Micrognathozoa: A New Class With Complicated Jaws Like Those of Rotifera and Gnathostomulida

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ABSTRACT A new microscopic aschelminth-like animal, *Limnognathia maerski* nov. gen. et sp., is described from a cold spring at Disko Island, West Greenland, and assigned to Micrognathozoa nov. class. It has a complex of jaws in its pharynx, and the ultrastructure of the main jaws is similar to that of the jaws of advanced scleroperalian gnathostomulids. However, other jaw elements appear also to have characteristics of the trophi of Rotifera. Jaw-like structures are found in other protostome taxa as well—for instance, in proboscises of kalyptorhynch platyhelminths, in dorvilleid polychaetes and aplacophoran mollusks—but studies of their ultrastructure show that none of these jaws is homologous with jaws found in Gnathostomulida, Rotifera, and Micrognathozoa. The latter three groups have recently been joined into the monophylum Gnathifera Ahlrichs, 1995, an interpretation supported by the presence of jaw elements with cuticular rods with osmiophilic cores in all three groups. Such tubular structures are found in the fulcrum of all Rotifera and in several cuticular sclerites of both Gnathostomulida and Micrognathozoa. The gross morphology of the pharyngeal apparatus is similar in the three groups. It consists of a ventral pharyngeal bulb and a dorsal pharyngeal lumen. The absence of pharyngeal ciliation cannot be used as an autapomorphy in the ground pattern of the Gnathifera because the Micrognathozoa has the plesiomorphic alternative with a ciliated pharyngeal epithelium.

The body of *Limnognathia maerski* nov. gen. et sp. consists of a head, thorax, and abdomen. The dorsal and lateral epidermis have plates formed by an intracellular matrix, as in Rotifera and Acanthocephala; however, the epidermis is not syncytial. The ventral epidermis lacks internal plates, but has a cuticular oral plate without ciliary structures. Two ventral rows of multiciliated cells form a locomotory organ. These ciliated cells resemble the ciliophores present in some interstitial annelids. An adhesive ciliated pad is located ventrally close to a caudal plate.

As in many marine interstitial animals—e.g., gnathostomulids, gastrotrichs, and polychaetes—a special form of tactile bristles or sensoria is found on the body. Two pairs of protonephridia with unicellular terminal cells are found in the trunk; this unicellular condition may be the plesiomorphic condition in Bilateria. Only specimens with the female reproductive system have been found, indicating that all adult animals are parthenogenetic females. We suggest that 1) jaws of Gnathostomulida, Rotifera, and the new taxon, Micrognathozoa, are homologous structures; 2) Rotifera (including Acanthocephala) and the new group might be sister groups, while Gnathostomulida could be the sister-group to this assemblage; and 3) the similarities to certain gastrotrichs and interstitial polychaetes are convergent. J. Morphol. 246:1–49, 2000.

KEY WORDS: Micrognathozoa; new class; Gnathifera; *Limnognathia*; taxonomy; ecology; phylogeny; Arctic; Greenland; freshwater meiofauna; cold spring

The rich vegetation near the more than 1,000 homothermic springs of Disko Island, West Greenland, has been considered to contain a southern relict element from a period with a warmer climate when the ranges of southern elements in the flora extended beyond the island of Disko (Porsild, 1920; Kristensen, 1987). In the same way the marine faunal elements in Greenland springs have been regarded as relicts from a postglacial hypsithermal period, when many springs were below sea level (Kristensen, 1977).

Measurements of the abiotic parameters in more than 100 homothermic springs on Disko Island indicate that the springs can be separated into at least three different types (Kristensen, 1982). The marine faunal elements are only found in warm electrolyte-

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rich, radioactive springs ("salt springs"). The dominant type of spring on Disko Island is an electrolyte-poor spring with only "normal freshwater" species. The "southern" vegetation around the springs is a result of the so-called "greenhouse effect" brought about by the snow and ice cover in winter.

Compared with the "warm" springs of Disko Island, very few investigations had been carried out in cold springs, which may be frozen up to 8 months of the year. The freshwater fauna of the cold (heterothermic) springs differ greatly from those of the homothermic springs. The flora and fauna of a cold spring (Isunngua/Mudderbugten) was compared with that of the relatively cold homothermic springs (Sullorsuaq/Kvandalen) during a field course in Arctic Biology, 1994, at Disko Island. To our surprise, we found a new type of animal in the cold spring (Kristensen, 1995; Kristensen and Funch, 1995) epiphytic on water mosses. Here we fully describe the new taxon, discuss its phylogenetic position, and correct some misconceptions about this unique animal (see Ahlrichs, 1997; Herlyn and Ehlers, 1997).

The first specimens of the new species were collected in 1979 at Disko Island. These three specimens were then labeled Rotifera because they were not observed alive and were strongly contracted in the formaldehyde/glycerol preparation. Later, we recognized the complicated jaw apparatus. The new animal has several superficial similarities to monogonont rotifers, especially in the pharyngeal apparatus. Therefore, we compared the extremely complex jaw apparatus with the more simple mastax of several species of Monogononta by scanning electron microscopy (SEM). Recently, excellent SEM analysis of the sclerite system of the rotifer mastax has been published (Markevich, 1989; De Smet, 1996, 1997). Surprisingly the jaw apparatus of the new animal clearly has elements similar to the scleroperalian gnathostomulids (Kristensen and Nørrevang, 1977, 1978; Herlyn and Ehlers, 1997), especially in the main jaws (articularium and dentarium). We compare the lamellarization of the dentarium and the fibularization of the apophysis in the family Gnathostomulidae (Riedl and Rieger, 1972) with similar structures in the main jaws of the new animal.

Gnathostomulida was described by Ax (1956) as an order of Turbellaria (Platyhelminthes). Later, Riedl (1969) established a new phylum for the gnathostomulids and Sterrer (1972) confirmed the new status of the group, but he also mentioned that gnathostomulids share characteristics with both Platyhelminthes and Aschelminthes. In the excellent review article of Lammert (1991) the Gnathostomulida was also included in the Aschelminthes. Zoology textbooks, such as Ruppert and Barnes (1994), follow the idea of Sterrer et al. (1985) and Lammert (1991) and include the Gnathostomulida in the Aschelminthes. Nielsen (1995) included them in Annelida. Littlewood et al. (1998) were the first to sequence 18S ribosomal DNA from a species of Gnathostomulida. In comparing this sequence to that of other phyla they came to the conclusion that the Gnathostomulida is a member of a Nematoda + Chaetognatha clade. Zrzavý et al. (1998) came to another conclusion. They used the 18S ribosomal DNA data of Gnathostomulida from Littlewood et al. (1998) and combined these with morphological characters in a total-evidence approach. Analysis of this huge dataset indicated a monophylum Neotrichozoa consisting of Gnathostomulida and Gastrotricha. So far, the only agreement is that gnathostomulids are protostomian worms.

Recently, Rieger and Tyler (1995) and Ahlrichs (1995) cited ultrastructural evidence for a sister-group relationship of Gnathostomulida with Rotifera-Acanthocephala. Ahlrichs (1995) established a new monophylum Gnathifera for these groups. Later, Ahlrichs (1997) included our new taxon as "New group A" in his phylogenetic diagram of Gnathifera. He cited our unpublished Danish report from the Arctic Field Course (Kristensen and Funch, 1995). Unfortunately, the same new taxon was also mentioned as "New group 1" (Kristensen, 1995). "New group A" for our unnamed taxon and its inclusion in Gnathifera were mentioned again in the article of Herlyn and Ehlers (1997). They argued that the trophi of rotifers and the cuticular jaws of gnathostomulids are homologous. Their conclusion was based on a transmission electron microscopy (TEM) investigation of the pharynx of the scleroperalian gnathostomulid, *Gnathostomula paradoxa*. Recently, Sørensen (2000) made the first SEM-investigation of the two main type jaws ("compact" and "basket" type) within the Gnathostomulida. His study gives further support for a closer relationship with rotifers by virtue of similarities with the jaws and trophi of advanced monogonont rotifers. Sørensen shows that the pseudofulcrum of *Rastrognathia macrostoma* resembles the fulcrum in the rotiferan family Dicranophoridae. The cuticular elements, the sclerofibrillae, are almost identical in the two groups. Our new taxon from Greenland belongs to Gnathifera and supports the monophyly of this taxon comprising the Gnathostomulida, Rotifera (including *Seison*), and Acanthocephala. Consequently, Gnathostomulida is not as closely related to the Turbellaria (Platyhelminthes) as suggested by Ax (1956, 1985, 1989, 1996).

Finally, we considered other possibilities for a phylogenetic relationship for the "New Group 1," because we doubt that Aschelminthes is monophyletic, a hypothesis that has gained support from recent phylogenetic analyses using morphological data (Nielsen et al., 1996; Sørensen et al., 2000), molecular data and combined data (Giribet et al., 2000). There are similarities in outer morphology—e.g., the ventral trunk ciliation and sensoria—between certain gastrotrichs (Hyman, 1951; Ruppert, 1991b) and the new animal. The phylogenetic significance of these structures is briefly discussed.

The free-swimming chordoid larva of Cycliophora (Funch and Kristensen, 1995; Funch, 1996) has a gastrotroch of compound cilia as in the ventral ciliation of the new animal. This is probably an adaptation to crawling and not a homology. We discuss the hypothesis of polyphyletic origin of Aschelminthes (Winnepenninckx et al., 1995) in the context of groups, such as the interstitial polychaete families Diurodrilidae and Dorvilleidae (see Kristensen and Niilonen, 1982; Eibye-Jacobsen and Kristensen, 1994) having ventral ciliophores both on the head and abdomen, as does our new animal. Furthermore, the family Dorvilleidae has a cuticular jaw in the pharyngeal apparatus. The recently described species of dorvilleid, *Neotenotrocha sterreri,* has a swimming behavior like that of a rotifer and a jaw apparatus similar to the trophi of rotifers. In light of the similarities between *Neotenotrocha*, "New Group 1," and Rotifera the old theory of Semper (1872) that the rotifers are simply neotenic annelids is discussed.

MATERIALS AND METHODS

Substrate and Abiotic Factors in the Isunngua Spring

The sources of the Isunngua spring are located 1,030–1,120 m from the coast at 50 m above sea level in a cold moor vegetation. The outflow runs through two more moors at 315–345 m and 200–296 m from the coast. Between the last two moor areas and the sea the outflow runs in a 30–50 cm-deep water channel in well-sorted sand from the Cretaceous. The outlet from the second moor is the type locality at a position: 69°43.799'N and 51°56.549'W. The spring temperature on 5 August 1994 was 5°C, the conductivity 62 μmho, and the pH in the field was 6.4. The radioactivity was only slightly higher than the background.

The new animal was found only in the last two moors and the outlets from the moors. More than 100 specimens were collected epiphytic in the mosses on 22 July 1994, 25 July 1994, 5 August 1994, and 20 May 1995. About 5 kg moss and soil were collected 5 August 1994 and carried to Copenhagen and kept in culture in a 4°C refrigerator with constant light and airing. The animals reproduced in the moss culture until 28 February 1997, when an unknown fungus overgrew the culture. The moss species on which the new animal lives epiphytically comprise the following species: *Aulocommium palustre*, *Calliergon sarmenfosum* (the dominant species), *Drepanocladus intermedius*, *Paludella squarrosa*, and *Tomenthypnum nitens* (soil species).

Living Materials

Mosses with the rhizoids kept at 4°C were squeezed in spring water for detritus and meiofauna and the rinsings decanted through a 32-μm mesh net. The epiphytic meiofauna was placed in spring water or distilled water in Petri dishes at room temperature and inspected under a stereomicroscope using magnifications of ×40–×100. After about a half hour the new animal could be observed free-swimming in the water column. The animals adhere strongly to Pasteur pipettes, so Irwin loops were use to transfer specimens to the microslides, where they were mounted in spring water and photographed in the differential interference contrast microscope (DIC, Nomarski technique) or videotaped with a camera mounted on a Zeiss phase contrast microscope.

The holotype (Fig. 1) and one paratype (Fig. 2) were drawn using camera lucida (magnification ×2,000). These habitus drawings were made at 4°C, when the animals were still alive but had been slightly squeezed. The measurements of total length and width of living specimens are about 20% larger than the same animal later fixed with osmium-tetroxide vapor. The drawings and measurements of all sensoria, ciliophores, adhesive ciliated pad, and the dorsal plates could only be made on living but slightly squeezed animals (Figs. 3, 4). Some animals from the culture were prepared for SEM (Figs. 5, 6) and a prominent oral plate that was not included in the drawings was observed.

Some animals laid eggs in small salt cellars and some of these eggs were prepared for SEM (Fig. 7).

Wholemount Preparations

Twenty-eight specimens were fixed with a drop of 4% formaldehyde (buffered with borax) in the spring water or with 1% osmium-tetroxide vapor directed onto the hanging drop of water on the microslides. Thereafter the specimens were mounted with a coverslip in the fixative and the fixative was replaced by 2% glycerol in distilled water that evaporated to glycerol over several days; the mounts were finally sealed with Glyceel®.

Seven living mounted specimens were treated with 2% sodium hypochlorite, using the technique described by Riedl and Rieger (1972) for isolating the jaws of Gnathostomulida. The specimen was mounted under a coverslip in spring water in a squeezed preparation. Thereafter, sodium hypochlorite was sucked in with bibulous paper. The jaws and the basal plate (Figs. 8, 9) were strongly resistant to oxidation; however, the outer oral plate, the different lamellae, ligaments, and symphyses became transparent and disappeared under the bleaching process.

The photomicrographs were taken during the bleaching process of the jaw apparatus (Figs. 10–15). After bleaching, the tissue debris was removed with distilled water, glycerol was added, and the coverslip was sealed with Glyceel®. All wholemounts were examined with phase contrast and DIC optics and drawn using a camera lucida. For drawings of

the pharyngeal apparatus and the complex jaw system (Figs. 8, 9), a Wild drawing tube mounted on a Wild M20 microscope was used, allowing a drawing magnification of ×10,000.

Electron Microscopical Techniques

Adult specimens and eggs used for SEM were fixed in 4% buffered formaldehyde or osmium-tetroxide vapor. Specimens were transferred through an acetone dehydration series and were critical-point dried using carbon dioxide; thereafter, they were mounted on aluminum SEM stubs with double-sided tape.

A new technique was developed for SEM observations of the jaw apparatus. For this, several living animals were placed in a small drop of distilled water directly on the aluminum SEM stub. When the distilled water evaporated, the dried animals adhered strongly to the SEM stub. The soft tissue was then removed by 2% sodium hypochlorite and the cleaned jaw apparatus was rinsed several times with distilled water. All SEM preparations were sputter-coated with gold and examined in a JEOL JSM-840 scanning electron microscope (Figs. 16, 17).

For TEM, six adult animals were fixed in a mixture of three aldehydes, a so-called trialdehyde fixation (Lake, 1973), in 0.1 M sodium cacodylate buffer (see Kalt and Tandler, 1971). All specimens were postfixed in 1% osmium-tetroxide with 0.1 M sodium cacodylate buffer for 1 h at 20°C. After fixation, the animals were dehydrated in an ethanol series, transferred to propylene oxide, and finally embedded in epoxy resin type TAAB 812®. The ultrathin serial sections were stained with uranyl acetate and lead citrate (Reynolds, 1963). TEM examinations (Figs. 18–33) were performed with a JEOL JEM-100SX transmission electron microscope.

DESCRIPTION

Phylum: Gnathifera Ahlrichs, 1995

Micrognathozoa, new class

Diagnosis. Acoelomate metazoans with bilateral symmetry and epidermal dorsal and lateral intracellular plates. Epidermis cellular, not syncytial. Body divided into a head with the pharyngeal apparatus, an accordion-like thorax, and an abdomen with a dorsal anus, which may be functional only periodically. Ventral epidermis with thick glycocalyx and two rows of multiciliated ciliophores. Ventral mouth opening surrounded by a cuticular oral plate. Epidermis of the mouth cavity and the pharyngeal apparatus also with a cuticle. Jaw apparatus with one unpaired and nine paired major sclerites. Females with one pair of ovaries. One egg per clutch. Two pairs of protonephridia with monociliated terminal cells. Direct development.

Etymology. *Micro, gnathos* and *zoa* are Greek for "small," "jaws," and "animal," referring to the small animal with complex jaws.

Limnognathida, new order

Diagnosis. Same as the class and with a life-cycle with free-living individuals in freshwaters.

Limnognathiidae, new family

Diagnosis. Same as the class.

Limnognathia gen. nov.

Diagnosis. Same as the class.

Type species. *Limnognathia maerski*, new species by designation (Figs. 1, 2).

Etymology. *Limnos* and *gnathos* are Greek for "freshwater" and "jaws," referring to the habitat being freshwater; feminine gender.

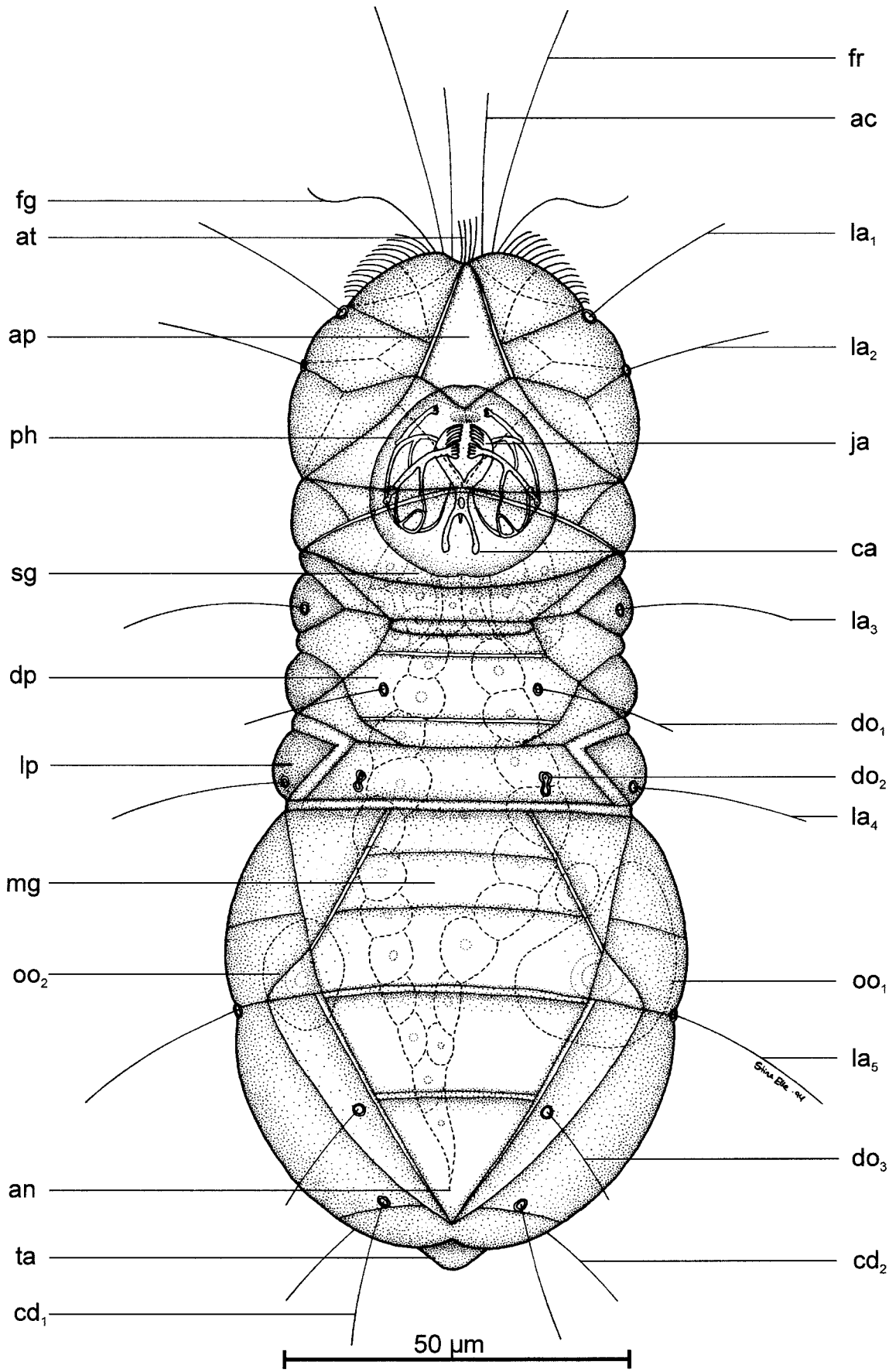
Limnognathia maerski sp. nov.

Diagnosis. Mature females 105–152 μm long, juveniles 85–107 μm long, ovoid sculptured egg 40 × 30 μm. Stiff sensoria consisting of: 1) one pair of apicalia, 2) one pair of frontalia, 3) five pairs of lateralia, 4) three pairs of dorsalia, and 5) two pairs of caudalia, each with a ring-shaped epidermal socket. Second dorsalia (Fig. 1, do_2) on the thorax are double and may lack cilia.

Etymology. To honor Maersk McKinney Møller, who sponsored the new research vessel *Porsild* for the Arctic Station. The new animal was discovered during the maiden trip with the new research vessel in 1994.

Type material. The holotype (MIC 0001, ZMUC) is an adult female with two unsculptured oocytes. This wholemount slide, together with 19 paratypes (MIC 0002-MIC 0020, ZMUC), is deposited in the Zoological Museum of Copenhagen, Denmark. Seven sodium hypochlorite-treated jaws are also deposited on microslides (MIC 0021-MIC 0023, ZMUC). Three paratypes and two jaw apparatuses are located on SEM stubs. Six paratypes are ultrasectioned and located on 105 grids. The type material is placed in ZMUC and a single paratype is in the National

Fig. 1. Holotype of *Limnognathia maerski* nov. gen. et nov. sp. Dorsal view of the slightly squeezed living female from the spring at Isunngua, Disko Island, W. Greenland. A few details from study after the animal was fixed are included. ac, apicalia; an, anus; ap, apical plate; at, apical cilia tuft; ca, cauda of the main jaws (ja); cd_1-cd_2, caudalia; do_1-do_3, dorsalia; dp, dorsal plate; fg, flagellar head structure; fr, frontalia; la_1-la_5, lateralia; lp, lateral plate; mg, midgut; oo_1-oo_2, oocytes; ph, pharyngeal apparatus; sg, salivary gland; ta, tail (pygidium).


fr
ac
fg
at
la1
ap
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ph
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ca
sg
la3
dp
do1
lp
do2
la4
mg
oo2
oo1
la5
do3
an
ta
cd2
cd1
50 µm

Figure 1

Museum of Natural History, Smithsonian Institution (USNM), Washington DC, USA.

Type locality. The type material was extracted from living water mosses from the cold Isunngua spring (69°43.799'N, 51°56.549'W), located at the eastern corner of Disko Island, West Greenland (Figs. 34–39). The living material was collected by the authors on 22 July 1994, 25 July 1994, 5 August 1994, and 20 May 1995. The holotype was from the 5 August 1994 collection.

Additional material. Three strongly retracted animals had been mounted on microslides in 1979. This material is from a cold homothermic spring close to Lymnaea lake (69°42.297′N, 52°11.435′W) in the valley of Sullorsuaq/Kvandalen (Fig. 36). The specimens were collected during a survey for tardigrades in July 1979 (Kristensen, 1982).

Description of the Holotype (Adult Female)

The holotype was observed alive and after fixation with 1% osmium-tetroxide and preparation of a glycerol wholemount. The total length of the living animal was 142 μm and the maximum width of the abdomen was 55 μm. The drawing (Fig. 1) is in dorsal view. The body seems segmented or divided into a two-parted head, accordion-like thorax, and ovoid abdomen with a small retractile pygidium (tail).

External anatomy. The whole dorsal part (dorsum) of the animal is covered with plates. The animal bears sensoria on all parts of the body. As in many marine interstitial animals, e.g., gnathostomulids, gastrotrichs, and polychaetes, these tactile bristles (stiff, adjoined cilia) consist of more than one cilium. In each sensorium the cilia seem to emerge from one cell. However, this observation is not yet confirmed with TEM. In the holotype, two to three adjoined stiff cilia arise from a circular reinforcement, the socket. Still, when the bristle disappears under fixation the socket can be recognized as a ring with a pore in the middle. The tactile sensoria are always found in pairs, serially arranged on the body. The arrangement is consistent in all investigated specimens of *Limnognathia maerski*, including the holotype. On the anterior part of the head are four pairs of bristles. A pair of long frontalia (fr, 34 μm long) and a pair of shorter apicalia (ac, 23 μm long) are located on the frontal margin of the head. These sensoria are directed forward. Two pairs of laterally oriented sensoria (la_1 and la_2, 22 μm long) are located between the sutures of the first two lateral headplates. The posterior part of the head lacks bristles. The thorax has two pairs of lateralia (la_3 and la_4, 25 μm long) and two pairs of dorsalia (do_1, 18 μm long and do_2, without ciliary structure). They are located on middorsal plates. The posterior pair (do_2) lacks the external ciliary structures in the holotype, and the socket for the sensory structure is a double structure (8-shaped) and has a pore in the anterior part of the structure.

The abdomen has a single pair of lateralia (la_5, 24 μm long) located in a constriction that divides the abdomen into two parts. Furthermore, a shorter pair of dorsalia (do_3, 15 μm long), which is oriented dorso-caudally, is located on two thin dorso-lateral plates close to the triangular anal plate. Dorso-caudally, a pair of caudalia (cd_1, 19 μm long) and ventro-caudally another pair of caudalia (cd_2, 22 μm long) are located. Both pairs are oriented caudally and the sockets of the ventro-caudal bristles are located on the two caudal plates, which can be seen only in the ventral view (Fig. 2), and therefore are not seen in the holotype. All sensoria break off easily and in the wholemount preparation of the holotype only the sockets of the sensoria can be observed.

Three other ciliary structures could be observed on the head of the holotype when it was alive: 1) One small apical cilia tuft (at) was observed between the two apicalia. It consists of four short, stiff cilia, although eight cilia were seen in some paratypes. The cilia are about 8 μm long and are not adjoined, but it seems that they arise from the same epidermal cell. 2) Close to the base of each frontalium, a long single flagellum-like structure (30 μm long) is present (fg). This structure has the typical stroke of a flagellum. 3) Between the frontalia and the first pair of lateralia, a broom of short cilia is present. These cilia are perhaps a part of the ventral preoral cilia field (see Fig. 2, pc). These cilia persist in the wholemount preparation.

The characteristic dorsal plates can best be investigated in the head. Here it is clearly seen that each plate comprises 3–4 epidermal cells. The borders between cells are shown as broken lines in Figure 1. An exception seems to be the thick apical plate (ap), which strongly reflects light in DIC microscopy and seems to be formed by a single giant epidermal cell. The forehead is separated from the posterior part by a constriction. The forehead consists of eight plates and the posterior part consists of five plates.

The thorax has thick dorsal plates (dp) and more flexible lateral plates (lp). The lateral plates work like an accordion and the thorax can change shape rapidly from a broad and solid structure to a thinner, longer, and flexible structure. Between the dorsal and lateral plates, up to five smaller rod-like to rhomboid plates are located.

The abdomen is subdivided dorsally with a sulcus (incomplete transverse furrow). The plates are large and there are no distinctions between dorsal and

→

Fig. 2. *Limnognathia maerski* nov. gen. et nov. sp. Ventral view of living paratype (slightly squeezed) from the spring at Isunngua, Disko Island. ab, abdomen; ad, adhesive ciliated pad; cd_1-cd_2, caudalia; cp, caudal plate; rb_1-rb_2, refractive bodies; ey, eye structure ?; fg, flagellar head structure; hc, head ciliophore; he, head; me, mid-ventral sensorium; mo, mouth opening, oo_1-oo_2, oocytes; pc, preoral cilia field; ph, pharyngeal apparatus; pr_1-pr_2, protonephridia; tc, trunk ciliophore; th, thorax (neck).

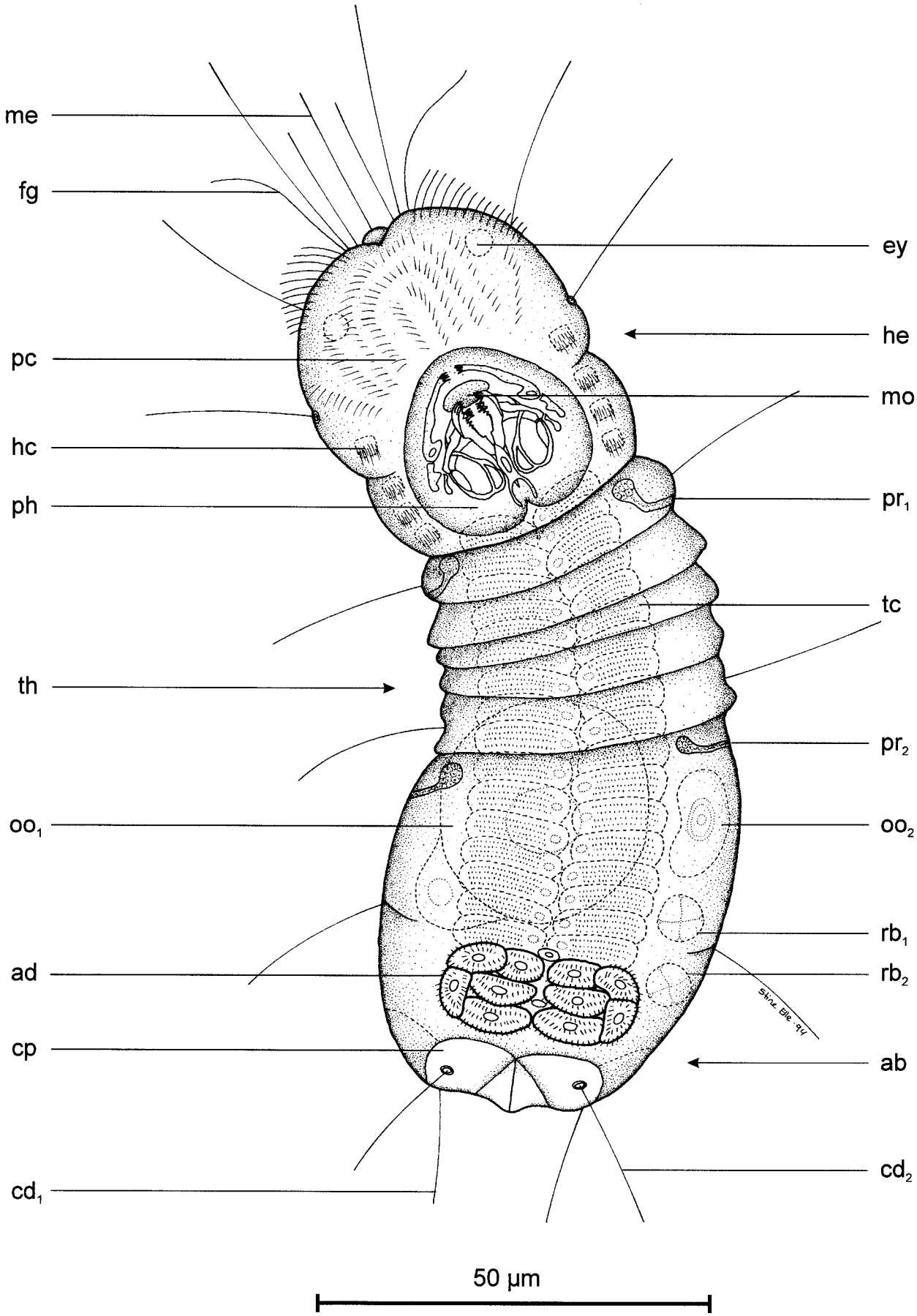

me
fg
ey
he
pc
mo
hc
ph
pr1
tc
th
pr2
oo1
oo2
rb1
ad
rb2
cp
ab
cd2
cd1
50 µm

Figure 2

lateral plates. The dorsal triangular anal plate is thick and seems to lack an anal opening.

Internal structures. The thick dorsal plates obscure many of the internal structures in the holotype. Some of these structures could be seen in the allotype from the ventral view (Fig. 2).

The most conspicuous structure in the holotype and all the paratypes is the large pharyngeal apparatus (25 × 23 μm) located in the head. From the dorsal view all three pairs of jaw-like structures (ja) can be seen, but the subunits in these complex structures can only be distinguished in sodium-hypochlorite-treated animals. The cross-striated muscles are not drawn in Figure 1 to avoid complicating the figure. The muscles are best analyzed with TEM. The ovoid mouth opening is surrounded by a true cuticle and therefore it can be seen from the dorsal view through the transparent tissue. The pharyngeal apparatus will be described in detail below. The large salivary glands (sg) seem to open inside the midgut.

The digestive system continues with a short esophagus, which leads to a large midgut, consisting of large transparent endoderm cells. The midgut totally lacks ciliary structures. The anus has been difficult to locate. It seems that *Limnognathia maerski* has only a temporary opening. In the holotype, the anal plate covers the rectum and anus.

The ovary is paired and two large oocytes dominate the abdomen. A third right oocyte is seen close to the large oocyte (oo_1). A gonopore was not observed.

Description of Allotype

The animal was observed for several hours at 4°C by DIC optics in a cooling room at Arctic Station. Later, observations were continued on the wholemount glycerol preparation fixed by 1% osmium-tetroxide vapor. The drawing was made with camera lucida in Greenland of the ventral view (Fig. 2). Details of the pharyngeal apparatus were added later. The total length of the living animal was 127 μm; maximum width of the abdomen was 47 μm.

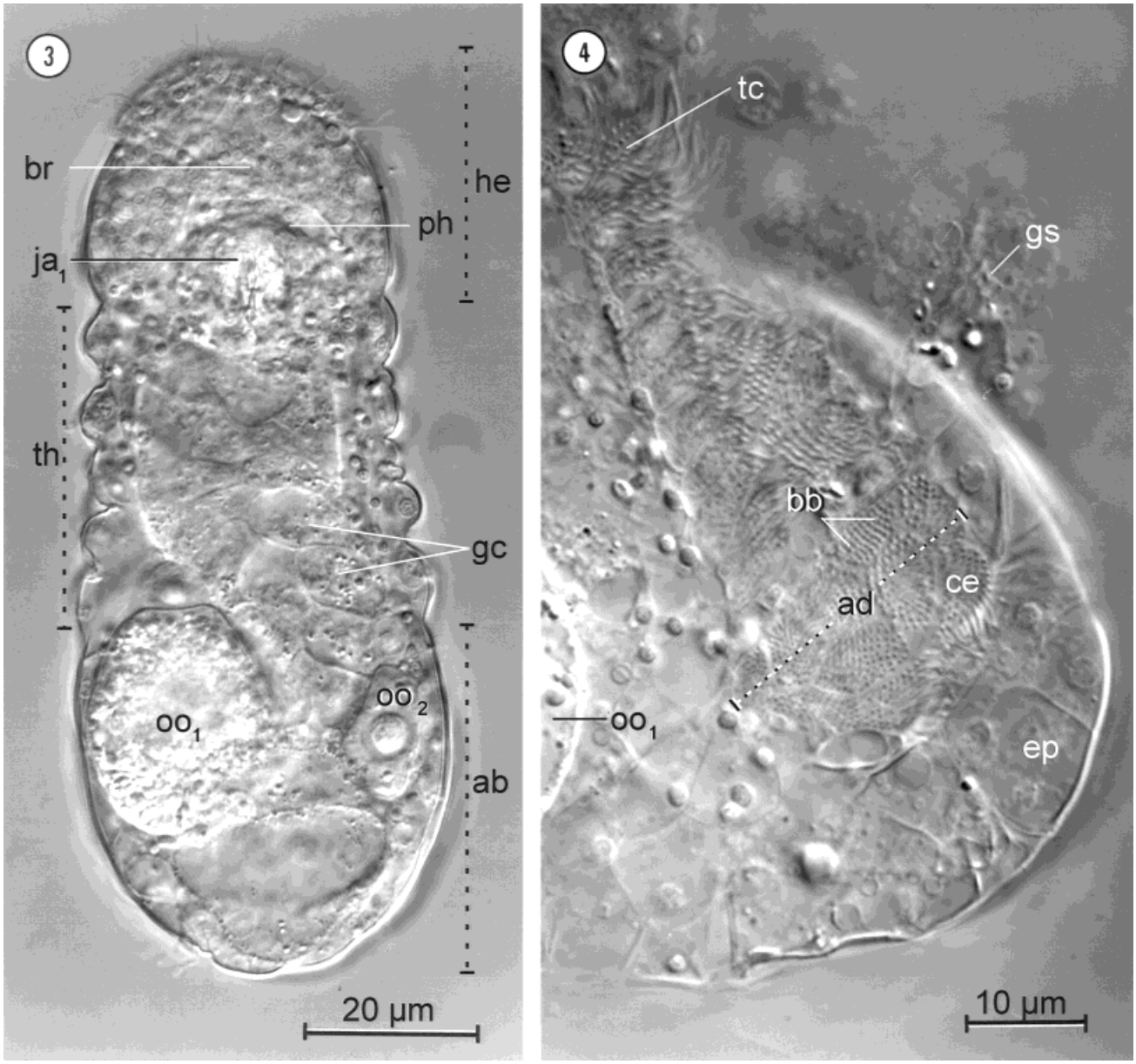
External gross anatomy. The entire ventral part (ventrum) of the animal lacks intracellular plates, except for the two caudal plates (cp), which in the living animal can be seen ventrally; the pair of caudo-ventral caudalia (cd_2) is located on these plates. Furthermore, a large cuticular oral plate is present, but it was only observed after the animal was fixed.

It is clearly seen from a ventral view that the head is divided into two parts. A sulcus separates the forehead from the posterior part. The accordion-shaped thorax is divided by five annulations (transverse furrows), which are very flexible and therefore disappear after fixation. The abdomen has a small sulcus, but it does not continue as a transverse furrow to the midventral part side. In a few paratypes, a very thin furrow was seen in the anterior part of the abdomen. This furrow was not as distinct as the annulations on the thorax.

The oval mouth opening (Fig. 2, mo) is located midventrally on the anterior edge of a spade-shaped oral plate. The large oral plate is not drawn in Figure 2, but is seen clearly on the SEM micrograph of a paratype (Fig. 6, op). The width of the oral plate in the allotype is 21 μm, and the length is 24 μm. The oral plate consists of true cuticle and lacks ciliation (Figs. 25, 27, op). In retracted animals the oral plate reaches far posterior to the edge of the ventral thorax. In the allotype preparation the shape of the mouth is a small oval opening, as in all wholemount preparations of *Limnognathia maerski*. Therefore it was very interesting to see the behavior of the living allotype. In a relaxed swimming position, the mouth opening has the typical oval form, but when the animal feeds the two ventral jaw elements (ja_1) can be protruded out through the mouth as two small arms to grasp the substrate. Furthermore, if the animal swallows some unwanted items the large cross-striated muscles in the head retract the dorsal part of the forehead, which is lifted upward and backward. Consequently, most of the pharyngeal apparatus with the whole jaw apparatus could be seen to stick out of the mouth. Several fast movements of all the jaws elements often accompany this behavior. The movements consist both of a snapping reaction and of turning the cauda of the main jaws (Fig. 1, ca) forward and backward. We called this action of the jaws a "vomit" behavior. One of the paratypes sectioned for TEM is fixed with the forehead lifted upward and the jaws protruded.

Ciliophores and ventral ciliation. The ventral ciliation is well developed and all body parts have a complex ciliation, unlike ciliation in taxa such as Gastrotricha, Rotifera, and Gnathostomulida. The forehead is covered ventrally by rows of single cilia. The rows of cilia are formed as arcs, leading particles directly to the mouth opening. From the mouth opening itself several stiff sensory cilia stick out like a broom.

Lateral to the oral plate a quite different ciliation exists. Four pairs of specialized ciliated areas were seen in the allotype. One pair is located at the forehead and three pairs are located at the posterior part of the head. The cilia in each area are stiff and they arise from a single rectangular cell. The cilia are not bounded by a common membrane, as in the gastrotrichs, but they move in unison nevertheless. Similar multiciliated cells in other invertebrates are called ciliophores (Kristensen and Niilonen, 1982; Eibye-Jacobsen and Kristensen, 1994). The ciliophores are large epidermal cells with numerous cilia whose basal bodies are ordered in regular rows. The head ciliophores in *Limnognathia maerski* are located in the same position as the metastomial ciliophores in the two interstitial polychaetes *Diurodrilus westheidei* and *Neotenotrocha sterreri*.

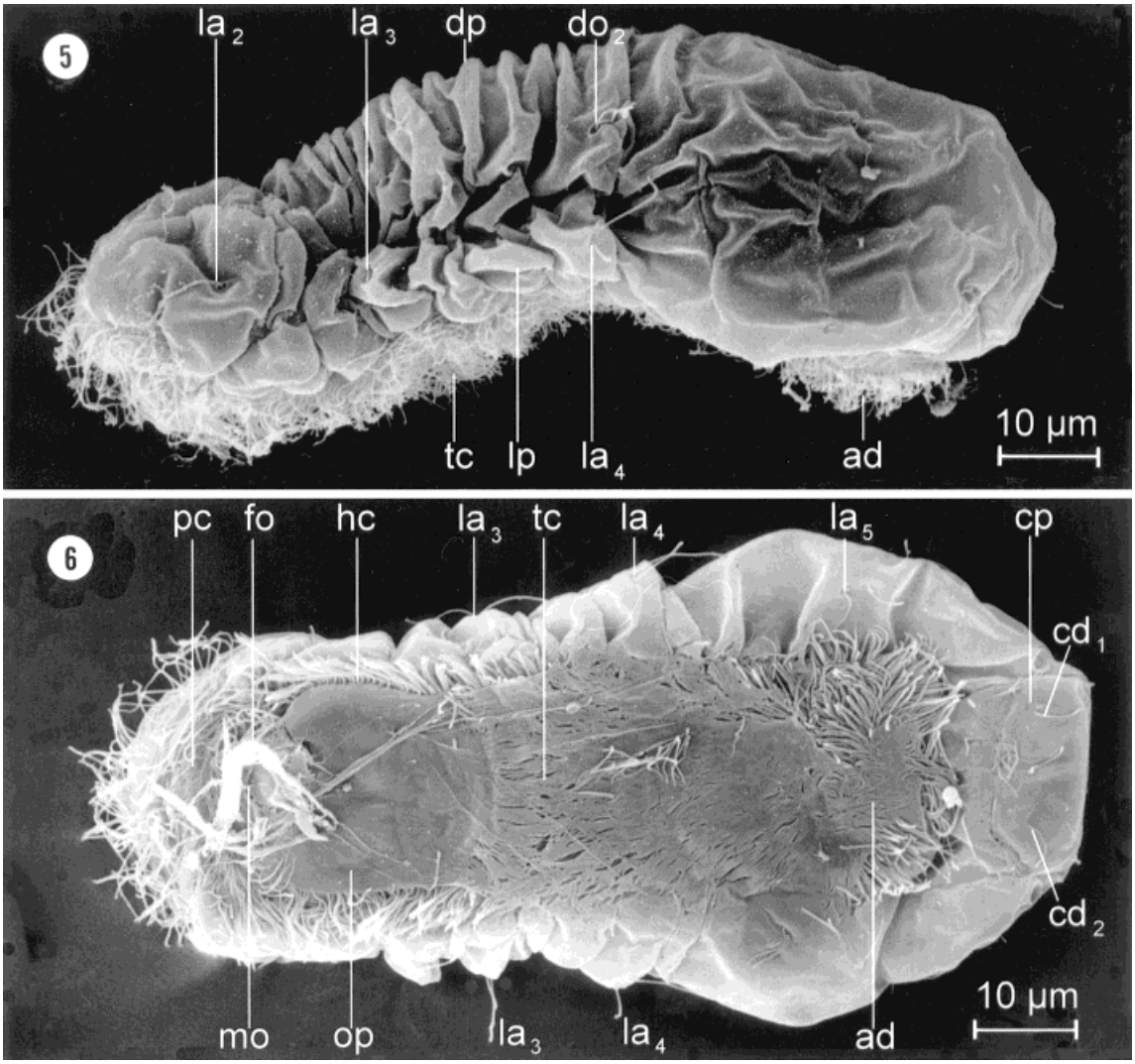


Figs. 3, 4. *Limnognathia maerski* nov. sp. et nov. sp. Micrographs (DIC) of live specimens. Fig. 3: Habitus photo of the whole animal divided into head (he), thorax (th), and abdomen (ab). Note the different size of the oocytes (oo_1 and oo_2). br, brain; gc, midgut cells; ja_1, ventral jaw; ph, pharyngeal apparatus. Fig. 4: Ventral view of strongly squeezed caudal part. ad, adhesive ciliated pad consisting of ten ciliated cells (ce); bb, basal bodies in rows; ep, unciliated epidermal cells; gs, glue secretion; oo_1, oocyte; tc; trunk ciliophore.

The dominating ventral ciliation in *Limnognathia maerski* is located on the thorax and the abdomen. Two rows of trunk ciliophores exist. The length of each ciliophore is about 5 μm and the width is about 15 μm. The cilia are ordered in four rows. There are about 20 cilia in each row. In the allotype there exist 18 pairs of trunk ciliophores, ten pairs on the thorax and eight on the abdomen. There is no difference between the ciliophores of the thorax and those of the abdomen.

Just behind the last abdominal ciliophores is a large adhesive ciliated pad (Fig. 2, ad). The pad consists of two paired groups of five cells. The cilia in the pad are also stiff, but they are longer than the cilia in the ciliophores and they do not beat in unison. In the strongly squeezed paratype (Fig. 4, gs), a secretion is squeezed out from a pore midventral between the two clusters of cells. We assume the secretion is adhesive. This assumption was supported by live observations of the allotype. The animal stuck to the substrate with the posterior ventral part when we tried to remove it with a Pasteur pipette.

The action of the trunk ciliophores was also observed in the living allotype. The trunk ciliophores



Figs. 5, 6. *Limnognathia maerski* nov. gen et nov. sp. Females. SEM. From culture, Copenhagen. Fig. 5: Lateral view of a slightly shrunken specimen showing the dorsal (dp) and lateral plates (lp). The locomotory cilia are ventral ciliation with trunk ciliophores (tc) and an adhesive ciliated pad (ad) posteriorly. Note the position of one of each of some of the paired sensoria: lateralia (la_2-la_4) and dorsalia (do_2). Fig. 6: Ventral view of animal with anterior preoral cilia field (pc), head (hc) and trunk ciliophores (tc), and a posterior adhesive ciliated pad (ad). The mouth (mo) is partly covered by food (fo) or detritus and situated anterior to the oral plate (op). Note the position of some of the paired lateralia (la_3-la_5) and caudalia (cd_1-cd_2). cp, caudal plate.

are locomotory organs and they beat when the animal crawled on the substrate (mosses) and when the animal was swimming. It could be that the preoral ciliary field is used together with the head ciliophores as a broom to collect small detritus particles. This theory was not supported by live observations, however. It seems that the animals feed by grasping food particles directly by the ventral jaws. The anterior ciliation instead could be involved in swimming.

Internal structures. The few internal structures that could be seen in the living allotype included two pairs of protonephridia (Fig. 2, pr_1-pr_2), one pair in the thorax and another pair in the anterior part of the abdomen. The flame cells could be observed beating inside the lateral protonephridium, but the nephridiopore could not be located (see later, "Excretory Structures").

The oocytes in the two ovaria are similar to the oocytes in the holotype, but the right oocytes are further developed and larger (diameter = 28 µm). In the left side of the abdomen of the allotype two characteristic globular bodies are located. We call them refractive bodies (Fig. 2, rb_1-rb_2). Their function is unknown, but they are only seen in mature females with large oocytes or sculptured eggs.

Other Paratypes

More than 100 specimens were observed alive from the cold spring of Isunngua. Many of them

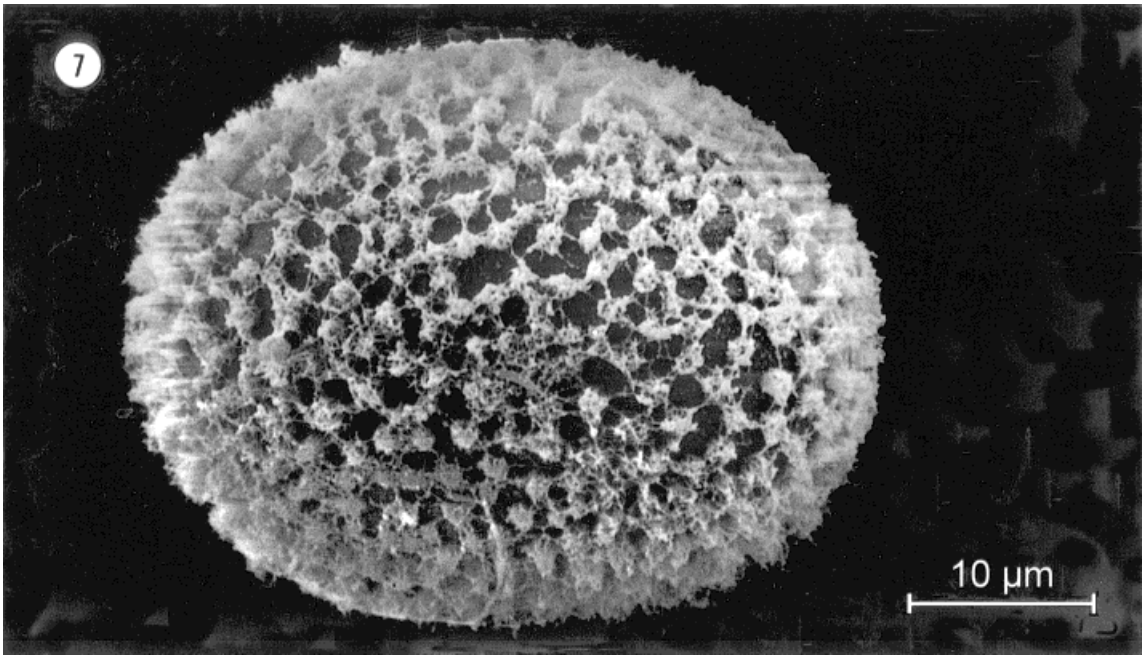


Fig. 7. *Limnognathia maerski* nov. gen. et nov. sp. SEM. A newly laid sculptured egg from a female. From Isunngua, 25 July 1994.

were used for video recordings, or squeeze or jaw preparations. This material does not exist any longer, but has been documented with video recordings, drawings, and micrographs (Figs. 3, 4). Three paratypes were used for SEM (Figs. 5, 6) and six for TEM. The TEM material will be treated under "Ultrastructural TEM Morphology." The holotype, allotype, and an additional 27 specimens were drawn using a camera lucida, measured, and then kept as wholemount preparations on glass microslides.

The length of the adult animals ranges from 105–152 μm, with an average length of 123.3 μm (n = 23). We defined adult females as those where both ovaries with oocytes are present. The length of the juveniles, being those without any signs of gonads, ranges from 85–107 μm, with an average length of 93.0 μm (n = 7). When we compared juveniles with adults no differences in the jaws, sensoria, or dorsal plates were observed. The number of rows of the double ciliophores varied, however. A minimum number of ten rows was observed in the small juveniles, while a maximum of 18 rows was seen in the allotype. It is therefore likely that development is direct, although we never observed a juvenile hatching from an egg. The ciliophores can be observed easily using DIC optics (Fig. 4). In the SEM preparations we made (Fig. 6) the trunk ciliophores (tc) and the adhesive ciliated pad (ad) were covered with a thin layer of mucus or glue, and all the unique characters could not be distinguished. The head ciliophores (hc) are organized as compound cilia. The sensoria are often lost in SEM preparations (Figs. 5, 6) and only the sockets are seen (cd_1). If the sensorium is present, it could be observed that the structure consists of more than one stiff cilium (Fig. 6, la_3).

Development

The smallest female (105 μm) had two small oocytes, one in each ovary. Usually there is only one large oocyte and a smaller oocyte in the opposite ovary (Fig. 3). A large animal (152 μm) had developed two large oocytes (right oocytes, d = 45 μm; left oocytes, d = 39 μm). In several animals the female gamete had developed a chorion or eggshell. We call this gamete an egg. The egg can be enormous relative to the small size of the animal, and the egg may occupy most of the abdomen. Only one egg develops per clutch. The smallest egg with a chorion had a diameter of 31 μm and was unsculptured. The largest egg in the ovary had a diameter of 48 μm and was sculptured, with osmiophilic dots at the surface.

We could provoke the females to lay the egg. When we kept the animals at room temperature (20°C) the animals left the detritus or the moss leaves and swam around in the water column. When we transferred the specimens to small watch glasses the animals would lay eggs at the bottom. The egg-laying behavior was difficult to observe, but it seemed that the animal bent ventrally, and that the egg burst out from the ventral side close to the adhesive pad. All eggs were of the same size and were quite sticky. We recognized two types of eggs. One type was unsculptured and burst easily, and the second type was sculptured (Fig. 7) and could be removed more easily from the watch glass. The free

egg is oval (30 × 40 μm) and other observed eggs ranged in size from 40–60 μm at the longest axis. The unsculptured eggs could be abortive, while the sculptured eggs were capable of further development. The justification for this assumption is that the whole population of adult females would deliver the oocytes and eggs when they were kept at high temperature. We did not observe any cleavage in the eggs. Furthermore, we never found eggs with jaws of juveniles inside.

We looked very intensively for smaller males both in Greenland and in the culture in Copenhagen. Unfortunately, we never observed any, and therefore, we expect that the species reproduces by parthenogenesis, at least during the summer. We cannot rule out that the animal is hermaphroditic. The two refractive bodies (Fig. 2, rb) may be a part of the male reproductive system. The smallest juvenile (85 μm) was observed alive. The head was relatively large (35 μm) and the thorax weakly developed. As mentioned above, it had only ten rows of ciliophores and it may be the ciliophores of the thorax that are lacking. It moved exactly like the adults and it already had a full set of jaws in the pharyngeal apparatus. The animals were kept in a moss culture in a 4°C refrigerator for more than 2 years. During this period only seven juveniles were observed and measured.

Fine Structure of the Jaw Apparatus

The fine structure of the "true" cuticularized parts of the pharyngeal apparatus is important for a discussion of the phylogenetic position of *Limnognathia*. We therefore describe the isolated cuticularized parts (sclerites) of the pharyngeal apparatus in this section.

A complete investigation of the ultrastructure of the whole pharyngeal apparatus, e.g., cuticular elements, epidermal cells, and the mesodermal cross-striated muscles, is needed. The only way to do that is to combine SEM and TEM techniques (Fiege, 1990). A less sophisticated technique is to serial-section the entire pharyngeal apparatus and make three-dimensional reconstructions of the location of all cuticular elements, the nuclei of the epidermal cells, and the cross-striated muscles. This technique has been used with great success in the eutardigrades (Eibye-Jacobsen, 1997). Unfortunately, this technique is time-consuming and requires single-hole grids for serial sectioning. We sectioned six pharyngeal apparatuses, but we used the more secure 200-mesh grids. The additional information obtained from the TEM study was crucial to understanding the general organization of the pharyngeal apparatus; for example, that the cuticularized parts are extracellular and that the jaw apparatuses are built in the same way as the jaws of gnathostomulids. The results from our TEM study of the pharyngeal apparatus will be treated under the section on ultrastructure.

In this section, we use the technique described by Riedl and Rieger (1972) for gnathostomulids and thereafter combine light microscopical observations with SEM micrographs. We first observed living, squeezed animals by DIC optics (Figs. 10,11). A few animals were treated with OsO_4-vapor and were strongly squeezed (Fig. 12). This allowed us to see the dentarium and articularium of the main jaws (ja_2). Soon it became clear that some parts of the cuticular jaw apparatus were hidden in the epidermal tissues and the muscles. In order to reveal the hidden parts, whole animals were treated with 2% sodium hypochlorite (Figs. 13–15). This bleaching process allowed us to see the main cuticular parts, the basal plates, the three pairs of jaws, and the complex structure of the fibularium with its many fenestrae and fibulae. These structures are strongly resistant to the bleaching process. Unfortunately, all ligaments, the oral plate, and the lamellae orales dissolved immediately. Consequently, the ventral jaws (ja_1) and the accessory sclerites (as_1, as_2) were lost in all sodium hypochlorite preparations. It soon became obvious that the fine structure of the cuticularized pieces was too small and complex to be fully resolved with light microscopy. A modified SEM technique of Koehler and Hayes (1969) was therefore introduced (Figs. 16, 17). In this technique the soft tissue was also removed with sodium hypochlorite. The three-dimensional configurations were added to the two schematic drawings (Figs. 8, 9), which were made with the camera lucida technique. When the DIC micrographs (Figs. 10–15) were compared with the SEM micrographs (Figs. 16, 17), it was obvious that both techniques have their advantages. When we used DIC it was possible to observe the living animals first and later to treat the animals with sodium hypochlorite. This affords the opportunity to observe the cuticular elements in situ attached to epidermal cells, ligaments, and muscles. We never observed all cuticular parts together using SEM and the thin lamellae of the fibularium were twisted, so it gave the impression that the structure is much more flattened compared to similar structures in living specimens.

On the other hand, many details were seen only with SEM and these structures are still not well understood, e.g., the free teeth between the main jaws (ja_2) and the dorsal jaws (ja_3), the pseudodentes (p.de), the trochanter (tr), and the spinula (sp) of the dorsal jaws (see Figs. 16, 17). We have to admit that our interpretation is preliminary and we may have misinterpreted several structures, but otherwise we hope the readers will understand that the complexity of the jaw apparatus of *Limnognathia maerski* is far beyond what is seen in other invertebrates such as gnathostomulids, rotifers, and dorvilleid polychaetes. For a comparison of the fine structure

of their jaw apparatuses and that of *Limnognathia*, see below.

We used a new terminology for the more important cuticular parts of the jaw apparatus. We employed the Latin nomenclature in many general structures, such as apophysis, fenestra, trochanter, etc. Unfortunately, this nomenclature has been used in gnathostomulids, and to some extent in rotifers as well. We are still not sure if these structures are homologous in the three groups, but the ultrastructure of some of the cuticular elements (Rieger and Tyler, 1995) indicates that this is the case. The following description of the main elements 1) the basal plates, 2) the lamellae orales, 3) the ventral jaws, 4) the main jaws, 5) the two fibularia, and 6) the dorsal jaws is given from the ventral perspective to the dorsal view (Figs. 8–17).

Basal Plates (ba)

A pair of molar-like structures (Figs. 9, 16, ba) is located on the ventro-caudal part of the mouth opening. These two structures can be extruded out from the lower lips when the animal forages. The molar structure is triangular in shape, with five cusps of heavily sclerotized material. The two molars are the first elements to be seen in optical sections with DIC techniques (Figs. 11, 13) in ventral view. Each molar is 2 μm wide and 1.5 μm high. The outer cuticular part of the basal plates is fixed to thin jointed lamella plates, which continue inside the lower part of the mouth cavity. The thin plates were not observed with DIC optics, but were clearly seen in specimens treated with sodium hypochlorite (Figs. 8, 9, 16, 17). The thin lamellar parts are 4 μm long and are fused rostrally with a 1.5 μm-long suture. Two large cross-striated muscles seem to attach close to the molar portion of the basal part. In connection with the basal plates, five additional dentes oralis (de.o) are seen in the SEM micrographs (Figs. 16, 17). These structures were not observed by DIC optics and were consequently omitted from Figure 9. They could have been hidden by the outer cuticular rim of the mouth opening itself.

Lamellae Orales

Lamellae orales were observed only in living animals and in the TEM micrographs. They consist of two halfcone-shaped structures on the rostral part of the mouth cavity. In ventral optical sections the lamellae orales are seen as two arched upper lips (Fig. 11). These structures may constitute a continuous, folded cuticular membrane covering the upper part of the mouth cavity. By DIC techniques (Fig. 11) the lamellae orales are seen as two arcs with delicate striation. At least 13 folds are observed in each arc. The lamellae orales support the dorsal mouth cavity and prevent cavity collapse during feeding. The lamellae orales quickly disappear in the sodium hypochlorite bleaching process and the collapsed lamellae can be seen as an extra, membrane-like structure outside the cleaned jaw apparatus (Figs. 13–15).

Ventral Jaws (Pseudophalangia)

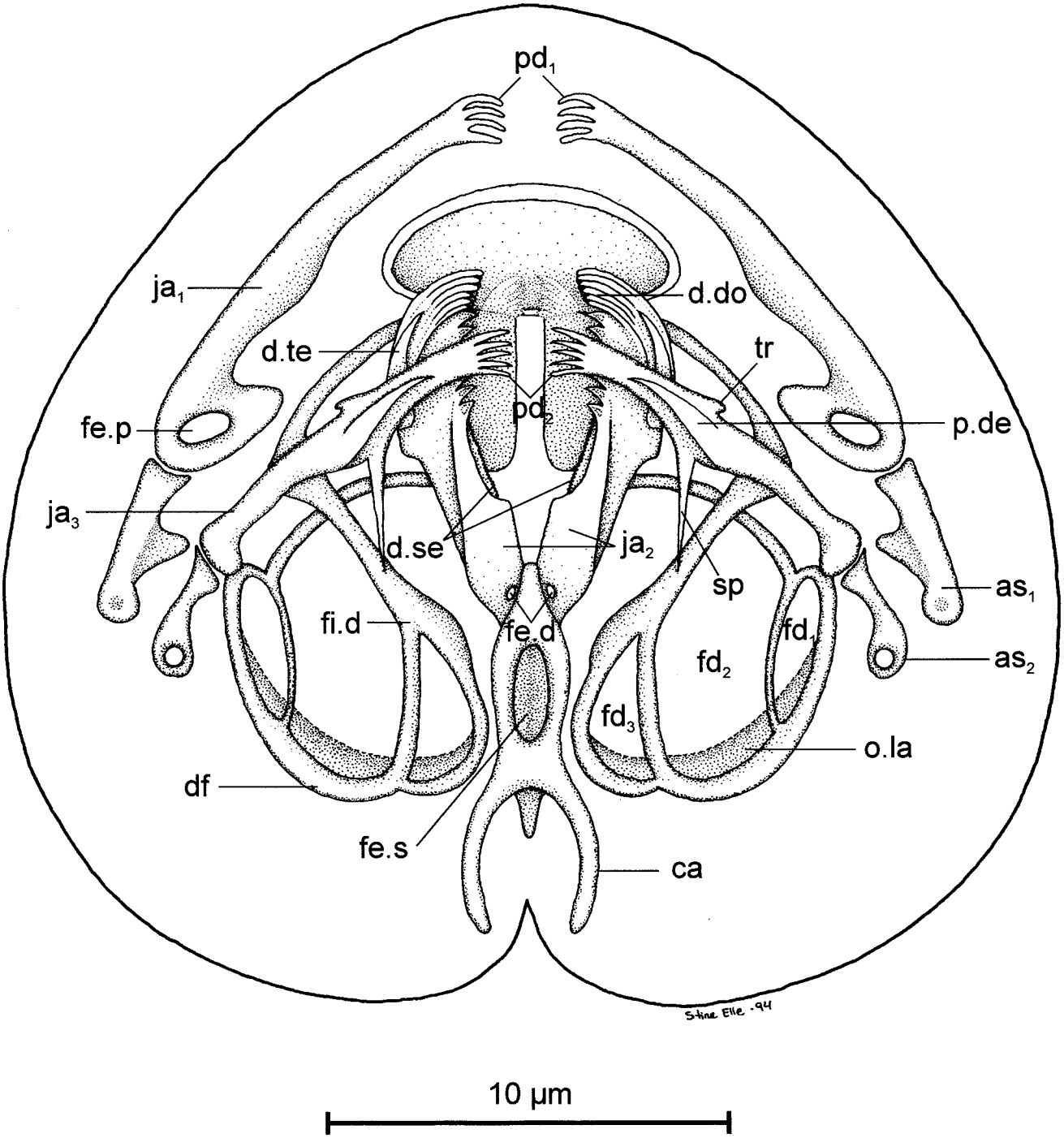
The ventral jaws consist of two pairs of strongly cuticularized elements, the pseudophalangia (ja_1) and the accessory sclerites (as_1). These two elements are joined with a ligament and a ball-and-socket joint. Furthermore, a ligament connects the pseudophalangium to the fibula ventralis of the fibularium. Both ligaments and the large cross-striated muscle disappeared when sodium hypochlorite was added, and the pseudophalangium was found independent from the rest of the jaw apparatus. The accessory sclerite, on the other hand, stays attached to the lateral part of the fibularium (Figs. 13, 14, 16, 17), even after the pseudophalangium disappears. The link between the pseudophalangium, the fibularium, and the accessory sclerite is much more complicated than we have drawn in the squeezed preparation of the jaw apparatus (Figs. 8, 9).

The pseudophalangium is a large sclerite and it can be quite moveable, facilitated by the large cross-striated muscles. The muscle attachment is formed as a fenestra pseudophalangialis (fe.p) at the swollen base of the sclerite. Both pseudophalangia can be protruded through the mouth opening under foraging. In relaxed swimming behavior the two pseudophalangia would be located latero-rostral of the mouth opening (Figs. 8, 9). The tip of each pseudophalangium consists of four large digits; in a few specimens a fifth smaller digit also was observed as a dorsal thumb. The length of the pseudophalangium is 12 μm and the length of the accessory sclerite is 5 μm. Both structures are a hollow tube-like structure in cross section (see below).

Main Jaws

The main jaws are pincer-like. The pincer consists of the paired dentarium and the unpaired articularium with the symphysis. Dorso-caudally the main structure again is split into a symmetrical cauda (Figs. 8, 9, 12, 15–17, ca), which is the apodeme for the posterior pharyngeal muscle sac. Ventro-caudally the symphysis (Figs. 9, 17, sy) continues as an unpaired element. The fibularium, the largest element in the jaw apparatus, attaches to the main jaws laterally. The fibularium could be regarded as a part of the main jaws, but will be treated separately because of its complexity.

The dentarium is the teeth-bearing distal part of the pincer. Each branch of the pincer has an arc-shaped arrangement of teeth which can be observed from a lateral view (Fig. 12). The ventral arc (arcus ventralis) is prominent and possesses five large teeth, the dentes ventrales (Fig. 9, d.ve).



Figs. 8, 9. *Limnognathia maerski* nov. gen. et nov. sp. Semi-schematic drawings of the two pharyngeal apparatuses based on DIC and SEM techniques. The drawings are constructed from two different animals. Fig. 8: Dorsal view. Fig. 9: Ventral view. as_1, accessory sclerite of ventral jaw (ja_1); as_2, accessory sclerite of dorsal jaw (ja_3); ba, basal plate; ca, cauda; d.do, dentes dorsales; df, dorsal fibularium; d.me, dentes mediales; d.se, dorsal serratum; d.te, dentes terminalis; d.ve, dentes ventrales; fd_1-fd_3, three fenestrae of dorsal fibularium; fe.d, fenestra dentarialis; fe.p, fenestra pseudophalangialis, fe.s, fenestra symphysis; fi.d, fibula dorsalis; fi.v, fibula ventralis; fl_1-fl_4, four fenestrae of lateral fibularium; fv_1-fv_2, two fenestrae of ventral fibularium; ja_1, ventral jaw (pseudophalangium); ja_2, main jaw consisting of dentarium and articularium; ja_3, dorsal jaw; lf, lateral fibularium; l.or, lamellae oralis; mo, mouth opening; o.la, outer lamella of fibularium; pd_1, pseudodigit of ventral jaw; pd_2, pseudodigit of dorsal jaw; p.de, pseudodentes on the dorsal jaw; sp, spinula of dorsal jaw; sy, symphysis of main jaw; tr, trochanter of dorsal jaw, vf, ventral fibularium.

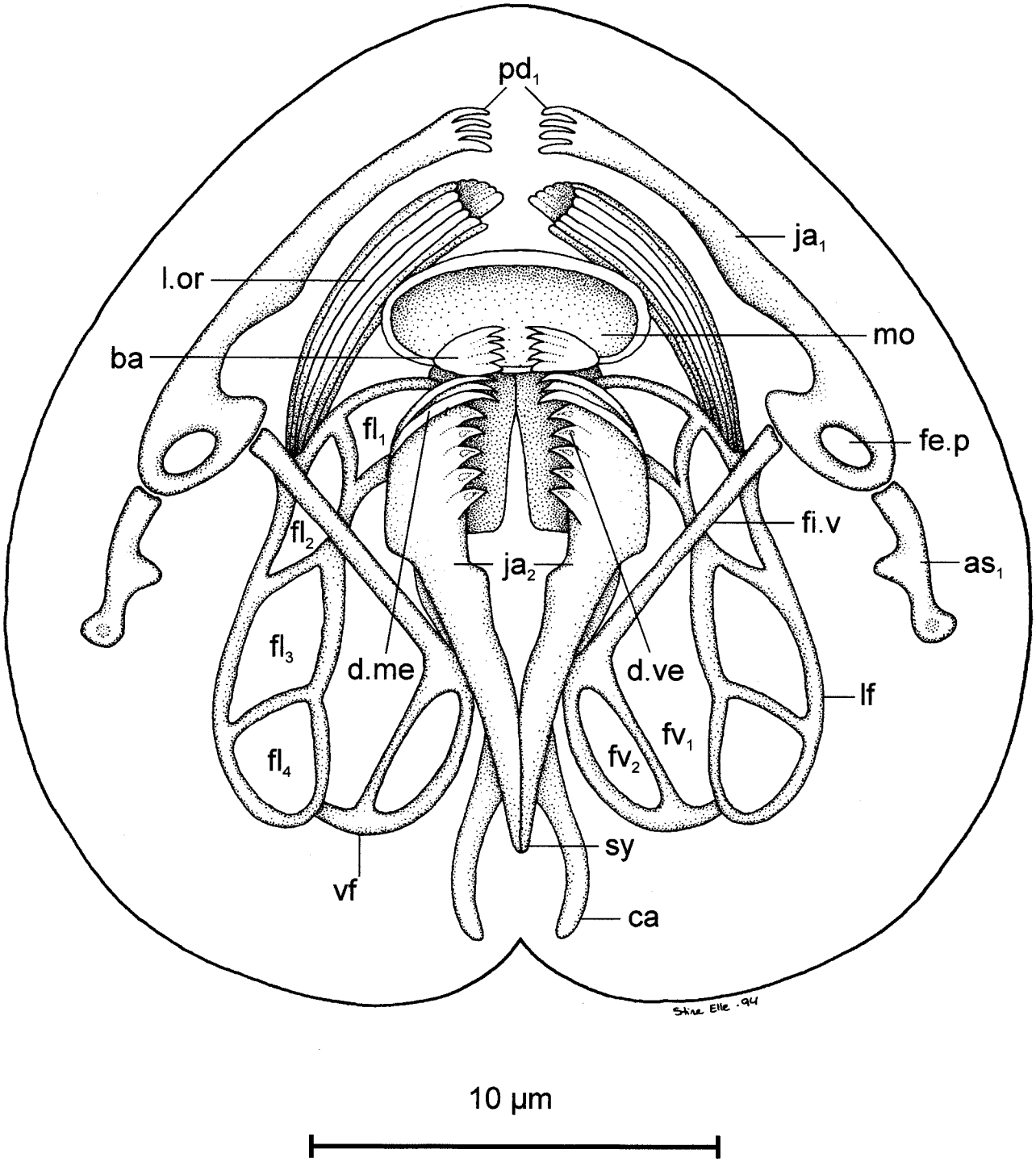
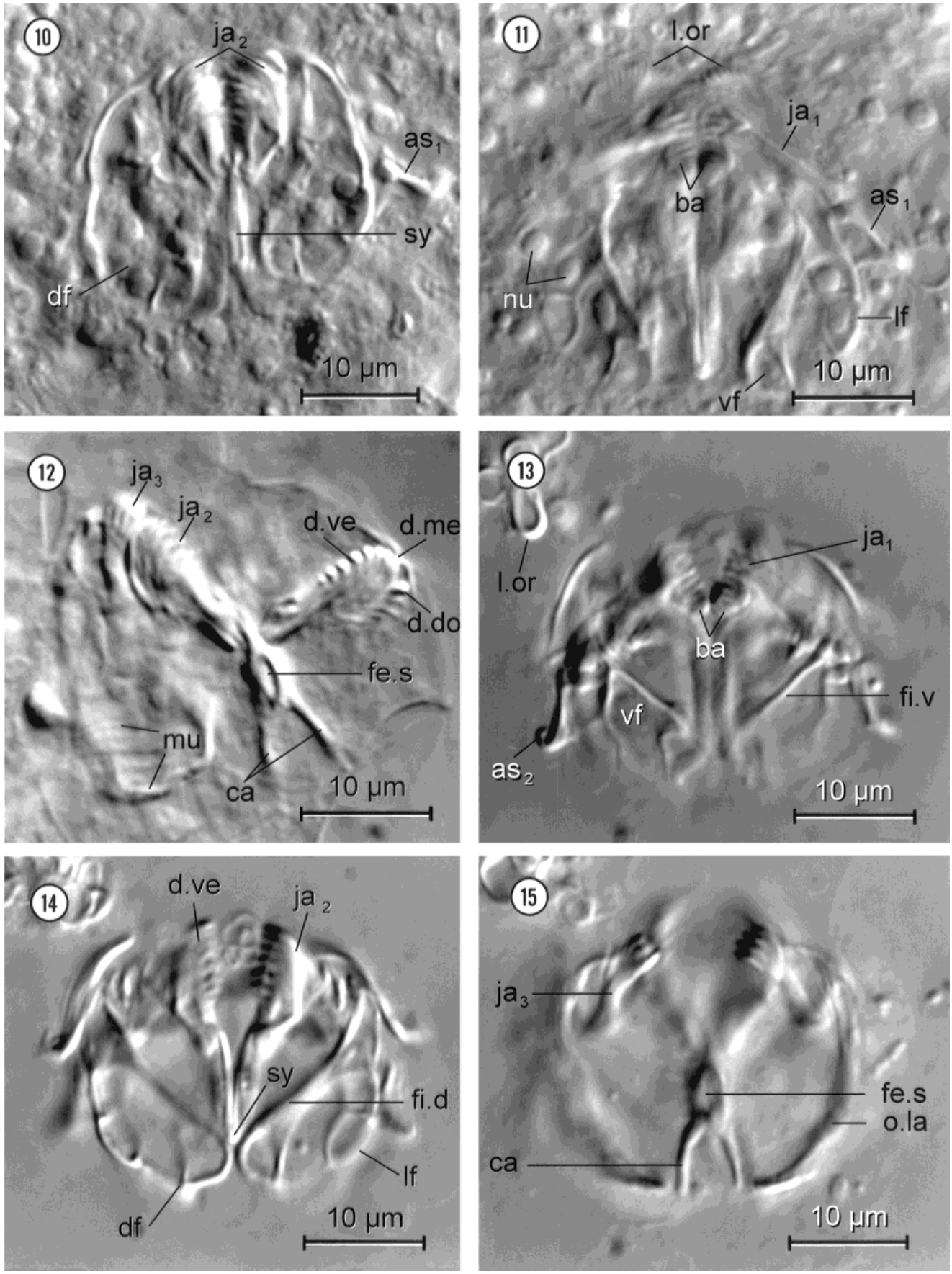


Figure 9

The dentation of the median part of the arc (Fig. 9, d.me) remains beyond the resolution of the light microscope (Fig. 12, d.me). With SEM, however, it seems that three small dentes mediales are present, and in TEM preparations even more teeth are seen in cross section. The structure of the dorsal arc is much more difficult to interpret. When the dorsal arc (arcus dorsalis) is observed in the light microscope with DIC optics (Fig. 12, d.do), it seems to consist of a large tooth, the dentes terminalis, followed by five tread-like structures. When cleaned jaws are observed with SEM, it is seen that the dentes terminalis are not connected to the dorsal arc, but lie free at the



Figures 10–15

surface (Fig. 16, d.te). We still believe it is the same structure as seen by DIC optics, because the dentes dorsalis (d.do) are also located superficially as thin tread-like teeth on the dorsal arc.

Caudal to the arc-structure, the dorsal edge of the dentarium has a serrated appearance. This dorsal serratum (Figs. 8, 16, d.se) consists of 20 small saw teeth on each side of the pincer-arms. Just before the symphysis the dentarium has a pair of small holes (Figs. 8, 16, fe.d). The symphysis continues ventrally as an unpaired caudal part (Figs. 9, 14, 17, sy). The dorsal part of the articularium is more complicated. Behind the articulation of the two arms in the dentarium, is a 4 μm-long single piece of the articularium. This lamella structure is penetrated by a large hole, the fenestra symphysis (Figs. 8, 12, 15, 16, fe.s). The articularium has two symmetrical cauda (ca). We believe that this structure is the apodeme for the large caudal muscle-sac.

The Two Fibularia

The fibularium consists of cuticularized fibulae and fenestrae. Each fenestra is a window with cellular tissue surrounded by cuticle. The cellular part is involved in forming the extracellular fibulae. The unique fibularium is perhaps only a part of the apophysis of the main jaws as seen in some gnathostomulids and rotifers, but in *Limnognathia* the structure has developed to an extreme degree. The fibularium in *Limnognathia* is a three-dimensional structure that totally surrounds the main jaws as three compartments: the ventral, lateral, and dorsal fibularia.

The ventral fibularium is characterized by a strong fibula ventralis (Figs. 9, 13, fi.v). This fibula runs across the ventral fibularium as a straight bar and it connects the basal parts of the pseudophalangium. The epidermal cells in the ventral fibularium have large nuclei and abundant cytoplasm (Figs. 10, 11). There are at least five ventral fenestrae with large cells. The lateral fibularium has four fenestrae whose cells have large nuclei. Each nucleus nearly fills the lateral fenestra (Fig. 11). The fibulae of the lateral fibularium are thin. We could only recognize three fibulae of the dorsal fibularium. The fibula dorsalis (Figs. 8, 14, 16, 17, fi.d) is thick and strongly sclerotized.

The outer limit of the fibularium consists of a thick lamella that can be seen as a nearly circular dorsal structure (o.la) by DIC optics (Fig. 15) or as a robust structure by SEM (Figs. 16, 17). The fibularium is attached to the dentarium rostrally but is embedded in the cellular tissue as an apophysial structure in its caudal part.

Dorsal Jaw System

The pharyngeal channel passes through the two arcs of the main jaws and continues dorso-caudally to a short esophagus. Before entering the esophagus the food particles must pass through the third jaw system (ja_3). The dorsal jaws are in many ways similar to the pseudophalangia. These dorsal cuticular structures consist of a pair of arm-shaped rods (Figs. 8, 15–17, ja_3). Rostrally, each arm has four large digits, a smaller dorsal thumb-like digit, and several small teeth, which could not be seen by light microscopy. The caudal part of the dorsal jaw also extends a ligament to an accessory sclerite (Fig. 8, as_2). The ligament disappears with sodium hypochlorite treatment and the pair of accessory sclerites is therefore missing in the SEM preparation (Figs. 16, 17).

The dorsal jaws are surely serially homologous with the ventral jaws, but have a more complicated structure. The jaws have a dorsal trochanter (tr) for the attachment of a large striated muscle and a midventral spinula (sp) attaching the fibula dorsalis (fi.d). Furthermore, the caudal part is attached to the outer lamina of the fibularium (Fig. 16, o.la). The paired dorsal jaw system is also connected to the fibularium in the sodium-hypochlorite-treated animals (Figs. 15–17).

Ultrastructural TEM Morphology

The ultrastructural observations by TEM are based on trialdehyde-fixed materials (Figs. 18–33). A few artifacts were clearly seen by TEM. The spacious body cavity between the digestive system and the epidermal cells (see Figs. 18, 22) is an artifact of inappropriate fixation. The same osmotic problem may be seen in the dorsal epidermal cells with the

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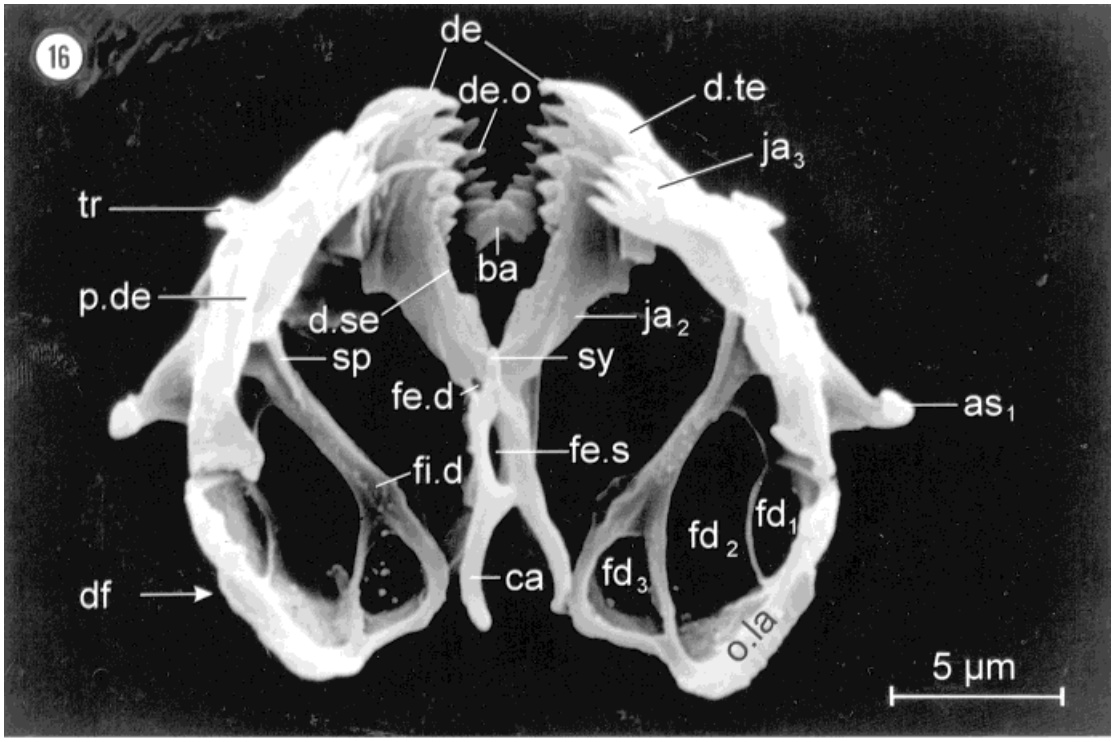
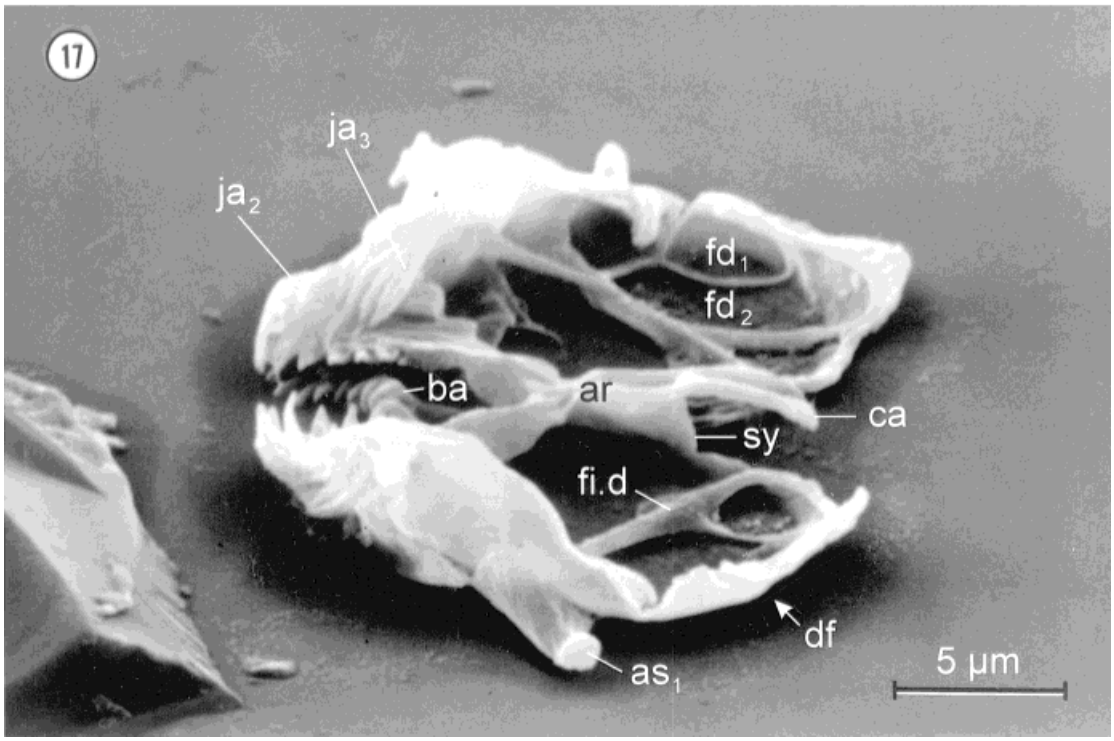
Figs. 10–15. *Limnognathia maerski* nov. gen. et nov. sp. DIC micrographs of jaws. Figs. 13–15. The jaw apparatus of the same animal as Figure 11 treated with sodium hypochlorite. The dissolved lamellae oralis (l.or) is seen at the upper left corner in all three photos. Fig. 10: Living animal, middorsal optical section, strongly squeezed preparation. The dorsal fibularium (df) is attached to the main jaws (ja_2). Note the symphysis (sy) of the articularium in the middle and the accessory sclerite (as_1) of the ventral jaw to the right. Fig. 11: Living animal, ventral optical section focused on ventral jaws (ja_1) with the accessory sclerite (as_1), lamellae oralis (l.or), and basal plates (ba). The fenestrae of both the ventral (vf) and lateral fibularium (lf) contain several nuclei (nu). Fig. 12: Strongly squeezed, osmium-fixed animal. To the left a large cross-striated muscle (mu) seems to attach to the dorsal jaw (ja_3). The main jaws (ja_2) are twisted. The arcs of the dentarium with teeth (d.do, d.me, d.ve) are seen to the right. The articularium with a large fenestra symphysis (fe.s) and the two symmetrical arms of the cauda (ca) are recognized. Fig. 13: Ventral optical section focused on the basal plates (ba), ventral jaw (ja_1), accessory sclerite (as_2), and the strong fibula ventralis (fi.v) in the ventral fibularium (vf). Fig. 14: Midventral optical section focused on the ventral arc of dentarium with teeth (d.ve), the symphysis (sy) of the articularium, the lateral (lf) and the dorsal fibularium (df) with the strong cutilarized fibula (fi.d). Fig. 15: Dorsal optical section focused on the dorsal jaw (ja_3), the outer lamella (o.la) of the fibularium and the symmetrical arms of the cauda (ca). fe.s, fenestra symphysis.





Figs. 16, 17. *Limnognathia maerski* nov. gen. et nov. sp. SEM. Jaw apparatus treated with sodium hypochlorite. The dorsal fibularium (df) is twisted ventrally compared to the view in Figure 14. Note also: The ventral jaws (pseudophalangia), the accessory sclerite, and lamellae oralis are lacking. Fig. 16: Dorsal view. Fig. 17: Lateral view. ar, articularium of main jaws (ja_2); as_1, accessory sclerite to ventral jaw (which is lacking); ba, basal plates; ca, cauda; de, dentarium of main jaws (ja_2); de.o, dentes oralis; d.se, dorsal serratum; d.te, dentes terminales; fd_1-fd_3, fenestrae of dorsal fibularium; fe.d, fenestra dentarialis; fe.s, fenestra symphysis; fi.d, fibula dorsalis; ja_2, main jaws consisting of articularium (ar) and dentarium (de); ja_3, dorsal jaw with pseudodentes (p.de), trochanter (tr) and spinula (sp); o.la, outer lamella; sy, symphysis.

plates and the nearly mature egg (Fig. 22). The thick glycocalyx may also become separated from the ventral ciliated cells, but otherwise the trialdehyde fixation was excellent, especially for low magnifications of different cell structures.

Integumentary Structures

The main integumentary structures in all adult females can be divided into the nonciliated dorsal epidermis (dorsal and lateral plates) with an intracellular matrix layer and into the ventral locomotory/feeding organ with multiciliated epidermal cells covered with a glycocalyx. The glycocalyx may be thin and simple or, as on the adhesive ciliated pad, thick and complex. Furthermore, the epidermal sensory cells (sensoria) consist of monociliary to multiciliary cells without a cuticle. The only external cuticular structure is the ventral oral plate, which is formed by nonciliated epidermal cells. Other epidermal cells in the pharyngeal apparatus produce hard cuticular parts (Fig. 23), but the ultrastructure of these is treated in the section "Digestive System," below. All epidermal structures are nonsyncytial. Syncytia were not observed at all in *Limnognathia maerski*.

Dorsal and Lateral Intracellular Plates

The dorsal epidermis in *Limnognathia maerski* is cellular, unciliated, and generally 2–5 μm thick (Fig. 18, ep). In the sutures between the plates the epidermis is much thinner, being less than 0.2 μm thick. A very thin glycocalyx (120 nm) covers the cell membranes of the epidermis (Fig. 21). Cell constancy (eutely) might be present in the epidermis. Two to four cells form each dorsal plate. The pronounced apical plate seems to be formed by a single large epidermal cell. The cell borders can be seen by DIC optics through the plate structure as dotted lines (Fig. 1). The dotted structure can be interpreted by TEM observations. It consists of two junctions (Fig. 26, dj) with a distance of about 0.5 μm, where the two cells form some interdigitations. The cell processes from one cell extend deeply into the apposing cell. Two other junctional complexes are present between neighboring epidermal cells. Between the two middorsal epidermal cells, which form the first dorsal plate in the thoracic region (Fig. 18, dp), a very characteristic cell junction complex is present (Fig. 21). Distally, the two cells form a large intercellular space (ic) distal to a gap junction, which can be up to 0.5 μm long. A unique type of septate junction is present between several dorsolateral epidermal cells (Fig. 19, zj) on the borders between plates (see review, Green and Bergquist, 1982). The junction consists of three to seven bridges, which open up like a zipper when the animal is treated with sodium hypochlorite, and the plates are separating. We named this new type of junction the zipper junction. The nucleus of the dorsal epidermal cells is round to oval and has heterochromatin close to the nuclear membrane. The cytoplasm in these epidermal cells is osmiophobic, with few vesicles and mitochondria (Figs. 18, 19).

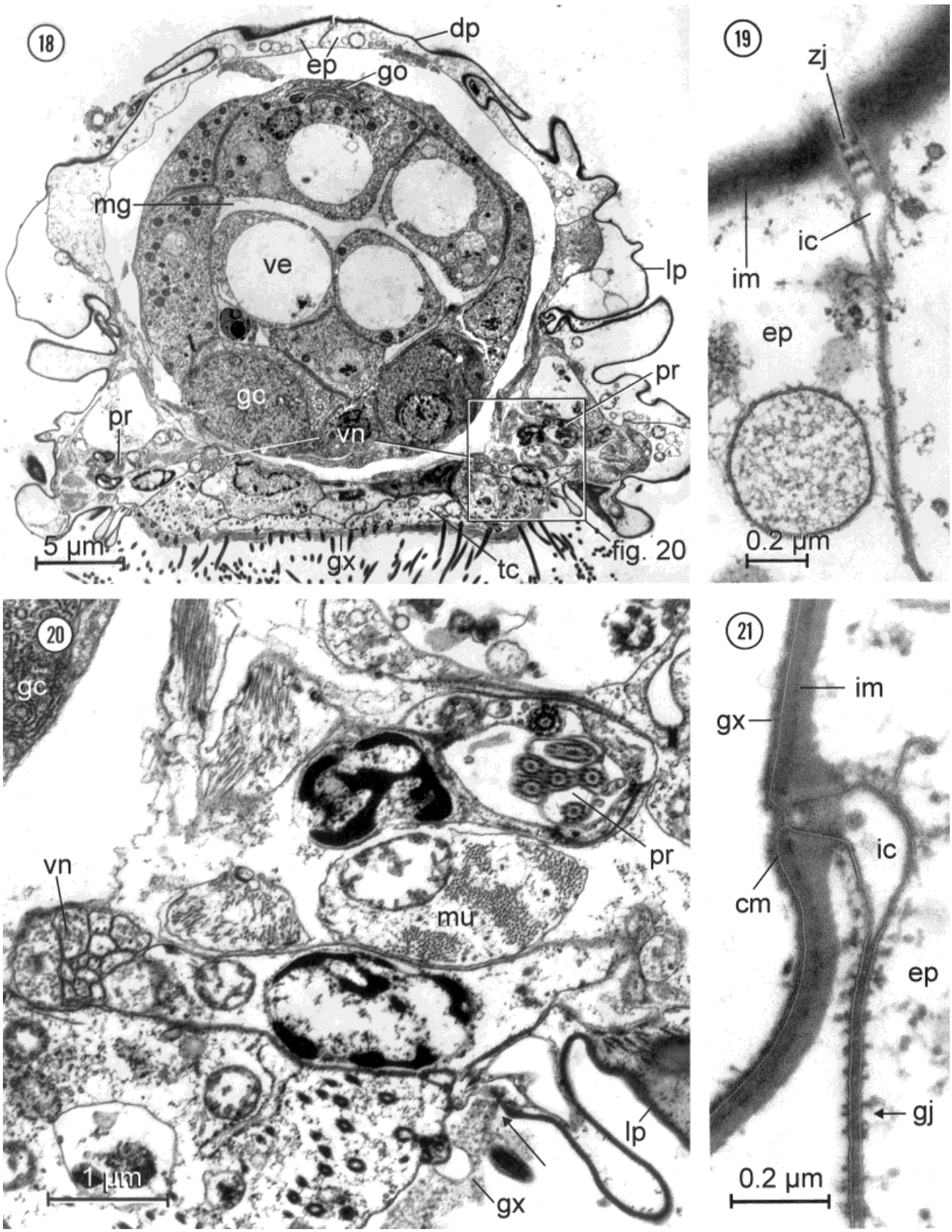
The dorsal and lateral plates are situated inside the epidermal cells. A plate consists of a conspicuous intracellular matrix layer (Figs. 19, 21, im). The matrix layer can be differentiated into a strongly osmiophilic outer layer and a less osmiophilic inner layer. The intracellular-matrix layer may range in thickness from 0.1–0.3 μm. It has been very difficult to observe the outer cell membrane of the epidermal cell (Figs. 19, 26), but with high magnification the cell membrane is seen as a typical unit membrane consisting of two osmiophilic layers with an osmiophobic layer in between (Fig. 21, cm). The plate structure is clearly located beneath the cell membrane, and is therefore intracellular.

The lateral plates are formed like the dorsal plates (Figs. 18, 20, lp) but they seem to be more flexible than the thick dorsal plates. The lateral plate continues to the ventral side, where the border between the plate and the ciliated ventrum is easily observed (Fig. 20). Usually, the lateral epidermis forms one to two folds in the junction between the plate structure and the stiff, flat ventrum.

Ventral Ciliated Epidermis

The ventrum has four quite different ciliated structures: the preoral cilia field, the head ciliophores, the trunk ciliophores, and the adhesive ciliated pad (Figs. 2, 5, 6, 18, 22). The preoral ciliary field consists of five rows of single cilia on both sides of the head and two rows of fronto-lateral rows of shorter cilia. The ciliary roots are relatively short. These cilia are associated with a labyrinth of extracellular membranes. Similar membrane structures are seen in the pharyngeal apparatus associated with the pharyngeal cilia. The cilia may work as a broom during foraging, but they are clearly also involved in swimming behavior. The four pairs of head ciliophores are located lateral to the oral plate. These ciliophores consist of rectangular cells with stiff compound cilia. The ciliary roots are relatively long.

The pairs of 18 trunk ciliophores are a unique character for *Limnognathia maerski*. They are the locomotory organ when the animal is creeping on the substrate. Each cell has four rows of cilia, each with about 20 stiff compound cilia. The beat of one row of compound cilia is synchronized. Each cilium is surrounded at its base by an extracellular ring of cuticle. The same feature is seen in the adhesive ciliated pad (Fig. 32, cu). Often, four ciliophores are seen in cross section. There exist only two rows of cells, but they overlap slightly, so the distal part of the anterior row is also cut. The ciliophores are all covered with a thick glycocalyx (Fig. 18, gx).



Figures 18–21

The adhesive ciliated pad consists of ten cells (Fig. 4). The cilia are ordered in register like those of the trunk ciliophores. The cross-striated ciliary root passes through the entire cell. The ciliary root may attach to the nucleus, which lies proximally in the cell. The glycocalyx is thick and is ordered in a characteristic network of osmiophilic granules with thin fibers (Fig. 32, gx).

Oral Plate

The oral plate was not visible by DIC optics in the living animals observed in Greenland. The jaw structure and the pharyngeal bulb completely overshadowed the outer cuticular plate. Later, the thin but large oral plate was discovered using SEM (Fig. 6, op) and TEM (Fig. 25, op). The oral plate is very flexible and can be transformed from a flat plate into a cone-shaped structure when the ventral jaws are protruded. The internal part of the oral plate is osmium-negative, as are the main elements in the jaws. The tubular subunits are seen between two unit membranes similar to plasma membrane (Fig. 27, tu).

Sensory Structures

The tactile bristles consist of stiff cilia arising from a ring-shaped socket (Fig. 5, do_2, la_3). This reinforcement consists of a thickening in the intracellular matrix layer. The pore in the middle where the cilia arise is naked, covered only with the cell membrane. The cilium may be surrounded by very short microvilli. It seems likely that all stiff cilia lack the central doublet of microtubules. There may be only one stiff cilium or up to three long, stiff, and adjoined cilia, as in the lateralia. Other sensory structures comprising cilia are located around the mouth opening. The ciliary structures in the pharynx (Figs. 22, 23, ci) may also have a sensory function.

A pair of round vesicular structures in front of the head was observed both in the allotype (Fig. 2) and in several paratypes (Fig. 40). They are osmiophilic and may be lipoid eye granules. The granules disappear in wholemount preparation, but an eye structure similar to a phaosome was observed with TEM just in front of the pharyngeal structure.

Glands and Secretion

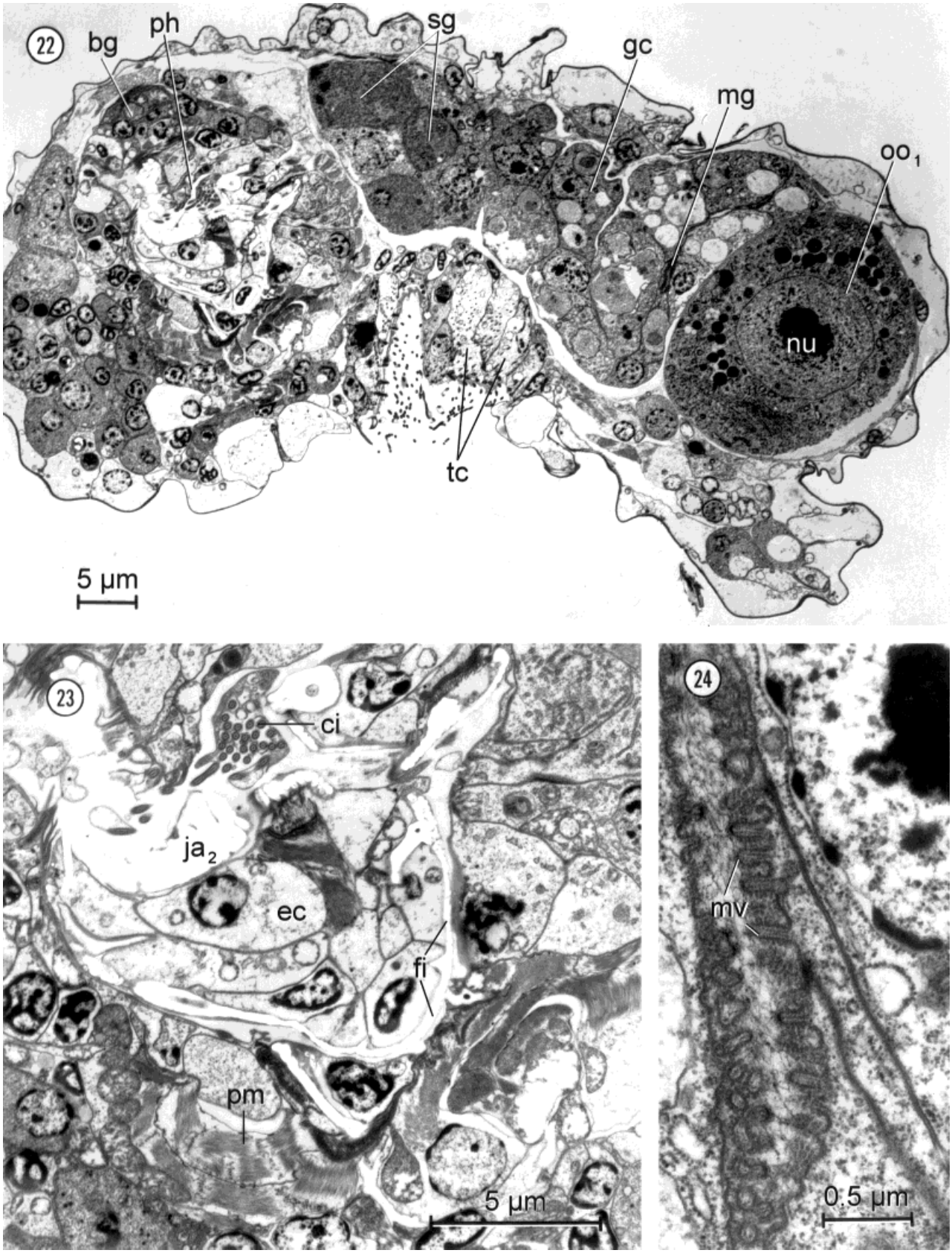
Limnognathia maerski is poor in epidermal glands. The only epidermal gland complex is associated with the adhesive ciliated pad. It is located in the tail (Fig. 29, ag) and consists of two cells filled with large vesicles. These cells are large, up to 10 μm. The substructure in the glands does not resemble the duo-gland systems in other meiofaunal animals; it looks more like mucous glands as found in kinorhynchs. The glands seem to open just behind the adhesive ciliated pad. In a strongly compressed animal the glandular material is extruded (Fig. 4, gs). The glands related to the digestive system will be described in that section.

Nervous System

Brain. The large cerebral ganglion occupies most of the head in front of the pharyngeal apparatus (Fig. 3, br). It is slightly bilobate. The neuronal cell bodies form the outer rim of the brain and surround the central neuropile (Fig. 29, br). The basal laminae are poorly developed in the head, so it is unclear whether the brain is intra- or subepithelial in position. The perikarya of the outer rim consist of about 175 round cell bodies, each with a large nucleus. The heterochromatin forms several clusters inside the nucleus. The commissural connections between the two brain lobes are prominent. The commissure consists of neuropile fibers, which contain vesicles ranging in diameter from 40–100 nm. A ventral longitudinal nerve cord extends from each brain lobe. Other nerve cords were not observed, but they may be present because nerves were observed in the pharyngeal apparatus.

Ventral nerve cords. A pair of ventral longitudinal nerve cords extends lateroventrally to the pharyngeal apparatus. Each consists of about 15–17 nerve fibers filled with neurovesicles (Fig. 20). The ventral nerve cords are deeply submerged and they seem to be subepidermal, but again the basal lamina is hard to locate. It is therefore very difficult to state the position of the whole nervous system. The two nerve cords may be associated with a double ganglion in the thorax and a caudal ganglion close to the tail (pygidium).

Figs. 18–21. *Limnognathia maerski* nov. gen. et nov. sp. TEM. Fig. 18: Cross section of the animal just behind the pharyngeal apparatus. Note the large vesicle (ve) and the Golgi apparatus (go) inside the midgut cell (gc). The midgut (mg) lacks cilia. The dorsum is covered with epidermal cells (ep) forming the dorsal plates (dp) and lateral plates (lp), while the ventrum consists of trunk ciliophores (tc) that are only covered with glycocalyx (gx). The two ventral nerve cords (vn) are located close to the lateral protonephridia (pr). Fig. 19: High magnification of a new type of septate junction, the zipper junction (zj), between two dorsolateral epidermal cells. The plate, or intracellular matrix layer (im), is formed inside the epidermal cell (ep). ic, intercellular space. Fig. 20: A close-up of Figure 18 showing a midgut cell (gc), the right protonephridium (pr), and the right ventral nerve cord (vn). The arrow indicates the junction between the lateral plate (lp) and the ciliated ventrum that is only covered with glycocalyx (gx). The muscle (mu) is obliquely cross-striated. Fig. 21: High magnification of junction between two middorsal epidermal cells showing the intracellular matrix layer (im) inside the epidermal cell (ep). The two epidermal cells form a gap junction (gj) after a distal intercellular space (ic). The cell membrane (cm) is only covered with a very thin glycocalyx (gx).



Figs. 22–24. *Limnognathia maerski* nov. gen. et nov. sp. TEM. Fig. 22: Latero-longitudinal section of the whole animal. Note the pharyngeal apparatus (ph), the salivary gland (sg), the midgut (mg), and nearly mature oocyte of the right side (oo_1). The buccal gland (bg) is located dorsal of the pharyngeal lumen. Only a few trunk ciliophores (tc) are cut. Fig. 23: Close-up of the pharyngeal apparatus seen in Figure 22. Note the true cross-striated pharyngeal muscles (pm), the negative staining of the main jaws (ja_2), and the cilia (ci) in the pharynx. The epithelial cells (ec) lie in clusters surrounded by the fibularium (fi). Fig. 24: The lumen of the midgut with microvilli (mv) from the endodermal cells.

Musculature

The musculature consists of fiber-form muscle cells. Myosyncytia are not present. The somatic muscles consist of several longitudinal fiber cells attaching to various parts of the trunk and fibers running through the entire trunk, attaching in the caudal end close to the tail. The dorsoventral muscles attach through the epidermal cells on the edge of lateral plates. Circular muscles were not observed. Minute muscle fibers attaching one epidermal plate to another contract the thorax. Such contractions were observed in many living animals. The muscles of the pharyngeal apparatus are classically cross-striated (Fig. 23, pm), while many of the somatic muscles in the trunk are obliquely striated (Fig. 20, mu). The number of sarcomeres varies from one to five in the pharyngeal muscles. The length of each sarcomere is about 2 μm. A continuous Z-line is absent. Instead, there are five to seven Z-discs at sites where the Z-components would be expected.

All the pharyngeal muscles observed attach to epidermal cells (Fig. 25, mu). We never observed muscles attaching directly to the cuticle. The epidermal cells may have both microtubules and thread-like fibers in the muscle attachment (Fig. 28, mt). Furthermore, the somatic muscles never attach directly on the dorsal and lateral plates. The connection is always through an epidermal cell (Fig. 26, ep), which contains the intracellular plate structure. The somatic muscles (Fig. 26, mu) may either lack a basal lamina or the lamina is very diffuse and thin. Since embryological data are missing, ideas on the origin of the musculature are based solely on the location of the musculature, proximally to the epidermal cells and the basement membrane (two basal laminae). When the basal lamina is lacking or is diffuse, as in *Limnognathia maerski*, the origin of the musculature remains uncertain. Myoepithelial muscle cells were not observed either in the body wall or associated with the sclerites of the pharyngeal apparatus. We therefore suggest that all muscles of *L. maerski* are of mesodermal origin.

Digestive System

Mouth opening. The oval mouth opening (Figs. 2, 9, mo) is located ventrally on the frontal edge of the oral plate (Fig. 6, mo). We observed living animals with a slightly protruded mouth cone, which suddenly protruded most of the pharyngeal apparatus out of the mouth (see Discussion). To be capable of that the whole rim of the mouth must be flexible. The flexible part is the upper lip, which can be expanded enormously. The lower lip is attached to the basal plate complex and cannot be everted. A multicellular, ciliary organ surrounds the anterior margin of the mouth and may, in fact, constitute part of the buccal cavity (Figs. 25, 27, 29, bc). These cilia stick out of the mouth when the animal is foraging. They do not beat like locomotory cilia, but only move rigidly. They may be chemoreceptors.

Pharyngeal apparatus. The fine structure of the hard part of the jaw apparatus has been described under "Description." Here we will deal with the cellular parts and the ultrastructure of the solid parts of the pharyngeal apparatus.

The pharyngeal apparatus of *Limnognathia maerski* consists of the following structures: epithelial cells forming the cuticular structures, gland cells covering the buccal cavity, cuticular jaw elements (sclerites), the buccal nervous system, sensory or ciliated cells, and the pharynx musculature.

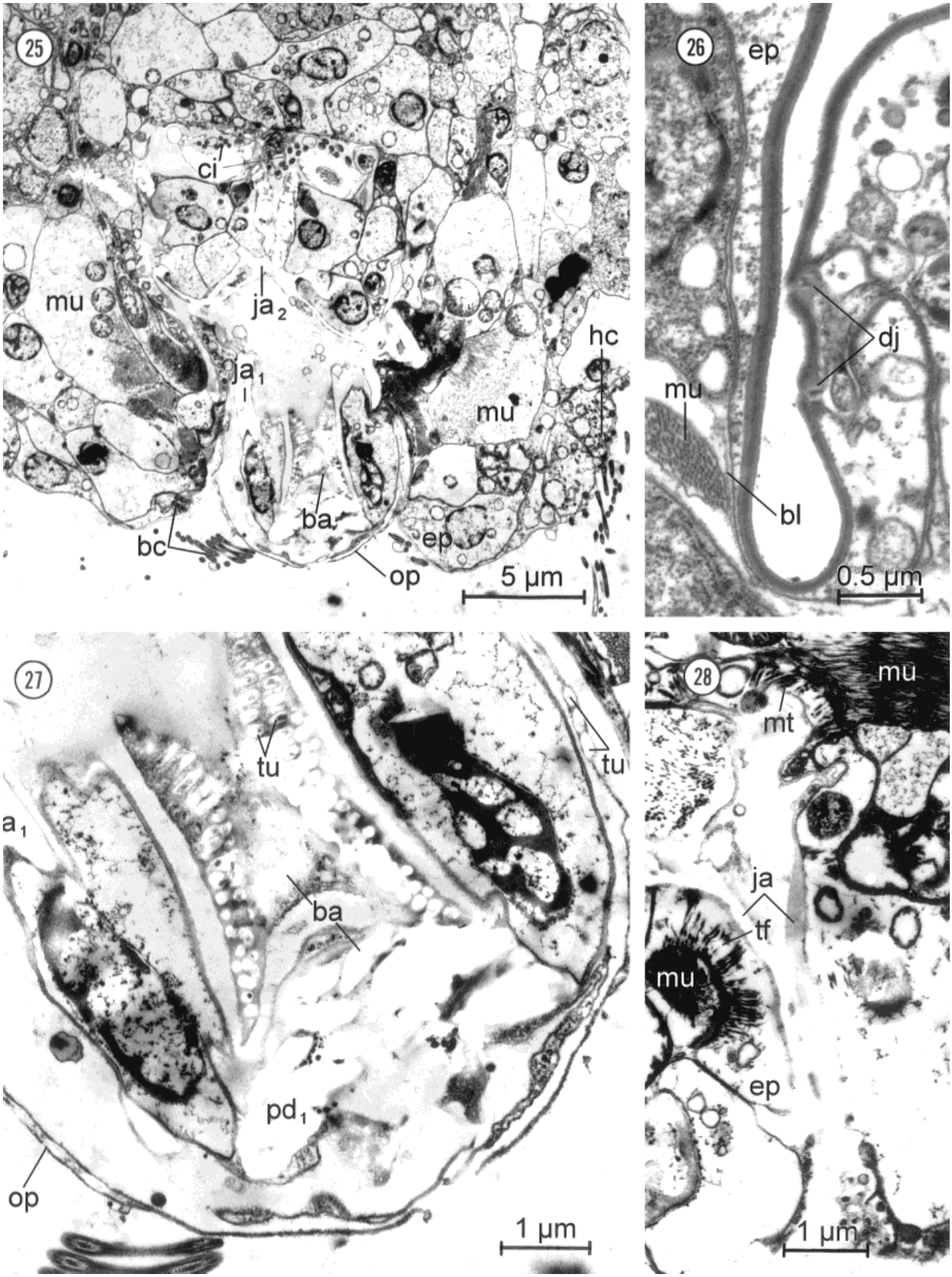
The epithelial cells in the pharyngeal bulb secrete a true cuticle that covers most of the mouth cavity and the pharyngeal lumen itself; furthermore, these cells also form the cuticular sclerites (the jaw system). Some epithelial cells lie inside the sclerites (Fig. 27, ja_1) and connect to other epithelial cells or muscle cells through fenestrae. Other epithelial cells lie in clusters surrounded with thin cuticle, as in the fibularium (Fig. 23, fi). In adult specimens, the epithelial cells lack any indication of secretory activity, suggesting that generation of the cuticle only happened once (already in the egg?) and that the cuticle is not molted. The shape of cuticle-generating cells ranges from round in the fibularium to elongated inside the jaw elements. There are only a few organelles and their cytoplasm is osmiophobic. The nucleus shows greater areas of condensed chromatin than in cells with a secretory activity. All features suggest that these cells are resting cells.

Unicellular buccal glands seem to be indistinct, but several glandular cells are scattered around the anterior part of the mouth cavity. One large dorsal gland of about 20 cells covers the upper lumen of the pharynx (Fig. 22, bg). These cells have enlarged perinuclear and cytoplasmic cisternae, several mitochondria, and numerous secretory vesicles—features indicating secretory activity.

The buccal nervous system is not well investigated but two large buccal nerves (each with 17 nerve fibers) run laterally between the two halves of the fibularium and the main jaws. A buccal ganglion may be present but was not observed.

Limnognathia maerski has overwhelming numbers of pharyngeal solid parts, the sclerites. The main parts consist of two ventral basal plates fused posteriorly, two dorsal lamellae orales, two fibularia, and three sets of jaws, where the main jaws are fused and consist of a dentarium and an articularium. Furthermore, most of the lumen of the pharynx is covered with a thin cuticle or glycocalyx.

By DIC optics we observed that each half of the thin lamellae orales (Figs. 9, 11, l.or) consists of vertical rows of 12 longitudinal cuticular rods or tubes. Therefore, it was not surprising to discover by TEM that all other sclerites have the same features, e.g., the fused part of the basal plate (Fig. 27, tu). The tubes consist of lucent, osmiophobic material



Figures 25–28

surrounding a dense osmiophilic core. The tubular substructures are embedded in true cuticle, so in cross section the sclerite appears porous and in longitudinal section the sclerite is striated due to the many vertical rows of cuticular tubes. How these structures are generated is still uncertain, but in one sclerite we observed microvilli in the tubes. It seems that the epithelial cell microvilli secrete the tubes. Later, when the microvilli are retracted they leave a dense core in the middle. In fact, we observed the osmiophilic microfibers in the middle of these microvilli.

The tubular substructure may be hidden in the sclerotized part of the sclerite, e.g., the pseudodigits of the ventral jaws (pseudophalangia) and in molar parts of the basal plates. The "arm" of the pseudophalangium is hollow in the middle. This lumen still contains the cells that generated the sclerite. The tubular substructure can be observed as a striation in the lamellae orales and the symphysis by DIC optics. While the diameter of the tubes in these two sclerites is up to 0.4 μm, the diameter of the tubes in all other cuticular elements is about 0.2 μm.

Ciliated epithelial cells (Fig. 23, ci) without cuticle are located dorsal of the two jaw halves of the dentarium (Fig. 23, ja_2). Microvilli are rarely observed in the pharyngeal lumen. Only one type of microvillus is present, short with an electron-dense core, being similar to the microvilli of the gut epithelium (Fig. 24, mv).

The muscular part of the pharyngeal system is observed as three-lobed by light microscopy. One median, caudal lobe attaches to the two arms of the cauda and two lateral lobes attach to the three sets of jaws (Fig. 12, mu). By TEM we observed many smaller muscle bundles attaching to the accessory sclerites of both the ventral and the dorsal jaws. The fine structure of all these muscles is beyond the scope of this article, and serial sections of the whole pharyngeal bulb are needed to determine the true location of each muscle bundle. A capsular muscle observed in several gnathostomulids seems not to be present in *Limnognathia maerski*. The whole pharyngeal bulb is surrounded only by a weakly developed basement membrane.

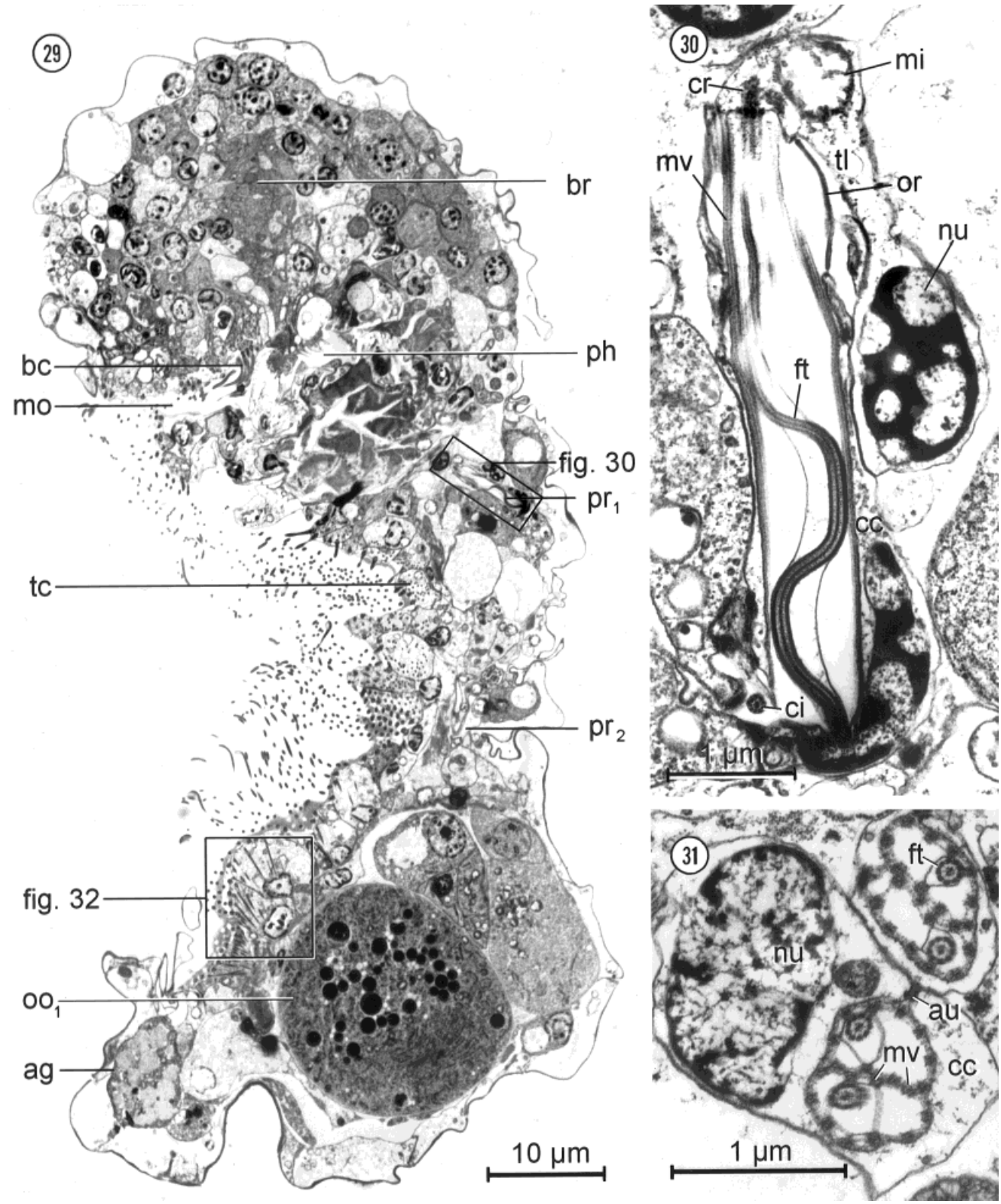
Esophagus. The esophagus is dorsal of the pharyngeal apparatus. It is very short and lacks cilia or microvilli. The esophageal lumen is usually closed, but a sphincter muscle was not observed. The esophagus is surrounded laterally by a pair of large salivary glands. It seems that the salivary glands do not open into the pharyngeal apparatus or into the esophagus. They open into the lumen of the midgut. Further investigations are needed before it can be decided if the salivary glands are homologous with stomach glands in other Gnathifera.

Salivary glands. The so-called salivary glands are located latero-posteriorly of the pharyngeal apparatus (Fig. 1, sg) and they interdigitate with the midgut cells. The cytoplasm of the salivary glands is osmiophilic and numerous granules and vesicles are present (Fig. 22, sg). The large nuclei are lobed, with a centrally positioned nucleolus. The salivary gland cells have the ultrastructure of highly active cells that export enzymes to the digestive system.

Midgut. The midgut fills up most of the thoracic region and the anterior part of the abdomen (Figs. 1, 3, 18). The midgut consists of cells with large vesicles and a Golgi apparatus (Fig. 18, ve, go) that surround the lumen of the midgut and peripheral cells with smaller vesicles and giant nuclei. These nuclei can reach a diameter of 4 μm and are the second-largest nuclei in the entire animal. Only the nucleus of the oocyte may be larger, at up to 10 μm in diameter. In well-fed animals the lumen of the midgut is a labyrinth of crevices (Fig. 18, mg), but in other animals an oval lumen is present in cross section. All midgut cells have a brush border of small microvilli, only 0.2 μm long (Fig. 24, mv). Each microvillus has an osmiophilic core of two to three fibers, and from the surface of the microvillus numerous fine osmiophilic fibers extend out into the lumen of the midgut.

Rectum and anal system. *Limnognathia maerski* lacks a true cuticle-lined hindgut, as seen in many other invertebrates with a cuticle, except for Gnathostomulida and some Gastrotricha that also lack the permanent anus. Instead, the gut cells decrease in size posteriorly and end on the edge of the triangular anal plate (Fig. 1, an). The six most caudal cells, the rectal cells (Fig. 33, rc), differ from the midgut cells. They lack the microvilli and only slightly interdigitate with the epidermal cell. The lumen in the gut disappears and only a lacunar system exists between the cells. Two small muscles terminate at the edge of the dorsal anal plate, and may be involved in opening what we interpret as a periodically functioning anus. There is no basement membrane between the rectal cells, the two muscles, and the epidermal cells. Because basal laminae are poorly developed in any case in *Limnognathia*

Figs. 25–28. *Limnognathia maerski* nov. gen. et nov. sp. TEM. Pharyngeal apparatus and epidermal cells. Fig. 25: Cross section through the cuticular oral plate (op) and the anterior of the pharyngeal apparatus with basal plates (ba), the ventral jaw (ja_1) and the main jaws (ja_2); bc, buccal cilia; ci, cilia in the pharynx; ep; epidermal cell; hc, head ciliophores; mu, muscle. Fig. 26: The interdigitated junction (dj) between two latero-dorsal epidermal cells. Note that the matrix layer is intracellular in the epidermal cell (ep). The muscle (mu) is surrounded by a diffuse basal lamina (bl). Fig. 27: Close-up of the pharyngeal apparatus seen in Figure 25. The jaw structures (ba, basal plate) and (pd_1, pseudodigit) are extracellular, as is the oral plate (op) surrounding the mouth opening. The tubular elements (tu) with the dense core are seen both in the basal plate and the oral plate. The left ventral jaw (ja_1) has an epidermal cell inside the cuticular structure. Fig. 28: Muscle-epidermal junction attached to the jaw elements (ja). Note that the epidermal cells (ep) have both microtubuli (mt) and thread-like (tf) fibers (tonofilaments) in the muscle-attachment. The muscle (mu) is cross-striated.



Figs. 29–31. *Limnognathia maerski* nov. gen. et nov. sp. TEM. Brain, pharyngeal apparatus, ventral trunk ciliophores, and the excretory system. Fig. 29: Latero-longitudinal section of the whole animal. ag, adhesive gland; bc, buccal cilia; br, brain; mo, mouth; oo_1, oocyte; ph, pharyngeal apparatus; pr_1-pr_2, protonephridia; tc, trunk ciliophores. Fig. 30: Close-up of the anterior protonephridium seen in Figure 29. Note the terminal cell (tl) with flagellum (ft), ciliary root (cr) and microvilli (mv). The canal cell (cc) forms the outer rods (or) of the weir. The nucleus (nu) of the terminal cell is located laterally on the canal. mi, mitochondria. Fig. 31: Canal cell (cc) with nucleus (nu), autodesmosome (au), and two flagella (ft), each surrounded by 9–10 microvilli (mv).

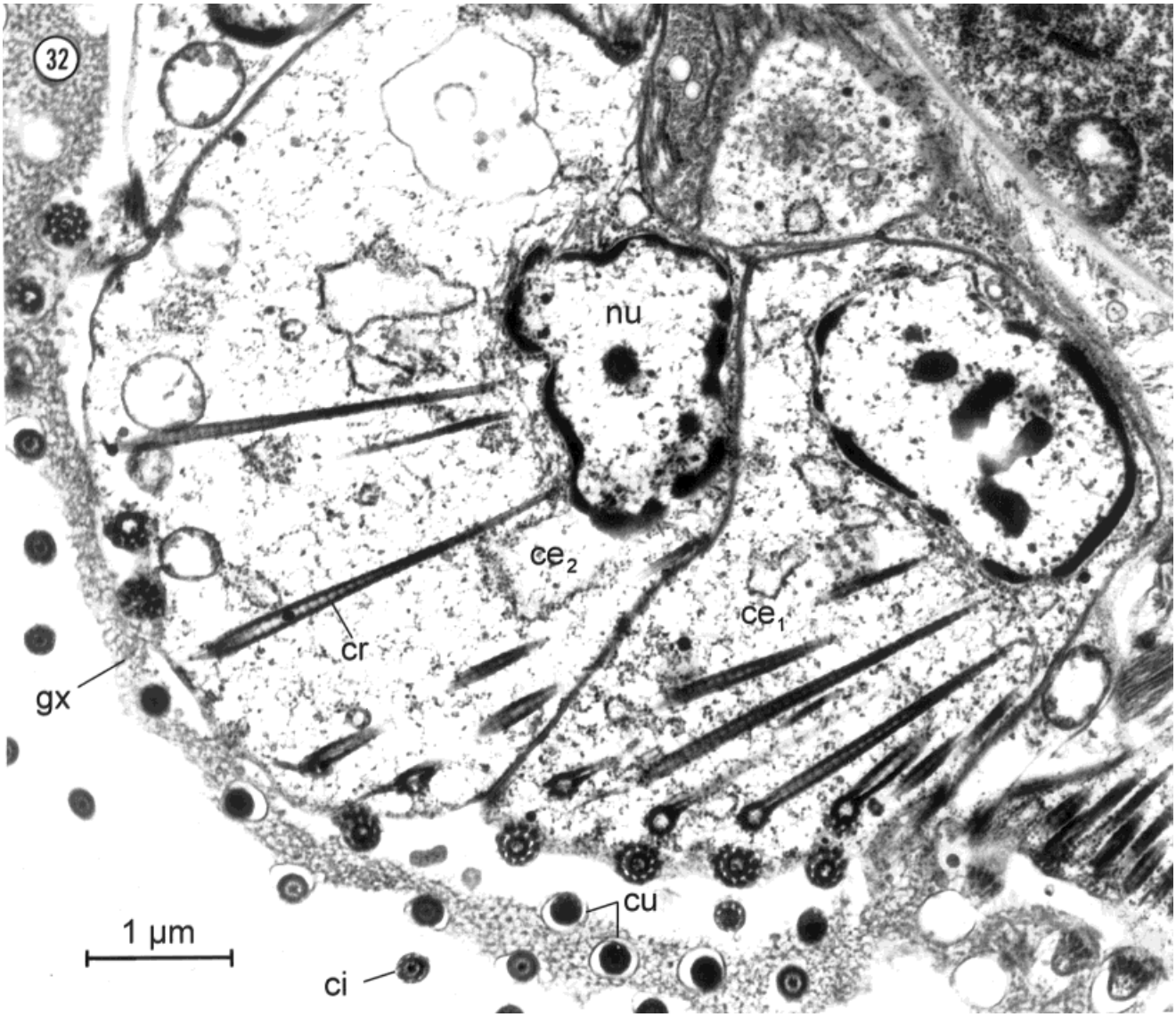


Fig. 32. *Limnognathia maerski* nov. gen. et nov. sp. TEM. The adhesive ciliated pad, where two (ce_1 and ce_2) of the ten adhesive cells are shown. The nucleus (nu) is located proximally in the cell. The cells are multiciliated; each cilium (ci) has a long ciliary root (cr). The cell surface is covered by a thick glycocalyx (gx). Each cilium is surrounded proximally with a tube-shaped cuticle (cu).

maerski, it is unclear whether these rectal cells are ectodermal or mesodermal in origin, but they differ from the epidermal cells in having many vesicles and mitochondria. In this way they look more like the midgut cells. Defecation was never observed.

Body Cavity

There is no coelom in the context of an extracellular fluid-filled space (pseudocoel) or of mesodermal lining cells (eucoelom). In nearly all cross and longitudinal sections the animal is totally compact (Figs. 29, 33), but in a few cross and longitudinal sections a cavity is observed between the epidermis and the midgut (Figs. 18, 22). This may be an artifact of fixation and the shrinking it causes. The extracellular matrix between the epidermis and mesodermal muscles consists of a very thin to diffuse basal lamina (Fig. 26). A distinct basement membrane was never observed between mesodermal cells and the endodermal part of the digestive system or the ectodermal ventral nerve cord (Fig. 20, vn). All mesodermal cells are fixed and amebocytes are not present. We therefore believe that the micrognathozoans are fundamentally acoelomate.

Excretory Structures

The excretory system of Micrognathozoa consists of two pairs of protonephridia lying ventrolateral to

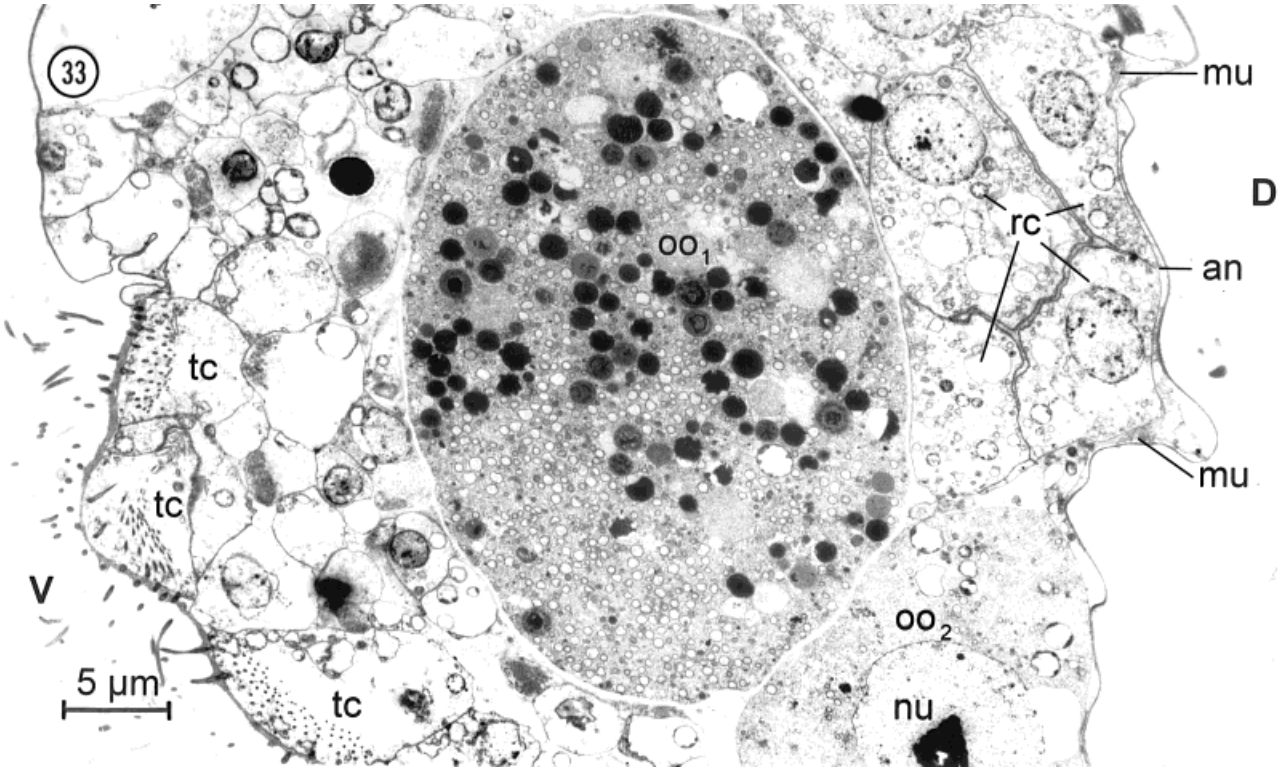


Fig. 33. *Limnognathia maerski* nov. gen. et nov. sp. TEM. Cross section of the animal in the anal region. an, temporary anus; D, dorsal; mu, muscle; nu, nucleus; oo_1, mature oocyte; oo_2, secondary oocyte; rc, rectal cells; tc, trunk ciliophores; V, ventral.

the gut, one pair in the thoracic region and the other anterior in the abdominal region (Figs. 2, 29, pr_1-pr_2). In *Limnognathia maerski*, the protonephridium consists of four terminal cells, two canal cells, and a single nephridiopore cell. The nephridiopore cell opens laterally between two lateral plates. All cells in the protonephridium are monociliated, so in a cross section in the distal part of the protonephridium, seven cilia are seen—four flagella-like cilia from the four terminal cells, two short cilia from the two canal cells, and one cilium close to the wall of the nephridiopore cell (Fig. 20).

The terminal cell forms a cylindrical weir apparatus or ciliary pit (Fig. 30), which consists of nine to ten stiff microvilli (inner rods of the weir) and the single flagellum in the middle. Between the microvilli the weir is filled with an extracellular striated material (Fig. 31). This material is formed inside large, coated vesicles in the cytoplasm of the terminal cell and is released by the vesicles into the weir apparatus. The striated material may be homologous with the fenestration lamina in other invertebrates. The terminal cell contains a few mitochondria and an abortive ciliary root (Fig. 30, cr). The cell body and nucleus of the terminal cell are located laterally on the weir, as is the nucleus of the canal cell, a cell that engulfs the weirs from two different terminal cells. The weirs lie in pairs in the proximal part of the canal cell (Fig. 31). The canal cell also has its own flagellar structure, which enters the lumen just after the weir. The canal cell surrounds the two weirs and closes itself with an autodesmosome (Fig. 31, au). In a few cases the canal cell also is involved in formation of the weir. The canal forms the outer rods (Fig. 30, or) that surround two flagella and the two sets of inner rods (Figs. 30, 31, mv). This interpretation could be wrong. The terminal cell may have two flagella, as in some Turbellaria and all known Kinorhyncha, but in the six animals investigated there were always four terminal cells and only two canal cells. The nephridiopore cell is not well investigated (Fig. 20). It looks very similar to the canal cell and it also forms a canal by closing on itself with an autodesmosome. The two weirs do not reach to the nephridiopore cell, but four of the six cilia are the flagella from the four terminal cells; two are shorter cilia from the canal cell and one is the cilium from the nephridiopore cell. In the wall of the nephridiopore these seven ciliary structures often may be seen (Fig. 20, pr). If our interpretation is correct, all cells in the protonephridium are monociliated.

Reproductive Components

After five years of investigation, we found only mature females of *Limnognathia maerski* and we

assume that the species reproduces by parthenogenesis, as do many other freshwater meiofaunal animals, such as bdelloid rotifers and echiniscid heterotardigrades. There is a small chance the animal could be a hermaphrodite (see below, "refractive bodies") or we may have overlooked dwarf males in our culture.

The investigated part of the reproductive system is anatomically simple. There always exist paired, compact ovaries in mature animals (Figs. 1–3). The ovary seems to consist only of oocytes; nongerminal sheath cells and oviducal cells were not observed. Usually only one egg of the two oocytes matures per clutch; just before oviposition this egg may fill one-third of the entire abdomen. The mature ovum is located ventro-posteriorly (Fig. 22). In the final stage of vitellogenesis, when a thin eggshell is developed, it lies in direct contact with the rectal cells (Fig. 33). Oviposition has not been thoroughly examined. Video documentation failed just when the eggs were laid, but we suggest that oviposition may occur through a permanent ventral pore just behind the adhesive pad, and not through the temporary dorsal anus as we first thought.

Vitellogenesis may be autosynthetic, i.e., yolk is synthesized within the oocyte from low-weight molecules that diffuse across the thin oolemma directly from the midgut. Evidence for that is seen at earlier stages of vitellogenesis before the eggshell is formed (Fig. 22). Here the oocyte is in direct contact with the midgut. The midgut contains large vesicles in the region where it contacts the oocyte. The oocyte has an enlarged nucleus with a nucleolus, many mitochondria, and prominent endoplasmic reticulum. This clearly indicates that the oocyte itself is very active in the formation of the osmiophilic yolk granules.

The mature egg itself also forms the eggshell. Two types of eggshell surfaces were observed on newly laid eggs. One type of eggshell is smooth and sticky; the other is strongly sculptured (Fig. 7). We first thought that the unsculptured, sticky egg was an abortive egg, considering that we had introduced them into the depression slides at too high temperatures. Later, both types of eggs were found in the ovary of different specimens. It seems that smaller females lay sticky eggs, while larger females lay sculptured eggs. Thus, there is a possibility that there exist two types of eggs, as in gastrotrichs and rotifers. The sticky eggs may be quick-developing summer eggs (tachyblastic) and the sculptured eggs may be resting winter eggs (opsiblastic).

Two refractive bodies found in close contact with the mature egg (Fig. 2, rb_1-rb_2) may be accessory organs. The function of these two structures is not understood, and more TEM investigations are needed. We believe that it is unlikely that these are part of a male reproductive system, as found recently in freshwater chaetonotid gastrotrichs. There is no evidence for modified spermatozoa inside these two bodies.

Live Observations

More than 100 living specimens of *Limnognathia maerski* have been observed, both in Greenland and in the moss culture in Copenhagen. Usually the animals were collected by an Irwin loop when they were swimming. If we tried to collect animals sitting on the substrate, they adhered very strongly to the substrate with their adhesive pad. Color photos (Figs. 40, 41) were taken immediately after the animals were removed from the culture in Copenhagen. These photos showed that the body shape was flexible; the thorax especially could contract like an accordion, but it could also constrict to a narrow, neck-like shape, and then the animal looked nearly like a figure eight.

Video technique was used to observe swimming and crawling behaviors. The swimming behavior was very characteristic, because the animal moves in a spiral. It is a slow movement, different from that of monogonont rotifers, which are common in the cold spring from Isunngua. It seems that the trunk ciliophores are used both in swimming and in crawling on the substrate. The preoral ciliary field is not the main locomotory organ, as we first expected. Specimens were never observed moving backwards, as nearly all gnathostomulids do, when they reverse their long cilia. Kept cool at 4°C and mounted on a depression microslide with a cover slip, the animals could be observed alive for about 5 h. When the animal crawls on the substrate, e.g., pieces of moss leaf or detritus, it moves by slow gliding, using the ciliophores in unison. The gliding is caused by the trunk ciliophores in exactly the same way as the ciliophores in *Diurodrilus* (Kristensen and Niilonen, 1982), or as the two rows of ventral ciliation in some of the chaetonotid gastrotrichs (Ruppert and Barnes, 1994). The gastrotrich genus *Chaetonotus* was also represented in the same moss samples as *Limnognathia*, but these animals were easily distinguished because *Chaetonotus* has scales and lacks jaws in the pharyngeal apparatus.

The foraging behavior of *Limnognathia maerski* was also observed several times. It consists of a rhythmic oscillation of the head from side to side. It seems like food items, which could be blue-green bacteria or diatoms, are collected with the preoral ciliary field (Fig. 2, pc) and picked up by the pseudophalangia. The pseudophalangia may be extruded out of the mouth opening, and it was observed that they could pick up a single diatom, which later was macerated by the main jaws. Whole diatoms were never observed in the midgut in any of the type specimens. The vomit behavior, which was described under "the allotype," is very similar to the same behavior in one species of gnathostomulids, *Problognathia minima* (see Sterrer and Farris, 1975). Most

of the pharyngeal apparatus is protruded out through the mouth, followed by a fast snapping reaction of the main jaws. Defecation was never observed in any of the animals, and it was first thought that the anus was located ventrally. Later, when we had located the dorsal anal tissue by TEM investigations, we could also see by DIC optics that the midgut continues dorsally, directly into a closed rectum, which ends on the edge of the most posterior triangular dorsal plate. There is no permanent functional anal pore. Such a pore would only open to the exterior, when defecation is happening. A closed anal pore is found in several other meiofaunal groups. The anus may be secondarily closed, as in some loriciferans and in all Heterotardigrada. All Eutardigrada have a permanent open cloaca and defecation behavior is very easy to observe. The chance of observing defecation behavior in living *Limnognathia* is small, and we did not succeed in making that observation, although we videoed the animals for hours.

DISCUSSION

Comparison Between Micrognathozoa and Gastrotricha

The phylum Gastrotricha consists of only about 500 species of microscopical acoelomate worms. The phylum is divided into two orders, the marine Macrodasyida and the marine and freshwater Chaetonotida. The phylum is one of the best TEM-investigated groups in the world (see the excellent review of Ruppert, 1991b). The so-called primitive groups of gastrotrichs may have monociliated epidermal cells, a plesiomorphic character shared with all gnathostomulids (Rieger, 1976). Superficially, Micrognathozoa look like chaetonotid gastrotrichs. Like all gastrotrichs, the chaetonotids have only ventral locomotory cilia, with which they glide smoothly over the substrate. In some chaetonotids the locomotory cilia are arranged in two longitudinal bands, as in *Limnognathia maerski*. Furthermore, the ciliary bands of chaetonotids are grouped in cirri like the ciliophores of *Limnognathia*. The cilia (including the locomotory cilia) of gastrotrichs are always surrounded by the exocuticle (Ruppert, 1991b) and this has been considered a good autapomorphy for the phylum. The cilia of the adhesive pad of *Limnognathia* clearly have a cuticular membrane most proximally, but this membrane does not continue all the way out on the cilia (Fig. 32). The similarities between the Micrognathozoa and Gastrotricha seem to be only superficial, and are not supported by ultrastructural evidence. The differences in the pharyngeal apparatus and the integumental structures are so large that the other similarities must be considered convergence. All gastrotrichs have a myoepithelial pharyngeal bulb with a triangular lumen that is lined by a layer of cuticle. This type of pharyngeal system with a pharyngeal channel running through the center of the bulb is very different from the jaw system and the ventrally placed pharyngeal bulb seen in both Gnathostomulida and Micrognathozoa. Furthermore, in the two last-mentioned groups the bulb consists of an epithelial and a muscle layer. The epidermis of gastrotrichs is covered with a true cuticle, which in some gastrotrichs may be specialized to form scales or spines. There is no evidence for an intracellular plate structure, as in Micrognathozoa and Rotifera. We therefore consider gastrotrichs to be members of the Cycloneuralia clade (Nielsen, 1995) and Gastrotricha is not the sister-group to Gnathostomulida (Wallace et al., 1996; Cavalier-Smith, 1998; Zrzavý et al., 1998), but to the Introverta clade, which includes Nematoda, Nematomorpha, Priapulida, Kinorhyncha, and Loricifera (Nielsen et al., 1996; Sørensen et al., 2000).

Comparison Between Micrognathozoa and Gnathostomulida

The phylum Gnathostomulida consists of microscopical, acoelomate worms that live exclusively in marine sediments, especially those with a high content of hydrogen sulfide and a lack of oxygen. The approximately 80 described species may be found in cryptic habitats such as stromatolites, cold seeps, or deep in the sediments, avoiding oxygen (for review, see Sterrer, 1998). The monociliated epidermis and protonephridia, the blind tubular gut system and acoelomate body condition (Lammert, 1991) may support the theory of an ancient origin of Gnathostomulida and they may have survived from the Precambrian, when oxygen was nearly lacking (Fenchel and Riedl, 1970; Boaden 1975, 1989). However, the complex cuticular jaws and extremely complex hermaphroditic gonads do not support the theory that gnathostomulids should be the evolutionary bridge between a cnidarian ancestor and the Platyhelminthes (Ruppert and Barnes, 1994). The phylogenetic position of Gnathostomulida has been very turbulent. Haszprunar (1996a) regarded them to be the "most primitive" of all spiralian Metazoa, while Nielsen (1995) considered them to be specialized polychaetes. Before presenting a comparison between the new group, Micrognathozoa, and Gnathostomulida, we therefore outline a short historical review of the "enigmatic" Gnathostomulida (see Sterrer et al., 1985).

Gnathostomulida was described by Ax (1956) as an order of Turbellaria, but the group was known since the 1920s by Remane (unpublished). Riedl (1969) established a new phylum for the gnathostomulids and Sterrer (1972) supported this, but he also mentioned that gnathostomulids share characteristics with both Platyhelminthes and Aschelminthes. Ax (1985, 1989, 1996) could not give up his first impression and placed the Platyhelminthes (flatworms) and Gnathostomulida as sister taxa in

the clade Plathelminthomorpha. This theory was supported by four synapomorphies: 1) hermaphroditism, 2) direct transfer of sperm and internal fertilization, 3) thread-like sperm, and 4) lack of mitosis in somatic cells. The three first-mentioned autapomorphies are very weak characters because they are observed in many other lines of Metazoa, and the fourth character may be a plesiomorphic character. The monociliated epidermal cells (Rieger, 1976; Rieger and Mainitz, 1977) may also be a plesiomorphic character present in all gnathostomulids and some gastrotrichs, e.g., *Chordodasys* and *Xenodasys* (Rieger, 1976; Ruppert, 1991b).

Sterrer et al. (1985) questioned the link between the Platyhelminthes and Gnathostomulida on the basis of the ventral pharyngeal bulb, the true cross-striated muscles, and the presence of a periodically functioning anus (Knauss, 1979). Kristensen and Nørrevang (1977, 1978) indicated similarities between polychaetes (Dorveillidae) and gnathostomulids in jaw formation and structure. They also stated that the similarities between the mastax of Rotifera (Koehler and Hayes, 1969) and Gnathostomulida seem rather more inconclusive (see below, "Comparison Between Micrognathozoa and Annelida"). The molecular data (18S ribosomal DNA) are not really helping to clarify the phylogenetic position of Gnathostomulida. Littlewood et al. (1998) indicate a relationship with the Gnathostomulida and a Nematoda + Chaetognatha clade. Both Zrzavý et al. (1998) and Giribet et al. (2000) offer phylogenetic hypotheses based on combined morphological and molecular data. Although the datasets in the two analyses are quite similar, their analyses differ and Zrzavý et al. (1998) suggest a close relationship between the Gnathostomulida and Gastrotricha (Neotrichozoa), while Giribet et al. (2000) suggest that Gnathostomulida is a sister-group to Cycliophora + Syndermata. Recently, Rieger and Tyler (1995) and Ahlrichs (1995, 1997) published ultrastructural evidence indicating a sister-group relationship of Gnathostomulida with Rotifera-Acanthocephala. Ahlrichs (1995) established a new monophylum Gnathifera for the three mentioned groups. Sørensen (2000) used a new SEM technique to clean both the rotifer trophi and the jaws of gnathostomulids. His observations support a close relationship between Gnathostomulida and Rotifera. The TEM investigation of pharyngeal epithelium, pharyngeal musculature, and the pharyngeal jaws has already shown the same evidence (Herlyn and Ehlers, 1997). In the same study, Herlyn and Ehlers (1997) suggest that the absence of pharyngeal ciliation is an autapomorphy for the Gnathifera. We disagree with that interpretation. Pharyngeal cilia in Rotifera are clearly present (see fig. 26, Clément and Wurdak, 1991), and cilia are present in the pharyngeal epithelium of Micrognathozoa as well (Fig. 23). Gnathostomulida, however, seems to lack the ciliation in the pharyngeal epithelium; instead, they have thick microvilli (fig. 13, Kristensen and Nørrevang, 1977). It is therefore interesting that *Limnognathia maerski* has both cilia and microvilli (Figs. 23, 29) in the pharyngeal epithelium.

In this research, we used the same SEM technique as described by Sørensen (2000) for Gnathostomulida and also used in Rotifera (De Smet, 1996, 1997) to clean the cuticular elements of *Limnognathia*-pharyngeal apparatus. It should therefore be easy to compare the solid parts of the pharyngeal system in the three groups. Unfortunately the comparison is *not* easy. The largest dissimilarity is between the "compact" type of gnathostomulid jaws found in the order Filospermoidea. Several similarities are present between the jaws of Micrognathozoa and the "basket type" of gnathostomulid jaws found in the order Bursovaginoidea. Especially, basket jaws of the suborder Scleroperalia are very similar to the main jaws of *Limnognathia*. The basket type is also more similar to the trophi (jaws) of Eurotatoria than the compact jaws. These findings are very confusing because the compact type of jaws has always been considered as the plesiomorphic condition within the Gnathostomulida (Sterrer, 1972). If the compact type is the plesiomorphic condition, several elements of rotiferan trophi and micrognathozoan jaws may have been formed by convergence, so that they look similar to the basket jaws, or the ground pattern for both rotiferan trophi and micrognathozoan jaws was also present in the stem group of Gnathostomulida. Then the compact jaws in filospermoid gnathostomulids are a secondary reduction. Within the Rotifera we have the same problem. The trophi of the marine genus *Seison* (Order Seisonidea) are very aberrant and are not easy to compare with the trophi of the Eurotatoria (Ahlrichs, 1995; Segers and Melone, 1998).

The similarities between the isolated jaws of the genus *Gnathostomula* and the main jaws of *Limnognathia* are really astonishing (Riedl and Rieger, 1972). There are similarities, which are so convincing that they may be homologies. Similarities are seen, not only in the dentarium, where three arcs of teeth are present in both genera, but also in the articularium. The articularium consists of two symphysis lamellae that are joined by a symphysis. The cuticularized part of the main jaws in *Limnognathia* ends in a paired cauda system, as in *Gnathostomula*. In *Limnognathia* the caudae are symmetrical, as in, e.g., *Gnathostomula microstyla* (see Riedl and Rieger, 1972). The fibularium of *Limnognathia* may be better understood if we follow the theory (Riedl and Rieger, 1972) of fibularization of the involucrum in the family Gnathostomulidae. The fibularium may be homologous to the apophysis and the involucrum system of Gnathostomulida. If so, then the fibularium is developed to an extreme degree in *Limnognathia*. As in gnathostomulids, each fenestra contains a single cell, but instead of three fenestrae (in each jaw) in Gnathostomulida there exist at least

eight fenestrae in *Limnognathia* on both sides of each main jaw element (Figs. 8, 9). It is very difficult to find counterparts in the gnathostomulid jaws to the other sclerites in *Limnognathia* jaws. The basal plates of *Limnognathia* are paired but partly fused. The basal plate of gnathostomulids consists of a single plate, but may be secondarily fused of several elements. The two lamellae orales in *Limnognathia* have the same substructure as the lamellae addentalis in *Valvognathia pogonostoma*. They may in fact constitute a continuous, folded cuticle, covering a large part of the pharyngeal lumen. The ventral jaws and dorsal jaw elements are not observed in any Gnathostomulida. The complexity of the jaw of *Limnognathia* is greater than that of jaws of any invertebrate group and far beyond the complexity of Gnathostomulida. Unfortunately, they are also among the smallest jaws, so subunits in the sclerites can only be observed by high-resolution SEM.

The new group Micrognathozoa supports the theory that the jaws of gnathostomulids are homologous with rotiferan trophi. The jaws of *Limnognathia* have several elements that are similar to those of gnathostomulids; other elements may be closer to some of the sclerites of rotiferan trophi.

A closer relationship (sister-group relationship) between Micrognathozoa and Gnathostomulida was supposed when we discovered *Limnognathia* in 1994 (Kristensen and Funch, 1995). This was based on the SEM investigations of the main jaws of *Limnognathia* and the jaws of scleropleralian gnathostomulids and also on behavioral observations of *Problognathia minima* and *Limnognathia maerski*.

Problognathia minima was described from Castle Harbour of Bermuda (Sterrer and Farris, 1975). It is a very small, plump gnathostomulid that lacks the ability to swim backwards. Furthermore, the manner in which *Problognathia* protrudes the pharyngeal apparatus (Sterrer and Farris, 1975) closely resembles the "vomit" behavior we observed in *Limnognathia*. The two refractive bodies of the allotype (Fig. 2, rb) were thought to be bursae or prebursae, like the round bursa of *Problognathia*. Unfortunately, we still do not have any TEM photographs of refractive bodies, but after investigations of more than 50 living specimens of *Limnognathia* it seems that the bodies are glandular structures and there is no evidence that *L. maerski* should be a hermaphrodite. The new SEM investigations of the trophi of planktonic Rotifera (Sørensen and Kristensen, 2000) and the TEM investigations of *L. maerski* may indicate that the Rotifera is the sister-group to Micrognathozoa, and not the Gnathostomulida.

Comparison Between Micrognathozoa and Rotifera

The rotifers were discovered just after the invention of the microscope, but Leeuwenhoek could not distinguish them from Protozoa and they were included in the group Infusoria (for historical review, see Hyman, 1951). Today more than 1,500 species have been described, the majority from freshwater environments, e.g., zooplankton, algae, and mosses (Nogrady et al., 1993). Several species of the class Bdelloidea may enter into cryptobiosis and can then be found in extremely dry habitats such as the Namib desert, where they form anhydrobiotic tuns under quartz stone (unpublished observations), or on the surface of glaciers, where they may have survived for many years in a cryobiotic stage in the cryoconite holes (De Smet and Van Rompu, 1994). Still, the active stage always needs water and all rotifers are aquatic animals. The marine species of rotifers have generally been neglected. In many textbooks rotifers are only treated as freshwater animals, although planktonic rotifers (e.g., the genus *Synchaeta*) are very important in nutrient recycling in all marine systems. Recently, marine rotifers of the class Seisonida have been placed centrally in phylogenetic discussions of the origin of the Rotifera (Ahlrichs, 1993a, 1997; Ricci et al., 1993; Segers and Melone, 1998). The monophyly of the Rotifera has been questioned and the class Seisonida (only one genus *Seison*) has been placed as a sister-group to the exclusively parasitic phylum Acanthocephala (Ahlrichs, 1995, 1997; Herlyn and Ehlers, 1997). The new theory to include the Acanthocephala as an ingroup inside the Rotifera is based primarily on ultrastructural observations of the integumentary structures. Storch and Welsch (1969) first discovered several ultrastructural similarities between the rotiferan and acanthocephalan integuments. Later, the monophylum Syndermata was proposed for Rotifera and Acanthocephala (see Ahlrichs, 1995), based on several other ultrastructural characters. Molecular data strongly support such a monophyletic group (Winnepenninckx et al., 1995, 1998) but the interrelationship inside the clade remains unclear (Garey et al., 1998; Welsch, 2000). Unfortunately, we cannot compare important morphological characters present in Micrognathozoa with potential homolog characters in Acanthocephala because Acanthocephala totally lacks an alimentary tract, including the pharyngeal apparatus (Dunagan and Miller, 1991). We can only state that Micrognathozoa lacks a syncytial epidermis, one of the proposed autapomorphies for the Syndermata clade.

One of the autapomorphies for all Rotifera is the complicated jaw apparatus (the mastax) that always contains cuticular parts elements, the trophi. The plesiomorphic condition for the trophi is the condition in which the trophi (singular: trophus) comprise four different kinds of cuticular elements called sclerites (Markevich, 1989). Before we compare the trophi of Rotifera and the jaw apparatus of Micrognathozoa, we should briefly define the four primary cuticular elements of the rotiferan trophi.

The central parts of the trophi consist of two paired sclerites, the rami. The rami are held together posteriorly by a flexible cuticular ligament, so the two sclerites can be moved independently by muscles. Each ramus is formed by three epithelial cells (Markevich, 1989). The epithelial cells form chambers inside the sclerites, and each of these opens to the surface of the sclerite by a small window, or fenestra. At least two windows, basifenestra and sub-basifenestra, are used today in systematic studies of monogonont rotifers (Markevich, 1989; De Smet, 1997). According to Markevich (1989), a third window, the fenestrulum, might be present apically on the ramus. Each ramus may have a strong dentition going all the way down to the symphysis, but often the distal part of the ramus is smooth.

The unpaired fulcrum is positioned caudally to the symphysis of the rami. By light microscopy and SEM (Sørensen, 2000) the fulcrum appears striated, which is caused by longitudinally arranged tubes. This striation may be present in other sclerites, but often the microtubes are embedded in a homogeneous matrix layer and the subunits cannot be observed by SEM. The fulcrum serves as the cuticular muscle attachment for the large abductor muscles, which are responsible for moving the rami like forceps.

The paired unci are positioned apically or ventroapically to the paired rami. Each uncus may be a single large tooth or a large plate with several teeth. The uncus seems to lack windows or fenestra. The unci and the rami are responsible for handling the food and may crush food particles in different ways.

The last pair of primary sclerites is the manubria. They are located lateral to the rami. Each manubrium is attached to the uncus by a thin ligament. An extensor muscle attaches to the posterior part of the uncus and the caudal part of the manubrium is responsible for the movement of these two sclerites. The manubrium is formed by three epithelial cells, each lying in a chamber or on the surface of the manubrium. If all three chambers with fenestra are present the openings are referred to as the anterior, the median, and the posterior fenestra.

Secondary sclerites may be present in some monogonont trophi as well in the *Seison* trophi. The secondary sclerites form the epipharynx (De Smet, 1997; Segers and Melone, 1998) or pseudoepipharynx (*Seison*; Ahlrichs, 1995). At least in the genus *Dicranophorus*, the paired epipharynx consists of striated plates. The location of the epipharynx may vary from one species to another (De Smet, 1997), but often it is found anterior to the unci. Two pairs of accessory sclerites may be present between the uncus and the manubrium. These sclerites are the supramanubrium and the intramalleus.

In principle, all cuticular elements of Rotifera are formed by tubular rods with a dense osmiophilic core (fig. 27, Clément and Wurdak, 1991; fig. 1, Rieger and Tyler, 1995), as in Gnathostomulida and Micrognathozoa, but in different species the sclerites may be reduced or highly modified, so the tubular subunit can only be observed in the unpaired fulcrum (Segers and Melone, 1998; Sørensen and Kristensen, 2000). When the fulcrum is reduced, as in the class Bdelloidea, the tubular subunits are difficult to recognize, but we still believe that the tubular subunits were present in all sclerites in the stem species of Rotifera, and they are an autapomorphy for Gnathostomulida, Rotifera, and Micrognathozoa. There is no evidence that the cuticular sclerites may molt or that the juveniles of rotifers have fewer sclerites than the adults. The embryo inside the rotiferan egg already has all elements. In the same way, the cuticular elements of the gnathostomulid jaw may degenerate (Sterrer, 1968), but molting of the cuticular elements is not observed in gnathostomulids or in *Limnognathia*.

It is not easy to homologize the jaw elements of *Limnognathia* with the sclerites of rotiferan trophi. The complexity of the *Limnognathia* jaw is much greater than that of any known rotiferan trophi. At least seven paired elements and one unpaired cuticular element are present in *Limnognathia* jaws. The greatest similarity is found in the trophi of the family Lindiidae, which have the cardate type of trophi (Nogrady et al., 1993). Cardate trophi are similar to the virgate trophi that are common in marine monogonont rotifers (*Synchaeta*; Sørensen and Kristensen, 2000). The virgate type may be the plesiomorphic type of trophi with Eurotatoria. Like the trophi of *Synchaeta*, where the manubria, unci, and fulcrum curve (Koste, 1978), the jaw apparatus of Micrognathozoa has a globular form.

Using the genus *Lindia* trophi as a model may be dangerous if the cardate trophi are an advanced type within the Rotifera. We had the same problem with *Gnathostomula* jaws within the Gnathostomulida. The comparison between the trophi of *Lindia* and *Limnognathia* is as follows: 1) The unci and the ventral jaws (pseudophalangia) are homologous; 2) The manubria and the accessory sclerites attached to the ventral jaws are homologous; 3) The rami and the dentarium of the main jaws are homologous; 4) The fulcrum and the articularium of the main jaws are homologous; 5) The epipharynx and the lamellae orales are homologous; 6) The fibularia are elements of rami, which have developed to an extreme degree in *Limnognathia*; 7) The dorsal jaws with accessory sclerites are an autapomorphy for Micrognathozoa; and 8) The basal plates are absent in Rotifera (present in Gnathostomulida).

There are several problems with these eight statements. The major problem is the main jaws. They look much more like the jaws of gnathostomulids. The fulcrum of the rotiferan trophi is never split into two symmetrical arms, like the cauda of *Gnathostomula* and *Limnognathia*. Furthermore, the dentes terminales (Fig. 8, d.te) may be homologous with the uncinal teeth, and then the fibularium may be the

manubrium (Ahlrichs, pers.com.). More extensive SEM investigations of the jaws using higher magnifications may clarify these questions. The synapomophies for Rotifera, Gnathostomulida, and Micrognathozoa are very limited, but a possible unique character is that the epithelial cells lie inside the sclerites and form openings to the outside via the windows or fenestra. Recently Sørensen (2000) has shown that the fenestra dorsalis in *Rastrognathia macrostoma* also contains a cell. All fenestrae of *Limnognathia maerski* have the same feature (Figs. 10, 11).

Except for the possible homology of the cuticular jaws of *Limnognathia* and the trophi of rotifers, there are very few similarities in the ultrastructure of the two groups. In fact, the micrognathozoans lack structures that could be interpreted as homologous to the corona, the germovitellarium, the retrocerebral complex, and the syncytial origin of many tissues, as is seen in rotifers. The intracellular skeletal lamina found in Syndermata and in Micrognathozoa is probably homologous, in spite of the lack of this structure on the ciliated ventrum of *Limnognathia*.

The integument of *Limnognathia* exhibits a distinctive intracellular dense matrix layer (Fig. 21, im) that resembles the intracytoplasmic skeletal lamina of rotifers (Clément and Wurdak, 1991). The skeletal lamina in rotifers has been considered homologous to a similar structure in the integument of Acanthocephala, and this shared feature has been used to suggest a sister-group relationship between Rotifera and Acanthocephala (Storch and Welsch, 1969). But in Rotifera and Acanthocephala the intracellular lamina are in a syncytial epidermis, while in Micrognathozoa the dense matrix is within a cellular epidermis. The cellular epidermis of the Micrognathozoa is likely to represent the plesiomorphic condition as it is found in most invertebrates.

Otherwise, the apical position of an electron-dense, intracytoplasmic lamina in the epidermis, just beneath the cell membrane, is shared in all three groups. The lamina could be considered a specialized terminal web, with a skeletal function for somatic muscle attachment in the absence of a true extracellular cuticle. Such a molting cuticle is missing in all three groups. Other integumental structures in Micrognathozoa are not similar to those of Rotifera. Micrognathozoa has multiciliated epidermal cells, as in Rotifera, and, typically, rotifers possess a corona consisting of a circumapical band and a buccal field. Micrognathozoa has no structure that could be considered homologous to the rotiferan corona. The locomotory cilia in Micrognathozoa are without exception ventral, and they extend to the posterior trunk. A ciliated band that encircles the anterior is not present. Furthermore, the cilia of the corona in Rotifera are restricted to the anterior end, although some rotifers, such as species belonging to the family Dicranophoridae, have a ventral anterior ciliated field of some extent.

An organ similar to the characteristic germovitellarium of rotifers is not present in Micrognathozoa. Both Rotifera and Micrognathozoa have protonephridia, but their ultrastructure differs. The rotiferan terminal cell is multiciliated (Ahlrichs 1993a,b), while the micrognathozoan terminal organ is monociliated. The stomach of Eurotatoria is typically ciliated; in some species it is syncytial. The micrognathozoan gut is neither; it consists of an epithelium with a brush border of microvilli extending into the gut lumen. The stomach of Seisonida also exhibits a brush border of microvilli (Ricci et al., 1993; Ahlrichs, 1995). Most rotifers have a dorsal cloaca, although members of the genus *Asplanchna* have no anus. The condition with a temporary anus in Micrognathozoa is not known in rotifers.

Comparison Between Gnathifera Ahlrichs, 1995 and Annelida

The gross anatomy of the body of *Limnognathia maerski* appears similar to that of some interstitial polychaetes (cf. Westheide, 1990). As in many marine interstitial animals, e.g., turbellarians, gnathostomulids, gastrotrichs, and interstitial polychaetes, tactile bristles or sensoria are present on the body of Micrognathozoa. It is well known that these sensory structures have developed by convergence in the different phyla as an adaptation to living in the interstitium between sand grains (Swedmark, 1964; Ax, 1966). The problem with *L. maerski* is that it is neither marine nor interstitial. It has been overlooked that many freshwater and epiphytic meiofaunal animals also have tactile bristles, e.g., chaetonotid gastrotrichs and aelosomatid annelids. The tactile bristles always consist of compound cilia, but they may have a different origin. The tactile bristles of gnathostomulids are all formed from several monociliary cells. In annelids, however, the bristle may be formed by either a single multiciliary cell or by several monociliary cells. The long stiff bristles (apicalia, frontalia, dorsalia, lateralia, and caudalia) of *L. maerski* consist of one to three long cilia. The lateralia, dorsalia, and caudalia have a ring-shaped to oval opening, the aperture, through the epidermal cell. Such an aperture often is found in the dorsal antenna of loricated rotifers (*Notholca ikaitophila*; Sørensen and Kristensen, 2000) but until now has not been observed in annelids. However, the repetition of the tactile bristles on the body is not found in rotifers, but is present in several interstitial dorvilleid polychaetes (*Neotenotrocha sterreri*; Eibye-Jacobsen and Kristensen, 1994) and in the exclusively interstitial annelid order Diurodrilida (see *Diurodrilus westheideri*; Kristensen and Niilonen, 1982), and in *L. maerski*. Furthermore, the lateral tactile bristles (lateralia) are similar in pattern in both annelids and *L. maerski*. Three trunk dorsalia are present serially in *L. maerski*, and they are always paired. In contrast, the dorsalia in *Diu-*

rodrilus are located only on the protostomium (head) and the dorsalia may be paired (two pairs) or unpaired (two single middorsal structures). We believe that tactile bristles have developed by convergence in several meiofaunal groups, including the Annelida and Micrognathozoa, but the similarities are surprising.

More surprising is the presence of ciliophores in both *Diurodrilus* and *Limnognathia.* Ciliophores are large, ventral epidermal cells with numerous cilia to which basal bodies are attached in regular rows. The beat of the cilia in one row is synchronized and the ciliary roots are very long in both *Diurodrilus* (fig. 14, Kristensen and Niilonen, 1982) and *Limnognathia* (Fig. 20). The cilia are not bounded by a common membrane, as in gastrotrichs (Ruppert, 1991b).

The prostomial ciliophores of *Diurodrilus* have a very specific pattern and have been used in species determination. These ciliophores may be used as a broom to collect small detritus particles. In *Diurodrilus,* three rows of prostomial ciliophores exist: an anterior, a central, and two lateral groups. The anterior rows of prostomial ciliophores have rectangular bases, while the large central and the small lateral groups vary considerably. In addition, three to six pairs of metastomial ciliophores usually flank the pharyngeal apparatus. Kristensen and Niilonen (1982) stated that these prostomial and metastomial (= peristomial) ciliophores may be an autapomorphy for the genus *Diurodrilus.* However, head ciliophores have also been found recently in dorvilleid polychaetes (Eibye-Jacobsen and Kristensen, 1994) and now in *Limnognathia*. Both protostomial and peristomial ciliophores are present in the small dorvilleid, *Neotenotrocha sterreri*. Here the bases of the ciliophores are rectangular. The rows of both the protostomial and peristomial ciliophores are not only located on the ventral side, as in *Diurodrilus* and *Limnognathia*, but the two rows of ciliophores in *N. sterreri* continue onto the dorsal side as well. It is too early to state whether the head ciliophores are homologous in the two annelid species.

The preoral ciliary field (Fig. 2, pc) in *Limnognathia* superficially looks like ciliophores, but the cilia are not placed in regular rows or groups. The most surprising findings in *Limnognathia* are the lateral ciliophores that flank the pharyngeal apparatus (Fig. 2, hc), exactly in the same way as in *Diurodrilus* (fig. 2 in Kristensen and Niilonen, 1982). The head ciliophores of both *Limnognathia* and *Neotenotrocha* have a rectangular base, while only the anterior group of ciliophores of *Diurodrilus* has this very characteristic configuration. This presumed homology between the head ciliophores of Micrognathozoa and Annelida probably represents a plesiomorphic condition, because it is very difficult to believe that the head ciliophores of Micrognathozoa and Annelida could be convergent structures. Consequently, head ciliophores have been secondarily reduced several times independently in various taxa, and that is not a very parsimonious hypothesis.

Trunk ciliophores are only found in *Diurodrilus* and *Limnognathia.* The 18 trunk ciliophores of *Limnognathia* consist of double cells (Figs. 2, 4). All ciliophores are very similar, with four rows of cilia in each cell. The first rows of trunk ciliophores are located just behind the oral plate (Fig. 6). In living and slightly contracted animals (Fig. 2), the first row may overlap the oral plate, but the trunk ciliophores are always located on the thorax and the abdomen, and not on the head. Just behind the last row of trunk ciliophores is located the adhesive ciliated pad (Figs. 2, 4, 32). The cilia of the pad may be modified trunk ciliophores, which have changed from a locomotory function to being adhesive. The adhesive glands seem to open in a midventral position between the two clusters of five large ciliated cells. In *Diurodrilus* the number of trunk ciliophores is constant within the species, but shows marked differences between the species. The plesiomorphic condition seems to be 15 trunk ciliophores, which means three ciliophores on each trunk segment. Each ciliophore has ten rows of cilia ordered in a very strict way. In *Diurodrilus westheidei* there exist an anterior postpharyngeal (ppc) and a posterior preanal ciliary field (see fig. 2, Kristensen and Niilonen, 1982). The preanal ciliary field closely resembles the adhesive ciliated pad in *Limnognathia*, but in *Diurodrilus* the ciliary pad is not adhesive. The adhesive glands (duo glands) are located in the so-called toes. By DIC optics it looks as though the trunk ciliophores of *Diurodrilus* consist of only one element (not double, as in *Limnognathia*). However, by TEM technique it is seen that there may be up to four ciliophores forming the specialized ciliated areas, which form the locomotory organs ("Membranellen," Ax, 1967). In an as-yet undescribed *Diurodrilus* species from Australia with dorsal plates, the first two rows of trunk ciliophores are still not fused. These ciliophores consist of four ciliary fields. The three to nine rows of ciliophores comprise two cells, and the last (posterior) row nine is reduced to a single ciliophore. The rows of trunk ciliophores in the genus *Diurodrilus* may vary considerably, and ciliophore pattern is used to determine the different species (Mock, 1981; Villora-Moreno, 1996). The number of rows of trunk ciliophores may be related to the dorsal reinforcements or to the so-called dorsal cuticular plates. These structures were described for the first time by Ax (1967) in *D. ankeli*. They are absent in *D. westheidei* and in *D. minimus*, and are present as very delicate structures (Mock, 1981) in *D. subterraneus*. Species with strong dorsal reinforcements seem to have fewer rows of ciliophores. The rows of ciliophores may be reduced to nine and it is always the posterior rows that are reduced. In the new species of *Diurodrilus* from Queensland (Australia), the dorsal reinforcements as well as the fine structure of the trunk ciliophores can only be

observed when the animal is alive. The dorsal plates are not only present on the prostomium and metastomium (= peristomium) as described in *D. ankeli*, but are also present on all five trunk segments. In wholemount preparations, the so-called cuticular plates disappear, as in *Limnognathia*. New TEM investigations are greatly needed to determine whether the dorsal reinforcements in *Diurodrilus* may also be intracellular matrix plates, as in *Limnognathia*.

In the classic work of Ax (1956), in which he described the gnathostomulids, he stated that "Archiannelida" and Gnathostomulida could not be related because the "Archiannelida" lack jaws (p. 553, Ax, 1956). Many new forms with jaws in the so-called "Archiannelida" have been found since 1956 (for review, see Westheide, 1982; Westheide and von Nordheim, 1985). Today the group "Archiannelida" has been abandoned by nearly all scientists and the different families are included in the class Polychaeta. The "Archiannelida" with jaws are all included in the polychaete family Dorvilleidae (Eibye-Jacobsen and Kristensen, 1994).

Ax (1956) cited Remane (unpubl.) as the first person to have stated that gnathostomulids were related to annelids ("Archiannelida"). Later, Kristensen and Nørrevang (1977, 1978) came to the same conclusion when they described the pharyngeal apparatus of the gnathostomulids *Rastrognathia macrostoma* and *Valvognathia pogonostoma*. The muscular part of the pharyngeal apparatus is similar to that found in various annelids, being ventral, with the pharyngeal tract passing dorsally to the muscular bulb. In Turbellaria, to which gnathostomulids were first assigned (Ax, 1956), the pharyngeal muscles surround the pharynx that passes through the middle of the muscular pharyngeal bulb. As a consequence, Kristensen and Nørrevang (1977, 1978) followed the idea of Riedl (1969) and regarded Gnathostomulida as a separate phylum, clearly unrelated to Platyhelminthes, thinking that gnathostomulids were closely related to annelids. They based their hypothesis on the formation and structure of the jaw apparatus of the dorvilleid polychaete, *Ophryotrocha* sp. These TEM investigations were never published because the fixation was of poor quality.

Later, Kristensen and Niilonen (1982) found similarities between the Diurodrilidae and Gnathostomulida. In some gnathostomulids the pharyngeal area is provided with glands, which, with respect to number and location, may be compared to the glands of the Diurodrilidae. Some aberrant spermatozoa of gnathostomulids (suborder Scleroperalia) have microvillar feet that superficially look like the mushroom-shaped bodies from the mature spermatozoa of *Diurodrilus subterraneus*. The similarity, however, is due to convergence. When Kristensen and Eibye-Jacobsen (1995) made a thorough TEM investigation of spermiogenesis of *D. subterraneus*, they also noted important differences at the functional level. The spermatozoa of *D. subterraneus* are flagellar and the mushroom-shaped bodies are not involved in their movement. Those spermatozoa of *Gnathostomula* that have been investigated (Graebner, 1969) are aflagellar, and movement is performed with the aid of microvilli, which look like the mushroom-shaped bodies. The theory regarding the Gnathostomulida–Annelida relationship proposed by Kristensen and Nørrevang (1977) was totally neglected by other scientists, except for Nielsen (1995), who denied gnathostomulids phylum status and incorporated them in Annelida as "a highly specialized polychaete group."

A few years ago the finding of Remane's hypothetical "archiannelids with jaws" (*Neotenotrocha sterreri*) would have supported Nielsen's theory about the gnathostomulids as polychaetes. Today we know that the jaws of dorvilleids (Purschke, 1987) are built quite differently from those of the gnathostomulids (Herlyn and Ehlers, 1997). When Eibye-Jacobsen and Kristensen (1994) described *N. sterreri*, they abandoned the sister-group relationship between gnathostomulids and annelids. Instead, they included the new taxon in the polychaete family Dorvilleidae. They followed the new concepts (see Westheide, 1987) that neoteny and progenesis may have a major role in the adaptive radiation of interstitial annelids and the characteristics of the interstitial dorvilleids may be included, although *Neotenotrocha* did not "fit" into any of the hypothetical reduction series for interstitial polychaetes (Westheide and Riser, 1983; Westheide, 1984, 1987). It has been thought that the loss of setae and parapodia happened after the loss of jaws and other elements of the pharyngeal apparatus. Suddenly, *Neotenotrocha* turned up with a full set of jaw elements, but with reduced parapodia and without polychaete setae. It appears that the archiannelid problem will never be resolved by morphological data. That all so-called archiannelids are only very advanced polychaetes is very easy to show in a hypothetical reduction series, but the "true" evolutionary events of archiannelids may be more complex.

Unfortunately, it is possible that some "archiannelids" may be primarily adapted to interstitial life very early in animal evolution, e.g., Diurodrilidae and Lobatocerebridae (Rieger, 1980), whereas Protodrilidae, Saccocirridae, Protodriloidae, Nerillidae, and interstitial Dorvilleidae are secondarily modified polychaetes. It is not possible for morphological data alone to show the difference between primary adaptation to interstitial life or secondary adaptation happening several times in several phyla without any close relationship. In fact, a modern cladistic analysis would have difficulties helping us with a new animal such as *Limnognathia maerski*. In gross anatomy it clearly exhibits characters of an interstitial annelid, e.g., tactile bristles, ciliophores, and the body plan.

The hypothesis that Micrognathozoa could be an interstitial polychaete is not supported by our TEM and SEM observations, but this theory should be examined in the light of the old theory of Semper (1872), who proposed that Rotifera are only simple neotenic annelids. When Semper discovered the aberrant rotifer *Trochosphaera* in 1859, he stated that this rotifer was of the primitive ancestral type, and all rotifers developed by neoteny from a trochophore larva of an annelid. This theory was abandoned after it was clearly shown that *Trochosphaera* is a very specialized floscularicean rotifer (Hyman, 1951). Still, the Annelida–Rotifera relationship should not be ruled out in any discussion. Today, most scientists believe that rotifers evolved from a small, ciliated, creeping ancestor (Ruppert and Barnes, 1994) and not from a trochophore-like ancestor. The well-developed buccal field seen in some *Dicranophorus* species may represent a vestige of the ancestral ciliation, the neurotroch. Many vermiform phyla have ventral ciliation, but the similarities between the neotenous polychaetes, e.g., *Neotenotrocha*, are very convincing. The behavior of *N. sterreri* is rotifer-like, especially swimming and foraging behavior. The jaw apparatus consists superficially of a trophi-like structure, or perhaps it is more similar to the jaws of *Limnognathia maerski*. The pincer-like mandibles of *Neotenotrocha* superficially resemble the main jaws (ja_2) of *Limnognathia*, the free denticles of the maxillary apparatus resemble the pseudophalangia (ja_1), and the basal plates resemble the molar structure (also called the basal plates) in *Limnognathia*. Without our TEM knowledge of dorvilleid-jaws (Purschke, 1987), the rotiferan trophi (Clément and Wurdak, 1991; Rieger and Tyler, 1995; Kleinow and Wratil, 1996) and gnathostomulid jaws (Herlyn and Ehlers, 1997), we could not resolve the type of *Limnognathia* jaws. The tubular substructures of the *Limnognathia* jaw are seen clearly in Figure 27, and such substructures are present in the fulcrum of all Rotifera and in the articularium (lamellae symphyses) of Gnathostomulida, but never in dorvilleid Polychaeta. The explanation for the similarity in gross morphology of the jaws of dorvilleid polychaetes (*Neotenotrocha*) and micrognathozoans (*Limnognathia*) is convergence. Furthermore, *Neotenotrocha* is an advanced neotenic polychaete, and its jaws can very easily be placed into a reduction series from complex dorvilleid jaws (Westheide, 1982) to the situation in which jaws are totally lost, as in dinophilids (Eibye-Jacobsen and Kristensen, 1994).

When Ahlrichs (1995) established the monophylum Gnathifera (Gnathostomulida + Rotifera + *Seison* + Acanthocephala), he indicated Platyhelminthes as the sister-group. The monophyly of Platyhelminthes and Gnathifera may be seen in a new light after the discovery of the Micrognathozoa. *Limnognathia maerski* has several characters in common with both Gnathostomulida/Rotifera and interstitial Annelida. Except for the tactile bristles (compound cilia), *Limnognathia* shows no similarities with the Platyhelminthes. The tactile bristles are seen in many meiofaunal groups, and these sensory structures may be an adaptation to small size or to an interstitial way of life (Ax, 1966). Platyhelminthes lack all the characters found in the pharyngeal apparatus of Gnathifera, and today several authors (Nielsen, 1995; Sørensen et al., 2000) place Platyhelminthes as an ingroup in Euspiralia under the monophylum Parenchymia (Platyhelminthes + Nemertini). To remove the Platyhelminthes as the sister-group to Gnathifera and place them as an ingroup in Euspiralia causes a new problem: What is the sister-group to the monophylum Gnathifera? There are two possibilities: 1) the Euspiralia is the sister-group to Gnathifera or 2) the Aschelminthes is a monophylum and Cycloneuralia (Gastrotricha + Nematoda + Nematomorpha + Priapulida + Kinorhyncha + Loricifera) is the sister-group to Gnathifera.

Limnognathia shares some characters in common with Euspiralia, especially Annelida, which are not seen in the other members of Gnathifera. These characters are the ciliation of the pharyngeal epithelium and ventral ciliation (ciliophores). It is too early to state that these characters are plesiomorphic.

Monophyletic, Paraphyletic, or Polyphyletic Origin of Aschelminthes

The Aschelminthes currently consist of two clades: Cycloneuralia (Gastrotricha, Nematoda; Nematomorpha, Priapulida, Kinorhyncha, and Loricifera) and Gnathifera (Gnathostomulida, Eurotatoria, Seisonida, Acanthocephala, and the new group, Micrognathozoa). Other groups have been suggested to belong to Aschelminthes, such as Tardigrada (Ruppert and Barnes, 1994), Entoprocta (Hyman, 1951; Bartolomaeus, 1993), and Chaetognatha (Nielsen, 1995). Although we find these ideas interesting, we will not discuss them further in this section.

The phylum Aschelminthes was proposed by Grobben in 1910 (see Hyman, 1951) to cover an assemblage of animal groups with the so-called pseudocoel or pseudocoelom. The five groups were Rotifera, Gastrotricha, Kinorhyncha, Nematoda, and Nematomorpha. Later, Hyman (1951) included the Acanthocephala and Entoprocta as "the pseudocoelomate Bilateria." Still, in many textbooks the name "Pseudocoelomata" is used for Aschelminthes, inspired by Hyman's coelom theory: "primitive" Acoelomata without coelom, Pseudocoelomata with a persisting blastocoel, and "advanced" Eucoelomata with "true coelom." Unfortunately, the coelom characters alone have no value in the interpretation of metazoan phylogeny, at least not at the higher levels such as classes or phyla (Haszprunar, 1996b). Bartolomaeus (1993) has excellently discussed the coelom concept on the basis of his new

ultrastructural studies. Rotifera, Acanthocephala, large Nematoda, and one family of Loricifera (Pliciloricidae) are pseudocoelomate. In Priapulida, all three body cavity conditions exist: acoelomate, pseudocoelomate, and a body cavity with an incomplete epithelial lining (eucoelomate) in *Meiopriapulus fijiensis* (see Storch et al., 1989). Other Aschelminthes are acoelomate, e.g., Gnathostomulida, Gastrotricha, many small Nematoda, and some Loricifera and Kinorhyncha (Kristensen, 1995).

Several authors have been aware of the pitfall of recognizing the Pseudocoelomata (Aschelminthes) as a monophylum because they require special consideration in terms of convergent evolution (Ruppert, 1991a). The so-called pseudocoelom is the embryonic blastocoel that persists in the adult organism, regardless of the time at which it first appeared during ontogeny. Rieger (1976) regarded the pseudocoelom or pseudocoel as a neotenic condition secondarily derived from coelomate ancestors rather than a plesiomorphic character in metazoan evolution (see Brusca and Brusca, 1990; Haszprunar, 1996b; Valentine, 1997). Others still believe that Aschelminthes constitute a monophyletic group (Nielsen, 1995), but they now avoid the term Pseudocoelomata. When the phylum Loricifera was described (Kristensen, 1983), it was assigned to Aschelminthes on the basis of at least one synapomorphic character shared with each other phylum of Aschelminthes, except for the Gnathostomulida. Some of these characters have later been recognized as not synapomorphic but clearly convergent, e.g., the similarities of the lorica of rotifers with dorsal antennae and the lorica of Nanaloricidae with flosculae are not homologous but convergent. The Loricifera clearly belongs to the Cycloneuralia-clade and the Rotifera to the Gnathifera-clade.

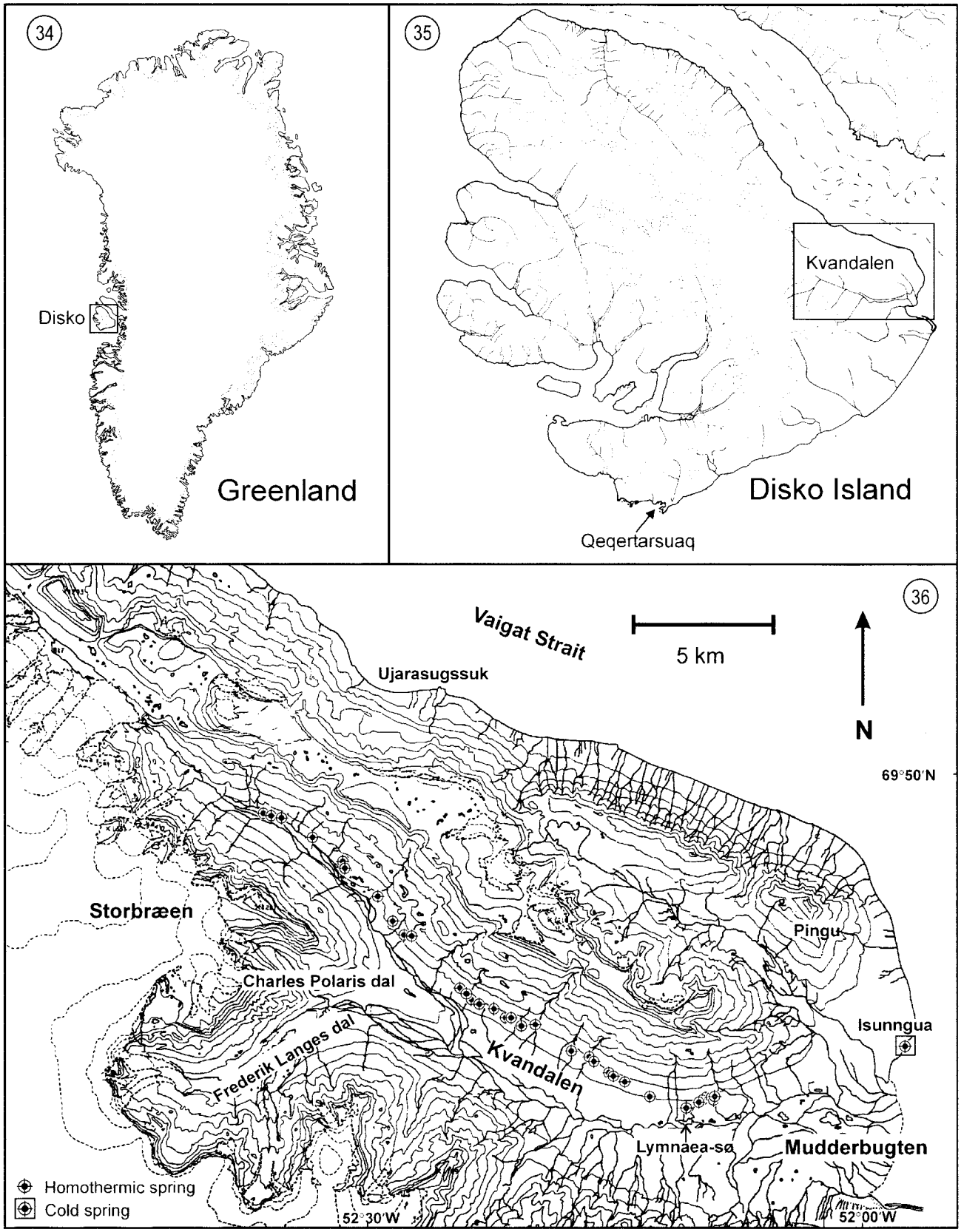
Likewise, it can be difficult to answer the question about the phylogenetic position of Micrognathozoa: is the new animal *Limnognathia maerski* an aschelminth? The ultrastructure of the jaw clearly indicates affinities with the Gnathifera. It lacks the syncytia in the epidermis and therefore cannot be included in Syndermata (Rotifera and Acanthocephala), but it has an intracellular matrix in the dorsal plates, as in many Rotifera. The ventrum of *L. maerski* has multiciliated cells and cannot be included in Gnathostomulida (epidermal cells are always monociliated), but *L. maerski* has a temporary dorsal anus like *Gnathostomula*. The monociliation of the terminal cell in the protonephridia and the ciliated pharynx may be plesiomorphic characters shared with other Euspiralia.

The question about monophyly, paraphyly, or polyphyly of Aschelminthes is very difficult to answer (Winnepenninckx et al., 1995; Wallace et al., 1996) and there exists considerable disagreement between morphological and molecular data. When we described Cycliophora (Funch and Kristensen, 1995, 1997), we placed the new group as a sister-group to the Entoprocta/Ectoprocta clade in Euspiralia (Protostomia). We argued that the chordoid larva of *Symbion pandora* is a modified trochophore (Funch, 1996) in our conclusion. Later, Winnepenninckx et al. (1998) used the 18S ribosomal DNA dataset to include Cycliophora in the Rotifera/Acanthocephala clade. In a new cladistic approach built only on morphological data, Sørensen et al. (2000) placed Entoprocta as a sister-group to Cycliophora in the Euspiralia. Ectoprocta is placed in the Deuterostomia by these authors.

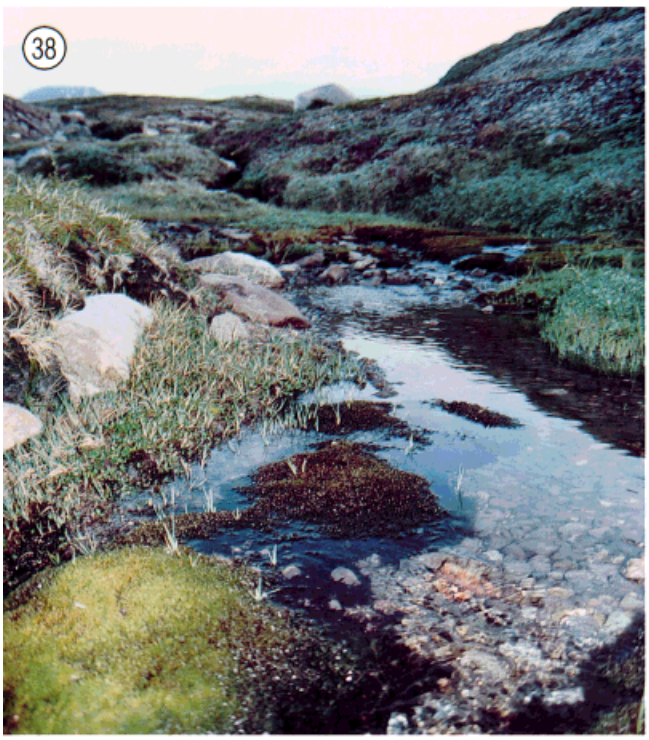
While nearly all morphologists discussed the monophyletic or paraphyletic nature of Aschelminthes (Lorenzen, 1985; Ehlers et al., 1996; Nielsen et al., 1996; Neuhaus et al., 1996), new molecular data may indicate a polyphyletic origin of Aschelminthes (Aguinaldo et al., 1997). The new phylogenetic analysis of 18S ribosomal DNA sequences indicates a close relationship between Panarthropoda (Tardigrada, Onychophora, Arthropoda) and molting Aschelminthes (Nematoda, Nematomorpha, Priapulida, Kinorhyncha [and Loricifera?]). Ecdysis (the molting cycle) has only arisen once in the Animal Kingdom. The new clade is called Ecdysozoa and contains all molting animals (Garey and Schmidt-Rhaesa, 1998). The Aschelminthes should then be a polyphyletic group. This new theory could explain why Tardigrada may have characters from both Aschelminthes and Euarthropoda. The theory is also supported by fossil evidence from the Cambrian (Conway Morris, 1989; Kristensen, 1991). Some segmental worms, e.g., *Opabinia*, could be aschelminths with a segmental cuticle, e.g., as the kinorhynchs. Unfortunately, the clade Ecdysozoa is not supported by many morphological data, e.g., the Gastrotricha, belonging to the Cycloneuralia (Nielsen et al., 1996), do not molt. Schmidt-Rhaesa et al. (1998) suggest Gastrotricha as a sister-group to Ecdysozoa based on the synapomorphic characters: triradiate muscular sucking pharynx, terminal mouth opening, and bilayered cuticle with a trilaminate epicuticle. Most morphologists have placed Gastrotricha as a sister-group to the Nematoda/Nematomorpha clade. The new animal *Limnognathia maerski* does not resolve the problem about the Aschelminthes phylogenetic relationship, which still remains unclear.

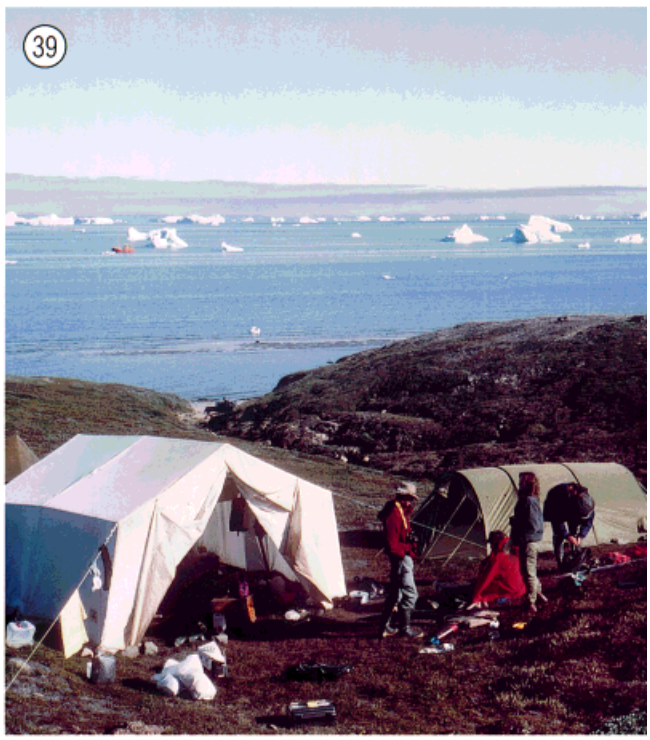
Additional Meiofauna of the Isunngua Spring

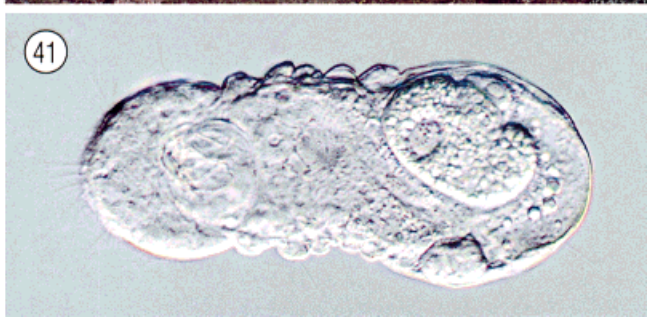
The usually characteristic fauna of warm homothermic springs (Kristensen, 1987) was not found in the cold spring of Isunngua (Figs. 34–36). Instead, a cold water fauna seen in some mud volcanoes and cold homothermic springs was found. There is a rich fauna of turbellarians in the spring, including the kalyptorhynch genus *Geoplana*. The gastrotrich fauna is relatively poor; only one species, *Chaetonotus* sp., was found. The rotifer fauna in the sediment



Figs. 34–36. Maps of the type locality for *Limnognathia maerski*. Fig. 34: Map of Greenland indicating Disko Island. Fig. 35: Map of Disko Island showing Kvandalen. Fig. 36: Close-up of Mudderbugten (The Bay of Mud) and Kvandalen (The Valley of Angelika) showing the type locality, the cold spring at Isunngua, the Lymnaea lake where *Limnognathia maerski* was collected in 1979, and all the known homothermic springs in Kvandalen.







Figs. 37–41. The type locality and living *Limnognathia maerski* nov. gen. et sp. Fig. 37: The first sledge expedition from Arctic Station to Isunngua (5 April 1978). Fig. 38: The type locality for *Limnognathia maerski*. Note the moss cushions where more than 100 specimens were collected 25 July 1994. Fig. 39: The base camp for the Arctic Field Course in 1994. The small cold Isunngua spring was used for drinking water and the spring can be seen just behind the camp (22 July 1994). Fig. 40: *Limnognathia maerski* nov. gen. et nov. sp. Ventral view of living *Limnognathia maerski*. The so-called eye spots can be seen as round vesicles just in front of the pharyngeal bulb. The animal is 115 μm long. Fig. 41: *Limnognathia maerski* nov. gen. et nov. sp. Dorsal view of a slightly squeezed animal. The sensoria located on the head and the nucleus in the large oocyte are clearly seen. The animal is 110 μm long.

Figs. 42–46. Search for *Limnognathia maerski* year-round. Fig. 42: Isunngua spring in autumn (27 August 1996). Fig. 43: Digging down to the type locality (5 May 1998). Fig. 44: The spring was totally frozen beneath 1.5 m snow cover (5 May 1998). Note the research vessel *Porsild* in the Vaigat Strait and Arveprinsens Ejland more than 50 km away. Fig. 45: The third moor and Isunngua spring early in the spring (20 May 1995). Only one juvenile was collected. Fig. 46: Breaking the sea ice to reach Isunngua (20 May 1995). Note the Cretaceous sand on the beach of Isunngua and the mountain Pingu in the background.

consists of *Notholoica squamula* f. *lapponica, Lepadella patella*, and *Encentrum unicinatum*. The rotifer fauna in the water mosses in the spring consists of *Colurella obtusa*, *Lepadella acuminata, Lecane lunaris, Trichocerca weberi, T. longiseta*, and *T. rattus rattus*.

The tardigrade fauna is very interesting because two of the species were originally described from a mud volcano (*Eohypsibius nadjae*) and a cold homothermic spring near Isunngua (*Microhypsibius minimus*). The other members of the tardigrade fauna are the following species: *Amphibolus nebulosus, A. weglarskae, Dactobiotus ambiguus, Dactobiotus* nov. sp., *Diphascon scoticum, Hypsibius arcticus, H. dujardini, Isohypsibius elegans, Macrobiotus echinogenitus, M. richtersi*, and *Murryon pullari*. All the mentioned species are very common in freshwater (lakes, ponds, or rivers) or in wet soil at Disko Island. The copepod *Bryocampus arcticus* was rare, as well as other members of the meiofaunal crustaceans. The marine fauna element found in the radioactive salt springs at Disko Island (Kristensen, 1977, 1982, 1987) was not present in the Isunngua spring.

Ecological Remarks

The senior author visited the Isunngua area the first time by a sledge expedition from Arctic Station in the winter of 1978 (Fig. 37). The temperature at noon was measured as −29°C on 5 April 1978. The spring of Isunngua has abiotic parameters similar to other small springs dominated by melt water at Disko Island: the temperature varies throughout the year and the springs have a very low conductivity (43–63 μmho). The pH (6.4) is low because the spring is running through three moor systems. After the last moor, the spring runs in a deep channel of well-sorted Cretaceous sand (Figs. 38, 39, 42). There exists a rich interstitial meiofauna (e.g., the rotifer *Notholca squamula*) in this former tropical sand, but the taxon *Limnognathia maerski* was only found associated with the spring mosses. The radioactivity in the spring is very low (about 40–60 counts per second). In the salt springs at Disko Island the radioactivity may be up to 1,500 cps (Kristensen, 1987). The cold spring of Isunngua is totally frozen during the long Arctic winter. At Disko Island this means that all springs, which are not homothermal, are frozen from about early November to early May. The type locality was visited twice in May (Figs. 43–46). Both in 1995 and 1998 spring came early to Disko Island and we could reach the remote place by the research vessel *Porsild* without any problem with sea ice. On 21 May 1995, the spring was not covered with snow and the water temperature was already 4°C, while the air temperature was 1.5°C. The meiofauna in the spring was already activated. Seven species of monogonont rotifers and five species of tardigrades were found in the spring mosses, but only a single specimen of *Limnognathia maerski* was found. The length of the specimen measured only 84 μm. It is absolutely the smallest specimen we have ever found. The animal was transparent and it could be a newly hatched juvenile. It could indicate that *Limnognathia* survives the Arctic winter as eggs, a different life strategy than some of the other meiofaunal animals in the spring, which clearly overwinter in all different stages of the life cycle, e.g., the large tardigrade *Amphibolus nebulosus*, which was present in the spring as eggs, juveniles, and adults.

The spring was revisited on 5 May 1998. The air temperature was −2.2°C. The spring was covered with nearly two meters of snow (Figs. 43, 44). The crew of *Porsild* dug down to the Isunngua spring; the spring was totally frozen and the ice temperature in the spring was −0.1°C. Frozen moss samples were carried to the Arctic Station at Qeqertarsuaq. Several species of rotifers, including two species of bdelloids and four species of monogononts, were observed. The very rare tardigrade *Eohypsibius nadjae* (see Bertolani and Kristensen, 1987) and a species of the gastrotrich genus *Chaetonotus* were also present in the frozen samples. Not a single specimen of *Limnognathia* was found. This finding supports the theory that with *Limnognathia* overwintering as eggs, the eggs hatch early in the summer and the maximum of the population will be in July–August. Late in August the population begins to decline. The two different eggs (unsculptured and sculptured) found in August 1994 may be summer eggs and overwintering eggs (Fig. 7). The so-called unsculptured, abortive eggs laid by several females may not be induced by high temperature in the culture, but could instead be true summer eggs. The sculptured eggs could then be winter eggs.

Temperature data recorders placed in the submerged mosses at the type locality in the winter of 1996/97 from 26 October to 1 May showed that *Limnognathia maerski* endures minimum temperatures (−2.7°C) at the end of October. Typical winter temperatures in the frozen mosses are remarkably stable and range between −1.0 to −0.5°C beneath the protective snow cover, with an average for the whole period of 0.8°C. Summer temperatures were measured between 2 July and 16 August 1997, each for 4.5 h. The average temperature in this period was 8.7°C. The highest temperatures are in the afternoon and evening (maximum temperature: 14.2°C was measured on 6 July 1997, 6 PM). Low summer temperatures are typically in the morning (minimum temperature: 4.6°C was measured on 16 August 1997, 6 AM). We attempted to record temperatures for an entire year, but the recorder was lost. Probably the powerful melting water flushed the recorder out into the sea. Although *Limnognathia maerski* lives in Arctic springs, the temperatures encountered are moderate. Adaptations to cope with low temperatures do not seem to be important for

the adults. However, adaptation to attach firmly to the substrate is important. *Limnognathia* probably endures powerful currents caused by melting water in the spring. Eggs are probably the stages that disperse over longer distances and they might survive low temperatures.

Zoogeography

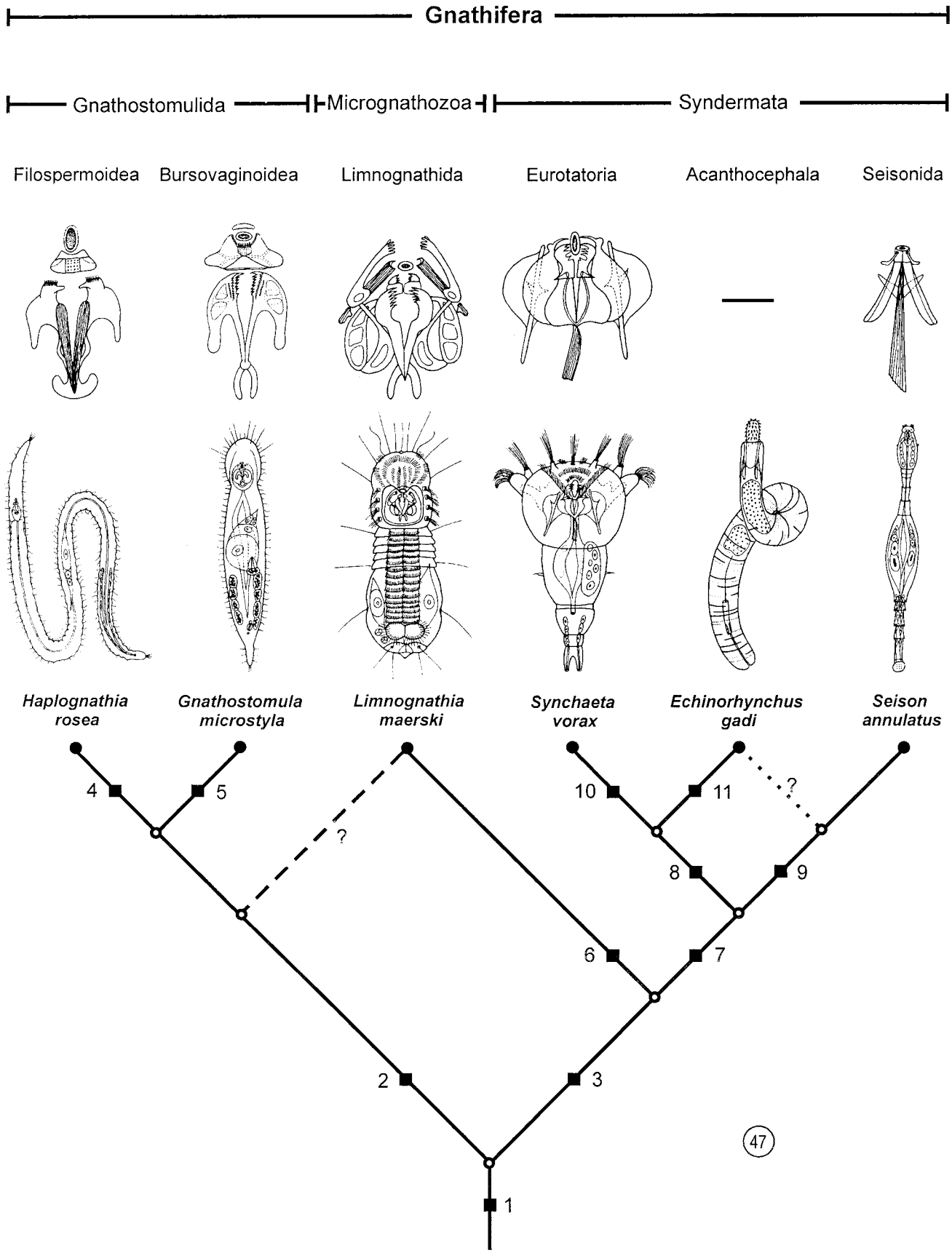
If rather similar vermiform phyla had diverged already in the deep Precambrian (Wray et al., 1996; Sterrer, 1998), as some molecular data indicate, the zoogeographic data cannot help us in the phylogenetic discussion, e.g., the cosmopolitan distribution of some low-water species of Gnathostomulida and Tardigrada without obvious mechanisms for long-range dispersal only tell us that they are groups that go back to the supercontinent Pangaea in early Mesozoic time. The earlier supercontinent Rodinia (see McMenamin, 1998) in the Precambrian would not have any influence on worldwide distribution of recent species. Recently, what seems to be a member of Micrognathozoa was found in Antarctica (De Smet, pers. com.). The bipolar distribution of Micrognathozoa may indicate that the group is very ancient. The problem is that the spring at Isunngua is young compared with other springs throughout the world. In the last glaciation the Isunngua spring was, as were many of the homothermic springs at Disko Island, about 100 meters below sea level. When glaciation ended in the area about 6,900 years ago, the land masses rose and subsequently the spring was left above sea level. This uplifting of the land masses happened so slowly that the marine forms of animals could adapt to freshwater in some of the homothermic springs (Kristensen, 1977). The new taxon, *Limnognathia maerski,* is found in a cold spring that is frozen for nearly half of the year. It cannot go into anhydrobiosis, as do many of the other members of the meiofauna in the spring. Thus, it has a very low dispersal range. The animal may be a marine relict in the spring, but there are no other marine animals in the Isunngua spring. More than 20 years research for marine interstitial meiofauna just outside the spring in Disko Bay (Kristensen and Niilonen, 1982; Kristensen and Nørrevang, 1982) did not produce any Micrognathozoa. The discovery of three specimens of *Limnognathia* from 1979 in the valley of Sullorsuaq/Kvandalen may indicate that the new taxon belongs to an ancient true freshwater fauna. The source of the cold homothermic spring at Sullorsuaq (Fig. 36) is about 100 meters above sea level, and there is no evidence to assume that the springs were under sea level during the last glaciation. The finding of a "missing link" between the marine gnathostomulids and the marine/freshwater rotifers in a remote Arctic spring is very enigmatic, and we have no explanation as to why the animal was not discovered before.

Phylogenetic Position of Micrognathozoa

The discovery of the new taxon, *Limnognathia maerski,* gave the impetus to the proposal of the homology of gnathostomulid and rotifer jaws (Rieger and Tyler, 1995) and the phylogenetic discussion of the validity of the monophylum Gnathifera (Ahlrichs, 1997; Herlyn and Ehlers, 1997). When we observed the first living specimens of *L. maerski* in 1994, it became clear to us that it was a unique animal (Kristensen, 1995; Kristensen and Funch, 1995). We have therefore discussed these new findings of morphological characters with several of our colleagues and a new computer-assisted cladistic analysis of all Metazoa will soon be published (Sørensen et al., 2000). In this cladistic analysis both the phylogenetic position of Cycliophora and Micrognathozoa will be treated. Here, we discuss only the phylogenetic position of Micrognathozoa within the monophylum Gnathifera (see Fig. 47).

We consider the possession of cuticular jaws with tubes (rods) as a synapomorphy for all Gnathifera (Fig. 47, apomorphy 1). Jaw-like structures are found in other taxa as well, for instance, in the probosces of kalyptorhynch turbellarians, in dorvilleid polychaetes, and aplacophoran mollusks, but studies of their ultrastructure show that none of these jaws are homologous with the jaws in Gnathostomulida, Micrognathozoa, and Rotifera (Rieger and Tyler, 1995; this article). We are uncertain of the origin of the basal plates in Gnathifera. A single cuticular basal plate could belong to the ground pattern of Gnathifera; consequently, basal plates were lost in the stem lineage of Syndermata and became paired and modified in the stem lineage of Micrognathozoa. A second alternative is that paired basal plates were present in the ancestor of Gnathifera, and were completely lost in Syndermata. A third alternative is that the basal plates in Gnathostomulida and Micrognathozoa are not homologous. We consider the loss of the digestive system, including the pharyngeal apparatus, as an autapomorphic character for Acanthocephala.

The presence of a monociliated cellular epidermis has been postulated to be present in the stem species of the Bilateria (Ehlers, 1985; Ax, 1985), but Sørensen et al. (2000) regard that a multiciliated epidermis evolved in the ancestor of Bilateria. Monociliation in Gnathifera is only found on the overall surface of the body in Gnathostomulida and in the protonephridia of both Gnathostomulida and Micrognathozoa. Rieger (1976) interpreted complete monociliation with microvilli surrounding the cilium as a plesiomorphic character. However, the long flagellar-like ciliation present in all gnathostomulids may be interpreted as an autapomorphic character for gnathostomulids. Absence of motile cilia on the dorsal and lateral sides and ventral multiciliated epidermal cells may be interpreted as a synapomorphy of Micrognathozoa and Syndermata (Ro-


Gnathifera
Gnathostomulida
Micrognathozoa
Syndermata
Filospermoidea
Bursovaginoidea
Limnognathida
Eurotatoria
Acanthocephala
Seisonida
Haplognathia rosea
Gnathostomula microstyla
Limnognathia maerski
Synchaeta vorax
Echinorhynchus gadi
Seison annulatus
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?
10
11
?
8
9
6
7
2
3
1
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Figure 47

tifera + Acanthocephala). We consider the reduction of ventral ciliation in Eurotatoria + Seisonida and the total absence of epidermal ciliation in Acanthocephala (Dunagan and Miller, 1991) as apomorphic characters. Ventral ciliation may be a plesiomorphic character for *Limnognathia*, while the presence of ventral ciliophores is an autapomorphy for Micrognathozoa. The thick dorsal and lateral epidermal plates in Micrognathozoa are not cuticular but are formed by an intracellular matrix layer, as in Syndermata. We consider this character as a synapomorphy for Micrognathozoa and Syndermata (Fig. 47, apomorphy 3). The syncytial epidermis is an autapomorphy for the clade Syndermata, which includes the Eurotatoria, Acanthocephala, and Seisonida. The new taxon, Micrognathozoa, lacks syncytia.

We also consider the presence of separate sexes as a plesiomorphic character in Gnathifera, and the presence of hermaphroditism and a complex reproductive system in Gnathostomulida as autapomorphic characters. Furthermore, we follow the idea of Sterrer (1972) that the presence of a filiform spermatozoon with one flagellum and an acrosome is a plesiomorphic character, and that all other types of spermatozoa are derived from this type.

Figure 47 shows our phylogeny of the main groups of Gnathifera. It differs somewhat from Ahlrichs (1997) and Herlyn and Ehlers (1997). The two question marks indicate two other possibilities for relationships in Gnathifera. The ultrastructure of the main jaws of *Limnognathia maerski* is similar to the jaws of advanced scleroperalian gnathostomulids, especially in the jaw apophyses and the paired cauda. We have therefore previously suggested that Gnathostomulida and Micrognathozoa were closely related (Kristensen and Funch, 1995). New SEM data on the jaws of *Haplognathia rosea* and *Rastrognathia macrostoma* (see Sørensen, 2000) made us consider that some of these jaw characters could belong to the ground pattern of Gnathifera, e.g., the longitudinally arranged rods in the lamellae symphysis of gnathostomulids might be homologous with the striation present in the fulcrum of Eurotatoria (for review, see Markevich, 1989) and Seisonida (Segers and Melone, 1998). Other characters, such as the presence of a paired cauda, might be convergently developed in some species of *Gnathostomula* and *Limnognathia*. But it is too early to conclude that the paired fibularia of *Limnognathia* and the fibularization of the jaw apophyses of *Gnathostomula* belong to the ground pattern of Gnathifera. A consequence of fibularization belonging to the ground pattern of Gnathifera is that the so-called solid type (arc type) of jaws in some gnathostomulids represents a derived type. However, this contradicts the most accepted classification of Gnathostomulida (Sterrer, 1972), where the compact arc type is considered to have the plesiomorphic characters and the row type with fibularization has the apomorphic characters. Sterrer's classification of the jaws is strongly supported by other characters, e.g., the sperm types and the fine structure of the reproductive organs. We therefore suggest that Micrognathozoa is the sister-group to the taxon Syndermata, and not Gnathostomulida. An important synapomorphy for the clade Micrognathozoa + Syndermata is the presence of an intracellular matrix within the epidermis. The micrognathozoans do not belong to Syndermata (Eurotatoria + Acanthocephala + Seisonida), because *Limnognathia* totally lacks the syncytia in the epidermis (Fig. 47, apomorphy 7).

Lorenzen (1985) argued that the characteristic sacs, lemnisci in acanthocephalans, were in fact present in bdelloid rotifers but had been overlooked. Based on this and other morphological similarities Lorenzen suggested that acanthocephalans were merely aberrant bdelloid rotifers. The first phyloge-

Fig. 47. Phylogeny of the Gnathifera. The cladogram is modified from Ahlrichs (1997), Herlyn and Ehlers (1997), and Sørensen et al. (2000). Our suggestion is shown with solid lines. The broken line with a question mark denotes the suggestion of Ahlrichs (1997) and the dotted line with a question mark is our alternative suggestion (Kristensen and Funch, 1995). Ventral views of the animals and their jaws. From left to right: *Haplognathia rosea* (Filospermoidea, Gnathostomulida); *Gnathostomulida microstyla* (Bursovaginoidea, Gnathostomulida); *Limnognathia maerski* nov. gen et nov. sp. (Micrognathozoa); *Synchaeta vorax* (Monogononta, Eurotatoria); *Echinorhynchus gadi* (Palaeacanthocephala, Acanthocephala); *Seison annulatus* (Seisonida, Rotifera). The black squares 1–11 mark syn- and autapomorphies. 1. Gnathifera. Cuticular jaws with tubes composed of lucent material surrounding an electron-dense core. 2. Gnathostomulida. Hermaphrodites with complex reproductive system. Pharyngeal apparatus with muscle capsule. 3. Micrognathozoa + Syndermata. Epidermis with intracellular matrix, motile cilia absent on the surface of dorsal and lateral trunk regions. 4. Filospermoidea. Elongation of the body with a long and slender rostrum. Compact jaws with 8–12 longitudinally arranged rods in lamellae symphyses. 5. Bursovaginoidea. Fibularization of the jaw apophyses. Myosyncytia in the pharyngeal apparatus. Complex female organs with a bursa and often a vagina. Male opening with an injectory penis often consisting of 8–12 intracellular rod-like structures. Spermatozoon strongly modified, lacking flagellar or centriolar structures. 6. Micrognathozoa. Ventral locomotory organ consists of two rows of multiciliated cells (trunk ciliophores). Ventral epidermis covered only with a thin glycocalyx, except for the large cuticular oral plate. Dorsal and lateral intracellulary plates present. The solid parts of the jaws are very complex, with paired basal plates, paired lamellae orales, paired fibularia, and three sets of paired jaws. 7. Syndermata. Syncytial epidermis; epidermal cells with apical crypts. Eutelic epidermis. Spermatozoon with anteriorly inserted flagellum. 8. Eurotatoria + Acanthocephala. Spermatozoon without acrosome. 9. Epizoic. The head ciliation consists of a few tufts of stiff bristles. The trophi form an anterior cuticular sheath. Presence of spermatophore. 10. Eurotatoria. Corona, unpaired retrocerebral glands, female reproductive organs with vitellarium. 11. Acanthocephala. Endoparasitic with a two-host life cycle. Epidermal lacunar system. Proboscis with intraepidermal hooks. Females with a uterine bell.

netic analyses of the relationship of acanthocephalans to rotifers, using nuclear 18S ribosomal DNA and mitochondrial 16 ribosomal RNA genes, gave strong support for a sister-group relationship between Acanthocephala and Bdelloidea (Garey et al., 1998). Ahlrichs (1997) argued that ultrastructural features such as the presence of dense bodies in the spermatozoa and an epidermis with similar bundles of filaments in both Acanthocephala and Seisonida gave support for a sister-group relationship (Fig. 47, dotted line), a point of view supported by Herlyn and Ehlers (1997). We are in favor of a monophyletic taxon Eurotatoria consisting of the two classes Monogononta and Bdelloidea based on a number of morphological characters (Fig. 47). This point of view is supported by a computer-generated cladistic analysis of Animalia (Sørensen et al., 2000), where the sister-group to Eurotatoria is Acanthocephala. The loss of the acrosome in the spermatozoon is a synapomorphy supporting this relationship (Fig. 47, apomorphy 8). Thus, acanthocephalans are in fact large parasitic rotifers. A recent phylogenetic study by Welch (2000) using a nuclear protein-coding gene and comprising sequences for several species of rotifers actually supports our interpretation, and the same conclusion was made by García-Varela et al. (2000) in their molecular study of Acanthocephala and Eurotatoria.

In the classical taxonomy of rotifers the order Seisonidea is placed in the class Digononta, also containing Bdelloidea (Beauchamp, 1965; Nogrady et al., 1993). Segers and Melone (1998), on the other hand, treat Seisonidea as an independent class of rotifers. We consider the presence of paired gonads as a plesiomorphic character for Gnathifera and suggest that Digononta is paraphyletic. Seisonidea is instead the sister-group to Eurotatoria + Acanthocephala. The two marine species of *Seison* are very aberrant rotifers, especially in trophi. In fact, different studies disagree in labeling the sclerites consistently (see Segers and Melone, 1998).

CONCLUSION

The jaws of Gnathostomulida, Rotifera, and the new group Micrognathozoa are homologous structures. The ultrastructure of cuticular elements of the jaws in all three groups has a tubular substructure. The tube-like rods consist of an osmiophilic core surrounded by an osmiophobic layer of cuticle. The rods may be present only in special areas or the whole jaw apparatus is built of parallel-layered rods. The three mentioned groups + Acanthocephala have been included in the monophylum group Gnathifera. The absence of pharyngeal ciliation cannot be used as an autapomorphy of the Gnathifera, as suggested by Herlyn and Ehlers (1997). The Micrognathozoa has a ciliated pharyngeal epithelium, as do several interstitial polychaetes. Furthermore, the pharyngeal epithelium is covered with a thin cuticle, as in Rotifera.

The new animal, *Limnognathia maerski* nov. gen. et sp., is probably closely related to both Rotifera and Gnathostomulida. Rotifera + Acanthocephala and Micrognathozoa might be sister-groups, while Gnathostomulida may be the sister-group to this assemblage. The monophyly of Plathelminthomorpha (Platyhelminthes and Gnathostomulida) and Platyhelminthes (see Ax, 1996; Haszprunar, 1996a) is doubtful. The discovery of *Limnognathia maerski* supports the sister-group relationship of Gnathostomulida and Syndermata (Rotifera-Acanthocephala clade), as first stated by Rieger and Tyler (1995) and later supported by Ahlrichs (1995, 1997) when he established the monophylum Gnathifera. The new molecular data of *Gnathostomula* based on 18S ribosomal DNA are interesting, but also confusing. The data of Littlewood et al. (1998) supported a relationship with Gnathostomulida and the Nematoda-Chaetognatha clade, while the data of Zrzavý et al. (1998) supported an old theory about a sister-group relationship between Gastrotricha and Gnathostomulida (Neotrichozoa). The old theory was based on morphological evidence, such as the presence of monociliated epidermal cells in all Gnathostomulida and some Gastrotricha (Rieger, 1976), but we consider the monociliation in the epidermal cells and the terminal cells of the protonephridium as a plesiomorphic character in all Euspiralia, as did Rieger (1976), and so not sufficient to support the monophyly of Gnathostomulida + Gastrotricha. Before we can abandon the clade Neotrichozoa, however, we need to have molecular data on Micrognathozoa. The ultrastructural similarities of the new animal to certain gastrotrichs and interstitial polychaetes may be convergent, or may show the plesiomorphic condition in Euspiralia.

Autapomorphies for Micrognathozoa are the following: on the dorsum, the presence of dorsal plates sclerotized by an intracellular matrix, serially homologous tactile bristles consisting of one to three cilia, and zipper-junctions between all dorsal epidermal cells; on the ventrum are 18 trunk ciliophores and paired ventral head ciliophores flanking the pharyngeal apparatus, a posterior adhesive ciliated pad, and sclerotization only in an oral plate; all other ventral cells lack cuticle and sclerotization. The complexity of the cuticular pharyngeal elements is greater than in other members of Gnathifera and its special features constitute good autapomorphies.

Limnognathia maerski cannot be included in any existing group without difficulty. Therefore, we have established a new class, Micrognathozoa, for the new freshwater animal, indicating that it has the same rank as Gnathostomulida and Syndermata in the Gnathifera. We suppose a closer relationship (sister-group relationship) to the Syndermata than to Gnathostomulida, and we support the theory of monophyly of Gnathifera.

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LITERATURE CITED

Aguinaldo AMA, Turbeville JM, Lindford LS, Rivera MC, Garey JR, Raff RA. 1997. Evidence for a clade of nematodes, arthropods and other moulting animals. Nature 387:489–493.

Ahlrichs WH. 1993a. Ultrastructure of the protonephridia of *Seison annulatus* (Rotifera). Zoomorphology 113:245–251.

Ahlrichs WH. 1993b. On the protonephridial system of the brackish-water rotifer *Proales reinhardti* (Rotifera, Monogononta). Microfauna Mar 8:39–53.

Ahlrichs WH. 1995. Ultrastruktur und Phylogenie von *Seison nebaliae* (Grube 1859) und *Seison annulatus* (Claus 1876). Göttingen: Cuvillier Verlag. p 310.

Ahlrichs WH. 1997. Epidermal ultrastructure of *Seison nebaliae* and *Seison annulatus*, and a comparison of epidermal structures within the Gnathifera. Zoomorphology 117:41–48.

Ax P. 1956. Die Gnathostomulida, eine rätselhafte Wurmgruppe aus dem Meeressand. Abh math-naturw Kl Akad Wiss Mainz 8:1–32.

Ax P. 1966. Die Bedeutung der interstiellen Sandfauna für allgemeine Probleme der Systematik, Ökologie und Biologie. Veröffentl. d. Inst. f. Meeresforschung Bremerhaven, 2. Sonderband: 15–66.

Ax P. 1967. *Diurodrilus ankeli* nov. spec. (Archiannelida) von der nordamerikanischen Pazifikküste. Ein Beidrag zur Morphologie, Systematik und Verbreitung der Gattung *Diurodrilus*. Z Morphol Oeko Tiere 60:5–16.

Ax P. 1985. The position of the Gnathostomulida and Plathelminthes in the phylogenetic system of the Bilateria. In: Conway Morris S, George JD, Gibson R, Platt HM, editors. The origins and relationships of lower invertebrates. Oxford: Clarendon Press. p 168–180.

Ax P. 1989. Basic phylogenetic systematization of the Metazoa. In: Fernholm B, Bremer K, Jörnvall H, editors. The hierarchy of life. Amsterdam: Elsevier Science Publishers. p 229–245.

Ax P. 1996. Multicellular animals: a new approach to the phylogenetic order in nature, vol. 1. Berlin: Springer-Verlag. p 225.

Bartolomaeus T. 1993. Die Leibeshöhlenverhältnisse und Nephridialorgane der Bilateria-Ultrastruktur, Entwicklung und Evolution. Habilitationsschrift. Göttingen: Universität Göttingen. p 293.

Bertolani R, Kristensen RM. 1987. New records of *Eohypsibius nadjae* Kristensen, 1982, and revision of the taxonomic position of two genera of Eutardigrada (Tardigrada). In: Bertolani R, editor. Biology of tardigrades. Modena: U.Z.I. 1, Mucci. p 359–372.

Boaden PJS. 1975. Anaerobiosis, meiofauna and early metazoan evolution. Cah Biol Mar 15:367–378.

Boaden PJS. 1989. Meiofauna and the origins of the Metazoa. Zool J Linn Soc 96:217–227.

Brusca RC, Brusca GJ. 1990. Invertebrates. Sunderland, MA: Sinauer Associates. p 992.

Cavalier-Smith T. 1998. A revised six-kingdom system of life. Biol Rev Camb Philos Soc 73:203–266.

Clément P, Wurdak E. 1991. Rotifera. In: Harrison FW, Ruppert EE, editors. Microscopic anatomy of invertebrates, vol. 4. Aschelminthes. New York: Wiley-Liss. p 219–297.

Conway Morris S. 1989. Burgess Shale faunas and the Cambrian explosion. Science 246:339–346.

De Smet WH. 1996. Rotifera. The Proalidae (Monogononta), vol. 4. In: Dumont HJF, editor. Guides to identification of microinvertebrates of the continental waters of the world, vol. 9. Amsterdam: SPB Academic. p 102.

De Smet WH. 1997. Rotifera. The Dicranophoridae (Monogononta), vol. 5. In: Dumont HJF, editor. Guides to identification of microinvertebrates of the continental waters of the world, vol. 12. Amsterdam: SPB Academic Publishing. p 325.

De Smet WH, Van Rompu EA. 1994. Rotifera and Tardigrada from some cryoconite holes on a Spitsbergen (Svalbard) glacier. Belg J Zool 124:27–37.

Dunagan TT, Miller DM. 1991. Acanthocephala. In: Harrison FW, Ruppert EE, editors. Microscopic anatomy of invertebrates, vol. 4. Aschelminthes. New York: Wiley-Liss. p 299–332.

Ehlers U. 1985. Das phylogenetische System der Plathelminthes. Stuttgart: Gustav Fischer.

Ehlers U, Ahlrichs W, Lemburg C, Schmidt-Rhaesa A. 1996. Phylogenetic systematization of the Nemathelminthes (Aschelminthes). Verh Dtsch Zool Ges 89:8.

Eibye-Jacobsen J. 1997. Development, ultrastructure and function of the pharynx of *Halobiotus crispae* Kristensen, 1982 (Eutardigrada). Acta Zool (Stockholm) 78:329–347.

Eibye-Jacobsen D, Kristensen RM. 1994. A new genus and species of Dorvilleidae (Annelida, Polychaeta) from Bermuda, with a phylogenetic analysis of Dorvilleidae, Iphitimidae and Dinophilidae. Zool Scr 23:107–131.

Fenchel T, Riedl RJ. 1970. The sulfide system: a new biotic community underneath the oxidized layer of marine sandy bottoms. Mar Biol 7:225–268.

Fiege D. 1990. Morphology of the wings of euthecosomatous pteropods. Am Malac Bull 8:27–36.

Funch P. 1996. The chordoid larva of *Symbion pandora* (Cycliophora) is a modified trochophore. J Morphol 230:231–263.

Funch P, Kristensen RM. 1995. Cycliophora is a new phylum with affinities to Entoprocta and Ectoprocta. Nature 378:711–714.

Funch P, Kristensen RM. 1997. Cycliophora. In: Harrison FW, Woollacott RM, editors. Microscopic anatomy of invertebrates, vol. 13. Lophophorates, Entoprocta, and Cycliophora. New York: Wiley-Liss. p 392–462.

García-Varela M, Pérez-Ponce de León G, de la Torre P, Cummings MP, Sarma SSS, Laclette JP. 2000. Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. J Mol Evol 50:532–540.

Garey JR, Schmidt-Rhaesa A. 1998. The essential role of "minor" phyla in molecular studies of animal evolution. Am Zool 38: 907–917.

Garey JR, Schmidt-Rhaesa A, Near TJ, Nadler SA. 1998. The evolutionary relationships of rotifers and acanthocephalans. Hydrobiologia 387/388:83–91.

Giribet G, Distel DL, Polz M, Sterrer W, Wheeler WC. Triploblastic relationships with emphasis on the acoelomates, and the position of Gnathostomulida, Cycliophora, Plathelminthes and

Chaetognatha; a combined approach of 18S rDNA sequences and morphology. Syst Biol (in press).

Graebner I. 1969. Vergleichende elektronenmikroskopische Untersuchung der Spermienmorphologie und Spermiogenese einiger *Gnathostomula*-Arten: *Gnathostomula paradoxa* (Ax 1956), *Gnathostomula axi* (Kirsteuer 1964), *Gnathostomula jenneri* (Riedl 1969). Microskopie 24:131–160.

Green CR, Bergquist PR. 1982. Phylogenetic relationships within the Invertebrata in relation to the structure of septate junctions and the development of "occluding" junctional types. J Cell Sci 53:279–305.

Haszprunar G. 1996a. Plathelminthes and Plathelminthomorpha — paraphyletic taxa. J Zoo Syst Evol Res 34:41–48.

Haszprunar G. 1996b The Mollusca: coelomate turbellarians or mesenchymate annelids? In: Taylor JD, editor. Origin and evolutionary radiation of the Mollusca. Oxford: Oxford University Press. p 1–28.

Herlyn H, Ehlers U. 1997. Ultrastructure and function of the pharynx of *Gnathostomula paradoxa* (Gnathostomulida). Zoomorphology 117:135–145.

Hyman LH. 1951. The invertebrates: Acanthocephala, Aschelminthes, and Entoprocta. The pseudocoelomate Bilateria. New York: McGraw-Hill. p 572.

Kalt MR, Tandler B. 1971. A study of fixation of early amphibian embryos for electron microscopy. J Ultrastruct Res 36:633–645.

Kleinow W, Wratil H. 1996. On the structure and function of the mastax of *Brachionus plicatilis* (Rotifera), a scanning electron microscope analysis. Zoomorphology 116:169–177.

Knauss EB. 1979. Indication of an anal pore in Gnathostomulida. Zool Scr 8:181–186.

Koehler JK, Hayes TL. 1969. The rotifer jaw: a scanning and transmission electron microscope study. I. The trophi of *Philodina acuticornis odiosa*. J Ultrastruct Res 27:402–418.

Koste W. 1978. Rotatoria, Die Rädertiere Mitteleuropas. Textband + Tafelband. Berlin, Stuttgart: Gebrüder Borntraeger. p 673.

Kristensen RM. 1977. On the marine genus *Styraconyx* (Tardigrada, Heterotardigrada, Halechiniscidae) with description of a new species from a warm spring on Disko Island. Astarte 10: 87–91.

Kristensen RM. 1982. New aberrant eutardigrades from homothermic springs on Disko Island, West Greenland. In: Nelson DR, editor. Proceedings of the Third International Symposium on the Tardigrada. Johnson City, TN: East Tennessee State University Press. p 203–220.

Kristensen RM. 1983. Loricifera, a new phylum with Aschelminthes characters from meiobenthos. Z Zool Syst Evolut Forsch 21:163–180.

Kristensen RM. 1987. The "southern" flora and the "marine" fauna elements in the homothermic springs on Disko Island, West Greenland. In: Petersen GH, editor. Grönland Exkursion, 2.-25. August 1987. Kiel: Institut für Polarökologie. p 202–226.

Kristensen RM. 1991. 6. New higher taxa — presented by their discoverers. Loricifera — a general biological and phylogenetic overview. Verh Dtsch Ges 84:231–246.

Kristensen RM. 1995. Are Aschelminthes pseudocoelomate or acoelomate? In: Lacivelli G, Valvassori R, Candia Carnevali MD, editors. Body cavities function and phylogeny. Selected symposia and monographs. U.Z.I. 8. Modena: Mucci. p 41–43.

Kristensen RM, Eibye-Jacobsen D. 1995. Ultrastructure of spermiogenesis and spermatozoa in *Diurodrilus subterraneus* (Polychaeta, Diurodrilidae). Zoomorphology 115:117–132.

Kristensen RM, Funch P. 1995. En ny aschelminth med gnathostomulid-lignende kæber fra en kold kilde ved Isunngua. In: Ehrhardt C, editor. Arktisk Biologisk Feltkursus, Qeqertarsuaq/ Godhavn 1994. Copenhagen: University of Copenhagen. p 73–82.

Kristensen RM, Niilonen T. 1982. Structural studies on *Diurodrilus* Remane (Diurodrilidae fam.n.), with description of *Diurodrilus westheidei* sp.n. from the Arctic interstitial meiobenthos, W. Greenland. Zool Scr 11:1–12.

Kristensen RM, Nørrevang A. 1977. On the fine structure of *Rastrognathia macrostoma* gen. et sp. n. placed in Rastrognathiidae fam. n. (Gnathostomulida). Zool Scr 6:27–41.

Kristensen RM, Nørrevang A. 1978. On the fine structure of *Valvognathia pogonostoma* gen. et sp. n. (Gnathostomulida, Onychognathiidae) with special reference to the jaw apparatus. Zool Scr 7:179–186.

Kristensen RM, Nørrevang A. 1982. Description of *Psammodrilus aeificator* sp. n. (Polychaeta), with notes on the Arctic interstitial fauna of Disko Island, W. Greenland. Zool Scr 11:265–279.

Lake PS. 1973. Trialdehyde fixation of crustacean tissue for electron microscopy. Crustaceana (Leiden) 24:244–246.

Lammert V. 1991. Gnathostomulida. In: Harrison FW, Ruppert EE, editors. Microscopic anatomy of invertebrates, vol. 4. Aschelminthes. New York: Wiley-Liss. p 19–39.

Littlewood DTJ, Telford MJ, Clough KA, Rohde K. 1998. Gnathostomulida — an enigmatic metazoan phylum from both morphological and molecular perspectives. Mol Phylogenet Evol 9:72–79.

Lorenzen S. 1985. Phylogenetic aspects of pseudocoelomate evolution. In: Conway Morris S, George JD, Gibson R, Platt HM, editors. The origins and relationships of lower invertebrates. Oxford: Clarendon Press. p 210–223.

Markevich GI. 1989. Morphology and principal organization of the sclerite system of the rotifer mastax. In: Biologiya, Sistematika i Funksionalnaya Morfologiya Presnovadnyk Zhivotnyk. Trudy Inst. Biol. Vnutrenney Vod. Acad. Nauk S.S.S.R:27-82 (in Russian).

McMenamin MS. 1998. The garden of Ediacara. Discovery of the first complex life. New York: Columbia University Press. p 295.

Mock H. 1981. Zur Kenntnis von *Diurodrilus subterraneus* (Polychaeta, Dinophilidae) aus dem Sandhang der Nordseeinsel Sylt. Helgol Wiss Meeresunters 34:329–335.

Neuhaus B, Kristensen RM, Lemburg C. 1996. Ultrastructure of the cuticle of Nemathelminthes and electron microscopical localization of chitin. Verh Dtsch Zool Ges 89:221.

Nielsen C. 1995. Animal evolution: interrelationships of the living phyla. Oxford: Oxford University Press. p 467.

Nielsen C, Scharff N, Eibye-Jacobsen D. 1996. Cladistic analyses of the animal kingdom. Biol J Linn Soc Lond 57:385–410.

Nogrady T, Wallace RL, Snell TW. 1993. Rotifera. In: Dumont HJF, editor. Guides to the identification of the microinvertebrates of the continental waters of the world, vol. 4. Rotifera. The Hague: SPB Academic Publishing. p 142.

Porsild MP. 1920. The flora of Disko Island and the adjacent coast of West Greenland from 66°-71° N Lat. with remarks on phytogeography, ecology, flowering, fructation and hibernation. Meddr Grønland 58:1–156.

Purschke G. 1987. Anatomy and ultrastructure of ventral pharyngeal organs and their phylogenetic importance in Polychaeta (Annelida). IV. The pharynx and jaws of the Dorvilleidae. Acta Zool (Stockholm) 68:83–105.

Reynolds ES. 1963. The use of lead citrate at high pH as an electronopaque stain in electron microscopy. J Cell Biol 17:208–212.

Ricci C, Melone G, Sotgia C. 1993. Old and new data on Seisonidea (Rotifera). Hydrobiologia 255/256:495–511.

Riedl RJ. 1969. Gnathostomulida from America. Science 163:445–452.

Riedl RJ, Rieger RM. 1972. New characters observed on isolated jaws and basal plates of the family Gnathostomulidae (Gnathostomulida). Z Morph Tiere 72:131–172.

Rieger RM. 1976. Monociliated epidermal cells in Gastrotricha: significance for concepts of early metazoan evolution. Z Zool Syst Evolut Forsch 14:198–226.

Rieger RM. 1980. A new group of interstitial annelids (Lobatocerebridae, nov. fam.) and its significance for metazoan phylogeny. Zoomorphology 95:41–84.

Rieger RM, Mainitz M. 1977. Comparative fine structure study of the body wall in Gnathostomulida and their phylogenetic position between Platyhelminthes and Aschelminthes. Z Zool Syst Evolut Forsch 15:9–35.

Rieger RM, Tyler S. 1995. Sister-group relationship of Gnathostomulida and Rotifera–Acanthocephala. Invert Biol 114:186-188.

Ruppert EE. 1991a. Introduction to the aschelminth phyla: a consideration of mesoderm, body cavities and cuticle. In: Harrison FW, Ruppert EE, editors. Microscopic anatomy of invertebrates, vol. 4. Aschelminthes. New York: Wiley-Liss. p 1–17.

Ruppert EE. 1991b. Gastrotricha. In: Harrison FW, Ruppert EE, editors. Microscopic anatomy of invertebrates, vol. 4. Aschelminthes. New York: Wiley-Liss. p 41–109.

Ruppert EE, Barnes RD. 1994. Invertebrate zoology, 6th ed. Philadelphia: Saunders College Publishing. p 1056.

Schmidt-Rhaesa A, Bartolomaeus T, Lemburg C, Ehlers U, Garey JR. 1998. The position of the Arthropoda in the phylogenetic system. J Morphol 238:263–285.

Segers H, Melone G. 1998. A comparative study of trophi morphology in Seisonidea (Rotifera). J Zool Lond 244:201–207.

Semper C. 1872. Zoologische Aphorismen, III. Trochosphaera. Z Wiss Zool 22:310–322.

Sterrer W. 1968. Beiträge zur Kenntniss der Gnathostomulida. I. Anatomie und Morphologie des Genus *Pterognathia* Sterrer. Ark F Zool 22:1–125.

Sterrer W. 1972. Systematics and evolution within the Gnathostomulida. Syst Zool 21:151–173.

Sterrer W. 1998. Gnathostomulida from the (sub)tropical northwestern Atlantic. In: van der Steen LJ, editor. Studies on the natural history of the Caribbean region, vol. 74. Amsterdam: Publications Foundation for Scientific Research in the Caribbean Region: 142. p 1–186.

Sterrer W, Farris RA. 1975. *Problognathia minima* n. g., n. sp., representative of a new family of Gnathostomulida, Problognathiidae n. fam. from Bermuda. Trans Am Microsc Soc 94: 357–367.

Sterrer W, Mainitz M, Rieger RM. 1985. Gnathostomulida: enigmatic as ever. In: Conway Morris S, George JD, Gibson R, Platt HM, editors. The origins and relationships of lower invertebrates. Oxford: Clarendon Press. p 181–199.

Storch V, Welsch U. 1969. Über den Aufbau des Rotatorienintegumentes. Z Zellforsch 95:405–414.

Storch V, Higgins RP, Morse MP. 1989. Internal anatomy of *Meiopriapulus fijiensis* (Priapulida). Trans Am Microsc Soc 108: 245–261.

Swedmark B. 1964. The interstitial fauna of marine sand. Biol Rev 39:1–42.

Sørensen MV. 2000. An SEM study of the jaws of *Haplognathia rosea* and *Rastrognathia macrostoma* (Gnathostomulida), with a preliminary comparison with the rotiferan trophi. Acta Zool (Stockholm) 81:9–16.

Sørensen MV, Kristensen RM. 2000. Marine Rotifera from Ikka Fjord, SW Greenland, with the description of a new species from the rare mineral ikaite. Meddr Grønland (Bioscience) 51: 1–46.

Sørensen MV, Funch P, Hansen AJ, Willerslev E, Olesen J. 2000. On the phylogeny of Metazoa in light of Cycliophora and Micrognathozoa. Zool Anz (in press).

Valentine JW. 1997. Cleavage patterns and the topology of the metazoan tree of life. Proc Natl Acad Sci 94:8001–8007.

Villora-Moreno S. 1996. Ecology and distribution of the Diurodrilidae (Polychaeta), with redescription of *Diurodrilus benazzi*. Cah Biol Mar 37:99–108.

Wallace RL, Ricci C, Melone G. 1996. A cladistic analysis of pseudocoelomate (aschelminth) morphology. Invert Biol 115: 104–112.

Welch DBM. 2000. Evidence from a protein-coding gene that acanthocephalans are rotifers. Invert Biol 119:17–26.

Westheide W. 1982. *Ikosipodus carolensis* gen. et sp. n. an interstitial neotenic polychaete from North Carolina, U.S.A., and its phylogenetic relations within Dorvilleidae. Zool Scr 11:117–126.

Westheide W. 1984. The concept of reproduction in polychaetes with small body size: adaptation in interstitial species. In: Fischer A, Pfannenstiel H-D, editors. Polychaete Reproduction. Fortschr Zool 29:265–287.

Westheide W. 1987. Progenesis as a principle of meiofauna evolution. J Nat Hist 21:843–854.

Westheide W. 1990. Polychaetes: interstitial families. In: Kermack DM, Barnes RSK, editors. Synopsis of the British Fauna (new series). Oestgeest: Universal Book Services/Dr. W. Bachhuys. p 152.

Westheide W, Riser NW. 1983. Morphology and phylogenetic relationships of the neotenic interstitial polychaete *Apodotrocha progenerans* n. gen., n. spec. (Annelida). Zoomorphology 103:67–87.

Westheide W, von Nordheim H. 1985. Interstitial Dorvilleidae (Annelida, Polychaeta) from Europe, Australia and New Zealand. Zool Scr 14:183–199.

Winnepenninckx B, Backeljau T, Mackey LY, Brooks JM, De Wachter R, Kumar S, Garey JR. 1995. 18S rRNA data indicate that Aschelminthes are polyphyletic in origin and consist of at least three distinct clades. Mol Biol Evol 12:1132–1137.

Winnepenninckx B, Backeljau T, Kristensen RM. 1998. Relations of the new phylum Cycliophora. Nature 393:636–638.

Wray GA, Levinton JS, Shapiro LH. 1996. Molecular evidence for deep Precambrian divergences among metazoan phyla. Science 274:568–573.

Zrzavý J, Stanislav S, Kepka P, Bezdék A. 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. Cladistics 14:249–285.