

## **Loricifera, a new phylum with Aschelminthes characters from the meiobenthos<sup>1</sup>**

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### **Introduction**

The meiobenthos from subtidal coarse sand or shelly gravel is a highly diverse assemblage of microscopic metazoans whose existence today largely reflects the pioneering research by ADOLF REMANE in the 1920's. Recently, RIEGER (1980) described a new group of interstitial worms, the Lobatocerebridae, with superficial similarities to the phylum Turbellaria, from this kind of sediment. The fine structure (RIEGER 1981) of the Lobatocerebridae suggests, however, that this aberrant worm is a member of the phylum Annelida, having some affinities to the class Oligochaeta.

During the last seven years, I have studied several interstitial taxa from shelly gravel, especially archiannelids and tardigrades (KRISTENSEN and RENAUD-MORNANT, in press; KRISTENSEN and HIGGINS, in press) from the Arctic to the tropics. In several of these samples I have found an undescribed animal with characters indicating affinities with, but not belonging to, any of the described phyla of the polyphyletic group referred to as the Aschelminthes (GROBBEN 1908) or Pseudocoelomata (HYMAN 1951).

The most recently discovered phylum of animals, the Gnathostomulida, was described first as an order of the Platyhelminthes by Ax (1956), although it was known since the 1920's by REMANE and his colleagues. Fourteen years ago this taxon was elevated to phylum level (RIEDL 1969). The description of the gnathostomulids by Ax (1956, 1960) was significant in that it revealed a completely new type of organization.

The purpose of this paper is to describe a new phylum of metazoans found in the interstitial spaces of shelly gravel. This description is based on observations of both living and preserved material using light microscopy (LM) and on preserved material especially prepared for scanning electron microscopy (SEM). Future publications will deal with fine structure described from transmission electron microscopic (TEM) observations.

Previously unpublished information about three other phyla Nematomorpha, Kinorhyncha and Priapulida are used in the phylogenetic discussion in this publication. Recently, two authors have independently come to the same conclusions, that the Nematomorpha, Kinorhyncha and Priapulida are closely related. MORRIS (1977) has based his conclusions on fossil priapulid worms, especially from Burgess Shale fauna, and MALAKHOV (1980), basing his conclusions on embryology and larval morphology, has concluded that the Priapulida, Kinorhyncha and Nematomorpha be united into the phylum Cephalorhyncha.

<sup>1</sup> This work is posthumously dedicated to the memory of my teacher, Dr. phil. S. L. TUXEN, whose sudden death took place the 15th of June 1983, while this publication was in press.

When VAN DER LAND (1968) described the aberrant priapulid, *Tubiluchus*, he concluded correctly that this group has more characters in common with priapulids than with the sister group, the kinorhynchs, but he also concluded that the priapulids and the kinorhynchs belong to the same phylum, the Rhynchohelminthes Lang (1953). The discovery of two more aberrant genera of priapulids, *Maccabeus* (POR 1973) and *Meiopriapulidus* (MORSE 1981), has not provided any more significant information about a close relationship between priapulids and kinorhynchs. The new phylum described in this paper was predicted by HIGGINS (1961) in his unpublished doctoral thesis. This new phylum possesses a combination of characters found in several other related phyla, especially the Rotatoria, Kinorhyncha, Priapulida and Nematomorpha; it also has a new kind of buccal apparatus superficially similar to that of the phylum Tardigrada but which, in combination with other characters, establishes its uniqueness and justifies the establishment of a new phylum, the Loricifera.

### Historical background for the discovery

Inspired by the standing discussion of the phylogenetic position of the phylum Gnathostomulida (RIEDL 1969; STERRER 1972) I started an intensive exploration (KRISTENSEN and NÖRREVANG 1977, 1978) of the Danish gnathostomulids near Helsingør, Denmark between October 1975 and March 1976. In a coarse sandy bottom (10–12 m depth) rich in organic matter, we found an interstitial community rich in species and high in diversity. Most of the animals were undescribed. Two specimens, in particular, (x- and y-animals) were so aberrant that I sectioned them both for TEM. The y-animal, a small specimen (700 µm), was an undescribed representative of R. RIEGER's Lobatocerebriidae. The x-animal was a very small (80 µm) rotifera-like animal. I observed the only live specimen, using a phase contrast microscope, before I decided to fix it for TEM. When I made ultrathin sections of the epon-embedded animal, most of the tissue fell off the block and, unfortunately, was lost.

In 1976–79, while scientific leader of the Danish Arctic Station, W. Greenland, I continued my investigations of Arctic meiobenthos from a shell-gravel habitat at 100–110 m depth just outside the harbour at Godhavn. In 1978, again I found these new animals and noted that all were loricate larvae with no evidence of a reproductive system.

Influenced by VAN DER LAND's and POR's findings of the priapulids *Tubiluchus corallicola* Van der Land, 1968 and *Maccabeus tentaculatus* Por, 1973, I looked for a mature worm-like animal in the sediment for more than three years without results. In 1980, I received a sample of coarse coralline sand from the Chesterfield Reefs, Coral Sea. Together with a small undescribed interstitial enteropneust, I found a larger larval stage (150 µm) of the new animal. To my surprise, I realized that it could not be a priapulid larva, as I suspected earlier, because it had two very large caudal appendages and a special locomotory system on the anteroventral part of the lorica. Unfortunately, there were no mature animals in the sample. In April 1982, while working at the Marine Biological Station in Roscoff, France, I obtained a huge sample (more than 100 kg) of nearly clean shelly gravel from a depth of 25–30 m, taken with a Sanders dredge for the purpose of looking for marine tardigrades. "Unfortunately", it was my last day of work and time would not permit my using the standard  $MgCl_2$ -extraction method; instead, I osmotically shocked the entire sample with fresh water and fixed the decanted detritus and meiobenthos in buffered formalin. I was delighted to find that this material contained more than 20 species of tardigrades (KRISTENSEN and RENAUD-MORNANT, in press) instead of the 3 species I had been able to extract using the  $MgCl_2$ -technique. Most important, however, I found a complete series of life history stages of the new animals.

In June–July, 1982, while teaching a course in Arctic Biology in W. Greenland, my students and I repeated this same procedure on a shelly gravel sample with equally rewarding

results: mature specimens of the new animal, a different species than found in Roscoff, were found. The new animals were not the worm-like priapulids I had searched for previously without success, but a small, loricate metazoon which appeared to be some kind of ectoparasite. Perhaps I failed to see this stage previously because the animals were attached to a host animal and released only because of the fresh water shock treatment; the larval stages, on the other hand, were probably free-living.

The description of the larva, postlarva and the adult in this publication will be based on my material from Roscoff, France, the Azores Islands (one specimen from the Barlett Expedition) and material collected from a site about 6 miles east of Fort Pierce, Florida by me and my colleague, R. P. HIGGINS. Although the sorting of this latter material is not complete at this time, preliminary examination suggests that the animals from the three localities are conspecific. The animals from the Arctic and Denmark represent two other undescribed species, but the Danish specimens have been lost and cannot be described. The larva and the exuvium from the Coral Sea, perhaps, represent another genus. Additional material from both arctic and tropical localities will be sought before further descriptions are prepared. Another specimen belonging to this phylum has been known to R. P. HIGGINS since 1974 from a sample of sandy sediment taken off the coast of North Carolina (COULL et al. 1977). This specimen, so far undescribed because it also was suspected to be a juvenile priapulid, will be described in a later publication.

### Materials and methods

The description of the new phylum is based on material collected as follows: 1. from the upper surface layer of shelly gravel known as "Sable Dentalium", collected by a Sanders dredge, near Roscoff, France ( $48^{\circ}43'N - 03^{\circ}54'W$ ), at 25–30 m depth; 2. from shelly gravel, collected by a rock dredge with canvas bag, near Azores Islands ( $37^{\circ}13'N - 28^{\circ}44'W$ ) at 480 m depth (Barlett Expedition); and 3. from a mixed sediment of quartz sand and shelly gravel (Amphioxus sand) with a Higgins anchor dredge at 15 m depth, 6 miles east of Fort Pierce, Florida, U.S.A. ( $27^{\circ}29.5'N - 80^{\circ}10.7'W$ ).

The first and third samples were immersed in freshwater for a few seconds in order to shock the meiofauna osmotically thereby causing their release from the substrate and to allow subsequent extraction by decantation of the detritus and meiofauna through a 62  $\mu$ m mesh net. The second sample, consisting of dry shelly gravel, was immersed in fresh water overnight. Most of the dry animals regained their original shape; one larva was found. The specimens were fixed in 4 % formalin buffered with Borax. Photomicrographs were made of the specimens mounted in 4 % formalin and using a Zeiss interference contrast microscope. Formalin fixed material was stained by osmium tetroxide vapor before it was used to prepare specimens for SEM examination. The animals were cleaned ultrasonically before being transferred through an ethynol dehydration series culminating in absolute ethynol. Specimens were dried in a critical point depression apparatus using carbon dioxide. They were mounted on aluminium SEM stubs and coated with gold-platinum. SEM examinations were made with a Cambridge Stereoscan 250 Mk2.

The type-material of the specimens from Roscoff was first mounted in formalin which was slowly replaced by ethynol-glycerin. After the ethynol had vaporized, the glycerin wholemount preparation was sealed with Murrayite. The holotype (female), allotype (male), paratype with extruded buccal canal and two larvae were illustrated with the aid of a camera lucida. Some observations seen only by SEM were added to the illustrations.

The specimens from Roscoff and Azores Islands will be deposited in the Zoological (ZM) Museum of Copenhagen, Denmark and the Museum National d'Histoire Naturelle (MNHN) Paris, France. The specimens from Fort Pierce, Florida will be deposited in the National Museum of Natural History, Smithsonian Institution (USNM) Washington, D.C. U.S.A.

## Results

### Diagnosis

#### LORICIFERA phylum novum

Etymology: from the Latin *loricus* (corset) plus *fero* (carry, bear).

Diagnosis of adult: Small, marine bilaterally symmetrical aschelminth-like animals (Fig. 6) with spiny head ("introvert") and thorax ("neck") retractible in lorica ("abdomen"); with 8 – (9)<sup>1</sup> oral stylets surrounded by a membrane (Fig. 11); stylets only free at tip. Cuticularized buccal canal long, flexible, capable of telescopic extrusion (Fig. 3B). Sexual dimorphism apparent in first row of scalids (*clavoscalids*); no differences apparent in next six rows of scalids, the spinoscalids (Fig. 9). Last two rows of appendages on introvert tooth-like. Thorax with tricoscalids with basal plates. Lorica consisting of 6 plates (Fig. 10) with anteriorly oriented spine-like edges (Fig. 8). No tubuli or adhesive tubes present. Several glands open directly on surface of lorica (Fig. 7). Flosculae present posteriorly on lorica. Saccate gonads and gut open terminally.

Diagnosis of larva: Very small rotifera-like animals (Fig. 14) with spiny head (introvert) and pharyngeal tube (mouth cone) without armature; 5 – (6)<sup>2</sup> segmented thorax, first two ventral plates enlarged to form closing apparatus for retractible head. Thorax not retractible into lorica. Lorica longitudinally folded. Locomotory organ (Fig. 17), consisting of (2)<sup>2</sup>–3 spines, located ventrally on the anterior part of the lorica. Two appendages (Fig. 15), or toes, each with a series of leaf-like structures (mucros) located caudally. Anus situated slightly dorsal. Flosculae (Fig. 18) on dorsal surface of three of five additional posterior loric plates. Sensory setae (Figs. 18, 19) present on edges of caudal plates. Development by a series of larval stages, with moults. Metamorphosis via a postlarval stage, an intermediate form between free-living larva and ectoparasitic or sedentary adult.

#### Nanaloricida order novum

Diagnosis: same as the phylum.

#### Nanaloricidae family novum

Diagnosis: same as the phylum.

Type genus: *Nanaloricus* genus novum

Etymology: from the Latin *nano* (dwarf) plus *lorica* (corset); masculine gender.

Diagnosis: same as the phylum.

Type species: *Nanaloricus mysticus* species novum.

Etymology: from the Latin adjective *mysticus* (secret), masculine gender.

Diagnosis of adult: Mature female 230–235  $\mu\text{m}$  long; mature male 227–235  $\mu\text{m}$  long. Female with two segmented clavoscalids, each with many small tubercles on the first segment, tip of second segment spine-like. Male with two ventral clavoscalids similar to those of female and three pairs of dorsal clavoscalids divided into primary, secondary and tertiary branches. Tertiary branches of mid-dorsal pair enlarged and club-shaped.

<sup>2</sup> Characters in parenthesis refer to taxa known to the author but which are not being included formally in this publication because there has not been sufficient material for adequate description.

Diagnosis of postlarva: 195  $\mu\text{m}$  long with eight clavoscalids of female type. Six plates in lorica as in adult. Each plate with 2–4 longitudinal ridges as in larvae.

Diagnosis of Higgins-larva: First instar 120–135  $\mu\text{m}$  long. With many globular coelomocytes containing yolk material. Second instar (158–185  $\mu\text{m}$ ) with four clusters of mesodermal cells caudally. Introvert with eight clavoscalids, at least in large larvae; five rows of spinoscalids and two rows of tooth-like scalids dorsally; single middorsal clavoid sense organ; hooked-shaped ventral spinoscalids (Fig. 16) of nematomorph larva-type and two to four leaf-like sense organs located midventrally.

## Morphology

### Description of holotype (female)

Measured from the tip of the oral stylets to the anus, the holotype is 235  $\mu\text{m}$  long. The maximum width (of lorica) is 90  $\mu\text{m}$ . The animal has the mouth cone, introvert, and thorax totally extended from the lorica (Figs. 1, 3 A).

**External morphology:** The mouth cone is surrounded by eight oral stylets, each with a separate rostral opening where the stylet can be extruded when the large stylet muscles contract. The stylets are rigid. The basal part of each stylet is furcate. Each stylet is 60  $\mu\text{m}$  long. The terminal mouth opening is separated from the stylet opening and can be telescopically retracted inside the animal. The mouth cone is slightly separated from the introvert by a naked, unarmed area. The introvert has nine rows of appendages. The first row consists of six clavoscalids which are oriented slightly forward. The two ventral clavoscalids present in immature female, male and postlarva are lacking in the holotype (and other mature females). The next eight rows of scalids project posteriorly when

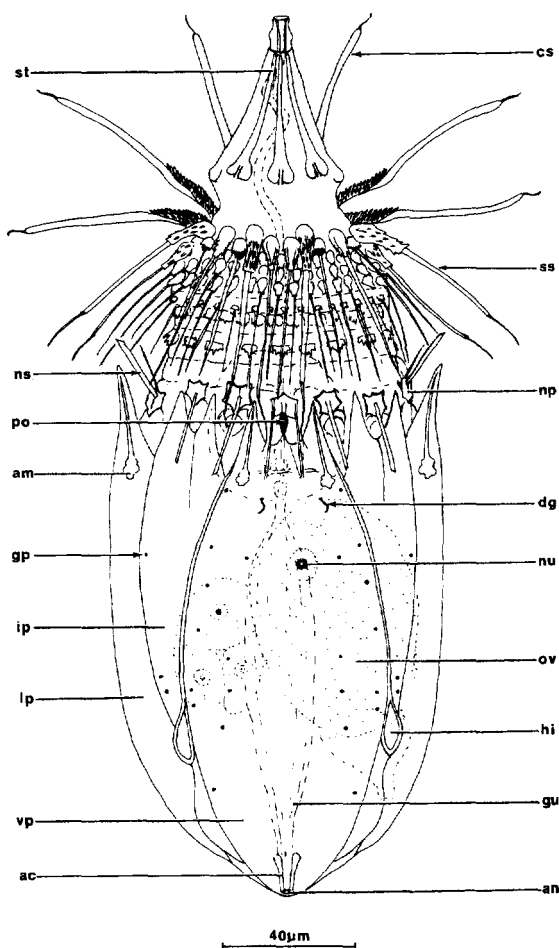


Fig. 1. *Nanaloricus mysticus* sp. nov. Holotypic female, ventral view. ac = anal cone; am = armature in the spine of lorica; an = anus; cs = clavoscalid; dg = duct of epidermal gland; gp = glandular pore opening; gu = gut; hi = lorical hinge; ip = intermediate plate of lorica; lp = lateral plate of lorica; np = neck plate; ns = neck scalid; nu = nucleus of ovum; ov = ovum; po = neck pore; ss = spinoscalid; st = oral stylet; vp = ventral plate of lorica

the head is extended, as in kinorhynchs. The scalids lack the strongly circular symmetry of priapulids. None of the rows (circlets) has the 25 elements as CALLOWAY (1975) has found in all priapulids. The fine structure of the spinoscalids is much more like that of kinorhynchs. Spinoscalids of the first five rows have a bulbous base, those of the last row have a toothed base. Spinoscalids can be segmented as in first two rows, or unsegmented as in the next four rows. The highest number of scalids is found in the third through fifth rows where they number about 34–35; there are about 30 scalids in the sixth row. The last two rows of appendages lack the spine and only consist of the dentate structure as found in priapulids. When the mouth cone and the introvert are withdrawn into the lorica, the scalids fold together as the ribs of an umbrella closing the wrong way, as described for the kinorhynch (BOYKIN 1965), but the mouth cone and the oral stylets merely retract.

The thorax (or neck) is separated externally from the introvert only by a slight constriction. The thorax consists of two segments, the first is unarmed, the next has two rows of plates with an appendage of the trichoscalid type found in kinorhynchs. Each of two lateral plates also has a very thin accessory spine. A very large pore or invagination is located on the midventral plate. The thorax is the transition zone between the introvert and the very strongly cuticularized abdomen which is covered by the lorica. No segments can be seen in this part of the animal. As already noted, the lorica consists of six longitudinal plates with several small pores communicating with epidermal glands in a distinctive pattern. Scanning electron microscopy reveals that the lorica is not smooth as indicated by LM, but has the same characteristic sculpture as found in the larva, but much less distinct. Muscle attachment areas are seen especially on the dorsal plates and, more caudally, on the lateral plates. A hinge or joint is present on both sides of the large ventral plate. The only sense organs seen on the lorica are a single floscula on the dorsal plate and four others on each lateral plate. The anterior edges of the lorica have a distinctive series of 15 hollow spines. When the introvert is retracted inside the lorica, the lorica is very similar to that of the rotifer *Notholca*.

**Internal morphology:** The buccal canal consists of a large, thin, cuticularized tube, curving several times within the mouth cone. In the introvert, the buccal tube is supported ventrally by two accessory stylets which may have openings into the buccal canal. The accessory stylets have a trifurcate basal area which is attached to the large pharynx bulb. In the area of the accessory stylets, there is a ventral, very thin cuticular plate and two lateral ligaments. The lateral ligament may be muscle, but the cross striations seen in other muscles of the introvert are not apparent. The buccal tube enters the pharynx bulb mesially and a large pharynx apophysis is present. The pharynx bulb has five placoids located at the points of the radial muscle attachment. A thin cuticular lining can be seen between the placoids. The pharynx bulb can be retracted inside the lorica or can slide forward so as to lie totally inside the introvert.

After the pharynx bulb, the digestive system continues with a short oesophagus and a straight midgut. No ciliated structures can be seen in the midgut, which appears to have a thin cuticular lining. The midgut of the holotypic female is empty and has eight or nine longitudinal folds. Paratypes have the midgut filled up by a white amorphous material and lack the longitudinal folds. The gut ends in a short rectum and a strongly cuticular anal cone. A pair of large salivary glands, opening into the buccal canal dorsally, are the only glands associated with the digestive system. The reproductive system consists of paired, saccate ovaria, but only one egg is fully developed. The right ovarium of the holotype contains the huge egg seen best from the ventral view (Fig. 1) and the left ovarium has five small oocytes. The presence of a small seminal receptacle in the area of the hinge of the lorica is suspected.

The nervous system consists of a very large dorsal brain which nearly fills half of the introvert. Each circle of scalids is innervated separately by the brain. The clavoscalids, especially, have a strong innervation which connects with eight circumoral ganglia nearly fused with the brain. A large ventral ganglion in the thorax innervates the tricoscalids.

Several ganglia may be present in the abdomen but the thickness of the lorica makes it impossible to see these clearly; only a large ventral, caudal ganglion is obvious. The caudal ganglion innervates the dorsal floscular system, the only sensory system seen on the lorica. Each floscula consists of five micropapillae (pentamere) or microvilli.

The muscular system is very complex and only TEM investigations will provide an adequate description. The very large retractor muscles of the introvert (Fig. 3 A) show that *Nanaloricus* has cross-striated muscles as in the kinorhynchs. A very large ligament or diaphragm can be seen between the thorax and the abdomen as in the larva of *Paragordius* (MONTGOMERY 1904).

### Description of allotype (male)

Measured from the tip of the oral stylets to the anus, the allotype is 230  $\mu\text{m}$  long; its maximum width is 70  $\mu\text{m}$  measured on the lorica before it was distorted by the cover glass in order to make it easier to observe the internal structure. The camera lucida drawing (Fig. 2) is based on a slightly compressed animal (Figs. 2, 4).

**External morphology:** The only external sexual dimorphic character noted was the difference in the clavoscalids as already mentioned in the diagnosis. The 20 clavoscalids of the allotype and five paratypes all have the same characteristic pattern. Although there are 20 clavoscalids, they are derived from the eight primary clavoscalids found in the larva. These eight primary elements persist in the postlarvae and were seen also in two immature females (Figs. 6–11). The middorsal, dorsolateral, and ventrolateral pairs of clavoscalids are divided into three branches called the primary, secondary and the tertiary clavoscalids. Each of the ventral pair of clavoscalids has only one branch and are of the same type found in the female. The tertiary branches

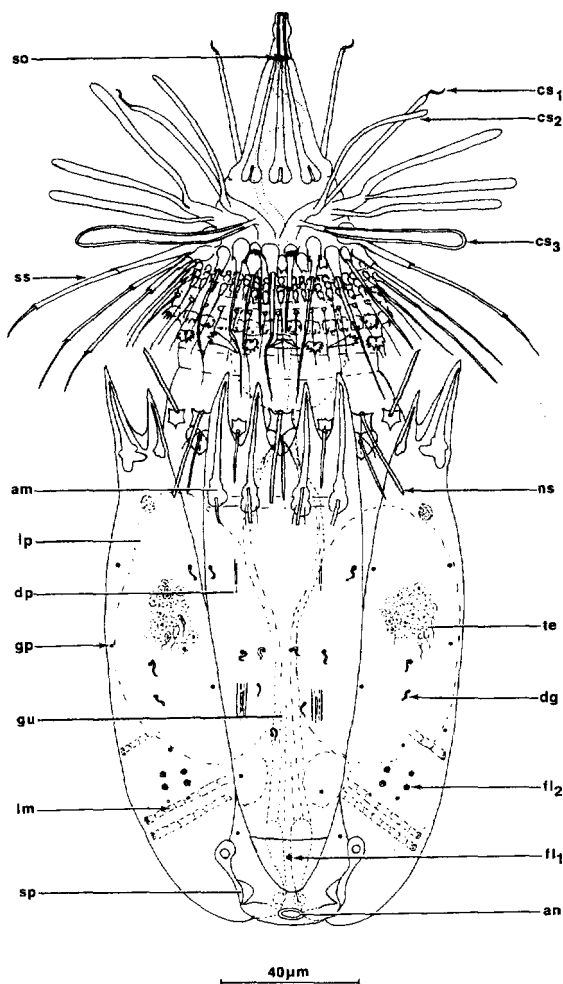


Fig. 2. *Nanaloricus mysticus*, nov. sp. Allotypic male, dorsal view. am = armature in the spine of lorica; an = anus; cs<sub>1</sub> = primary scaldid; cs<sub>2</sub> = secondary scaldid; cs<sub>3</sub> = tertiary scaldid; dg = duct of epidermal gland; dp = dorsal plate of lorica; fl<sub>1</sub> = dorsal floscula; fl<sub>2</sub> = lateral floscula; gp = glandular pore opening; gu = gut; lm = lorical muscle; lp = lateral plate of lorica; ns = neck scaldid; so = stylet opening; sp = penile spine?; ss = spinoscalid; te = testis

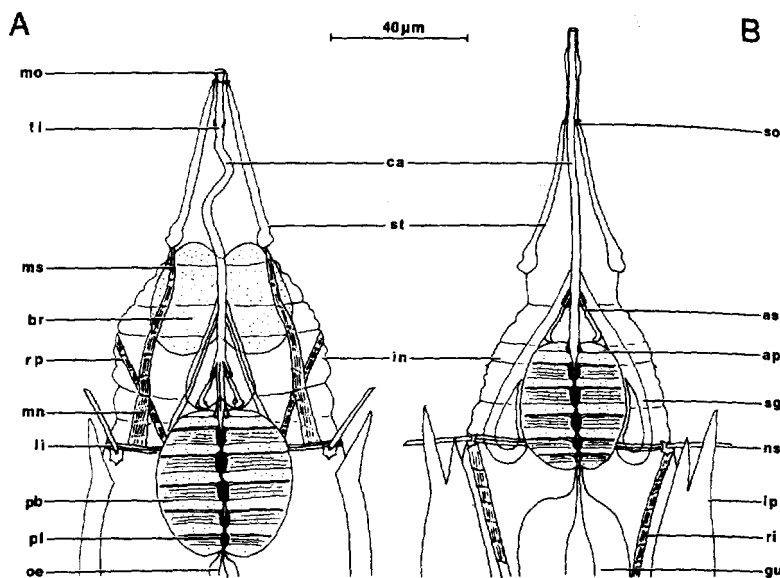
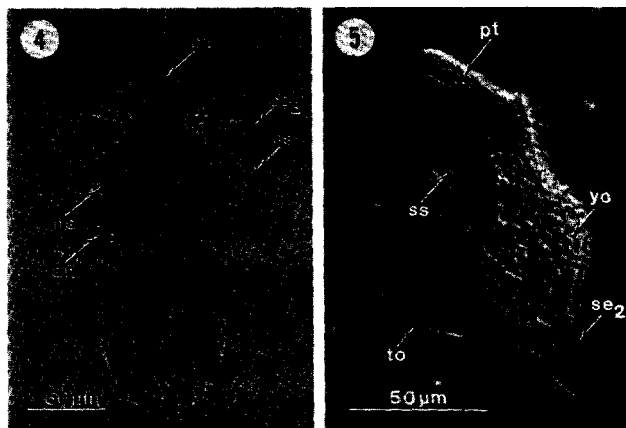


Fig. 3. *Nanaloricus mysticus*, nov. sp. A: Anterior end of holotypic female, ventral view. B: Anterior end of paratype male with extruded buccal canal, dorsal view. Note the scalids are not drawn. ap = pharyngeal apophysis; as = accessory stylet; br = brain; ca = buccal canal; gu = gut; in = introvert; li = ligament; lp = lateral plate of lorica; mn = neck muscle; mo = mouth opening; ms = stylet muscle; ns = neck scalid; oe = oesophagus; pb = pharyngeal bulb; pl = placoid; ri = introvert retractor muscle; rp = pharyngeal bulb retractor muscle; sg = salivary gland; so = stylet opening; st = stylet; tl = telescopic point of buccal canal



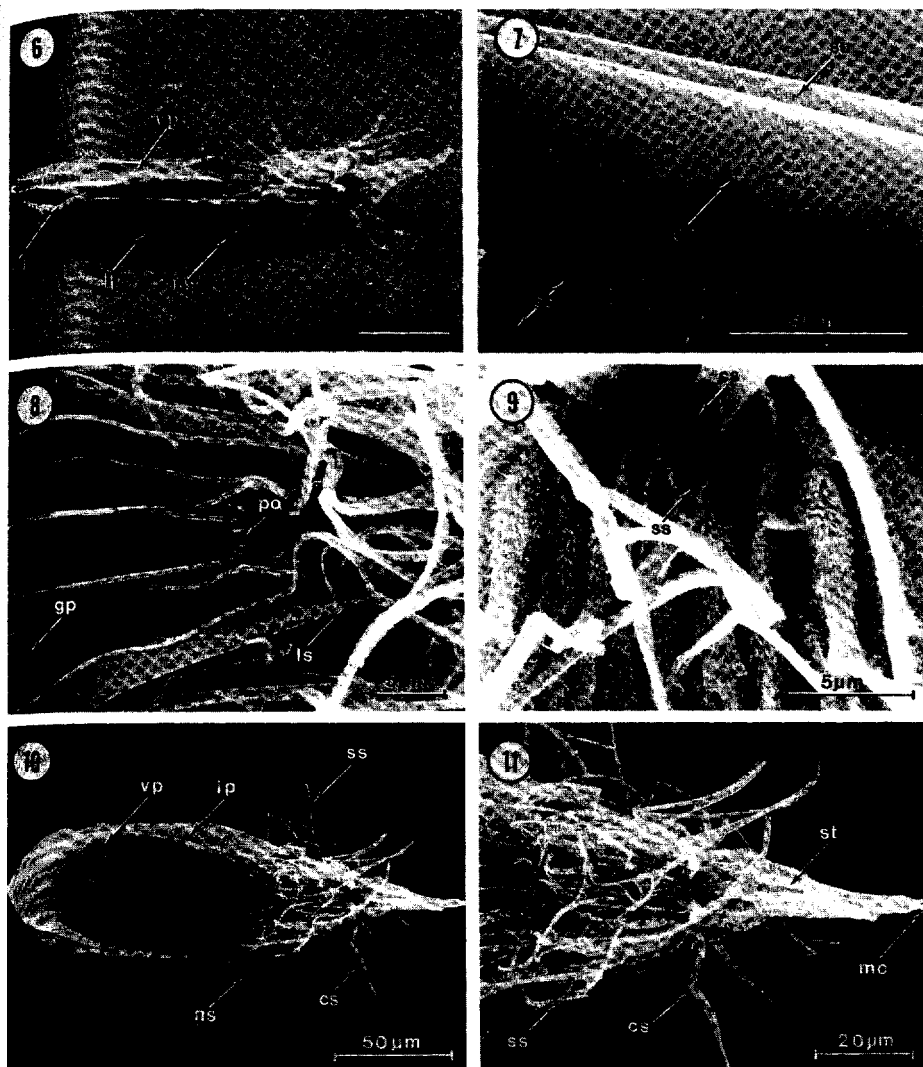
Figs. 4–5. *Nanaloricus mysticus*, nov. sp. Light microscopic observations. Fig. 4. Paratype male, ventral view. am = armature in the spine of lorica; ca = buccal canal; cs<sub>3</sub> = tertiary clavoscalid; ns = neck scalid; ss = spinoscalid. – Fig. 5. Higgins-larva, dorsal view. (An interference phase contrast micrograph). pt = mouth cone; se<sub>2</sub> = second sensory seta; ss = spinoscalid; to = toes; yo = yolk material

of the middorsal clavoscalids are enlarged and club-shaped. The pair of ganglia which innervate these scalids are enlarged also, and send a large sensory dendrite out to the base of the tertiary clavoscalids. Inside the club-shaped part of the scalids, there is a modified ciliary structure. The clavoscalids of the male have a sensory function, presumed to be olfactory. The branches of the dorsolateral and ventrolateral clavoscalids are all more or less club-shaped, too.

**Internal morphology:** The reproductive system consists of two large dorsal

testes which occupy about half of the coelom in the abdomen. They contain small round headed sperm, only slightly modified from the primitive type of sperm found in animals with





Figs. 6–11. *Nanaloricus mysticus*, sp. nov. SEM-investigations of paratype young female. Fig. 6. Lateral view. – Fig. 7. Loric sculpture. – Fig. 8. Anterior end of ventral plate. – Fig. 9. Different types of scalids. – Fig. 10. Ventral view. – Fig. 11. Introvert and mouth cone. cs = clavoscalid; gp = glandular pore opening; hi = lorical hinge; ip = intermediate plate of loric; lp = lateral plate of loric; ls = lateral spine of ventral plate; mo = mouth opening; ns = neck scalid; po = neck pore; sc = lorica sculpture; ss = spinoscalid; st = oral stylet; su = suture between ventral and intermediate plate; vp = ventral plate of loric

external fertilization (FRANZEN 1956). A pair of cuticular structures are found on the edges of the lateral plates of the loric. These structures could be a pair of penile spines, but the females have a similar but less pronounced structure in the same area; a TEM investigation is necessary to resolve their function. However, *Nanaloricus* appears to have internal fertilization.

For a full description of the external and internal morphology of the male, see the holotypic female.

## Description of Higgins-larva

External morphology (with comments on the function): The few observations of living specimens show that the mouth cone can be inverted into the introvert which, in turn, can be inverted into the thorax when the animal is disturbed or at rest. Two ventral plates function as a closing apparatus. There is no evidence that the thorax is retracted into the abdominal region within the lorica. Active larvae extend and withdraw the introvert, exclusive of the mouth cone, in the same way as in the Kinorhyncha (REMANE 1928); the

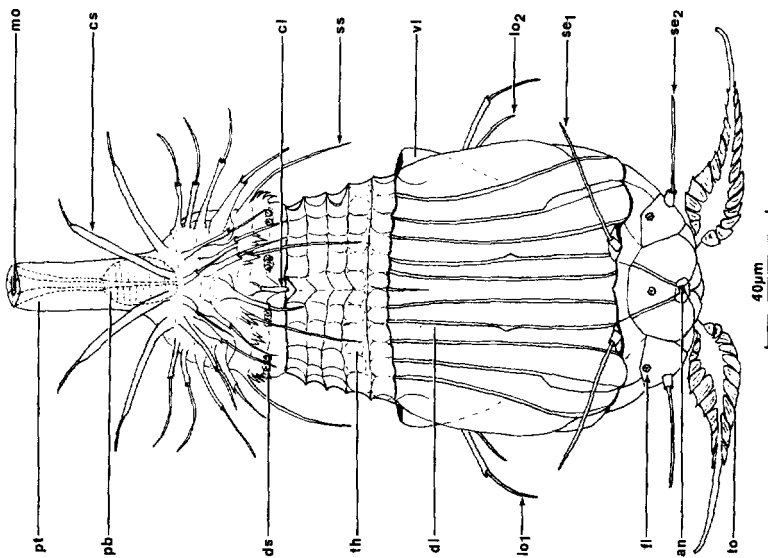


Fig. 13. *Nandaloricus mysticus*, nov. sp. Dorsal view of Higgins-larva (last instar). an = anus; cl = club-shaped sense organ; cs = clavoscalid; dl = dorsal loric; ds = double scalid; fl = floscula; lo<sub>1</sub>-lo<sub>2</sub> = first to third locomotory spines; mo = mouth opening; pb = pharyngeal bulb; pt = mouth cone; se<sub>1</sub>-se<sub>2</sub> = first and second sensory seta; ss = spinoscalid; th = thorax; to = toe; vl = ventral loric

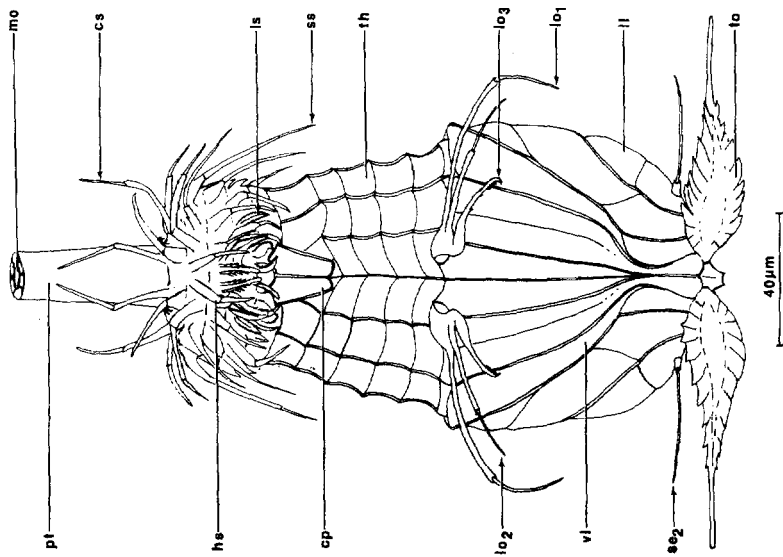
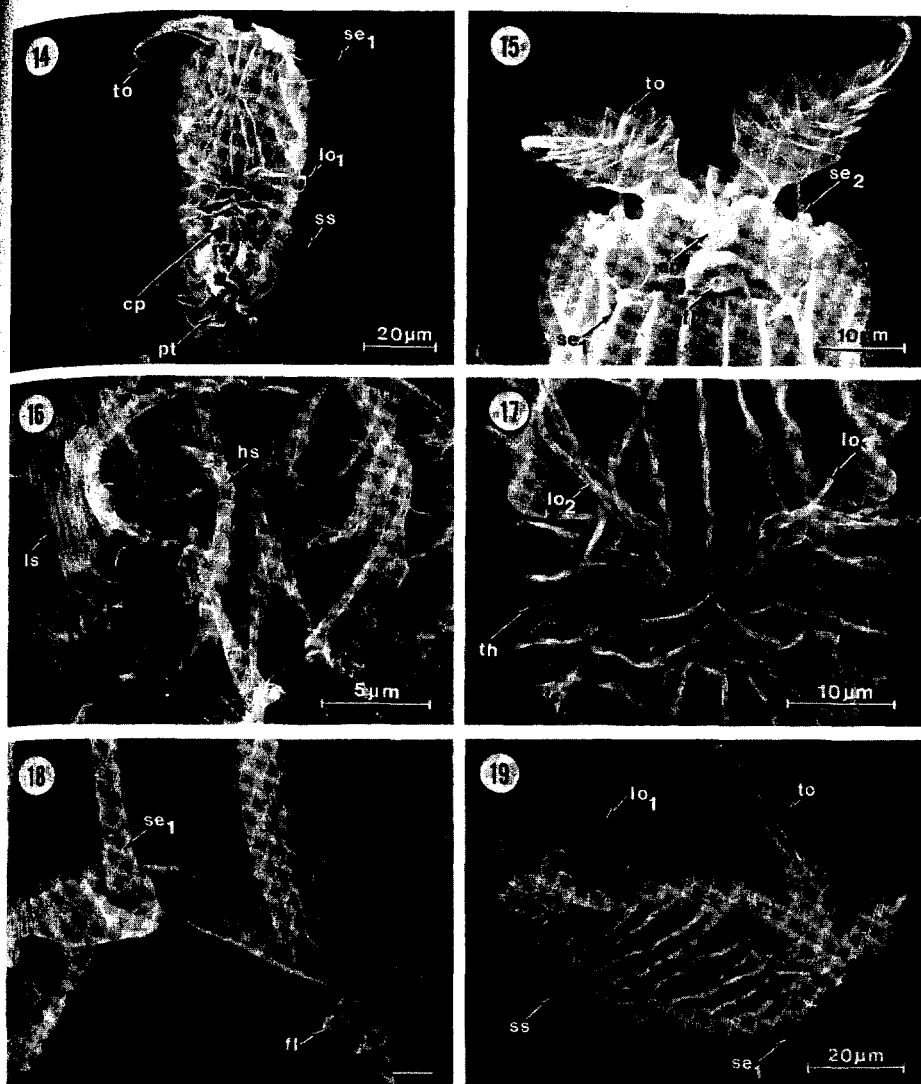


Fig. 12. *Nandaloricus mysticus*, nov. sp. Ventral view of Higgins-larva. cp = closing apparatus; cs = clavoscalid; hs = hook-shaped scalid; ll = lateral loric; lo<sub>1</sub>-lo<sub>3</sub> = first to third locomotory spines; ls = leaf-shaped sensory scalid; mo = mouth opening; pt = mouth cone; se<sub>1</sub> = second sensory seta; ss = spinoscalid; to = toe; th = thorax or neck; vl = ventral loric



Figs. 14–19. *Nanaloricus mysticus*, nov. sp. SEM-investigations of Higgins-larva. Fig. 14. Ventral view. – Fig. 15. Dorsal view of caudal end. – Fig. 16. Ventral scalids. – Fig. 17. Ventral view of thorax (neck), anterior part of lorica and locomotory spines. – Fig. 18. Lateral floscula and first sensory seta. – Fig. 19. Lateral view. Note the animal is mounted on the head. an = anus; cp = closing apparatus; hs = hook-shaped scalid; fl = floscula; lo<sub>1</sub>–lo<sub>3</sub> = first to third locomotory spines; ls = leaf-shaped scalid; pt = mouth cone; se<sub>1</sub>–se<sub>2</sub> = first and second sensory setae; ss = spinoscalid; th = thorax or neck; to = toe

mouth cone of the kinorhynch is protruded, not everted. However, the adults of *Nanaloricus* do protrude the mouth cone in a similar manner. The organization of the anterior portion of the larva is much more kinorhynch-like than any other animal, but it differs from that of the adult. The protruded mouth cone in fixed specimens (Figs. 12–24) is the product of the fresh water shock treatment described earlier.

The mouth cone is unarmed and slightly conical. The mouth opening is terminal and very large. The introvert is bilaterally symmetrical and lacks the quincuncial arrangement of the

scalids found characteristically in priapulids (CALLOWAY 1975) and also in kinorhynch (HIGGINS 1983). Seven rows of scalids are present as in kinorhynch, but those on the ventral surface may break the otherwise uniform sequence so a full circlet is not recognizable. At least the last instar of the Higgins-larva (Fig. 13) has eight clavoscalids of the female type. The first instar has a more stylet-like clavoscalid (Fig. 12). Dorsally, the next four rows of scalids are typical spinoscalids, and the two last rows are more dentate. The seventh row consists of four double teeth. Middorsally, between the sixth and seventh row of teeth, a single club-shaped sense organ is present. Four of the six dorsal teeth in the sixth row have a long, thin flexible spine (modified spinoscalid) as well (Fig. 13).

Ventrally, the scalids are more or less hook-shaped. The two to four leaf-like sense organs (Fig. 16) found in this same area have similar counterparts in some Rotifera (REMANE 1928).

The thorax (or neck) consists of five rows of plates. The ventral plates are much larger than the dorsal plates. Totally, there are 30 ventral plates in addition to the two closing plates. Dorsally, there are about 60 plates, but only the two last rows have well defined limits. The system is similar to the bellows of an accordion (Fig. 17). The thorax can be retracted to half its length when the animal is disturbed. Ventrally, between the thorax and the abdomen, there is a locomotory organ consisting of three pairs of spines. Superficially, these look like the so-called lateral antennae of rotifers. The first two are scalid-like and might have a sensory as well as locomotory function. The third pair are claw-like distally and used as grasping organs as the animal moves slowly over the substrate.

The larval lorica is not as strongly cuticularized as it is in the adult. It consists of four large elements, one dorsal, one ventral, and two lateral, all of which have prominent longitudinal folds. On the caudal end, surrounding the anus, there are five additional dorsal plates. The surface of the lorica has a distinct sculpturing, easily seen by LM. With the higher magnification of the SEM, the sculpturing (Fig. 13) appears as a honeycomb structure. This sculpturing is much less pronounced on the adult lorica (Fig. 7).

The larvae observed in this study (57 specimens) all have two pairs of sensory setae or cirri dorsally (Figs. 12–15; 18–19), each with a flexible, jointed basal area or cirrophore. The sensory setae probably are mechanoreceptors. Each of three additional lorical plates has a floscula. The lateral flosculae (Fig. 18) consist of six papillae while the middorsal flosculae have five papillae as found in the adults. Perhaps the most notable structures seen on the larva are the two caudal appendages or toes (Figs. 12–15, 19). Laterocaudally they articulate with the abdomen in a ball and socket joint and, thus, are able to move in nearly all directions. The toes have a series of leaf-like structures (mucros) which change direction in unison. The distal portion of each toe is used when the larva is slowly climbing around, but the entire structure is used to propel the animal in a swimming manner. The swimming has two phases, the first is a kind of paddling, which can increase moderately to propel the animal faster. The second phase involves an extremely rapid movement – so rapid as to prevent detailed observation.

**Internal morphology:** A funnel-shaped buccal canal extends from the terminal mouth opening straight to the pharynx bulb. When the larvae are subjected to the osmotic stress of treatment with fresh water, the pharynx bulb is positioned at the base of the protruded mouth cone. In live animals it always was seen in the thorax or the posterior end of the introvert. The very short oesophagus connects directly with a very thin, delicate midgut. The digestive system terminates with a short rectum and slightly dorsal anus (Figs. 13, 15). The first instar of the larva has a coelom with yolk-containing coelomocytes (Fig. 5). The last instar has fewer coelomocytes, and the caudal part of the coelom is dominated by four very large clusters of mesodermal cells. At the base of the toes, two small glands, presumably of adhesive function, are present. The nervous system consists of a very large brain which nearly fills the entire introvert. A large ventral ganglion innervates the locomotory spines, and a less pronounced ganglion innervates the toes, dorsal setae, and flosculae.

The larvae are found in at least two sizes (120–135  $\mu\text{m}$  and 158–185  $\mu\text{m}$ ). Development is by moulting and several exuvia were found of both larval stages. Inside each was a round yellow amorphous material, similar to that found in the exuvia of kinorhynch. Only two postlarvae were found. With the exception of an indistinctly folded lorica, as in the larva, all other characters appear as they do in the adult.

### Material examined

Type-material: The holotype (Fig. 1) is an adult female with one well-developed egg. It was collected by R. M. KRISTENSEN, 15 Mar 1982 from shelly gravel substrate near Roscoff, France (48°43'N – 03°54'W) at 25–30 m depth. The allotype is an adult male with well-developed testis, other data are the same as for the holotype. Paratypes include 2 females, 5 males, 2 postlarvae, 57 larvae and 5 exuvia, other data are the same as for the holotype. Six adults of undetermined sex were fixed for TEM study.

Additional paratypes: 1 larva from shelly gravel substrate collected during the BARLETT Expedition, 31 Jan 1975, near the Azores Islands (37°13'N – 28°44'W) at 480 m depth; 1 male, 2 larvae and 1 exuvium were collected by R. M. KRISTENSEN and R. P. HIGGINS, 4 May 1983, from a shelly gravel substrate 6 miles offshore Fort Pierce, Florida (27°29.5'N – 80°107'W).

### Ecological remarks

*Nanaloricus mysticus* was collected from the upper surface layer of shelly gravel near Roscoff, France (type-locality) at a depth of 25–30 m; from the same kind of sediments near the Azores Islands at 480 m; and from Fort Pierce, Florida, U.S.A. at 15 m. All three sediment samples consisted of coarse material with very little silt. The meiofauna was extremely rich and the macrofauna was poorly represented.

In the samples from Roscoff and Fort Pierce, tardigrades, for the first time in a meiofaunal investigation, were the dominant group both in numbers of species and specimens; 22 species were found in the Roscoff sample and 25 species in the Fort Pierce sample. Furthermore, the only known semipelagic tardigrade, *Tholoarctus natans* Kristensen and Renaud-Mornant, in press, was present in all three samples. Until now, this species has been found only where the current of the water is strong; the tardigrade probably can be considered as an indicator species for this shelly gravel habitat just as *Amphioxus* has been an indicator for the same kind of sediment but where currents are not strong.

The kinorhynch fauna in the samples is also unique, e. g. most of the species have very long spines. HIGGINS (1967) has noted that the spine length of kinorhynch from sediment increase as the mean grain size increases.

The nematode fauna is represented by aberrant genera with locomotory spines, e. g. *Draconema* sp. and *Desmoscolex* sp. Both nematodes and harpacticoid copepods are relatively less abundant in the coarse sediment than in the finer sediment where these two groups usually dominate.

Studies of the "soft-bodied" meiobenthos from coarse shelly sediments already have shown that this kind of sediment has a very diverse and aberrant interstitial fauna (RIEGER 1980).

### Discussion

The description of the new phylum, Loricifera, is based on one species, *Nanaloricus mysticus*, from the Atlantic, but two other undescribed species of *Nanaloricus*, one from the Arctic (W. Greenland) and another from the temperate region (Denmark), and undescribed

taxa from Chesterfield Reefs, Coral Sea and off the coast of North Carolina, U. S. A., show that the phylum has a world-wide distribution and is not necessarily uncommon.

Why the phylum has not been discovered previously perhaps has two answers. The first is that the animal often may be present in a sample, but not extracted by the normal meiofaunal collection methods. Both larvae and the adults adhere tightly to the substrate; the free-living larva, with its abdominal appendages grasps tenaciously to sediment particles while the adult may be ectoparasitic or commensal on another meiofaunal taxon. They release their hold on the substrate when subjected to the osmotic shock of introducing coarse sediment of a brief, fresh water rinse. The other reason could be that both the larva and the adult superficially look like a marine rotifer and, hence, may be disregarded. The rotifer *Notholca*, especially, could be mistaken for the adult of *Nanaloricus*. The loricated Rotifera are abundant in the same samples in which *Nanaloricus* is found as was the case with that sample taken in Fort Pierce, Florida, U. S. A. (May, 1983). My first thought was that there were several hundred specimens of the new phylum present, but a closer examination, using phase contrast optics, showed that only three specimens of *Nanaloricus* were present, the rest were rotifers. Furthermore, the latter three specimens of Loricifera were noticed primarily because their exposure to fresh water had everted their heads in characteristic manner.

The similarities between loricate rotifers and the Loricifera also will be better understood phylogenetically once ultrastructural studies, especially studies of the cuticle of the Loricifera, are complete. The Rotifera do not have a real cuticle, only an intracellular formation (KOEHLER 1966). The loriceran cuticle probably is homologous to that of the Kinorhyncha and Priapulida. Together with these latter two phyla, the Loricifera develop by a series of moults, which includes shedding a series of exuvia, but moulting is also present in Arthropoda, Tardigrada and Nematoda. The cuticle of the Arthropoda and Nematoda is analogous, but two theories exist about the cuticle of Tardigrada. CROWE et al. (1970) postulated a homology between the cuticle of Tardigrada and Nematoda, while KRISTENSEN (1976) postulated a homology between the cuticle of Tardigrada and Arthropoda. The similarities between the flexible buccal canal with its pair of accessory stylets as found in the adults of Loricifera, and the flexible buccal canal with stylets and stylet supports found in the tardigrade genus *Diphyscon*, are remarkable. Furthermore, the pharynx bulb in both *Diphyscon* and *Nanaloricus* has a cuticular lining consisting of disc-like placoids. Again, TEM investigations of the pharynx bulb of *Nanaloricus* will be necessary before one can conclude whether or not these similarities are homologous or analogous. RUPPERT (1981) recently has shown that homologies exist in the myoepithelial foreguts (pharynx bulb) in the superphylum Aschelminthes. He also has concluded that the pharynx of tardigrades shares some unique similarities with the aschelminth phyla, but because of a lack of sufficient comparative ultrastructural data for the pharynx of tardigrades, he could not conclude whether or not these similarities were homologous or analogous. My own unpublished investigation of the pharynx bulb of the marine tardigrade *Actinarctus* shows that the tardigrades do not have a myoepithelial foregut as found in the aschelminth phyla. Loricifera is clearly related to the aschelminths, and the similarities between the tardigrade *Diphyscon* and *Nanaloricus* are an excellent example of convergence.

MORRIS showed that an evolutionary line exists from an acanthocephalan proboscis to a nematomorph introvert (MORRIS 1977, Fig. 33). He also gives very good evidence for a homology between the "proboscides" (= introverts) of archaepriapulids via modern priapulids to the kinorhynchs. In my opinion, there is no homology between the acanthocephalan proboscis and that of the priapulids; the entire organization of these two groups is different. The new phylum Loricifera suggests that both MORRIS (1977) and MALAKHOV (1980) may be correct concerning homologies between the priapulids, kinorhynchs and Nematomorpha. The Loricifera may be the "missing link" between these three phyla. Interestingly enough, HUUS (1931) has shown that the marine nematomorph larva of *Nectonema munidae* has an elongate, curving canal of the same kind as the buccal

canal of *Nanaloricus*. When MONTGOMERY (1904), MUHLDOERF (1914) and HUUS (1931) failed to see the pharynx bulb in the nematomorph larva, possibly they were accustomed to its absence in other parasites such as the Acanthocephala. The pharynx bulb in the nematomorph larva probably is homologous with the pharynx bulb of *Nanaloricus*. What these three authors called the "gland" is the pharynx bulb and the gland duct is the buccal canal. My hypothesis is supported by the fact that the so-called gland in *Paragordius* (MONTGOMERY 1904, Fig. 30) has a triradiate lumen, possibly lined with cuticle, and it is developed in the same way as the pharynx bulb is in other aschelminths.

Several similarities between the nematomorph larva and the adult of Loricifera could be homologies. For example, they both have a ligament or diaphragm between the thorax (neck) and the abdomen; the stylet type is the same; the spicules of the *Paragordius* larva and the scalids of *Nanaloricus* are similar; all investigated nematomorph larvae also have the large mesenchyme cells found in the late stage of the *Nanaloricus* larva.

The sister group of the Loricifera is probably the Kinorhyncha, with which, in general organization, they share several unique characters such as the type of mouth cone with oral stylets; the first row of blunt scalids or clavoscalids; and the presence of a ventral closing apparatus. However, TEM investigations of both Kinorhyncha and Loricifera are necessary to support this hypothesis.

Superficially, the larva of *Nanaloricus mysticus* looks like that of a priapulid, especially *Tubiluchus* (KIRSTEYER 1976). The genus *Tubiluchus* now consists of two species *T. corallicola* and *T. remanei* (VAN DER LAND 1982). Recently I examined both adults and larvae of both species and compared them with one specimen of the aberrant genus *Maccabeus* Por, 1973 (= *Chaetostephanus* Salvini-Plawen 1974). Furthermore, I have examined the larva, postlarva and the adult of *Priapulius caudatus* from Greenland. I believe, as CALLOWAY (1975) very instructively indicated, that all priapulids have the same basic body-plan. This priapulid body-plan includes 25 longitudinal rows of introvert scalids and pectinate teeth on the anterior pharynx. None of these characters are present in Loricifera. A special sensory organ or floscula was first described from *Tubiluchus* (VAN DER LAND 1968, 1972) and is now known to be present in *Nanaloricus*, but this characteristic structure is also present in kinorhynch, although here it is modified (HIGGINS 1983, Fig. 327, sensory spot), but even more interesting is the presence of this same structure on the caudal end of a loricate rotifer, *Keratella* sp. (KRISTENSEN unpubl.).

It is difficult to make any statement about the phylogenetic position of Loricifera. The presence of both a lorica in a very aberrant, free-living larva and a sedentary adult could indicate that the adult is a neotenic form of a priapulid or kinorhynch-like animal. This hypothesis, however, is contradicted by the fact that Loricifera also shares characters with Nematomorpha, Rotifera and perhaps also Tardigrada. Thus, the new group might be an ancestral form, and characters such as the lorica and toes in the larva are plesiomorphous. Finally, one hypothesis which is sure to be popular with those scientists still subscribing to the Aschelminthes as a taxon is since the Loricifera share characters with nearly all subordinate aschelminth taxa this indicates that the Aschelminthes is a monophyletic taxon which should have the rank of phylum, and what I consider as the aschelminth phyla should be ranked as classes.

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### Summary

1. A new phylum, Loricifera, in the Animal Kingdom is described from the meiobenthos. All stages of the type species *Nanaloricus mysticus* gen. et sp. nov. is found from shelly gravel, Roscoff, France (25–30 m depth) and Fort Pierce, Florida, U. S. A. (15 m depth). The aberrant larva, called a Higgins-larva, is found also at 480 m depth in shelly gravel near the Azores Islands.
2. Two other undescribed species of *Nanaloricus* have been found in "Amphioxus sand" near Helsingør, Denmark (10–12 m) and Godhavn, W. Greenland (110 m depth). A single Higgins-larva and an exuvium perhaps belonging to another genus were found intertidally in coralline gravel from the Chesterfield Reefs, Coral Sea. Still another representative of this phylum has been found off the coast of North Carolina, U. S. A.
3. The larvae, postlarvae and the adults have a spiny introvert and a lorica. At least two larval stages (100–135  $\mu$ m and 158–185  $\mu$ m) are present. Female and male have the same size (227–235  $\mu$ m), but sexual dimorphism is present in the first row of eight scalids, the clavoscalids. Paired ovaria are present in the female and paired testes are present in the male.
4. The Higgins-larva has a simple unarmed mouth cone and an introvert with seven to eight rows of scalids. The first row has eight clavoscalids. The next four to five rows consist dorsally of spinoscalids similar to those found in kinorhynch. The two last rows of dorsal scalids are of the priapulid-type. Ventrally, all scalids are hook-shaped as found in the larva of the Nematomorpha. Two to four sense organs, trichoscalids, are found ventrally. One club-shaped sense organ is present middorsally on the introvert. The neck or thorax consists of five to six rows of plates. Two ventral plates in the first row are large and constitute a closing apparatus, when the introvert is retracted. The lorica of the larva is strongly folded longitudinally. Dorso-caudally, five small plates are present. Three of the plates each have single floscula. Caudally two pairs of long sensory hairs are present. A midventral locomotory organ, consisting of three pairs of spines, used as legs, is present on the anterior edge of the lorica. One pair of toes with appendages (mucros), used primarily in swimming, are located at the caudal end.
5. The adult has eight to nine oral stylets surrounding a mouth cone, a flexible thin cuticular buccal canal, and an ovoid pharynx bulb with five rows of placoids. The buccal canal has two internal buccal supports or accessory stylets. The gut is straight and simple. The female has six to eight clavoscalids. The male has 20 clavoscalids. The next six rows are spinoscalids of the kinorhynch type, the last two rows are toothed scalids found in priapulids. The lorica consists of a ventral and a dorsal plate, and two pairs of lateral plates. Caudally, nine flosculae are present. The spiny introvert or head and the two "segmented" thorax or neck can be retracted into the lorica, the anterior part has large spines extending rostrally. The reproductive system of the adult is unique in its organization. Superficially the *Nanaloricus* looks like a rotifer when the spiny head (introvert) is retracted. The type of buccal apparatus is not like any other within the aschelminth complex. The oral stylets are found in Kinorhyncha. Phylogenetically the *Nanaloricus* is most closely related to kinorhynch and priapulids but the type of oral stylets are only found in the larvae of Nematomorpha.

### Zusammenfassung

#### *Loricifera: Ein neuer Stamm mit Aschelminthen Charakteren aus dem Meiobenthos*

1. Aus dem Meiobenthos wird ein Vertreter eines neuen Tierstammes, Loricifera, beschrieben. Alle Stadien der Typenart, *Nanaloricus mysticus* gen. et spec. nov. sind in einem aus Muschelschalen bestehenden Sand bei Roscoff (Frankreich) in einer Tiefe von 25–30 m und bei Fort Pierce (Florida, USA) in einer Tiefe von 15 m gefunden worden. Die aberrant gestaltete Larve („Higgins-Larve“) wurde in 480 m Tiefe auf muschelschaligen Sand in der Nähe der Azoren gefunden.
2. Zwei weitere noch unbeschriebene Arten von *Nanaloricus* sind im sogenannten „Amphioxus-Sand“ in der Nähe von Helsingør (Dänemark) in 10–12 m Tiefe gefunden worden und weiter noch ein Exemplar einer Higgins-Larve und eine Exuvie (die vielleicht zu einer anderen Gattung gehören) im Korallensand von Chesterfield Reefs (Coral Sea). Schließlich liegt noch ein Exemplar von der Küste von North Carolina (USA) vor.
3. Sowohl die Larve als auch die Postlarve und die Erwachsenen sind durch einen mit Stacheln besetzten Introvert und eine Lorica ausgezeichnet. Die Entwicklung verläuft über mindest zwei Larven-



- stadien. Männchen und Weibchen sind gleich groß, jedoch besteht ein Sexualdimorphismus in der Gestalt der 8 Stacheln der ersten Reihe (Clavoscaliden). Die Gonaden (Ovarien bzw. Hoden) sind paarig entwickelt.
4. Die Higgins-Larve hat einen unbewaffneten Mundcorus und einen Introvert mit 7–8 Stachelreihen. Die erste Reihe besteht aus 8 Clavoscaliden, die nächsten 4–5 Reihen werden dorsal von Spinoscaliden gebildet, die denen der Kinorhynchen ähneln. Die letzten beiden Dorsalreihen sind Priapuliden-artig entwickelt. Ventral sind alle Scaliden hakenartig, wie bei den Larven der Nematomorpha, gestaltet. Als Sinnesorgane finden sich 2–4 Trichoscaliden auf der Ventralseite und ein keulenförmiges Organ auf der Dorsomedianseite des Introverts. Die „Nackenregion“ (oder der „Thorax“) wird von 5–6 Reihen von Platten gebildet, wovon zwei ventrale Platten als Verschlussapparat fungieren, wenn der Introvert eingezogen ist. Die Loricula der Larven ist in Längsrichtung stark gefaltet. In der Dorsocaudalregion finden sich 5 schmale Platten, von denen 3 eine Floscula aufweisen. Caudal stehen zwei Paar lange Sinneshaare. Am Vorderende der Loricula stehen 3 Paar Stacheln, die wie Beine als Fortbewegungsorgane eingesetzt werden. Am Caudalende findet sich ein Paar Zehen mit plattenförmigen Anhängen (Mucros), die ein Schwimmen ermöglichen.
5. Bei den Adulten stehen 8–9 Stilette um den Mundcorus. Die Intima des Pharynx besteht aus einer dünnen, biegsamen Cuticula, die im eiförmigen Bulbus 5 Reihen cuticularer Platten bildet. Im Bukalkanal sind zwei akzessorische Stilette ausgebildet. Die Cuticula des Weibchens ist mit 6–8, die des Männchens mit 20 Reihen von Clavoscaliden besetzt. Die daran anschließenden 6 Stachelreihen tragen Spinoscaliden vom Kinorhynchen-Typ, die letzten beiden Reihen gezähnte Scaliden, wie bei Priapuliden. Die Loricula ist aus je einer dorsalen und ventralen Platte und aus je einem Paar lateraler Platten zusammengesetzt. Caudal sind 9 Flosculae vorhanden. Der bestachelte Introvert (Kopf) und der zweigliedrige „Thorax“ (Nacken) können in die Loricula zurückgezogen werden. Wenn der bestachelte Introvert eingezogen ist, wirkt *Nanaloricus* bei oberflächlicher Betrachtung wie ein Vertreter der Rotatoria. Die Buccalregion ist nicht wie bei anderen Gruppen der Aschelminthes gestaltet. Die Mundstilette erinnern an entsprechende Strukturen bei den Kinorhynchen. Phylogenetisch weist *Nanaloricus* die engsten Beziehungen zu den Kinorhynchen und Priapuliden auf, der Typ ihrer Oralstilette gleicht allein denen der Nematomorpha.

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