Comparative Spermatology of the Crustacea Entomostraca

1. Subclass Branchiopoda

By KARL GEORG WINGSTRAND

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Synopsis

The ultrastructure of the spermatozoa was studied in 71 species of branchiopod Crustacea, representing 23 out of the 24 acknowledged families of the subclass. All species have nonflagellate spermatozoa without an acrosome, and a mitochondrial "Nebenkern" is never formed. Anostraca, Notostraca, and Conchostraca have almost identical, amoeba-like spermatozoa, whereas Cladocera show excessive variation of this basic type. The size varies from 1 μ in *Scapholeberis mucronata* to 60 – 80 μ in some sidids and onychopods. Great variation is seen with regard to extracellular coats, morphology of pseudopodia, presence of axial rods in the pseudopodia, polarity of the cell, morphology of the cell surface, microtubules, endoplasmic reticulum, and spermatogenesis.

The most complicated spermatozoa were found in *Moina brachiata*, whose sperm have elaborate, branched pseudopodia, each supported by a tubular nuclear diverticulum which contains a bundle of chromatin tubules. Other surprising specializations are the filament bundles in *Podon* and *Evadne*. These consist of thin tubules which measure about 80 Å in diameter and belong to an unknown class of filaments.

In general variation of the spermatozoa is in agreement with established systematics, but in many cases spermatozoan morphology contributes to the solution of doubtful points of systematics and phylogeny. The monophyly of the Branchiopoda including the Anostraca is strongly supported, the isolated position of the Haplopoda and the Holopediadae is underlined, and the Macrothricidae are shown to be remnants of a basal radiation of the Anomopoda and appear to be a paraphyletic unit.

Spermatozoa are also useful for systematics on the species level, e.g. in the genera *Ceriodaphnia* and *Simocephalus*. *Simocephalus exspinosus* and *S. congener* can be clearly distinguished, and *Ceriodaphnia pulchella* and *C. quadrangula* are also shown to have very different spermatozoa.

KARL GEORG WINGSTRAND
Institute for Comparative Anatomy
University of Copenhagen
Universitetsparken 15
2100 Copenhagen Ø, Denmark

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TABLE 1
Systematic position of the branchiopod species investigated.
System according to Flössner (1972).
The arbitrary subgrouping, used for practical reasons in the descriptive text of the present paper, is shown to the right.

	Number of species	Subgroups in the text (and number of species)	Page
SUPERORDER ANOSTRACA			
Fam. Branchinectidae	2	Anostraca (10)	12
Fam. Artemiidae	1		
Fam. Branchipodidae	2		
Fam. Streptocephalidae	1		
Fam. Thamnocephalidae	1		
Fam. Chirocephalidae	2		
Fam. Polyartemiidae	1		
SUPERORDER PHYLLOPODA			
Order Notostraca			
Fam. Triopsidae	5	Notostraca (5)	15
Order Diplostraca (= Onychura)			
Suborder Conchostraca			
Fam. Cyzicidae	2	Conchostraca (5)	17
Fam. Leptestheriidae	1		
Fam. Limnadiidae	1		
Fam. Cyclestheriidae	–		
Fam. Lynceidae	1		
Suborder Cladocera			
Division Haplopoda			
Fam. Leptodoridae	1	Haplopoda (1)	18
Division Eucladocera			
Superfam. Sidoidea (= Ctenopoda)			
Fam. Sididae	3	Sididae (3)	20
Fam. Holopedidae	1	*Holopedium* (1)	22
Superfam. Chydoroidea (=Anomopoda)			
Fam. Daphniidae	18	*Daphnia* (7)	23
		Ceriodaphnia (5)	25
		Simocephalus (4)	27
		Scapholeberis (2)	30
Fam. Moinidae	3	*Moina* (3)	30
Fam. Bosminidae	3	*Bosmina* (3)	35
Fam. Macrothricidae	1	*Streblocerus* and *Ilyocryptus* (2)	36
		Macrothrix (1)	38
		Ophryoxus (1)	38
Fam. Chydoridae			
Subfam. Eurycercinae	1	*Eurycercus* (1)	39
Subfam. Aloninae	9	Aloninae (9)	40
Subfam. Chydorinae	4	Chydorinae (4)	42
Subfam. Sayciinae	–		
Superfam. Polyphemoidea (= Onychopoda)			
Fam. Polyphemidae	1	*Polyphemus* (1)	44
Fam. Podonidae	2	Podonidae (2)	45
Fam. Cercopagidae	1	*Bytotrephes* (1)	47

General Introduction

The present investigation was originally started in the hope of adding some new arguments to the discussion on the phylogeny and systematics within the "lower" crustaceans. Inspiration actually came from a previous work, in which spermatozoan structure was found significant for the old problem of pentastomid relationships (Wingstrand 1972, 1974). Later, the great variation and fascinating cytology of entomostracan spermatozoa prompted me to extend the material and to make a monographic study of this subject, which has been largely ignored by previous investigators.

Up to now about 200 species of Entomostraca have been studied more or less thoroughly. Each subclass (Branchiopoda, Copepoda, Ostracoda, Cirripedia, etc.) appears to have its own basic type of spermatozoon, but within each subclass variations of this basic type are found to the genus or species level. The pattern of variation generally agrees well with established systematics, but in some cases the structure of the spermatozoa contributes to the solution of doubtful points of phylogeny.

Since the material of branchiopod spermatozoa appears to be sufficient for a reliable review, this subclass is dealt with separately here. Publication of results from the other subclasses will be postponed until they are better covered by material.

Previous literature on branchiopod spermatozoa

Older investigators correctly describe branchiopod spermatozoa as nonflagellate, but the restricted resolution of the light microscope made further analysis difficult. The smallest spermatozoa were drawn as granules or rods, with a more or less distinct nucleus, e.g., in *Daphnia*, *Simocephalus*, *Ceriodaphnia*, *Eurycercus*, *Camptocercus*, *Chydorus*, and some *Moina* species (Weismann 1880, Retzius 1909, Mortimer 1936, Scourfield 1947, Ojima 1954, Zaffagnini 1965, Goulden 1968). The somewhat larger spermatozoa of "Euphyllopods" *(Artemia, Branchinecta, Triops, Lynceus)* were drawn as simple, amoeba-like cells (Kozubowski 1857, Sars 1896, v. Zograf 1906, Longhurst 1955, Fautrez-Firlefyn & Fautrez 1955, Wolfe 1971). The large spermatozoa of onychopod and ctenopod Cladocera were described as vesicular cells with a comparatively small nucleaus *(Sida, Diaphanosoma, Latona:* Sars 1865, Weismann 1880; *Evadne, Podon, Polyphenus, Bytotrephes:* Leydig 1860, P. E. Müller 1867, Claus 1877, Weismann 1880, Zacharias 1884, Retzius 1909). Pseudopodia emitted by these large spermatozoa were seen in *Sida* and the onychopods, located at one or both ends of the elongate cell. They were seen immediately after the spermatozoa had been suspended in water and disappeared some minutes later, when the cells tended to round up and disintegrate. The significance of these processes therefore remained somewhat uncertain (Weismann 1880, Zacharias 1884).

Pseudopodia were also described by Weismann (1880) in the large spermatozoa of *Moina brachiata*, which were depicted as small heliozoans with slender processes all the way round (compare Goulden 1968). The spermatozoa of *Leptodora* were described by Weismann (1874) as long, slender filaments, coiled up inside a testicular

cell. Weismann later (1880) realized that this was a mistake, and that the "host cell" itself was the spermatozoon proper.

Ultrastructural investigations are few. Brown (1966, 1970 a, b) describes the spermatozoa of *Artemia salina* as rounded cells with plentiful cytoplasm, a distinct nucleus, some endoplasmic reticulum (ER) and mitochondria. Small processes suggestive of the slender pseudopodia seen with phase contrast in living spermatozoa were present all over the surface. Delavault & Bérard (1974) and Bérard (1974) followed spermatogenesis in *Daphnia magna* and showed that the rod-shaped spermatozoa have a very dense cytoplasm, a distinct nucleus, and are surrounded by a well-organized extracellular coat. Finally, Garraud de Loubresse (1967) described the remarkable crystalline bodies in the elongate mitochondria of *Tanymastix* spermatozoa.

The nonflagellate nature and the more or less amoeba-like appearance of branchiopod spermatozoa was amply corroborated by the present investigation, which, in addition, revealed a rather unexpected diversity with regard to detailed cytological structure (Fig. 8).

Material and methods

The material includes 71 species of branchiopods, i.e. about half of the species recorded in northern Europe plus some Mediterranean and American forms, and covers 23 of the 24 families of the world fauna (see Table 1, page 4). The only family not represented in the material is the Cyclestheridae among the Conchostraca. It is particularly important that all three subdivisions of the classical "Euphyllopods" (Anostraca, Notostraca, Conchostraca), as well as all major subdivisions of the Cladocera are well represented. Detailed records of the material will be given under each main heading in the descriptive part.

Most samples were brought living to the laboratory, where males of desired species were picked out and collected in a small drop of water. An excess of fixative was added, and the animals were decapitated or (large species) completely opened with iridectomy scissors as soon as they stopped moving.

Fixatives used were:

1% Os (OsO_4) with veronal acetate buffer according to Palade (1952) (1 – 2 hours, + 4°C).

2% Os in 0.1 M cacodylate buffer, pH 7.4, 1 hour, + 4°C.

2% Gla (glutaraldehyde) in phosphate buffer, pH 7.4, 2 – 3 hours at room temperature. Usually the concentration was 0.05 M with regard to PO_4, rarely *(Artemia)* 0.124 M.

3-A trialdehyde mixture according to Kalt & Tandler (1971), 2 – 3 hours, room temperature or outdoor temperature (see Lake 1973).

When fixation in the field was necessary, the entire plankton sample was fixed in 3-A for 2-3 hours, transferred to buffer (0.1 M cacodylate and 0.1 M. sucrose, pH 7.4) and transported in buffer to the laboratory, where the sample could be sorted and the animals decapitated. All aldehyde-fixed material was post-fixed for 1-2 hours in 2% OsO_4 with 0.1 M cacodylate, pH 7.4, at + 4°C, rinsed in cacodylate buffer, and transferred via 30% and 50% alcohol to 70% where it was stored at ÷ 20°C.

For the few marine forms, salt water from the locality (or a mixture of salt water: distilled water 2:1) was used in preparation of the trialdehyde mixture instead of distilled water.

Most specimens were satisfactory both after fixation in osmium tetroxide and in aldehydes. The trialdehyde mixture was very convenient for field work. Useful results were obtained with material which had been kept in buffer at + 4°C for a week or even months before osmification. In animals with intact cuticle (plankton samples), excellent results were obtained with trialdehyde, whereas osmium tetroxide or glutaraldehyde alone gave very poor results.

All material was embedded in epon and the sections for EM were contrasted with uranyl acetate and lead citrate. Epon sections 1-2μ thick and stained with toluidine blue – borax, were used for light microscopical orientation.

A few species proved particularly difficult to fix properly. The mature sparmatozoa of *Holopedium* virtually exploded during repeated attempts to fix them with osmium tetroxide, but tolerable results were obtained with aldehydes. Also some "Euphyllopods", particularly *Lepidurus*, but also *Cyzicus*, *Artemia*, *Tanymastix* and *Steptocephalus* caused some difficulties: fixation in aldehydes and osmium tetroxide gave fairly different pictures, the aldehydes causing some shrinkage and coarse precipitation, whereas material fixed in osmium tetroxide often showed membrane breakage. Rapid dissection in the fixative proved essential for keeping these artifacts down at a tolerable level.

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So many people have helped me to carry out this investigation that it is impossible to mention all of then. Dr. U. Røen, of this institute, has been my main source of information about freshwater localities and literature, and helped me with identification of many critical forms. Collecting material has been favoured in different ways by numerous people: Mr. A. Adolfson, Tenhult, Sweden; Dr. Gunnar Anderson, Limnological Institute, Lund, Sweden; Dr. Chanan Dimentman, Dept. of Zoology, Hebrew University, Jerusalem; Mr. Vagn Holme Frederiksen, this institute; Dr. N. Holland, Scripps Institution, California; Mr. Poul Jeppesen, Zool. Museum, Copenhagen; Miss Åse Jespersen, this institute; Mr. J. Just, Zool. Museum, Copenhagen; Mr. Jørgen Lützen, this institute; Dr. Claus Nielsen, Marine Biol. Laboratory, Helsingør; Mr. Jens Petter Nielsen, Zool. Institute, Blindern, Oslo; Dr. N. Rieder, Karlsruhe; Mr. R. Møbjerg Kristensen, this institute; Dr. T. Wolff, Zool. Museum, Copenhagen; and several students of Zoology. I am most grateful for this help, and also for facilities put at my disposal when collecting material at the marine biological stations at Espegrend, Bergen, Norway; Kristineberg, Sweden; and Helsingør, Denmark, and at the Limnological Station at Aneboda, Sweden.

Finally I want to thank Dr. B. Tandler, Cleveland, Ohio, for valuable tips about fixation, and I am very grateful to Mrs M. E. Petersen, Zoological Museum, Copenhagen, for revising the English text.

General features of the mature branchiopod spermatozoa

The branchiopod spermatozoa lack all the characteristic features usually assoiated with the concept "spermatozoon" except that they are used to fertilize the eggs. They are rounded or elongate nonflagellate cells with a distinct, membrane-bound nucleus (Fig. 1). The mitochondria of the mature spermatozoon are often typical, with distinct cristae, but may be small and modified or even absent in some species, particularly among anomopods. The two centrioles are always present in spermatids and are preserved in the mature spermatozoa of the euphyllopods. No acrosome was found in any species. Fautrez-Firlefyn & Fautrez (1955) interpreted a PAS-positive granule in *Artemia* spermatozoa as an acrosome vesicle, but this interpretation could not be confirmed ultrastructually.

The simplest structure of the spermatozoon is found in the classical euphyllopods and *Holopedium*, which have amoebalike spermatozoa measuring about 5 μ in diameter (Pls. 1-3). Cladoceran spermatozoa are far more variable and often very complicated. Their size varies from about 1 μ to 80 μ, and the plasm contains a variety of filaments, rods, tubules and specialized kinds of endoplasmic reticulum (Fig. 8). The surface is often covered by specialized and complicated extracellular coats, and often protudes into axopod or filopod-like processes.

Probably most of these spermatozoa are capable of some kind of amoeboid movements, although hardly of locomotion. Many produce pseudopodia-like processes while still inside the

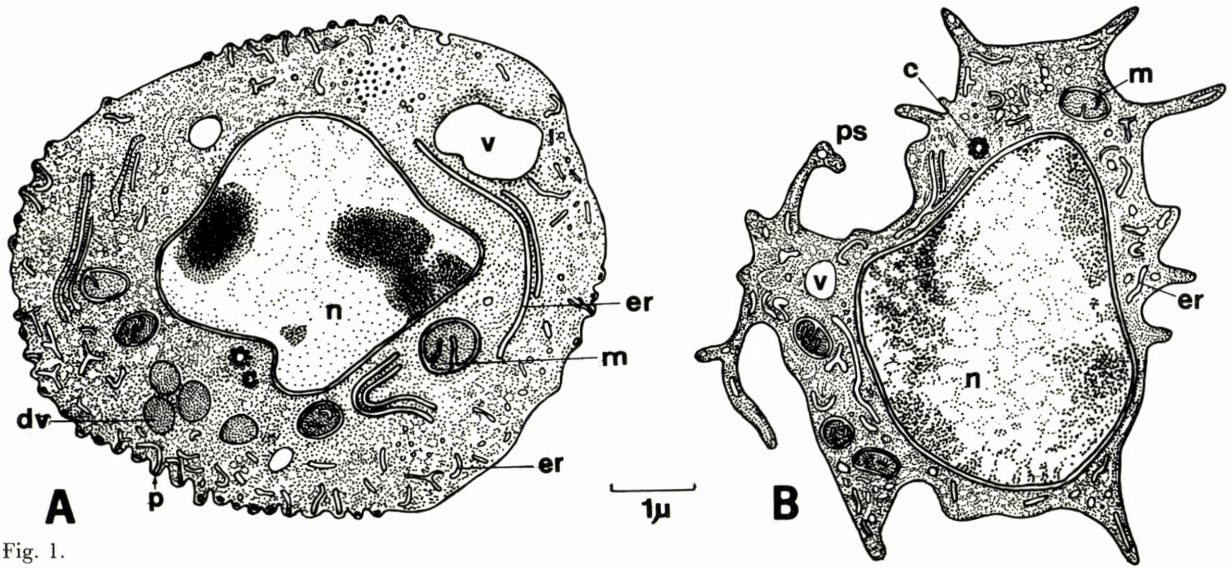

Fig. 1.

Diagrams of mature anostracan spermatozoa. A. *Artemia salina* (L.). B. *Polyartemia forcipata* Fischer.

Legends: c = centriole, dv = dark vacuoles, er = endoplasmic reticulum, m = mitochondria, n = nucleus, p = papilla with blind end of reticulum tube, ps = pseudopodia, v = light vacuoles.

male ducts. In other species, as *Artemia* and onychopod Cladocera, such pseudopodia appear when the spermatozoa are forced out of living males into the surrounding water (Leydig 1860, Weismann 1880, Zacharias 1884, P. E. Müller 1867, Brown 1966, 1970 a, b). The significance of these pseudopodia in fertilization is unknown, but it appears reasonable to assume that they establish membrane contact with the eggs (Brown 1970 b). However, when spermatozoa are observed in water under a light microscope, these pseudopodia disappear within few minutes, and the cells round up and tend to disintegrate. It is therefore difficult to say what is physiological and what is pathological in the activities of these pseudopodia, and their ultrastructural appearance is accordingly variable and often confusing.

In order to avoid errors inherent in such degeneration I had to restrict descriptions and comparisons to spermatozoa lying inside the male, in the testicular lumen or the spermoduct.

Main types of spermatogenesis

With the single exception of *Leptodora*, all branchiopods have paired testicles lying in the hemocoel on each side of the intestine. The shape of the testicles varies from sac-like or tubular with no or a few lobes in Cladocera and Anostraca to strongly lobulated and ramified in Notostraca and Conchostraca. There is usually a distinct lumen, lined by a typical epithelium which contains vegetative cells and germinal cells. Only in onychopod Cladocera do the testicles appear compact, and the lumen and the epithelial surface may be difficult to define in mature males.

The vegetative cells of the testicular epithelium are usually large cells which reach from the basement membrane to the epithelial surface, where their inflated ends form a continuous lining of the lumen (Fig. 2). The germinal cells originally lie in the interstitia between the vegetative cells and divide there, but great differences were found with regard to the location of the maturing spermatids. Since this may be important for comparisons, I shall mention some of the characteristic types.

A. *Maturation in cysts* (Fig. 2A). In this case,

maturing spermatids form multicellular nests inside the epithelium, located in cyst-like dilatations of the intercellular space. In each such cyst the spermatids develop synchronously and are probably all descendants of a single original germ cell. At maturation the cyst opens into the testicular lumen and the spematozoa are liberated.

This cystic type of testicle is predominant in the Anostraca and occurs also in the cladocerans *Holopedium*, *Ilyocryptus*, and *Streblocerus*. The notostracans *Lepidurus* and *Triops* and the conchostracan *Lynceus* have a similar type of spermatogenesis, but the cysts are not so clearly delimited and regular in type.

Theoretically, only mature spermatozoa would be expected in the testicular lumen of these species, and this was actually observed in specimens of *Siphonophanes*, *Branchinecta*, and *Branchipus*. But in other species a variable number of immature cells must be liberated, for the lumen contains a mixture of mature and immature cells. Even spermatocytes in 2nd meiosis and non-separated pairs of spermatids were frequent in the testicular lumen of *Chirocephalus* and *Artemia*. Such animals may be said to approach the following type, although many cysts develop typically to full maturation.

B. *Maturation in the lumen* (Fig. 2B). In this case no distinct cysts are formed in the epithelium, which is low, cuboidal or even squamous in some parts of the wall. Spermatids, in part also spermatocytes, are continuously liberated into the lumen, where maturation takes place. During this process the cells may stick to the wall and may even be attached to neighbouring vegetative cells by means of junctional zones as in *Moina*.

This lumenal type of maturation was found in the conchostracans *Cyzicus*, *Imnadia* and *Leptestheria* and in the cladocerans *Sida*, *Diaphanosoma* and *Moina*. In *Cyzicus*, *Imnadia* and *Leptestheria* all the segmental dilations of the testicle appear to produce spermatids, while in the sidids and *Moina* the germinal zone is restricted to the anterior end of each testicle.

C. *Maturation in vacuoles* (Fig. 2C). This type of maturation is characteristic of and restricted to the anomopod Cladocera (except *Moina*, *Ilyocryptus* and *Streblocerus)*. In these crustaceans the vegetative cells of the testicular wall are exceptionally large ("cellules géantes", Bérard 1974). Their basal ends are in contact with the basement membrane, and their distal ends form the continuous lining of the testicular lumen.

Spermatocyte divisions produce small clusters of spermatids lying deep in the epithelium near the basement membrane. The spermatids spread and sink into individual pouches formed by the walls of the surrounding giant cells. Later these pouches are pinched off, and by a process similar to phagocytosis each spermatid becomes enclosed in a "private" vacuole in the plasm of the vegetative cell.

Inside this vacuole the spermatid develops to maturity while the vacuole migrates through the plasm and towards the testicular lumen, where it eventually causes the plasma membrane of the cell to bulge into the lumen. The final opening of the vacuole and the liberation of the mature sperm is a process identical with exocytosis.

D. *Various aberrant types*. The onychopods, which have a compact-looking testicle and very large spermatozoa, are difficult to classify with regard to spermatogenesis, for great differences were noted between the genera. They are therfore described separately in the systematic account. I have also given up trying to classify the types af spermatogenesis found in *Latona* and *Leptodora*.

Comments on spermatogenesis. Of the types described, the cystic type (A) and the lumenal type (B) of maturation are obviously closely related. In some Anostraca a mixture of both is seen in the same testicle, where some spermatids are liberated before maturation, whereas others remain in the cysts until mature, The presence of this general type (A or B or both) in all three groups of euphyllopods and in a few Cladocera suggests that it is primitive in the Branchiopoda.

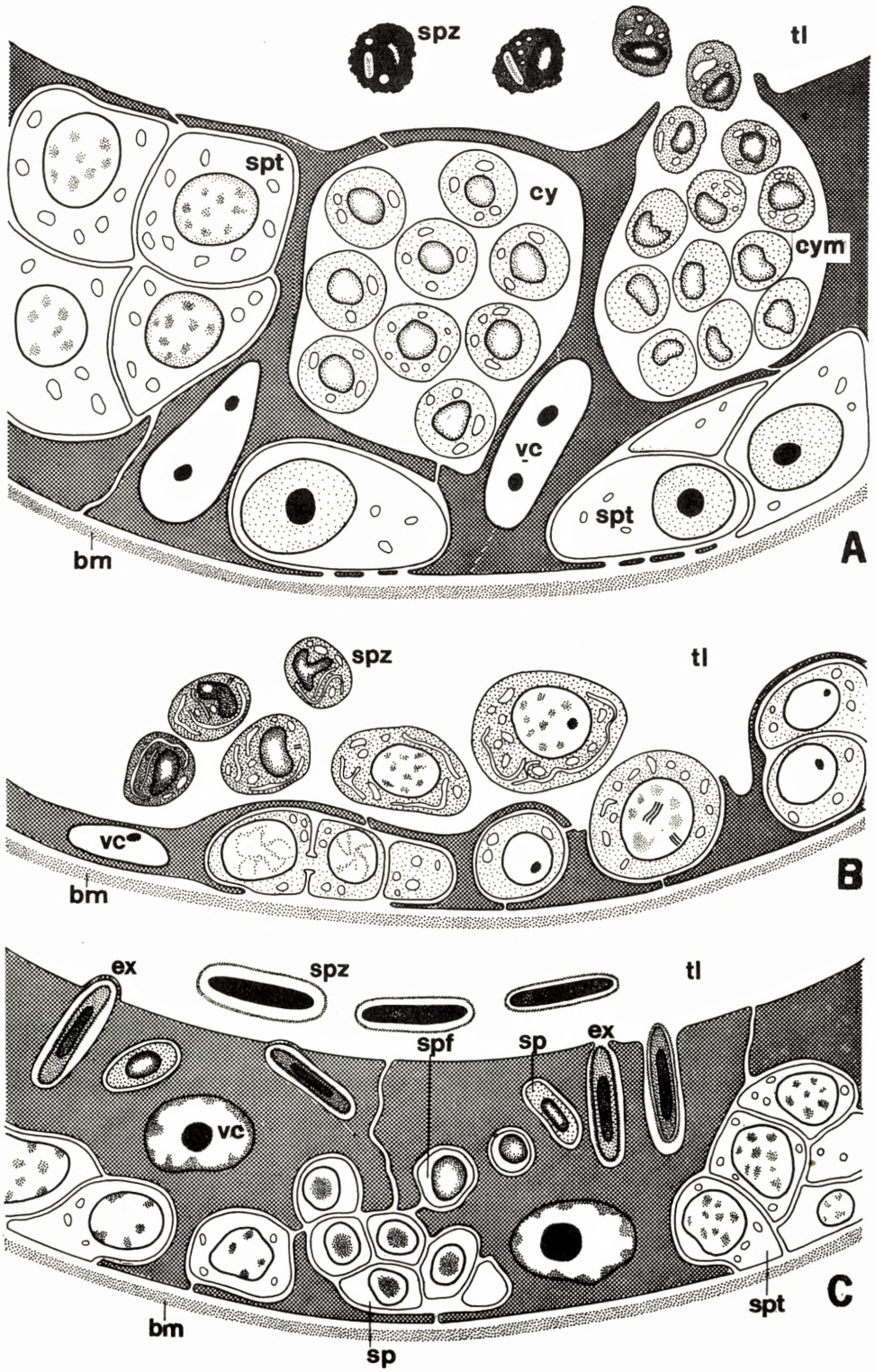
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Fig. 2.

Main types of spermatogenesis in Branchiopods.

A. Cystic type as in *Siphonophanes grubei*. Clusters of spermatids mature inside cystic dilations (cy) of the intercellular space between vegetative cells (VC).

B. Lumenal type as in *Cyzicus* sp. Spermatids, partly also spermatocytes, are liberated into the testicular lumen (tl) and mature there.

C. Vacuolar type as in *Daphnia magna*. Spermatids are phagocytosed by vegetative cells (spf) and mature inside "private" vacuoles (sp). When mature they are exocytosed by the begetative cell (ex) and arrive into the testicular lumen (tl).

Legends to all figures: bm = basement membrane of testicular epithelium, cy = extracellular cyst, cym = mature cyst opening into the testicular lumen, ex = intracellular vacuoles with mature spermatozoa, opening into the testicular lumen by exocytosis, sp = spermatids, spf = spermatids being phagocytosed, spt = spermatocytes, spz = spermatozoa, tl = testicular lumen, vc = vegetative cell nucleus.

The plasm of vegetative cells is hatched.

Maturation in vacuoles (Type C) is restricted to the anomopod Cladocera. It was first described by Bérard (1974), for *Daphnia magna*. Such maturation in intracellular vacuoles is certainly rare in the animal kingdom; the only parallel cases I know of are the halacarine mite *Copidognathus* (V. Holme Frederiksen, pers. comm.), and bdellid and thrombidiform mites (Alberti & Storch 1976b, Witte & Storch 1974). It is most probably an apomorphic feature, envolved in some ancestral anomopods (see Fig. 8).

The existence of this type of spermatogenesis was doubted by Rosen-Runge (1977), who suggested that Bérard's description of intracellular vacuoles containing spermatids depend on misinterpretation of other, more complicated patterns. However, having seen hundreds of spermatids enclosed in private vacuoles in most species of anomopoda, I agree completely with Bérard's interpretation. The membrane of the vacuole wall is closely attached to the cell membrane of the spermatid until the final stages of maturation, and no connections with the surface of the vegetative cell are seen (see Pls. 9:43, 10:52, 13:64, 16:78).

Descriptive Part

Superorder Anostraca

Material

Fam. Branchinectidae

Branchinecta paludosa (O. F. Müller). Godhavn, Greenland, 16. VII. 73, ♂♂, 1 % Os (Coll. U. Røen).

B. ferox (Milne-Edwards). Laboratory culture of mud collected at Gush Etzion, Israel, 11. VI. 73, ♂♂, 3-A, 2 % Os (Coll. Ch. Dimentman).

Fam. Artemiidae

Artemia salina (L.). Culture from commercial eggs in 1972, 1973, 1974, all with ♂♂ and ♀♀, 3-A, 1 % Os, 2 % Os, Gla with 0.05 and 0.124 M phospate buffer.

Fam. Branchipodidae

Branchipus schaefferi Fischer. Culture of mud from Nahal-Zin, Negev, Israel, 2. VII. 73, ♂♂, 3-A, 2 % Os (Coll. Ch. Dimentman). – Laboratory culture of sand from Zeiselmauer, NW Vienna, fixed in 1976, ♂♂, 3-A (Coll. and cultured by N. Rieder, Karlsruhe).

Tanymastix stagnalis (L.). Ottenby, Öl., Sweden, 5. X. 74, ♂♂, 3-A, 2 % Os.

Fam. Streptocephalidae

Streptocephalus torvicornis (Waga). Culture of mud from Ramle, Israel, 21. II. 73, ♂♂, 3-A, 2 % Os (Coll. Ch. Dimentman).

Fam. Thamnocephalidae.

Branchinella spinosa (Milne-Edwards). Laboratory culture of dry mud from Sebhka Zima, Morocco, 12. IV. 77, 1 ♂, 3-A (Coll. Å. Jespersen).

Fam. Chirocephalidae

Chirocephalus bairdi (Brauer). Culture of mud from Khirbet Kharaik, Israel, 6. III. 75, ♂♂, 3-A, 2 % Os (Coll. Ch. Dimentman).

Siphonophanes grubei (Dybowski). Dyrehaven, N of Copenhagen, 16. IV. 73, ♂♂, 1 % Os, Gla.

Fam. Polyartemiidae

Polyartemia forcipata Fischer. Ivarlakko, Sarek, Swedish Lapland, 2. VIII. 1977, ♂♂, 3-A (coll. Å. Jespersen).

In Anostraca the tubular testicles extend on each side of the intestine from a level above to one behind the penes (see Sars 1896, tab. VII; Linder 1941, fig. 166; Wolfe 1971). The spermoduct, which passes from the anterior end of each testicle down to the penis of the same side, may be dilated into a seminal vesicle. As far as possible, mature spermatozoa were studied in the spermoduct and the seminal vesicles.

Mature anostracan spermatozoa are simple, amoeba-like cells with a rounded or lobate outline, and with a distinct, membranebound nucleus (Fig. 1, Pls. 1-2). Although they are smaller than early spermatids the condensation of plasm and chromatin during spermatogenesis is insignificant to moderate. There is a distinct nucleolus which in *Artemia* sometimes consists of concentric laminae. Mitochondria with normal cristae are present, but the matrix contains some dark matter in most species. In *Tanymastix* some mitochondria are elongate and contain a spindle-shaped, crystalline rod (Garreaud de Loubresse 1967). The rod shows a cross-striation with about 110 Å periodicity (Pl. 2:12). Such intramitochondrial crystals have not been seen in other branchiopods; those in *Tanymastix* look like the crystalline elements in metamorphosed mitochondria of some insects and gastropods (See Favard & André 1970).

A pair of centrioles is probably always present, for one or two are frequently seen in sections of mature spermatozoa of all species. Ribosomes, granular ER and Golgi stacks are seen in spermatids but tend to disappear in mature sperm. Clusters of glycogen-like granules occur regularly in the spermatozoan plasm of *Streptocephalus* but are scattered and infrequent in other species. Round vacuoles with a heterogeneous, some-

times very dark, content are often seen. Some of them could be lysosomes. The structure of the cell surface and of the smooth ER varies from species to species and will be dealt with separately.

Spermatogenesis is of the cystic type (Fig. 2A). Since the mature spermatozoa differ very little from early spermatids in most species, the morphological changes during maturation are slight: some condensation of nucleus and plasm; reduction of ribosomes, granular ER and Golgi stacks; and differentiation of the cell surface and of the smooth ER to the state typical of mature cells.

Branchinecta paludosa and *B. ferox* (Pl. 1:1-2). In both species the cell surface is smooth and appears lobulate, lacking the small papillae typical of some of the following species. Actually, in *B. paludosa*, the spermatozoon extends into true lobes, often with a narrower base. In *B. ferox* there are instead numerous sac-like depressions of the cell surface, shaped like incompletely cut off, spherical vesicles (Plate 1:1). The smooth ER is much better developed in *B. ferox*, and consists of numerous flat sacs which tend to form a layer just under the plasma membrane.

Artemia salina. Spermatozoa believed to be mature are 5-6 μ in diameter, have an irregular, often lobate nucleus and dark plasm, in which an extensive system of irregular endoplasmic tubules can be seen (Fig. 1A). The general outline is ovoid or spherical, with densely set small, low papillae. Each papilla has a tuft of extracellular coat material on the top and contains the blind end of a tubule of the smooth ER (Pl. 2:9). Mitochondria with normal cristae are present, but are often swollen and disarranged in material fixed in osmium tetroxide.

Spherical vacuoles, often with a dark and heterogeneous content, sometimes with a homogeneous medium-contrast content, are seen in most spermatozoa in the sections. Such vacuoles could be identical with the "acrosome" described by Fautrez-Firlefyn & Fautrez (1955) in their light microscopical study of *Artemia* sperm. This presumed acrosome is said to be a PAS-positive, round or disc-shaped body lying near the cell surface. However, Brown (1970) and Wolfe (1971), found no PAS-positive structure in *Artemia* sperm. Since the vacuoles observed have no contact with the nucleus and lack the ultrastructural features by which an acrosome could be identified, I cannot accept them as acrosomes.

The picture obtained agrees well with figures of *Artemia* spermatozoa published by Brown (1970 a and b). The astonishing fact is, however, that such cells are rare in the testicular lumen of some males and usually make up less than half of the cells in the spermoduct in my material. The cells which predominate appear immature, have a larger cell body, large spherical nucleus, less dense plasm and are often devoid of superficial papillae. However, it appears reasonable to accept the smaller dark cells as mature, since they have developed the papillary system typical of the species.

The high frequency of immature cells is difficult to explain. It is improbable that all the males should be immature, for more than ten males from different breeds with egg-bearing females were sectioned. The species is certainly heterogeneous, including both parthenogenetic and sexual populations, but in sexual populations true fertilization appears to be necessary for egg development, i.e., the males must have functional spermatozoa (Barigozzi 1957, Stefani 1964). Also Brown (1970 b) found immature spermatids among the mature spermatozoa in the spermoduct of *Artemia*.

Brown (1966, 1970 a and b), using phase contrast, observed that when *Artemia* spermatozoa from living males were forced out into the water they extended filamentous pseudopodia ("arms") about twice as long as the diameter of the cell body. In Brown's material, when the spermatozoa were inside the male, these pseudopodia were poorly developed or represented only by the papillae on the cell surface.

Branchipus schaefferi (Pl. 1:5). The sperm fluid in this species is remarkable in containing large flakes of a granular matter. The spermatozoa are round or ovoid, 5-6 μ in diameter, with a large nucleus (3-4 μ), well-developed mitochondria, centrioles, and a moderately developed smooth ER consisting of small vesicles and tubules. The surface is typical of the species. Papillae containing the blind end of an endoplasmic tube are present but are not so numerous and strictly organized as in *Artemia* (Pl. 2:11). In *Branchipus* some of the papillae are extended into long filamentous psedopodia which in sectioned material are seen as variously shaped profiles in the testicular fluid. Unique are the dark, thickened and slightly convex plaques, about 1 μ in diameter, which are scattered over the cell surface (Pl. 1:5). High magnification of such plaques show an apparently thickened plasma membrane supported on the plasmatic side by a dark layer of slightly granular appearance. Immature cells have smaller such plaques (0.5 μ) and a thicker layer of amorphous matter on the plasmatic side.

Tanymastrix stagnalis (Pl. 1:6). The spermatozoa are very similar to those of *Artemia*, 4-5 μ in diameter, with a fairly dark plasm, a lobate and dense nucleus, and a richly developed smooth ER consisting of flattened sacs and irregularly branching small tubules. Some af the latter end in typical papillae on the cell surface as in *Artemia* (Pl. 2:10). Some mitochondria of normal type are present, whereas others are elongated and contain a chrystalline body as described on p. 12 (Pl. 2:12). The sperm fluid contains numerous bodies of granular structure (Pl. 1:6).

Streptocephalus torvicornis (Pl. 2:7). The sperm fluid is packed with small granules and also contains large dark globules which sometimes attain the same size as the spermatozoa (5 μ) (Pl. 2:7); bundles of fine filaments are also common. The spermatozoa have a large, somewhat lobate nucleus, a richly developed system of tubular ER, normal mitochondria, and a simple cell surface which may form a few broad, lobe-like protuberances. Clusters of glycogen-like granules are common in the plasm.

Branchinella spinosa (Pl. 2:8). The spermatozoa in the seminal vesicles are packed together into strings and clumps, separated by large globules (or vesicles) of non-contrast matter. The spermatozoa are 5-6 μ, oval or irregularly isodiammetrical, with a central nucleus and large, round mitochondria (pl. 2:8). Mitochondria and dark plasm are concentrated around the nucleus, whereas the periphery of the cell is occupied by light, almost empty-looking spaces with little contrast. The cell surface exhibits some characteristic dark "plaques" about 1 μ in diameter; 2-8 such plaques are seen in each section of a cell (Pl. 2:8, insets). They have a complicated structure: immediately under the cell membrane there is a disc of dark, homogeneous matter, covered on the plasmatic side by a very dark, honeycomblike structure in which the tubules are oriented at right angles to the cell surface. Some medium-contrast cytoplasm delimits each plaque from the light peripheral spaces in the cell. Small (0.08 μ) round marginal vescicles, part of them opening to the exterior, are irregularly scattered in a single layer under the cell surface between the plaques. No papillae of the *Artemia* type are present.

Chirocephalus bairdi and *Siphonophanes grubei* (Pl. 1:3-4). The two species have almost identical spermatozoa, about 5 μ in diameter, rounded or oval, with a round nucleus, normal mitochondria and centrioles. The smooth ER consists of flat sacs which sometimes form a sheath around the nucleus, and an extensive system of irregular, branching tubules. The latter extend under the plasma membrane as a regular system of uniformly spaced, roughly parallel units. Numerous elongate marginal vesicles (0.2 μ) form an irregular zone just under the plasma membrane and

appear to be in constant communication with the exterior through pores, for the communication is frequently seen in sections (Pl. 1:4). The surface is covered by coat material, which keeps the small scattered granules of the sperm fluid away from the nearest 0.2 μ around the cell (Pl. 1:3).

Polyartemia forcipata (Fig. 1B). The spermatozoa are 5-7 μ large, and are more irregular in shape than those of other species. The entire cell surface is elevated into broad, cone-shaped and fairly variable pseudopodia. Centrioles, mitochondria with cristae, and smooth ER in the shape of irregular tubules and vesicles are present. The tubules of smooth ER are usually also seen in the pseudopodia, but these differ distinctly from the pseudopodia of Artemiidae and Branchipodidae by being much larger, more irregular, and attaching to the cell body with a broad base.

Comments on the anostracan spermatozoa. The morphology of the spermatozoa is fairly constant: 5 μ large, nonpolar, nonflagellate cells without an acrosome and with no or slight specializations of nucleus, mitochondria and centrioles. Specific radiation is mainly seen in the smooth ER and the structure of the cell surface.

Most variations, e.g., in the smooth ER, are ill-defined and difficult to use in phylogenetic discussion. It should be noted, however, that *Branchinecta paludosa* and *B. ferox* differ strikingly with regard to the ER, although they are certainly closely related (Pl. 1:1-2).

The cell surface exhibits some more clear-cut differences. The presence of the small characteristic papillae (prospective pseudopodia) on the surface of *Artemia*, *Tanymastix* and *Branchipus* (Pl. 2:9-11) might be accepted as an argument for the monophyly of the families Artemiidae and Branchipodidae, for such papillae are definitely lacking in the other families. This fits well with Linder's (1941) opinions. He regarded the Artemiidae and Branchipodidae as closely related, particularly because of similar structure of the penes and the ovisacs, and was uncertain whether they should be maintained separate or not (op. cit. pp. 204-206).

Similarly, the superficial dark "plaques" in *Branchinella spinosa* and *Branchipus schaefferi* can be used as an argument for a close phylogenetical relationship between the Thamnocephalidae and the Branchipodidae. This is supported by other morphological features, mainly in the head appendages and genital organs, and Linder (1941) actually regards the families Artemiidae, Branchipodidae and Thamnocephalidae as a fairly well-defined subdivision of the Anostraca with more distant relations to the Streptocephalidae. I realize that the papillae and the plaques support two alternative phylogenetic interpretations (Branchipodidae either monophyletic with the Artemiidae or the Thamnocephalidae), but a discussion of these details is certainly premature until more species have been examined.

Order Notostraca

Material

Fam. Triopsidae

Lepidurus arcticus (Pallas). Godhavn, Greenland, 28. VIII. 76, 3-A (osmified 8 months later), ♀♀ (Coll. R. Møbjerg Kristensen).

L. apus apus (L.). Dyrehaven, N. of Copenhagen, 26. IV. 73, ♀♀, 1 % Os.

L. apus lubbocki Brauer. Culture of dry mud from Zerachia, Israel, 1974, 3 ♂♂, 3-A, 2 % Os (Coll. Ch. Dimentman).

Triops cancriformis cancriformis (Bosc). Laboratory culture of sand from Zeiselmauer, NW Vienna, Austria, fixed in 1976, ♀♀, 3-A (coll. and part of culturing by N. Riedel, Karlsruhe).

T. c. mauretanicus Ghigi. 20 km S of Casablanca, Morocco, temperary pool, 10. IV. 77, ♂♂, 3-A (Coll. Å. Jespersen, J. Lützen and J. Bresciani).

According to Longhurst (1955), most notostracan populations in northern and temperate Europe are hermaphroditic whereas most Mediterranean and tropical forms are bisexual. This is beautifully illustrated by the present material. The temperate *Lepidurus apus apus* is hermaphroditic whereas the

closely related *L. a. lubbocki* from Israel is bisexual, and the temperate *Triops cancriformis cancriformis* is hermaphroditic, whereas the North African *T. c. mauretanicus* is bisexual. *Lepidurus arcticus* from Greenland is hermaphroditic.

For a long time it was uncertain how reproduction takes place in the "unisexual" populations of Europe, and many scientists thought them to be parthenogenetic, like some conchostracans and cladocerans. But Longhurst (1954, 1955), in agreement with Bernard (1899), stated that the "females" in the "unisexual" populations had a hermaphroditic gonad, with testicular vesicles scattered among the egg follicles. The present investigation confirmed this for *Lepidurus a. apus*, *Triops c. cancriformis*, and *L. arcticus*. It also showed that the testicular vesicles and the spermatozoa of the hermaphroditic forms were almost identical with those of males in the bisexual *Triops c. mauretanicus* and *Lepidurus a. lubbocki*.

In all notostracans a long, ramified gonad extends on each side of the intestine through most of the body, from a few segments behind the head to a point about halfway down the abdomen. In females the terminal ramifications of the ducts carry a number of stalked ovarian follicles. In typical males from bisexual populations the end branches of the gonad carry several testicular vesicles, each consisting of a surrounding germinal epithelium and a central lumen with spermatozoa, sometimes also spermatids.

The hermaphroditic specimens have a predominantly female gonad but single sperm vesicles are scattered among the egg follicles. These testicular follicles are not numerous and are often difficult to find, since they are small in comparison with egg follicles and often situated near the base of such follicles. But they were present in all three "unisexual" species. Some of these vesicles are similar to those of normal males, others are quite small, reduced to one or a few sperm cysts, located in the prismatic epithelium of the gonadal duct and bulging out very little or not at all from its wall.

Mature spermatozoa of all five species are structurally simple cells, 6-7 μ in diameter and with an irregular round or ovoid contour (Pl. 3:14-15). The nucleus is small, about 2 μ, and somewhat lobate. The plasm is coarsely granular in *Triops* and more finely granular in *Lepidurus*, with few organelles: a few long, thread-like mitochondria with distinct cristae, and a pair of centrioles located near the nucleus. In *Triops* there are, in addition, some flattened sacs of smooth ER.

The spermatozoa of the three *Lepidurus* forms are not significantly different: the contour is more even than in *Triops*, the plasm is smooth and there is practically no ER (Pl. 3:14). In the two *Triops* forms the spermatozoa are more irregular in shape, the plasm has a coarse structure, the nucleus is a little larger and there is some smooth ER in the form of flat sacs, but there is no difference between the hermaphroditic and the bisexual forms (Pl. 3:15).

No axonema with doublet tubules was ever seen in the spermatozoa, but a loose tuft or bundle of about 20 single 200 Å microtubules is present in most spermatozoa of *Triops c. cancriformis* and *T. c. mauretanicus*. In some cases the microtubules seem to originate around the centrioles but are never continuous with centriolar tubules. No such plasmatic microtubules were seen in *Lepidurus*.

Spermatogenesis was easily followed in the fairly thick germinal epithelium in the sperm vesicles, where spermatocytes and spermatids lie in the interstitia (Pl. 2:13). The changes in cell morphology during spermatogenesis are slight: the early spermatids are somwhat larger in size than mature spermatozoa and contain free ribosomes and granular ER which are lost during final maturation. The nucleus is large and vesicular in spermatocytes and spermatids, and is reduced in size during maturation.

In *Lepidurus* the germ cells are numerous and make up a major part of the thick germinal epithelium. They lie in the interstitia between the long and irregular vegetative cells, which reach from the basement membrane to the lumi-

nal surface. Sometimes clusters of spermatids are formed, reminiscent of the sperm cysts of the Anostraca, but the cysts contain few cells and are less clearly delimited from the surroundings. In other parts of the testicle spermatids and spermatozoa are irregularly liberated into the lumen (Pl. 2:13). In *Triops* the cysts are somewhat better delimited but still small, and irregular liberation of spermatozoa into the lumen occurs in some parts of the testicles.

An unexpected feature is the high frequency of degenerating spermatozoa in the gonads. In *Ledpidurus* the normal spermatozoa are found mainly in the periphery of the testicular vesicles, where they may be mixed with maturing spermatids. The central parts of each follicle are often filled with thousands of degenerate spermatozoa and cell debris (Pl. 2:13). Such cells look empty, their plasm is reduced to scattered dark blocks, the cell membrane and the nuclear menbrane are wrinkled, often broken, and the nuclear content is irregularly precipitated. In *Triops c. cancriformis* there are also many degenerate spermatozoa in the testicular vesicles, such spermatozoa are characterized by irregular precipitation of the plasm as large, dark blocks, and by breakage of cell membranes. Only the material of *Triops c. mauretanicus* shows a moderate frequency of degenerate cells. It is difficult to understand the biological significance of all this degeneration. It has been shown (Longhurst 1955) that copulation is necessary for reproduction in the bisexual forms, so the sperm probably functions in a normal way. The degeneration is hardly caused by adverse culture conditions, for it was equally strong in specimens of *Lepidurus a. apus* and *L. articus* caught in the field and fixed directly. Nor is it probable that the remarkable features are fixation artifacts, for the normal spermatozoa and the surrounding tissue look well-fixed, and different fixation methods caused only minor differences.

Suborder Conchostraca

Material

Division Spinicaudata

Fam. Cyzicidae

Cyzicus sp. Culture of dry mud from Gush-Etzion, Israel, 11. VI. 73, ♂♂, 3-A, 2% Os (Coll. Ch. Dimentman). – I have not been able to identify the material with any of the many species described by Daday (1915). It is almost identical with *Caenestheria syriaca* Daday, 1915, but the males lack the tuft of bristles at the base of the terminal tubercle of the 2nd leg, which is said to be characteristic (See also Hartland-Rowe 1967).

C. hierosolymitanus (Fischer). Temporary pool 20 km S of Casablanca, Morocco, 10. IV. 77, 3-A (Coll. Å. Jespersen).

Fam. Leptestheriidae

Leptestheria dahalacensis (Rüppel). Culture of sand from Zeiselmauer, NW Vienna, Austria, cultured and fixed in 1977, ♂♂, 3-A (Coll. and cultured by N. Rieder, Karlsruhe).

Fam. Limnadiidae

Imnadia yeyetta Hetz. Culture of dry sand from Zeiselmauer, NW Vienna, Austria, cultured and fixed in 1977, ♂♂, 3-A (Coll. N. Rieder, Karlsruhe).

Fam. Cyclestheriidae. (No material).

Division Laevicaudata

Fam. Lynceidae

Lynceus brachyurus brachyurus O. F. Müller. Dyrehaven, N of Copenhagen, 15. V. 73, ♂♂, 1% Os.

The number of spermatozoa present in the testicular lobules of all the specimens of spinicaudate conchostracans examined was moderate, whereas the testicle of *Lynceus* was virtually crowded with them.

Mature spermatozoa (Pl. 3:16-19) are simple, amoeba-like cells, varying in size from 3-4 μ *(Cyzicus)* through 4-5 μ *(Lynceus, Imnadia)* to 5-6 μ *(Leptestheria)*. In all species the spermatozoa contain distinct mitochondria with cristae, the centrioles are preserved in the mature cell, and there is no distinct surface coat. In many features, however, there are differences between the species examined.

Cyzicus sp. and *C. hierosolymitanus*. The small spermatozoa are regularly shaped, oval or round. *Cyzicus* sp. from Israel has no pseudopodia at all (Pl. 3:17), whereas one or a few simple plasmatic extensions were seen on some spermatozoa of *C. hierosolymitanus*. The plasm is fairly dense and contains some flat sacs of smooth ER, which are best developed and tend to be concentrically arranged in *Cyzicus* sp. The nucleus is lobated, irregular, and its contents are moderately condensed and dark, although uniformly dispersed.

Leptestheria dahalacensis (Pl. 3:18) has irregularly shaped spermatozoa with thin, filamentous pseudopodia, which reach several microns into the testicular fluid. The pseudopodia appear to be simple extensions of the plasm, covered by the cell membrane, and are devoid of axial filaments. The cytoplasm is dense, so the ER and the nuclear membranes are sometimes difficult to see. The ER has the shape of flat sacs as in *Cyzicus*. The chromatin is condensed to a large, dark body.

Imnadia yeyetta has irregularly rounded or elongate spermatozoa without pseudopodia (Pl. 3:19). The ER is poorly developed and the nucleus and the plasm are without distinct specializations.

Lynceus brachyurus (Pl. 3:16) has smooth even spermatozoa without pseudopodia. The nucleus is ovoid and the chromatin is condensed to form a characteristic half-moon attached to the nuclear envelope. The smooth ER is irregular and consists of varcuoles and small sacs of variable shape.

Spermatogenesis in *Lynceus* is of the cystic type (see Fig. 2A). The epithelium is high and the spermatids form cysts with synchronous development. They are liberated when mature. In the spinicaudate Conchostraca spermatogenesis is definitely of a lumenar type (Fig. 2B). The epithelium is low, distinct cysts are not formed, and spermatocytes and spermatids are liberated into the lumen and mature there.

Comments. Conchostracan spermatozoa are of the same simple amoeba-like type as those of the Anostraca, the Notostraca, and a few Cladocera, particularly *Holopedium* (cf. Pl. 1 and 3). Although there is some variation from species to species in all three groups of euphyllopods, there are no consistent spermatological differences between Anostraca, Notostraca and Conchostraca. The spermatozoa of *Imnadia* (Pl. 3:19), e.g., fall well within the range of variation seen in Notostraca (Pl. 3:15) and Anostraca (Pl. 1), and are almost identical with the spermatozoa of the cladoceran *Holopedium* (Pl. 4:23).

The Conchostraca are usually regarded as closely related to the Cladocera and are placed together with this group in an order Onychura or Diplostraca (Kaestner 1959, Flössner 1972, Brooks 1966). The Lynceidae (Laevicaudata) was regarded by Linder (1945) as a specialized branch of the Conchostraca with some features in common with the Notostraca, and the Cyclestheriidae among the Spinicaudata have several features in common with the Cladocera. The uniform appearance of the spermatozoa in all these groups certainly indicates that the mentioned groups are phylogenetically related, but the uniformity also makes the spermatozoa almost useless for discussions of the phylogenetical relationships between the individual groups.

Section Haplopoda

Material

Fam. Leptodoridae

Leptodora kindti (Focke). Lyngby Sø, Zealand, 29. IX. 72, 19. X. 72, and 14. X. 73, many ♂♂ each time, 1 % Os, Gla, 3-A.

As described by Weismann (1874) the testicle of *Leptodora* is unpaired, consisting of symmetrical lateral lobes and an unpaired isthmus in the 2nd abdominal segment. The unpaired condition of the testicle appears to be unique among branchiopods. The testicle walls are thin, except in parts of the lateral lobes, where a high germinal

epithelium is seen in immature males. During maturation this epithelium bulges out into the lumen of each lobe, forming irregular masses of large nutritive cells with plentiful ergastoplasm. The spermatids lie in the interstitia between these cells and are not liberated into the lumen until fully mature.

Mature spermatozoa are large, transparent cells with a rounded or ovid shape. Well-fixed spermatozoa are usually 17-25 μ. Smaller dimensions are found but such cells are almost always wrinkled and distorted, probably as a result of fixation hazards. The nucleus is 2.5 – 2.7 μ, moderately dense and located eccentrically near the plasma membrane (Pl. 4:20). The cell is filled with large, smooth-walled and strongly flattened vesicles, which tend to form densely packed hemispheres and whorls. Between these empty-looking sacs there is almost no normal plasm. Recognizable protoplasm is only present as a narrow zone around the nucleus and in some (perhaps immature) cells as a thin rim under the plasma membrane. A few small (0.5 μ) mitochondria with dilated cristae are seen in the scanty plasm surrounding the nucleus.

Early spermatids inside the epithelium are smaller (5 – 7 μ), with a relatively large nucleus (2.5 μ), nuclear pores, numerous ribosomes, some granular ER, and normal mitochondria. More advanced spermatids show progressive development of the smooth ER in the shape of flat sacs which form stacks or concentric sheaths around the nucleus (Pl. 4:21). The walls of the sacs are often seen to be continuous with the outer nuclear membrane, a feature present also in mature sperm. Meanwhile the cell grows and most organelles disappear before the cell is liberated into the lumen as a mature spermatozoon.

The development distinctly shows that the remarkable cells described are the spermatozoa, as suggested already by Weismann (1880). However, for some time I was uncertain whether these cells with their unusual vesicular appearance were normal spermatozoa or in some way degenerate. I therefore sectioned about 15 males of different sizes and from different samples and used three different fixatives (to account for possible artifacts). The cells found in the testicular lumen of the mature males were always of the vesicular type described above as mature spermatozoa. Such cells could also easily be identified in a large number of males from living samples, when the testicular segments were squashed and studied under phase contrast. No doubt, therefore, they are the true spermatozoa.

Comments on Leptodora spermatozoa. The spermatozoa of *Leptodora* are specialized in a way which is unparalleled in other branchiopods. The reduction of the normal plasm and its substitution by the large flat sacs of ER probably gives *Leptodora* spermatozoa their great transparancy. This is unique. In other branchiopods which have a well developed smooth ER (e.g., some euphyllopods, *Simocephalus*, *Macrothrix* and the onychopods) the pattern is completely different.

The increase in size during maturation, seen in *Leptodora* spermatids distinguishes this genus from the euphyllopods and the anomopod Cladocera, but a similar and even greater increase in size is seen in most ctenopods and onychopods.

The unique structure of the *Leptodora* spermatozoa fits with the prevailing view that the group Haplopoda is a highly independent evolutional line (Woltereck 1919, Wagler 1926-27). Eriksson (1934), referring to the several remarkable features of *Leptodora* (great size, straight abdomen with distinct segments, nauplius larva), suggests that it should be derived directly from the Conchostraca, independently of the Cladocera, and Flössner's (1972) system, where the Haplopoda and the Eucladocera are regarded as sister groups, also involves great independence of these groups.

Family Sididae

Material

Sida crystallina (O. F. Müller). Lyngby Sø, Zealand, 21. IX. and 29. IX. 72, ♂♂, 1 % Os. – Tenhultssjön, Sm., Sweden, 10. X. 73, ♂♂, 3-A (Coll. W. Adolfsson). – Teglgårdssøen, Hillerød, 17. X, 73, ♂♂, 2 % Os – Lyngby Sø, Zealand, 12. X, 73, ♂♂, 3-A.

Diaphanosoma brachyurum (Liévin). Lyngby Sø, Zealand, 21. IX. 72, ♂♂, 1 % Os, Gla – Furesøen, Zealand, 10. XI. 72, ♂♂, 1 % Os – Värmen, Sm., Sweden, 25. X. 77, ♂♂, 3-A.

Latona setifera (O. F. Müller). Løg Sø, Zealand, 29. IX. 74, 1 ♂, 2 % Os – Kap Farvel, Greenland, 12. VIII. 72, ♂♂, formalin, for light microscopy (Coll. U. Røen).

The testicles of all three species are long tubes lying on each side of the intestine and open out behind the 6th pair of legs, on papillae *(Sida)* or on well-developed copulatory organs *(Latona, Diaphanosoma)* (see Weismann 1880, Lilljeborg 1900). The germinal zone is restricted to the anterior end of the testicular tubes, where spermatocytes and spermatids, embedded in plasmatic processes of vegetative cells, fill up the tube and make it compact (See Weismann 1880, Taf. VIII). In *Sida* and *Diaphanosoma* the spermatids are liberated into the lumen when they are still small and undifferentiated, and grow to large size and attain great cytological complication during their stay in the lumen. In *Latona* there is no or little further differentiation and growth of spermatozoa in the lumen. In all species the posterior part of the testicular tube is thin-walled with a low, squamous epithelium and appears to serve as a reservoir for mature sperm.

Sida crystallina.

As described by Weismann (1880), the spermatozoa of *Sida* are large, elongate cells, 20 – 30 μ broad and about 80 μ long. One or, usually, both ends are split up into dense tufts of pseudopodia-like processes (Fig. 8, Pl. 5).

The contents of the large cells appears very much "diluted". The nucleus is round or ovoid, abot 9 μ in diameter, with a distinct nucleolus, and is approximately central in the cell. The main body of the cell looks empty, and contains very little stainable matter. A zone around the nucleus contains some vestiges of ER and some membrane-bound vesicles with a dark or heterogeneous contant, probably lysosomes. Small mitochondria with distinct cristae are scattered throughout the cytoplasm but are rare. In the end regions of aldehyde-fixed spermatozoa, 250 Å microtubules are visible; they are longitudinally oriented in the cell and continue into the pseudopodia-like ramifications. The cell membrane is usually uncomplicated but is sometimes supported by a single layer of flat endoplasmic sacs (Pl. 5:26).

The pseudopodia are thick at the base, but repeated ramification results in a great number of thin end-branches with a complicated structure. The proximal parts of pseudopodia are united by weblike plasmatic bridges and are in fact only separated by deep furrows (Pl. 5:26). This structural pattern was seen by Weismann (1880), who described it as a "longitudinal striation" of the plasm.

The stem pseudopodia and their thicker branches have a very simple structure (Pl. 5:26). They contain a few mitochondria and microtubules, and the cell membrane is supported by flattened sacs of smooth ER. These sacs do not extend into the thinner branches.

The end-branches with a diameter of 0.5 μ or less look different, partly because their plasm is darker, partly because their cell membrane is elevated to form regularly spaced ridges with a complicated structure (Pl. 5:25-26). The cell membrane on the top of these ridges is supported by some amorphous dark matter on the plasmatic side, and is covered by some filamentous coat material on the outer side. Elsewhere around the cell there is no coat of this kind.

Spermatogenesis starts in the germinal epithelium, where meiotic divisions produce round spermatids of moderate size (about 11 μ). These have a large nucleus, nuclear pores, numerous free ribosomes and normal large mitochondria. Such

spermatids are early liberated into the lumen, where they grow and develop a very dense system of rough ER. During final maturation many spermatids, perhaps all, are attached to the testicular wall by a placenta-like structure: a flattened surface of the spermatid is invaginated by numerous short, villi-like projections from the wall cell. Final maturation includes reduction of the rough ER, the free ribosomes and the nuclear pores, as well as elongation of the cell and development of the tufts of pseudopodia.

Diaphanosoma brachyurum

The mature spermatozoa of *Diaphanosoma* (Fig. 8, Pl. 6) are best described as large vesicles, approximately spherical, with a width of 60-75 μ. As described by Weismann (1880) there are only 10 – 14 of these large, mature spermatozoa in each testicle, arranged in a single row through the testicular tube. Weismann also remarks that the spermatozoa are somewhat deformed during the peristaltic movements of the intestine. Under a binocular dissecting microscope these spermatozoa are easily seen in the living animal as a row of clear vesicles on each side of the intestine.

The spermatozoan nucleus is moderately large (about 9 μ), almost perfectly spherical, and is centrally located in the cell. The space between the nuclear membrane and plasma membrane, where the protoplasm would be expected, looks empty and is easily mistaken for testicular lumen in EM. This picture is independent of the mode of fixation. The only organelles found here are very small (0.2 μ) mitochondria and some small irregular vesicles, but both are rare and found mainly near the cell wall, where a narrow zone of somewhat denser matter underlies the plasma membrane.

The cell wall is elevated into a system of complicated sharp ridges, about 0.4 μ high. The plasma membrane appears rather thick and dark, partly because of the presence of a distinct amorphous coat on its outer side. The interior of the ridges is filled with medium-contrast matter, in which 250 Å microtubules can be seen after aldehyde fixation. The microtubules are always parallel with the cell surface.

Spermatogenesis of these remarkable spermatozoa begins in the germinal epithelium in the anterior end of the testicle. Early spermatids are small (5 – 8 μ) and have the usual equipment, with a large nucleus, a distinct nucleolus, nuclear pores, polysomes and simple plasma membrane. During spermatogenesis the diameter increases to 60 – 75 μ, i.e., the volume increases about 1000 times. The growth advances far before the cell leaves the germinal epithelium, which is poorly delimited in mature males. Differentiation of the cell surface begins early, when the cells are about 15 μ in diameter. First a single layer of vesicles appears under the cell membrane. The wall of these vesicles appears thickned by addition of dark matter on the plasmatic side, like the cell membrane, and the lumen contains stainable matter reminiscent of the coat substance on the cell surface. It is therefore possible that the vesicles have arisen by invagination of the plasma membrane, but they are completely closed in the spermatids of this stage. Later the vesicles become elongate, with the axis perpendicular to the cell surface, and are separated from each other by a single row of microtubules (Pl. 6:28-29). Finally all the vesicles fuse with the cell membrane and open to the outside, leaving between them the ridges characteristic of the mature spermatozoon. Since the height and density of the ridges vary from one spermatozoon to another, one is lead to believe that the folds are a membrane reserve for further dilatation of the cell.

Latona setifera

Although *Latona* must be related to *Diaphanosoma* (Sars 1865), perhaps standing near the ancestral form of the latter (Eriksson 1934, p. 148), there are considerable differences in the spermatozoa of the two genera. Weismann (1880) noticed this difference, stating that those of *Latona* are much

smaller (23 μ) than those of *Diaphanosoma* (according to Weismann 49 – 55 μ, actually up to 60 – 75 μ).

My material contains only one mature male fixed for electron microscopy. This specimen gave a good picture of spermatogenesis but contained only few mature spermatozoa in the testicular lumen. Light microscopical comparisons with the abundant formalin material from Greenland and with Weismann's measurements show, however, that the few spermatozoa studied in EM must be mature.

The spermatozoa are rounded, 17 – 20 μ in epon sections (Fig. 8, Pl. 4:22). The nucleus is spherical, approximately central in the cell, and measures about 5 μ in diameter. There is a distinct nucleolus. Unlike *Diaphanosoma* spermatozoa, those of *Latona* are filled with structures: numerous smooth-walled vesicles of different sizes, large mitochondria with numerous cristae, and a large granular body of glycogen-like structure which fills about half of the cell (Pl. 4:22). The plasma membrane has a distinct coat on its outer side and is irregularly wrinkled into broad, low folds, but these folds do not show apparent similarity to the ridges of *Diaphanosoma*.

Spermatogenesis is simple and takes place while the spermatids lie in the germinal epithelium at the anterior end of the testicle. The cells grow from an original size of 6 – 7 μ to 15 – 20 μ and the glycogen bodies are deposited at the end of the growth period. During growth the spermatids have the normal organelles: numerous polysomes and ribosomes, some granular ER, nuclear pores and a large nucleolus. These structures disappear or, in case of the nucleolus, are reduced in size during maturation.

Comments on the spermatozoa of the Sididae

The great increase in size during spermatogenesis is the most striking common feature in the three investigated sidids. During the enormous growth in *Sida* and *Diaphanosoma* the protoplasm seems to increase its volume mainly by water uptake, for the mature spermatozoon appears almost empty. In *Latona* the increase in size is more moderate, and a large glycogen body fills much space in the mature sperm. Many other features are incomparable in the three genera and must have envolved independently in each of them, e.g., the pseudopodia in *Sida* and the ridge system in *Diaphanosoma*.

Family Holopedidae

Material

Holopedium gibberum Zaddach. Lake Värmen, Sm., Sweden, 2. XI. 72, ♂♂, 1 % Os; 25. X. 73, ♂♂, 3-A. – Lake Fiolen, Sm., Sweden, 14. XI. 74, ♂♂, 3-A, 2 % Os.

Sars (1865, p. 65) says that the testicles of *Holopedium* are filled with "large clear cells". This must be a mistake, for in my material only small dense spermatozoa with a diameter of 5 – 6 μ were seen, and I have strong reasons to conclude that these were mature. About 20 ♂♂ out of the three samples mentioned above were sectioned for electron microscopy, and in addition 10 more were sectioned for light microscopy. In most cases the testicle contained only few spermatozoa in the lumen, but these were all of the size given above. In three specimens the testicle was virtually full of spermatozoa of the said size all the way back to the genital opening. This certainly shows that the spermatozoa never grow larger.

Another result obtained from these technical efforts is that OsO_4, both in 1 % and 2 % solution, is useless as a fixative for mature spermatozoa of *Holopedium* as it causes extensive swelling and membrane damage. The 3-A mixture, when used on the same fresh samples, works well although perhaps causing some shrinkage (Pl. 4:23).

The spermatozoa accepted as mature are irregularly rounded or elongate with some broad, lobe-like projections (Pl. 4:23). The nucleus is rounded and relatively large (3 μ) compared with the cell size (5 – 6 μ) and contains a small nucleolus. Flattened sacs of smooth ER are present

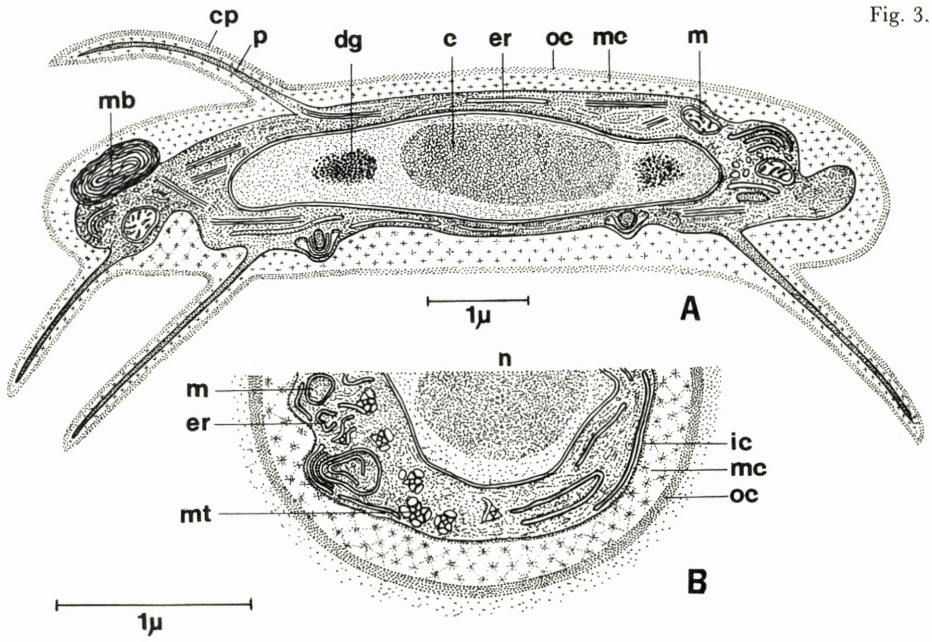

Fig. 3. Diagram of spermatozoa of *Daphnia longispina* O. F. Müller.
A. longitudinal section.
B. cross section.
Legends: c = carysome-like body, cp = extra-cellular coat on pseudo-podium, dg = clump of dark granules in nucleus, er = endoplasmic reti-culum, ic = inner layer of extra-cellular coat, m = mitochondria, mb = myelin body, mc = middle layer of extra-cellular coat, mt = microtubules, sur-rounded in a stellate fasion by unknown tubules, n = nucleus, oc = outer layer of extracellular coat, p = pseudopodium.

although not dominating, and in some cases a myelin-like lamellated body was seen. The mito-chondria have a dark matrix and distinct, some-what dilated, cristae. The ground plasm in the cell is fairly dark, somewhat granular and the plasma membrane is simple and uncomplicated.

Spermatogenesis is of the cystic type (Fig. 2A). Each cyst contains ten or more spermatids. There is no recognizable change of size during matura-tion, and the young spermatids differ from mature spermatozoa mainly in their darker plasm, which contains numerous ribosomes.

Comments on the spermatozoa of Holopedium. Sperma-tozoan structure and spermatogenesis in *Holoped-ium* are hardly distinguishable from those of euphyllopods. As, e.g., in Anostraca the sperma-tozoa are simple, amoeba-like cells and are formed in typical cysts in the testicular wall. There is no increase in size during maturation of spermatids as in the Sididae and the Onycho-poda, nor is there any reduction in size as in most Anomopoda.

Genus Daphnia

Material

Daphnia (Ctenodaphnia) magna Straus. Emdrup Sø, Copenhagen, 21. VIII. 72 and 20. IX. 72, ♂♂, 1 % Os. – Ottenby, Öl., Sweden, 4. X. 74, ♂♂, 3-A. – Sebhka Zima, Morocco, 12. IV. 77, ♂♂, 3-A, (coll. Å. Jespersen).

D. (Ctenodaphnia) atkinsoni Baird. Laboratory culture of sand from Gush Etzion, Israel, collected 11. VI. 73 (Coll. Ch. Dimentman), ♂♂, 2 % Os.

D. (Ctenodaphnia) lumholtzi G. O. Sars. Marrakech, Morocco, 29. XII. 76, ♂♂, 3-A (coll. Å Jespersen and J. Lützen).

D. (Daphnia) curvirostris Eylmann. Søborg, Copen-hagen, 26. IX. 74, ♂♂, 2 % Os, 3-A.

D. (Daphnia) longispina O. F. Müll. Søborg Mose, Co-penhagen, 18. X. 72. ♂♂, 1 % Os.

D. (Daphnia) galeata Sars. Lyngby Sø, Zealand, 19. X. 72 and 19. XI. 72, ♂♂, 1 % Os.

D. (Daphnia) cucullata Sars. Lyngby Sø, Zealand, 12. X. 73, ♂♂, 3-A.

The tubular testicles of the *Daphnia* species, lying on each side of the intestine, have thick walls consisting mainly of the very large, vegetative cells ("cellules géantes", Delavault & Gerard

1974). Spermatogenesis is of the vacuolar type (Fig. 2C).

Mature spermatozoa of the *Daphnia* species are rod-shaped, cylindrical, 6 – 9 μ long and 1 – 2 μ thick, somewhat shorter and thicker in species of *Dapnia s. str.* than in species of *Ctenodaphnia* (Fig. 3, Pl. 7). They are covered by a thick layer of characteristic coat material outside the cell membrane.

The nucleus is elongate, central, and has the usual double nuclear evelope (Pl. 7:32), but it may be difficult to resolve clearly because of the density of plasm and nuclear content. The central parts of the nucleus are occupied by a large, dark-staining caryosome-like body, and all species so far examined contain an ill-defined heap of dark granules in the light plasm near each end of the nucleus (Pl. 7:31). This is best seen in the species of *Daphnia s. str.*, which, in general, have less condensed spermatozoa than the species of *Ctenodaphnia*.

The dense plasm always contains irregular membranes which may be remnants of the smooth ER seen in spermatids. A myelin-like body with numerous concentric membranes appears to be characteristic of all mature spermatozoa in the testicular lumen (Pl, 7:31). Mitochondria were clearly identified in mature spermatozoa of all species, but they are small, with few irregular cristae, and may be difficult to see in the dense plasm.

Typical 250 Å microtubules were regularly seen in aldehyde-fixed material of spermatids but were not seen in mature sperm, except in the spermatozoa of *D. longispina*. In these, a few (about 10) microtubules were seen in each cross section, surrounded in a stellate way by four tubes of unknown significance (Pl. 7:32, inset).

The cell surface is somewhat irregular and is covered by a single unit membrane. Scattered, slender pseudopodia-like processes, simple tubes about 800-900 Å in diameter, extend from the end regions of the spermatozoa in all species except *D. magna*. In *D. longispina* and *D. cucullata* these pseudopodia extend far into the testicular fluid, surrounded by a sheath of the characteristic, stratified coat of the spermatozoon. In *D. lumholtzi*, *D. curvirostris* and *D. atkinsoni* these processes are shorter and keep inside the general contour of the coat.

The coat or capsule consists of medium-contrast matter, appearing as filaments or fine granules, like a mucoid substance. The coat is distinctly stratified and consists of: 1) an outer, more compact layer, about 0.2 μ thick, 2) an intermediate light zone, about 0.5 μ thick, and 3) a very thin (0.02 μ) filamentous layer adhering to the plasma membrane (Pl. 7:32, see also Delavault & Bérard 1974).

The intermediate zone (2) shows characteristic differences between the species. In *D. magna*, *D. lumholtzi*, *D. atkinsoni* and *D. curvirostris* this zone looks almost empty, with only scattered, irregular filaments or granules. In *D. longispina*, *D. galeata* and *D. cucullata* this zone contains small stellate bodies of dark matter, of equal size and arranged in a regular crystalline pattern. The center to center distance tetween these bodies varies from 650 to 900 Å both in transverse and longitudinal sections of the spermatozoon (Pl. 7).

Spermatogenesis. Development of the spermatids to mature sperm inside the vacuoles in the vegetative cells (cf. Fig. 2c, vacuolar type) is characterized by contraction and condensation of the cells and elaboration of the coat. In *D. magna* the newly formed spermatids are rounded cells with a diameter of about 5 μ. They have a large vesicular nucleus, nuclear pores, centrioles, normal mitochondria, many ribosomes and some ER. They are engulfed by the large vegetative cells and continue development inside individual vacuoles. During further development the cells condense and elongate to end up as dark rods, slightly more than 1 μ thick and 7-8 μ long. During elongation typical 250 μ microtubules are visible in the plasm. The cytoplasmic organelles finally disappear with the exception of some

membranes of the smooth ER and some mitochondria. The latter are soon so distorted that they are difficult to recognize in the dark, condensed plasm.

The coat begins to form just before the spermatozoa are liberated into the lumen (Fig. 2c). Early spermatids fill out their vacuoles almost completly, so their membrane is closely attached to that of the vacuole. Later spermatids are separated from the wall of the vacuole by a broad cleft. The outer layer of the coat begins to form in this cleft while the spermatid is still inside the vacuole. It is first seen as a poorly defined dark layer on the cell surface. The intermediate light layer also begins to appear before liberation of the spermatozoon, as a series of light globules separating the dark layer from the cell membrane. Real increase in thickness of this light layer is not seen until the spermatozoon is lying free in the lumen.

Comments on the spermatozoa of the Daphnia species. The characteristic rod-shape of the spermatozoa of *Daphnia* is a rare feature within the Branchiopoda. The only additional cases are found within the genus *Ceriodaphnia* and *Simocephalus*, which are regarded as closely related to *Daphnia*, and in *Moina macrocopa*. Other features of the daphnian spermatozoa such as decrease in size during maturation, condensation of nucleaus and plasm, and presence of a differentiated coat, are shared by most anomopod spermatozoa. With regard to coat structure the *Daphnia* species fall into two well-defined groups as far the present material shows, those with a noncrystalline coat *(D. magna, D. atkinsoni, D. lumholtzi* and *D. curvirostris)*, and those with a crystalline coat *(D. longispina, D. galeata, D. cucullata)*. The former group is also characterized by two strong combs with long teeth on the abdominal claw, whereas the latter group has small uniform teeth and inconspicuous combs. The commonly used systematics subdivides the genus in a different way: into subgenus *Ctenodaphnia* and *Daphnia s. str.*, the latter including *D. curvirostris* (Wagler 1936, Brooks 1967, Flössner 1972). The sperm coat and the combs rather indicate that *D. curvirostris* belongs to *Ctenodaphnia*. However, there are strong arguments also for the present systematics, and at any rate a critical evaluation of the spermatological arguments must be postponed until more species of *Daphnia* have been studied ultrastructurally.

It should also be mentioned that Scourfield (1947) in his description of *D. ambigua* states that the spermatozoa of this species are thin, flattened, oval plates, and that they differ from the spermatozoa of other *Daphnia* that he had seen. This most remarkable statement certainly calls for an ultrastructural examination.

Genus Ceriodaphnia

Material

Ceriodaphnia reticulata (Jurine). Faurholm Mose, S of Hillerød, Zealand, 12. IX. 74, ♂♂, 2 % Os.

C. megops Sars. Lake Fenen, Sm., Sweden, 3. X. 74, ♂♂, 3-A.

C. pulchella Sars. Teglgårdssøen, Hillerød, Zealand, 7. X. 73 and 17. X. 74, ♂♂, 2 % Os, 3-A. The specimens agree closely with Lilljeborg's (1900, Tab. 28) descriptions of *C. pulchella*, but differ from Flössner's (1972) descriptions in two respects: The criss-crossing of dorsal anal spines is indistinct in ♀♀ and absent in ♂♂, and the 1st antenna of ♂♂ is very short and blunt, witout basal papilla on the terminal seta.

C. quadrangula O. F. Müller, var. *hamata* Sars. Store Grib Sø, Zealand, 24. X. 74, ♂♂, 3-A.

C. laticaudata P. E. Müller. Pond at Zoological Central Institute, Copenhagen, 27. VIII., 30. VIII., and 1. IX. 72, ♂♂, 1 % Os. – Faurholm Mose, S of Hillerød, Zealand, 12. IX. 74, ♂♂, 2 %, Os. – Søborg, Copenhagen, 26. IX. 74, ♂♂, 2 % Os.

The testicle is very similar to that in the genus *Dapnia*.

Mature spermatozoa are more or less regularly rod-shaped, surrounded by a coat of medium-contrast, mucoid matter (Pl. 8). The spermatozoon and its nucleus are strongly condensed, so details are often difficult to resolve if the contrast is too intense. The plasm contains a few mito-

chondria, some membranes of unknown origin, and microtubules which are arranged in patterns characteristic of the single species. Spermatogenesis is of the vacuolar type (Fig. 2c) and is identical with that of the *Daphnia* species.

The differences between the species examined are very striking.

Ceriodaphnia quadrangula var. *hamata* has long, slender, rodlike spermatozoa with a circular cross section (6-7 × 0.6 μ). Numerous longitudinal microtubules are irregularly scattered in the cross section (Pl. 8:40). The cell coat consists of an inner dark granular layer (0.16 μ) and an outer, lighter, poorly delimited layer (0.12 μ).

C. reticulata has somewhat shorter (4.2 – 4.7 μ) and thicker (1.6 – 2.1 μ) spermatozoa with a circular cross section in which irregularly scattered microtubules are seen (Pls, 8:37 and 10:51). The inner, dark layer of the coat is about 0.3 μ thick with distinct radial stripes. It is delimited from the outer light layer by a membrane-like condensation (cm in Pl. 8:37). The light, external layer is only about 0.07 μ thick.

C. laticaudata. The spermatozoa are 4.9 – 6.0 μ long, flattened rods, which measure 2 × 0.4 μ in cross section (Pl. 8:36, 38). If seen from the flat surfaces the ends are rounded. The microtubules form a well-defined marginal bundle all along the periphery of the flattened, disc-like cell (Pl. 8:36, inset). The 0.6 μ thick coat is not differentiated into zones like that of the preceding species but has a distinct, membrane-like layer of dark bodies about 0.3 μ from the cell surface.

C. pulchella. The rod-shaped spermatozoa are 5.5 – 6.8 μ long. The cross section is characteristic, showing the cell extended into a longitudinal fold on one side of the rod (Pl. 8:39). The height of the fold is about the same as the diameter of the rod proper (0.7 μ). The microtubules are always found in a single layer, asymmetrically covering one side of the nucleus. There are no microtubules in the fold. The coat consists of an inner dark layer (0.1 μ) and an outer light layer (0.18 μ).

C. megops. The contrast is poor in my preparations, but it can be stated that the spermatozoa are very irregular, somewhat elongate (2.5 – 3.3 × 1.2 – 1.3 μ), with the nucleus located near one end. An indistinct and irregularly delimited coat, 0.3 μ thick, covers the surface.

Two of the species have very characteristic paracrystalline inclusions in the sperm fluid. In *C. pulchella* these are 0.15 μ thick plates with a dark central lamella and, on both sides of this, a lighter zone with a palisade-like arrangement of the light greyish matter (Pl. 8:39). In *C. quadrangula* var. *hamata* there are large, somewhat irregular, blocks about 5 μ in diameter, consisting of alternating thin dark and thick light lamellae (Pl. 8:40) which correspond in structure to the components seen in *C. pulchella*. These crystal-like structures are identical after osmium and aldehyde fixation. They form already inside the vacuoles in the nutritive cells when the coat begins to appear and the spermatozoon is ready to be liberated into the lumen. The paracrystalline bodies actually look like the coat substance of the two species and may be excess substance of this kind. Its presence in the vacuoles where the coat secretion is initiated supports this opinion.

Comments on the spermatozoa of Ceriodaphnia. The general morphology of the spermatozoa is similar to that of the *Daphnia* species and also of *Simocephalus congener:* small size, elongate shape, strong condensation of nucleus and plasm, and thick coat. Characteristic of the genus – in contrast to the *Daphnia* species – is the absence of pseudopodia, and the regular occurence and distinctness of longitudinal microtubules in the mature sperm.

All species examined have their own, clear-cut type of spermatozoon. This may be useful for defining these rather critical species. Thus, for instance, the population of *C. pulchella* differs

strikingly from *C. quadrangula* var. *hamata*, although these forms are so similar morphologically that the specific of *pulchella* has been questioned. These two species are also characterized by clearly different types of crystalline inclusions in the sperm fluid (Pl. 8:39, 40). *C. laticaudata is* unmistakably characterized by the flattened shape of the spermatozoa and the marginal bundle of microtubules.

Genus Simocephalus

Material

Simocephalus vetulus (O. F. Müller). Zoological Central Institute, Copenhagen, small pond, 12. VI. 72 and 8. VII. 74, ♂♂, 1 % Os, 2 % Os.

S. exspinosus (Koch). Søborg, Copenhagen, 27. IX. 74, ♂♂, and ♀♀, together with the following species, 2 % Os.

S. congener Schoedler. Faurholm Mose, S of Hillerød, Zealand, 12. IX. 74, ♂♂ and ♀♀, 2 % Os. – Søborg, Copenhagen, 27. IX. 74 ♂♂ and ♀♀, together with preceding species, 2 % Os.

S. serrulatus (Koch). Store Grib Sø, Zealand, 17. X. 74, ♂♂, 3-A, 2 % Os.

The paired testicles of *Simocephalus* are similar to those of the genera *Daphnia* and *Ceriodaphnia*, and spermatogenesis is of the vacuolar type (Fig. 2:C).

Simocephalus vetulus and *S. serrulatus* have very similar, spherical spermatozoa, although those of *S. serrulatus* are larger (3 – 3.5 μ) than those of *S. vetulus* (2 – 2.5 μ). The cell body extends into numerous slender radiating pseudopodia, about 1 μ long (Fig. 4A, Pl. 9). Inside the cell there is a distinct polarity: the nucleus is dislocated to one side, leaving place for a *cell centre* filled with criss-crossing tubules. Outside this cell centre is a *vacuolated zone* filled with large irregular sacs of smooth ER. The vacuoles are separated from the cell membrane by a more *compact cortex*.

The round nucleus contains large dark blocks of chromatin. It lies close to the cell membrane but is separated from it by a large myeloid body consisting of numerous, densely packed concentric membranes. The myeloid body is comparatively small in *S. serrulatus* but is large in *S. vetulus*, where it usually grows out to form a long rod or shaft, covered by the cell membrane. This can be seen in phase contrast of living cells. The myeloid body is formed after the spermatozoa have been liberated into the lumen. A small indentation of the nuclear membrane next to the cell surface is seen in some late spermatids still inside their vacuoles. This indentation is filled with concentric membranes which grow out to the myeloid body after the cell is liberated.

As a permanent structure with constant relations to the nucleus and the cell membrane, the myeloid body could perhaps be interpreted as a strongly modified acrosome. However, its structure is fundamentally different from that of acrosomes, and it has no relation to the Golgi apparatus. Moreover, similar myeloid bodies occur in variable locations in other branchiopods. It is therefore more probable that the myeloid bodies are a kind of residual bodies, formed by cytoplasmic components which degenerate at maturation.

The cell centre develops in the immediate neighbourhood of the centrioles, which lie near the nucleus (Pl. 9:43). The area is filled by two kinds of tubular structures: straight microtubules, 250 Å thick, and irregular, branching and bending tubules with thinner walls. The typical straight microtubules radiate from the centre of the cell to the cell surface, where each of them forms the axis of a pseudopodium (Pl. 9:44). In *S. serrulatus* some pseudopodia contain more than one microtubule in their axis. In mature spermatozoa of both species the microtubules become associated with some dark matter, so the single microtubules may be indistinct in the dark rod which is formed. The mitochondria concentrate in the periphery of the cell centre. They decrease in size and tend to lose their cristae, so they may be difficult to identify in the mature spermatozoon.

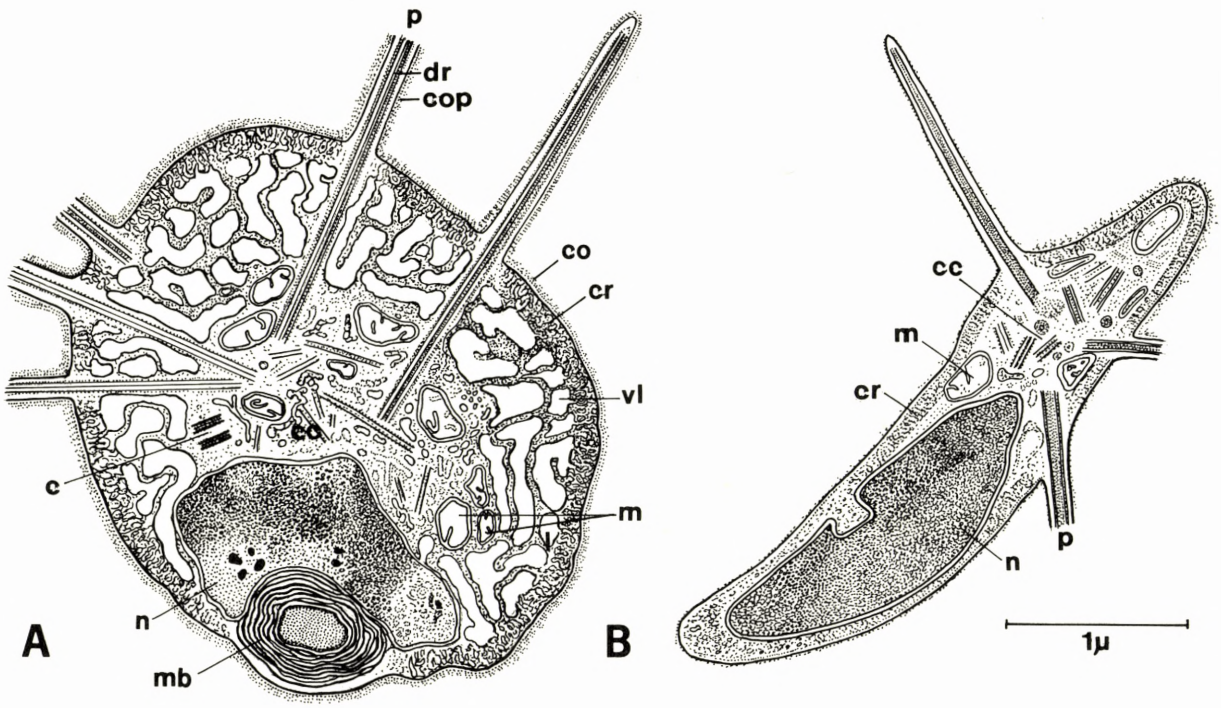

Fig. 4.

Diagrams of *Simocephalus* spermatozoa. A. *Simocephalus serrulatus* (Koch). B. *S. congener* Schoedler.
Legends: c = centriole, cc = cell centre, co = coat, cop = coat on the pseudopodium, cr = cortical zone of plasm, dr = dark rod in pseudopodium, m = mitochondria, mb = myelin body, n = nucleus, p = pseudopodium (axopodium), vl = vacuole in the vacuolated zone of cytoplasm.

The vacuolated zone appears in late spermatids by accumulation of irregular vesicles of smooth ER. The cortex arises at the same time in the superficial plasm, which is invaded by a dense system of irregular, branching tubules, communicating with the vacuoles of the vacuolated zone. Peripherally these tubules have a radial course and appear to attain contact with the plasma membrane without directly opening trough it (Fig. 4A, Pl. 9:43).

The mature spermatozoa are surrounded by an indistinct coat, about 0.35 μ thick, with very little contrast. In *S. serrulatus* there is also a narrow, dark zone (0.04 μ) attached to the plasma membrane (Pl. 9:41).

During maturation there is a considerable decrease in size, in *S. vetulus* from 4 – 4.5 μ in young spermatids to 2 – 2.5 μ in mature spermatozoa. The membrane of young spermatids is closely attached to the wall of the vacuole, but later a broad cleft appears, into which the axopodia grow out. The development of the other organelles has been mentioned above.

Simocephalus exspinosus and *S. congener* are usually treated as variants of a single species, for only large females show characteristics clear enough to distinguish the two forms (Flössner 1972). The best character is the numerous spines in the comb on the abdominal claw (18 – 30) in *congener*, whereas *exspinosus* has larger and fewer (9 – 12) spines (Pl. 10:45, 48). I managed to get a pure population of *S. congener* from Faurholm Mose. The type of sperm in this form could be established with full certainty in many males and proved very characteristic: rod-shaped, and

clearly different from the round spermatozoa of the other *Simocephalus* species (Pl. 10:47).

Another population from Søborg contained females of both *congener* and *exspinosus s. str.*, but the males, as expected, were of one kind only as far as external morphology was concerned (Pl. 10:46, 49). With regard to spermatozoa, however, the males of this population belonged to two distinct types. The difference is distinct in the light microscope, one type having round, the other rod-shaped spermatozoa. In EM the rod-shaped spermatozoa were identical with those from the pure *congener* population from Faurholm Mose. The males with such rod-shaped spermatozoa are therefore accepted as belonging to *S. congener*, and those with round spermatozoa as *S. exspinosus s. str.*

Simocephalus congener. The mature spermatozoa (Fig. 4B, Pl. 10:47) are 3.3 – 4 μ long rods, about 0.8 μ thick, with a rounded cross section. They are distinctly polar with the large, elongate nucleus filling 2/3 of the cell from one end. The other end is filled with irregular dark rods which arise as 250 Å microtubules in the spermatids. Some of these straight rods extend into the pseudopodia (Fig. 4B), which are restricted to this non-nuclear end of the cell. The space with irregular rods certainly corresponds to the "cell centre" in spermatozoa of other species. Under the cell membrane there is a 0.05 μ broad, coarsely granular zone, comparable to the cortex of the spermatozoa of other species, although distinct tubules appear to be lacking. Nothing comparable to the vacuolated zone of other species could be found, but there is probably an extracellular coat, for a 0.2 μ broad zone outside the cell membrane is free from precipitated sperm fluid.

S. exspinosus s. str. has small (1.3 μ) spherical spermatozoa with a number of slender (0.05 μ) pseudopodia radiating to all sides (Pl. 10:50). The nucleus is strongly lobulate, so 8–10 lobules may be cut it one section (Pl. 10:52). It is asymmetrically placed in the cell, but its lobulation makes this asymmetry less pronounced. Each pseudopodium contains a dark axis which can be follwed into the central parts of the cell, where a "cell centre" with more or less confluent, densely packed rods is seen. In spermatids this cell centre contains some 250 Å microtubules and some irregular tubes of ER. The mitochondria, distinct in spermatids, are difficult to identify in the strongly condensed mature spermatozoa. A cortical zone with some irregular tubules and a coarse granulation is present but is restricted to a 0.1 μ thick layer in mature spermatozoa.

Spermatogenesis is of the vacuolar type (Fig. 2C, Pl. 10:52). The strong reduction in size of *S. exspinosus* spermatozoa has proceeded rather far before the spermatids are included in vacuoles. They are about 1.8 μ when being inclosed and are reduced to about 1.3 μ during final maturation.

Comments on the spermatozoa of the genus Simocephalus. The spermatozoa of *Simocephalus* spp. differ from those of other Daphniidae in several features: the polar structure, the cell centre, and the tubular axis of the pseudopodia. However, these features are present in other Anomopoda (*Ophryoxus gracilis*, most Chydoridae) and are therefore probably primitive (plesiomorphous) in *Simocephalus*, derived from some anomopod ancestor. The absence of these features in *Daphnia*, *Ceriodaphnia*, and *Scapholeberis* must therefore be the result of progressive reduction.

On the other hand, the vacuolated zone in the spermatozoa of *Simocephalus vetulus* and *S. serrulatus* lacks a counterpart in other Cladocera and must be an advanced (apomorphous) feature evolved in the genus *Simocephalus*. It indicates a close relationship of the two species.

Genus Scapholeberis

Material

Scapholeberis mucronata (O. F. Müller). Lyngby Sø, Zealand, 29. IX. 72, ♂♂, 1 % Os. – Furesø, Zealand, 19. IX. 72, ♂♂, 1 % Os.

S. aurita (Fischer). Emdrup Sø, Copenhagen, 18. IX. 72, ♂♂, Gla, 1 % Os.

The structure of the testicle is as in daphniids, and spermatogenesis is of the vacuolar type (Fig. 2C).

The mature spermatozoa of both species are structurally simple, irregularly isodiammetrical cells. Those of *S. aurita* are much larger (2 – 3 μ) than those of *S. mucronata* (1 – 1.3 μ) (Pl. 11:53). Pseudopodia are present in both species. Those of *S. aurita* are numerous, conical and fairly broad, whereas those of *S. mucronata* are fewer, thinner and more filamentous. The nucleus is dense and its membranes are sometimes indistinct, particularly in the strongly condensed, compact spermatozoa of *S. mucronata* (Pl. 11:53, inset). A myeloid body appears to be present in all mature spermatozoa. Mitochondria and a few microtubules are seen in *S. aurita*, but in the compact spermatozoa of *S. mucronata* only a few vesicles of unknown significance were seen.

A poorly defined coat, measuring 0.3 μ in *S. aurita* and 0.15 μ in *S. mucronata*, is present along the cell surface. It has a filamentous appearance in contrast to the surrounding sperm fluid, which is granular.

Spermatogenesis implies considerable reduction of the size of the spermatids, from ca. 2.5 μ to 1.3 μ in *S. mucronata* and from 4 – 5 μ to about 2.5 μ in *S. aurita*. Centrioles, mitochondria, microtubules, and ER are distinct in spermatids but are reduced *(S. aurita)* or disappear completely *(S. mucronata)* during maturation, when the spermatids contract.

Comments on the spermatozoa of Scapholeberis. The very small, cytologically simple spermatozoa of *Scapholeberis* have very little in common with those of other daphniids, although an approach towards a similar simple type is seen in *Simocephalus exspinosus*. However, the small spermatozoa of the Bosminidae have almost exactly the same appearance. (Pl. 13:63). In all cases the loss of organelles is correlated with the extreme decrease in size of the spermatozoa. As will be discussed later this decrease in size and recuction of structure must be secondary, for there are strong arguments indicating that ancestral anomopods had more complicated spermatozoa. Although the derivation of *Scapholeberis* spermatozoa from the type seen in *Simocephalus exspinosus* could be imagined, all such comparisons are hampered by the striking lack of structures.

Family Moinidae

Material

Moina brachiata (Jurine). Pond at Slaglille near Sorø, Zealand, 12. VIII. 74. Kept in culture until ♂♂ appeared (2. IX. 74), 3-A, 2 % Os.

M. micrura Kurtz. Laboratory culture of dry mud from near Gay's Cave, Barbados, 1976, ♂♂, 3-A, (coll. T. Wolff and J. Just).

M. macrocopa (Straus), ssp. *americana* Goulden. Laboratory culture of dry mud from drain ditch at Del Mar, San Diego County, California, USA, 22. VI. 77, ♂♂, 3-A (coll. N. Holland).

The testicles of the three species are wide, elongate sacs, each continuous posteriorly with a spermoduct which can be expanded by sperm and serve as a kind of seminal vesicle (Weismann 1880). The genital opening of *M. brachiata* has a remarkable situation on the flanks of the abdomen proper, near the region where it passes over into the postabdomen, and not so far from the proximal end of the row of feathered setae. This situation, which was checked by scanning electron microscopy, is supposed to be typical of most *Moina* species (Goulden 1968). The proximal situation of the genital opening resembles that in sidids, which have a ventrolateral male genital opening behind the 6th pair of legs. However, *Moina macrocopa* and *M. belli* Gurney have a distal genital opening ventral to the base of the

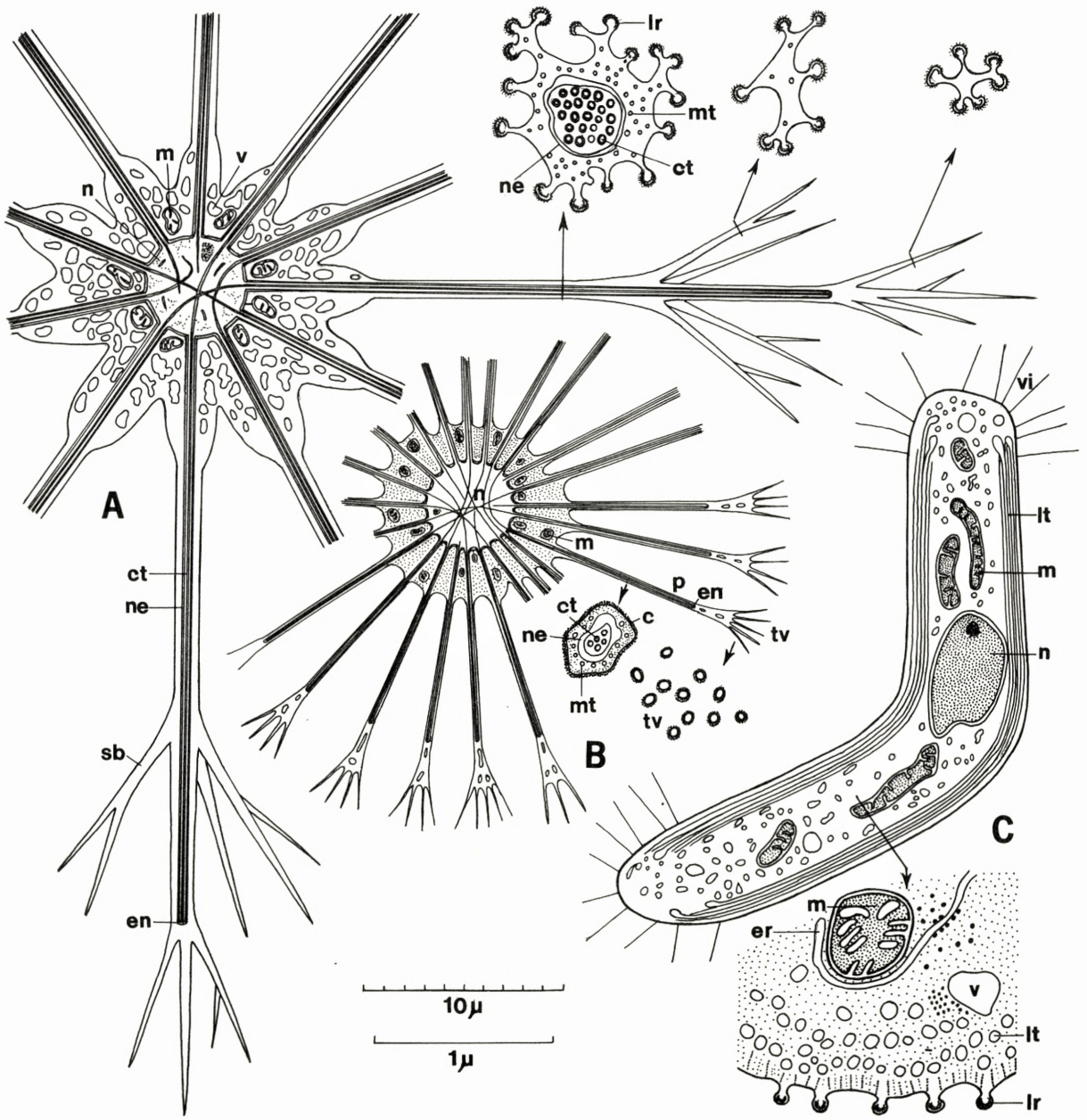

Fig. 5.

Diagrams of the spermatozoa of three *Moina* species, based upon light microscopy and electron microscopy. See Pls. 11 and 12. The 10 μ scale is for the three main figures, the 1 μ scale is for the details.

A. *Moina brachiata* (Jurine). B. *M. micrura* Kurtz. C. *M. macrocopa* (Straus).

Legends: ct = chromatin tubules, en = end of nuclear diverticulum, er = endoplasmic reticulum, lr = longitudinal ribs, lt = longitudinal tubules, m = mitochondria, mt = microtubules, n = nucleus, ne = nuclear envelope, p = pseudopodium, sb = side branches, tv = terminal villi, v = vacuoles, vi = villi.

abdominal claw, as in the macrothricids. At any rate, all moinids differ strikingly in this respect from the daphniids, which have a distal genital opening dorsal to the base of the abdominal claw.

The walls of the testicle are thin, covered by a low, endothelium-like cell layer, except anteriorly, where patches of high germinal epithelium are found. This contains clusters of germinal cells in the interstitia, and small clusters of spermatids are often seen bulging into the testicular lumen. Weismann (1880) believed that these clusters of spermatids develop inside large, clear cells called spermatoblasts, but this could not be confirmed in the present investigation.

In the three species examined maturation of the spermatids is of the lumenal type (see Fig. 2B). As described by Weismann, the young spermatids disperse in the testicle and become attached to the walls; they are filled with ER and ribosomes, so they appear almost black in light microscopical preparations stained with toluidine blue (Pl. 11:54). The spermatids of *M. macrocopa* remain attached with one end until they are rod-shaped and almost mature, whereas those of *M. brachiata* and *M. micrura* are detached before development of pseudopodia and reduction of the ribosomes (see Pl. 11:54).

Mature spermatozoa of the three species are so complicated and different, that each species must be dealt with separately.

Moina brachiata. The mature spermatozoa have a rounded cell body, 10 – 15 μ in diameter, and a corona of radiating, branched pseudopodia, about 35 μ long (Fig. 5A). The total diameter, pseudopodia included, is therefore 80 – 90 μ.

The large, spherical *nucleus* (ca. 5 μ) contains one or a few nucleolus-like clumps of dark material. The nuclear membrane is evaginated into numerous narrow cylindrical processes over its entire surface (Fig. 5A, Pls. 11, 12). Each of these nuclear diverticula contains a bundle of straight tubules which have an outer diameter of 400 – 600 Å and 100 – 150 Å thick walls. Development indicates that the tubules are formed by condensation of chromatin during spermatogenesis. Proximally the bundles of chromatin tubules continue through the nucleus, following a straight or arched course (Pl. 11:55). No distinct endings of tubules were ever seen inside the nucleus, so it it is supposed that most bundles pass through the nucleus and into a nuclear diverticulum on the opposite side.

The *plasm of the cell body* is strongly vacuolated, with large empty sacs delimited by smooth unit membranes. Large mitochondria with numerous cristae and dark matrix are present in a single layer next to the nuclear envelope. The surface of the central cell body is mainly made up of the densely set, conelike bases of the pseudopodia. These basal cones have a normal cell membrane and lack the ridges characteristic of the peripheral parts of the pseudopodia.

Each *pseudopodium* has a fairly thick straight shaft, about 15 μ long, which extends from the conelike base to the first branching-point (Fig. 5A). Light microscopy of epon sections shows that several side branches are emitted at each level, both at this proximal branching-point and at the distal (terminal) one. Also the side branches are straight and may have a few smaller secondary branches.

A cross section through the shaft of the pseudopodium shows the structure of its axis (Pl. 12:59): a central bundle of chromatin tubules surrounded by the two membranes of the nuclear envelope. Sometimes there are two or three bundles of tubules which, then, share a common outer nuclear membrane but have separate inner ones. The continuity of these nuclear membranes with those of the central nucleus can be seen in longitudinal sections through the base of a nuclear diverticulum (Pl. 11:58).

The plasm outside the nuclear cylinder in the pseudopodium shaft usually contains longitudinal microtubules and, rarely, a few sacs of smooth ER. The microtubules are of the common 200-250 Å class and are thus much thinner than the chroma-

tin tubules, which measure 400 – 600 Å in diameter (Pl. 12:59).

The cell membrane of the shaft is elevated into 10 – 20 longitudinal ridges of characteristic appearance (Pl. 12:59). The edge of each ridge is expanded to form an almost cylindrical, rodlike structure. This cylindrical edge of the ridge appears dark, for its cell membrane is supported by an underlying, probably amorphous, plate of similar thickness as a unit membrane, and there is a distinct layer of filamentous coat substance on the outside. Frequent observations of incompletely separated ridges of this kind indicate that they branch.

The axis (nuclear diverticulum) is restricted to the main stem of each pseudopodium. Its blunt end, where the chromatin tubules terminate in contact with the inner nuclear membrane of the end wall, has been seen several times in EM sections. The exact location of this blind ending of the diverticulum was not definitely established, but it must be close to the terminal ramification of each pseudopodium. It remains certain that the nuclear diverticulum is unbranched and restricted to the main shaft of each pseudopodium, and that the side branches and terminal ramifications lack an axis.

The ridges on the surface of the side branches and terminal branches are identical with those of the thick stem but are fewer in number (Pl. 12:59, inset). The extreme tips of these branches appear to have the thickened and coated type of cell membrane all the way round and are circular or symmetrically lobate in cross sections. The ridges appear to fuse distally and form the tip.

Maturation of the spermatids implies considerable increase in size. Early spermatids before or just after settling on the wall of the testicle are 7-8 μ in diameter. The spherical, free spermatids in the testicular fluid are about 15 μ. The central cell body of mature spermatozoa is 10 – 15 μ, but if the corona of radiating pseudopodia is included, the spermatozoa occupy a space about 80 – 90 μ across.

The cytological differentiation begins in the attached spermatids with development of a dense system of branched ergastoplasmic tubules and of abundant ribosomes throughout the cytoplasm. Some of the tubules appear to expand and form the large vacuoles of the mature spermatozoon, whereas the number of ribosomes is reduced in the late spermatids which lie free in the fluid.

Development of the chromatin tubules starts after the spermatids have been liberated into the lumen. First a number of indistinct primordial bundles are seen criss-crossing through the fairly dense nucleoplasm (Pl. 11:57). Each bundle consists of several filaments, which are about 100 Å thick and have a distinct cross-striation with a periodicity of 100 Å. No doubt these cross-striated filaments are transformed into chromatin tubules, but details could not be followed, although tubules and cross-striated filaments exist for some time side by side in the same bundles (see Pl. 11:57, inset). During the formation of the tubules there is a strong decrease in the density of the nucleoplasm, which, in the mature spermatozoa, is almost clear, with scattered filaments and clumps of stainable matter.

The primordial filament bundles in the nucleus end at the nuclear envelope, which is pushed out to form a small cone-shaped diverticulum at the place of contact (Pl. 11:57). It is therefore probable that the bundles of filaments (later tubules) are mechanically active in pushing out the nuclear diverticula.

Moina micrura. (Fig. 5B, Pls. 11:56, 12:60). Mature spermatozoa of *M. micrura* consist of a central cell body, 6 – 8 μ in diameter, and a corona of numerous pseudopodia which are about 10 – 12 μ long. The entire spermatozoon, including the pseudopodia, is thus 30 – 35 μ in diameter. As in *M. brachiata* the nucleus emits numerous cylindrical diverticula, each containing chromatin tubules which end as axial rods in the pseudopodia.

The general type of spermatozoon is thus the same as in *M. brachiata*, but those of *M. micrura* are considerably smaller and differ in many details.

The *nucleus*, about 3.5 μ, is spherical and emits narrow cylindrical diverticula, which appear to be much more numerous than in *M. brachiata* although it in difficult to make an exact comparison (see Pl. 11:55 and 56). The nuclear diverticula which form the axial rods of the pseudopodia are not so thick and contain fewer (3 – 11) chromatin tubules than those of *M. brachiata* (up to 40, usually 10 – 30 tubules in one diverticulum). The chromatin tubules themselves are of similar thickness in the two species.

The *plasm of the cell body* is not vacuolated as in *M. brachiata* and contains numerous small mitochondria in the spaces between the numerous nuclear diverticula (Pl. 11:56).

The *pseudopodia* of *M. micrura* are simple thin rods without side branches and end with a peculiar "terminal brush" (Fig. 5B, Pl. 12:60). The main stem contains a nuclear diverticulum as an axis and scattered microtubules are present in the plasm. Its surface has no ribs like those typical of *M. brachiata*, but an almost continuous layer of dark matter is attached to the inner side of the cell membrane all the way round. The nuclear diverticulum in the centre ends about 1 μ proximal to the "terminal brush". The distal end of the pseudopodium expands before reaching the flat end surface, to which several pointed, finger-like villi are attached (Pl. 12:60, inset). These villi appear very dark, having a distinct coat outside and some amorphous dark matter inside the plasma membrane.

Moina micrura is thus similar to *M. brachiata* with regard to the presence and structure of the pseudopodial axis but is completely different with regard to all other details of pseudopodial structure.

Moina macrocopa (Fig. 5C, Pl. 12:61, 62). The spermatozoa of *M. macrocopa* are completely different from those of the preceding two species. They are somewhat flattened rods, bent in the middle so the two equal arms form an angle of about 135° (Fig. 5C, Pl. 12:61, inset). The distance from end to end is 40 μ, and the thickness of the rod is 4 $\times$ 6 μ. Both ends are covered by sparse, hairlike villi, whereas the main middle portion has some low, longitudinal ridges not visible in the light microscope.

The *nucleus* is situated in or near the bend of the spermatozoon. It has an ordinary structure and lacks all the diverticula and chromatin tubules which are so characteristic of the preceding species.

The *cytoplasm* is remarkably vesicular, containing numerous smooth-walled vacuoles, probably smooth ER, and normal mitochondria, at least some of which are long rods (Pl. 12:61, 62). The peripheral plasm under the cell membrane is differentiated into a kind of cortex, which covers the main, middle part of the spermatozoon but is absent from the end region, where the villi are attached. The cortex is characterized by two fairly regular layers of longitudinal tubules (deep cortex), by a more superficial zone of non-tubular filaments (outer cortex) and by the longitudinal ridges of the surface (Fig. 5C, Pl. 12: 61, 62). The tubules of the deep cortex are about 800 Å thick and somewhat irregular both in cross and longitudinal sections (Pl. 12:62). They can hardly be compared with ordinary microtubules of the 250 Å class, which appear to be lacking in these spermatozoa. The filaments in the outer cortex are longitudinal and in cross sections appear to form more or less distinct radial septa.

The longitudinal ridges on the surface have an expanded, roughly cylindrical margin (Pl. 12:62). The cell membrane of the cylinder is supported on the plasmatic side by a thin lamella of dark, amorphous matter. These ridges are therefore similar to those seen on the pseudopodia of *Moina brachiata* spermatozoa, but I have not seen the characteristic coat typical of the latter.

In the *end regions* the vesicular plasm extends to the cell-membrane and there is no cortex, nor

superficial ridges. The hairlike villi, which are scattered over the surface, are about 3 μ long and only 600 Å thick. They contain a dark, amorphous axial rod, about 150 Å thick. The rod could not be followed into the plasm but appears to end at the base of the villus.

Maturation. Differentiation of the spermatozoa of *M. macrocopa* takes place while the spermatids are attached to the wall of the testicle. The originally rounded cells become elongated, one end remaining in contact with the cells of the testicle wall. Also the cortical structures and the hairlike villi develop before the spermatid is detached. This is a clear difference from the course of development of spermatids of *M. brachiata*, which are detached before the final maturation of most organelles begins.

Comments on the spermatozoa of Moina. The spermatozoa of the three examined species of *Moina* are by far the most complicated and strange variants seen in the Branchiopoda. Actually they do not seem to fit at all into the anomopod system. But light microscopy of the spermatozoa of other *Moina* species, summarized by Goulden (1968), suggests a reasonable explanation.

According to Goulden's account, most *Moina* species, including the presumed primitive types such as *M. australiensis* Sars and *M. tenuicornis* Sars, have small spherical spermatozoa. The exact size is usually unknown, but those of *M. wierzejskii* Richard are drawn by Goulden (1968, fig. 19) with a diameter of about 5 μ. Larger spermatozoa are found only in discrete subgroups within the genus. Thus, the spectacular and large spermatozoa with a corona of radiating pseudopodia such as found in *M. brachiata* and *M. micrura* are present also in *M. hartwigi* Weltner, which is referred to the same sub-group. The bent, rodlike spermatozoa found in *M. macrocopa* are reported also in *M. belli* Gurney, but appear somewhat smaller in the latter species (Goulden 1968, fig. 34), and some large spherical spermatozoa with about 20 μ diameter are reported from *M. hutchinsoni* Brehm (Goulden, 1968, fig. 38).

This all indicates that the evolution of the strange and large spermatozoa in the three examined species has taken place within the genus *Moina*, and that the starting point in ancestral moinids was a small spherical cell, perhaps of more normal anomopod type. Goulden (1968) also used the spermatological characters to support his phylogenetical discussion. However, it is obvious from the present investigation that ultrastructural studies of spermatozoa of several species can reveal numerous details which can be used to strengthen the phylogenetical conclusions. It is, for instance, quite evident that *M. brachiata* and *M. micrura* are more closely related to each other than to *M. macrocopa*, for they share the radiating pseudopodia supported by nuclear diverticula which contain chromatin tubules, and such structures are not present in *M. macrocopa*. It would also be completely unrealistic to suppose that these complicated structures, never seen in other animal cells, have arisen independently in each of the two species.

Family Bosminidae

Bosmina (Bosmina) longirostris (O. F. Müller). Søborg, Copenhagen, 18. X. 72, ♂♂, 1 % Os. – Ermelunden, near Copenhagen, 19. X. 72, ♂♂, 1 % Os.
B. (Eubosmina) longispina Leydig. Lake Fiolen, Sm., Sweden, 15. XI. 74, ♂♂, 3-A.
B. (E.) coregoni Baird. Lake Värmen, Sm., Sweden, 25. X. 73, ♂♂, 3-A. – Lake Fenen, Sm., Sweden, 3. X. 74, ♂♂, 3-A.

The testicles of bosminids are of the common anomopod (daphnian) type, and maturation of the spermatids takes place inside "private" vacuoles in the large nutritive cells of the testis in all three species (see Fig. 2C).

The mature spermatozoa are small, amoeba-like, with irregular, broad, pseudopodia-like lobes. (Pl. 13:63). The spermatozoa of *Bosmina longirostris* are elongate (3 × 1.4 μ) and are distinctly larger than those of *B. longispina* and

B. coregoni, which are 1.7-2 μ in diameter. The compact nucleus is somewhat elongate (max. length 1.2 μ) in *B. longirostris*, whereas the other two species have rounded nuclei (diam. 0.9 μ). The margin of the nucleus may be invaginated, and there are often a few lighter spots in the dark nuclear content. The cytoplasm is finely granular, with a few dark bodies and some membrane-bound vesicles, but no other distinct organelles. There is a broad light coat outside the simple cell membrane, visible mainly because it is light in contrast to the heavily stainable (and after fixation hard) sperm fluid.

Development of the spermatozoa has not been studied in detail, but maturation inside "private" vacuoles in the nutritive cells was seen in all species.

Comments on the spermatozoa of Bosmina. The spermatozoa of *Bosmina* are, together with those of *Simocephalus exspinosus* and *Scapholeberis* spp., the smallest seen in branchiopods. In these genera the structure of the spermatozoa is extremely simple, obviously depending on lack of organelles. The spermatozoa of the said genera therefore show a superficial similarity, but this may well be a case of convergence. The difference in shape and size of spermatozoa between *B. longirostris* and the other two species may reflect a difference between the subgenus *Bosmina s. str.* and *Eubosmina*, the latter including *B. longispina* and *B. coregoni*. However, since the spermatozoa are small and difficult to section because of the compact sperm fluid, this may not be a useful method in systematical work with the group.

Family Macrothricidae (gen. Ilyocryptus and Streblocerus)

Material

Ilyocryptus agilis Kurtz. Løg sø, Zealand, 16. VIII. 74, ♂♂, 3-A, 1 % Os.

Streblocerus serricaudatus (Fischer). Hjortesølet, N. of Hillerød, Zealand, 28. IX. 73. Cultivated in laboratory till ♂♂ appeared, fixed 2. IX. and 3. X. 73, 3-A, 1 % Os.

Streblocerus has elongate, sac-like testicles on each side of the intestine, like other anomopods. This may be true also for young males of *Ilyocryptus*, but in the mature males investigated the testicles are extremly voluminous, filling out the entire ventral part of the body and meeting each other at the midline, below the intestine.

The mature spermatozoa of the two species are flat discs, somewhat thicker in the central part, where the nucleus is located (Fig. 6C, Pl. 13:65-66). Those of *Streblocerus* are 3 – 4 μ in diameter × 0.6 μ thick, those of *Ilyocryptus* are 5 × 0.4 μ. The nucleus is central, flattened, with a greatest diameter of about 2.3 μ in both species. The spermatozoa of *Streblocerus* are cytologically simple, containing only a few vesicles and some microtubules in the granular cytoplasm. Those of *Ilyocryptus* contain many spherical mitochondria with dark matrix in a zone peripheral to the flattened nucleus and, outside these, a marginal bundle of about 40 – 50 microtubules. Each of these microtubules is about 250 Å thick. The bundle of microtubules forms an uninterrupted ring all the way round, just inside the margin of the disc. The microtubules are embedded in medium-contrast, slightly granular matter (Pl. 13:67). The cell membrane is uncomplicated and without distinct coat structures.

Spermatogenesis. Unexpectedly, maturation in these two species turned out to be of the cystic type (Fig. 2A) and is thus completely different from that of other anomopods. The clumps of spermatids formed by spermatocyte divisions near the basement membrane remain together in cystlike dilatations of the interstitia of the testicular epithelium (Pl. 14:71). They remain in these cysts until maturation is completed and are not enclosed in "private" vacuoles as in other anomopods. The cyst wall is formed by the surrounding vegetative cells, which also form the wall between the cyst lumen and the testicular lumen. The latter wall becomes extended and thin over the advanced cysts and finally opens, freeing the spermatozoa into the testicular lumen.

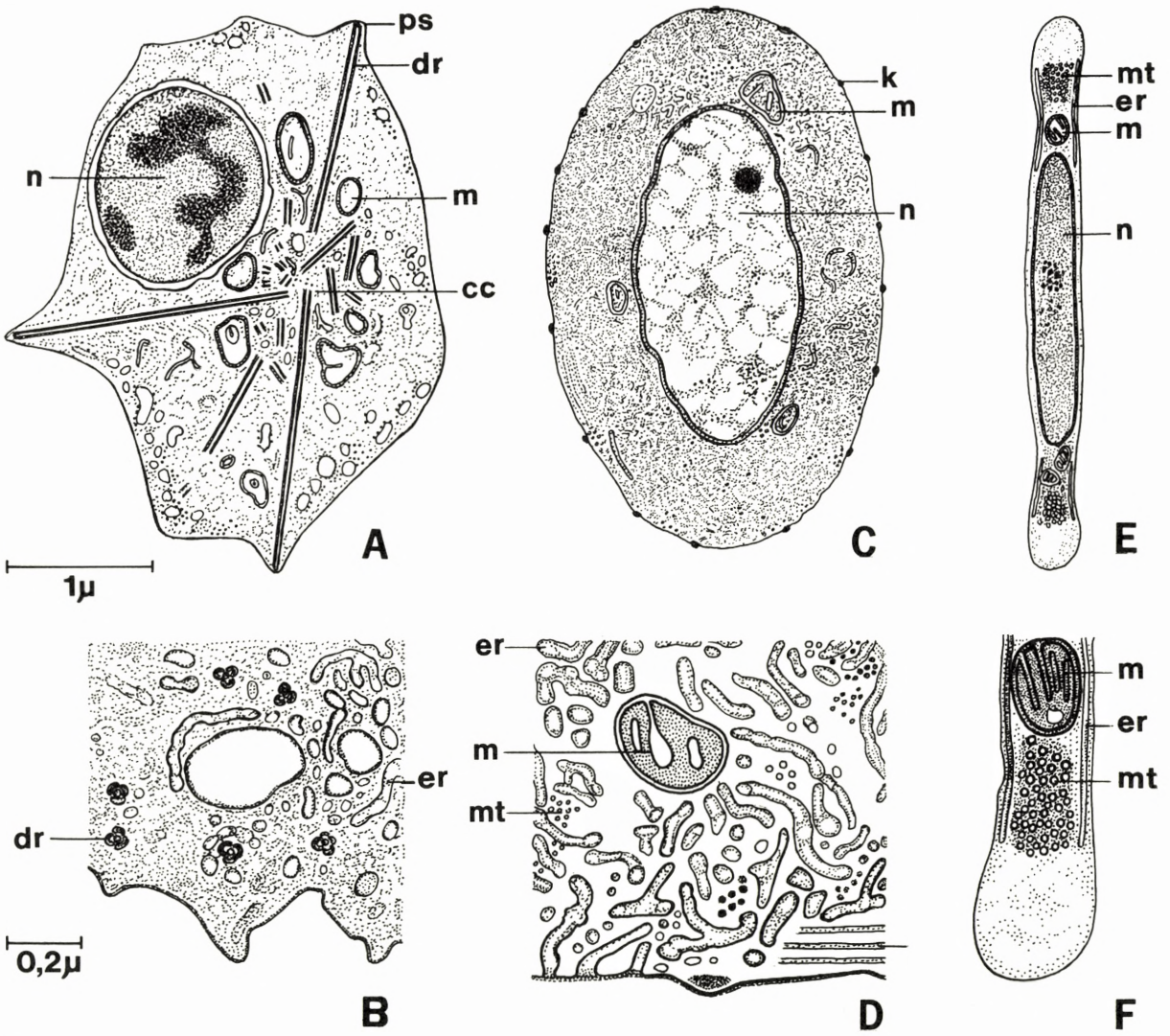

Fig. 6.

Diagrams of spermatozoa of macrothricids: A-B. *Ophryoxus gracilis* G. O. Sars. C-D. *Macrothrix laticornis* (Jurine). E-F. *Ilyocryptus agilis* Kurtz.

Legends: cc = cell centre, dr = dark rods, in B in cross secrion, er = endoplasmic reticulum, k = "knobs' on cell surface in C and D, m = mitochondria, mt = microtubules, in E and F lying in dark matter, n = nucleus, ps = pseudopodium.

The number of spermatozoa formed in each cyst is high, in *Ilycryptus* probably about a hundred.

Comments on the spermatozoa of Ilyocryptus and Streblocerus. The cystic type of spermatogenesis seen in *Streblocerus* and *Ilyocryptus* comes very close to the type seen in Anostraca and *Holopedium*, and is fundamentally different from the vacuolar type seen in typical Anomopoda.

The mature spermatozoa of *Ilyocryptus* and *Streblocerus* are very similar and belong to a type which differs distinctly from types seen in normal anomopods. Sections of the spermatozoa of *Ceriodaphnia laticaudata* are superficially similar

to those of the two macrothricids, but in *C. laticaudata* the spermatozoa are rod-shaped like those of other *Ceriodaphnia* species, not flattened circular discs like those of *Ilyocryptus* and *Streblocerus*.

Family Macrothricidae (Macrothrix)

Material

Macrothrix laticornis (Jurine). Fælledparken, Copenhagen, 18. VIII. 74, cultivated in laboratory till ♂♂ appeared, fixed 2. IX. 74, 3-A, 2 % Os.

Macrothrix has normal anomopod testicles on each side of the intestine.

The spermatozoa are ovoid bodies measuring about 5-6 × 3 μ (Fig. 6B, Pl. 14:68), and are thus somewhat larger than those of the preceding anomopod species. The nucleus is central, rounded or elongate, 3 – 3.5 μ long. In the abundant cytoplasm there is a dense net of irregular, smooth-walled tubules with a diameter of about 330 Å (Pl. 14:69). These tubules form an irregularly branching and bending pattern, and some of them end in close contact with the cell membrane. Whether or not they open out could not be stated with certainty.

In addition, the cytoplasm contains a few mitochondria with a dark matrix, one or a few large, dark bodies, and some glycogen granules, which may form distinct clusters near the nucleus. In the peripheral cytoplasm under the cell membrane there are numerous straight microtubules of the common 250 Å class. These have a mainly longitudinal course and are parallel to the cell membrane.

The cell membrane shows some regularly spaced small knobs with a thickened cell membrane, supported by an underlying disc of dark substance (Pl. 14:69). There is no discernable coat on the outer side of the cell membrane.

Spermatogenesis is of the common anomopod type: individual spermatids are enclosed in vacuoles in the nutritive cells (Pl. 14:70).

Comments on the spermatozoa of Macrothrix. Although the spermatozoa of *Macrothrix* agree with typical anomopod spermatozoa with regard to size and type of spermatogenesis, they are distinctly different with regard to their cytological structure. The dense system of branching tubules in the plasm is similar to that seen in some onychopods and Anostraca, but is hardly paralleled within anomopods. The knobs on the cell surface have a remote resemblance to the "plaques" in some Anostraca. Also, there is very little similarity between *Macrothrix* spermatozoa and those of other investigated macrothricids.

Family Macrothricidae (Ophryoxus)

Material

Ophryxus gracilis G. O. Sars. Tenhultsjön, Sm., Sweden, 10. X. 73, 1 ♂, 3-A, (coll. W. Adolfson). – Lake Fenen, Sm., Sweden, 25. X. 73, 3-A, 1 ♂.

The testicles are of the daphnian type, with thick walls, and the final maturation of spermatids takes place inside "private" vacuoles in the plasm of giant nutritive cells (Pl. 13:64).

The mature spermatozoa are approximately isodiammetrical, about 4.5 μ across, and have a distinctly polar structure. The nucleus marks this polarity, lying near the cell membrane (Fig. 6A, Pl. 13:64). It is approximately spherical, with a diameter of 2 μ, and contains some irregular chromatin blocks suspended in a lighter nucleoplasm.

The cytoplasm contains a number of vacuoles, about 0.3 μ in diameter, with two limiting membranes. These are interpreted as vestiges of the mitochondria, which are well developed and typical only in immature spermatids. Some typical ER associated with some ribosomes is present in the free spermatozoa of one of the two specimens, but is more rare in the other.

The typical feature of these spermatozoa is a cell centre, containing the two centrioles and numerous tubules and rods (Pl. 13:64). It lies in

the middle of the cytoplasm, in close contact with the inner surface of the eccentric nucleus. The rods, about 900 Å thick, radiate out to the cell membrane and produce slight, conical elevations of the cell surface. Each rod arises around a 250 Å microtubule, which is distinct in immature cells. When mature, the rods have a triradiate pattern in cross sections, with several flattened tubules attached to a central microtubule with circular cross section (Fig. 6B, Pl. 13:64, inset). In most cases the central microtubule appears to have degenerated and the structure is too dense to be resolved in detail. A myeloid body lying close to the nucleus was seen in some mature spermatozoa. The cell membrane is simple and no particular coat structure was observed.

Spermatogenesis begins with the development of the cell centre with centrioles and radiating rods, while the nucleus is dislocated to near the cell surface. This takes place before the spermatids have been engulfed by nutritive cells. The final maturation takes place in vacuoles inside these nutritive cells (Pl. 13:64).

Comments on the structure of Ophryoxus spermatozoa. With regard to the spermatogenesis and structure of the spermatozoa, *Ophryoxus* is a typical anomopod. The mature spermatozoa with their eccentric nucleus, the cell centre and the radiating rods are almost indistinguishable from the spermatozoa of the Aloninae. The similarity extends to some details, e.g. the development of the radiating rods around typical 250 Å microtubules which tend to disappear in the mature sperm. A cell centre, radiating rods, and similar eccentricity are also found in the genus *Simocephalus* among the Daphniidae.

From a spermatological point of view, *Ophryoxus* is completely different from the other investigated macrothricids. *Macrothrix* has the anomopodan type of spermatogenesis but its spermatozoa are of a type unknown in typical anomopods. *Iliocryptus* and *Streblocerus* differ fundamentally from anomopods, and also from *Ophryoxus*, both with regard to type of spermatogenesis and structure of spermatozoa.

The spermatological features thus indicate that *Ophryoxus* is much more closely related to the typical anomopods than the other investigated macrothricids. The relatively isolated situation of the genus *Ophryoxus* within the Macrothricidae, and its closer relationship to daphniids and chydorids, is indicated also by external morphology (Sergeev 1970, 1973, Fryer 1974) and by the presence (in contrast to other macrothricids) of midgut coeca.

Subfamily Eurycercinae

Material

Eurycercus lamellatus (O. F. Müller). Lake Värmen, Sm., Sweden, 15. X. 73, ♂♂, 3-A. – Teglgårdssøen, Hillerød, Zealand, 24. X. 73, ♂♂, 3-A.

The testicles are of the common anomopod type: elongate sacs on each side of the intestine. Maturation of spermatids takes place in "private" vacuoles in the nutritive cells.

The mature spermatozoa are rounded, 2.5 – 2.7 μ in diameter, with short, blunt pseudopodia, and enveloped in a thick, cystlike coat (Pl. 15:72). The nucleus is rounded, 1.5 μ in diameter, and has an eccentric position near the cell wall. The chromatin is condensed to a large caryosome, filling most of the space inside the nuclear membrane. The peripheral layer of cytoplasm, including the pseudopodia, is granular with moderate contrast. The central parts of the cell contain some round, fairly atypical mitochondria, and a mixture of dark granules and densely packed membranes. Less condensed, probably younger, spermatozoa show these membranes more clearly, have more normal mitochondria with typical cristae, and may contain distinct remnants of the rods, which support the pseudopodia of spermatids.

The coat is approximately 0.6 μ thick and has an even, spherical shape. It consists of 1) a light,

seemingly empty space near the cell membrane, 2) a thick medium-contrast wall (0.4 μ) with faint radial striation, and 3) two thin external zones, the outermost one looking almost empty. The pseudopodia extend irregularly into the innermost, empty zone only.

Spermatogenesis. The meiotic divisions result in clusters of nondifferentiated spermatids lying in the deeper parts of the thick testicular wall, between the basal parts of the large nutritive cells. Cytogenesis of the spermatids proceeds far in these clusters, and only the final stages of spermatids are found enclosed in vacuoles in the vegetative cells. Pictures interpreted as such vacuoles in the process of opening and liberating spermatozoa into the lumen were often seen.

In the clusters of spermatids the nucleus tends to approach one of the cell walls and a cell centre develops in the middle of the cell body. This centre contains the centrioles, the mitochondria and the inner ends of some dark, radiating rods. In the periphery these rods push the cell membrane out into long, pointed pseudopodia (Pl. 15:73). The dark rods are formed around radiating bundles of 2-3 microtubules which have a diameter of 250 Å. During development some dark matter is attached to the rods, so the composite rod becomes about 900 Å thick. At the height of their development these rods contain 2-3 light, longitudinal canals, which represent the lumens of the microtubules, embedded in plentiful dark matter (Pl. 15:73, inset). This part of the development takes place when the spermatids lie together in clusters, and the pseudopodia extend between neighbouring spermatids.

The next stage implies enclosure of the spermatids in "private" vacuoles in the nutritive cells. During this process the pseudopodia are withdrawn and their dark axial rods are reduced to the remnants found in the free spermatozoa. Within the vacuole the cell is condensed to about half its original diameter, and the dark granules and packed membranes appear in the region of the cell centre.

The coat does not form until the spermatozoa are mature and are liberated into the lumen, but details could not be made out in the material at hand.

Comments on the spermatozoa of Eurycercus. With regard to almost every feature, the spermatozoa of *Eurycercus lamellatus* agree with those of the alonine chydorids. Among these, *Camptocercus rectirostris* and *Alona costata* have spermatozoa with a thick, spherical coat and contracted pseudopodia like those of *Eurycercus*. However, in *Eurycercus* the axial rods of the pseudopodia are reduced in the mature state, whereas the alonine chydorids retain the axial rods.

The general pattern seen in *Eurycercus*, particularly the eccentric nucleus, the cell centre, and the radiating rods, is a general feature in the Aloninae but is present in more or less modified forms also in the macrothricid *Ophryoxus* and the daphniid *Simocephalus*.

Subfamily Aloninae

Material

Camptocercus rectirostris Schoedler. Store Grib Sø, Zealand, 24. X. 74, ♂♂, 2 % Os.

Acroperus elongatus (Sars). Tenhultsjön, Sm., Sweden, 10. X. 73, ♂♂, 3-A (coll. W. Adolfson). – Möckeln, Sm., Sweden, 25. X. 73, ♂♂, 3-A.

Acroperus harpae (Baird). Hultsjön, Sm., Sweden, 2. XI. 72, ♂♂, 1 % Os. – Tenhultsjön, Sm., Sweden, 10. X. 73, ♂♂, 3-A, (coll. W. Adolfson). – Hornsviken, Öl., Sweden, 5. X. 74, ♂♂, 3-A. – Lille Grib Sø, Zealand, 24. X. 74, ♂♂, 3-A.

Alona costata Sars. Hornsviken, Öl., Sweden, 5. X. 74, 1 ♂, 3-A.

Alona quadrangularis (O. F. Müller). Lyngby Sø, Zealand, 19. X. 72, ♂♂, 1 % Os.

Alona affinis (Leydig). Greenland, formalin material, ♂♂ (coll. U. Røen). – Möckeln, Sm., Sweden, 25. X. 73, ♂♂, 3-A.

Rhynchotalona falcata (Sars). Store Grib Sø, Zealand, 24. X. 74, 2♂♂, 2 % Os.

Leydigia acanthoceroides (Fischer). Hillerød, Zealand, 5. IX. 73, 1♂, 1 % Os.

Graptoleberis testudinaria (Fischer). Store Grib Sø, Zealand, 24. X. 74, ♂♂, 3-A, 2 % Os.

The testicles of all species are of the common anomopod type, one elongate sac on each side of the intestine. Spermatogenesis, checked in all investigated species, is of the vacuolar type (Fig. 2C).

The morphology of the mature spermatozoa is remarkably homogeneous within the subfamily Aloninae. *Leydigia acanthoceroides* is the most extreme variant and will therefore be dealt with separately.

Mature spermatozoa. The cell is approximately isodiammetrical or somewhat elongate, in some species (*Camptocercus rectirostris* and *Alona costata*) with an almost even external contour, in the other species lobate or stellate with large, broad-based pseudopodial processes (Pl. 15:74-76, Pl. 16:82). The spermatozoa are small, the largest diameter being about 2-3 μ in most species. Some elongate and slender spermatozoa in *Acroperus elongatus* and *Alona affinis* may reach a length of 4 μ. The nucleus is eccentrically placed near the cell membrane. On its opposite side, in the middle of the plasm, is a cell centre from which numerous dark rods radiate to the periphery of the cell, ususally ending as axial rods in the pseudopodia-like processes. The rods arise as simple, straight, 250 Å microtubules in the spermatids but end as 700 – 900 Å thick dark rods by apposition of dark-staining matter (Pl. 16:79-81). In the rods of mature spermatozoa the original microtubule may become invisible, particularly in the peripheral part of the cell. The end of each rod is directly in contact with the cell membrane at the top of the pseudopodium.

The dark rods are surrounded by wide light spaces, which contain an open granulation. In several species these granules are so regularly spaced that the structure appears to be crystalline. Surrounding the light spaces are dark-staining areas containing strongly contracted mitochondria, closely packed membranes and variable amounts of granular, dark-staining matter.

The nucleus is rounded or elongate and strongly condensed (diameter only 1 – 1.5 μ). The chromatin is condensed to a dark granular body filling most of the space inside the nuclear membranes. Near the nucleus there is often a large myeloid body consisting of concentric membranes.

The coat on the surface is variable, very thick in the spherical spermatozoa of *Alona costata* and *Camptocercus rectirostris* (0.37 and 0.3 μ, resp.). In these two species a light outer zone is distinct from an inner darker zone with radial striation. In *Acroperus elongatus* the coat is similar but thinner (0.25 μ), and a bilaminate coat with an inner darker layer is also present in *Graptoleberis testudinaria* and *Rhynchotalona falcata* (0.2 and 0.18 μ, resp.). *Acroperus harpae* has a unilaminate coat (0.17 μ). *Alona affinis* has a very light coat (0.2 μ) with faint indications of several layers, and *Alona quadrangularis* has a very thin (0.04 μ) dark layer next to the cell membrane and a thick, light zone with ill-defined contours outside this, visible mainly as an empty space in the dense testicular fluid.

This standard description fits the majority of investigated species, but some variation was noted. It has already been mentioned that *Camptocercus rectirostris* and *Alona costata* have very thick, capsulelike coats and contracted pseudopodia. The same two species have also very little contrast between the light spaces around the rods and the dark cytoplasm with mitochondria, membranes, etc.

The most extreme diviation from the common alonine type was found in *Leydigia acanthoceroides* (Pl. 15:77). It differs in three respects: 1) there are no light spaces around the radial rods, 2) the rods are simple 250 Å microtubules without attached matter, and 3) there is no distinct coat.

Spermatogenesis was studied in its final stages in all investigated species. Cytodifferentiation begins in the young spermatids, which lie in clusters deep in the testicular wall. The nucleus moves to the cell wall and the two centrioles become located near its inward side, in the prospective

cell centre. Numerous 250 Å microtubules grow out from this centre to the cell membrane.

The final maturation takes place after the spermatids have been enclosed in "private" vacuoles in the nutrive cells (Pl. 16:78). It implies considerable condensation of the cytoplasm and the nucleus, development of pseudopodia, strong contraction of the mitochondria, packing of some ER membranes into dark bodies together with granular material, and apposition of dark matter on the radiating microtubules. The coat is formed when the mature spermatozoa are liberated into the lumen, for it is always present on the free spermatozoa but not (with rare exceptions, see below) on spermatids inside vacuoles (Pl. 16:78). In *Acroperus harpae* some partly opened vacuoles were seen with the spermatids still inside. These had developed an incomplete coat.

Comments on the spermatozoa of the Aloninae. The spermatozoa within this subfamily are so similar and characteristic that they strongly support the present classification. The eccentric nucleus and the radial rods formed around microtubules and ending as axes in the pseudopodia are common features in all species but are not completely unique, for the same structures are found in *Eurycercus lamellatus*, in the daphniid *Simocephalus* spp., in the macrothricid *Ophryxus gracilis*, and, in a modified form, in some Chydorines. The light spaces around the rods, so characteristic of most alonine genera investigated, have not been seen in other Cladocera and should therfore be regarded as a synapomorphic feature in the Aloninae. It is interesting that *Camptocercus rectirostris* and *Alona costata* approach *Eurycercus* with regard to the poor distinction of these spaces, with regard to the retraction of the pseudopodia, and with regard to the strong, capsulelike coat.

Leydigia acanthoceroides differs remarkably from the other Aloninae in the absence of a coat, absence of the light spaces around the rods, and absence of dark matter around the microtubules. This might indicate that it has a more isolated position within the subfamily. It may be significant that this genus differs from the other Aloninae also in an important external feature: the development of a tube-shaped penis between the posterior claws.

Subfamily Chydorinae

Material

Peracantha truncata (O. F. Müller). Lyngby Sø, Zealand, 19. X. 72, ♂♂, 1 % Os. – Lyngby Sø, Zealand, 12. X. 73, ♂♂, 3-A, – Tenhultsjön, Sm., Sweden, 10. X. 73, ♂♂, 3-A (coll. W. Adolfson). – Teglgårdssøen, Hillerød, Zealand, 7. X. 73, 2 ♂♂, 3-A. – Hornsviken, Öl., Sweden, 5. X. 74, ♂♂, 3-A.

Pleuroxus uncinatus Baird. Teglgårdssøen, Hillerød, Zealand, 17. X. 74, ♂♂, 3-A, 2 % Os.

Chydorus sphaericus (O. F. Müller). Lyngby Sø, Zealand, 19. X. 72, ♂♂, 1 % Os. – Hultsjön, Sm., Sweden, 2. XI. 72, 2 ♂♂, 1 % Os.

Disparalona rostrata (Koch). Lyngby Sø, Zealand, 19. X. 72, ♂♂, 1 % Os.

The testicles are sac-like and spermatogenesis is of the vacuolar type (Fig. 2C), as in other anomopods.

The mature spermatozoa are very variable within this subfamily, so each genus has to be treated separately.

Peracantha truncata. The spermatozoa are about 5 μ large, roughly isodiammetrical, with a central nucleus and broad, flat lobes all the way round (Pl. 17:86). The moderately condensed nucleus is surrounded by a body of normal, fairly dense cytoplasm with some contracted mitochondria and some smooth ER. The periphery of the cell, particularly within the broad lobes, is occupied by very light, seemingly empty spaces, which are transversed by radially oriented microtubules. These light spaces are not delimited from the normal plasm by any membrane and at the first glance would certainly be interpreted as fixation artifacts. However, the spaces are constantly present irrespective of the fixation used in animals from 5 different samples. Moreover,

the other structures, e.g., the cell membrane on the surface outside the spaces, appears unbroken and well fixed in the specimens. Finally, the development of the spaces can be followed step by step in the spermatids (Pl. 17:87).

The surface of the lobules carries numerous short, rodlike pseudopodia, each of which is filled with medium-contrast matter and contains a single 250 Å microtubule as an axis (Pl. 17:86, inset). Inwards these microtubules extend through the light spaces and appear to end in the denser plasm around the nucleus.

Spermatogenesis. The final maturation of the spermatids takes place in separate vacuoles in the giant nutritive cells. The cells do not change much until just before being liberated, when narrow light spaces appear just under the cell membrane (Pl. 17:87). These light clefts are from their origin transversed by radial microtubules, which are in contact with the even, uncomplicated cell membrane. The short, rodlike pseudopodia are probably formed by induction from these microtubules but do not appear until after the spermatid has been liberated into the lumen.

Pleuroxus uncinatus. The spermatozoa are about 3.3 μ, irregularly rounded, with a central nucleus and more or less pointed pseudopodia all the way round (Pl. 17:84). Each pseudopodium contains an axial microtubule, and the terminal part of the pseudopodium is filled with fairly dark, finely granular matter. The microtubules continue inwards to the level of the nuclear membrane. Some irregular light spaces, sometimes appearing around the microtubules, could be compared with the light spaces around rods in the Aloninae but are so variable and atypical that the homology appears doubtful.

The cytoplasm has moderate contrast, contains fairly normal mitochondria with distinct cristae and often some bodies consisting of dark-walled, anastomosing tubules of unknown significance.

Spermatogenesis inside individual vacuoles in the nutritive cells involves development of radial microtubules of the 250 Å class. They pass from the nuclear membrane to the cell membrane. Just before the spermatozoon is liberated, some dense matter is deposited as one or two large clumps around the peripheral part of each microtubule. The pseudopodia develop during liberation of the spermatozoa into the lumen, and the dark clumps disappear simultaneously, probably contributing to the dark content in the peripheral part of the pseudopodia.

Chydorus sphaericus. The spermatozoa are 3-5 μ in diameter, irregularly lobate with blunt, complicated, often branched processes (Pl. 17:85). A thick, rather dark coat fills out all spaces between the lobelike pseudopodia, so the external surface is comparatively even. The nucleus is round or elongate with a few large chromatin blocks. The protoplasm contains many small mitochondria with cristae but no other prominent organelles. The coat has a thin (0.04 μ light zone next to the cell membrane whereas the thick outer layer (0.2 – 0.5 μ) is dark.

Spermatogenesis. Maturation takes place inside vacuoles in the nutritive cells and involves only minor changes: some condensation of nucleus, reduction of cell size, and disappearance of the ribosomes. Pseudopodia and coat do not develop until the spermatid is about to be liberated. Microtubules were not observed during spermatogenesis.

Disparalona rostrata. The entire cell is about 1.5 – 3 μ in diameter, strongly irregular in shape and covered with irregularly branched pseudopodial processes (Pl. 16:83). The nucleus is moderately condensed, up to 2 μ long. The cytoplasm contains some well-preserved mitochondria with cristae, often a myeloid body, and often one or two dark bodies of anastomosing tubules (like those of *Pleuroxus)*. The most characteristic feature is the coat, which has the appearance of thin filaments, these filaments are attached

to the surface of the cell and extend into the surrounding fluid like long, irregular tufts. The filaments are about 60 Å thick and up to 0.5 μ long. Each filament shows an irregular cross-banding which may be interpreted as a coil with 120 Å periodicity.

Spermatogenesis. Maturation of spermatids inside individual vacuoles in the nutritive cells was verified in the few spermatids observed in the material. The pseudopodia and the remarkable coat do not develop until the spermatozoa are liberated, for some cells in the testicular fluid have these structures poorly developed, and a single late spermatid inside a vacuole had just begun to form pseudopodia but lacked a coat.

Comments on the chydorine spermatozoa. In contrast to the Aloninae, the chydorine Cladocera show great diversity with regard to their spermatozoa. A faint similarity with alonine spermatozoa can be traced in those of *Peracantha* and *Pleuroxus*, which both have some kind of pseudopodia supported by radial microtubules, and at a certain stage in the spermatogenesis in *Pleuroxus* some dark substance is deposited around these microtubules as in the alonines. But even in these two species the spermatozoa differ distinctly from the strict polar organization seen in the alonine spermatozoa. The distinction between the subfamilies is thus obvious.

It is interesting to see that this distinction at the spermatological level also holds for *Rhynchotalona falcata* and *Disparalona rostrata*, which previously were referred to the same genus *(Rhynchotalona)* (Pl. 16:82-83). The transfer of the species *rostrata* from the genus *Rhynchotalona* within the Aloninae to a genus (now *Disparalona)* within the Chydorinae, as originally suggested by Frey (1959), is beautifully supported by the morphology of the spermatozoa. *R. falcata* has typical alonine spermatozoa, whereas those of *D. rostrata* are completely different.

The material is hardly sufficient for a detailed discussion of relationships within the Chydorinae, but the available evidence indicates considerable heterogenity.

Family Polyphemidae

Material

Polyphemus pediculus (L.). Emdrup Sø, Copenhagen, 18. X. 72., ♂♂, 1 % Os. – Teglgårdssøen, Hillerød, Zealand, 7. X. 73, 1 ♂, 3-A (coll. O. Wetterberg). – Lake Fenen, Sm., Sweden, 3. X. 74, ♂♂, 3-A.

The elongate testicles of *Polyphemus* are beautifully shown in Leydig's "Daphniden" (1860, fig. 63). In sections they appear compact, filled with large, elongate spermatozoa and nutritive cells. The maturing and mature spermatozoa are not surrounded or enclosed by single nutritive cells but are bordered by several such cells and may even be in contact with other spermatozoa over part of their surface. The nutritive cells are almost as large as the spermatozoa and are filled with large vacuoles (about 2 μ) with a granular content (Pl. 18:88, 90). Earlier stages of spermatogenesis have not been studied.

The mature spermatozoa are elongate, 30 – 40 μ long and 15 – 20 μ broad (Fig. 7, Pl. 18:88). The surface is smooth, and the nucleus, more or less irregular in shape, has a variable location. Under low magnification the cytoplasm appears fairly dense in the EM, and is filled with organelles of different kinds (Fig. 7B, Pl. 18:89, 90): 1) numerous, irregular, branching and anastomosing tubules with a diameter of about 340 Å, 2) thick bundles of parallel, straight microtubules of the 250 Å class, 3) large aggregates of glycogen-like granules, visible also in the light microscope, 4) scattered mitochondria (about 1 μ) with distinct cristae, and 5) a marginal zone of irregular, empty-looking vacuoles with a maximum length af about 2 μ. The vacuoles form a single layer under the cell membrane. They are usually elongate, oriented at right angles to the cell surface, and many, perhaps all, are in close contact with the cell membrane with teir distal end. In some cases the vacuoles appear to open out, their membrane being continuous with the cell

membrane, but in all such cases there is a dark line across the vacuolar pore, probably some kind of coat substance, separating the vacuolar content from the exterior (Fig. 7 B).

Leydig (1860) observed that spermatozoa of Polyphemus emitted tufts of slender pseudopodia after being pressed out of the male into the water. Weismann (1880) was more inclined to interpret similar pictures as a result of the plastic, sticky nature of the protoplasm, whereas Zacharias (1884) in numerous experiments always saw the pseudopodia form when the sperm was liberated into the water. No pseudopodia were ever seen in my material, which only contained intratesticular spermatozoa. However, the marginal vesicles may constitute a membrane-reserve which is used when pseudopodia are formed.

Leydig (1860) and Weismann (1880) noticed that the spermatozoa in the testicle are surrounded by a granular mass, not present in *Bytotrephes*. This granular mass, obviously identical with the nutritive cells, is said to be pressed out into the water together with the spermatozoa.

Comments on the spermatozoa of Polyphemus. The spermatozoa of *Polyphemus* share important features with those of the other onychopods: large size, rounded – oval shape, smooth surface, smooth ER in the shape of irregular, anastomosing tubules troughout the plasm, and a zone of marginal vesicles just under the cell membrane. There are also differences: the bundles of 250 Å microtubules criss-crossing through the plasm are characteristic of *Polyphemus*. *Bytotrephes* has 250 Å microtubules but they do not form well organized large bundles as in *Polyphemus*, and *Podon* and *Evadne* have instead some unique filaments with a complicated cross section. The nutritive cells of *Polyphemus* differ from those of other onychopods in their prominent granulation.

The marginal vesicles in the onychopods may perhaps have a counterpart in the similar vesicles seen in some *Anostraca* and in *Diaphanosoma*. It is interesting that such marginal vesicles, sometimes

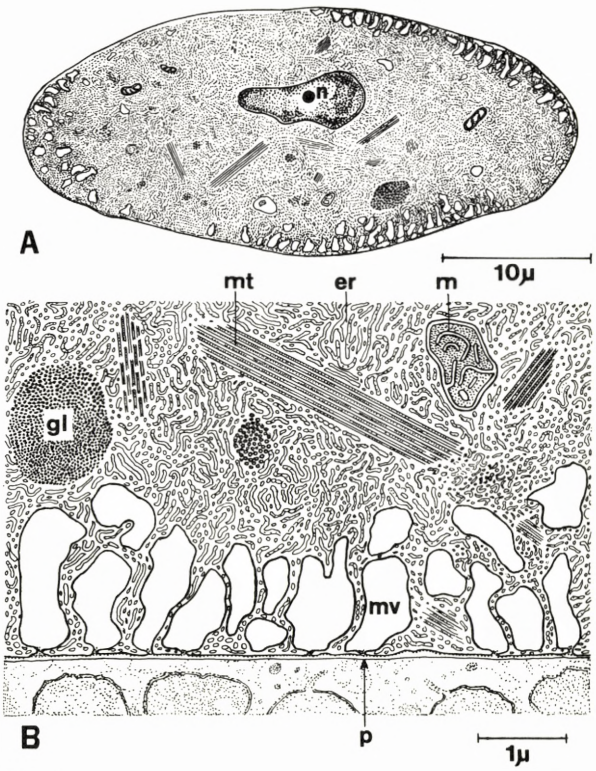

Fig. 7.

Diagrams of the spermatozoon of *Polyphemus pediculus* (L.). A. Mature spermatozoon. B. Detail of spermatozoan surface in contact with vegetative cell (below).

Legends: er = endoplasmic reticulum, gl = glycogen body, m = mitochondria, mt = microtubules, mv = marginal vesicles, n = nucleus, p = porelike opening of marginal vesicle, not closed by unit membranes.

complicated in shape, occur as "spongy chambers" in other aflagellate spermatozoa with rounded or elongate shape, e.g. nematodes, acarids, *Polyxenus*, and *Gymnarchus* (Afzelius & Baccetti, 1976).

Family Podonidae

Material

Podon leuckartii G. O. Sars. Nivå Bay, Øresund, 5. V. 75, ♂♂, 2 % Os.

Evadne nordmanni Loven. Øresund, 21. VIII. 72, ♂♂, 1 % Os. – Øresund, 21. IX. 73, ♂♂, 3-A.

The testicles are small globules lying dorsally on each side of the posterior intestine. Those of *Evadne* are easily visible under the transparent valves (Lilljeborg 1900, tab. 86). Young females may resemble the males, however, so the presence of male hooks on the 1st leg must be checked if confusion of sexes is to be avoided. In both species the testicles look compact, but the organisation differs considerably.

In *Podon* the large, ovoid spermatozoa lie irregularly scattered among large nutritive cells, as in *Polyphemus*, but the nutritive cells have no granules as in *Polyphemus* but are completely filled with densely packed cisternae of smooth ER, so the entire cell content looks like a gigantic Golgi apparatus.

In *Evadne* the immature testicles are filled with regular, polygonal cells, and no distinct difference between germinal cells and nutritive cells can be seen. Later this difference appears, and the regularly formed elongate spermatids are enveloped by individual nutritive cells, which become cuplike, each of them containing a spermatid in its cavity (Pl. 19:92, 93). The cupcells never close completely but remain open, and the spermatid inside each of them protrudes from the opening with a number of branching, pseudopodia-like processes. A very dark-staining cell membrane in the neck-like part of the cup cell is in contact with the spermatid. The large cup cells appear to have a parallel orientation and form a kind of epithelium attached to one side of the testicalar basement membrane. Pseudopodia protrude from the distal ends of the spermatids and intermingle with each other and with smaller cells which represent the epithelium of the opposite wall in the testicle (Pl. 19:92).

In each individual male the cup cells and spermatids were of approximately the same developmental stage, indicating simultaneous development. It was thus difficult to understand the sequence of events during early spermatogenesis.

Mature testicles of both species contain only a few, probably 10 – 20, spermatozoa. In *Evadne* the spermatozoa are always surrounded by thin lamellae of plasm, the remnants of the cup cells (Pl. 18:91).

Mature spermatozoa of Evadne could be described as gigantic, up to 70 μ long and 40 – 50 μ thick. The shape is oval with an even surface, and the wrinkled, somewhat irregular nucleus has a rather variable location within the cell (Pl. 18:91). A prominent nucleolus remains until late stages and is probably permanent. There are many large (2 – 3 μ), irregularly shaped mitochondria with numerous cristae. An extensive system of smooth ER in the shape of irregular, branching tubules and sacs is present troughout the cell. A distinct zone of marginal vesicles is present just under the cell surface. As in *Polyphemus* the narrow, almost tubular vesicles are often seen to contact the cell membrane and probably open through it.

The characteristic feature of these spermatozoa is the presence of fibre bundles which pass through the cytoplasm in a criss-cross arrangement. Each bundle consists of a number of fibres, usually 10 – 50, which have a comparatively straight course and belong to a hitherto unknown class of organelles. The cross section of each fibre is complicated, looking like an H or Y or forming still more complicated figures (Pl. 20:96). Under high resolution it can be seen that the fibres are composed of narrow tubules, about 80 Å in outer diameter and with a light, seemingly empty centre. Such tubules stick together forming plates as shown in the cross sections.

According to Claus (1877) the spermatozoa of *Evadne* show amoeboid movement when suspended in sea water, and his figures indicate that they are polar with pseudopodia emitted from one end. It may be that the pseudopodia extending from the opening of the cup cell inside the testicle are identical with those drawn from liberated cells by Claus.

Maturation of Evadne spermatozoa. At the time of enclosure in nutritive cells the spermatids are

still of moderate size, about 20 μ long and 15 μ broad. The nucleus is large and vesicular. The cytoplasm contains numerous mitochondria, ribosomes, and abundant ER. The prospective pseudopodia-forming end of the cell has a light plasm with few organelles at the time of enclosure, but is lobe-shaped with a simple outline. During the stay in the cup cell the pseudopodia, the marginal vesicles and the fibre bundles appear, whereas the ER is reduced and the ribosomes disappear. The simultaneous development in each testicle made it difficult to reconstruct exactly what happens.

Mature spermatozoa of Podon. The spermatozoa of *Podon* are smaller than those of *Evadne*, about 30 × 20 μ, and the irregular nucleus is central. The mitochondria are very large (up to 6 μ long and 1 μ thick) and have numerous parallel cristae (Pl. 19:94). The smooth ER and the marginal vesicles are approximately as in *Evadne*. The bundles of fibres are also similar to those of *Evadne*, each fibre consisting of several attached tubules with 80 Å outer diameter (Pl. 20:97). However, in *Podon* the tubules appear to be more loosely connected and the single filament has a simpler cross section.

Spermatogenesis was not studied in detail, but the spermatids are not enclosed in cuplike vegetative cells but are in contact with several such cells and with other spermatids during development.

Comments on the spermatozoa of the Podonidae. Both *Evadne* and *Podon* have very large spermatozoa with marginal vesicles like the other onychopods. A very specific feature for the podonids is the 80 Å microtubule which is the fundamental unit in the fibre bundles. Spermatogenesis is very different in the two examined forms, although the testicle has the same very compact structure as in other onychopods. *Evadne* is unique among branchiopods with regard to the cup-shaped nutritive cells, in which the spermatids are located.

Family Cercopagidae

Material

Bytotrephes longimanus Leydig, *ssp. balticus* Ischreyt. Lyngby Sø, Zealand, 8. IX. 72, ♂♂, 1 % Os, Gla. – Ibid., 15. IX. 72, ♂♂, 1 % Os. – Ibid., 29. IX. 72, ♂♂, 1 % Os.

As indicated in the drawings of Weismann (1880, fig. 21) and Lilljeborg (1900, tab. 92), the testicles are simple sacs lying on each side above the 2nd and 3rd pairs of legs. In sections the testicle appears compact, maturing spermatids and spermatozoa lying densely packed without intervening nutritive cells. The wall is lined by a single layer of very flat, low cells which may have a nutritive function, for the half-grown spermatids are attached to these cells with their base, which interdigitates with the wall cells by means of numerous small basal processes. The opposite, apical end of the same spermatids produces a remarkable, broad, pseudopodium-like lobe (Pl. 20:99). Such lobes from several spermatids are packed together in the centre of the testicle. The lobes have a homogeneous plasm without organelles, in contrast to the plasm in the cell body proper, which is crowded with tubules and other strutures.

Mature spermatozoa are approximately isodiammetrical, non-attached, and very large. The maximum diameter is about 55 μ (round cells) to 70 μ (elongate cells) (Pl. 20:98). The nucleus is approximately central, spherical, about 12 μ, with a large nucleolus. Such mature spermatozoa, lying in the centre of the testicle and in the spermoduct, lack the pseudopodium-like lobe seen in the spermatids.

The cytoplasm is virtually filled with small, 400 – 500 Å tubules which branch and bend. More ordinary-looking, straight microtubules with about 250 Å diameter are scattered in restricted areas of plasm. In spermatids, long straight bundles of such tubules radiate through the cell from the basal attachment (Pl. 20:99). There are also scattered glycogen-like granules and numerous 0.5 μ mitochondria with tubular

cristae. Mature cells have a zone of marginal vesicles just below the cell membrane. The vesicles are elongate, irregular tubes, about 1 μ long and 800 Å thick, with a roughly perpendicular orientation with regard to the cell membrane, but a clear, open communication through this membrane could not be seen.

Spermatogenesis. Development of the spermatids is characterized by their attachment to the wall, as described above. When the cells are less than 20 μ in diameter they lack the apical process and have numerous ribosomes but little tubular ER. The complicated system of tubules is always developed in the somewhat larger spermatids, which stick to the wall. This tubular system is present also in the mature spermatozoa in the spermoduct. Maturation involves considerable growth, reduction of the ribosomes, and development of the marginal vesicles.

Weismann (1880) observed that the living spermatozoa were very plastic and were torn out into long strings if caught by bristles, legs, etc. He regarded such pseudopodia-like strings as artifacts, and did not see them on undisturbed spermatozoa. The present investigation shows that pseudopodia are certainly not present on spermatozoa in the testicle.

Comments on the spermatozoa of Bytotrephes. With regard to the enormous size, the presence of marginal vesicles, and an abundant tubular type of smooth ER, the spermatozoa of *Bytotrephes* show significant similarity with those of the other onychopods. Spermatogenesis in *Bytotrephes* is unique with respect to the way the spermatids attach to the wall cells during spermatogenesis and with respect to the absence of nutritive cells in the testicular lumen. The lobe-like apical process of spermatids looks like the process which produces pseudopodia in the spermatids of *Evadne nordmanni*, but it certainly does not serve this purpose in *Bytotrephes*.

Comparative and Phylogenetic Part

The branchiopod type of spermatozoa

Looking over the results obtained one may conclude that the spermatozoa of all branchiopods share a number of important features:

1) Complete lack of axonema or flagellum at all developmental stages, although the centrioles are present and persist until late in spermatogenesis, in some cases even in the mature sperm.
2) Complete absence of acrosomal structures.
3) Presence of a well-defined nucleus with the typical double nuclear evelope.
4) No fusion of mitochondria into "Nebenkerne" has ever been seen in branchiopods. Mitochondria are usually preserved in the sperm but are sometimes small and degenerate. The matrix of the mitochondria is dense in some species, but intra-mitochondrial crystalline structures were seen in only one species, *Tanymastix stagnalis*.

In spite of the variation recorded it may thus be concluded that these features suffice to define a "Branchiopod type of spermatozoon", which is recognizable although subjected to modification throughout the subclass. This type, which is essentially amoeba-like, has a unique position within the Crustacea, as will be seen from the account below:

Copepoda have nonflagellate spermatozoa. The characteristic feature is the complete loss of the nuclear envelope during maturation (Brown 1966, 1970, Gupta 1964; author's unpublished material, comprising about 70 species).

Ostracoda have nonflagellate spermatozoa which preserve a typical nuclear evelope, but they have a prominent acrosomal apparatus and are in most cases extremely complicated. However, within the subfamily Asteropinae of the Myodocopa, the acrosome is reduced and the spermatozoa are simple rounded cells. This strongly approaches the conditions in the Branchiopoda but is clearly a case of convergence (Wingstrand, unpublished). Published reports of ostracod spermatozoa deal exclusively with cyprid Podocopa, and electron microscopy has only been used on some freshwater species (Zissler 1966, 1969 a, b, 1970; Gupta 1968; Reger & Florendo 1969 a, b; Tétard 1967; Reger 1970). My own unpublished material, in all about 60 species, includes also the Bairdiidae and Cytheridae of the Podocopa, and representatives of Myodocopa, Cladocopa, and Platycopa.

Branchiura have flagellate spermatozoa of a complicated and unique structure, including a "pseudoacrosome" which is nonhomologous with acrosomes of other animals with the exception of the Pentastomida, which have nearly identical spermatozoa (Wingstrand 1972, 1974).

Ascothoracida have simple, flagellate spermatozoa with an acrosome, actually the most primitive type of sperm seen in crustaceans (author's unpublished material of *Ulophysema oeresundense* Brattström).

Cirripedia have flagellate spermatozoa with anteriorly dislocated centrioles and a typical acrosome (Brown 1966, 1970, Turquier & Pochon-

Masson 1969, Bocquet-Védrine & Pochon-Masson 1969, Munn & Barnes 1970, Pochon-Masson 1970).

Mystacocarida have flagellate spermatozoa with anteriorly dislocated centrioles and an acrosome (Brown & Metz 1967, Brown 1970).

Cephalocarida have nonflagellate spermatozoa with a very large acrosome, and a long, straight bundle of filaments which begins behind the acrosomal vesicle, penetrates the nucleus and extends into a "posterior projection" behind the nucleus. This bundle looks very much like a post-acrosomal rod, well known from numerous bilaterian animals. The prominent acrosomal apparatus makes these spermatozoa malacostracan-like, a fact pointed out also by Brown & Metz (1967). All information about cephalocarid spermatozoa is from Brown & Metz (1967) and Brown (1970).

Malacostraca vary with regard to spermatozoan morphology, and the variations appear to be in good agreement with current systematics (Brown 1970). The spermatozoa of all species investigated so far are non-flagellate and have a prominent acrosomal apparatus.

Amoeba-like spermatozoa lacking both flagellum and acrosome are certinly rare in animals. Among non-crustacean arthropods the nearest approach to the branchiopod type is found in the proturan *Eosentomon* (Baccetti et al. 1975). This proturan has nonflagellate spermatozoa without an acrosome, but the spermatozoa have a very unique structure: they are shaped like flat, circular discs with a thick margin. The nucleus with its chromatin condensed into a ring in the periphery of the cell is unlike anything seen in branchiopods. Flagellum and acrosome are also completely reduced in *Tetranychus* urticae *(Acari)* (Alberti & Storch 1976), but in this species also the nuclear evelope is lost. Other acarid spermatozoa have a more or less well-developed acrosome.

Among non-arthropods, the best parallel to the spermatozoa of branchiopods is probably found in *Gymnarchus* and *Mormyrids* among teleost fishes (Mattei et al. 1967, 1972). In this case the spermatozoa are simple and actually rather similar to branchiopod spermatozoa. Other nonflagellate and acrosome-less spermatozoa, like those of some nematodes and gnathostomulids, are so specialized in other directions that comparisons with branchiopod spermatozoa must be fairly academic (See, e.g., Jamuar 1966, Lee & Anya 1967, Neill & Wright 1973, Pasternak & Samoiloff 1972, Shepherd et al. 1973, Wright et al. 1973, Wirth 1974, Graebner 1969).

Pattern of variation and current systematics

"*Euphyllopoda*". Within the classical "Euphyllopoda", including the Anostraca, Notostraca, and Conchostraca, there is a striking uniformity with regard to sperm structure (Figs. 1 and 8, Pls. 1 – 3). All of the 20 species examined have simple, amoeba-like spermatozoa, 3.5 – 6 μ in diameter. Small differences are found from species to species with regard to the condensation of nucleus and plasm, in the morphology of the cell surface, and in mitochondria and ER. Some features are even restricted to a few families and may be useful in discussions of taxonomic relationships, e.g., the structure of the cell surface in Branchipodidae and Artemiidae (p. 15). In general there are no significant differences between the spermatozoa of the major groups Anostraca, Notostraca, and Conchostraca. Spermatozoa of the conchostracan *Cyzicus* (Pl. 3:17) fit well in the series of anostracan spermatozoa (Pl. 1), and spermatozoa of the conchostracan *Imnadia* (Pl. 3:19) are almost identical with those of the notostracan *Triops* (Pl. 3:15).

It is reasonable to assume that these spermatological similarities between euphyllopods are homologies in the strict sense, i.e., that they depend on inheritance from a common ancestor. This ancestor must have lived in the lower Paleo-

zoicum, for the main branchiopod lines, or at least two of them, were distinct in the lower Devonian and must have separated in the Silurian or even earlier (Tasch 1963). It follows that the particular type of spermatozoon must have remained unchanged within each evolutionary line since early Paleozoic times. Spermatozoan morphology within the Euphyllopoda must therefore be characterized as extremely stable and conservative.

Cladocera. In contrast to the Euphyllopoda, the Cladocera show a really spectacular radiation with regard to sperm morphology, although the basic features of the amoeba-like sperm are retained (Fig. 8). The features subjected to variation are: 1) cell size, from 1 to 80 μ, 2) polarity of the cell, 3) the nucleus, its size, location, shape and degree of chromatin condensation, 4) presence and morphology of mitochondria, 5) presence and location of 250 Å microtubules, 6) presence and location of other types of tubules and filaments, 7) morphology of smooth ER, 8) myeloid bodies, glycogen, and other exceptional intraplasmatic structures, 9) presence and morphology of pseudopodia-like processes, 10) other specializations of the cell surface as plaques, marginal vesicles, etc., 11) morphology of extracellular coats, 12) type of spermatogenesis.

Modern taxonomists generally accept that the Cladocera are related to the Conchostraca, the two being treated as sister groups under the common name Onychura (or Diplostraca) and derived from a common developmental line (Calman 1909, Eriksson 1934, Brooks 1966, Kaestner 1967, Flössner 1972). If this is accepted it follows that cladoceran ancestors once must have had simple amoeba-like spermatozoa of the euphyllopod type. In recent Cladocera we mostly see more or less extreme modifications of this type (Fig. 8), but one cladoceran, *Holopedium gibberum*, actually has preserved the presumed primitive type, indistinguishable from that of euphyllopods (see Pl. 4:23 and Pl. 8).

Subdivisions of Cladocera. In general the variation of spermatozoa within the Cladocera is in good agreement with current opinions of systematics and evolution. First of all, each of the four major subdivisions of Cladocera has its own trend with regard to spermatozoan structure.

The *Haplopoda* (Genus *Leptodora)* have moderately enlarged hyaline spermatozoa filled with densely packed, empty-looking sacs of smooth ER, so the cell looks like a gigantic Golgi apparatus (Fig. 8, Pl. 4:20). This is unique among crustaceans and fits well with the agreed isolated position of the group Haplopoda (See p. 56).

The *Ctenopoda* (except *Holopedium)* are characterized by enormous spermatozoa (up to 70 – 80 μ), but variation of cytological detail makes the picture heterogeneous (Fig. 8). As discussed in a following chapter, this heterogeneity could mean that the sidid genera are less mutually related than usually supposed, and it is fairly obvious that *Holopedium* has a more independent state.

The *Anomopoda* are well characterized by their small spermatozoa (1-5 μ, somewhat more if rod-shaped), by the frequent occurence of axopods with characteristic and unique axial rods, and by their unique vacuolar type of spermatogenesis: the spermatids develop inside "private" vacuoles in the large nutritive cells (Fig. 2C). Only *Moina brachiata*, *M. micrura*, *M. macrocopa*, and the macrothricids *Streblocerus* and *Ilyocryptus* show interesting exceptions from this pattern, which will be discussed in the following chapters (Fig. 8).

The *Onychopoda* stand out as a fairly homogeneous group with regard to spermatozoa: gigantic cells (30 – 80 μ) with marginal vesicles and a dense plasmatic content of tubules and filaments. Some variation is found in the types of filaments and in the course of spermatogenesis.

Family and genus levels. Witin the Cladocera variation extends trough the lower taxa to the specific level. In many cases existent taxa can be clearly defined also using spermatological characters. A kind of filaments consisting of 80 Å tubules

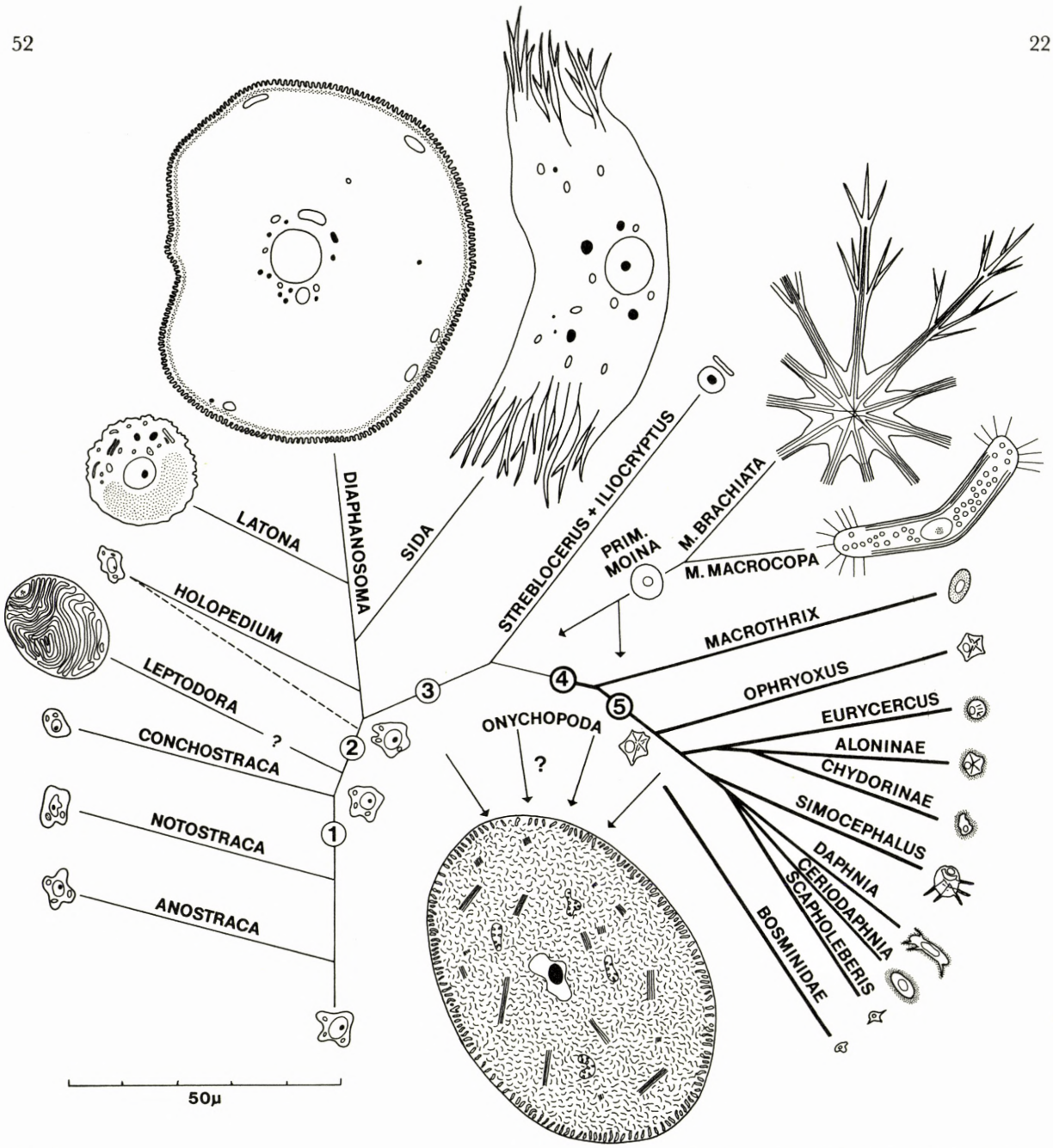

Fig. 8.

Diagram showing variation of spermatozoa within the Branchiopoda, and the presumed evolution of the group. The thick black lines indicates forms with the vacuolar type of sperm maturation (Fig. 2C). Magnification is the same for all spermatozoa. The figures indicate major events in the evolution of the group:

1. Bivalved shell, recurved abdomen, 2nd antenna natatory, male hooks on anterior legs.
2. Loss of nauplius larva, reduction to 6 pairs of limbs (may have occured earlier), reduction of abdominal segments.
3. Development of contrast between legs 1-2 and 3-4, beginning development of ephippium.
4. Evolution of vacuolar spermatogenesis (See Fig. 2C).
5. Evolution of heliozoan-like spermatozoa with cell centre, radiating dark rods and axopods.

The onychopod spermatozoon is that of *Evadne nordmanni* Lovén.

appears to be restricted to the spermatozoa of the Podonidae (p. 47), likewise the subfamily Aloninae can be defined on the basis of a characteristic sperm type (Pl. 15, p. 42). The genera *Daphnia*, *Ceriodaphnia*, and *Simocephalus* are well characterized by different sperm types, and there is little or no overlapping of the generic limits as defined on the basis of external morphology (Pls. 7 – 10). As a practical example may be mentioned that *Rhynchotalona falcata* has typical alonine spermatozoa, whereas *Disparalona rostrata* has a non-alonine (chydorine) type of sperm, although both species were referred to the same alonine genus *(Rhynchotalona)* before 1959. The removal of the species *rostrata* from the genus *Rhynchotalona* and its tranfer to another genus (now *Disparalona)* within the subfamily Chydorinae, originally founded on studies of head pores (Frey 1959) and on studies of limb structure (Smirnow 1966), is thus in good agreement with sperm morphology (Pl. 16:82, 83).

In other cases variation is great and seemingly irregular within a taxon. In such cases, e.g., in the family Macrothricidae and the subfamily Chydorinae, it is often felt that the taxon, as defined on external morphology, is heterogeneous. The case of *Macrothricidae* will be discussed in a following chapter. In the case of the Chydorinae I think that most taxonomists will consider this subfamily much less homogeneous than the Aloninae, which have a more constant sperm type (Pls. 15 – 17).

Species level. Differences in spermatozoan structure between species of the same genus, although sometimes small, can be very striking and can certainly be useful for defining difficult species. In the genera *Ceriodaphnia* and *Simocephalus* there are clear differences between the spermatozoa of most species, also in come cases *(Ceriodaphnia quadrangula* and *C. pulchella)* which are regarded critical (Pl. 8:39, 40, p. 26). Actually, in these two genera the phyletic changes in the spermatozoa seem to be stronger than the changes in external morphology. An example is *Simocephalus exspinosus* and *S. congener* which have completely diffirent spermatozoa but are difficult to distinguish morphologically (usually regarded variants of the same species, see p. 28, Pl. 10).

Comments. As evidenced above there is an interesting contrast between the classical "Euphyllopods" which have preserved the spermatozoa almost unchanged during their long and spectacular evolution, and the more modern group Cladocera, in which the spermatozoa have evolved as much or even more than the external morphological features. This somewhat subjective judgement only shows that spermatozoa behave like other morphological features: they are conservative in some evolutional lines, progressive in others. When used in phylogenetical discussions the spermatozoa must therefore be dealt with critically from case to case, using the same criteria as those used for other morphological features.

The monophyletic or polyphyletic origin of the Branchiopoda.

History of the problem. Opinions about the phylogenetical status of the Anostraca have changed considerably with time. The old concept of the Branchiopoda as a natural unit (Milne-Edwards 1834 – 40) was criticized by Linder (1941, 1945), who found profound differences between the Anostraca on the one side and the Notostraca, Conchostraca and Cladocera on the other. Preuss (1951, 1957) followed this line up, showing that the limbs of Anostraca and the other branchiopods have very different musculature and therefore may have become similar by convergence. The strongest argument for a monophyletic origin of branchiopods thus suddenly became doubtful and inconclusive. When Siewing in 1960 drew up a kind of status, there were few if any arguments left for a common origin of the Anostraca and the "Phyllopoda *s. str.*", i.e., Notostraca,

Conchostraca and Cladocera. A number of additional characters were previously used to support monophyly of the Branchiopoda: homonomous metamerism of the limbs, many segments, long heart with many ostia, relative independence of tritocerebrum, etc. However, these characters were shown to be plesiomorphous (primitive in crustaceans in general), and their presence in Anostraca and Phyllopoda actually does not show that these two are more closely related to each other than to other crustaceans. Other characters, particularly the leaflike (phyllopod) limbs and the morphology of the mouthparts, could be the result of convergence, whereas numerous differences dominated the picture: stalked eyes in Anostraca, no carapax in the Anostraca, 2nd antenna prehensile organ in male Anostraca, and differences in oogenesis. This caused some authors to treat the Anostraca as a crustacean subclass separate from the Phyllopoda (e. g., Remane 1954, and Kaestner in the first issue of his "Lehrbuch", 1959).

Dahl (1956 a, b) pointed out similarities in the development of the brain and the protocerebral sense organs between Anostraca and Phyllopoda *s. str.* and, while admitting that the two groups have gone far apart, was inclined to regard them as monophyletic. In a later paper (1963) Dahl's view had strengthened and he introduced into the discussion some new characteristics, shared by the Anostraca and Phyllopoda but not by other crustaceans: the 5 retinular cells of the ommata, the structure of the nauplius eye and the nauplius limbs. Sanders (1963) added several features which are restricted to anostracan and phyllopod nauplii. Some recent authors, e.g., Kaestner (2nd ed. 1967) and Flössner (1972), have acknowledged the weight of these arguments for a common origin of the Anostraca and Phyllopoda and have again placed the two groups as sister groups within a common crustacean subclass, the Branchiopoda.

Spermatological evidence. It appears to me that spermatozoan morphology speaks strongly in favour of a monophyletic origin of the Anostraca and the Phyllopoda *s. str.* It should be remembered that anostracan, notostracan, and conchostracan spermatozoa are indistinguishable, i.e., the slight variation in the groups is overlapping (p. 18, Pls. 1 – 3). Moreover, this particular branchiopod type of spermatozoon does not occur in other arthropods, except as an extreme variant within an ostracod subfamily. The Cladocera, clearly derived from the conchostracan line, do not disturb this picture, for though variable, their spermatozoa retain all the essential features of the euphyllopod sperm (intact nuclear membrane, no flagellum, no acrosome, no "Nebenkern"). Moreover, *Holopedium* among the Cladocera has simple spermatozoa indistinguishable from those of Euphyllopoda (Pl. 4:23).

It should be noted that differences between animals such as those found between the Anostraca and Phyllopoda (in leg musculature, eyestalks, etc.) are irrelevant for the problem of monophyletic development, for strictly monophyletic lines with a common ancestor are allowed to – and ususally do – develop profound differences. What matters is whether they share unique features, for which an origin in a common ancestor is the best explanation (synapomorphous features, Hennig 1966). It appears to me that the branchiopod type of spermatozoon is such a synapomorphous feature, inherited from and developed in a common branchiopod ancestor, and therefore indicating a strictly monophyletic development like the one indicated in Fig. 8. The only two alternative interpretations are discussed below.

Possibility of convergence. It may be objected that the branchiopod sperm, although structrually well defined, is characterized mainly by loss of features, and that such a simple gamete could have developed independently in the Anostraca and Phyllopoda by convergence. Superficially

seen, this would be a very simple evolutionary process: checking spermatogenesis at an early spermatid stage. But in nature such a change is certainly neither simple nor exclusively a loss of features, for it must be correlated with changes in the mechanism of fertilization from an acrosomal type of penetration to some amoeboid type of membrane fusion, and it requires positive specializations of copulatory organs, egg membranes and mating behaviour. The rare occurrence of similar spermatozoa in the animal kingdom supports this view. Furthermore, the great homogeneity of the spermatozoa in Anostraca, Notostraca and Conchostraca speaks against independent origin.

Possibility of plesiomorphous origin. Theoretically the branchiopod type of sperm could be a primitive feature in crustaceans in general, present already in the common crustacean ancestors, and its presence in Anostraca and Phyllopoda would be a case of symplesiomorphy (Hennig 1966). If so, its presence would not imply that Anostraca and Phyllopoda are more related to each other than to other crustaceans. This possibility can be practically ruled out, however, for there are strong reasons to believe that the common ancestors of the crustacean class had both flagellum and acrosome in their spermatozoa. Both or one of these structures are preserved in Ostracods (acrosome); branchiurans (flagellum and vestigial acrosome); ascothoracids, cirripeds and mystacocarids (flagellum and acrosome); cephalocarids and malacostracans (acrosome). Absence of these structures in ancestral forms would imply that the mentioned crustaceans had acquired flagellum and acrosome independently by convergence, which is highly improbable.

The statement that ancestral crustaceans had flagellate spermatozoa with an acrosome can certainly be extended to ancestral arthropods, for the said structures or one of them are preserved in most apterygotes, pterygotes, myriapods, chilopods and chelicerates (see Wingstrand 1972, pp. 60 – 61). It is even probable that acestral arthropods had the promitive type of external fertilization and the primitive type of spermatozoon always found in animals with such fertilization (Franzén 1956, 1970), for this is all preserved in *Limulus* and some pantopods (v. Deurs 1974). At any rate it is reasonable to assume that ancestral crustaceans, like ancestral arthropods in general, had flagellate spermatozoa with an acrosome, and that the amoeba-like phyllopod type of sperm was evolved within and restricted to the branchiopod line. That this type of sperm should be plesiomorphous in the branchiopods can therefore practically be excluded.

Relations to the Cephalocarida.

Sanders (1956, 1963) regarded the Cephalocarida as primitive Crustacea but actually closer to non-branchiopods than to branchiopods, mainly because their nauplius larva has none of the features characteristic of anostracan and phyllopod nauplii. Dahl (1956) was first inclined to accept a relationship between cephalocarids and branchiopods because there appeared to be similarities in the feeding apparatus, but later (1963) abandoned this view. Siewing (1960) was in favour of Dahl's first idea of a relationship between the Cephalocarida and Phyllopoda.

With regard to the spermatozoa it may be concluded that the cephalocarid *Hutchinsoniella* differs fundamentally from all branchiopods in the presence of a large acrosome (Brown & Metz 1967, Brown 1970). *Hutchinsoniella* can therefore not be derived from the branchiopod line above the branchingpoint between Anostraca and Phyllopoda (Fig. 8), for this would imply that cephalocarids should have evolved their large, malacostracan-like acrosome by convergence out of nothing. While this indicates that the Anostraca and the Phyllopoda are more closely related to each other than either of them is to the Cephalocarida, it does not exclude that the Cephalocarida come into the picture further back in the an-

cestry. But, as pointed out by Brown (1970), the absence of a flagellum and the presence of the large acrosome in cephalocarid sperm rather supports a relationship with malacostracans or ostracods – but without conclusive force.

The Haplopoda and the Onychopoda.

The Haplopoda and the Onychopoda, both being free-swimming and carnivorous, non-filtering animals, are superficially similar in the development of more or less cylindrical, prehensile legs. Sars (1865) united both in the order Gymnomera of the Cladocera, but this was criticized by subsequent authors, particularly Wesenberg-Lund (1904) and Eriksson (1934). The former played with the idea that the Haplopoda *(Leptodora)* were extremely specialized ctenopods, *Diaphanosoma* being the closest relative. Eriksson, like Wesenberg-Lund, found that the similarities between the Haplopoda and Onychopoda were fairly superficial and probably developed by convergence. Eriksson derived the Onychopoda from some unspecified Cladocera with the usual, recurved and unsegmented abdomen. *Leptodora* with its straight, segmented abdomen, great size, metanauplius larva, and other remarkable features was found so different from other Cladocera that Eriksson suggested its separation as an independent taxon Haplopoda, which was made a sister group of the Eucladocera (containing the remaining Cladocera). He found it probable that *Leptodora* had evolved independently from some conchostracan euphyllopods. This suggestion has been followed in some recent systems (Brooks 1966, Flössner 1972), whereas others have retained the Haplopoda within an undivided cladoceran taxon (e.g., Kaestner 1959, 1967).

The sperm structure cannot be used to extend our knowledge much on this point.

Haplopoda. Leptodora has unique spermatozoa, moderately large and filled with densely packed sacs of smooth ER (Pl. 4:20). The spermatozoa have nothing in common with those of other cladocerans, with the exception of the general branchiopod features. The study of the spermatozoa actually shows nothing about the origin of the group but only underlines its independence. Nevertheless I have introduced the haplopods into the diagram (Fig. 8) as a branch from the base of the cladoceran (or conchostracan) line, acknowledging that *Leptodora* has retained some primitive features not shared by the Eucladocera: metanauplius larva and a long, straight, segmented abdomen. Loss of the Metanauplius in the Eucladocera must be regarded as a synapomorphous features in this group and distinguishes them from the rest of the Onychura.

The Onychopoda. The sperm structure confirms that the Onychopoda form a natural, monophyletic unit: all species have gigantic spermatozoa with a smooth surface, marginal vesicles, and a dense cytoplasm filled with complicated tubular and filamentous structures (Pls, 18–20). No significant and unique features are shared with other Cladocera. The great size of the spermatozoa in sidids can hardly be referred to as a probable homology when the contents of the large cells are completely different. The complicated and dense pattern of tubular structures in the plasm of onychopods looks very much like that seen in *Macrothrix* (cf. Pls. 18:89 and 14:69). However, I hesitate to make a case out of this single feature, particularly because *Macrothrix* spermatozoa are so different from those of the Onychopoda in other respects: small size, superficial plaques, different spermatogenesis. In the diagram (Fig. 8), the uncertain origin of the Onychopoda is indicated by several unattached arrows.

Ctenopoda and the problem of Holopedium.

From a spermatological point of view the Ctenopoda (Sidoidea) is a heterogeneous group. *Holopedium*, which will be discussed separately below,

has spermatozoa of the same size (5 – 6 μ) and morphology as the classical euphyllopods (cf. Pl. 4:23 and Pls. 1–3). By contrast, the Sididae *(Sida, Diaphanosoma, Latona)* have very large spermatozoa (20 – 80 μ) which must be regarded as strongly specialized. But variation is large also within the Sididae, the single species having their own characteristics (Pls. 4 – 6). *Latona* appears to be least specialized, its spermatozoa are moderately large (20 μ) and well filled with organelles in addition to a large glycogen body, and the cell surface is simple. The spermatozoa of *Sida* and *Diaphanosoma* are gigantic, empty-looking vesicles, those of *Diaphanosoma* with a characteristically folded surface and those of *Sida* with complicated pseudopodia.

Nobody seems to have questioned the close mutual affinity of the genera assembled within the Sidoidea (Ctenopoda), but actually it is difficult to find critical arguments for a strict monophyletical origin of this group. The six uniformly built pairs of legs are usually used as a diagnostic feature for the Ctenopoda (including *Holopedium)*. But the homonomous metamerism of the legs and most of their detailed structure are certainly primitive features present also in the Conchostraca and therefore were probably present in ancestral Cladocera. The number six is certainly also primitive, shared by some recent Cladocera. Such obvious plesiomorphous features (Hennig 1966) can have been inherited independently from ancestral Cladocera or Conchostraca and are no proof for a common ctenopod ancestry.

Sididae. In fact there are a few, probably unique, features which may be apomorphous and which may support a monophyletic origin of the family Sididae: 1) the ridge on the shell, which closes the brood chamber, 2) the long, flagellum-like 1st antenna of the male. 3) In addition, the genera *Diaphanosoma* and *Latona* are characterized by a distinct and obviously homologous penis, so these two genera are, at any rate, monophyletic. 4) The large spermatozoa could, with some hesitation, be added to these apomorphous features of the Sididae, but the feature is not completely unique (see below). The heterogeneity of the family, as expressed in the spermatozoa, is paralleled in external morphology: no penis in *Sida*, "triforked" 2nd antenna in *Latona*, rostrum only in *Sida*, etc. This indicates considerable independence of the genera.

Large spermatozoa are present in the onychopods and haplopods *(Leptodora)*. In *Leptodora* the size of the spermatozoa is moderate (20 μ), as in *Latona*, but the structure of the spermatozoa in *Leptodora* is so different form anything seen in other Cladocera that I hesitate to draw any conclusions whatsoever (Pl. 4:20). The Onychopoda have giant spermatozoa with marginal vesicles and abundant tubular and filamentous structures in the cytoplasm. Their large size alone appears to be a weak argument for a relationship with the Sididae. Structures similar to marginal vesicles appear during spermatogenesis in *Diaphanosoma* but open later and contribute to the folded membrane pattern in this species. Anyhow since marginal vesicles are present in the Anostraca this character can well be plesiomorphous and therefore of little weight for relationships with the Sididae. *Latona* has many vesicles in the plasm but none of the characteristic tubular patterns seen in onychopods. Since also the spermatogenesis in sidids is different from that in the compact testicles of the onychopods, I do not feel that any conclusions are justified.

In agreement with the discussion above, the diagram (Fig. 8) shows the family Sididae as a monophyletic unit with the genera *Diaphanosoma* and *Latona* as a monophyletic subunit.

Holopedium is, with regard to its spermatozoa and also with regard to its spermatogenesis, a typical euphyllopod, and certainly never had specialized spermatozoa of the giant sidid type. It must therefore have been derived from near the base of the ctenopod stem or, perhaps, directly from a common cladoceran stem before the original

amoeba-like spermatozoa had begun to specialize (Fig. 8). I am uncertain about these two possibilities.

Eriksson (1934) had no doubt about a close relationship between *Holopedium* and the sidids, mainly on the basis of leg structure. He looked upon *Holopedium* as a specialized sidid and even considered family separation unnecessary. Recent taxonomists ((Brooks 1966, Flössner 1972) have maintained *Holopedium* in a separate family, obviously because of its numerous remarkable specializations: unbranched 2nd antenna in the female, small, immovable 1 st antenna in the male, gelatinous case, compressed body shape, etc.

Again the leg structure, usually referred to as showing a close relationship between the Sididae and Holopedidae, must be looked upon as a plesiomorphous feature. It can well have been inherited independently from ancestral Cladocera in the two families and does not show anything about their mutual relationships. Moreover, the legs of *Holopedium* differ clearly from those of sidids, e.g., in their elongate shape and in the absence of bristles on the lateral margin of the exópod (see Sars 1865, Lilljeborg 1900, Eriksson 1934). I am not able to exclude that the leg structure may include critical arguments for a monophyletic origin of the Sididae and the Holopedidae, i. e., that the two families share leg features not present in the Conchostraca and/or the Anomopoda, but I have not seen such a feature defined.

In the diagram (Fig. 8) I have therefore with some hesitation connected the *Holopedium* line to that of the sidids, but I have also with a broken line indicated the other possibility, that the Holopedidae have arisen directly from ancestral Cladocera. In the absence of positive arguments for a monophyly with the Sididae, the latter alternative is in fact more likely, and would imply that *Holopedium* should be moved into a taxon of its own, separate from the Ctenopoda (Sidoidea).

The Anomopoda and the problem of the Macrothricidae.

The Anomopoda (Chydoroidea) have long been regarded as a natural monophyletic unit, and this view is supported by some distinct apomorphous features. The principal anomopodan character is the contrast between the prehensile, more cylindrical legs one and two, and the following, flat, phyllopod limbs which may form a filtration pump. Almost as good a character is that the winter eggs are released from the female at a molt and are preserved in the exuvium, which may have a thickened carapace or a specialized ephippium serving as a reservoir. Other characters are probably also apomorphous in the Anomopoda but are less constant and may be lacking in some species, particularly in macrothricids: e.g., the abdominal processes which block the exit of the brood chamber in the majority of chydorids, daphniids and some macrothricids.

The spermatozoa of the Anomopoda show spectacular variation, but certain features make the group appear monophyletic also from a spermatological point of view. It should be noted, however, that the aberrant spermatology of the genus *Moina* is left for the following chapter and is not considered here, and that the macrothricids are considered separately below.

The main spermatological characteristics of the Anomopoda are:

1) The small size of the spermatozoa, about 1 – 5 μ, and the fact that they decrease strongly in size during the spermatid stage (some *Moina* species excepted). This quantitative feature is perhaps a weak argument in a phylogenetical discussion, also because sperm size in anomopods and euphyllopods overlaps.

2) The vacuolar type of spermatogenesis: the spermatids are enclosed in "private' vacuoles in the nutritive cells and are exocytosed into the testicular lumen when mature (p. 10, Fig. 2C). As discussed on p. 11 this feature is clearly apomorphous in the Anomopoda and a strong

argument for a monophyletic development of the group. In this as in other sperm features *Moina* is aberrant (next chapter), and the macrothricids *Ilyocryptus* and *Streblocerus* form another, probably more significant, exception (see below).

3) The wide distribution within the Anomopoda of a characteristic, heliozoan-like sperm type. Such spermatozoa are characterized by an excentric nucleus, which leaves place for a cell centre containing centrioles and tubular structures (Pls. 9–10, 13, 15–16). Dark straight rods radiate from this centre and continue as axes into axopods, which are more or less developed all around the cell. The dark rods arise as 250 Å microtubules, which are covered and sometimes completely replaced by some dark, amorphous matter in a way never seen in other cells (Pls. 15-16).

This unique and easily recognizable cell type is found in the Daphniidae *(Simocephalus)*, Macrothricidae *(Ophryoxus)* and in the Chydoridae (Eurycercinae, Aloninae, and, in a modified issue, in the chydorine genera *Pleuroxus* and *Peracantha)*.

The unique features of this spermatozoan type, particularly the remarkable development and the structure of the axial rods in the pseudopodia, make it unlikely that such spermatozoa should have evolved by convergence in independent evolutional lines. They must therefore have evolved within some ancestral anomopods (Fig. 8) and would be expected in all descendants, i. e., in all advanced members of the group. A secondary reduction of the heliozoan-like sperm pattern must therefore be postulated in the genera *Daphnia*, *Ceriodaphnia*, *Scapholeberis*, *Bosmina* and some chydorine genera, in which this pattern is vestigial or lacking. The fact that this pattern is reduced though still recognizable in some forms, e.g., *Simocephalus exspinosus* and *Peracantha truncata*, can perhaps make the postulate more realistic. It is obvious that the heliozoan pattern has been reduced in forms which have developed small or compact spermatozoa.

The Macrothricidae show significant variation also in fundamental spermatological features and have to be considered separately.

Ophryoxus is in all spermatological features a typical anomopod. Spermatogenesis is of the vacuolar type and the spermatozoa are of the heliozoan type and very similar to those seen in the Aloninae or *Eurycercus* (Pl. 13:64).

Macrothrix has the vacuolar, anomopod type of spermatogenesis but its spermatozoa are ovoid cells with superficial "plaques" and filled with a dense tangle of tubular structures (Pl. 14); they thus differ from anything else seen in the Anomopoda.

Ilyocryptus and *Streblocerus* have such remarkable spermatozoa that they actually fall outside the normal limits of variation in other Anomopoda. The spermatozoa are fairly small and shaped like flat discs with the flattened nucleus in the centre (Pl. 13:55, 56). More important is that spermatogenesis is of the cystic type (Fig. 2A, Pl. 14:71) never found in typical Anomopoda but present in many euphyllopods and *Holopedium*.

The simplest explanation for these remarkable differences is that the recent macrothricids are remnants of a basal radiation within the anomopods, as shown in Fig. 8. *Streblocerus* and *Ilyocryptus* must have departed from the common stem early, when the ancestral anomopods had developed the anomopod limb features but still had cystic spermatogenesis and amoeba-like sperm. Both genera have preserved the original, cystic type of spermatognesis, and have evolved the unique disc-like spermatozoa. The presence of the latter in both genera is the reason why thay are derived from a common stem in Fig. 8.

Macrothrix has been given a somewhat more recent branch in Fig. 8., departing from the common stem after the typical anomopod spermatogenesis of the vacuolar type had developed, but before the appearence of the heliozoan type of spermatozoon. This point can, of course, be discussed, for the original sperm type within the *Macrothrix* line is unknown. However, a change

from an amoeba-like simple sperm to the complicated *Macrothrix* sperm seems more simple and probable than a change from the very complicated heliozoan type of sperm to a *Macrothrix* sperm, for both are strongly specialized and have nothing in common.

Ophryoxus, finally, is in all spermatological respects a typical anomopod and is therefore placed as a sister group of the more advanced anomopods in Fig. 8.

This discussion of the macrothricids is based almost exclusively on spermatological features, but the results do not seem to be in serious conflict with current views among modern taxonomists.

Actually the opinions regarding the macrothricids have been much dependent on changes in the general ideas on evolution within the Cladocera. Some early authors derived the Anomopoda from the Ctenopoda, accepting the daphniids, with more free-swimming habits and exclusively filter-feeders, as a kind of intermediate forms closely related to ctenopods (e.g., Woltereck 1912, 1919; Behning 1912; Litynski 1916; Eriksson 1930). Wesenberg-Lund (1904, 1926) introduced the opposite idea that ancestral anomopods had been shore or bottom forms like the chydorids. He was supported by Eriksson (1934) who had changed opinion since his first paper in 1930 and admitted that anomopods could not be derived from recent ctenopods but must have had an independent origin in conchostracan-like ancestors. Freyer (1974) is definitely of the opinion that ancestral macrothricids (and ancestral Anomopoda) were bottom-dwelling forms and that *Ophryoxus* and *Acantholeberis* show extensive preservation of primitive features supposed to be present in ancestral anomopods. *Ophryoxus*, while preserving both a scraping device for collecting particles and a filtering apparatus, is a fairly good swimmer, and can according to Freyer (1974) show how the evolution of the daphniid filter-feeding type was initiated.

The diagram in Fig. 8 agrees in essential points with these views of Freyer and other modern carcinologists. The Macrothricidae are derived directly from ancestral anomopods and have preserved some of their features, e.g., recurved abdomen, 5–6 pairs of legs, the two foremost functionally and structurally different from the following ones, long movable 1st antenna, primitive ephippial structure, etc. *Ophryoxus* has preserved the 6 pairs of legs and some additional, probably primitive, features which would be expected in ancestors of daphniids and chydorids: elongate shape, intestinal coeca, long, movable postabdomen. It has also acquired some of the specializations which are found in these advanced Anomopoda: head shield, intestinal loop. Actually it has many limb features which must be expected in a primitive chydorid (Eriksson 1934), particularly if *Eurycercus* is regarded as the most primitive representative of the family (Smirnow 1968). This is in harmony with its place in the diagram, which was dictated by its spermatological features: vacuolar type of spermatogenesis and heliozoan-like sperm, features which are characteristic of the advanced Anomopoda.

Freyer (1934) stated that some macrothricid lines have specialized strongly and are actually very far from the basic structure of ancestral Anomopoda. This is beautifully paralleled by the unique and specialized spermatozoa in *Ilyocryptus*, *Streblocerus* and *Macrothrix*.

The crucial point is that the diagram in Fig. 8, constructed mainly on the basis of spermatology, indicates a paraphyletic origin of the Macrothricidae. It is obvious that *Ophryoxus* and the more advanced Anomopoda had a common ancestor with vacuolar spermatogenesis and heliozoan-like sperm. It is equally obvious that *Streblocerus* and *Ilyocryptus* cannot share this ancestor, for they have preserved the primitive, cystic spermatogenesis. Also *Macrothrix* is given a branch of its own in the diagram, because it is unlikely that its remarkable spermatozoa have evolved from the completely different and specialized heliozoan type.

This paraphyletic state of the Macrothricidae may or may not be in conflict with earlier views, for most authors have not committed themselves. I have searched the literature for just *one* good apomorphous character which could support a monophyletic origin of the Macrothricidae but have failed to find one. The well-developed, movable 1st antenna is used diagnostically but is obviously a primitive (plesiomorphous) feature and has certainly been taken over from euphyllopod-like ancestors. This is in agreement with the occurrence of movable antennae in *Moina*, *Bosmina* males and sidids. This character certainly does not show that the macrothricids have a common ancestor not shared by other Cladocera.

The Moina problem.

Until quite recently the genus *Moina* was referred to the Daphniidae, and Goulden (1968), who established the family Moinidae, is apparently still of the opinion that the two families are closely related. His discussion *(op. cit.* p. 11) ends in the conclusion that the Moinidae and the Daphniidae have developed from a common stem, and that this stem is separate from the one giving rise to the Macrothricidae, Chydoridae, and Bosminidae. His main (and important) argument for this opinion is the similarity in the structure of the advanced filtering mechanism of the 3rd and 4th legs in both families. But he also points out that the Moinidae share many (probably primitive) features with the Macrothricidae and even Sididae, and that the Daphniidae are far more specialized in many features than the other families. Goulden also admits that the specialization of the 3rd and 4th limbs, which holds the Daphniidae and Moinidae together, must have begun in the common anomopod ancestors and is present though less advanced also in the other families (see also Freyer 1968).

After having seen the strange spermatozoa in *Moina brachiata* and *M. micrura* – with nuclear diverticula as axes in the pseudopodia – and having realized that they have a lumenal kind of spermatogenesis, I was first inclined to deny any relationship to the Daphniidae, which have small spermatozoa and the vacuolar kind of spermatogenesis. However, the light-microscopical evidence indicates that these extreme spermatozoa as well as those of *M. macrocopa* have evolved as specializations in advanced developmental lines within the genus *Moina*, whereas the supposedly primitive species of the genus have small spermatozoa. It is thus possible that ancestral Moinidae had typical, small anomopod spermatozoa, which then have specialized dramatically within some lines.

The question is how the type of spermatogenesis should be evaluated phylogenetically. The presence of the ancient lumenal type of sperm maturation would make it necessary to derive moinids from the "lower" macrothricid level (left arrow from *Moina* in the diagram, Fig. 8). This would actually exclude a closer relationship with the Daphniidae, and the Moinidae would be an independent end product of one branch of a macrothricid radiation.

If, because of leg arguments, we want to derive the Moinidae together (monophyletically) with the Daphniidae, we must presume 1) that both originally had inherited the vacuolar type of maturation, typical of all advanced anomopods, 2) that this was given up in the genus *Moina* in favour of a lumenal type, almost identical with that of the Sididae and many Euphyllopoda. Since the vacuolar type of maturation must be regarded as a complicated feature, such to-and-fro-evolution appears improbable (right arrow from *Moina* in the diagram, Fig. 8).

On the other hand, we have to face the fact that something exceptional has happened in the examined moinids *(M. brachiata, M. micrura,* and *M. macrocopa)*, which have developed these strange and large spermatozoa. Furthermore, the size of these spermatozoa is incompatible with vacuolar maturation. The change of the sperma-

togenetic type could, therefore, be part of the very exceptional spermatozoan specialization which we know has occurred.

This latter somewhat circumstantial argument can not be used for the *Moina* species with small spermatozoa, which, therefore, will give us some better information about the origin of the Moinidae. An ultrastructural examination of the spermatogenesis in such species is therefore badly needed.

Abbreviations and Symbols

ER = endoplasmic reticulum
Sm. = Småland, part of southern Sweden.
Öl. = Öland, island east of southern Sweden.
Gla = glutaraldehyde fixative, 2 %, in 0.05 M phosphate buffer.
1 % Os = fixed in 1 % OsO_4 in veronal acetate buffer (Palade 1952).
2 % Os = fixed in 2 % OsO_4 in 0.1 M cacodylate buffer, pH 7.4.
3-A = fixed in trialdehyde according to Kalt & Tandler (1971).
♂♂ = In the lists of material, ♂♂ means that more than three males were examined ultrastructurally. If the material consists of three or less, the exact number is given (e.g., 1 ♂).

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Færdig fra trykkeriet juli 1978.

Plates

Spermatozoa of different Anostaca, shown in the same scale.

1. *Branchinecta ferox* (Milne-Edwards). Gush Etzion, Israel. Fix. 2 % Os. Note plentiful smooth ER and deep depressions from cell surface (x).

2. *Branchinecta paludosa* (O. F. Müller). Godhavn, Greenland. Fix. 1 % Os.

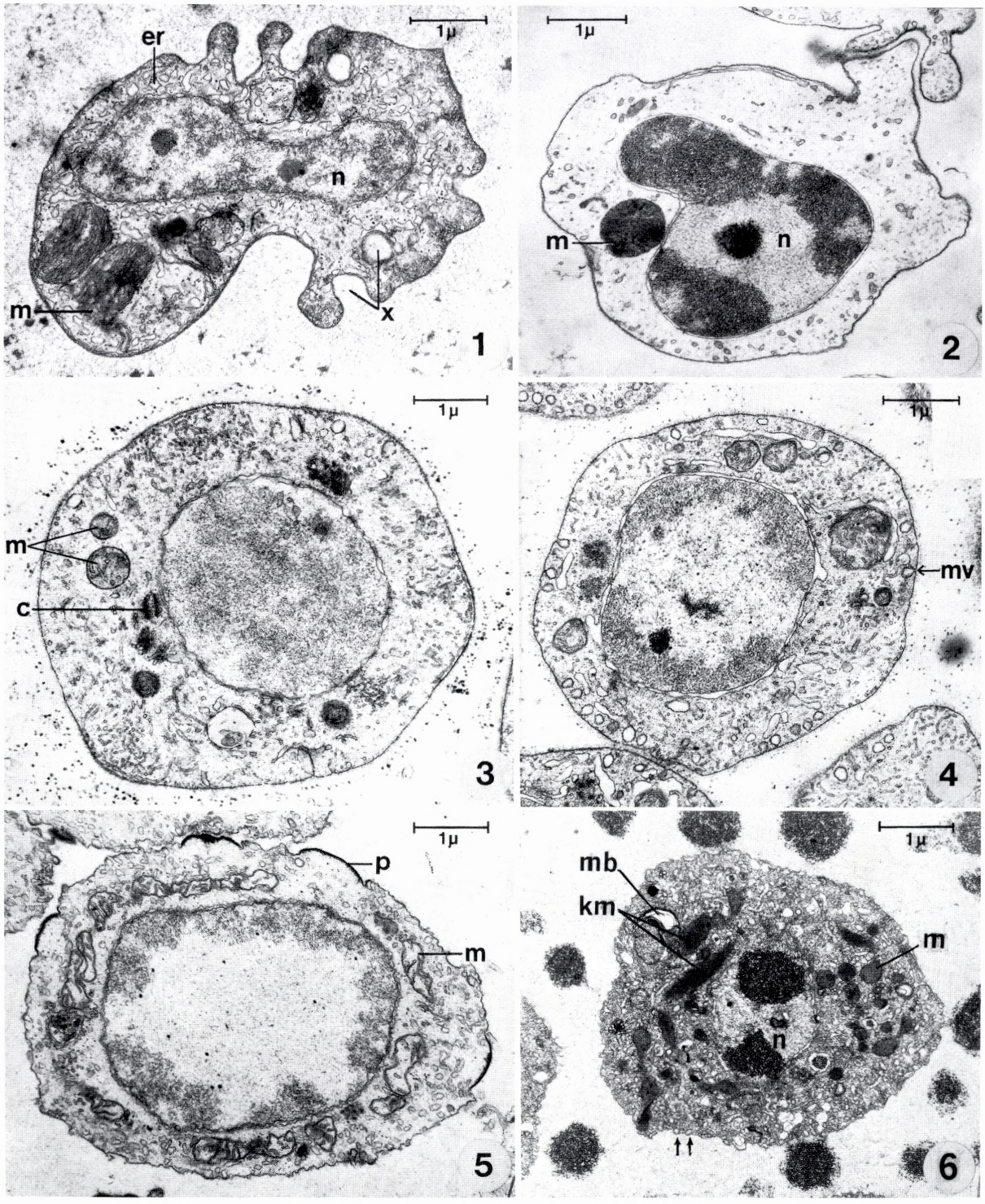
3. *Siphonophanes grubei* (Dybowski). Dyrehaven, Copenhagen. Fix. 1 % Os.

4. *Chirocephalus bairdi* (Brauer). Khirbet Kharaik, Israel. Fix. 2 % Os.

5. *Branchipus schaefferi* Fischer. Nahal-Zin, Israel. Fix. 2 % Os.

6. *Tanymastix stagnalis* (L.). Öland, Sweden. Fix. 3-A. Note crystalline inclusions in mitochondria (km) and numerous papillae on the surface (arrows).

Legends to all figures: c = centriole, er = endoplasmic reticulum, km = mitochondria with crystalline inclusions, m = mitochondria, mb = "myeloid body" with concentric membranes, mv = marginal vesicle with opening through cell membrane, n = nucleus, p = dark plaques of *Branchipus*, x = different aspects of depressions from the cell membrane.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7. *Streptocephalus torvicornis* (Waga). Ramle, Israel. Fix. 2 % Os. Mature spermatozoon in granular sperm fluid with globules (gl).

8. *Branchinella spinosa* (Milne-Edwards). Saline pond at Sebhka Zima, Morocco. Fix. 3-A. Mature spermatozoon with complicated dark plaques. Double magnification of such a plaque is seen in inset upper left, and tangential section of its honeycomb structure is seen in inset upper right.

9. *Artemia salina* (L.). Reared from commercial eggs. Fix. Gla with 0.124 M phosphate. Surface of mature spermatozoon, showing superficial papillae (arrows), containing a blind end of the tubular ER.

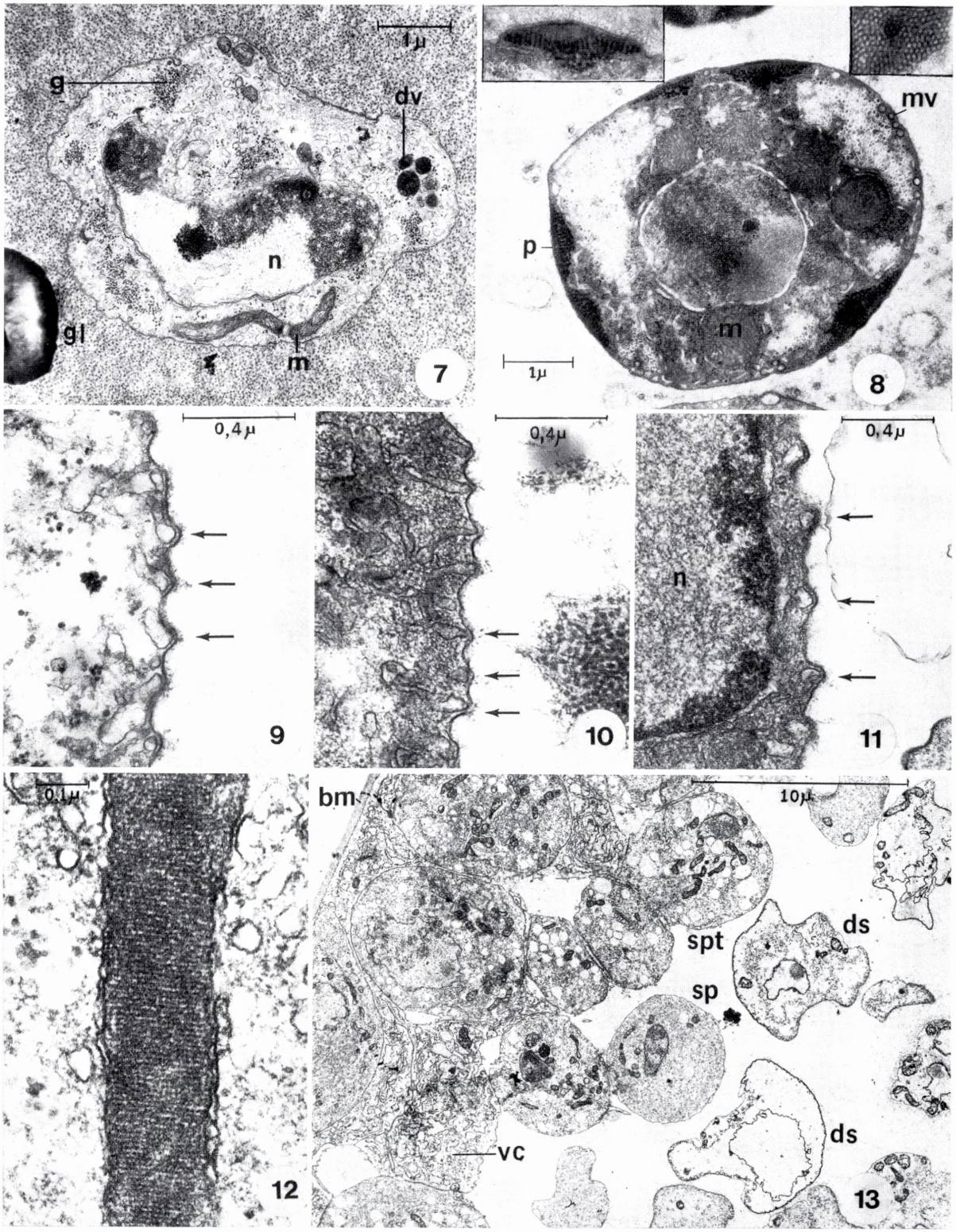
10. *Tanymastix stagnalis* (L.). Ottenby, Öland, Sweden. Fix. 3-A. Surface of spermatozoon for comparison with 9.

11. *Branchipus schaefferi* Fischer. Zeiselmauer, northwest Vienna. Fix. 3-A. Surface of spermatozoon for comparison with 9 and 10.

12. *Tanymastix stagnalis* (L.). Öland, Sweden. Fix. 3-A. Mitochondrion with crystalline matrix.

13. *Lepidurus apus lubbocki* Brauer. Zerachia, Israel. Fix. 2 % Os. Wall of testicle; in the lumen immature (spt), mature (sp) and degenerating (ds) spermatozoa.

Legends to all figures: bm = basement membrane, dv = dark vacuoles, ds = degenerating spermatozoa, g = glycogen, gl = dark globules in sperm fluid, m = mitochondria, mv = marginal vesicles, n = nucleus, p = plaques of *Branchinella*, sp = spermatozoa, spt = spermatids, vc = vegetative cell of testicular epithelium.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11
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12
bm
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spt
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vc
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13

Spermatozoa of Notostraca and Conchostaca

14. *Lepidurus a. apus* (Pallas). Dyrehaven, Copenhagen. Fix. 1 % Os.

15. *Triops c. cancriformis* (Bosc.). Zeiselmauer, NW Vienna. Fix. 3-A.

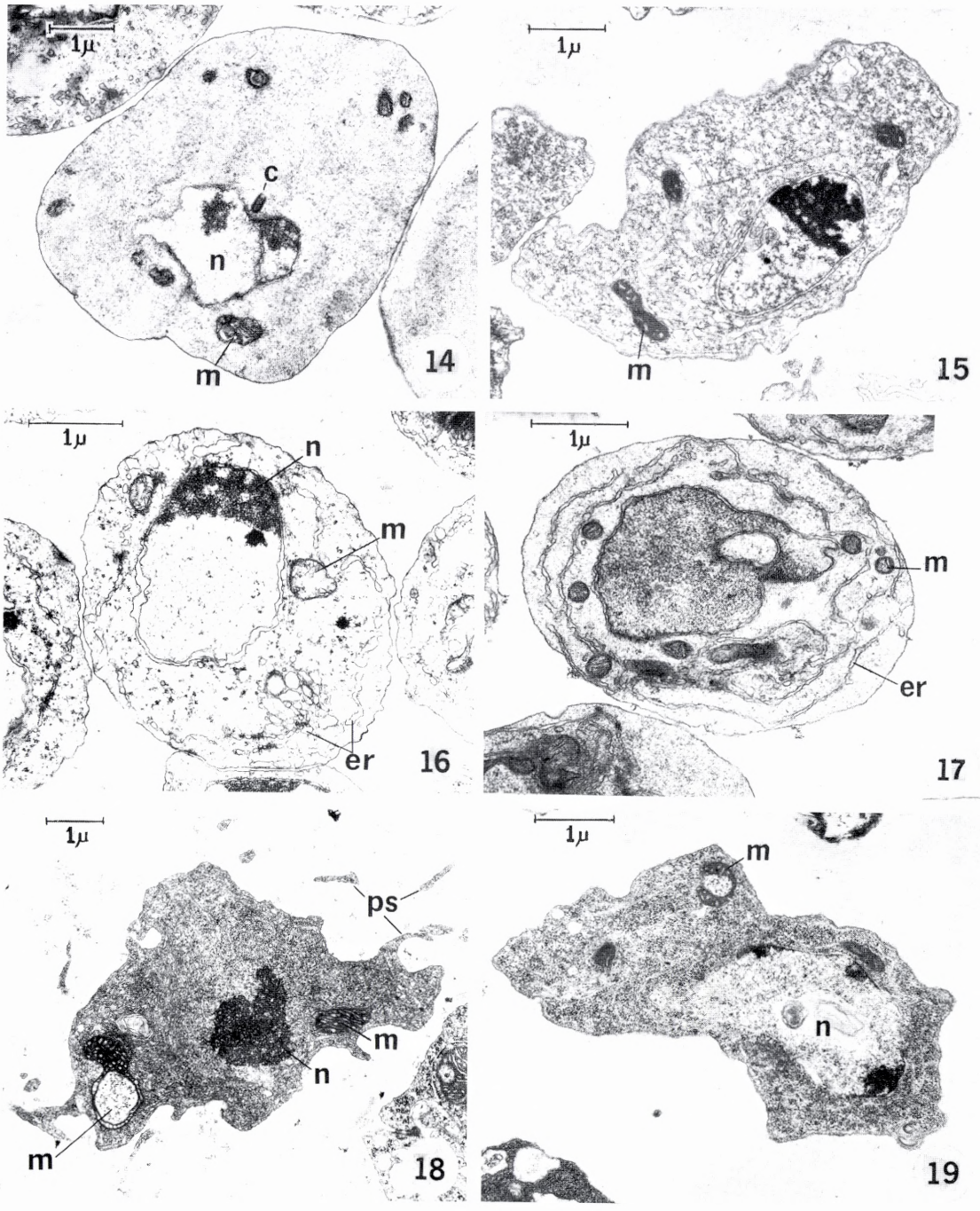
16. *Lynceus brachyurus* O. F. Müller. Dyrehaven, Copenhagen. Fix. 1 % Os.

17. *Cyzicus* sp. Gush-Etzion, Israel. Fix. 2 % Os.

18. *Leptestheria dahalacensis* (Rüppel). Zeiselmauer, northwest Vienna. Fix. 3-A.

19. *Imnadia yeyetta* Hertz. Zeiselmauer, northwest Vienna. Fix. 3-A.

Legends to all figures: c = centriole, er = endoplasmic reticulum, n = nucleus, m = mitochondria, ps = pseudopodia.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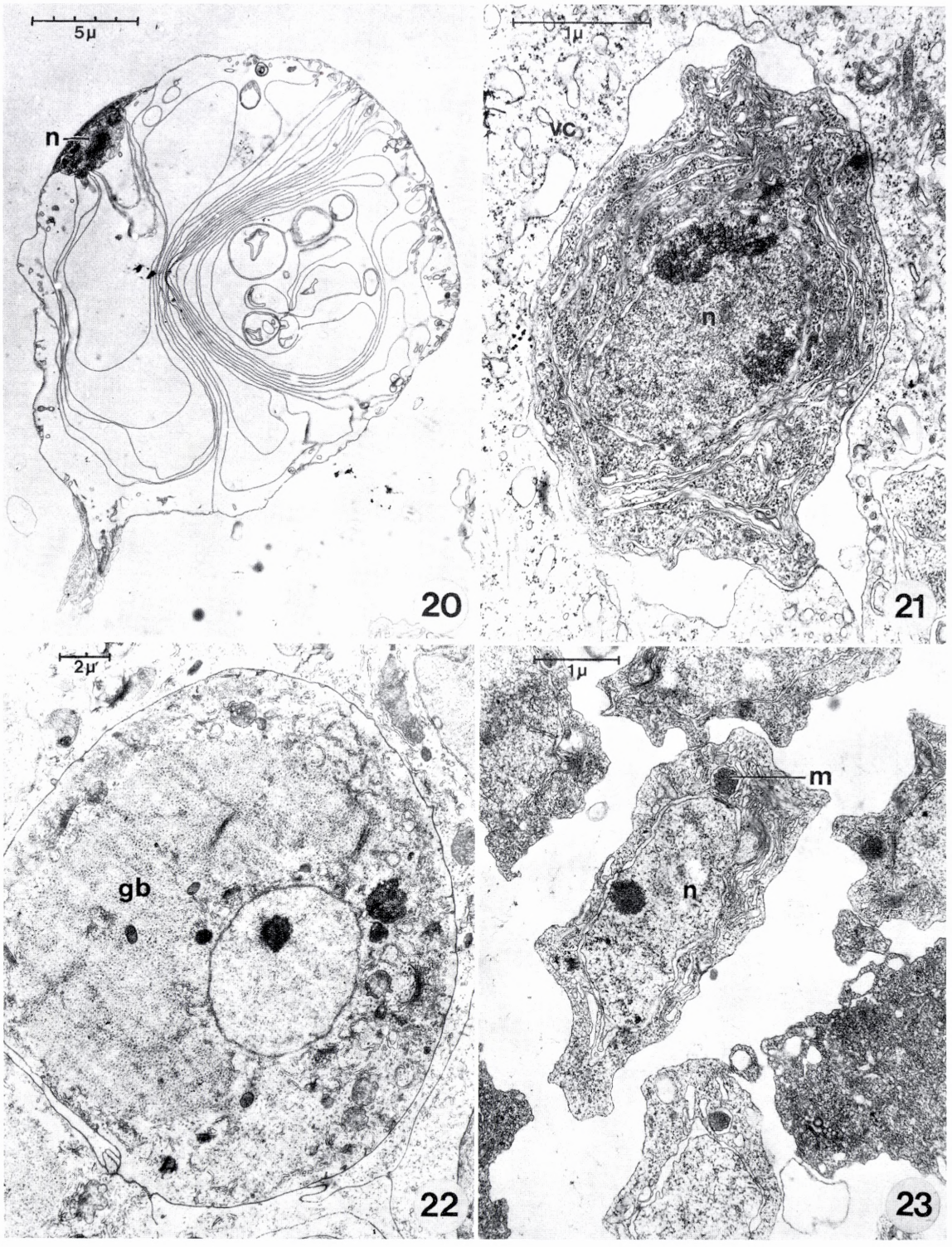
20. *Leptodora kindti* (Focke). Lyngby Sø, Zealand. Fix. 3-A. Mature spermatozoon in testicular fluid.

21. *Leptodora kindti* (Focke). Lyngby Sø, Zealand. Fix. 3-A. Spermatid with numerous ribosomes and beginning development of the ER sacs, lying between vegetative cells (vc).

22. *Latona setifera* (O. F. Müller). Løg Sø, Zealand. Fix. 2 % Os. Nearly mature spermatid. Note large glycogen body (gb).

23. *Holopedium gibberum* Zaddach. Lake Fiolen, Småland, Sweden. Fix. 3-A. Mature spermatozoon.

Legends to all figures: er = endoplasmic reticulum, gb = glycogen body, m = mitochondria, n = nucleus, vc = vegetative cell.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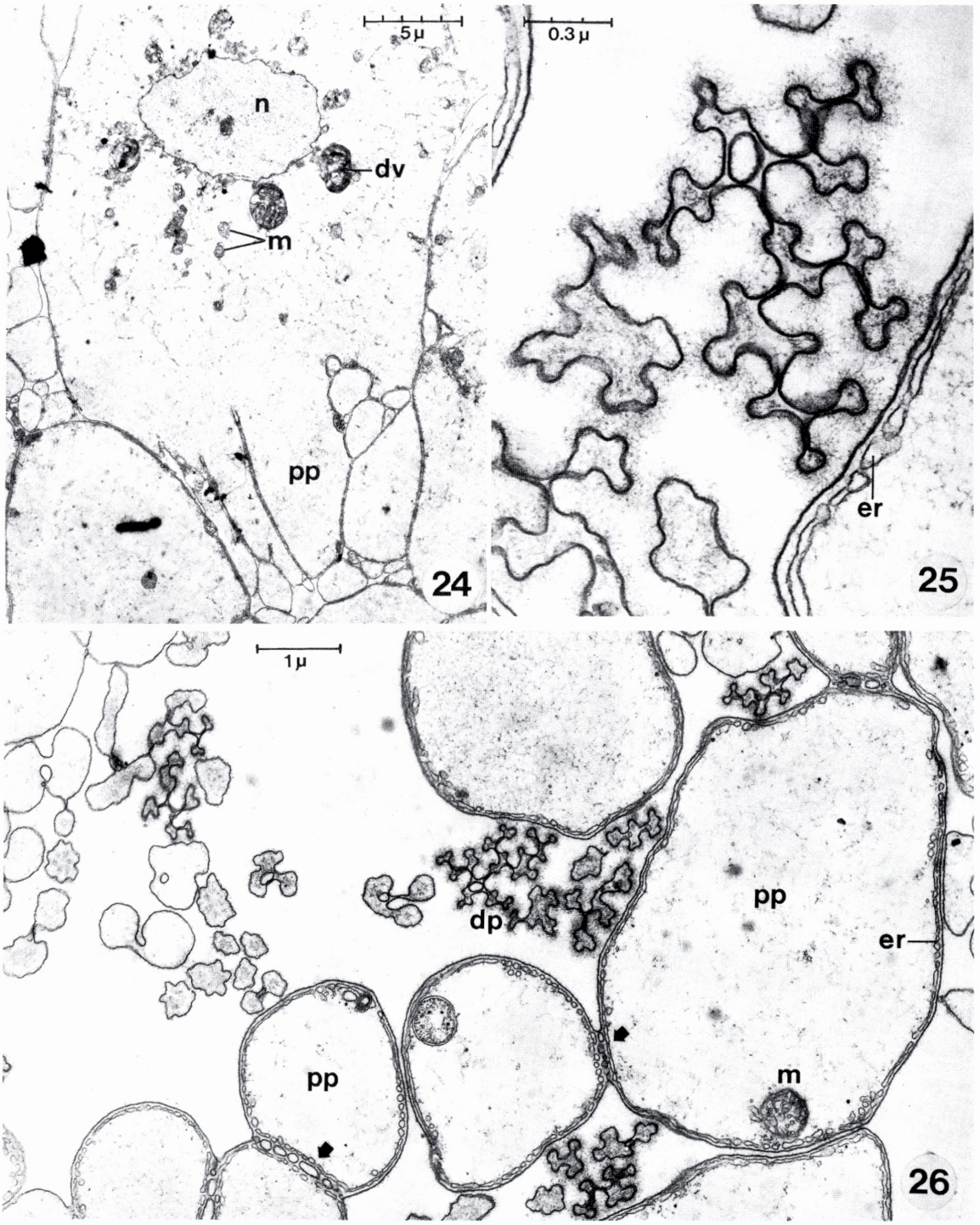
23

Mature spermatozoa of *Sida crystallina* O. F. Müller. Teglgårdssøen, Hillerød, Zealand. Fix. 2 % Os.

24. Part of cell body with lysosome-like dark vesicles (dv), nucleus (n), mitochondria (m), and proximal parts of pseudopodia (pp).

25. Cross sections of distal ramifications of pseudopodia. er = endoplasmic reticulum in basal part of pseudopodium seen in lower right corner.

26. Cross sections of pseudopodia. dp = distal ramifications, pp = proximal (basal) parts of pseudopodia, m = mitochondria, er = endoplasmic reticulum.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26

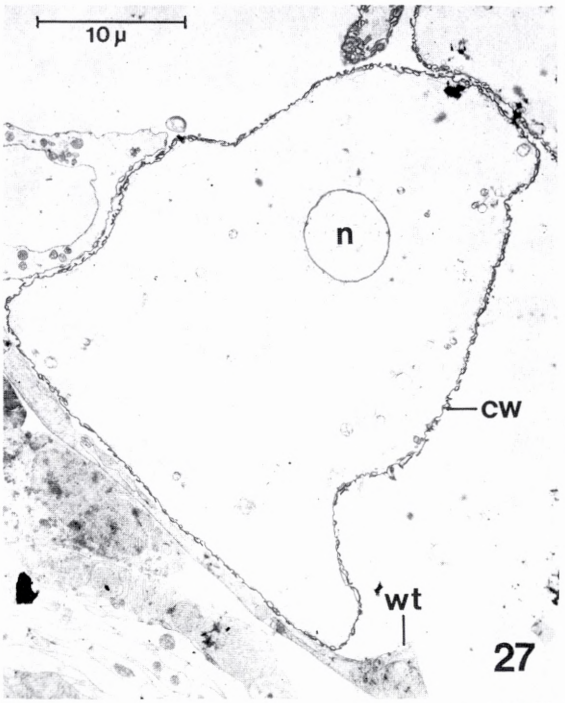
Diaphanosoma brachyurum (Lièvin).

27. Furesøen, Zealand. Fix. 1 % Os. Nearly mature spermatid, with vesicular nucleus (n), empty-looking plasm and complicated cell wall (cw). Mature spermatozoa are more than twice as large. wt = wall of testicle.

28. Furesøen, Zealand. Fix. 1 % Os. Cell walls of two spermatids. The cell wall is cut almost rangentially in (a), showing the separate vesicles, and perpendicularly in (b), showing communication of vesicles with the outside.

29. Lake Värmen, Småland, Sweden. Fix. 3-A. Two spermatids (a) and one mature spermatozoon (c). The upper spermatid still has but few vesicles. The lower spermatid has numerous vesicles, which open to the exterior (o). Microtubules (mt) in the walls between the vesicles are seen at lower left.

30. Furesøen, Zealand. Fix. 1 % Os. Young spermatid with fairly "normal" nucleus and nucleole, typical mitochondria (m) and some "dark vesicles" (dv).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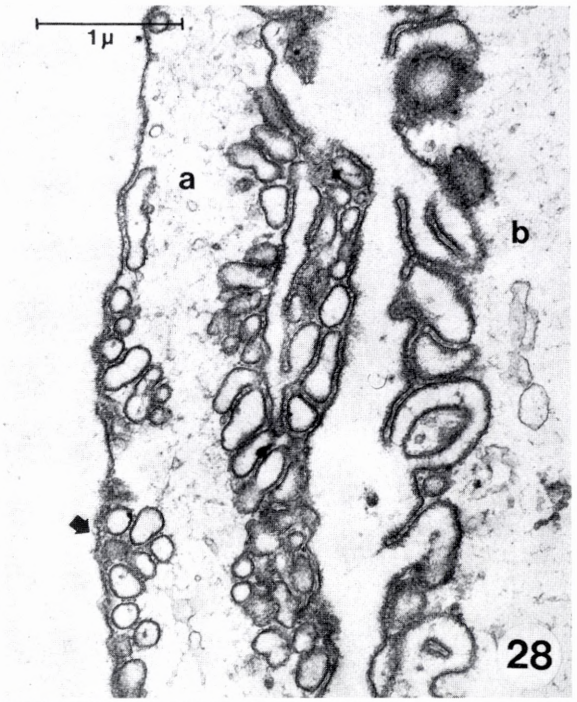
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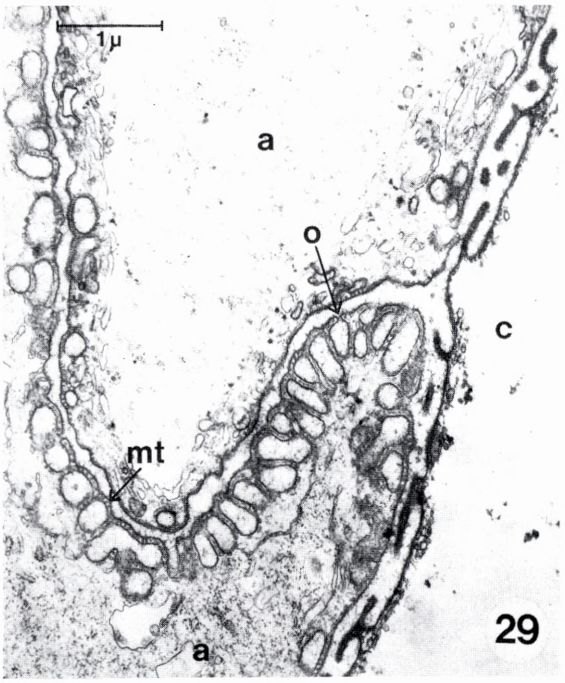
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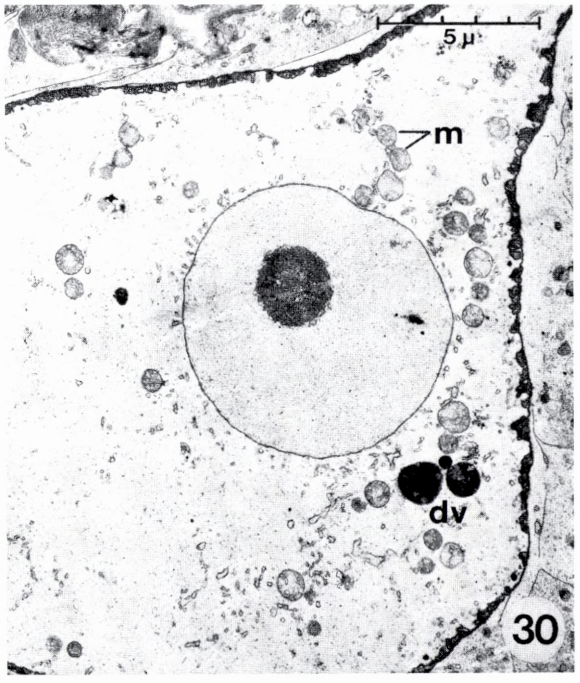
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31. *Daphnia longispina* O. F. Müller. Søborg Mose, Copenhagen. Fix. 1 % Os. Longitudinal section of rod-shaped spermatozoon.

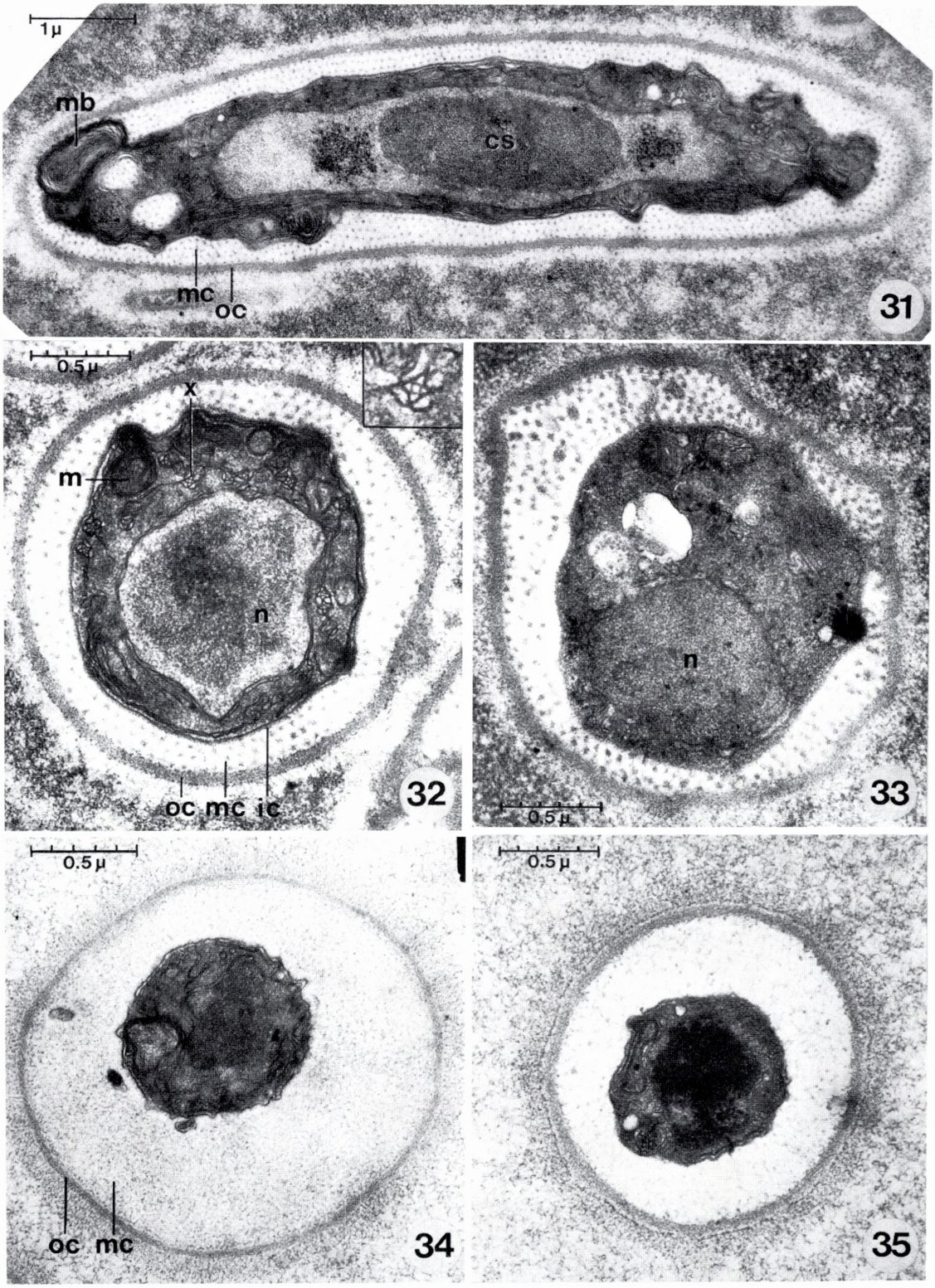
32. *Daphnia longispina* O. F. Müller. Søborg Mose, Copenhagen. Fix. 1 % Os. Cross section through nucleus of mature spermatozoon. Inset shows star-shaped cross section of tubular structures (x), enlarged three times that of the large figure.

33. *Daphnia cucullata* Sars. Lyngby Sø, Zealand. Fix. 3-A. Cross section through nucleus of mature spermatozoon.

34. *Daphnia atkinsoni* Baird. Gush Etzion, Israel. Fix. 2 % Os. Cross section through nucleus of mature spermatozoon.

35. *Daphnia magna* Straus. Emdrup Sø, Copenhagen. Fix. 1 % Os. Cross section through nucleus of mature spermatozoon.

Legends to all figures. cs = caryosome, ic = inner layer of the extracellular coat, m = mitochondria, mb = myelin-like body, mc = middle layer of extracellular coat, n = nucleus, oc = outer layer of extracellular coat, x = star-shaped cross section of tubular structures seen in *D. longispina*.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Variation of spermatozoan morphology within the genus *Ceriodaphnia*.

36. *Ceriodaphnia laticaudata* P. E. Müller. Faurholm Mose, South of Hillerød, Zealand. Fix. 2 % Os. Longitudinal section perpendicular to the flat surfaces of the flattened, rodshaped spermatozoon. Inset shows longitudinal section of cell margin with the bundle of microtubules (mb).

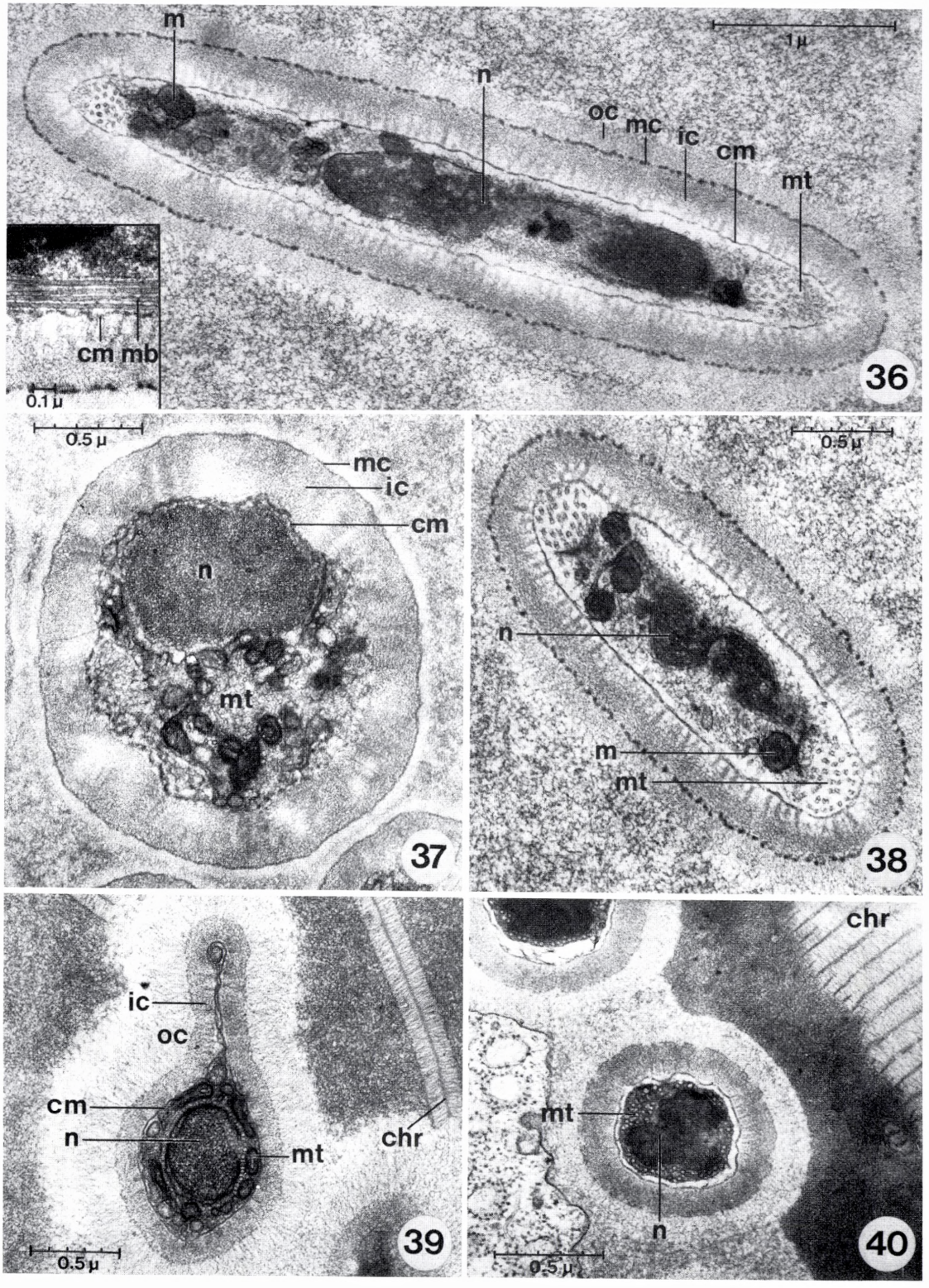
37. *Ceriodaphnia reticulata* (Jurine). Faurholm Mose, south of Hillerød, Zealand. Fix. 2 % Os. Cross section of short, rodlike spermatozoon.

38. *Ceriodaphnia laticaudata* P. E. Müller. Faurholm Mose, south of Hillerød, Zealand. Fix. 2 % Os. Cross section of flattened, rodlike spermatozoon.

39. *Ceriodaphnia pulchella* Sars. Teglgårdssøen, Hillerød, Zealand. Fix. 2 % Os. Cross section of rod-shaped spermatozoon. Note longitudinal ridge.

40. *Ceriodaphnia quadrangula hamata* Sars. Store Grib Sø, Zealand. Fix. 3-A. Cross section of rod-shaped spermatozoon.

Legends to all figures: chr = crystalline inclusions in sperm fluid, cm = cell membrane, ic = inner layer of extracellular coat, m = mitochondria, mb = bundle of microtubules, mc = middle layer of extracellular coat, mt = microtubules, n = nucleus, oc = outer layer of extracellular coat.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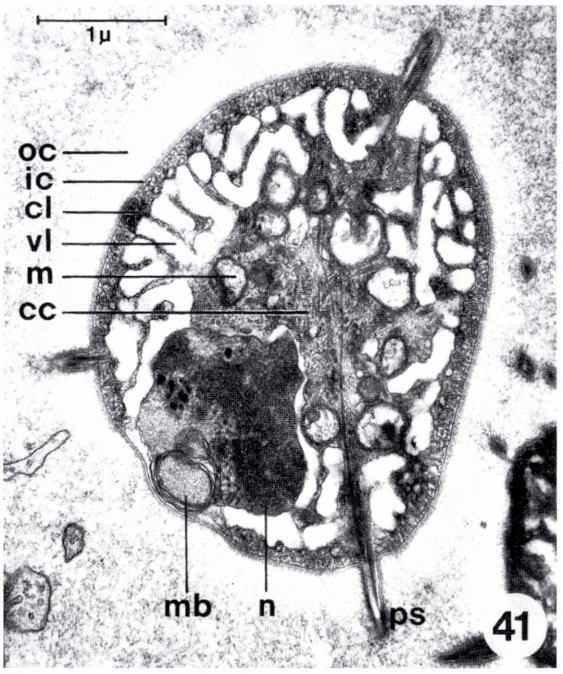
41. *Simocephalus serrulatus* (Koch). Store Grib Sø, Zealand. Fix. 2 % Os. Mature spermatozoon. Note radiation of axial rods from the cell centre (cc) into the pseudopodia (ps).

42. *Simocephalus vetulus* (O. F. Müller). Small pond at Zoological Central Institute, Copenhagen. Fix. 2 % Os. Mature spermatozoon with large myelin-like body (mb).

43. *Simocephalus vetulus* (O. F. Müller). Small pond at Zoological Central Institute, Copenhagen. Fix 2 % Os. Spermatid within vegetative cell. Note centriole (c) and tubules in cell centre and beginning development of vacuolated zone (vl).

44. *Simocephalus vetulus* (O. F. Müller). Small pond at Zoological Central Institute, Copenhagen. Fix. 1 % Os. Spermatozoon immediately after being liberated. Note axial tod going into the pseudopodium (ps).

Legends to all figures: cc = cell centre with tubules, centrioles, and mitochondria, cl = cortical layer with irregular, radiating tubules, ic = inner layer of extracellular coat, m = mitochondria, mb = myeloid body, n = nucleus, oc = outer layer of coat, ps = pseudopodia, vl = vacuolated layer of spermatozoan plasm.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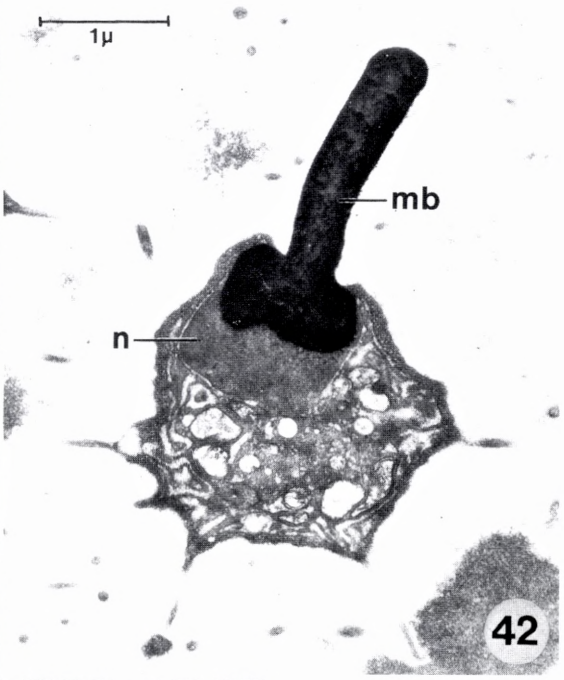
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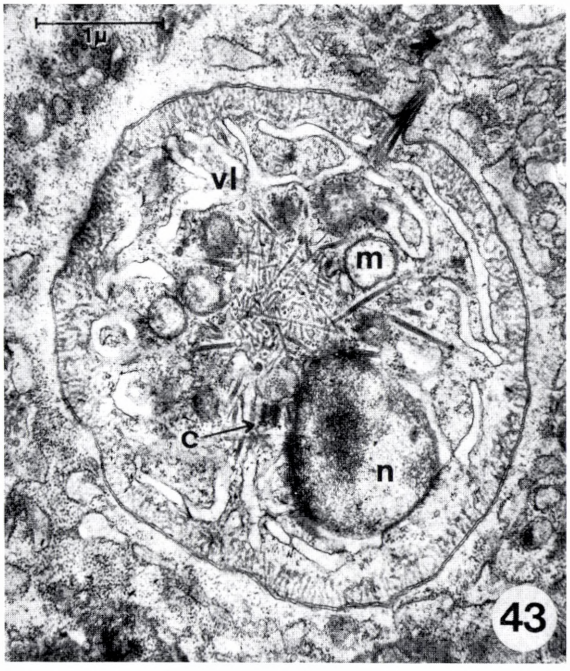
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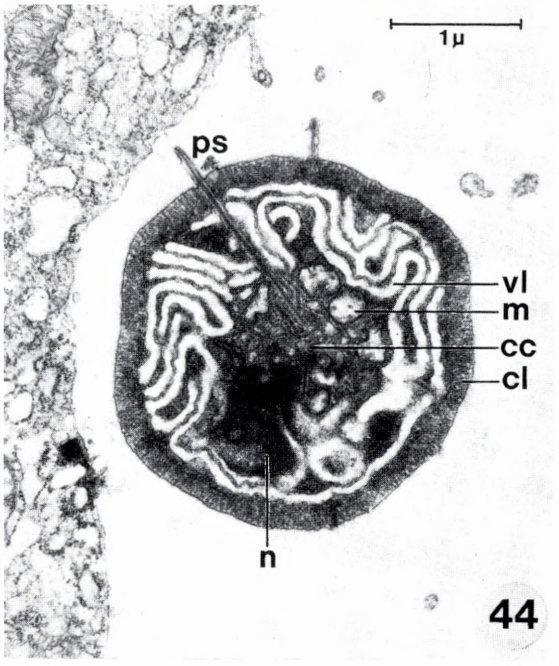
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45-47. *Simocephalus congener* Schoedler. Faurholm mose, Zealand.

45. Abdominal claw of large female with many relatively small teeth in the comb.

46. Abdominal claw of male. Comb with many slender teeth.

47. Fix. 2 % Os. Mature spermatozoon. Longitudinal section.

48-50. *Simocephalus exspinosus* (Koch). Søborghus, Copenhagen. For comparison with *S. congener* (45 – 47).

48. Abdominal claw of large female, with relatively few and strong spines in the comb.

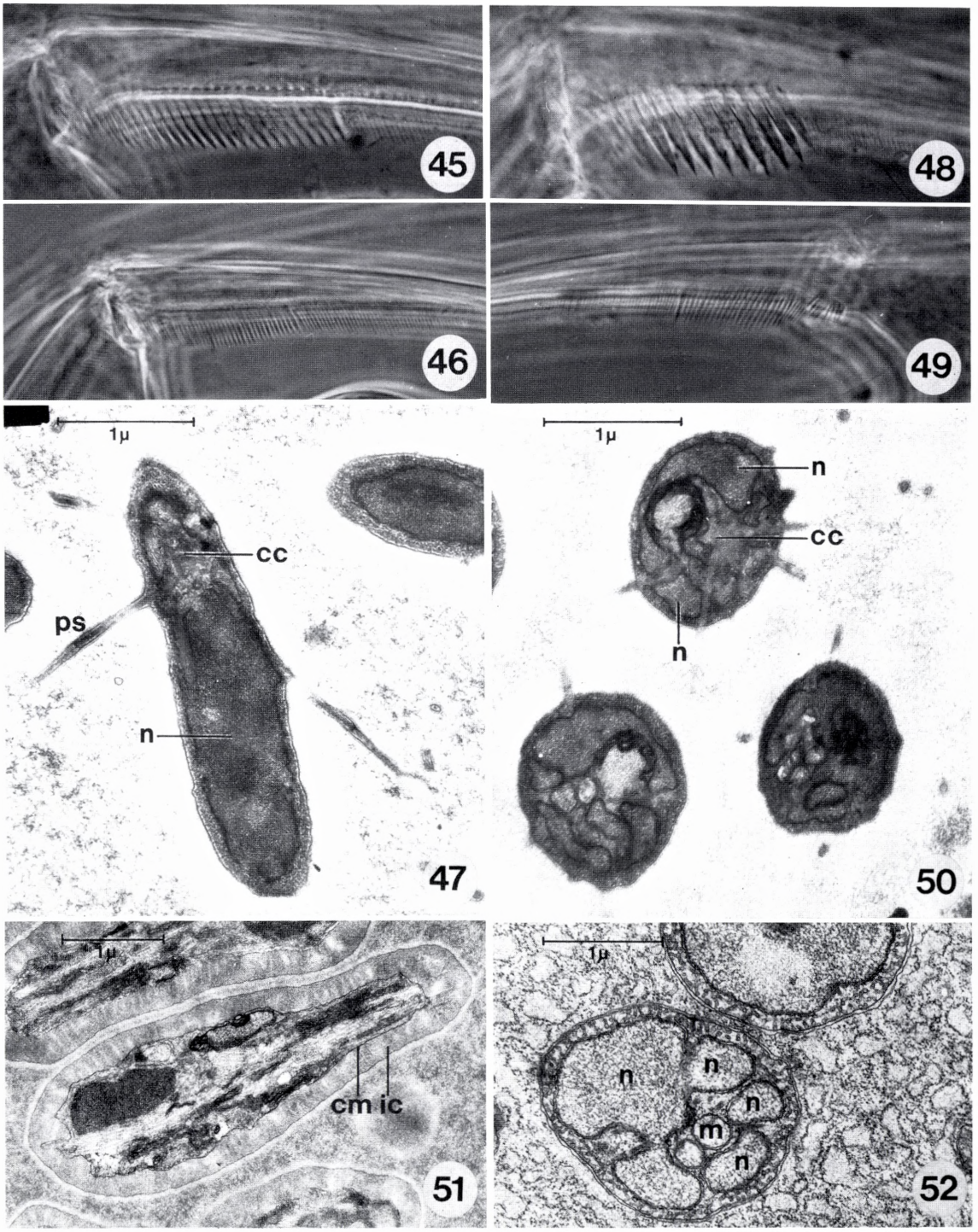
49. Abdominal claw of male. Comb essentially identical with that of male *S. congener* (46).

50. Fix. 2 % Os. Mature spermatozoa of spherical shape.

51. *Ceriodaphnia reticulata* (Jurine). Faurholm Mose, south of Hillerød, Zealand. Fix. 2 % Os. Longitudinal section of mature spermatozoon.

52. *Simocephalus exspinosus* (Koch). Søborghus, Copenhagen. Fix. 2 % Os. Spermatids with lobate nucleus (n), lying in vacuoles in nutritive cells. Note two cell membranes all the way round: that of the spermatid and that of the host cell.

Legends to all figures: cc = cell centre, cm = cell membrane of spermatozoon, ic = inner layer of extracellular coat, n = nucleus, ps = pseudopodium.


45
48
46
49
1μ
cc
ps
n
47
1μ
n
cc
n
50
1μ
cm ic
51
1μ
n
n
n
m
n
52

53. *Scapholeberis aurita* (Fischer). Emdrup Sø, Copenhagen. Fix. 1 % Os. Nucleus (n) and mitochondria (m) hardly visible in the dense plasm. Inset shows *Scapholeberis mucronata* (O. F. Müller). Lyngby Sø, Zealand. Fix. 1 % Os. The same magnification as large figure.

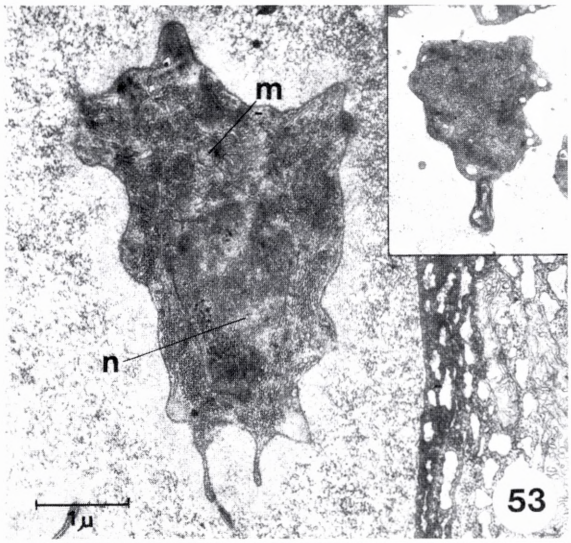
54. *Moina brachiata* (Jurine). Slaglille, near Sorø, Zealand. Fix. 2 % Os. Light microscopy. Shows fixed and free spermatids (spt) and mature spermatozoon (msp), the latter with pseudopodia (ps). The proximal branching point of the pseudopodium is marked by (bp).
55. *Moina brachiata* (Jurine). Slaglille, near Sorø, Zealand. Fix. 3-A. Chromatin tubules (ct) are seen entering nuclear diverticulum near top of figure.

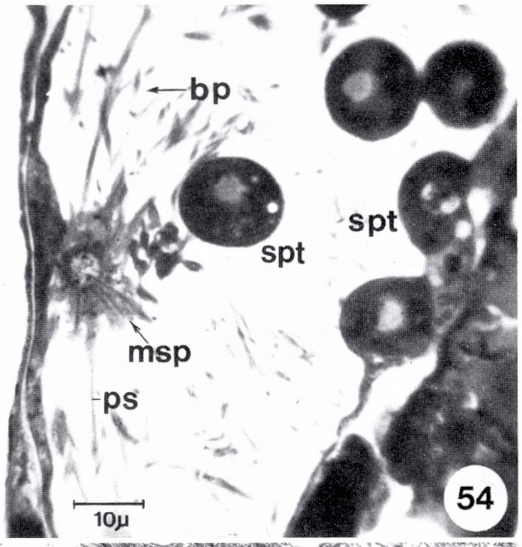
56. *Moina micrura* Kurtz. Barbados. Fix. 3-A. Nucleus with numerous bundles of chromatin tubules, some of wich ære also seen in pseudopodia inside nuclear diverticula.

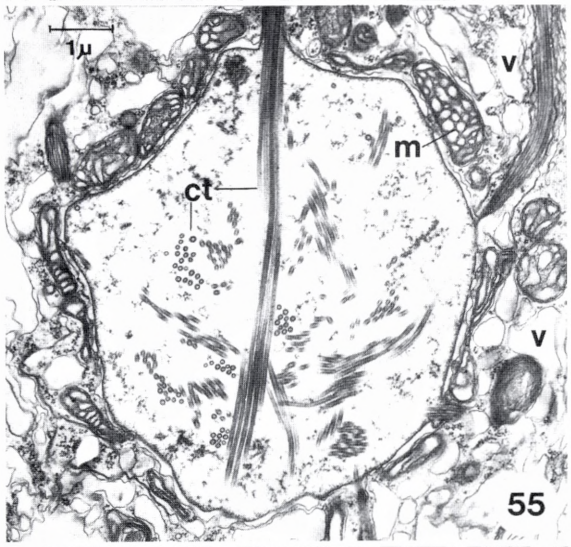
57. *Moina brachiata* (Jurine). Slaglille, near Sorø, Zealand. Fix. 3-A. Spermatid forming bundles of chromatin tubules (ct), one of which pushes the nuclear envelope out (beginning formation of nuclear diverticulum). Inset shows *Moina micrura*. Barbados. Fix. 3-A. Formation of bundle of chromatin tubules in spermatid. Cross-striated rods and some tubules are seen.

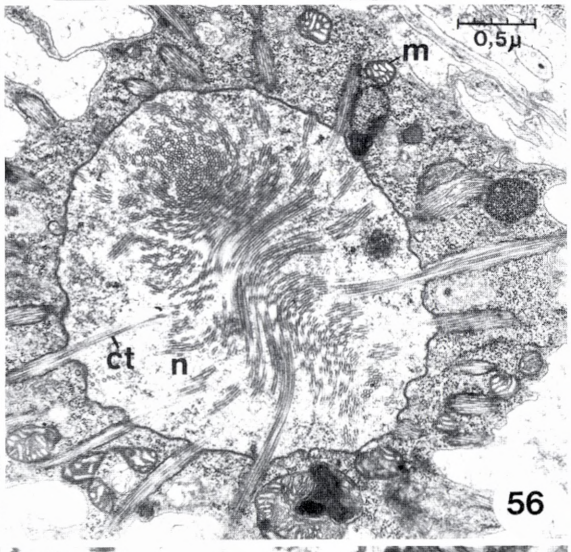
58. *Moina brachiata* (Jurine). Slaglille near Sorø, Zealand. Fix. 3-A. Bundle of chromatin tubules can be seen entering nuclear diverticulum, which is formed by the two membranes of the nuclear envelope.

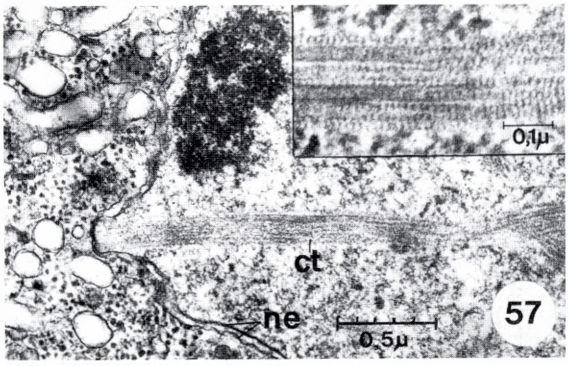
Legends to all figures: bp = branching point of pseudopodium, ct = chromatin tubules, m = mitochondria, msp = mature spermatozoon, n = nucleus, ne = nuclear envelope, ps = pseudopodia, spt = spermatids, v = vacoules in the plasm.

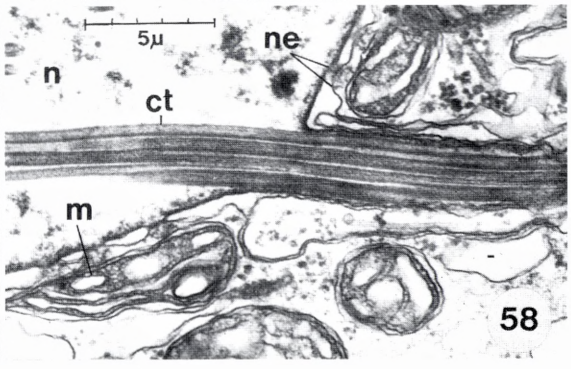

m
n
1µ
53


bp
spt
spt
msp
ps
10µ
54


1µ
v
m
ct
v
55


0,5µ
m
ct
n
56


0,1µ
ct
ne
0,5µ
57


5µ
ne
n
ct
m
58

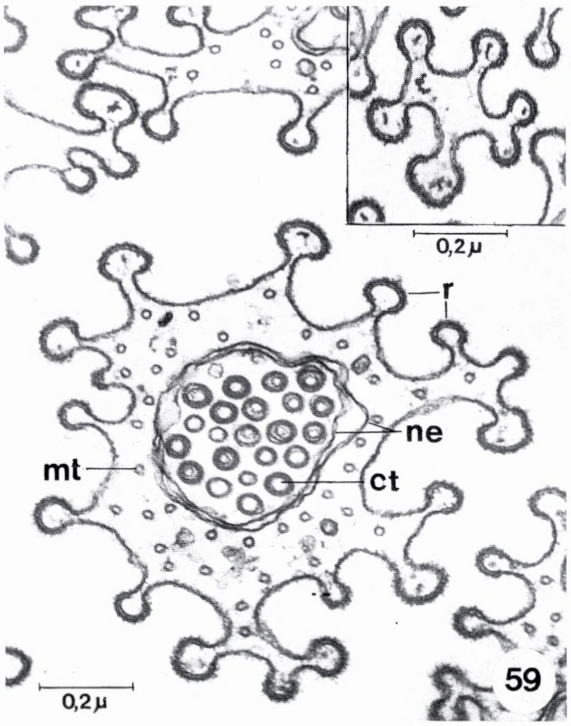
59. *Moina brachiata* (Jurine). Slaglille near Sorø, Zealand. Fix. 3-A. Cross section of stem of pseudopodium. Inset shows cross section of side branch of pseudopodium.

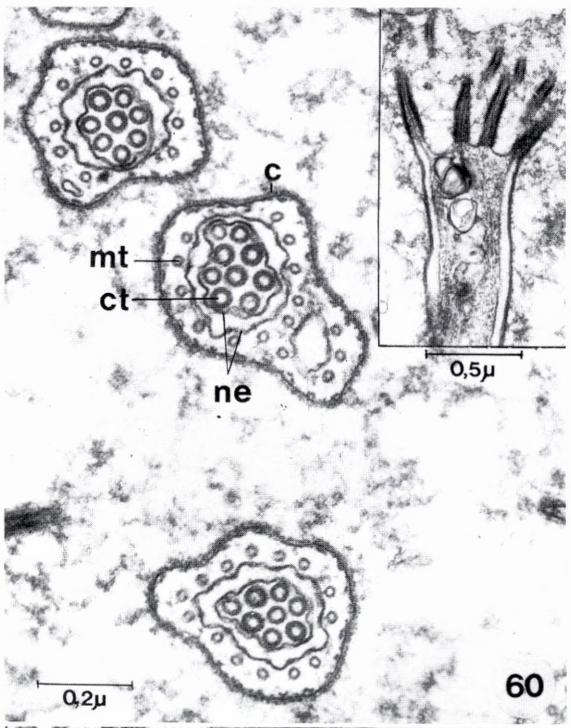
60. *Moina micrura* Kurtz. Barbados. Fix. 3-A. Cross section of pseudopodial stems. Inset shows longitudinal section through pseudopodial end, with fingerlike villi.
Diego County, U.S.A. Fix. 3-A. Cross section of spermatozoon through the nucleus (n). Inset shows phase contrast of living spermatozoa suspended in water.

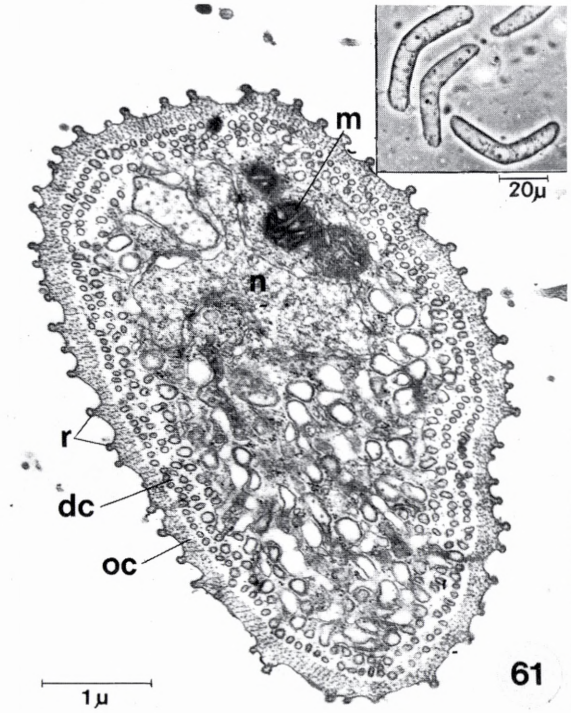
61. *Moina macrocopa* (Straus). Del Mar, San Diego County, U.S.A. Fix. 3-A. Cross section of spermatozoon through the nucleus (n). Inset shows phase contrast of living spermatozoa suspended in water.

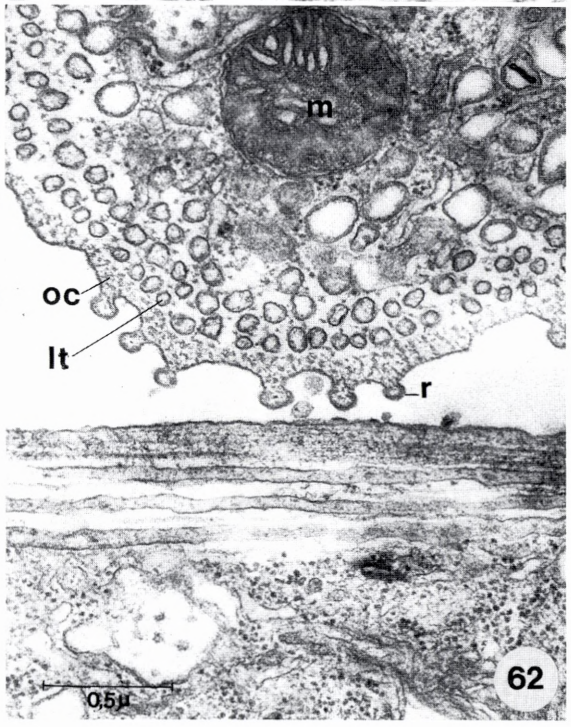
62. *Moina macrocopa* (Straus). Del Mar, San Diego County, U.S.A. Cross section (above) and longitudinal section (below) of mature spermatozoa. Note remarkable dimensions and irregular shape of the longitudinal microtubules in the deep cortex.

Legends to all figures: c = coat material, ct = chromatin tubules, dc = deep cortex with tubules, lt = longitudinal tubules, m = mitochondria, mt = microtubules, ne = nuclear envelope, oc = outer cortex with filaments, r = longitudinal ridges.


0,2 µ
r
ne
mt
ct
0,2 µ
59


c
mt
ct
ne
0,5µ
0,2µ
60


20µ
m
n
r
dc
oc
1 µ
61


m
oc
lt
r
0,5µ
62

63. *Bosmina longispina* Leydig. Lake Fiolen, Småland, Sweden. Fix. 3-A. Mature spermatozoon.

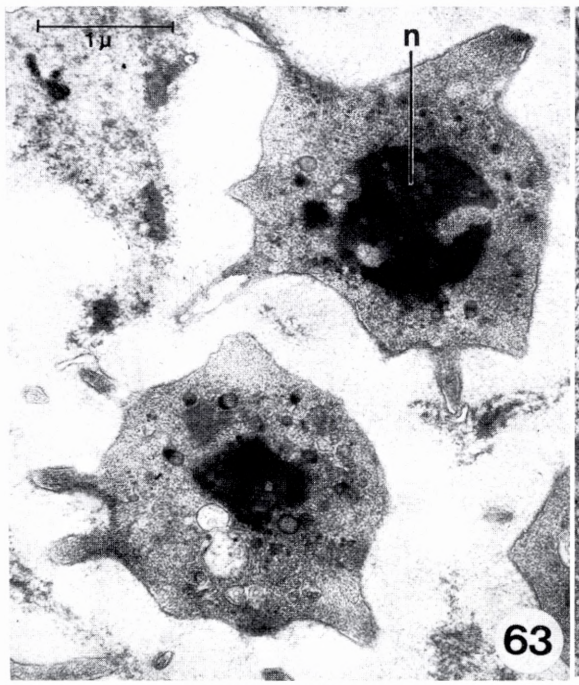
64. *Ophryoxus gracilis* G. O. Sars. Lake Fenen, Småland, Sweden. Fix. 3-A. Nearly mature spermatid in its vacuole in the vegetative cell. Cell centre (cc), degenerate mitochondria (m) and one radiating dark rod are seen. Inset shows cross section of dark rod in mature spermatozoon.

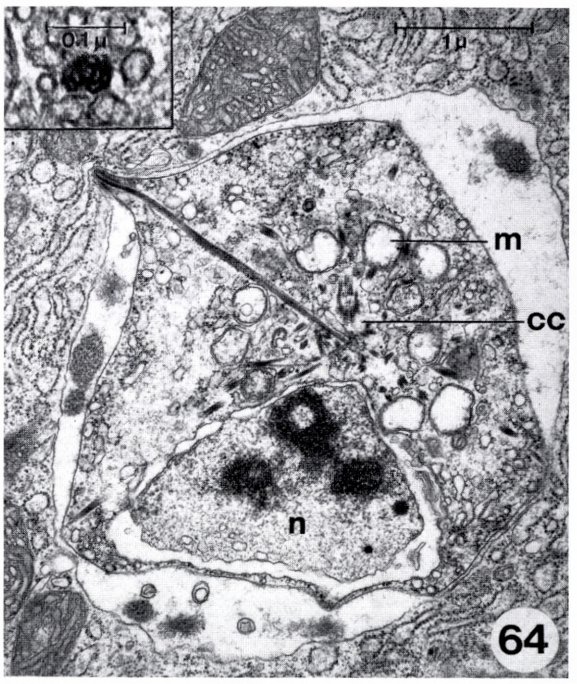
65. *Streblocerus serricaudatus* (Fischer). Hjortesølet, noth of Hillerød, Zealand, Fix. 3-A. Cross section of the disc-like mature spermatozoon.

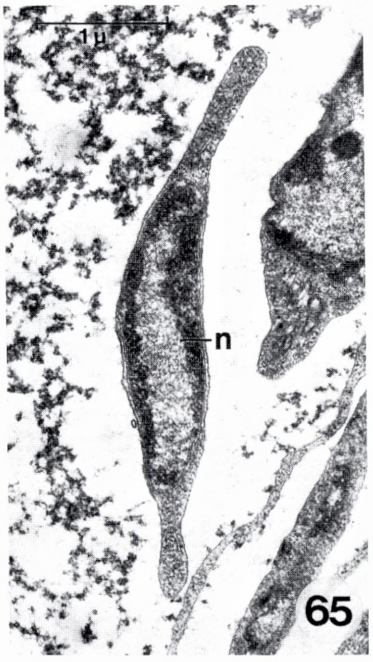
66. *Ilyocryptus agilis* Kurtz. Løg Sø, Zealand. Fix. 2 % Os. Cross section of mature, disc-like spermatozoa.

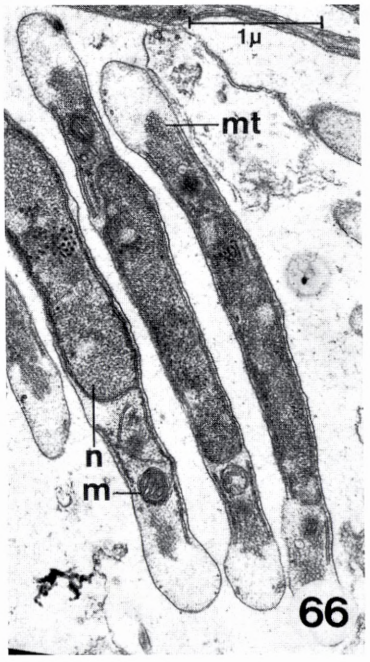
67. *Ilyocryptus agilis* Kurtz. Løg Sø, Zealand. Fix. 3-A. Margin of disc-like mature spermatozoon showing marginal bundle of microtubules (mt) in a darker matrix.

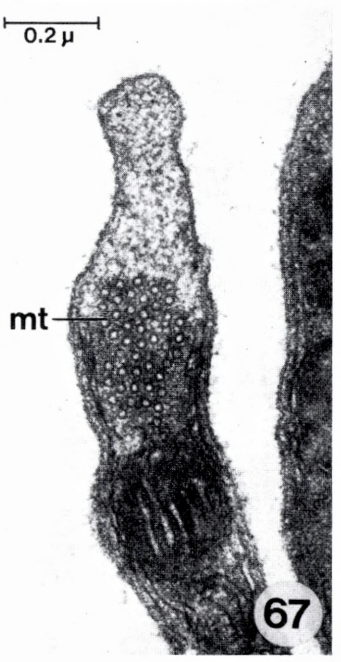
Legends to all figures: cc = cell centre, m = mitochondria, mt = microtubules, n = nucleus, v = vacuole in nutritive cell, containing the spermatid.


1 µ
n
63


0.1 µ
1 µ
m
cc
n
64


1 µ
n
65


1 µ
mt
n
m
66


0.2 µ
mt
67

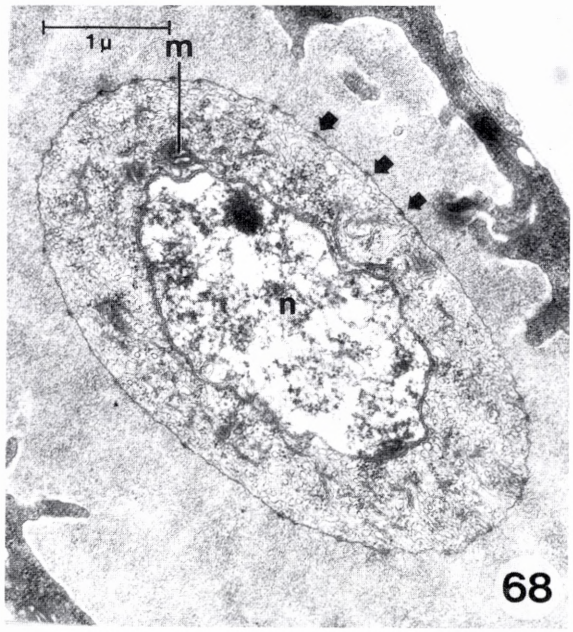
68. *Macrothrix laticornis* (Jurine). Copenhagen. Fix. 3-A. Mature spermatozoon. Arrows mark superficial "knobs", which are shown in higher magnification in 69.

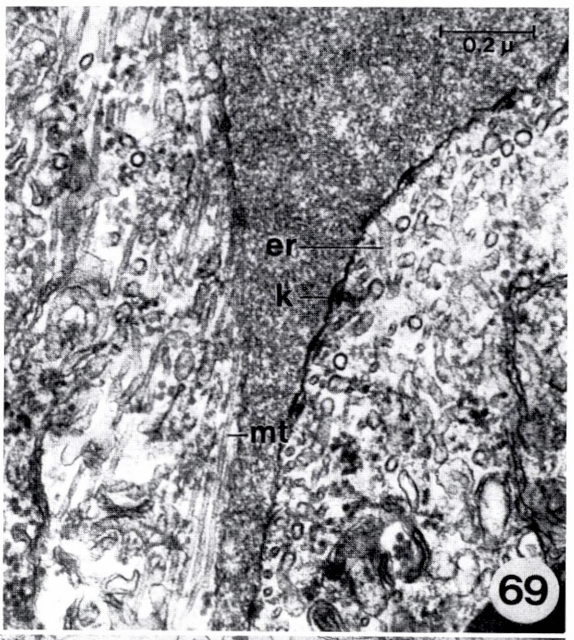
69. *Macrothrix laticornis* (Jurine). Copenhagen. Fix. 3-A. Superficial parts of mature spermatozoa, showing "knobs" (k), microtubules (mt) and endoplasmic tubules (er).

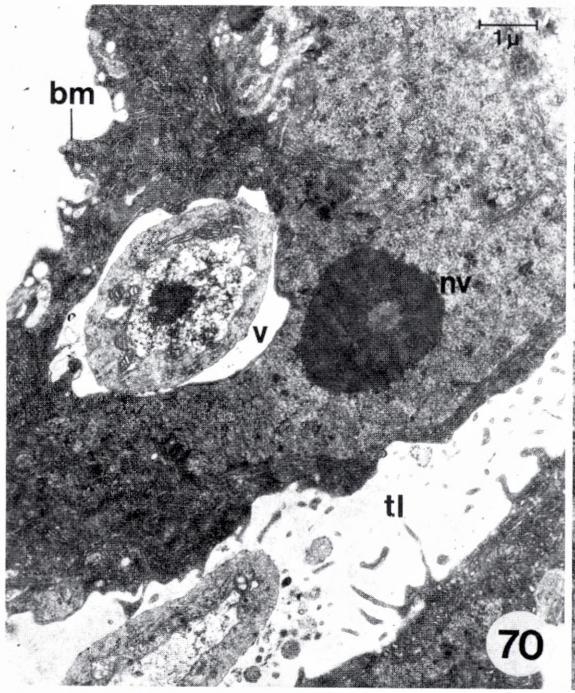
70. *Macrothrix laticornis* (Jurine). Copenhagen. Fix. 3-A. Late spermatid still inside vacuole (v) in nutritive cell.

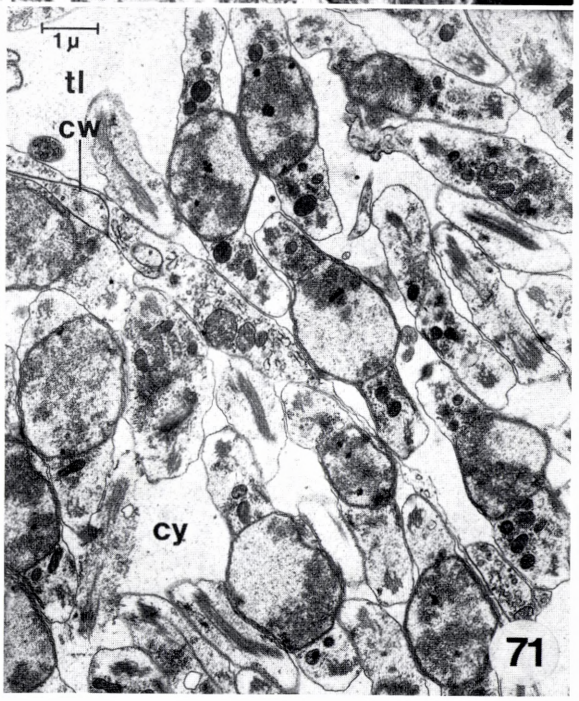
71. *Ilyocryptus gracilis* Kurtz. Løg Sø, Zealand. Fix. 2 % Os. Part of spermatogenic cyst (cy) and its wall (cw). The testicular lumen (tl, upper right) is filled with submature cells.

Legends to all figures: bm = basement membrane of testicular epithelium, cw = wall of spermatogenic cyst, cy = lumen of same, er = tubular endoplasmic reticulum, k = "knobs" on the surface of *Macrothrix*, m = mitochondria, mt = microtubules, n = nucleus, nv = nucleus of vegetative cell, tl = testicular lumen. v = vacuole in nutritive cell.


1µ
m
n
68


0,2 µ
er
k
mt
69


1µ
bm
nv
v
tl
70


1µ
tl
cw
cy
71

72. *Eurycercus lamellatus* (O. F. Müller). Teglgårdssøen, Hillerød, Zealand. Fix. 3-A. Mature spermatozoon. Vestigial dark rods are present in the cell centre (cc).

73. *Eurycercus lamellatus* (O. F. Müller). Teglgårdssøen, Hillerød, Zealand. Fix. 3-A. Pseudopodium of spermatid with dark axis (ax), extended between adjacent spermatids. Inset shows cross section of dark rod with star-shaped arrangement of dark matter.

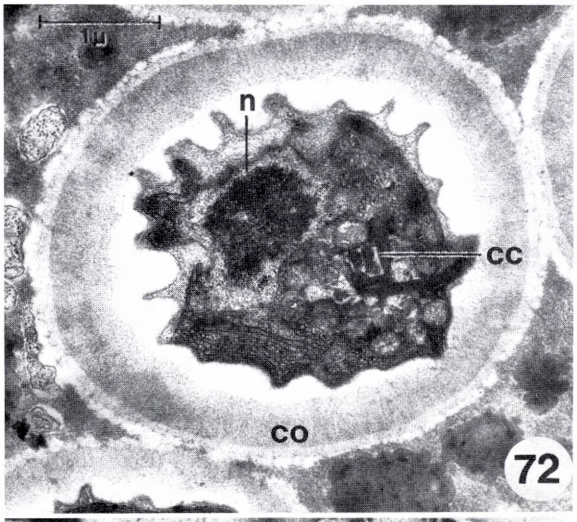
74. *Acroperus harpae* (Baird). Hultsjön, Småland, Sweden. Fix. 1 % Os. Mature spermatozoa.

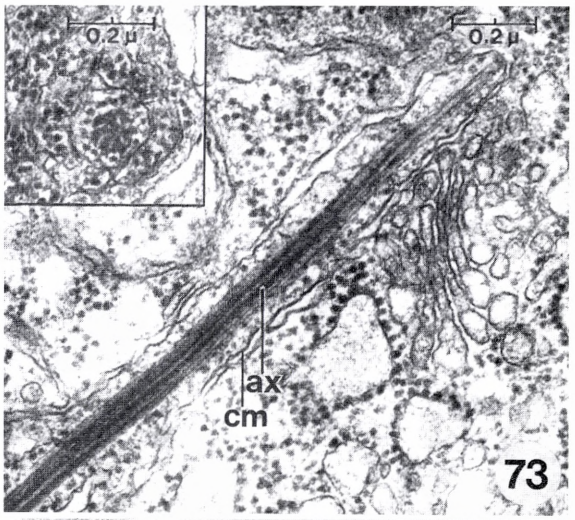
75. *Acroperus elongatus* (G. O. Sars). Lake Möckeln, Småland, Sweden. Fix. 3-A. Mature spermatozoon.

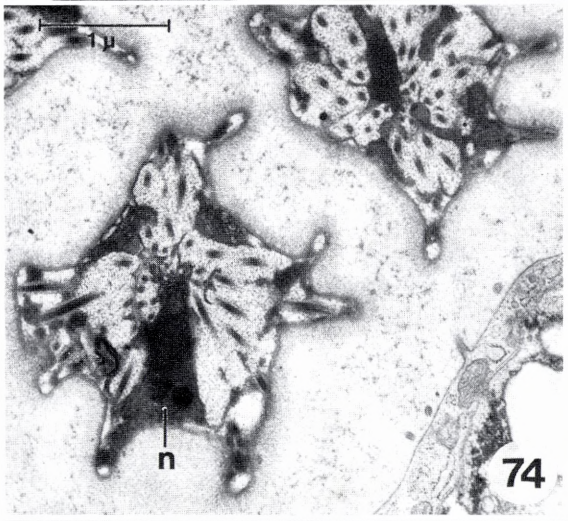
76. *Graptoleberis testudinaria* (Fischer). Store Grib Sø, Zealand. Fix. 3-A. Mature spermatozoon.

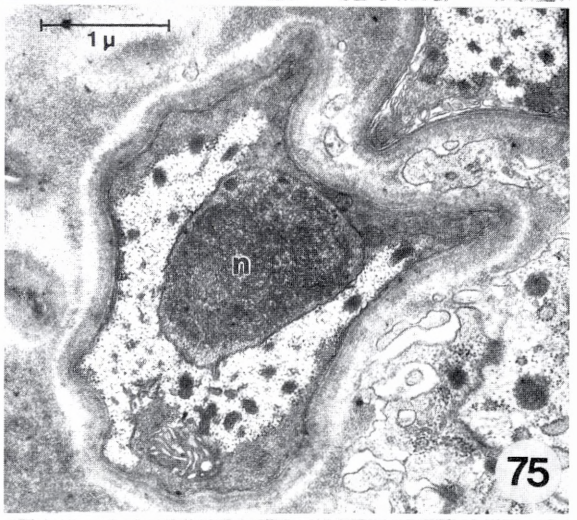
77. *Leydigia acanthoceroides* (Fischer). Hillerød, Zealand. Fix. 1 % Os. Mature spermatozoon.

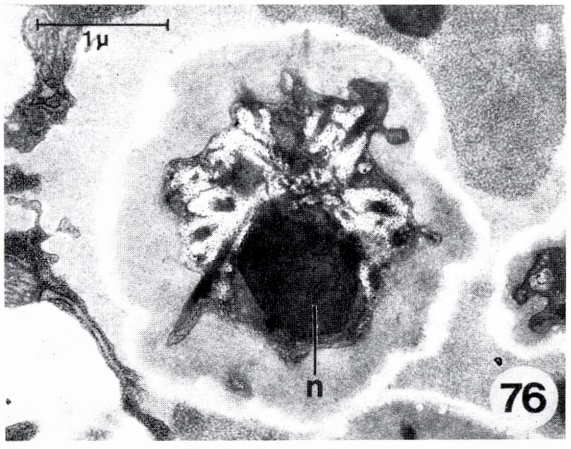
Legends to all figures: ax = dark axis in pseudopodium, cc = cell centre, cm = cell membrane of axopodium, co = extracellular coat, dp = dark plasm, dr = dark rods, lp = light plasm, n = nucleus, ps = pseudopodium.

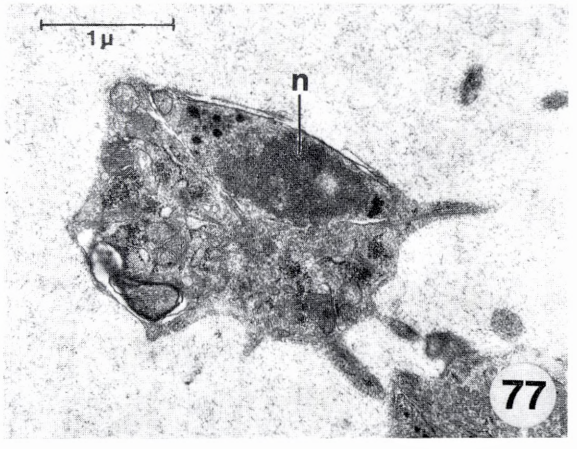

1μ
n
cc
co
72


0.2 μ
0.2 μ
ax
cm
73


1μ
n
74


1 μ
n
75


1μ
n
76


1μ
n
77

78. *Alona quadrangularis* (O. F. Müller). Lyngby Sø, Zealand. Fix. 1 % Os. Advanced spermatid in its vacuole (v) in a vegetative cell.

79. *Acroperus harpae* (Baird). Hultsjön, Småland, Sweden. 1 % Os. Spermatid. Developing cell centre with centriole (c), mitochondria (m), almost unmodified microtubules (t), and microtubules with some attached dark matter (periphery).

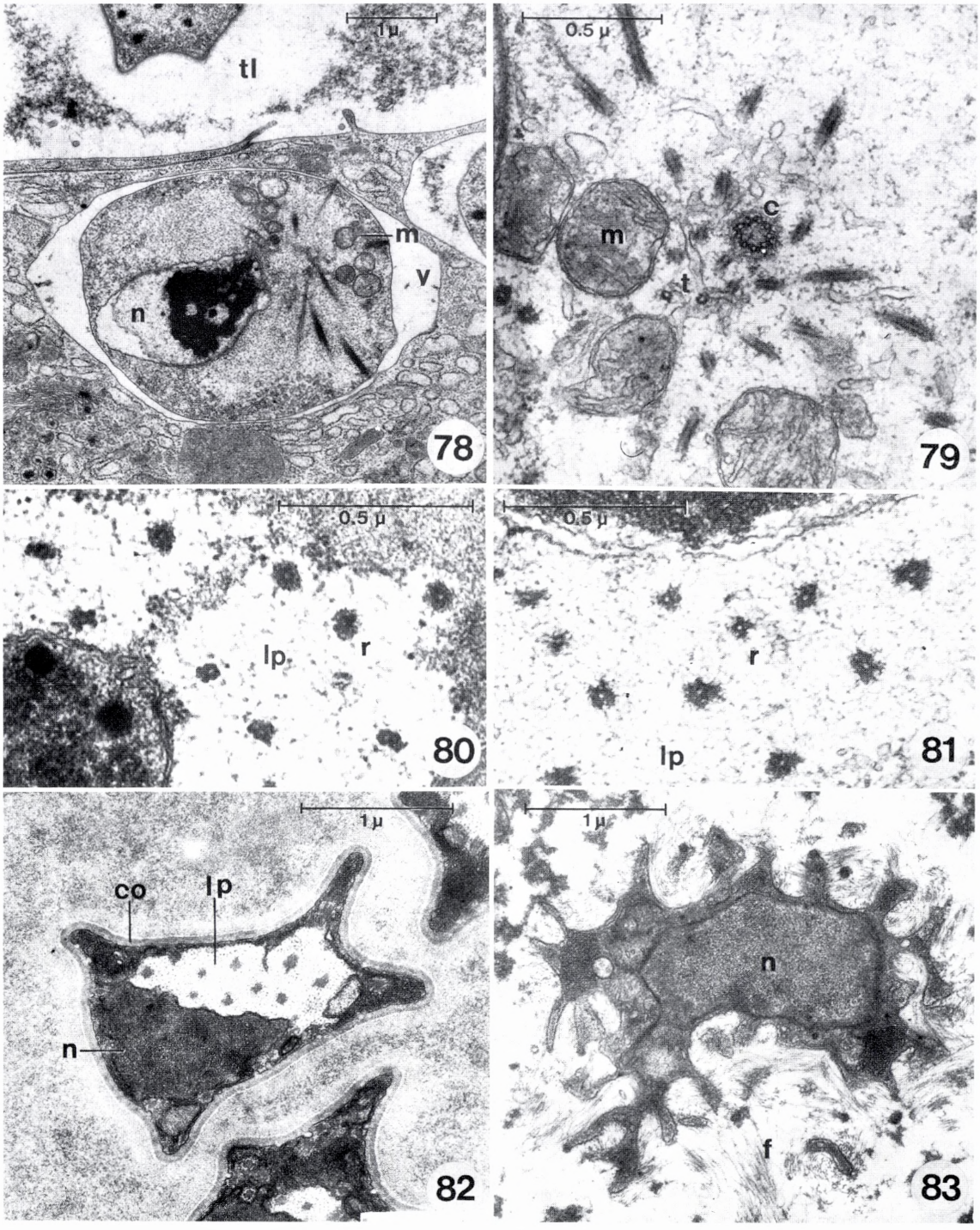
80. *Acroperus elongatus* (G. O. Sars). Lake Möckeln, Småland, Sweden. Fix. 3-A. Spermatid. Light plasm (lp) with dark rods (r). The microtubules have disappeared in most rods.

81. *Alona quadrangularis* (O. F. Müller). Lyngby Sø, Zealand. Fix. 1 % Os. Spermatid. Light plasm with rods. Plentiful dark matter surrounds the microtubules.

82. *Rhynchotalona falcata* (G. O. Sars). Store Grib Sø, Zealand. Fix. 2 % Os. Mature spermatozoon.

83. *Disparalona rostrata* (Koch). Lyngby Sø, Zealand. Fix. 1 % Os. Mature spermatozoon with a coat consisting of tufts of filaments.

Legends to all figures: c = centriole, co = coat, f = filamentous coat, lp = light plasm around rods, m = mitochondria, n = nucleus, r = rods, t = microtubules, tl = testicular lumen, v = vacuole in vegetative cell.


1 µ
tl
m
v
n
78
0.5 µ
m
c
t
79
0.5 µ
lp
r
80
0.5 µ
r
lp
81
1 µ
co
lp
n
82
1 µ
n
f
83

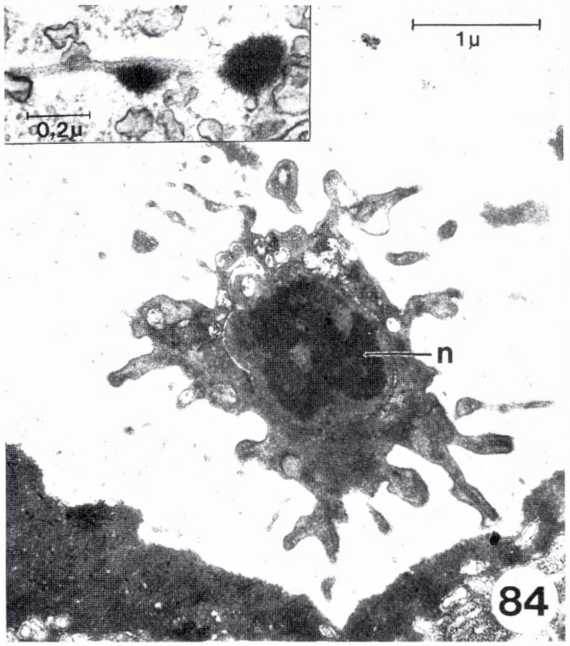
84. *Pleuroxus uncinatus* Baird. Teglgårdssøen, Hillerød, Zealand. Fix. 3-A. Mature spermatozoon. The inset shows clumps of dark matter attached to radiating microtubule in spermatid.

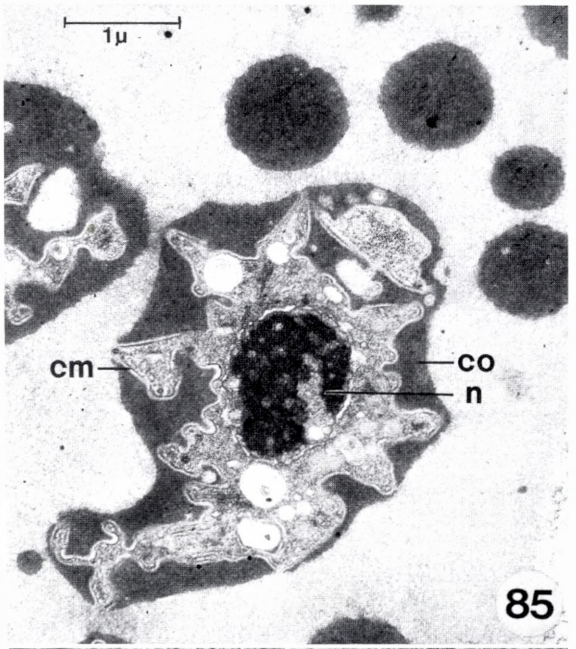
85. *Chydorus sphaericus* (O. F. Müller). Lyngby Sø, Zealand, Fix. 1 % Os. Mature spermatozoon.

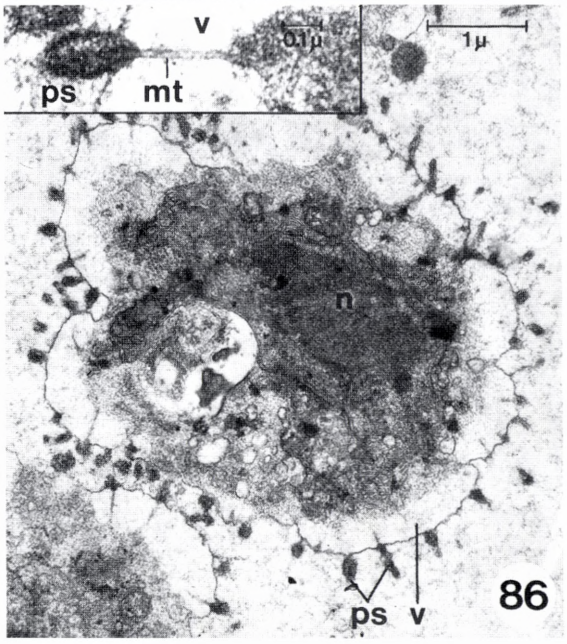
86. *Peracantha truncata* (O. F. Müller). Lyngby Sø, Zealand. Fix. 1 % Os. Mature spermatozoon. Inset shows microtubule (mt) passing radially through peripheral empty space (v) to short pseudopodium.

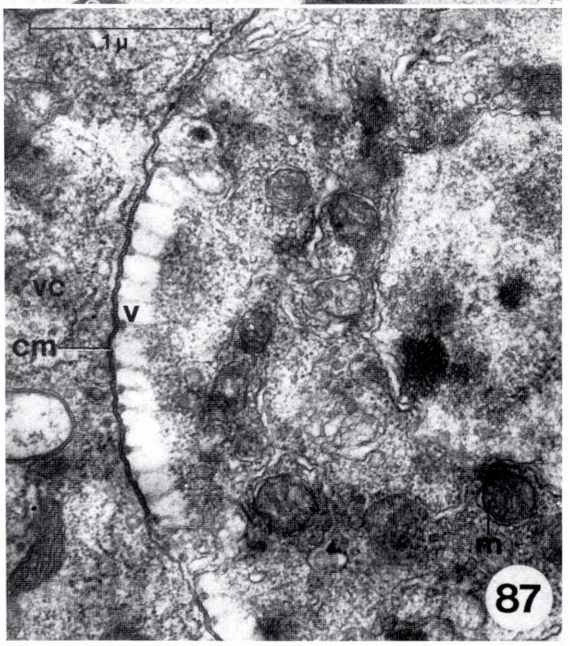
87. *Peracantha truncata* (O. F. Müller). Lyngby Sø, Zealand. Fix. 1 % Os. Spermatid in which peripheral empty spaces begin to appear (v), exposing radial microtubules. Note membrane of spermatid closely attached to membrane lining vacuole of vegetative cell (vc) at (cm).

Legends to all figures: cm = cell membranes, co = coat, mt = microtubule, n = nucleus, ps = pseudopodia, v = peripheral empty spaces (vacuoles) in *Peracantha*, vs = vegetative cell.


0,2µ
1µ
n
84


1µ
cm
co
n
85


v
0,1µ
ps
mt
1µ
n
ps
v
86


1µ
vc
v
cm
m
87

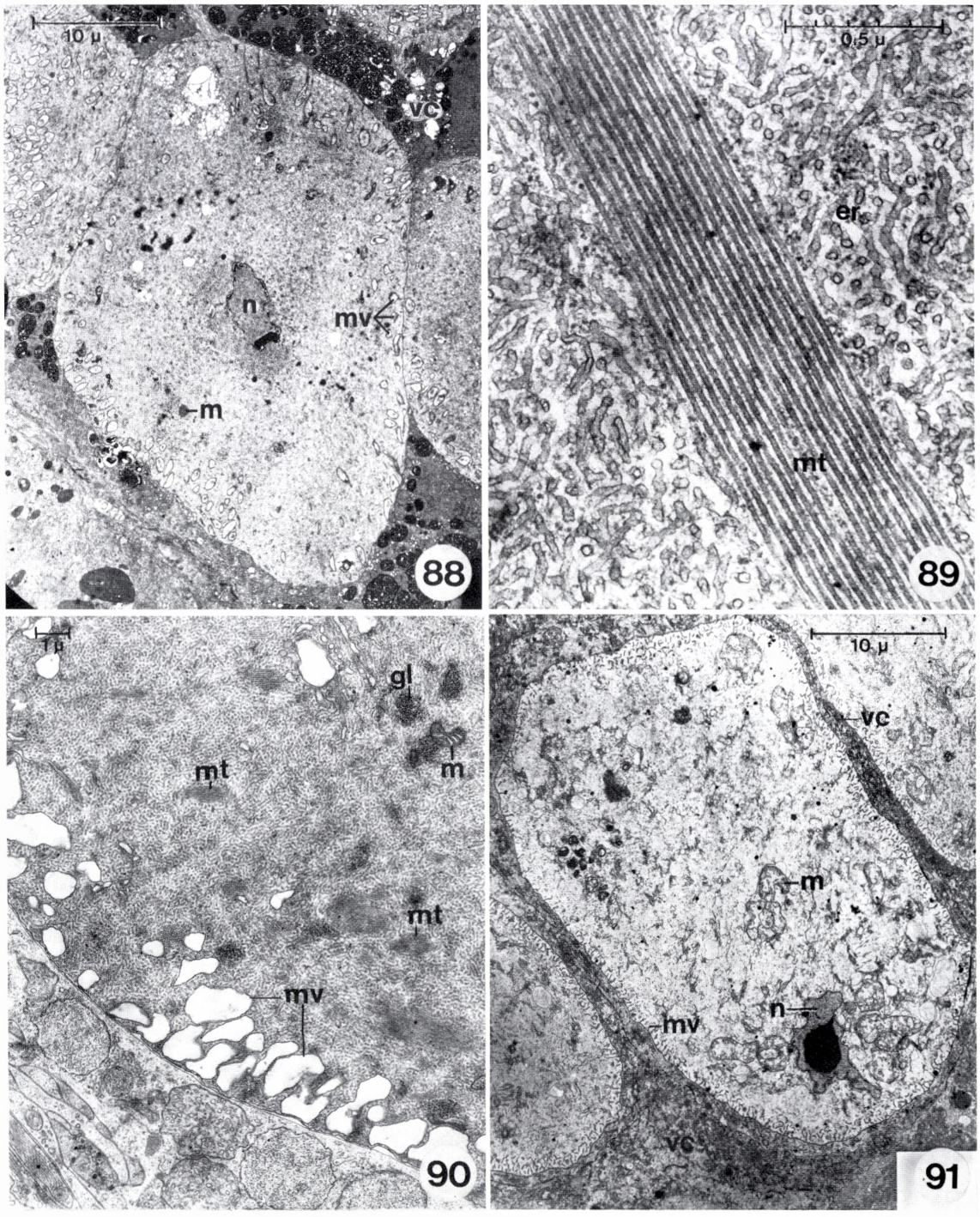
88. *Polyphemus pediculus* (L.). Lake Fenen, Småland, Sweden. Fix. 3-A. Mature spermatozoon surrounded by other spermatozoa and vegetative cells (vc).

89. *Polyphemus pediculus* (L.). Emdrup Sø, Copenhagen. Fix. 1 % Os. High magnification of section showing dense system of endoplasmatic tubules and a bundle of common microtubules.

90. *Polyphemus pediculus* (L.). Emdrup Sø, Copenhagen. Fix. 1 % Os. Part of mature spermatozoon with marginal vesicles.

91. *Evadne nordmanni* Lovén. Øresund. Fix. 3-A. Mature spermatozoon surrounded by remnants of vegetative cells.

Legends for all figures: er = endoplasmic reticulum, gl = glycogen granules?, m = mitochondria, mt = microtubules, mv = marginal vesicles, n = nucleus, vc = vegetative cells.


10 µ
vc
n
mv
m
88
0,5 µ
er
mt
89
1 µ
gl
m
mt
mt
mv
90
10 µ
vc
m
n
mv
vc
91

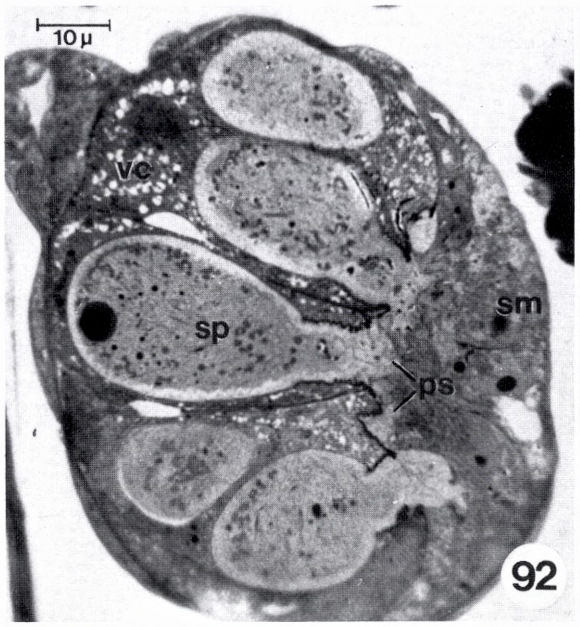
92. *Evadne normanni* Lovén. Øresund. Light microscopical picture of epon section, showing advanced spermatids (sp) in cup-shaped vegetative cells (vc). The epithelium of the opposite testicle wall is represented by small cells (sm).

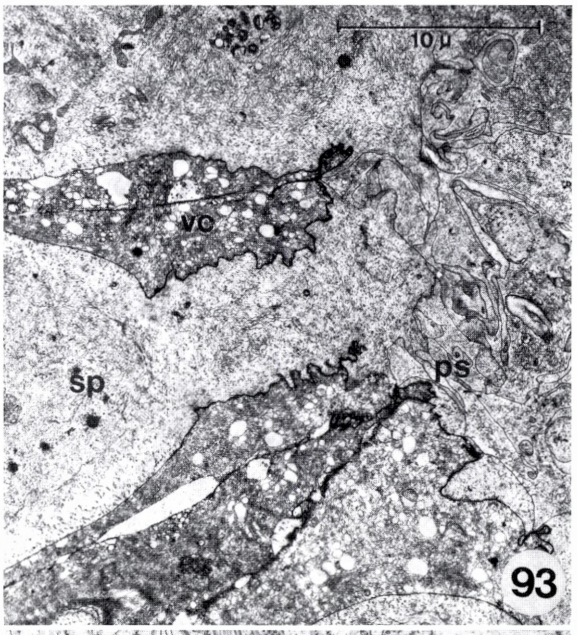
93. *Evadne nordmanni* Lovén. Øresund. Fix. 1 % Os. Necklike opening of cup-shaped vegetative cell (vc) and spermatid (sp). The pseudopodia of the spermatid form a tangle at (ps).

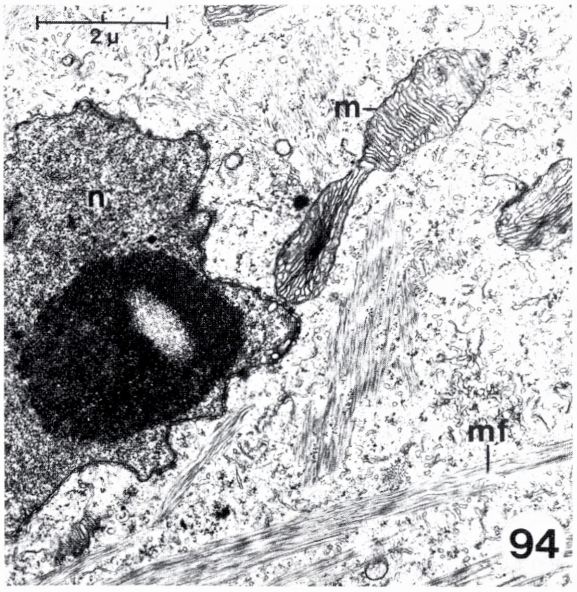
94. *Podon leuchartii* G. O. Sars. Øresund. Fix. 2 % Os. Region near nucleus (n) of mature spermatozoon, showing bundles of the remarkable, hollow microfilaments (See 97).

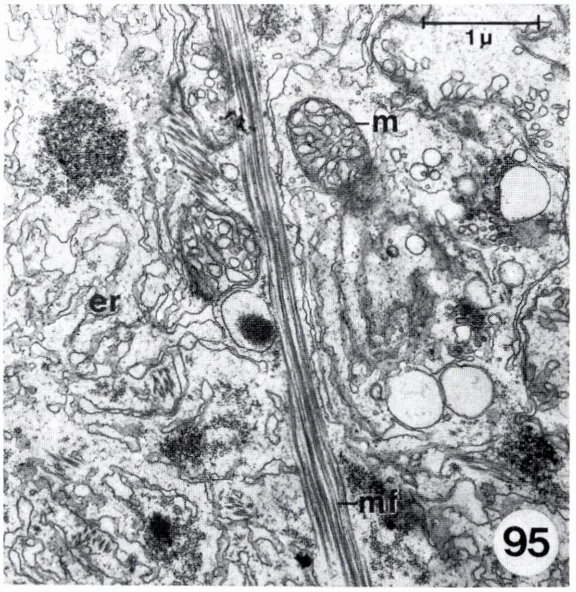
95. *Evadne nordmanni* Lovén. Øresund. Fix. 1 % Os. Part of plasm of mature spermatozoon with bundles of the remarkable, hollow 80 Å microfilaments (mf).

Legends to all figures: er = endoplasmic reticulum, m = mitochondria, mf = 80 Å hollow microfilaments, n = nucleus, ps = pseudopodia, sm = small cells of sterile testicular wall in *Evadne*, sp = spermatids, vc = vegetative cells.


10 µ
vc
sp
sm
ps
92


10 µ
vc
sp
ps
93


2 µ
m
n
mf
94


1 µ
m
er
mf
95

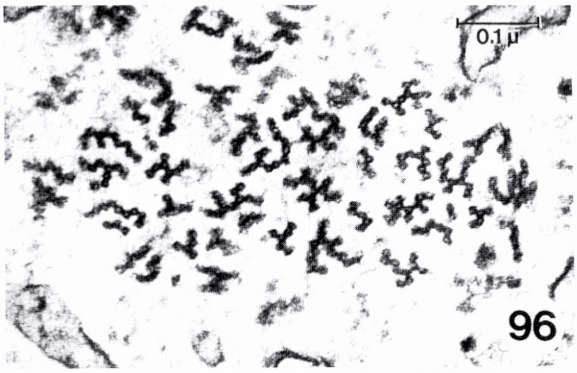
96. *Evadne nordmanni* Lovén. Øresund. Fix. 3-A. Mature spermatozoon. Bundle of the remarkable hollow microfilaments in cross section.

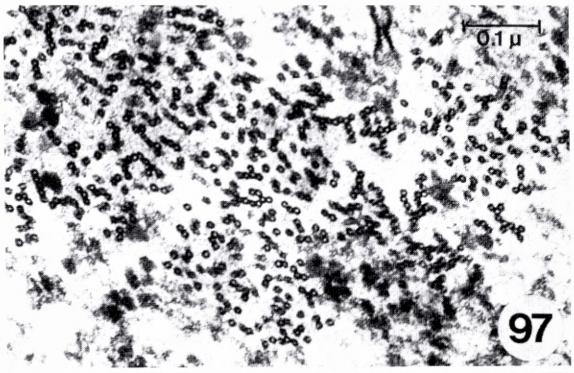
97. *Podon leuckartii* G. O. Sars. Øresund. Fix. 2 % Os. Mature spermatozoon. Cross section of bundle of the remarkable, hollow microfilaments.

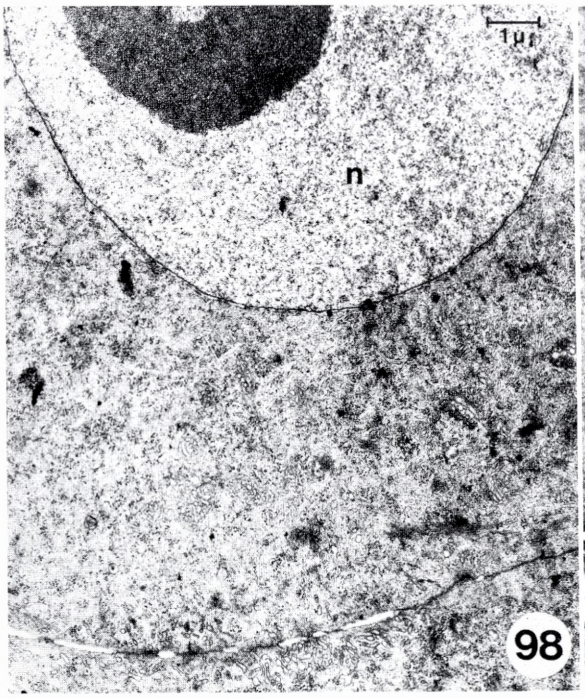
98. *Bytotrephes longimanus* Leydig, ssp. *balticus* Ischreyt. Lyngby Sø, Zealand. Fix. 1 % Os. Part of mature spermatozoon with vesicular nucleus (n), nucleus, and dense plasm.

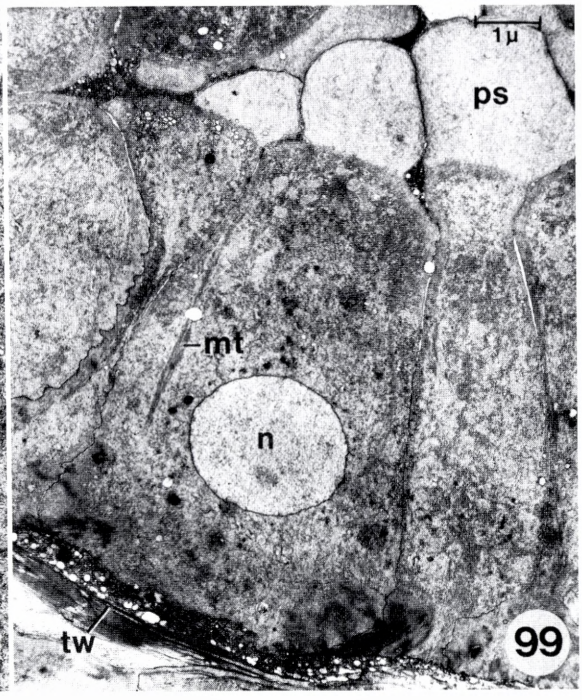
99. *Bytotrephes longimanus* Leydig, ssp. *balticus* Ischreyt. Lyngby Sø, Zealand. Fix. 1 % Os. Advanced spermatids with light, apical lobes (ps).

Legends to all figures: mt = bundle of microtubules of the common 250 Å class, n = nucleus, ps = light, pseudopodial lobes, tw = testicular wall.


0.1 µ
96


0.1 µ
97


1 µ
n
98


1 µ
ps
mt
n
tw
99

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