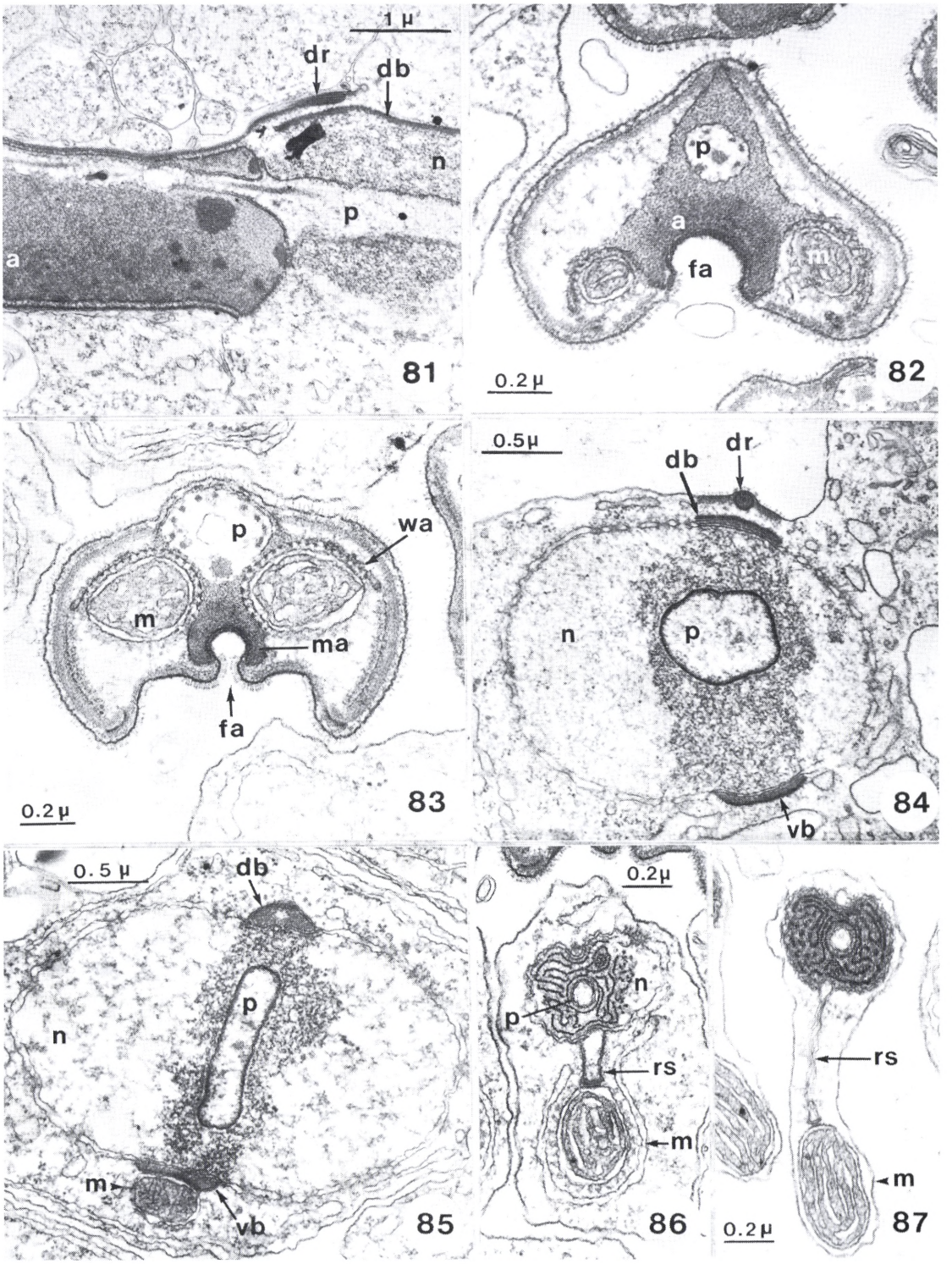
84. *B. cushmani* ♂. Fix. 3-A. Cross section of nucleus (n) of early spermatid. Age as Fig. 81. The perforatorium (p) and the dorsal and ventral bands of the nuclear envelope (db and vb) are seen; dr is the posterior extension of the dorsal rod of the acrosomal vesicle (Compare with Fig. 81). Chromatin condensation around perforatorium in nucleus.

85. *B. cushmani* ♂. Fix. 3-A. Cross section of nuclear region and perforatorium of spermatid, showing dorsal and ventral nuclear bands (db and vb). The mitochondrion (m) of the ventral side grows forwards in contact with the ventral band (vb).

86. *B. cushmani* ♂. Fix. 3-A. Cross section of nuclear region of late spermatid with lamellate condensation of the chromatin. Perforatorium (p) and ventral mitochondrion (m) visible. An elongated sac of endoplasmic reticulum (rs) connects nucleus and mitochondrion.

87. *B. cushmani* ♂. Fix. 3-A. Cross section of nuclear region of nearly mature sperm. Chromatin lamellae and perforatorium still present.

*Legends to all figures*: a = acrosomal vesicle, db = dorsal band on nuclear membrane, dr = dorsal rod of acrosome, fa = longitudinal furrow on acrosome, m = mitochondrion, ma = dark mantle on acrosomal furrow, n = nucleus, p = perforatorium, rs = sac of endoplasmic reticulum between nucleus and mitochondrion, vb = ventral band on nuclear membrane, wa = winglike extension of acrosomal vesicle covering the mitochondrion.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dr
db
n
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p
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m
0.2 µ
82
p
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m
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84
0.5 µ
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m
vb
85
0.2µ
n
p
rs
m
86
rs
m
0.2 µ
87

PLATE 14

Cytheracea, acrosomal crest.

88. *Cytheropteron latissimum* ♀. Fix. 2 % $OsO_4$ in bicarbonate buffer. Cross section of acrosomal region, with acrosomal crest (ac) and mitochondria (m).

89. *C. alatum* ♂. Fix. 3-A. Cross section of acrosomal region of mature sperm from vesicula seminalis. All sperm of *C. alatum* had an acrosomal crest different from that of *C. latissimum* (Fig. 88).

90. *Paradoxostoma variabile* ♀. Fix. 2 % $OsO_4$. Cross section of acrosomal region of mature sperm. Note acrosomal furrow (fa), acrosomal crest (ac), basal rod of the crest (br) and the dark longitudinal filaments in the coat (arrows).

91. *Cytherois fischeri* ♂. Fix. 3-A. Cross section of acrosomal region of nearly mature sperm showing normal mitochondria with cristae (m).

92. *Paradoxostoma* sp. from Barbados, ♂. Fix. 3-A. Cross section of acrosomal region of mature sperm showing the extremely complicated acrosomal crest (ac).

93. *Paradoxostoma ensiforme* ♂. Fix. 3-A. Cross section of acrosomal region of nearly mature sperm from testicle.

*Legends to all figures*: ac = acrosomal crest, br = basal dark rod in acrosomal crest, ec = extracellular coat, fa = longitudinal furrow on acrosomal region, m = mitochondria.

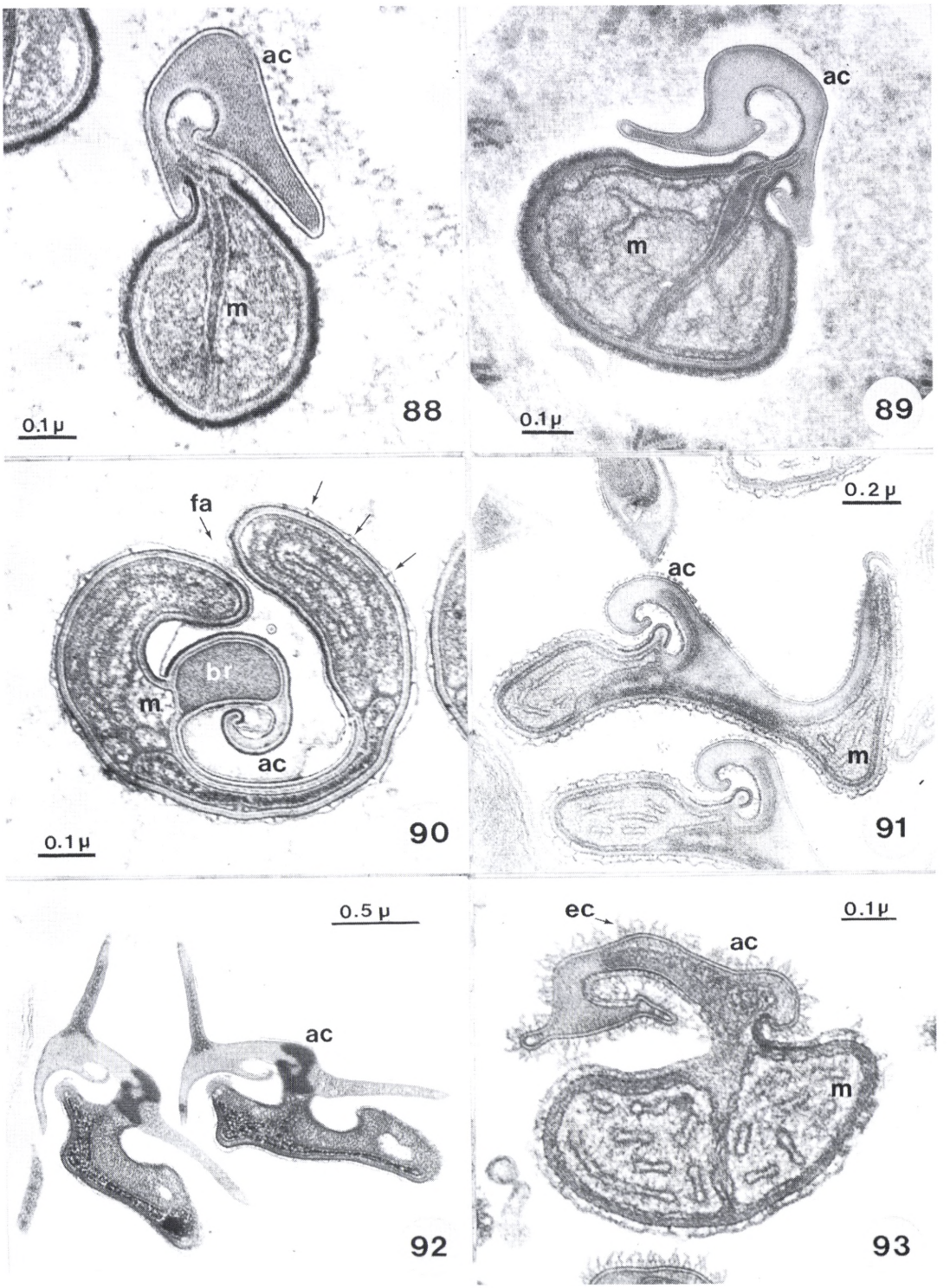

ac
m
0.1 µ
88
ac
m
0.1 µ
89
fa
br
m
ac
0.1 µ
90
0.2 µ
ac
m
91
0.5 µ
ac
92
ec
ac
m
0.1 µ
93

PLATE 15

Cytheracea, acrosomal crest.

94. *Loxoconcha impressa* ♂. Fix. 3-A. Cross section of acrosomal region of nearly mature sperm. Note acrosomal crest (ac) attached in the acrosomal furrow (fa). The rodlike bodies in the sperm fluid are not identified but are formed in vacuoles in the wall of the spermoduct.

95. *Leptocythere pellucida* ♂. Fix. 3-A. Cross section of acrosomal regions, the left one through the very point. Fully mature sperm. Bodies in the sperm fluid are globular.

96. *Xestoleberis aurantia* ♂. Fix. 3-A. Cross section through acrosomal region of nearly mature sperm. The acrosomal crest (ac) is of the typical *Xestoleberis* type.

97. *Hirschmannia viridis* ♂. Fix. 3-A. Cross section of acrosomal region of mature sperm. Acrosomal crest with distinct dark basal rod (br).

98. *Xestoleberis aurantia* ♂. Fix. 2 % $OsO_4$ with bicarbonate. Acrosomal region of mature sperm. Mitochondria (m) modified.

99. *X. depressa* ♂. Fix. 3-A. Cross section of acrosomal region of mature sperm. The acrosomal crest (ac) is somewhat different from that of *X. aurantia* (Fig. 98). "x" is an unpaired rod probably corresponding to the unpaired rod in other species but has a structure somewhat similar to the mitochondria.

*Legends to all figures*: ac = acrosomal crest, br = basal dark rod of acrosomal crest, ec = extracellular coat, fa = furrow on acrosomal region, m = mitochondria, x = intermitochondrial rod with a structure remarkably similar to that of the mitochondria.

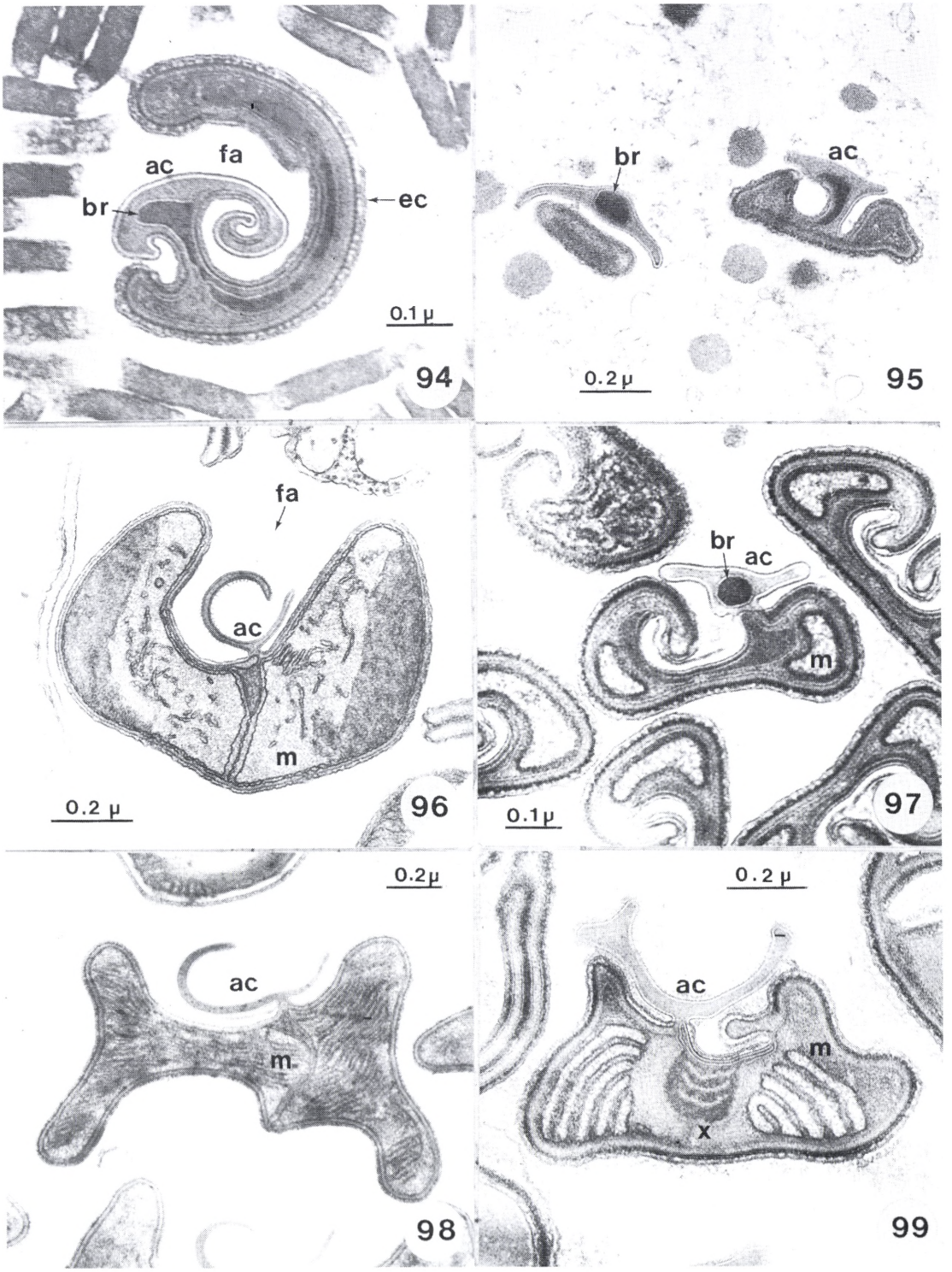

ac
fa
br
ec
0.1 µ
94
br
ac
0.2 µ
95
fa
ac
m
0.2 µ
96
br
ac
m
0.1µ
97
0.2µ
ac
m
98
0.2 µ
ac
m
x
99

PLATE 16

Cytheracea.

100. *Xestoleberis aurantia* ♂. Fix. $OsO_4$ with bicarbonate. One cross section of acrosomal region (lower left) and several cross sections of nuclear regions of mature spermatozoa. The nucleus lies in the free margin of a fold, attached between the segmented organelles A.

101. *X. aurantia* ♂. Fix. 3-A. Cross section through the transition from acrosomal to nuclear regions. The acrosomal crest is free and covers the dorsal side of the sperm like an apron.

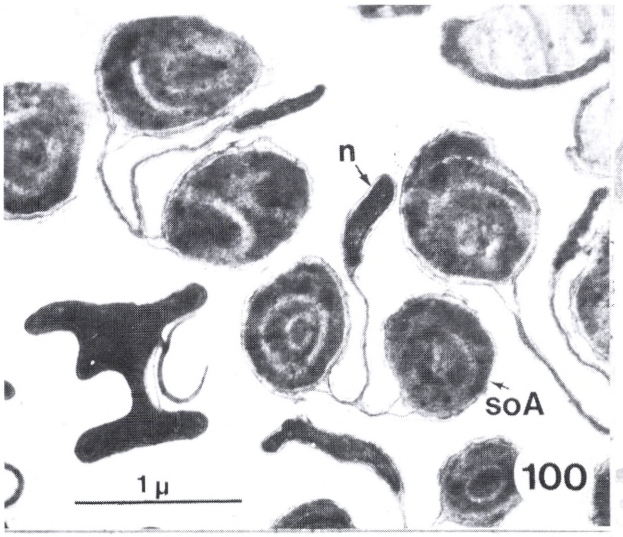
102. *Hirschmannia viridis* ♂. Fix. 3-A. Horizontal longitudinal section through the two segmented organelles B, compare with Fig. 108.

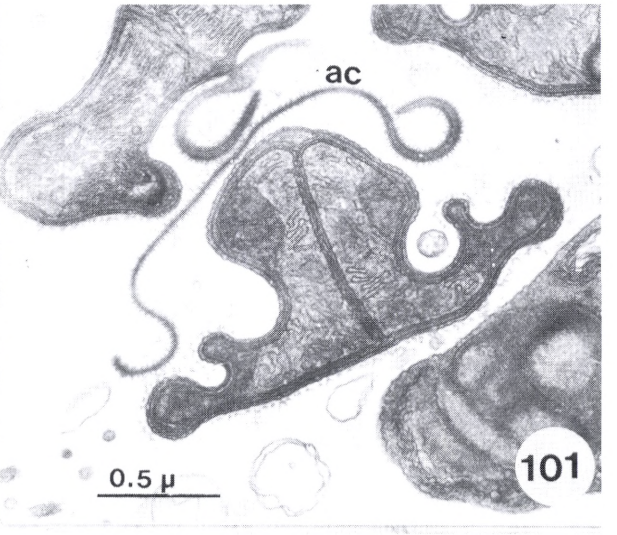
103. *Xestoleberis aurantia* ♂. Fix. 3-A. Longitudinal section through segmented organelle A of one side. Some sacs are filled with dark matter, other sacs not.

104. *X. aurantia* ♂. Fix. $OsO_4$ with bicarbonate buffer. Longitudinal section through mitochondrion in acrosomal region, showing densely set, probably metamorphosed cristae.

105. *Cythereis jonesi* ♂. Fix. $OsO_4$ with bicarbonate buffer. Half cross section of acrosomal region showing extracellular coat with regularly spaced dark rods (arrows).

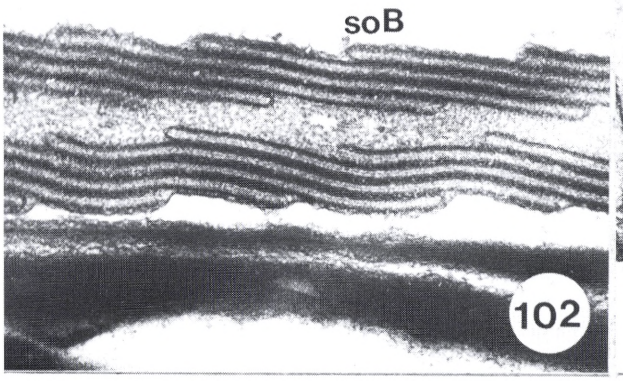
*Legends to all figures*: ac = acrosomal crest, fa = acrosomal furrow, m = mitochondria, n = nucleus, soA and soB = segmented organelles A and B.

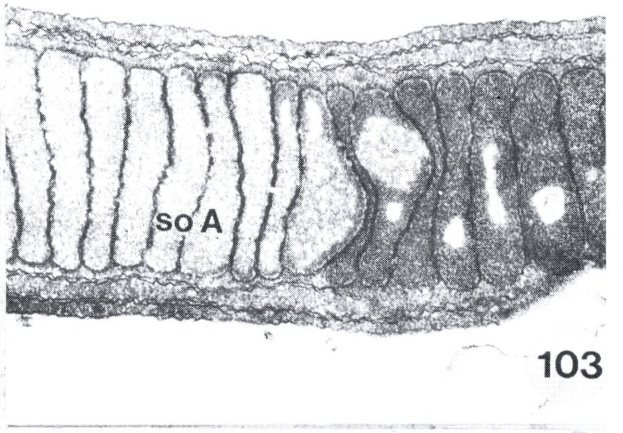


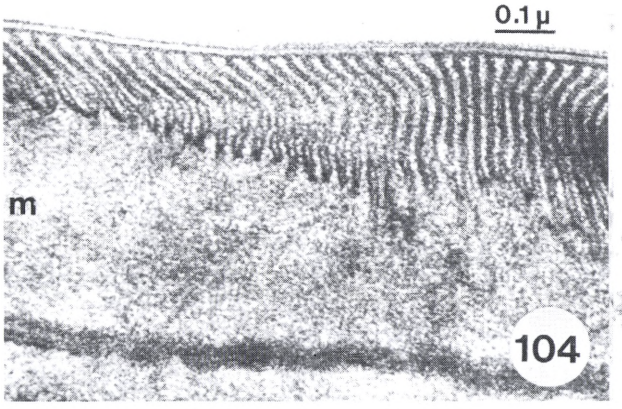




0.2 µ

0.5 µ



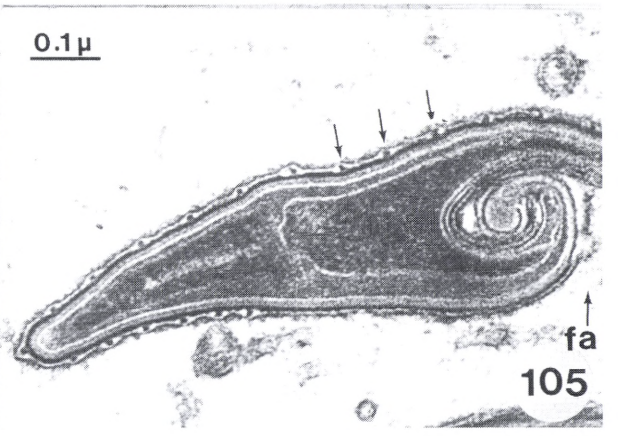



PLATE 17

Cytheracea, nucleus.

106. *Leptocythere pellucida* ♂. Fix. 3-A. Cross sections through nuclear regions of several spermatozoa. Only nuclei (n) are seen.

107. *Paradoxostoma variabile* ♂. Fix. 3-A. Cross section of nuclear region showing flat, spiralized nucleus (n) and segmented organelles B (soB).

108. *Hirschmannia viridis* ♂. Fix. 3-A. Cross section through nuclear region showing U-shaped nucleus (n) and the two plates of segmented organelles B (soB).

109. *Krithe barthonensis* ♂. Fix. 3-A. Cross section showing complicated nucleus (n) which partly surrounds segmented organelles A (soA). The Large mitochondria and segmented organelles B (soB) are from other spermatozoa.

110. *Cyprideis litoralis* ♂. Fix. $OsO_4$ with veronal acetate. Cross section of nuclear region with thin, U-shaped nucleus (n) partly surrounding segmented organelles A (soA), which have collapsed. Note regular configuration of segmented organelles B (soB). Compare with Fig. 112.

111. *Hemicythere oblonga* ♂. Fix. 3-A. Cross section of nuclear region showing nucleus (n) and segmented organelles A and B (soA and soB).

112. *Cyprideis litoralis* ♂. Fix. 3-A. Longitudinal section of nucleus (n) and segmented organelles A (soA). Compare with Fig. 110.

*Legends to all figures*: m = mitochondria, n = nucleus, soA and soB = segmented organelles A and B.

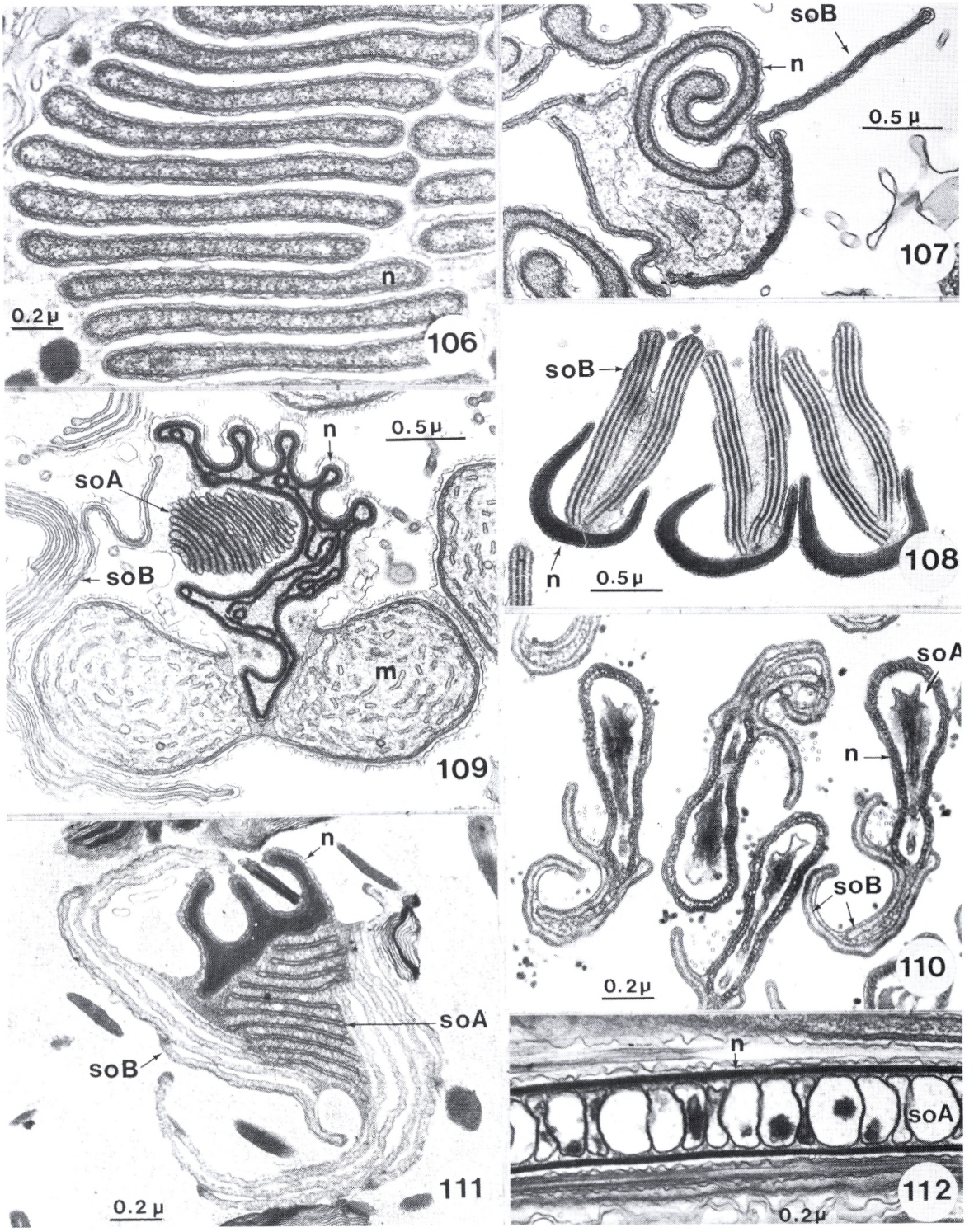

n
0.2 µ
106
soB
n
0.5 µ
107
n
0.5 µ
soA
soB
m
109
soB
n
0.5 µ
108
n
soA
soB
0.2 µ
110
n
soA
soB
0.2 µ
111
n
soA
112
0.2 µ

PLATE 18

Cytheracea, segmented organelles B.

113. *Xestoleberis aurantia* ♂. Fix. formalin, contrasted with uranyl acetate and spread on grid without sectioning. TEM picture. Tail fin with two plates of segmented organelles B, consisting of several imbricating flat vesicles. See cross section in Fig. 115.

114. *Loxoconcha impressa* ♂. Fix. 3-A and $OsO_4$ and dried for SEM. Tail fin with two rows of segmented organelles B, curved inwards as in Fig. 116.

115. *Xestoleberis aurantia* ♂. Fix. $OsO_4$ with bicarbonate. Double tail fin in cross section, each side consisting of segmented organelles B.

116. *Loxoconcha elliptica* ♂. Fix. 3-A. Double tail fin in cross section, showing segmented organelles B (soB) and metamorphosed mitochondria (m).

117. *Cytherura acuticostata* ♂. Fix. 3-A. Tail fin in cross section, showing segmented organelles B.

118. *Paradoxostoma ensiforme* ♂. Fix. 3-A. Tail end with double segmented organelles B (soB).

*Legends to all figures*: m = mitochondria, soB = segmented organelles B.

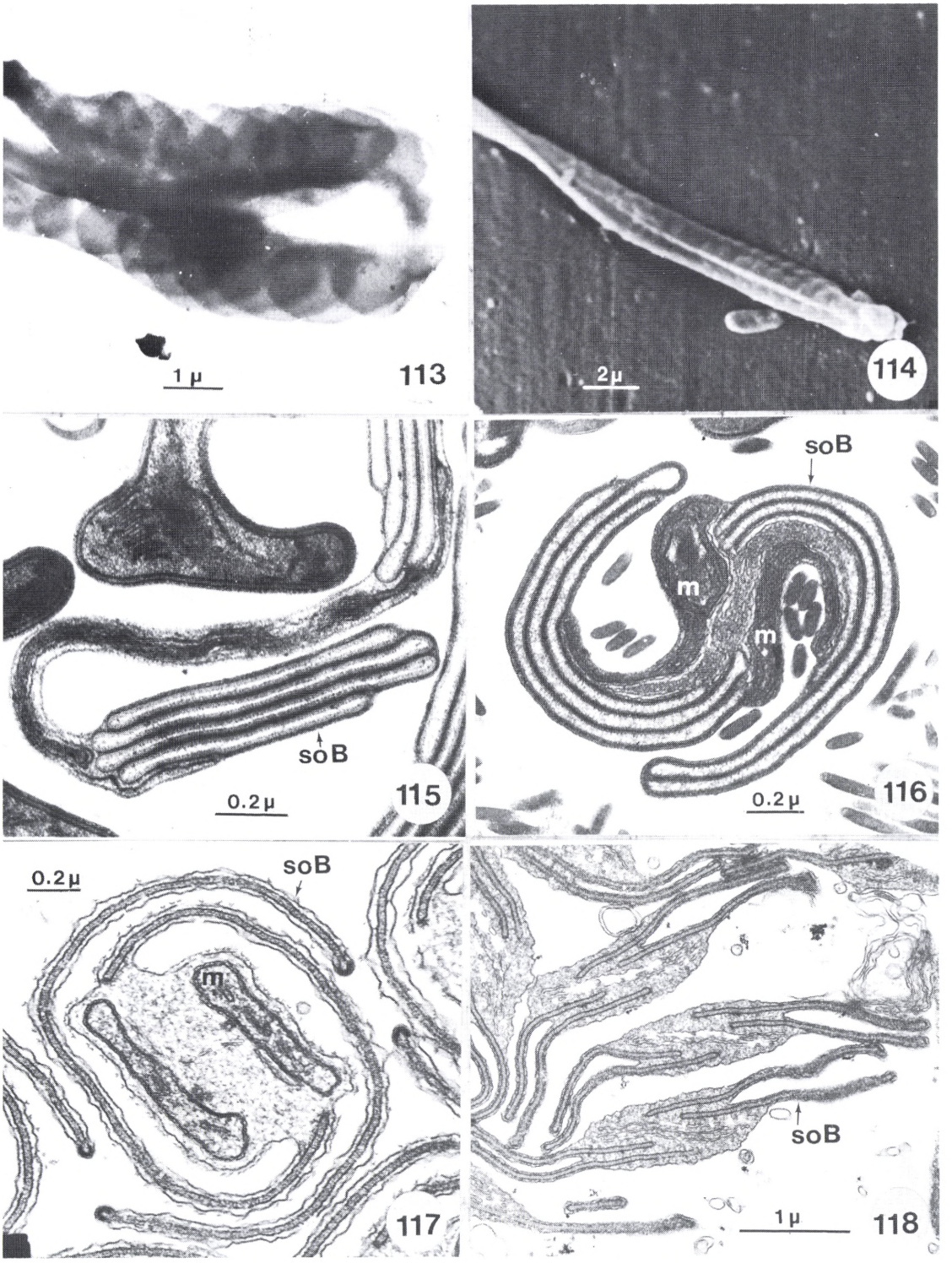

1 µ
113
2µ
114
soB
0.2µ
115
soB
m
m
0.2µ
116
0.2µ
soB
m
117
soB
1µ
118

PLATE 19

Cytheracea.

119. *Cythereis echinata* ♂. Fix. 3-A. Single sperm, osmified and freeze-dried for SEM. Micrograph shows "tail end" with numerous superficial, microvilli-like "haairs".

120. *Loxoconcha impressa* ♂. Fix. 3-A. Cross section of nuclear region with numerous microvilli-like plasmatic appendages (sm) attached along non-nuclear side of sperm, between the mitochondria.

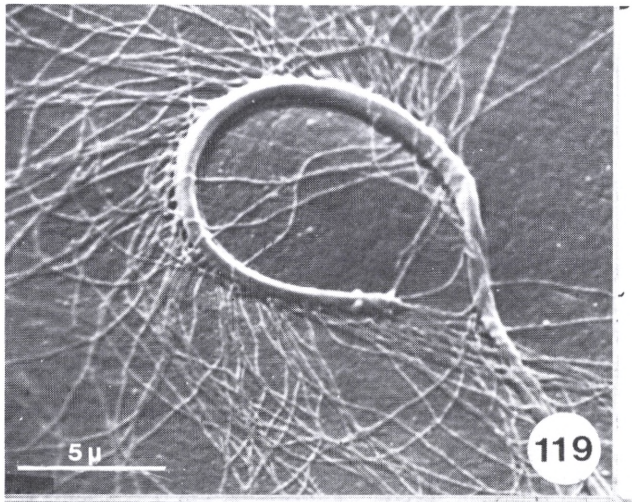
121. *Elofsonia baltica* ♂. Fix. 3-A. Single sperm, osmified and freeze-dried in benzene for SEM. The helical coiling, a feature rarely seen in cytherids, is distinct.

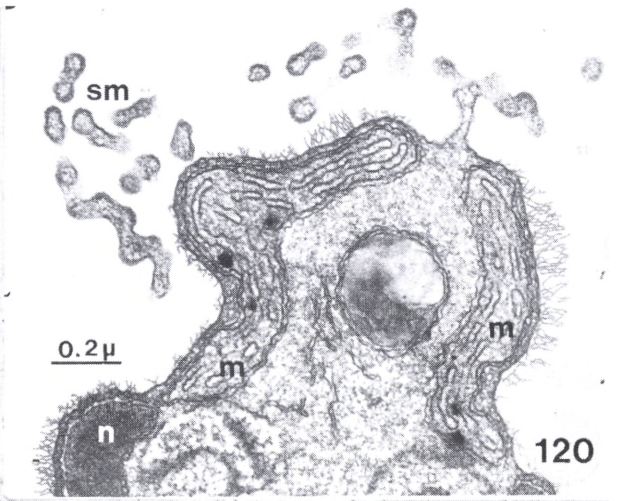
122. *E. baltica* ♂. Fix. 3-A. Section through testicle with early spermatids, showing paired development of Nebenkern (m).

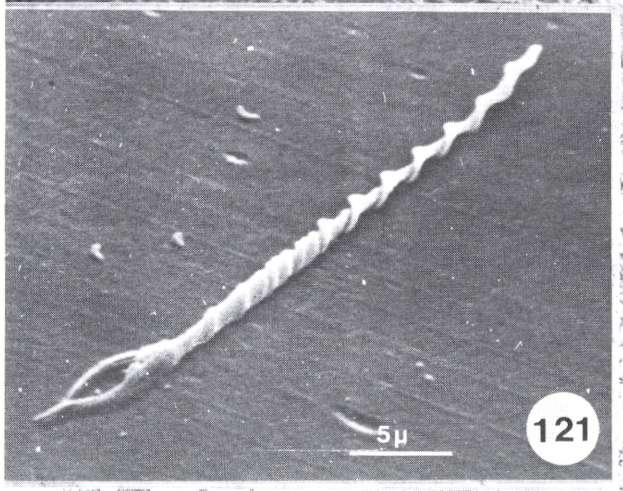
123. *Paradoxostoma variabile* ♂. Fix. 3-A. Early spermatid in testicle with mitochondrial Nebenkern (m), nucleus (n) and large acrosomal vesicle (a), the latter with labyrinthlike tubule pattern. Unknown body between the two mitochondria in the Nebenkern.

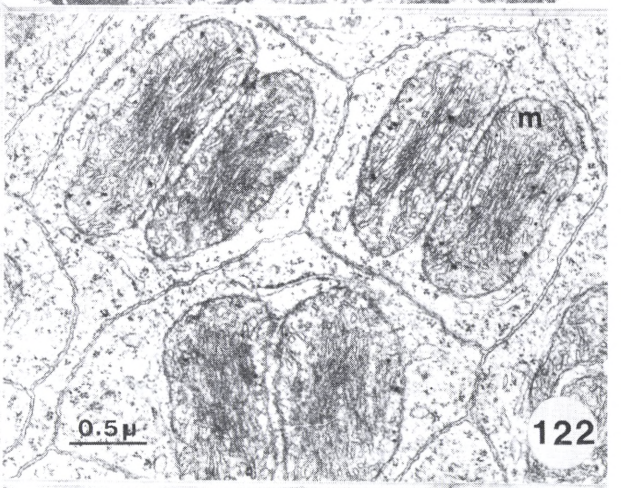
124. *Hemicythere oblonga* ♂. Fix. 3-A. Early spermatid with nucleus (n), acrosomal vesicle (a) and Golgi apparatus (g). The acrosomal vesicle is partly filled with labyrinthlike tubules, communicating with the lumen of the vesicle (al).

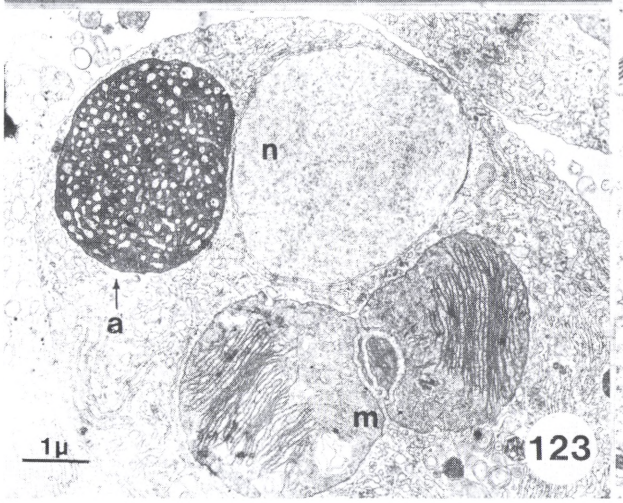
*Legends to all figures*: a = acrosomal vesicle, al = acrosomal labyrinth, i.e., netlike pattern of tubules in acrosomal vesicle, g = Golgi apparatus, m = mitochondria, n = nucleus, sm = superficial microvilli-like plasmatic appendages.


5µ
119


sm
0.2µ
m
m
n
120


5µ
121


m
0.5µ
122


n
a
m
1µ
123

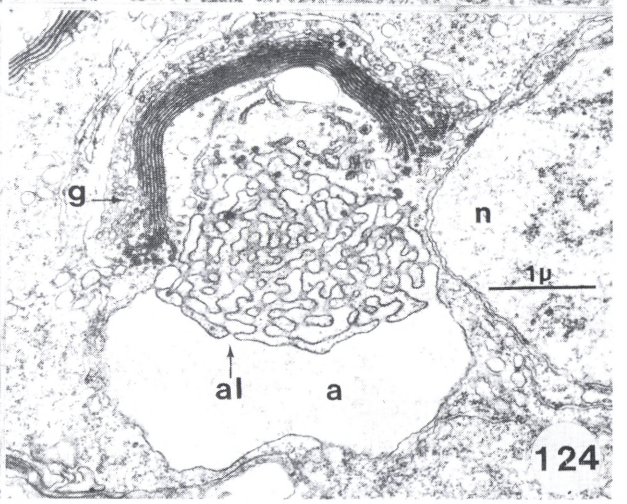

g
n
1µ
al
a
124

PLATE 20

Cytheracea.

125. *Paradoxostoma variabile* ♂. Fix. 3-A. Section of early spermatid with acrosomal vesicle filled with dark matter and containing acrosomal labyrinth of tubules.

126. *Xestoleberis depressa* ♂. Fix. 3-A. Cross section through end region of acrosomal process. The acrosomal vesicle is divided into several separate sacs, some of them empty, others filled with dark matter (a). "x" is an empty sac, probably belonging to another sperm. See Fig. 127.

127. *X. depressa* ♂. Fix. 3-A. Longitudinal section through acrosomal process of late spermatid, before spiralization begins. The acrosomal vesicle is subdivided (a). An invading mitochondrion (m) is seen. Compare with Fig. 126.

128. *X. aurantia* ♂. Fix. $OsO_4$ with bicarbonate buffer. Longitudinal section of acrosomal process. The acrosomal labyrinth (al) is still preserved near the nucleus (n) but peripheral parts of the acrosomal vesicle and the perforatorium are difficult to identify. The wall of the process is greatly thickened.

*Legends to all figures*: a = acrosomal vesicle, al = acrosomal labyrinth, m = mitochondrion, mc = membrane contact of acrosomal vesicle, n = nucleus, x = sac of acrosomal vesicle, probably from adjacent sperm.

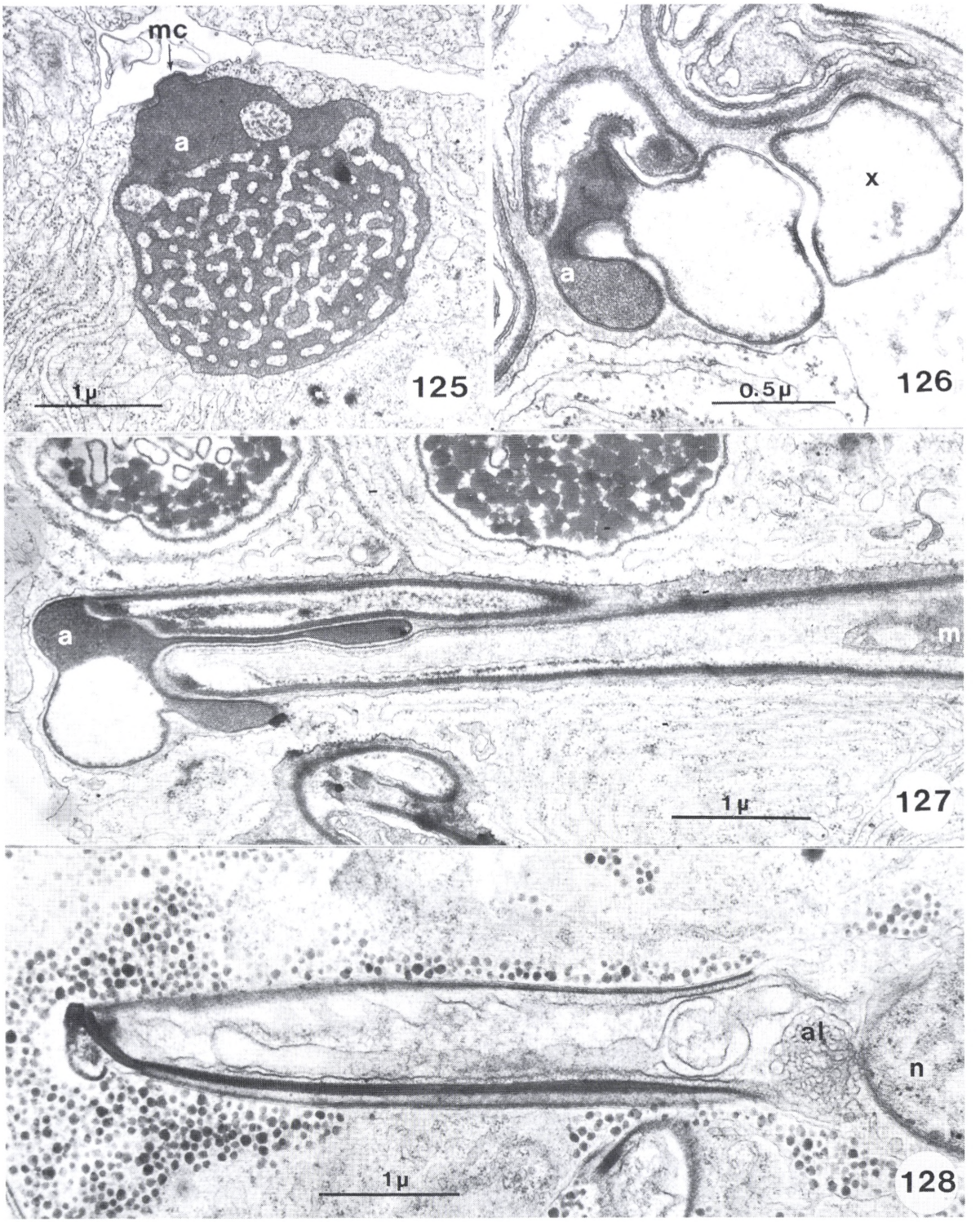

mc
a
1µ
125
a
x
0.5µ
126
a
m
1µ
127
al
n
1µ
128

PLATE 21

Cytheracea.

129. *Hirschmannia viridis* ♂. Fix. 3-A. Acrosomal vesicle of early spermatid with well developed acrosomal labyrinth (al). The thickening at the membrane contact of the acrosomal vesicle (mc) is distinct.

130. *Paradoxostoma variabile* ♂. Fix. 3-A. Acrosomal vesicle of early spermatid (a) with acrosomal labyrinth and dark matter in the lumen. Perforatorium (p) developing, still amorphous, invaginating the acrosomal vesicle but not the nucleus (n).

131. *Hirschmannia viridis* ♂. Fix. 3-A. Further development of the perforatorium (p) into the acrosomal vesicle (a), which is reduced to a tube with double walls. The nucleus (n) is not invaginated.

132. *Loxoconcha impressa* ♂. Fix. $OsO_4$ with bicarbonate buffer. Longitudinal section of acrosomal process. Further development of perforatorium (p), acrosomal vesicle (a), the two invading mitochondria (m) and appearance of the vanes (l), which are obliquely cut.

133. *Cytherois fischeri* ♂. Fix. 3-A. Longitudinal section of advanced acrosomal process with acrosomal vesicle (a) filled with dark matter. Tangential section of one of the "vanes" (l).

*Legends to all figures*: a = acrosomal vesicle, al = acrosomal labyrinth, l = vanes or "legs" (they look like legs in cross sections, see Pl. 22), m = mitochondria, mc = membrane contact of acrosomal vesicle, n = nucleus, p = perforatorium, so = segmented organelles.

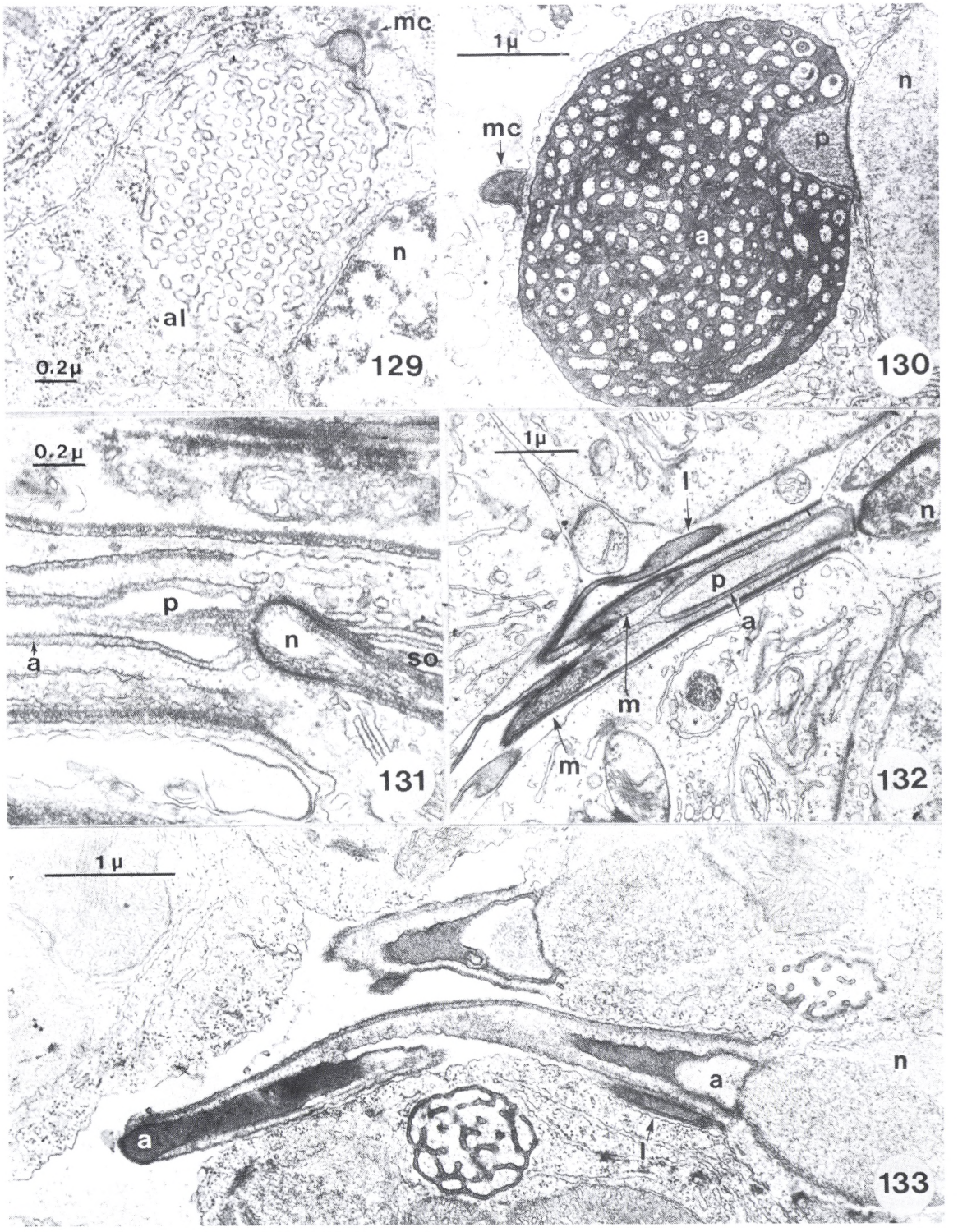

mc
n
al
0,2µ
129
1µ
n
mc
p
a
130
0,2µ
p
n
a
so
131
1µ
l
n
p
a
m
m
132
1µ
a
a
n
l
133

PLATE 22

Cytheracea.

134. *Loxoconcha elliptica* ♂. Fix. $OsO_4$ with veronal acetate buffer. Cross section of basis of acrosomal process, showing acrosomal vesicle (a) with acrosomal labyrinth (al) and the two vanes in the state of formation (l). The dark rod at the base of the vanes is distinct on both sides (rl).

135. *L. impressa* ♂. Fix. $OsO_4$ with bicarbonate buffer. Cross section of early acrosomal process with perforatorium (no distinct filament), acrosomal vesicle (a) and invading mitochondria (m) attached to the basal rods (rl) of the vanes (l).

136. *L. impressa* ♂. Fix. 3-A. Cross sections of four acrosomal processes with vanes (l) which look like legs in cross sections. Also seen are mitochondria (m), vestigial perforatorium (p), and basal rods of the vanes (rl).

137. *Elofsonia baltica* ♂. Fix. 3-A. Cross sections of acrosomal processes showing asymmetrical development of the vanes. In this species one of the vanes (dl) is swollen and different from the normal one (l).

138. *E. baltica* ♂. Fix. 3-A. Cross section of nuclear region of spermatid showing nucleus (n), mitochondria (m) and segmented organelles B arranged in an asymmetrical pattern, in which a comparable picture returns after rotation of the radius 180°.

139. *Loxoconcha elliptica* ♂. Fix. 3-A. Longitudinal section of nuclear region of spermatid, showing compressed, lamellar nucleus (n), mitochondria (m), and segmented organelles A and B (soA and SoB).

140. *L. impressa* ♂. Fix. 3-A. Cross section of late acrosomal process with acrosomal furrow (fa), acrosomal crest (ac) and dark mantle (ma) around acrosomal furrow.

141. *L. elliptica* ♂. Fix. 3-A. Cross section of nuclear region of spermatid, with flat, S-shaped nucleus (n), mitochondria (m), segmented organelles A and B (soA and soB), forming an asymmetrical pattern as in Fig. 138. A third kind of segmented organelle (sox), present in only a few species, is seen along one margin of the nucleus.

*Legends to all figures*: a = acrosomal vesicle, ac = acrosomal crest, al = acrosomal labyrinth, dl = deviating type of vane, fa = furrow on acrosomal region, l = vanes, m = mitochondria, ma = dark mantle of acrosomal furrow, n = nucleus, p = perforatorium, rl = dark rod of vanes, soA, soB and soX = segmented organelles A, B, and X, the latter rarely seen.

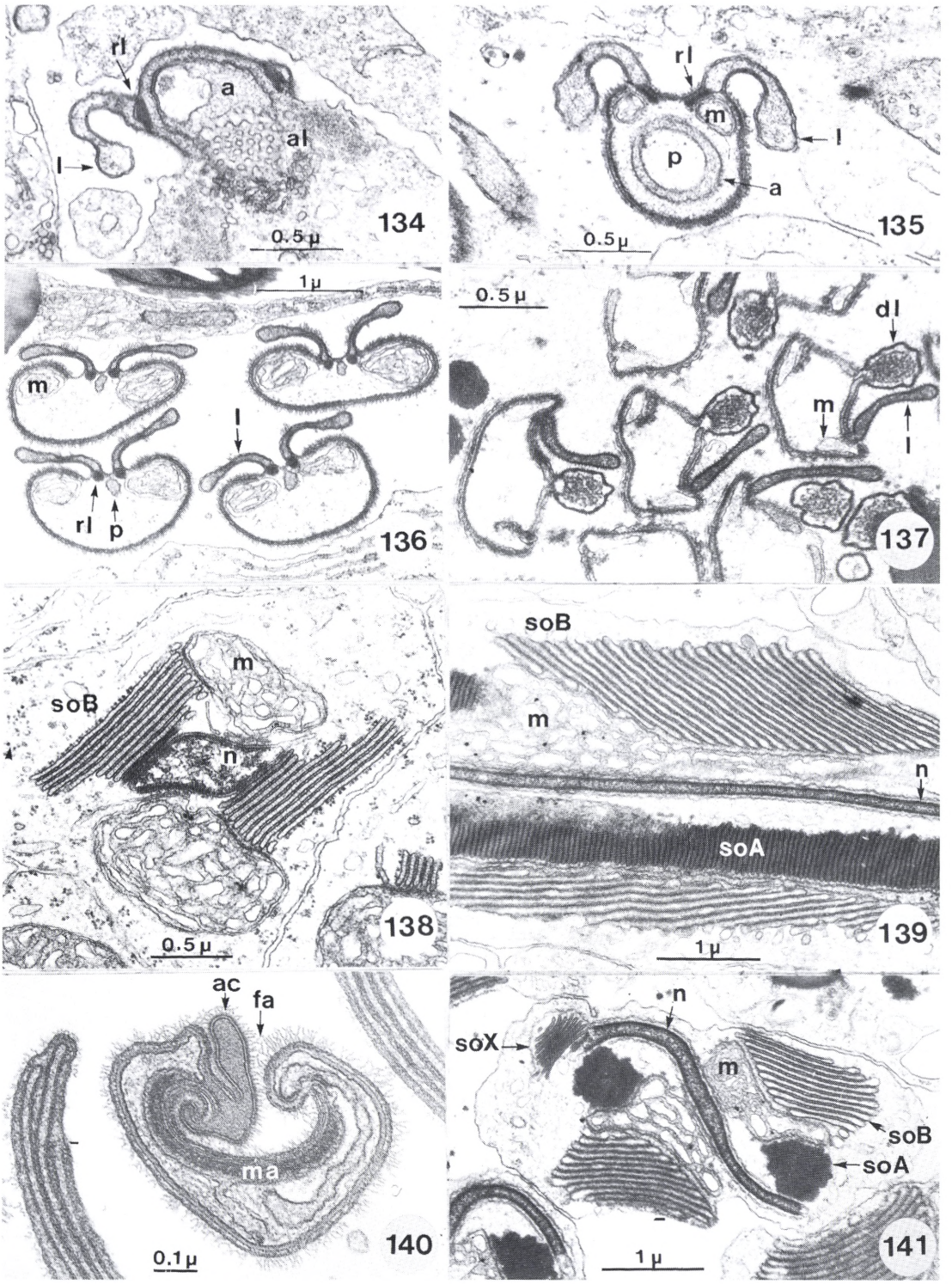

rl
a
al
l
0.5 µ
134
rl
m
l
p
a
0.5 µ
135
1 µ
m
l
rl
p
136
0.5 µ
dl
m
l
137
m
soB
n
0.5 µ
138
soB
m
n
soA
1 µ
139
ac
fa
ma
0.1 µ
140
n
soX
m
soB
soA
1 µ
141

PLATE 23

Cypridacea, anterior region.

142. *Eucypris cisternina* ♂. Fix. 3-A. Anterior end of mature sperm showing corkscrew arrangement of single ridge. To the right is a piece of posterior region. SEM.

143. *Cypria ophthalmica* ♂. Fix. 3-A. Anterior region of mature sperm showing terminal knob and absence of corkscrew pattern in this species. The axis shows slight coiling. SEM.

144. *Heterocypris* sp. ♀. Fix. 3-A. Anterior end of mature sperm with corkscrew pattern. SEM.

145. *Aglaiocypris* sp. ♂. Fix. 3-A. Cross section of anterior region of nearly mature sperm, surrounded by wall cells of vas deferens (vd). The nucleo-perforatorial rod (n) is surrounded by a dark sheath (sh) and several layers of extracellular coat substance (ec).

146. *Potamocypris* sp. ♂. Fix. 3-A. Cross section of mearly mature anterior region in vas deferens (vd). The nucleo-perforatorial sheath (sh) has two dark ridges (dr) and is surrounded by several layers of coat substance (ec).

147. *Chlamydotheca* sp. ♂. Fix. 3-A. Cross section of anterior region of nearly mature sperm in the vas deferens, surrounded by wall cells. The dark ridges of the nucleo-perforatorial sheath (sh) are paired (dr). The subdivision of the coat (ec) into a deciduous coat (dc) and a permanent coat is clearly marked by the surface pattern of the latter. The single coiling ridge (cr) of the corkscrew is attached to one of the dark ridges (dr) of the sheath.

*Legends to all figures*: cr = coiling ridge of the corkscrew, dc = deciduous coat, dr = dark ridge in the nucleo-perforatorial sheath, ec = extracellular coat, n = nucleo-perforatorium, pc = permanent coat, sh = nucleo-perforatorial sheath, vd = wall cells of vas deferens.

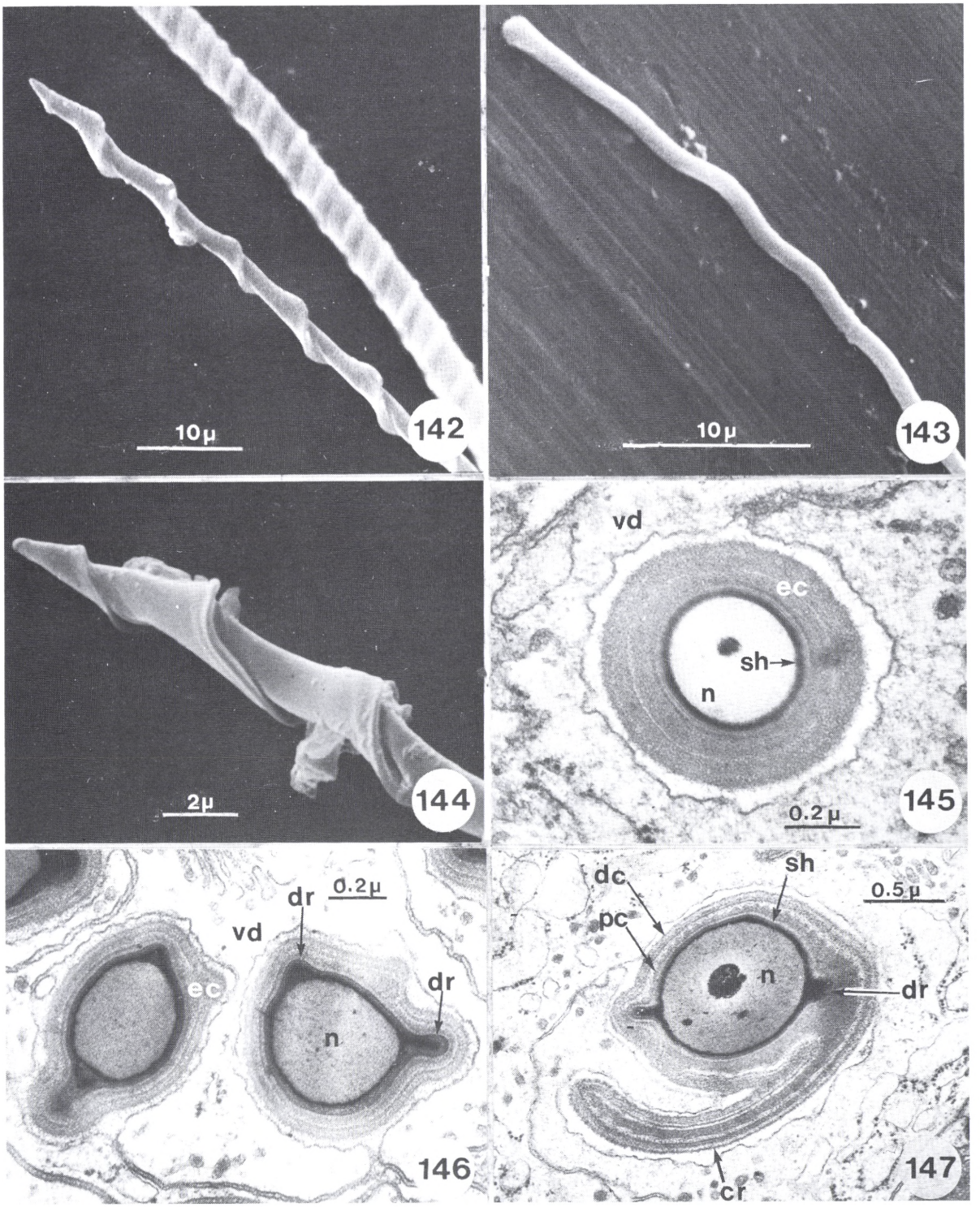

10µ
142
10µ
143
2µ
144
vd
ec
sh
n
0.2µ
145
dr
0.2µ
vd
ec
dr
n
146
dc
sh
0.5µ
pc
n
dr
cr
147

PLATE 24

Cypridacea.

148. *Ilyocypris* sp. ♀. Fix. 3-A. Cross section of anterior region of mature sperm in receptaculum of female. The nucleo-perforatorium (n) is only surrounded by its sheath and a layer of permanent coat (pc). The latter also forms the coiling ridge (cr) of the corkscrew. Note the characteristic surface structure of the permanent coat.

149. *Ilyocypris* sp. ♂. Fix. 3-A. Cross section of mature sperm lying in vas deferens. Note distinct boundary between deciduous coat and permanent coat, the latter showing the pattern seen on the surface in Fig. 148.

150. *Aglaiocypris* sp. ♂. Fix. 3-A. Cross section of posterior region of mature sperm in vas deferens. Note superficial ridges (rc) of deciduous and permanent coats and ventral "monorail" (mr) formed by the latter.

151. *Ilyocypris* sp. ♂. Fix. 3-A. Posterior region of mature sperm in vas deferens. The surface pattern of the permanent coat is not yet visible.

152. *Eucypris cisternina* ♂. Fix. 3-A. Cross section of posterior region of mature sperm from vas deferens. Note dorsal furrow (df), two lateral furrows (lf) and a ventral furrow with monorail (mr). The lateral furrows correspond to inward folds of the contractile organelles. They are followed by a valvelike crest (lv) of the permanent coat. All furrows are partly filled out by the deciduous coat. The structure (x) below the nucleus was believed by Gupta (1968) to be comprised of myelin.

153. *Potamocypris* sp. ♂. Fix. 3-A. Cross section of posterior region of mature sperm in vas deferens. Note the dorsal, lateral and ventral furrows.

*Legends to all figures*: co = contractile organelles, cr = coiling ridge of "corkscrew", dc = deciduous coat, not present on spermatozoa in female receptaculum, df = dorsal furrow, lf = lateral furrow, lv = lateral ridge (valve) along lateral furrow, m = mitochondria, mr = "monorail", inward ridge of permanent coat, n = nucleo-perforatorium, pc = permanent coat, rc = ridges on surface of permanent coat, x = myelin-like matter, according to Gupta (1968) an energy store.

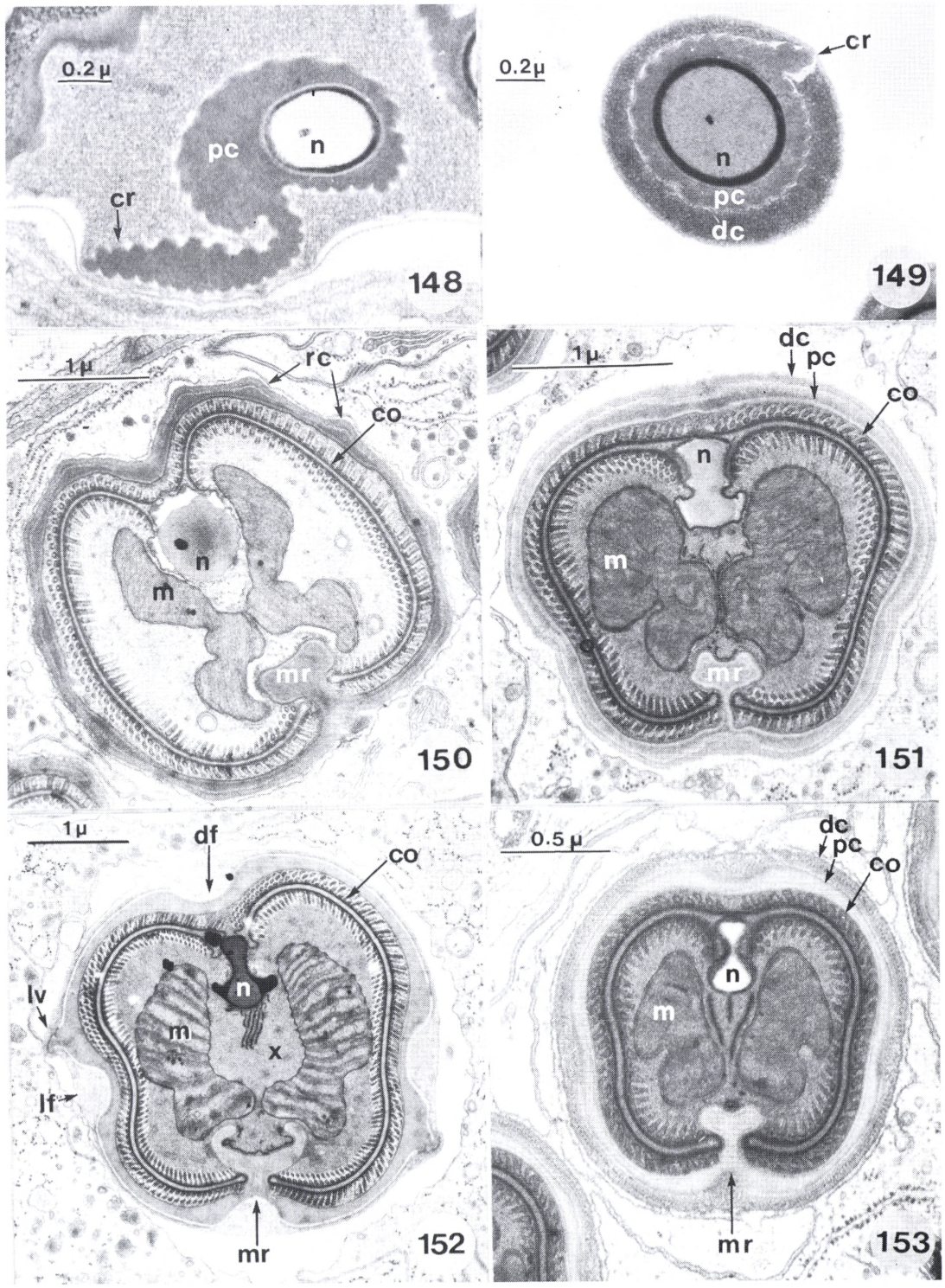

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PLATE 25

Structure of contractile organelles.

154. *Aglaiocypris* sp. ♂. Fix. 3-A. Cross section of mature sperm showing attachment of contractile organelles (co) to the nucleus (n). The two layers in the wall of the core-sac (cs) can be seen.

155. *Propontocypris litoricola* ♂. Fix. 3-A. Part of contractile organelle in cross section of sperm (see Fig. 175). Note very long pins (p) and the presence of filaments on the luminal side of the core-sac (f).

156. *Heterocypris* sp. ♀. Fix. 2 % $OsO_4$. Cross section of mature sperm in receptaculum showing relation between margin of contractile organelle, its core-sac (cs) and ventral monorail (mr). Core-sac with thin filaments (f) attached to its inner walls.

157. *Chlamydotheca* sp. ♀. Fix. 3-A. Tangential section of contractile organelle showing arrangement of cross-sectioned pins and the plasmatic bridges, about 25 Å thick, which connect the individual pins (br).

158. *Aglaiocypris* sp. ♂. FIx. 3-A. Mature sperm. Section shows double wall of core-sac (cs), the shape of the pins in axial section, and the mitochondria with parallel cristae.

159. *Chlamydotheca* sp. ♀. Fix. 3-A. Core-sac and longitudinally cut pins connected by plasma bridges near base (br).

*Legends to all figures*: br = plasmatic bridges between pins, f = longitudinal filaments in core-sac of contractile organelle, cs = core-sac of contractile organelle, co = contractile organelle, m = mitochondria, mr = "monorail" of coat, n = nucleo-perforatorium, p = pins.

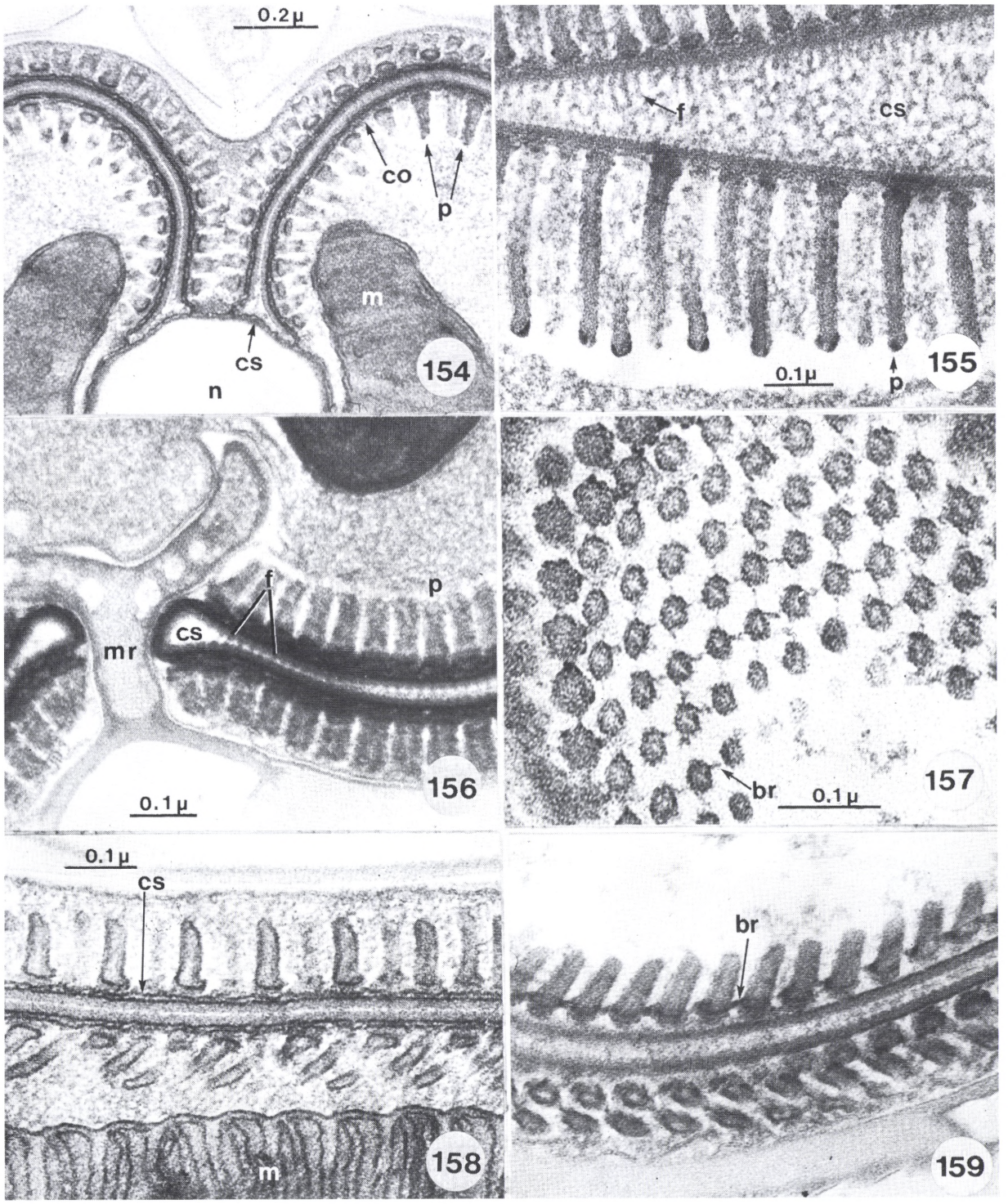
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PLATE 26

Cypridacea.

160. *Aglaiocypris* sp. ♂. Fix. 3-A. Longitudinal section of part of the contractile organelle (co), showing deciduous coat (dc), permanent coat (pc) and the cross-striated zone (cz) between the contractile organelles and the mitochondria (m).

161. *Macrocypria angusta* ♂. Fix. 3-A. Longitudinal section of part of mitochondrion, showing lamellar, transverse cristae (cr) which contain thin lamellae of dark matter.

162. *Ilyocypris* sp. ♂. Fix. 3-A. Longitudinal section of mature spermatozoon in vas deferens, showing deciduous coat (dc) and permanent coat with superficial ridges (sr).

163. *Chlamydotheca* sp. ♀. Fix. 3-A. Longitudinal section of mature sperm showing ventral monorail (mr), mitochondria (m) and ventral and lateral furrows of permanent coat (vf, lf). The furrows have microridges on the bottom and are accompanied by a valvelike ridge (va).

164. *Ilyocypris* sp. ♀. Fix. 3-A. Section through the receptaculum seminis. One normal spermatozoon with intact mitochondria and superficial ridges is visible, as well as several empty coats (ec) which only consist of the permanent coat with preserved superficial ridges (sr) and the ventral monorail (mr).

165. *Chlamydotheca* sp. ♀. Fix. 3-A. Longitudinal section of an empty coat from the receptaculum, with preserved dorsal, lateral and ventral furrows (df, lf, vf), the latter combined with the ventral monorail (mr). Note microridges in the furrows.

166. *Candona suchi* ♀. Fix. 3-A. Longitudinal section of empty coat from the receptaculum. Note large ventral and small dorsal monorail (mr, dmr) and the pairs of superficial ridges (compare with Fig. 8).

*Legends to all figures*: co = contractile organelles, cr = mitochondrial cristae with central lamella of dark matter (Fig. 161), cz = cross-striated zone (Fig. 160), dc = deciduous layer of coat, df = dorsal furrow of coat, dmr = dorsal monorail (in Fig. 166), ec = empty coats in receptaculum, lf = lateral furrows of coat, m = mitochondria, mr = ventral monorail, sr = superficial ridges of permanent coat, va = valvelike crest along superficial furrow (Figs. 163, 165), vf = ventral furrow on surface of permanent coat.

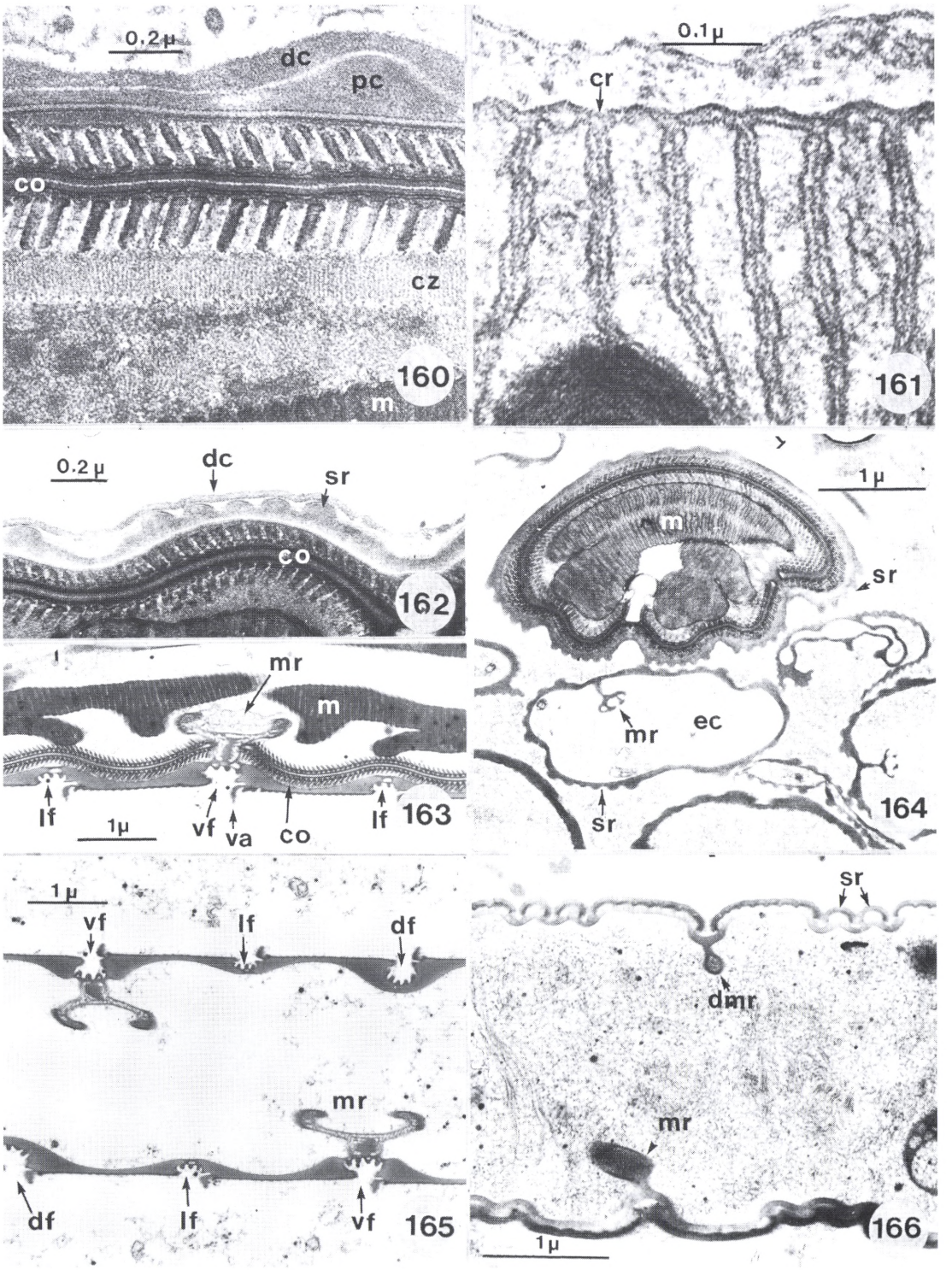
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PLATE 27

Surface structure and internal organelles.

167. *Ilyocypris* sp. ♂. Fix. 3-A. Longitudinal section of mature sperm in vas deferens. Distinct ventral furrow (v) marks the monorail between the wings of the contractile organelles. Dorsal furrow (d) marks space between the attachments of these organelles to nucleus (n), and a pair of lateral furrows (l) mark the inward bending of each organelle. All furrows coil in a parallel way around the sperm and return periodically on each side. Note deciduous coat (dc) and permanent coat (pc) and the surface ridges of the latter. Compare also with Textfig. 8.

168. *Aglaiocypris* sp. ♂. Fix. 3-A. Longitudinal section of mature sperm in vas deferens. As in Fig. 167, but *Aglaiocypris* has no lateral furrows over the even contractile organelles, and there are only five superficial coat ridges over each half organelle.

169. *Potamocypris* sp. Fix. 3-A. Longitudinal section of mature sperm in vas deferens. As in Fig. 167, but there are no small superficial coat ridges. Lateral furrows (l) are present over the inward bend of each contractile organelle.

*Legends to all figures*: co = contractile organelles, d = dorsal furrow, dc = deciduous coat, l = lateral furrow, m = mitochondria, mr = ventral monorail, n = nucleus, pc = permanent coat, st = superficial ridges on coat, v = ventral furrow.

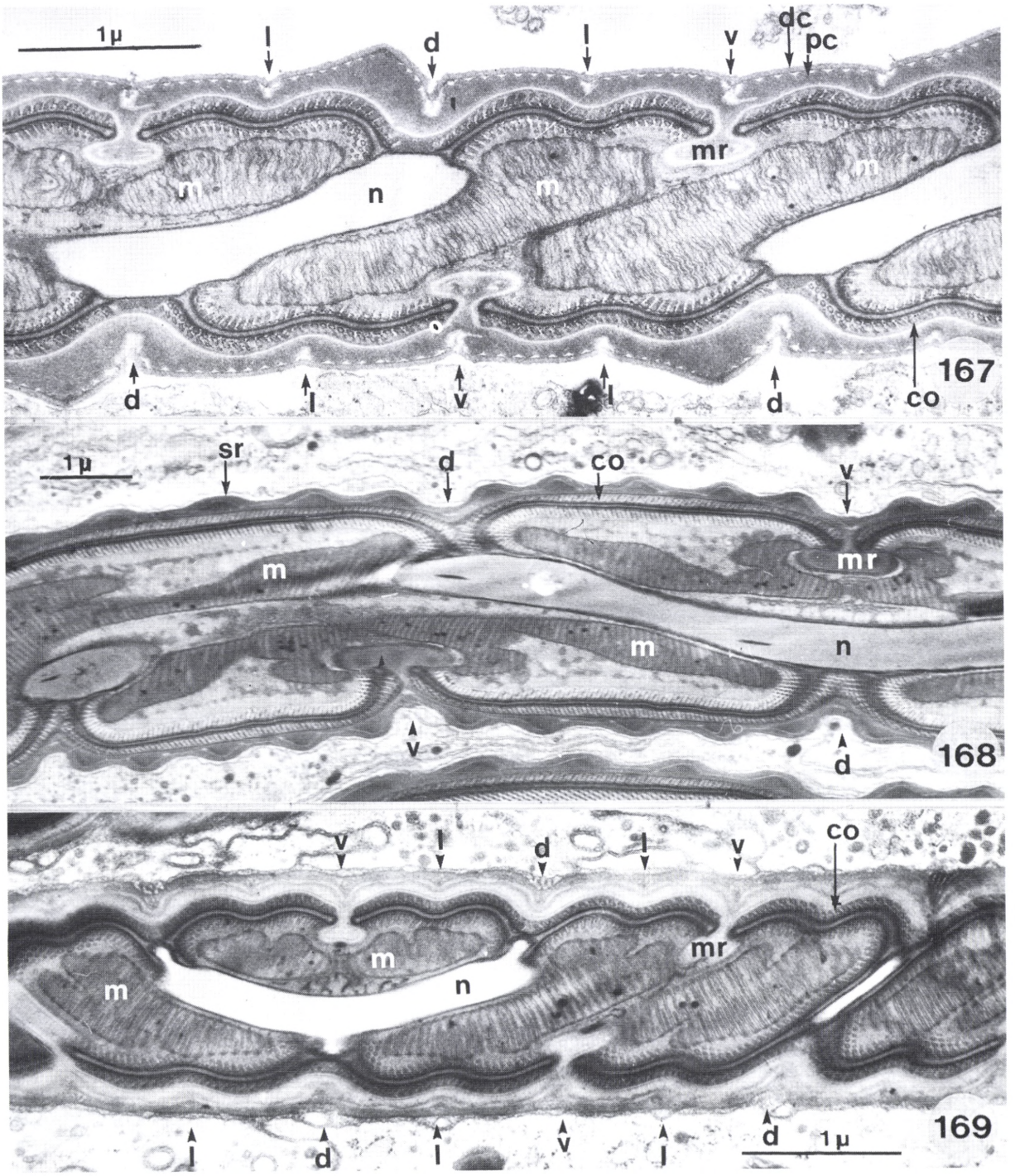
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PLATE 28

Cypridacea.

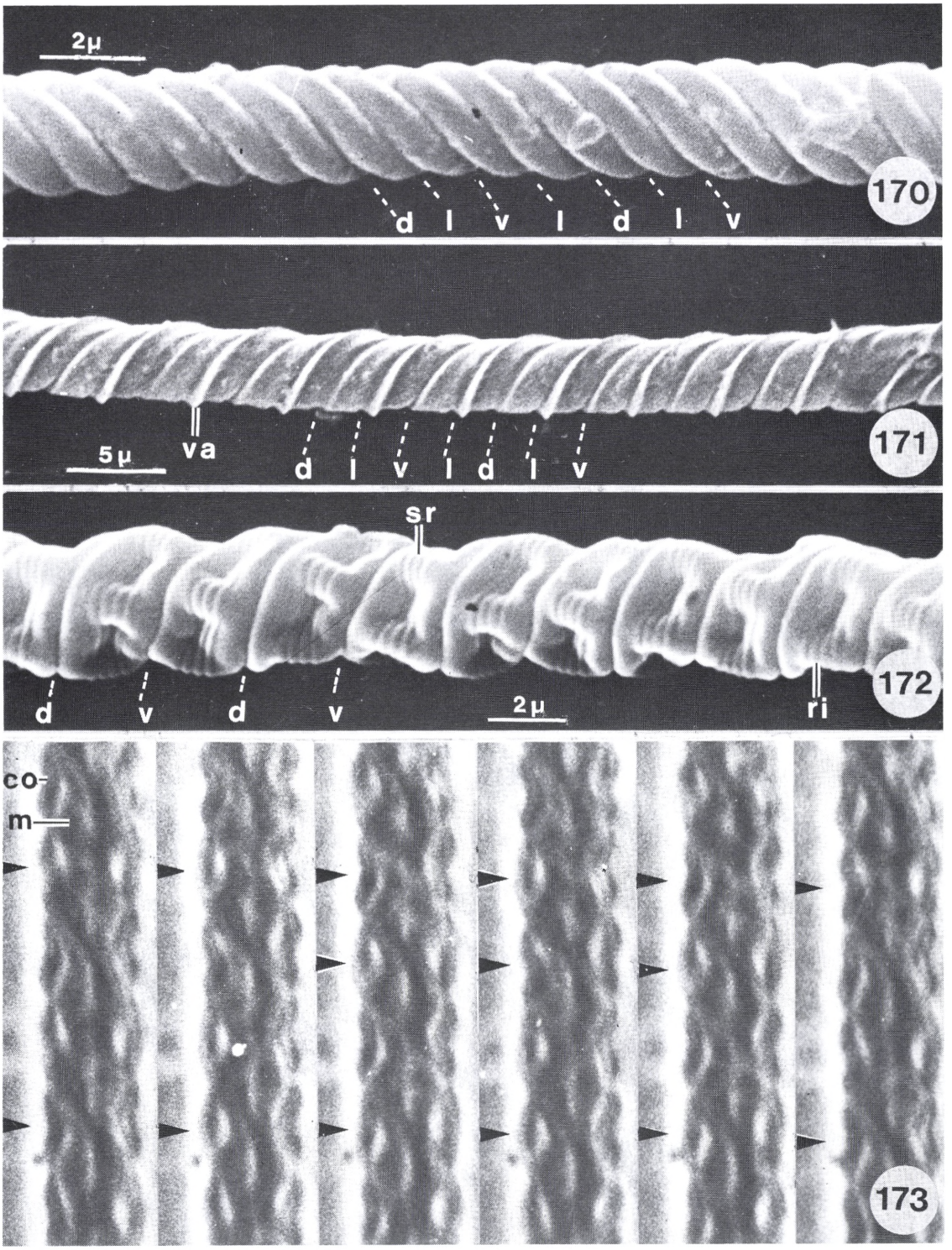
170. *Cypria ophthalmica* ♀. Fix. 3-A. Freeze-dried for SEM. Posterior ropelike region of sperm. Each coiling band corresponds to one half of a single wing of the contractile organelles as in *Potamocypris* (see Fig. 169) which was checked in longitudinal sections and TEM. The dorsal and ventral furrows are perhaps a little broader than the lateral ones. SEM photograph.

171. *Eucypris cisternina* ♀. Fix. 3-A. Posterior region of sperm. The lateral furrows are followed by a distinct valvelike ridge in this region, the dorsal and ventral furrows are not. One coiling band corresponds to half a wing of the contractile organelle (checked in sections). See also Fig. 152. SEM photograph.

172. *Candona suchi* ♀. Fixed in $OsO_4$ and dried for SEM. This species has no lateral furrows, and each coiling band corresponds to a single wing of the contractile organelle. Compare with Textfig. 8. The cross folds of the contractile bands (ri) may be produced or enlarged artificially during the drying but can illustrate the way such folds (ripples) move over the bands in the swimming sperm. SEM photograph.

173. *Candona suchi* ♀. Six successive frames from film of living sperm from the receptaculum, showing ripple movement. Frequency of exposures 24/second, and that of the ripple movement probably more. When ripples pass a certain point the contractile organelles bulge outwards and they contract again and form a slight depression when the ripple has passed. Small arrows indicate comparable levels of the sperm to facilitate interpretation. The sperm has only moved very little (compare the dust points below lowest arrows). See also Textfig. 8 and Fig. 172 above. Enlarged from film negative.

*Legends to all figures*: co = contractile organelles, d = dorsal furrow, l = lateral furrow, m = mitochondria, ri = ripplelike fold of contractile band, v = ventral furrow, va = valvelike ridge along coat furrows.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co-
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173

PLATE 29

Pontocypridid ostracods.

174. *Argilloecia cylindrica* ♀. Fix. 2 % $OsO_4$. Cross section of mature sperm in receptaculum. The mitochondria (m) are in contact ventral to the nucleus (n). They cover part of the contractile organelles (co). *Inset* shows tubular structure of the central core of the nucleo-perforatorium at a higher magnification (about 3 times that of the main figure).

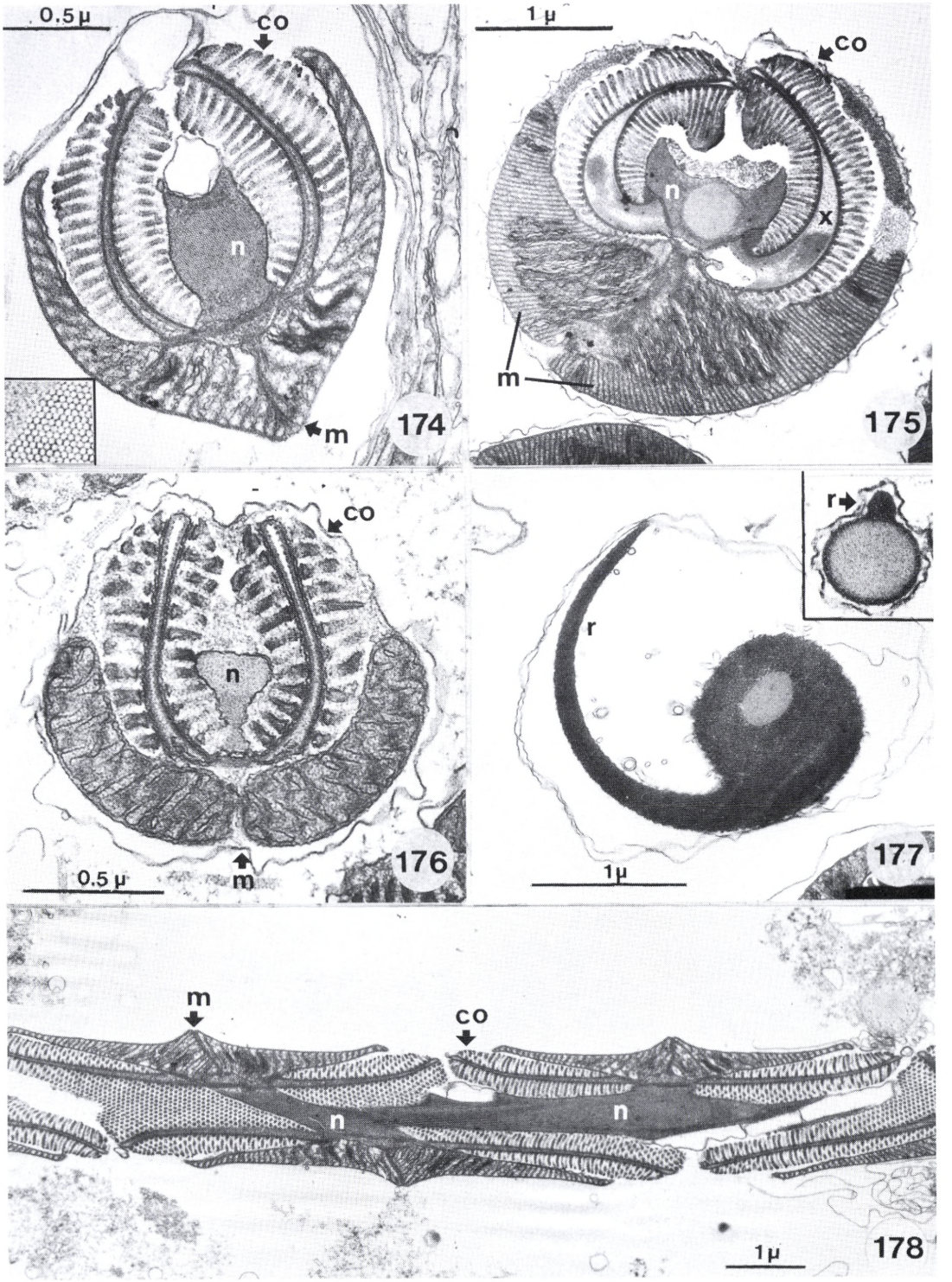
175. *Propontocypris litoricola* ♂. Fix. 3-A. Cross section of posterior region of mature sperm in the vesicula seminalis. Note absence of coat, the large pins of the contractile organelles (co) and the large lumen of the core of these organelles. The mitochondria (m) meet ventral to the nucleus.

176. *Pontocypris trigonellus* ♂. Fix. 3-A. Cross section of mature sperm in the vesicula seminalis. Note absence of coat and the contact of the mitochondria (m) ventral to the nucleus (n).

177. *Argilloecia cylindrica* ♀. Fix. 2 % $OsO_4$. Cross section of the large corkscrew at the anterior end in this species. *Inset* shows anterior region of sperm in *Pontocypris trigonellus*, with coiling ridge (r) and absence of external coat. Magnified as much as the main figure.

178. *Argilloecia cylindrica* ♀. Fix. 2 % $OsO_4$. Longitudinal section of mature sperm in receptaculum. Note that the mitochondria (m) cover most of the sperm surface and that the contractile organelles (co) only reach the surface in narrow zones.

*Legends to all figures*: co = contractile organelles, m = mitochondria, n = nucleus (nucleo-perforatorium), r = coiling ridge on the anterior region, x = large lumen of core of the contractile organelles.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co
n
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178

PLATE 30

*Propontocypris litoricola.*

179. *Propontocypris litoricola* ♂. Fix. 3-A. Free sperm in vesicula seminalis, showing the bulb (b) between anterior region (ar) and posterior region (pr). Light microscopy of fixed specimen.

180. *P. litoricola* ♂. Fix. 3-A. SEM picture of bulb (b) showing exit of the anterior region of the sperm (ar).

181. *P. litoricola* ♂. Fix. 3-A. Longitudinal section of bulb showing the thick layer of coat substance (c), the large, coiling mitochondria (m) and the coiling axis, which contains the nucleus and contractile organelles (ax).

182. *P. litoricola* ♂. Fix. 3-A. Cross section of bulb showing the large coiled mitochondria (m) and the axis with nucleus and contractile organelles (ax).

183. *P. litoricola* ♀. Fix. 3-A. Longitudinal section of sperm in receptaculum. Note the open coil of the contractile organelles (co), which reach the surface only in a narrow zone, alternating with the mitochondria (m).

*Lengends to all figures*: ar = anterior region, ax = the coiling axial complex of bulb, consisting of nucleus and contractile organelles, b = bulb, c = coat substance, co = contractile organelles, m = mitochondria, n = nucleus, pr = posterior region.

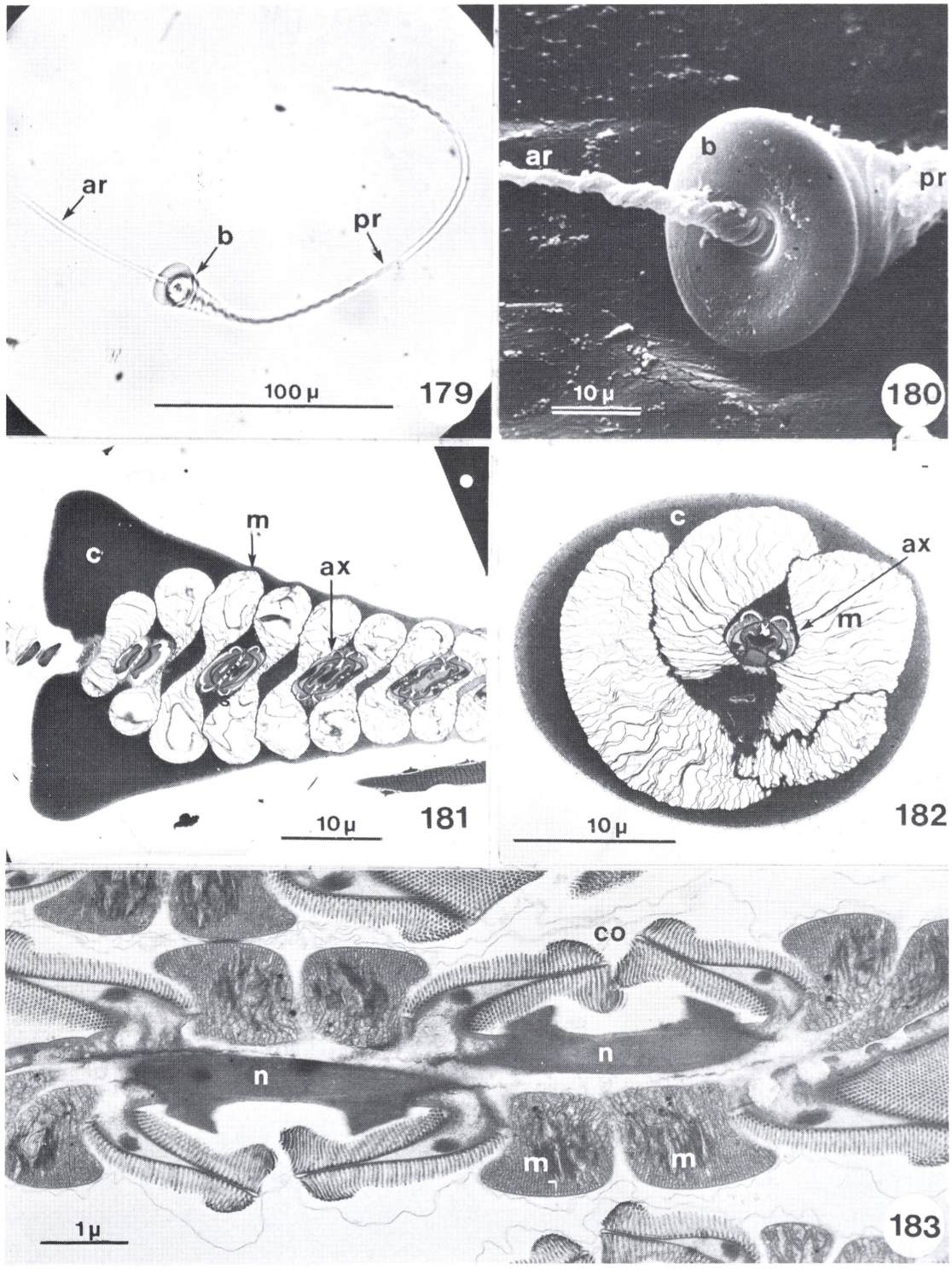
ar
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PLATE 31

Macrocyprididae.

184. *Macrocypris minna* ♂. Fix. 2 % $OsO_4$. Cross section of mature sperm in vesicula seminalis. The contractile organelles (co) extend straight laterally into the winglike borders of the sperm. A dark core in the nucleus (n) may be the perforatorium, although the surrounding membrane is lost.

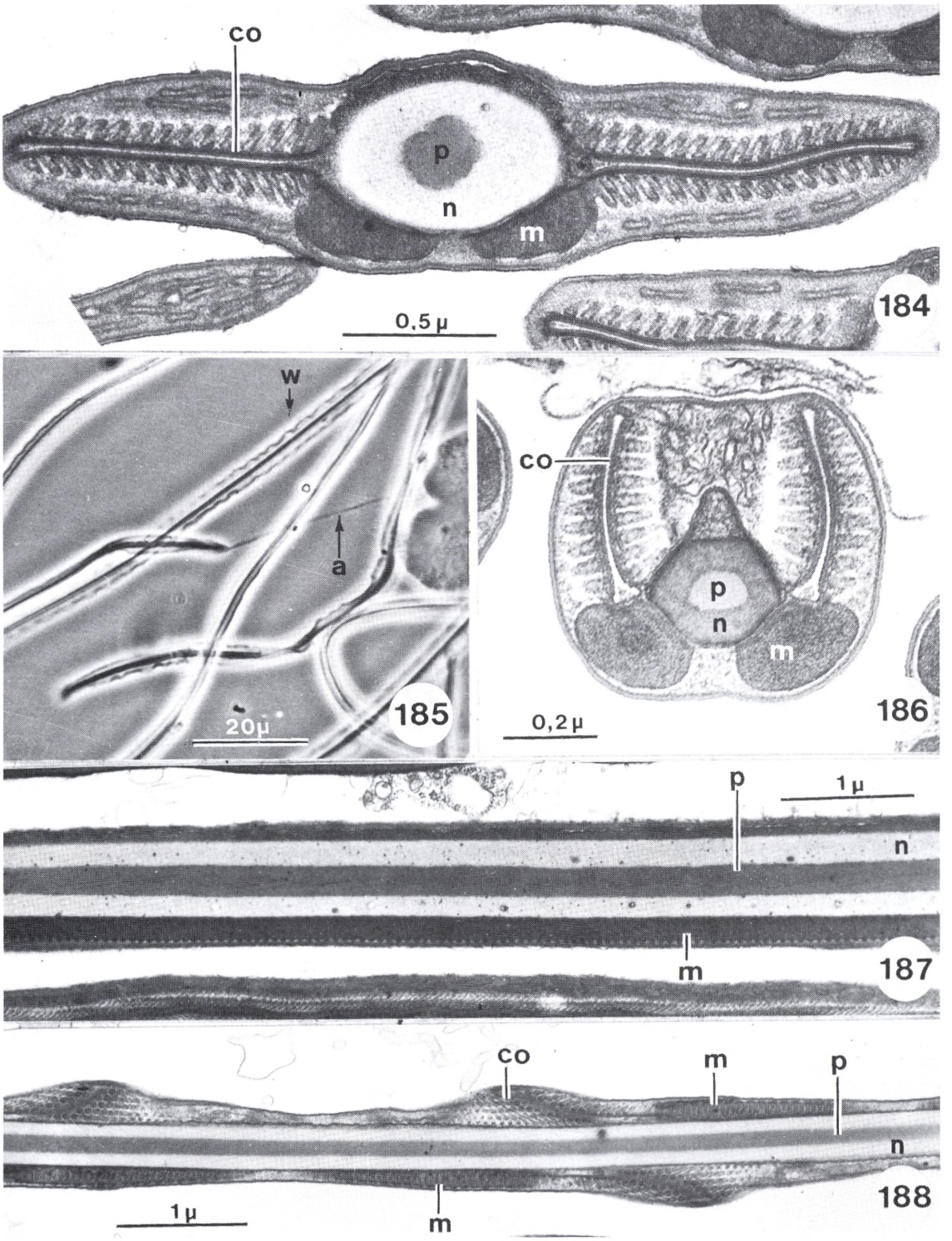
185. *M. minna* ♂. Fix. 2 % $OsO_4$. Light microscopy of fixed sperm from vesicula seminalis. Note wavy contour of lateral margins of the borders of the spermatozoon (w). The nucleo-perforatorial axis is distinct. A thin perforatorium-like process (a) was seen in some cases.

186. *Macrocypria angusta* ♂. Fix. 2 % $OsO_4$. Cross section of mature sperm from vesicula seminalis. Note the straight upward direction of the contractile organelles (co), the almost cylindrical mitochondria (m) and the light, perforatorium-like core (p) of the nucleus (n).

187. *Macrocypris minna* ♂. Fix. 2 % $OsO_4$. Longitudinal section showing absence of coiling, straight nucleus (n) and ?perforatorium (p), and one of the straight mitochondria (m).

188. *Macrocypria angusta* ♂. Fix. 3-A. Longitudinal section showing the straight nucleus (n) and ?perforatorium (p), and the slightly coiling contractile organelles (co) and mitochondria (m).

*Legends to all figures:* a = process from acrosomal region, similar to a reacted perforatorium, co = contractile organelles, m = mitochondria, n = nucleus, p = perforatorium-like central core in nucleus, w = wavy outline of of lateral borders of sperm.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187
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188

PLATE 32

Cross sections of posterior regions of cypridacean late spermatids, showing comparable situation of mitochondria, nucleus and contractile organelles.

189. *Aglaiocypris* sp. ♂. Fix. 3-A. Candonidae.

190. *Candona suchi* ♂. Fix. 3-A. Candonidae.

191. *Erythrocypris mytiloides* ♂. Fix. 3-A. Pontocyprididae.

192. *Macrocypria angusta* ♂. Fix. 2 % $OsO_4$. Macrocyprididae.

193. *Macrocypris minna* ♂. Fix. 2 % $OsO_4$. Macrocyprididae.

*Legends to all figures*: ch = chromatin accumulation in nucleus, co = contractile organelles, m = mitochondria, n = nucleus, p = perforatorium, in Figs. 190-193 surrounded by invaginated nuclear envelope, sc = part of dark string, associated with contractile organelle, sm = part of dark string associated with mitochondrion, t = double-walled tubule in the dark string.

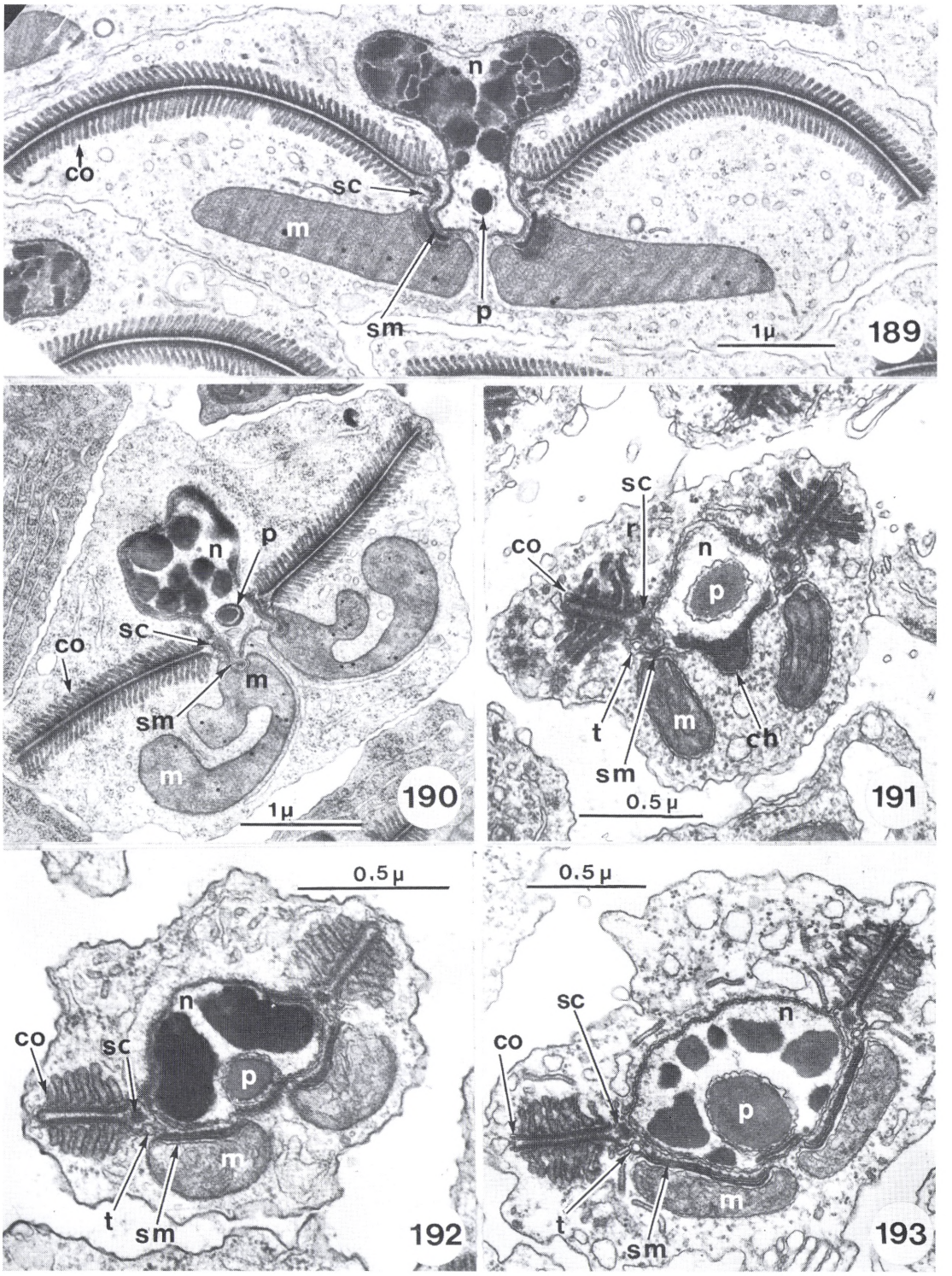
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PLATE 33

Cypridacea: acrosomal vesicle, perforatorium and dark strings.

194. *Macrocypris minna* ♂. Fix. 2 % $OsO_4$. Oblique longitudinal section of anterior end of early spermatid, showing cup-shaped acrosomal vesicle (a) and perforatorium (p). Dark string on one side (ds), hidden in a furrow of the nuclear surface, passes forwards in front of the mitochondria (m).

195. *M. minna* ♂. Fix. 2 % $OsO_4$. Anterior end of the nucleus (n), perforatorium (p), mitochondria (m) and dark strings of one side, in longitudinal section.

196. *Chlamydotheca flexilis* ♂. Fix. 3-A. Longitudinal section of the anterior end of nucleus (n), showing acrosomal vesicle (a), perforatorium with numerous tubules (p), perforatorial invagination of nucleus and both dark strings (ds).

197. *C. flexilis* ♂. Fix. 3-A. As Fig. 196 but shows dark matter being accumulated in acrosomal vesicle (a).

198. *C. flexilis* ♂. Fix. 3-A. Longitudinal section of anterior end of later stage than Fig. 197, showing acrosomal vesicle (a), perforatorium (p), beginning accumulation of chromatin blocks in the nucleus (n). The wall of the perforatorial tube ( = invaginated nuclear wall) has nuclear pores.

199. *C. flexilis* ♂. Fix. 3-A. Later stage than Fig. 198, dark matter in the acrosomal vesicle more abundant, perforatorium more compact.

200. *C. flexilis* ♂. Fix. 3-A. Longitudinal section of perforatorium (p) in advanced spermatid. The wall of the perforatorial tube still has nuclear pores.

201. *Macrocypris minna* ♂. Fix. 2 % $OsO_4$. Cross section of advanced, bandlike spermatid, with double, elongated Nebenkern (m), perforatorium and dark strings, each with a tubule (t).

202. *M. minna* ♂. Fix. 3-A. Somewhat younger stage than Fig. 201. Higher magnification showing one dark string (ds), its attachment to the mitochondrion (m) and its contained tubule (t) with double walls.

*Legends to all figures*: a = acrosomal vesicle, ds = dark strings, m = mitochondria, n = nucleus, p = perforatorium, pm = perforatorial membrane (invaginated nuclear membrane with pores), t = double-walled tubule in dark string, sc = part of string, associated with contractile organelles, sm = part of dark string associated with mitochondria. Asterisk marks the posterior limit of acrosomal vesicle.

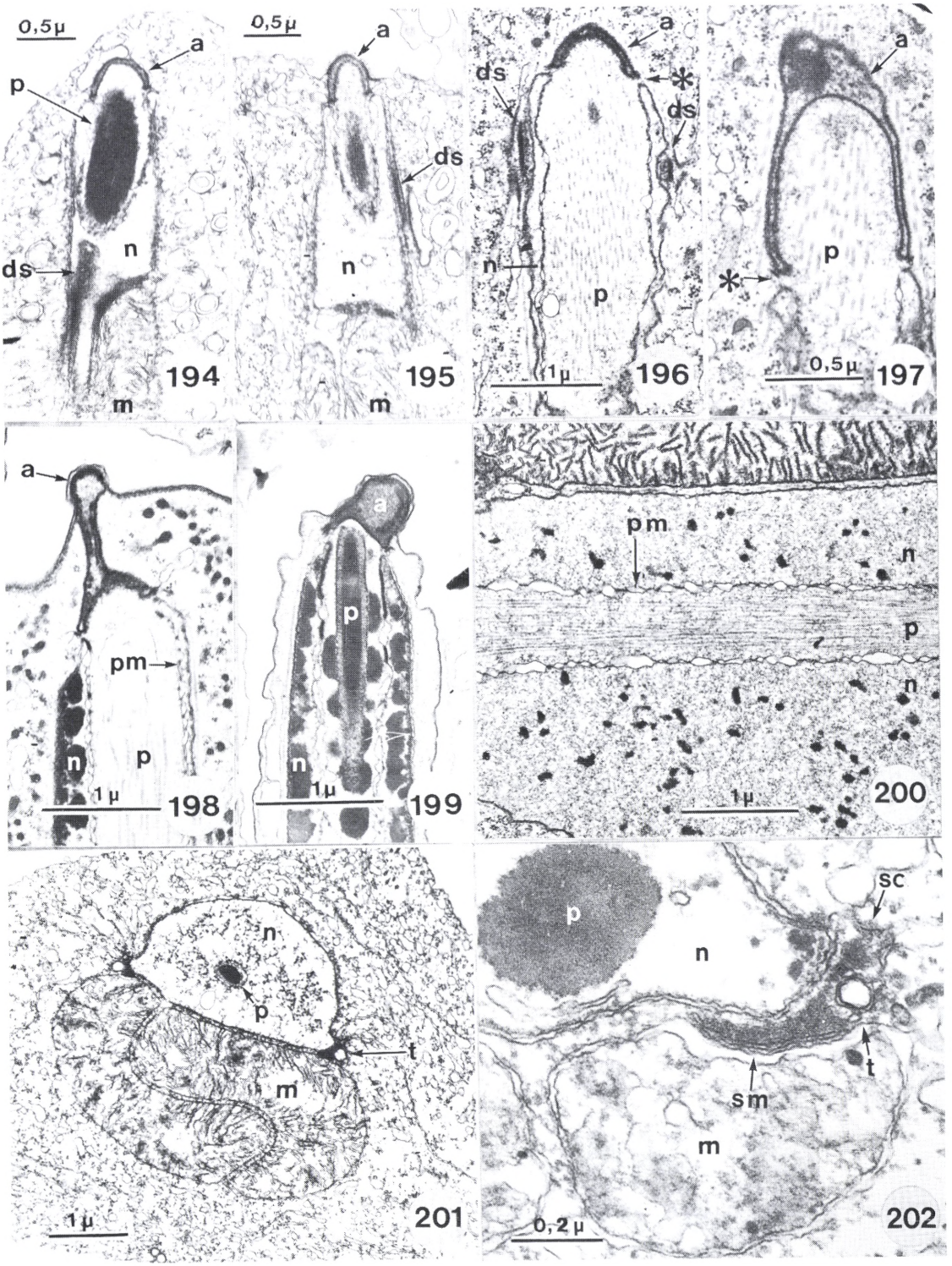
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PLATE 34

Development of contractile organelles.

203. *Chlamydotheca* sp. ♂. Fix. 3-A. Cross section of elongating spermatid, showing nucleus (n) with contained perforatorium (p) and a pair of dark strings (ds). Each core of the contractile organelle is seen as two dark ridges (cs) continuous with the outer nuclear envelope and the endoplasmic reticulum.

204. *Chlamydotheca* sp. ♂. Fix. 3-A. Cross section. Same stage as Fig. 203, greater magnification of one side. See text for Fig. 203.

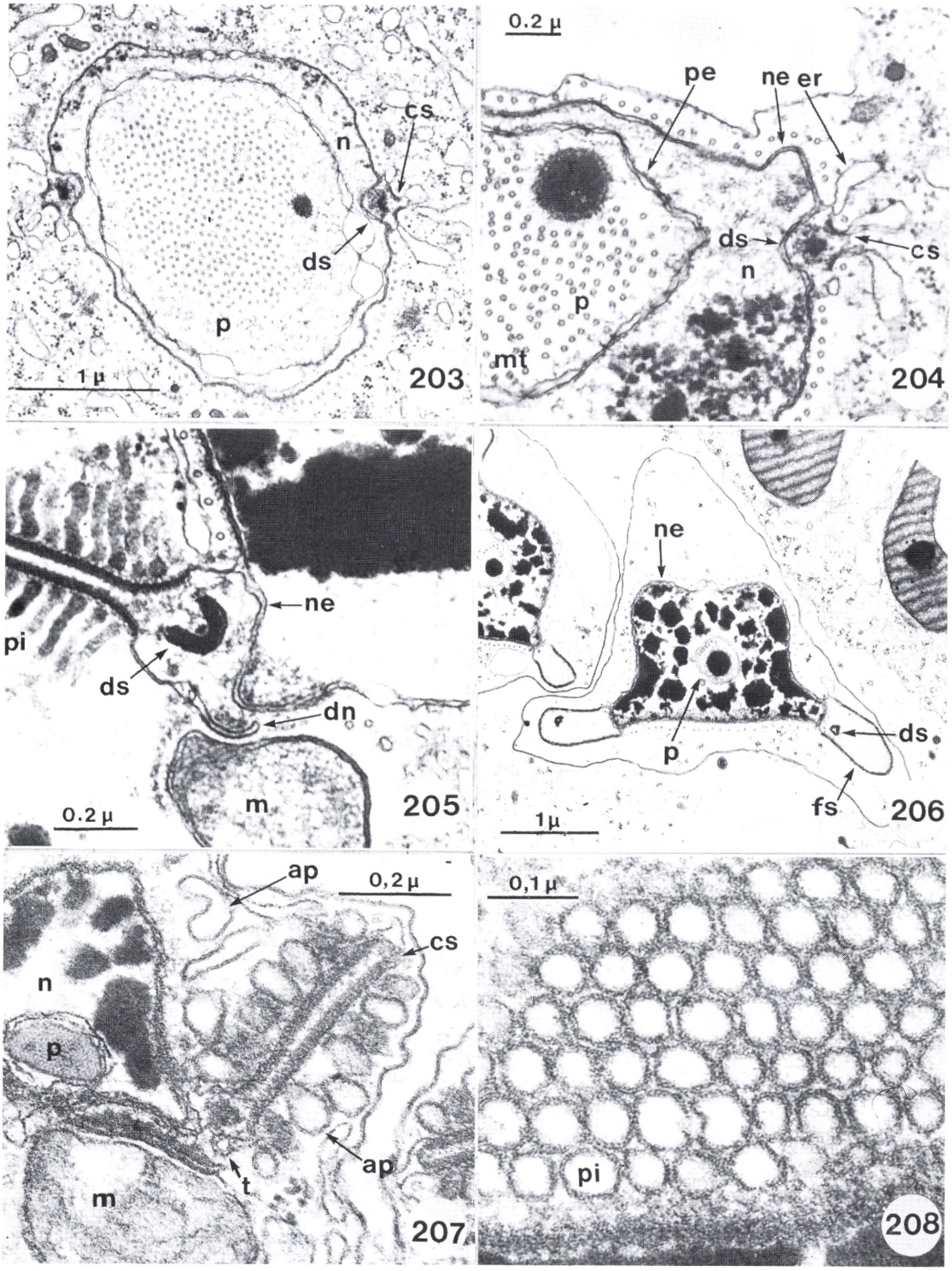
205. *Chlamydotheca* sp. ♂. Fix. 3-A. Cross section of more advanced stage. The core sac of the contractile organelle is formed and has typical pins (pi). Each of its walls is still double and continuous with the outer nuclear envelope. This envelope also has a diverticulum (dn) into the amorphous attachment matter of the mitochondria (m).

206. *Chlamydotheca* sp. ♂. Fix. 3-A. Cross section of anterior region of late spermatid. The perforatorium (p) is still present and has its envelope preserved. No contractile organelles form in this region but dark strings (ds) are present and are surrounded by short flat sacs (fs).

207. *Macrocypria angusta* ♂. Fix. 2 % $OsO_4$. High power picture of advanced stage showing perforatorium (p) still surrounded by envelope. The core-sac (cs) of the contractile organelles is distinctly double-walled. The pins are bladder-shaped, some free in the plasm, some in the state of attaching (ap).

208. *M. angusta* ♂. Fix. 2 % $OsO_4$. Tangential section of the surface of contractile organelle showing bladder-shaped pins (pi) in high magnification.

*Legends to all figures*: ap = newly formed pins in state of attaching, cs = core-sac of contractile organelle, dn = diverticulum of external nuclear membrane in attachment zone of the mitochondria, ds = dark strings, er = endoplasmic reticulum sacs, fs = flat sacs along anterior region, m = mitochondria, mt = microtubules, n = nucleus, ne = nuclear envelope, p = perforatorium, pe = perforatorial envelope, pi = pins, t = tubule with double walls, associated with the dark strings.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206
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208

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*Abstract, Summary.* – An abstract in English is compulsory. It should count 10-15 lines, outline main features, stress novel information and conclusions, and end with the author's name, title, and institutional and/or private postal address. – Papers in Danish may be provided with a summary in another language by agreement between author and editor.

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