An Introduction to Loricifera, Cyciophora, and Micrognathozoa

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SYNOPSIS. Loriciferans, cyclophorans and micrognathozoa are amongst the latest groups of animals to be discovered. Other than being microscopic, they have very different body plans and are not closely related. Loriciferans were originally assigned to the Aschelminthes. However, both new molecular and ultrastructural researches have shown that Aschelminthes consist of two unrelated groups, Cycloneuralia and Gnathifera. Cycloneuralia may be included in the Ecdysozoa, including all molting invertebrates, and Gnathifera are more closely related to Platyhelminthes. The phylum Loricifera shares many apomorphic characters (e.g., scalids on the introvert) with both Priapulida and Kinorhyncha, and can be included in the taxon Scalidophora, a subgroup of Cycloneuralia. Cyclophora was originally allied to the Entoprocta and Ectoprocta (Bryozoa) based on ultrastructural research. Subsequent molecular data show they may be related to Rotifer and Acanthocephala, within the taxon Gnathifera. The phylogenetic position of Cyclophora is therefore not settled, and more ultrastructural and molecular data are needed. Micrognathozoa is the most recent major group of animals to be described. They show strong affinities with both Rotifer and Gnathostomulida (within the taxon Gnathifera), especially in the fine structure of the pharyngeal apparatus, where the jaw elements have cuticular rods with osmiophilic cores. Furthermore the micrognathozoa have two rows of multiciliated cells that form a locomotory organ, similar to that seen in some gastrotrichs and interstitial annelids. This character is never seen in Rotifer or in the monociliated Gnathostomulida. Rotifer and Acanthocephala always have a syncytial epidermis (Syndermata). Micrognathozoa lack this characteristic feature. Therefore, they are postulated to be placed basally in the Gnathifera, either as a sister-group to Gnathostomulida or as a sister-group to Rotifer + Acanthocephala.

INTRODUCTION

Since the discovery of the Loricifera (Kristensen, 1983) 11 species have been described (Higgins and Kristensen, 1986; Kristensen and Shirayama, 1988; Todaro and Kristensen, 1998), and more than 80 recorded species await description (Kristensen, 1991a, b; Gad, 2000). The loriciferans seem to be one of the most abundant groups of meiofauna in the deep-sea, but also occur in mud on shallower water (Soetaert et al., 1998), and they may be one of the dominant meiofauna groups. Recently, Warwick (2000) has postulated that loriciferans are simply paedomorphic priapulids. This was based on the fact that the larvae of some loriciferans (Higgins and Kristensen, 1986) look superficially similar to the larvae of some priapulids (van der Land, 1970; Higgins et al., 1993). No comments were made concerning the unique adult features of loriciferans, including the cuticular buccal tube, myoepithelial pharyngeal apparatus with placoids (Kristensen, 1991a, b) and the very complex life cycle of the different species of loriciferans (Kristensen, 1999). Below I will discuss the different types of life cycles in loriciferans, totally different from all other known meiobenthic animals.

Cyclophora is the most recently described phylum, consisting of marine acelomate epibionts on setae of mouthlimbs of lobsters (Funch and Kristensen, 1995). Today three species are known, all in the genus Symbion. The only described species, Symbion pandora, has a complicated life history with several attached and several free-living stages. The life cycle varies between asexual and sexual reproduction (Funch and Kristensen, 1997). The asexual and the sexual parts of the life cycle each have a unique larva. The life cycle seems to be more complex than we originally thought (Funch and Kristensen, 1995), and accordingly the life cycle has been redrawn in this paper. Their phylogenetic position is still uncertain. Cyclophorans have been considered to be a sister-group to Entoprocta and Ectoprocta (Funch and Kristensen, 1995; Funch, 1996), a view mainly based on ultrastructural investigation, or alternatively as a sister-group to the clade Rotifer-Acanthocephala (Winnephenninckx et al., 1998), a conclusion arrived at on the basis of molecular evidence.

Micrognathozoa was described by Kristensen and Funch (2000) as a new class within the Gnathifera (see Ahrlich, 1995, 1997). This clade now contains Gnathostomulida, Micrognathozoa and Syndermata. Syndermata consists of all Rotifer (Eurotatoria and Seisonida) and the parasitic Acanthocephala. The sister-group relationship between Gnathostomulida and Rotifer + Acanthocephala was first mentioned by Rieger and Tyler (1995). The discovery of Micrognathozoa, in a cold spring from Greenland, strongly supports the above mentioned relationship (Ahrlich, 1997; Herlyn and Ehlers, 1997). Only one species, Limnognathia maerski, has been described.

CURRENT STATE OF KNOWLEDGE

Loricifera

Adult loriciferans are bilaterally symmetrical marine metazoa 108–485 µm long (Higgins and Kristensen,
The body is divided into five regions: mouth cone, head (intervert), neck, thorax and abdomen. The intervert of the adult has nine rings of scalids, which may have both sensory and locomotory functions. Two families, Nanaloricidae and Pliciloricidae, have been described, and two new families are under description. Nanaloricidae with one genus (*Nanaloricus*), is acoelomate both as larva and adult. The abdomen of the adult may be covered with a thick cuticular lorica consisting of 6 plates. Pliciloricidae with the two genera (*Pliciloricus* and *Rugiloricus*) may be acoelomate as larva, and pseudocoelomate as adult (Kristensen, 1995). The abdomen is usually covered with a thin lorica with many folds (plicae, in Higgins and Kristensen, 1986). The lorica of pliciloricids seems always to lack thick cuticular plates. All investigated loriciferans have chitin in the procuticle (Neuhaus et al., 1997).

The first loriciferans had already been collected in the seventies, but the first species, *Nanaloricus mysticus*, was described by me in 1983, from shell gravel off the coast of Brittany, France (Kristensen, 1983). The reason why it took so long from discovery to description was that these microscopic animals have a very complex life cycle, with a larva (Higgins-larva) which looks totally different from the adults (Kristensen, 1991a). Both the Higgins larvae and the adults may adhere strongly to sand grains or gravel by adhesive glands, so a new technique, freshwater-shocking on large samples of sand to release the animals from the bottom sediments had to be developed. Eleven species have been described, but about 80 other undescribed species have been collected, and many of the new species are found in deep-sea sediments (Gad, 2000). All known species are marine, microscopic, and live within the bottom sediments as adults. The Higgins-larvae can have very large swimming-toes (flippers) and have been taken free in the plankton. Alternatively, the larvae may be benthic and have tube-shaped toes with adhesive glands. Usually there exists a sexual secondary dimorphism in the first ring (clavoscalids) and last ring (trichoscalids) of scalids.

In the family Nanaloricidae the male has the mid-dorsal, dorsolateral and ventrolateral pairs of clavoscalids divided into three branches but the two midventral scalids are undivided, so the clavoscalids consist of 20 chemoreceptors; the female always has only eight primary clavoscalids (Kristensen, 1991a). Sexual dimorphism may also be apparent in the trichoscalids (Kristensen, 1991b). In the male of *Nanaloricus* the two lateroventral secondary elements are modified as claw-shaped organs, called claspers. The claspers are assumed to function in copulation. Recently sexual dimorphism has also been recognised in the trichoscalids of *Rugiloricus*, where the female has seven of the 15 trichoscalids reduced in length (Kristensen, unpublished).

In several species of *Rugiloricus* the larva develops a mature ovary (neoteny), and this produces between 4 to 12 larvae inside the old larval exuvium (Kristensen and Brooke, 2001). These neotenous larvae look exactly like the larva emerging from the free-laid egg. The *Rugiloricus*-larvae may be common for most of the year, and outnumber the adults by 100:1. Postlarvae, when they are present (seemingly absent in *Pliciloricus*), lack toes, setae and locomotory spines on the abdomen. Often the postlarva appears very similar to the adults, but it always has fewer scalids and a thinner lorical cuticle than the males and females, and it lacks the gonads. The life cycle of *Rugiloricus* is surprisingly variable (Fig. 1). It varies from one species to another and, more confusingly, may vary depending on the feeding condition of the larva in the same species.

Three types of life cycles have been discovered in *Rugiloricus*. In *R. carolinensis* the Higgins larva moults direct to a mature male or female (Fig. 1A, B, C and H1, H2). In *R. caulisculus* the Higgins larva moults into a postlarva without toes and gonads. The postlarva moults into the adult (Fig. 1A, B, C, G and H1, H2). In a new species of *Rugiloricus* from Faroe Bank (North Atlantic Sea) the Higgins larva (Fig. 1D) grows bigger than the adult and develops a large single ovary. This larva forms a cyst-like stage (Fig. 1E). Inside the cyst moulting occurs and, after forming several eggs from the ovary, the Higgins larva totally disintegrates. The eggs mature to neotensive larvae inside the cyst walls. The larvae escape later from the cyst (Fig. 1F). The neotensive larva moults several times and grows to a new very large larva, which again develops an ovary. This part of the life cycle, called the parthenogenetic life cycle (Fig. 1D, E, and F), recur and so explains why the Higgins larval can outnumber the adults by a factor of 100. Under certain unspecified conditions the Higgins larva moults to a postlarva, which again moults into the adult. It is unclear if other species of *Rugiloricus* also have this parthenogenetic life cycle, but there is some evidence that both *R. carolinensis* and *R. caulisculus* may also form neotenous larvae (Higgins and Kristensen, 1986; Kristensen, 1999;
Life cycles of the genus *Rugiloricus* (Loricifera)

50 μm
Kristensen and Brooke, 2001). Recently Gad (2000) has found neotenous larvae in several species of deepsea pliciloricid loriciferans.

The fine structure of the mouth cone, the introvert with scalids, and the circumenteric brain indicate that this meiofaunal phylum is monophyletic, related to Kinohynchna and Priapulida. The three groups are referred to as the Scalidophora (see Lemburg, 1995; Schmidt-Rhaesa et al., 1998). Autapomorphies for Loricifera may include the unique location in both males and females of the prootonephridia inside the gonads, the complexity of the spinosalids with muscles, and the special toe glands. The hexagonal arrangement in both adults and larvae of the internal armature in the buccal apparatus may be plesiomorphic, a shared character with Nematoda and Nematomorpha (Nematoidea, subgroup of Cycloneuralia). Annulation of the flexible buccal tube, telescoping of the mouth cone and three rows of placoids in the triradiated pharynx bulb are found only in Tardigrada and Loricifera, but these similarities between tardigrades and loriciferans may be either convergent or plesiomorphic to all Cycloneuralia and Tardigrada (Kristensen, 2002). The thick lorica consisting of plates seems to be developed only in the family Nanaloricidae, for which it is a convincing autapomorphic character. The plesiomorphic condition in both adults and larvae of the lorica seems to be a thin lorica with many plicae.

Unfortunately, no molecular data are yet available for loriciferans.

Cycliophora

The marine Cycliophora is the most recently described phylum in the animal kingdom. Only one microscopical species, Symbion pandora, has been described (Funch and Kristensen, 1995). The sessile stage had been known since the late sixties, but was never described (Funch and Kristensen, 1997). It is approximately 350 μm in length and 100 μm wide, and is attached with an acellular stalk and adhesive disc to crustacean mouth parts, which can be encrusted with thousands of individuals of the sessile stage. Investigations of mouth appendages of many decapod crustaceans world-wide have resulted in further discoveries of this new type of epibiont, with a very complex life cycle, living mainly on the setae of lobsters’ mouthparts.

The sessile stage is the feeding stage, and has a circular mouth surrounded by a ring of conducta used for filtering small food particles, such as bacteria or food particles from the host. Cycliophorans are acelolamate metazoans with bilateral symmetry and a well-differentiated cuticle, and the epidermis is cellular rather than syncytial. They cannot, therefore, be assigned to the Syndermata (Rotifera and Acanthocepha). The internal anatomy is relatively simple, with a U-shaped gut and the anus located outside the ciliated feeding apparatus, a similar arrangement to that found in Ectoprocta (anus outside the tentacular crown, Nielsen, 2001) and some sessile rotifers (anus outside the corona, Winnepenningkx et al., 1998).

It is the life cycle consisting of an asexual and a sexual generation (metagenesis) of the cycliophorans that makes the phylum unique (Fig. 2). The asexual part of the life cycle consists of two types of feeding animals. The first type houses the so-called Pandora larva, the second type houses the primary male and female inside a brooding chamber. Furthermore, the sessile animal shows internal budding, whereby it loses the head (buccal funnel) and entire digestive system, replacing them with a new bud arising from embryonic cells in the posterior part of the trunk. This process of internal budding is repeated several times in the life of the sessile animal and is coupled to the asexual formation of the Pandora larva. The Pandora larva forms a new feeding-stage, also by internal budding. It settles very close to the maternal individual, and in this way the epibiont can multiply very quickly and completely cover the mouth limbs of its host. Later, the feeding stages may switch to produce a primary male or a sexually mature female instead of the Pandora larva. This marks the beginning of the sexual part of the life cycle. The primary male is released from the brooding chamber without a penis or a true testis. It settles on a feeding animal. Only if there is a young female inside this feeding animal can the attached primary male develop one or two new secondary males inside by internal asexual budding. Each secondary male has a tubular cuticular penis and a single compartment with spermatozoa. The female is about double the size of the primary male, and she looks similar to the Pandora larva except for lacking internal budding; rather anteriorly she has a single large oocyte. Fertilisation of the egg occurs inside the female, perhaps just before or during the release of the female from the brooding chamber. She settles very close to her maternal feeding animal, and broods inside herself a new type of larva, the chordoid larva, so named after a mesodermal chordoid structure of approximate 50 stacked muscle cells (see Funch, 1996). The growing larva engulfs all her tissue, leaving only the cuticle. The chordoid larva hatches from the female exuvium, head first. It is a good swimmer and represents the dispersal stage. This larva settles on a new host and by internal budding forms a new feeding animal, so a new life cycle can begin. The complex life cycle of Symbion pandora as illustrated in Figure 2 differs from the original description only by the recognition of the free swimming secondary male (Fig. 2.17), where it can be shown that it develops by budding inside the primary male (Kristensen and Funch, 2001). We had not originally discovered the free-swimming secondary male because of its tiny size, only 37 μm long, so easily passing through our 63 μm net.

The type species was found on the Norway lobster, Nephrops norvegicus from Scandinavian waters. More recently two other and still undescribed species of the cycliophorans have been found on the mouth appendages of other lobsters such as Homarus gammarus.
Fig. 2. The modified life cycle of *Symbion pandora* indicating that the primary male develops two dwarf secondary males inside by budding. It is the secondary male (17) that fertilizes the female (18). The asexual part of the life cycle includes the sessile feeding stage, which forms the Pandora larva (3–11). The sexual part of the life cycle includes two types of sessile feeding stages, each forms a primary male and female, the secondary male and the chordoid larva (12–2). The chordoid larva is the dispersal stage (Modified from Funch and Kristensen, 1995, 1997).
(Europe) and *H. americanus* (North America). So far the cyclophorans have only been found on marine decapods from the Northern hemisphere. Records of cyclophorans from other crustaceans and nematodes all seem to be false alarms. All these records seem to represent chonotrich ciliates. Only by transmission electron microscopy it can be resolved that the ciliate only consists of one cell with several nuclei. Even so, the convergences between cyclophorans, and especially the chonotrich, *Isochonopsis kergueleni*, are surprising. In particular, this ciliate has a cryptogemmic budding like the cyclophorans (for detail, see Batisse and Crumeyrolle, 1988). Therefore the chonotrich ciliates often are recorded as cyclophorans on copepods or nematodes.

In the original description we stated that the Cyclophora are related to Entoprocta/Ectoprocta (Funch and Kristensen, 1995). The overall similarities with sessile rotifers (Collothecacea), and the presence of a dwarf male with the penial structure in both groups were considered to be superficial resemblances. This reluctance to ally cyclophorans and rotifers was based mainly on the ultrastructure of the epidermis covered with a cuticle and the lack of the mastax. Therefore rotifers have a mastax and a syncytial epidermis (Sørensen et al., 2000). These authors placed cyclophorans close to entoprocts on the basis of a careful cladistic approach. Unfortunately the phylogenetic relationships were only examined in the light of morphological evidence. The molecular data (study of 18S rRNA/DNA sequences) indicate that cyclophorans are a sister-group to the Syndermata which includes Rotifera and Acanthocephala (Winnepeninckx et al., 1998). Most recently Giribet et al. (2000) placed the cyclophorans within the Gnathifera. Their investigation was a combined approach based on both 18S rDNA sequences and morphology across the range of triploblasts, but their placement of Cycliophora was entirely dependent on our molecular data (1,542 base pairs of the 18S rRNA gene sequences) from alcohol-preserved material of Syncytostryma (see Winnepeninckx et al., 1998). The phylogenetic position of Cyclophora is, therefore, very uncertain, and new ultrastructural and molecular studies are urgently needed.

**Micrognathozoa**

A new microscopic animal, *Limnognathia maerski*, with complicated jaws, was found in a cold spring at Disko Island, Greenland in 1994, but first described by Kristensen and Funch (2000), where it was assigned to a new class, Micrognathozoa, in the Gnathifera (Ahlrichs, 1997). The clade Gnathifera now includes Gnathostomulida, Micrognathozoa and Syndermata (Eurotataria, Acanthocephala and Seisonida), see Figure 3.

*Limnognathia maerski* has dorsal plates formed by an intracellular matrix, as in Rotifera and Acanthocephala (Storch and Welsch, 1969), but ventrally the epidermis is “naked” only with a thin glycoalyx. The gross anatomy of the body consists of a two-part head, accordion-like thorax and ovoid abdomen with a small tail. The total length of the holotype was only 142 μm, and the maximum width of the abdomen was 55 μm. As in many marine interstitial animals, *e.g.*, gnathostomulids, gastrotrichs and polychaetes, special forms of tactile bristles or sensoria are found on the body. The locomotory organ consists of two rows of cells with cilia. These cells are similar to the ciliophores in annelids (Eibye-Jacobsen and Kristensen, 1994; Kristensen and Niilonen, 1982). An adhesive ciliary pad is located ventro-posteriorly. It consists of ten ciliated cells, which form a very sticky glue. The cells do not contain the two types of granulae which are characteristic in the duo-gland system (Fig. 16, Kristensen and Niilonen, 1982). Instead the ten cells are dominated by the cross-striated ciliary root passing through the entire cell. The cilia are ordered in register like those of the trunk ciliophores. The ciliary pad is very different from the adhesive toes of rotifers, gastrotrichs and annelids, and the structure may be a good autapomorphy for the micrognathozoans.

Because the new taxon lacks syncytia, a key character for the Rotifera and Acanthocephala (Syndermata, Ahlrichs, 1997), the Micrognathozoa cannot be included in Syndermata. Two pairs of protonephridiae are found in the trunk of *Limnognathia maerski*, the nephridiopore of which seems to open laterally on the abdomen. The terminal cells are monociliated, a pleiomorphic character Micrognathozoa shares with Gnathostomulida (Ax, 1996). Only the female reproductive system has been observed, suggesting that *Limnognathia maerski* reproduces by parthenogenesis, as do many other freshwater meiofaunal animals, such as gastrotrichs, rotifers and heterotardigrades. The reproductive system is anatomically simple, and it seems that the two ovaries derive nutrition directly from the midgut, a feature also seen in freshwater chaetonotoid gastrotrichs. There is a possibility that the female lays two types of eggs, as in limnic gastrotrichs and rotifers: a smooth egg may be quick-developing summer...
Most of the digestive system in micrognathozoans is anatomically very simple, except for the pharyngeal apparatus with large array of true cuticular elements, the sclerites. In fact, Linnognathia maerski has one of the most complicated jaw systems in microscopical animals, and it is still not well understood. Until now only the main parts have been described by SEM investigations (Kristensen and Funch, 2000). They consist of two ventral basal plates, each plate associated with large teeth (manus), two dorsal oral lamellae and two lateral pharyngeal lamellae, two fibularia with small teeth anteriorly, and three sets of jaws, where the main jaws (mid jaws) are fused and consist of a dentarium (basket formed), and an articulum (Sørensen, 2002). The posterior part of the main jaws has a paired cauda, as in some species of gnathostomulids (Riedel and Rieger, 1972). Furthermore, most of the lumen of the pharynx is covered with a thin cuticle, and several accessory sclerites attaching the ventral and dorsal jaws are still not fully described (Sørensen, 2001). All sclerites seem to be built of microtubules, consisting of lucent, osmophobic material surrounding a dense osmophilic core. This structure was first described by Rieger and Tyler (1995) in the jaws of Gnathostomulida and Rotifera. The rest of the digestive system is surprisingly simple. A short dorsal esophagus leads into the large midgut, which occupies most of the thoracic region and the anterior part of the abdomen. The midgut lacks cilia completely, and has only a brush border of short microvilli. A true cuticle-lined hindgut is lacking. The anus is located dorsally and opens only periodically, as seen in Gnathostomulida and some Gastrostricha that also lack a permanent anus (Knauss, 1979).

The ultrastructure of the micrognotozoan jaws is similar to jaws of advanced scleroperalian gnathostomulids (Rieger and Tyler, 1995; Herlyn and Ehlers, 1997). However, the jaw apparatus might also possess some of the characteristics of the rotiferan mastox (Koecher and Hayes, 1969). Jaw-like structures are found in other prostostome taxa as well, for instance in the proboscises of kalyptorhynch turbellarians, in dorvilleid polychaetes and aplacophoran molluscs, but studies of their ultrastructure (e.g., polychaetes, Purschke, 1987) show that none of these jaws is homologous with the jaws in Gnathostomulida, Rotifera, and Micrognathozoa (Sørensen, 2000; Sørensen et al., 2000).

Our proposed phylogeny of the clade Gnathifera is shown in Figure 3. The solid line is the suggestion of Kristensen and Funch (2000). The dotted line with a question mark denotes the suggestion of Ahlrichs (1997) that Acanthocephala and Seisonida are sister groups. The broken line with a question mark is our alternative suggestion (Kristensen and Funch, 1995) that Gnathostomulida and Micrognathozoa are sister groups. This preliminarily proposed idea was later confirmed by molecular data (Garey, personal communication). The black squares with numbers 1–11 mark synapomorphies: 1. Gnathifera. Cuticular jaws with tubes composed of lucent material surrounding on electron dense core. 2. Gnathostomulida. Monociliated epidermis. Hermaphrodites with complex reproductive system. 3. Micrognathozoa + Syndermata. Epidermis with apical intracellular matrix and dorsal and lateral trunk regions without motile cilia. 4. Filospermoidea. Elongation of the body with a long and slender rostrum. Compact jaws with 8 to 12 longitudinally arranged ridges in lamellae symphases. 5. Bursovaginoidea. Fibularization of the jaw apophyses. Complex female organs with a bursa and often a vagina. Male opening with an injectory penis often consisting of 8 to 12 intracellular rod-like structure. Spermatozoon strongly modified with lack of 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 glycoelastic. Cuticular oral plate and dorsal and lateral intracellular plates present. The solid parts of the jaws very complex with paired basal plates, each associated with denticulate manes, paired striated oral and pharyngeal lamellae, paired fibularia and three sets of paired jaws. 7. Syndermata. No motile trunk ciliation. Eutelic, syncytial epidermids with apical crypts. Spermatozoon with anterior inserted flagellum. 8. Eurotatoria + Acanthocephala. Spermatozoon with row of ciliates. Presence of spermatoophore. 10. Eurotatoria. Corona, unpaired retrocerebral glands, female reproductive organs with vitellarium. 11. Acanthocephala. Endoparasitic with a two-host life cycle. Epidermal lacunar system. Proscos with intraepidermal hooks. Females with a bursa and a terinial bell.

Molecular data are still not available for Micrognathozoa, but a study of 18S rRNA/DNA sequences is underway to be published (Garey, personal communication).

Conclusions

Loriciferans were originally assigned to the para- phyletic or polyphyletic group called Aschelminthes (Kristensen, 1983). Subsequently, both molecular (Winneppeninckx et al., 1995) and ultrastructural (Ehlers et al., 1996; Ahlrichs, 1997) studies have shown that Aschelminthes consist of two unrelated groups, Cycloneuralia and Gnathifera. Cycloneuralia may be monophyletic in origin, with the Scalidophora (Ehlers et al., 1996). Furthermore they have several plesiomorphic characters, such as a myoepithelial pharyngeal bulb, comparable to
the other Cycloneuralia, especially the Nematoda and Gastrotricha. The parasitic Nematomorpha, whose adults lack the pharyngeal bulb, has a larva with an introvert similar to Scalidophora. The loriciferans have, therefore, also been assigned to the Cephalorhyncha that includes Priapulida, Kinorhyncha and Nematomorpha (Malakhov, 1980; Adrianov et al., 1990). The phylogenetic position remains controversial, but the close relationship of loriciferans to kinorhynchs and priapulids can hardly be doubted (Ehlers et al., 1996; Neuhaus et al., 1996; Nielsen, 2001).

The idea that loriciferans are no more than “neotenous” or progenetic priapulids (Warwick, 2000) is based on misunderstandings of the supposed resemblances between adult loriciferans and the loricate larvae of priapulids. There are very few similarities, and it is incorrect that they are “virtually identical in morphology, except for the absence of gonads” (Warwick, 2000). It is correct that there are some similarities between the larvae of priapulids and loriciferans, but nearly all these similarities are plesiomorphic characters seen in all Cephalorhyncha (Nematoda, Nematomorpha, Priapulida, Kinorhyncha and Loricifera, see Nielsen, 2001). Furthermore if loriciferans are evolved from priapulids by progenesis, the larvae in loriciferans should not be present. This is the case in many progenetic polychaetes, e.g., Neotenotrocha sterreri (see Eibye-Jacobsen and Kristensen, 1994; Westheide, 1987), which secondarily are adapted to the interstitial way of life. Loriciferans do not lack larvae. In fact, they have several types of larva; ironically one type produces a mature ovary (i.e., neoteny). Therefore, I reject the hypothesis that the loriciferans are simply progenetic priapulids. Loricifera is a monophyletic group, and is the sister group to the Priapulida + Kinorhyncha clade, within the Scalidophora (Nielsen, 2001).

In our original description (Funch and Kristensen, 1995) the Cyclophora was related to the Entoprocta and Ectoprocta (Bryozoa) on the basis of ultrastructural research. Cladistic morphological analyses (Zrzavy et al., 1998; Sørensen et al., 2000) place the Cyclophora as sister-group to Entoprocta. On the other hand, Cavalier-Smith (1998) considered them to be members of the phylum Kamptozoa (=Entoprocta). This latter hypothesis should be rejected because it necessitates a total redefinition of the Entoprocta. As the name implies, entoprocts always have the anus inside the tentacular crown (Nielsen, 2001), whereas the cyclophorans have the anus outside the ciliated mouth ring (Funch and Kristensen, 1997). However, our molecular data (Winnepeninckx et al., 1998) show they may be related to Rotifera + Acanthocephala inside the taxon Gnathifera (Giribet et al., 2000). The phylogenetic position of Cyclophora is, therefore, not settled, and more ultrastructural and molecular research is needed.

Micrognathozoa is the latest described group of the new animals. They show strong affinities with both Rotifera and Gnathostomulida within the taxon Gnathifera, especially in the fine structure of the pharyngeal apparatus. Only in these three groups do the jaw elements have cuticular rods with osmiophilic cores. Such microtubular structures are found in the fulcrum of all Rotifera and in several cuticular sclerites of both Gnathostomulida and Micrognathozoa (Sørensen, 2000). Furthermore, the micrognathozoans have two rows of multiciated cells that forms a locomotory organ, similar to that in some gastrotrichs and interstitial annelids. This character is never seen in Rotifera, nor in the monociated Gnathostomulida. Rotifera and Acanthocephala always have synectia in the epidermis (Syndermata). Micrognathozoa lack this characteristic feature and, therefore, are postulated to be more basal in the Gnathifera, as a sister-group either to Gnathostomulida or Rotifera/Acanthocephala.

The jaws of Gnathostomulida, Rotifera, and Micrognathozoa, with their microtubes, are considered to be homologous structures. Limnognathia maerski is probably related to both Rotifera and Gnathostomulida. Syndermata + Micrognathozoa might be sister-groups, while Gnathostomulida could be the sister-group to this assemblage (Kristensen and Funch, 2000). Gnathostomulida was originally described as an order of the Platyyhelmintes (Ax, 1956). More recently Ax (1996) postulated a sister-group relationship of Gnathostomulida with Platyhelmintes (“Platyxhelmithomorpha hypothesis”). This hypothesis is rejected (Kristensen and Funch, 2000), and the finding of Limnognathia maerski supports the monophyly of Gnathifera (see Ahlrichs, 1997). The ultrastructural similarities of the new animal to certain chaetonotoid gastrotrichs and interstitial polychaetes may be either convergent or plesiomorphic features.

The discovery of Micrognathozoa has clearly thrown new light on the polyphyletic origin of the “so-called” Pseudocoelomata or Aschelminthes. Gnathifera are more closely related to other platyzoan aceolomates (Gastrorchtra and Platyhelmintes) than to introvertan pseudocoelomates. Cyclophora may be included in Platyyzoa, too (Giribet et al., 2000). Introverta (Cycloneuralia without Gastrorchtra) form part of the clade Edysisozoa (Aguiainalo et al., 1997). Already in 1991—before the molecular evidence for Edysisozoa—I had stated (Kristensen, 1991a) that some aschelminth groups (later named Scalidophora) and arthropods may be related. Therefore, similarities (i.e., chitin in procuticle) between Panarthropoda and Scalidophora could be true homologies. Proarthropoda (Tardigrada and Onychophora) could have developed from Scalidophora-like animals similar to Opabinia and Anomalocaris of the Cambrian Burgess Shale Fauna, and not from annelid-like animals without a moultung cuticle. The evidence for a clade Edysisozoa comprising all moulting invertebrates, is strongly supported by the discovery of Loricifera, especially the life cycles of the new pliciloricid loriciferans that indicate a close relationship between Panarthropoda and Scalidophora.

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