

On the phylogenetic position of Rotifera – have we come any further?

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Abstract

Rotifers are bilateral symmetric animals belonging to Protostomia. The ultrastructure of the rotiferan trophi suggests that they belong to the Gnathifera, and ultrastructural similarities between the integuments and spermatozoa as well as molecular evidence strongly suggest that rotifers and the parasitic acanthocephalans are closely related and form the clade Syndermata. Here we discuss the phylogenetic position of rotifers with regard to the gnathiferan groups. Originally, Gnathifera only included the hermaphroditic Gnathostomulida and the Syndermata. The synapomorphy supporting Gnathifera is the presence of pharyngeal hard parts such as jaws and trophi with similar ultrastructure. The newly discovered Micrognathozoa possesses such jaws and is a strong candidate for inclusion in Gnathifera because their cellular integument also has an apical intracytoplasmic lamina as in Syndermata. But Gnathifera might include other taxa. Potential candidates include the commensalistic Myzostomida and Cyclophora. Traditionally, Myzostomida has been included in the annelids but recent studies regard them either as sister group to the Acanthocephala or Cyclophora. Whether Cyclophora belongs to Gnathifera is still uncertain. Some analyses based on molecular data or total evidence point towards a close relationship between Cyclophora and Syndermata. Other cladistic studies using molecular data, morphological characters or total evidence suggest a sister group relationship between Cyclophora and Entoprocta. More molecular and morphological data and an improved sampling of taxa are obviously needed to elucidate the phylogenetic position of the rotifers and identify which phyla belong to Gnathifera.

Introduction

Our knowledge about the phylogenetic affinities of the Rotifera has increased a lot within the latest decade. Throughout time they have been considered relatives to the Infusoria, Crustacea, Tardigrada, Nematoda, Annelida, Mollusca, Gastrotricha and Platyhelminthes (Remane, 1929–1933; Hyman, 1951; Clément, 1985) and more recently as a member of the obviously polyphyletic group named ‘Aschelminthes’ (Ruppert & Barnes, 1994; Wallace et al., 1996). A close relationship between the

Rotifera and Acanthocephala has been broadly accepted since Storch & Welsch (1969, 1970) demonstrated the ultrastructural similarities in the integuments of the two groups, and today most taxonomists unite them in the taxon Syndermata Ahlrichs, 1995.

A possible homology between the jaws of rotifers and gnathostomulids was first suggested by Ax (1956) and Reisinger (1961). An increasing amount of data now supports a close relationship between Rotifera, Gnathostomulida, Micrognathozoa and Acanthocephala united in a group

named Gnathifera (Ahlrichs, 1995a, b, 1997; Rieger & Tyler, 1995; Haszprunar, 1996a; Melone et al., 1998; Kristensen & Funch, 2000; Sørensen, 2000, 2003; Jondelius et al., 2002; Sørensen & Sterrer, 2002; Zrzavý, 2003). Several synapomorphies have been proposed for Gnathifera, and even though some of these may be questionable (see Jenner, 2004), the presence of jaws with a unique ultrastructure appears to be a strong support argument for gnathiferan monophyly.

Some problems, however, still remain and new questions appear. The phylogenetic position of Gnathifera in the Metazoa is still uncertain and recently, new studies have questioned the monophyly of Gnathifera (Giribet et al., 2004). Cladistic analyses based partly or solely on molecular data imply that Gnathifera might be polyphyletic (Littlewood et al., 1998; Zrzavý et al., 1998; Peterson & Eernisse, 2001) or paraphyletic, for example, in respect to Cycliophora, Gastrotricha or Myzostomida (Cavalier-Smith, 1998; Giribet et al., 2000; Zrzavý et al., 2001; Giribet, 2002). Many studies based on morphological as well as molecular data have proposed that the Acanthocephala should be considered highly advanced rotifers (Lorenzen, 1985; Garey et al., 1996; Ahlrichs, 1997; Garey et al., 1998; Mark Welch, 2000; Herlyn et al., 2003) and most recently it was suggested that the newly described taxon Micrognathozoa (Kristensen & Funch, 2000) is sister group to the monogonont rotifers (De Smet, 2002).

Here we will discuss some of the conflicting proposals about rotifer relationships. We focus primarily on two newly described taxa, Cycliophora and Micrognathozoa, but also make some comments to the position of the Acanthocephala, Myzostomida, and Gnathifera within the Metazoa.

Discussion

Cycliophora – newly discovered taxon with rotifer affinities?

Among metazoans the Cycliophora is the most recently described major taxon. So far, only one marine species *Symbion pandora* Funch & Kristensen 1995 is known. This microscopic animal lives as a commensal on the mouth parts of the Norway lobster *Nephrops norvegicus* (Linné).

Throughout the metagenetic life cycle of *S. pandora* six different stages emerge of which the sessile feeding individual is the largest (approx. 350 μm) and most prominent one. It is the only stage with an alimentary tract and is permanently attached to the host with an adhesive disc (Fig. 1, ad). The body of the feeding individual has a bell-shaped buccal funnel (Fig. 1, bf) and an ovoid trunk. The buccal funnel carries a mouth ring (Fig. 1, mr₁) consisting of multiciliated cells with compound cilia. The feeding apparatus works as a downstream collecting system (Riisgård et al., 2000) that filters food particles generated from the feeding activities of the host. The mouth leads into a U-shaped gut that terminates in an anus that is situated close to the buccal funnel (Fig. 1, an). The entire alimentary apparatus is periodically replaced by internal budding (Fig. 1, ib). A fluid-filled body cavity is absent (Funch & Kristensen, 1997).

Three different stages develop in the brood chamber of the feeding individual (Fig. 1, bc). The asexual developed Pandora larva (Fig. 1, pl) is approximately 120 μm long and equipped with an antero-ventral ciliated disc, various frontal glands, long and stiff, sensory cilia, and an internal bud from which a new feeding individual will arise. The asexually developed male larva (Prometheus larva) is approximately 100 μm long and has an antero-ventral ciliated disc, various glands and several pairs of long, stiff cilia anteriorly. Several dwarf males arise from internal buds within this larva (Obst & Funch, 2003). The dwarf male, approximately 33 μm in length, has a complex morphology with two ciliated fields covering the anterior and ventral body. It has various sensory structures, gland complexes, a relatively large brain connected to a pair of ventral longitudinal nerves, and numerous muscle cells. The reproductive system of the male is located in the posterior part of the body and consists of an unpaired testis, several adjacent gland cells, and a ventral penis connected to some of the muscle cells. The female, which is also developed in the brood chamber of the feeding individual (Fig. 1, bc), grows to a size of about 150–190 μm , and is morphological like the Pandora larva. However, the female contains a single egg instead of a bud. After fertilization the embryo grows inside the female, nourished by the degenerating maternal tissue and develops into a chordoid larva with a size of about 200 μm and a

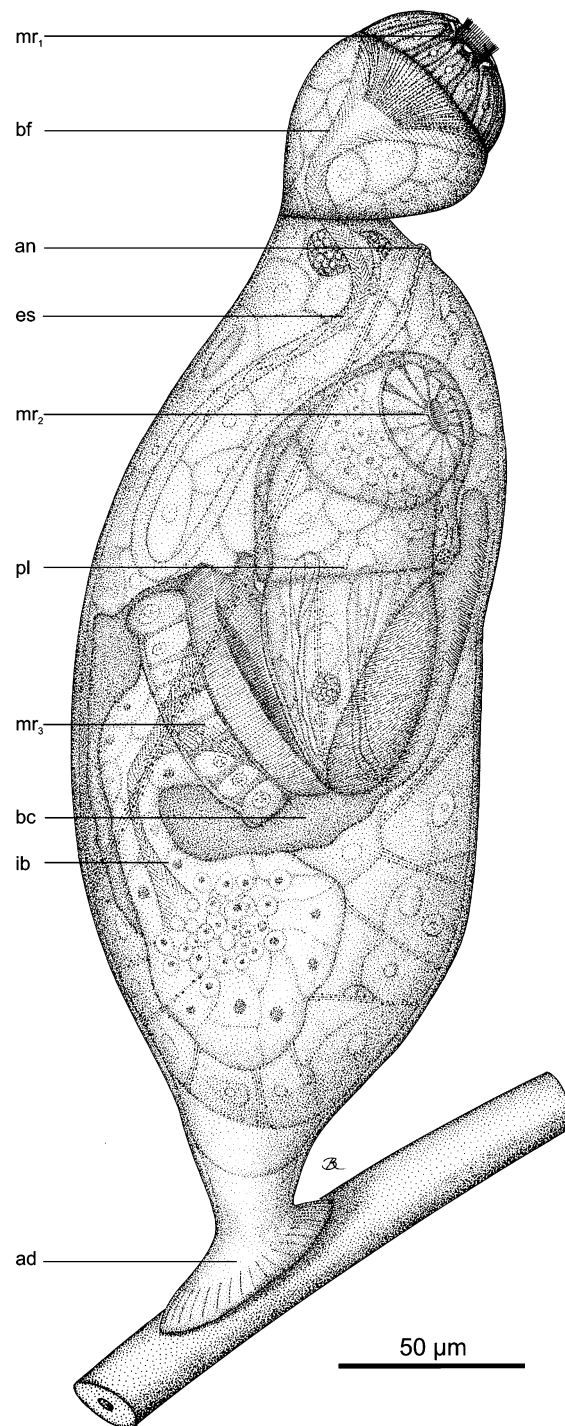


Figure 1. A young feeding stage of *Symbion pandora* (Cycliophora) on a seta from the host *Nephrops norvegicus*, lateral view. ad – adhesive disc; an – anus; bc – brood chamber; bf – buccal funnel; es – esophagus; ib – inner bud; mr₁ – functional mouth ring on feeding stage; mr₂ – mouth ring inside developing Pandora larva; mr₃ – developing mouth ring on inner bud; pl – Pandora larva. From Funch & Kristensen (1997).

complex morphology (Funch, 1996). The external ciliation of the chordoid larva consists of two anterior bands, two large ventral fields, and different sensory structures. The ciliated areas of the chordoid larva have been proposed to be homologous to those of a trochophore (Funch, 1996). Internally, the chordoid larva possesses a brain connected to a pair of ventral longitudinal nerves, a pair of protonephridia, a longitudinal rod of stacked muscle cells (chordoid organ), several gland and muscle complexes and one or two clusters of budding cells. Only a single host is known in the life cycle, and the chordoid larva is regarded as the dispersal stage between hosts.

Evaluation of the phylogenetic affinity between Cycliophora and Syndermata

In the original description of *Symbion pandora*, Funch & Kristensen (1995) proposed a close relationship between Cycliophora and Entoprocta and/or Bryozoa. Since then phylogenetic analyses have resulted in two competing hypotheses. Some analyses based on morphological data or total evidence support a Cycliophora–Entoprocta relationship (Zrzavý et al., 1998; Sørensen et al., 2000; Zrzavý, 2003), while others support a Cycliophora–Syndermata relationship (Peterson & Eernisse, 2001; Zrzavý et al., 2001). Analyses using molecular data or total evidence often favour a Cycliophora–Syndermata relationship (Winnepenninckx et al., 1998; Giribet et al., 2000; Peterson & Eernisse, 2001; Zrzavý et al., 2001). Based on both morphological and molecular data Zrzavý et al. (2001) proposed a monophyletic group including Cycliophora, Myzostomida, and Syndermata. This putative group was supported by three synapomorphies: (1) sperm with anteriorly inserted flagellum, (2) sperm without an accessory centriole, and possibly (3) the general tendency to live in association with crustaceans. However, it is not known if the flagellum in the cycliophoran sperm inserts anteriorly (Funch & Kristensen, 1997). An accessory centriole is apparently lacking in the sperm of *S. pandora*, but is also absent in several other taxa, i.e. some Gastrotricha and Platyhelminthes (Ferraguti & Balsamo, 1994; Ahlrichs, 1995b). Furthermore, the lack of an accessory centriole could be correlated with the evolution of the filiform sperm which probably

occurred more than once (Jenner, 2004). The association with crustaceans as a ‘possible support’ for Cycliophora, Myzostomida, and Syndermata being monophyletic (Zrzavý et al., 2001) is of dubious character. Most rotifers, acanthocephalans, and all myzostomids are not associated with crustaceans, and this synapomorphy would require numerous independent losses of symbiosis with crustaceans. One also may speculate whether the cycliophoran affiliation to the lobster’s mouth parts, the seisonid association with *Nebalia*, and the acanthocephalan endoparasitism in various crustaceans are so similar that they are products of one unique evolutionary event.

In a phylogenetic analysis using morphological data Peterson & Eernisse (2001) placed Cycliophora in a trichotomy with Rotifera and Gnathostomulida + Platyhelminthes, but Acanthocephala and *Seison* were not included. No unambiguous support for a Cycliophora–Rotifera relationship was found and the entire clade had a low Bremer and bootstrap support. Hence, none of the morphological analyses are able to produce consistent support for the suggested affinities between Cycliophora and Syndermata, even though they do share some superficial similarities.

Both the cycliophoran mouth ring and the rotiferan corona have bands of compound cilia that function as a downstream-collecting system, but this seems to be a general feature of ciliary suspension feeding protostomes (Riisgård et al., 2000; Nielsen, 2001). Also, the ability to retract the feeding structures differs, while the corona of rotifers can be retracted into the trunk, the mouth ring of cycliophorans cannot (Funch & Kristensen, 1997). The cycliophoran chordoid larva possesses one pair of multiciliated lateral pits and one paired dorsal ciliated organ, somewhat resembling the lateral and dorsal antennae in rotifers (Funch, 1996). In addition the sensory structures of rotifer and cycliophoran dwarf males have a somewhat similar morphology but their homology has yet to be assessed (see Obst & Funch, 2003).

Based on an ultrastructural study of the cycliophoran male and comparison with literature descriptions of rotiferan males, Obst & Funch (2003) argued that the presence of dwarf males in Rotifera and Cycliophora is a result of a convergent evolution. This is in agreement with the

generally accepted idea that dwarf males have evolved within Syndermata (Wallace & Colburn, 1989; Ahlrichs, 1997; Melone et al., 1998; Sørensen, 2002), since *Seison* (Ricci et al., 1993) and some monogonont rotifers (Wesenberg-Lund, 1923; Hermes, 1932) possess fully developed males. The ontogeny differs as well, while the males of monogonont rotifers develop from haploid eggs produced by mictic females (Wallace, 1999); cyclophoran males develop from budding cells inside a male larva. Also, the copulatory organ in monogonont males consists of several cell types (Aloia & Moretti, 1973; Gilbert, 1983), and sometimes bears a ciliated crown around the genital pore (Clément et al., 1983). The cuticular penis of *S. pandora* is more simple and without cilia (Obst & Funch, 2003). The external ciliation of the dwarf male of *S. pandora* is more extensive than in rotiferan males and consists of two separated ciliated fields (Obst & Funch, 2003). The corona of monogonont dwarf males usually consists of a single anterior terminal disk of cilia or a girdle surrounding bundles of cilia (Hyman, 1951).

In contrast to syndermates *S. pandora* has a true cuticle that is formed from the cellular epidermis. The regenerative powers between rotifers and cyclophorans differ as well; while rotifers are poor in regeneration and apparently lack cell divisions in the adults (Hyman, 1951), *S. pandora* is able to replace the alimentary apparatus periodically or develop individual stages from internal buds. Also cuticular jaws are absent in *S. pandora*.

In summary, the morphological data supporting cyclophoran-syndermate affinity are weak and since all molecular analyses including cyclophorans mentioned above, used the same partial 18S rDNA sequence, the resulting relationships have to be treated with caution. For example Zrzavý et al. (2001) found support for the Syndermata relationship and Zrzavý (2003) for the Entoprocta relationship. Both studies used total evidence with 18S rDNA data. In a recent cladistic study Giribet et al. (2004) used four molecular loci (18S rDNA, a fragment of 28S rDNA, the nuclear protein-coding gene histone H3 and the mitochondrial gene cytochrome *c* oxidase subunit I) and a dense sampling of gnathiferan taxa, but the phylogenetic position of Cyclophora was still unstable. All four loci tended to place Cyclophora with Syndermata. Ribosomal and nuclear loci

tended to place Cyclophora with Entoprocta. Alternatively, nuclear loci tended to place Cyclophora with Micrognathozoa and Syndermata. The support for closely related syndermates and cyclophorans is too weak (Fig. 6), and a relationship between Cyclophora and Entoprocta cannot be ruled out. Better comparative morphological studies of the possible homologies mentioned here are clearly needed to clarify a possible relationship between Cyclophora and Syndermata.

Micrognathozoa – sister group to Syndermata or aberrant rotifer?

Recently, a new microscopic taxon named Micrognathozoa was described from a cold spring on Disko Island, Greenland (Kristensen and Funch, 2000). At present it comprises a single species named *Limmognathia maerski* Kristensen and Funch and its possession of an intracytoplasmic lamina and complicated pharyngeal hard parts (Fig. 2, ja) suggest a close relationship with Rotifera.

With more than 15 paired or unpaired sclerites the micrognathozoan jaws are more complex than the pharyngeal hard parts found in any other microinvertebrate (Fig. 3). However, Kristensen & Funch (2000) demonstrated that the ultrastructure of the sclerites was very similar to that of the rotifer trophi. Based on a detailed comparison with the rotifer trophy, Kristensen & Funch (2000) proposed that the micrognathozoan main jaws and symphysis were homologous with the rotifer incus and that the micrognathozoan pseudophalangia and associated sclerites corresponded to the rotifer mallei, and these proposals were later supported by Sørensen (2003). Furthermore, Sørensen (2003) noted that the pharyngeal lamellae could be homologous with parts of the rotifer epipharynx, but it would be premature to draw this conclusion since the ground pattern of the rotifer epipharynx is not yet fully understood.

De Smet (2002) recently made a new attempt to understand the highly complicated jaws of Micrognathozoa. In a comprehensive description of the hard parts he pointed out several previously undescribed structures, such as the prominent brush on the main jaws and the circular platelet between the basal plates (Fig. 3, (cp), mj, vmj). Furthermore, he reinterpreted the already known

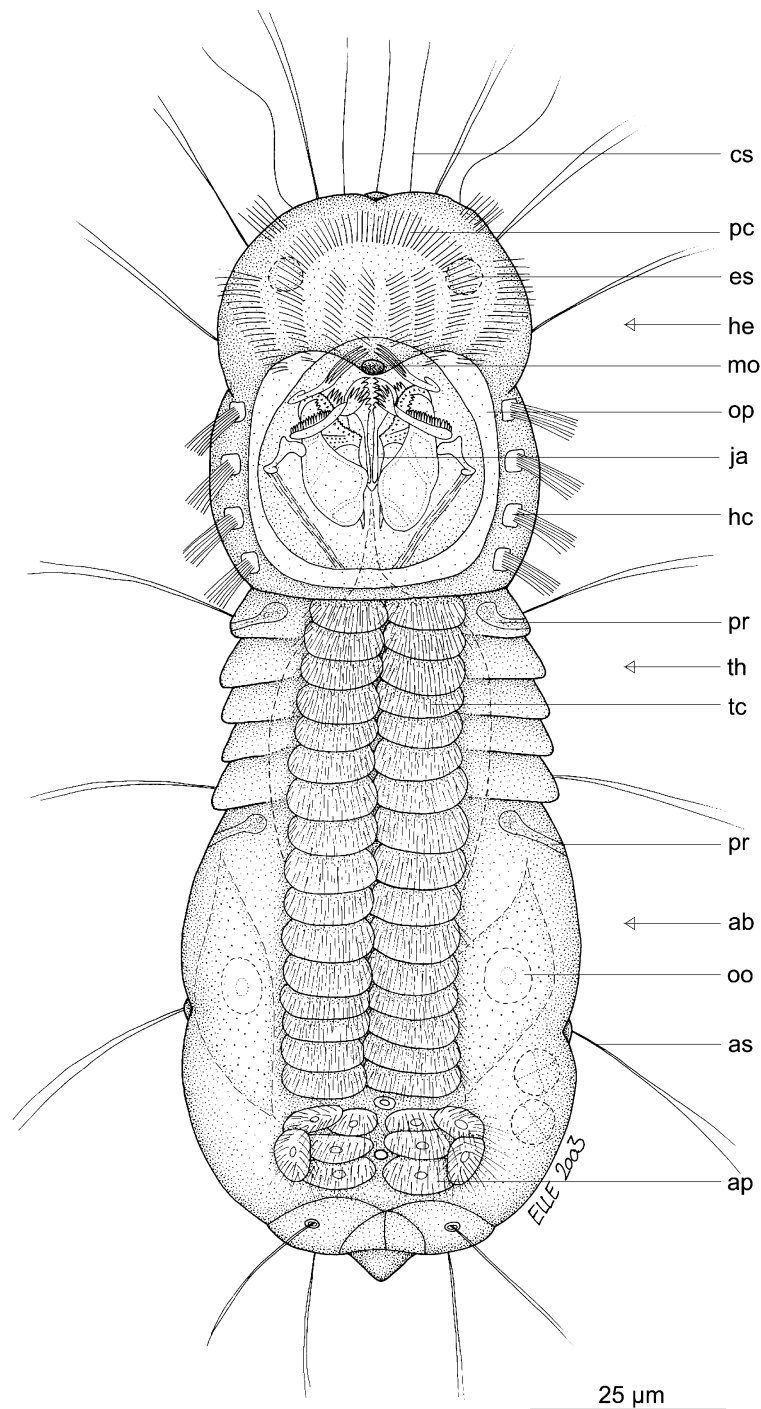
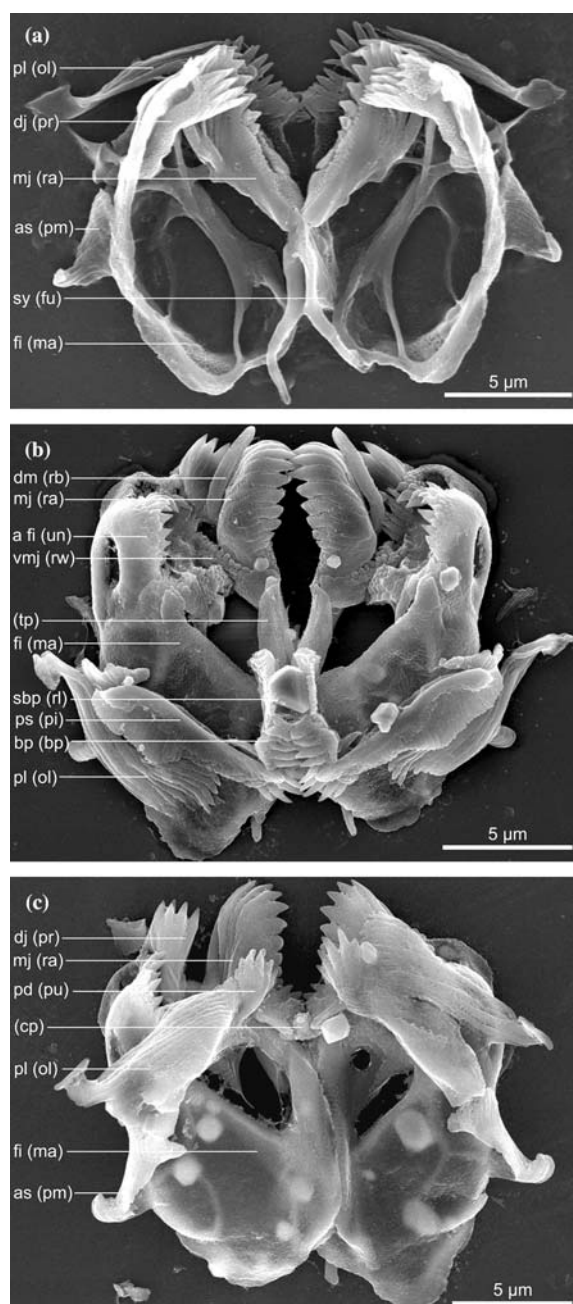


Figure 2. Drawing of *Limmognathia maerski* (Micrognathozoa), ventral view. ab – abdomen; as – abdominal sensory bristle; ap – adhesive pad; cs – cephalic sensory bristles; es – eyespot; hc – head ciliophore; he – head; ja – jaws; mo – mouth; oo – oocyte; op – oral plate; pc – preoral ciliary bands; pr – protonephridium; tc – trunk ciliophores; th – thorax. Courtesy of R. M. Kristensen, Zoological Museum, University of Copenhagen.



structures and made a detailed comparison with the rotiferan trophi. Based on these observations De Smet (2002) supports the previously proposed homology between the main jaws, plus symphysis (Fig. 3, mj, sy, vmj) and the rotifer incus, but rejects a possible homology between the pseudophalangia (Fig. 3, ps) including the associated

Figure 3. *Limnognathia maerski* (Micrognathozoa), SEM photographs of jaws. (a) Dorsal view. (b) Ventral view, note that basal plates, pharyngeal lamellae and pseudophalangia are tilted backwards. (c) Ventral view. Abbreviations are given *sensu* Kristensen & Funch (2000) and Sørensen (2003). Abbreviations in parenthesis are *sensu* De Smet (2002). a fi (un) – anterior part of fibularium (uncus); as (pm) – associate sclerite (pseudomanubrium); bp (bp) – basal plate (basal platelets); (cp) – (circular platelet); dj (pr) – dorsal jaw (pleural rod); dm (rb) – dentes medialis (rami brush); fi (ma) – fibularium (manubrium); mj (ra) – main jaw (ramus); ps (pi) – pseudophalangium (pseudintramalleus); pd (pu) – pseudodigits (pseuduncus); pl (ol) – pharyngeal lamella (oral lamella); sbp (rl) – shaft of basal plate (reinforced ligament); sy (fu) – symphysis (fulcrum); (tp) – triangular plate; vmj (rw) – ventral part of main jaw (reinforced web).

sclerites and the rotifer mallei, and homologizes instead the mallei and the micrognathozoan fibularia (Fig. 3, fi). Moreover, he compares several of the remaining micrognathozoan sclerites with the rotifer trophi and homologizes them with different epipharyngeal sclerites, and concludes that these similarities support ‘a sister-group relationship between Micrognathozoa and Rotifera Monogononta’ (De Smet, 2002).

Evaluation of the proposed relationship between Micrognathozoa and Monogononta

De Smet’s (2002) interpretation of the micrognathozoan jaws and suggested homologies with the rotiferan trophi are interesting in a phylogenetic as well as a comparative context and deserve some comment. The suggested homology between the fibularia (Fig. 3, fi) and the mallei are possible, but on the other hand it is difficult to find consistent morphological support for this assumption. De Smet (2002) interprets the anterior part of the fibularium as an uncus that is fused caudally with the manubrium, and notes that such an arrangement is not uncommon in rotifers. Fused unci and manubria are truly present in different taxa, for example, in all bdelloids and several monogonont taxa, such as *Birgea*, *Tylotrocha*, and Testudinellidae, but this character is nevertheless problematic. First, nothing indicates that fusion of the unci and manubria in *Birgea* and *Tylotrocha* is homologous with the arrangement found in Flosculariaceae and Bdelloidea. Second, the general appearance of the micrognathozoan fibularium differs significantly from the mallei in Bdelloidea

as well as in Flosculariacea and Ploima. In Micrognathozoa the fibularium has four chambers (exclusive the anterior-most one), whereas the rotifer manubrium has three or fewer. The fibularium is moreover connected with the main jaws via a unique structure named the reinforced web (Fig. 3, vmj (rw) (De Smet, 2002). Such an interconnection is not known in rotifers. Hence, from our point of view, nothing indicates that a homology between the malleus and fibularium is more likely than a homology between the malleus and the pseudophalangium with its associated sclerite. Nothing really favours the latter possibility, so at present this problem must be considered unresolved.

De Smet (2002) also homologizes the micrognathozoan dorsal jaws and pseudophalangia + associated sclerites (Fig. 3, dj, ps) with the rotiferan pleural rods and pseudomallei, respectively, but this assumption is questionable. First, the morphology of the epipharyngeal elements is very diverse, and our understanding of their basic patterns is still very limited. Thus, comparisons based on morphological similarities should be done with great care. The question can, however, be analyzed from a cladistic point of view. If *Limnognathia* is considered sister group to Rotifera, or even sister group to the Monogononta, and possesses pleural rods and pseudomallei that are homologous with those found in different ploimid taxa, it implies that these sclerites were present in the rotifer or monogonont ground plan. Pleural rods and pseudomallei are only present in some rotifers, and the structures do not necessarily co-occur in the same species. *Lindia* has pseudomallei, but lack pleural rods, while *Birgea* has pseudounci but lacks other pseudomallei sclerites. If all these elements should be present in the rotiferan ground plan it would require numerous secondary reductions, and if the presence of fused unci and manubria is added to this ground plan the number of necessary character transformations increases even more. Hence, we do not agree with the statement by De Smet (2002): 'that the jaws of *Limnognathia* can be homologized easily with the trophi of the monogonont Rotifera'. These uncertainties clearly demonstrate that it is premature to include Micrognathozoa as a subtaxon in Rotifera. It is furthermore noteworthy that Micrognathozoa deviate from monogonont rotifers at several points.

The integument is, as noted above, syncytial in both Acanthocephala and Rotifera, whereas the micrognathozoan epidermis is cellular. The structure of the integuments of Micrognathozoa and Syndermata could be explained by the following evolution: the ancestor of Micrognathozoa + Syndermata acquired a dense apical intracytoplasmic lamina that perhaps served as a cytoskeleton, and after the deviation of Micrognathozoa, the epidermis in the syndermate ancestor became a syncytium. Bender & Kleinow (1988) have shown that the intracytoplasmic lamina consists of keratin-like proteins in *Brachionus*. The integument of Acanthocephala also contains keratin (Dunagan & Miller, 1991), while the biochemical composition of the lamina in Micrognathozoa is unknown.

The ventral epidermis in *Limnognathia* is ciliated but posterior to the mouth the epidermis secretes a conspicuous cuticular oral plate (Fig. 2, op). This is in contrast to all known rotifers, where the ciliation of the epidermis is confined to the anterior corona and where no external cuticular oral plate is known. It is, however, interesting that Micrognathozoa has a ventral ciliation like in Gastrotricha. According to Rieger (1976) Gnathostomulida and Gastrotricha represent the most primitive bilaterians with respect to the construction of their integument, and Beauchamp (1909, 1965) actually suggested that both rotifers and gastrotrichs evolved from a ciliated crawling ancestor.

The gut epithelium of the micrognathozoans lacks cilia and has a brush border of microvilli as in Seisonidea (Ricci et al., 1993; Ahlrichs, 1995b) and Gnathostomulida (Lammert, 1991). The gut epithelium in monogonont rotifers is both ciliated and with microvilli, while a syncytial stomach epithelium seems to be autapomorphic for bdelloids (Clément & Wurdak, 1991; Melone et al., 1998). Of importance is also the fact that the micrognathozoan body plan is compact and has no fluid-filled extracellular compartment such as the often spacious pseudocoel in Syndermata (Wallace et al., 1996).

The Micrognathozoa possesses protonephridia (Fig. 2, pr) with two pairs of terminal cells. Interestingly, all the cells of these organs are monociliated as in Gnathostomulida and some Gastrotricha but contrary to all protonephridial

cells investigated in Syndermata (Bartolomaeus & Ax, 1992).

The presence of paired gonads in both Bdelloidea and Seisonidea has been used as an argument to unite these two groups in the Digononta (Remane, 1929–1933; Pennak, 1989), but male Acanthocephala (Fig. 4) and Micrognathozoa (Fig. 2) have paired gonads as well (Dunagan & Miller, 1991; Kristensen & Funch, 2000). The characteristic vitellarium present in Monogononta and Bdelloidea are absent in *Seison* (Wallace & Colburn, 1989; Ricci et al., 1993), Micrognathozoa (Kristensen & Funch, 2000), and Acanthocephala (Monks, 2001) supporting monophyletic Eurotatoria. While bisexual reproduction is obligatory in Acanthocephala and *Seison*, males are unknown in Bdelloidea and Micrognathozoa. Hypodermic insemination is well known from monogonont rotifers (Wallace, 1999), but it is probably also occurring in gnathostomulids belonging to the Filospermoidea, a group that lack a vagina, have penises that seem unable to deliver the sperm, and have sperm with a spiralled head that could work as a drill when they rotate and thus actively go through the integument (Knauss & Rieger, 1979; Mainitz, 1989).

The dorsal and lateral antennae of Rotifera (Nogrady et al., 1993) are not present in Micrognathozoa (Kristensen & Funch, 2000). Also micrognathozoans seems to lack structures homologous to the retrocerebral and pedal glands of rotifers (Kristensen & Funch, 2000). The special adhesive pad situated at the ventral posterior trunk of Micrognathozoa (Fig. 2, ap) is very different in morphology compared to the pedal glands of rotifers and the cement glands of acanthocephalans (Fig. 4, ce).

Thus, Micrognathozoa differs from the Syndermata at several points, and their inclusion in Rotifera would require numerous reversals in the evolution of Micrognathozoa. Therefore, we still consider a sister group relationship between Micrognathozoa and Syndermata as the most likely (Fig. 6). However, it should be noted that Giribet et al. (2004) recently analyzed the phylogenetic position of Micrognathozoa using four molecular loci. They suggested that Micrognathozoa might constitute an independent lineage from those of Gnathostomulida and Syndermata.

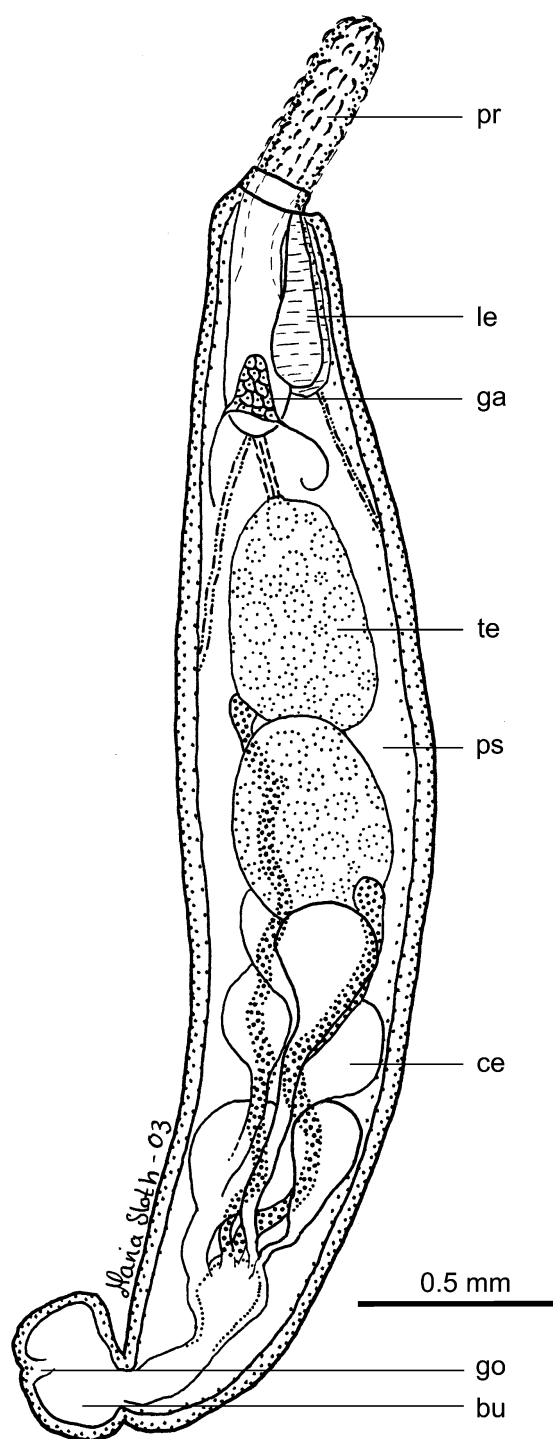


Figure 4. Drawing of male *Acanthocephalus dirus* (Acanthocephala). bu – bursa; ce – cement glands; ga – ganglion; go – gonopore; le – lemnisci; pr – proboscis; ps – pseudocoel; te – testes. Modified from Amin (1984).

Acanthocephala – gnathiferans without jaws and parasitic rotifers?

Adult acanthocephalans are endoparasites in the gut of vertebrates. They are unarticulated dioecious worms that attach themselves in the gut epithelium of the host with a retractile proboscis equipped with hooks (Fig. 4, pr). A general body cuticle is absent, collagen is present (Cain, 1970), and there is no trace of a digestive system. The epidermis is a syncytial tegument with a unique lacunar system. Males generally have paired testes and elaborate reproductive organs with cement glands, copulatory bursa, and penis (Fig. 4). Females develop free ovarian balls inside ligament sacs that sometimes rupture. The embryogenated eggs are sorted by the uterine bell. An acanthor larva develops from the fertilized egg and is capable of infecting an arthropod intermediate host. It invades the arthropod body cavity and develops into a larger acanthella stage in the right host. The growing acanthella develops the premature adult organs and inverts the proboscis. If the infected arthropod is eaten by a vertebrate the acanthella is capable of completing the life cycle by fixing itself to the gut epithelium of the vertebrate and grow into the adult (Hyman, 1951; Dunagan & Miller, 1991).

Evaluation of the phylogenetic position of Acanthocephala

Historically acanthocephalans were grouped with various parasitic worms such as Platyhelminthes, Nematoda, and Nematomorpha. Later they were often placed in the Aschelminthes together with Rotifera, Gastrotricha, Kinorhyncha, Nematoda, and Nematomorpha (Hyman, 1951; Ruppert & Barnes, 1994). More recent studies suggest that Aschelminthes is either paraphyletic or polyphyletic (Winnepenninckx et al., 1995; Ehlers et al., 1996; Ahlrichs, 1997). Although very different in morphology, acanthocephalans were early on suggested to be closely related to rotifers by Haffner (1950). This hypothesis gained further support from comparative ultrastructural studies on the syncytial integument (Storch & Welsch, 1969; Ahlrichs, 1997) and the sperm morphology (Ahlrichs, 1998; Ferraguti & Melone, 1999) and the clade Syndermata uniting Rotifera and

Acanthocephala was suggested by Ahlrichs (1995b). A monophyletic Syndermata is also supported in most analyses using molecular data (Garey et al., 1996; Garcia-Varela et al., 2000; Mark Welch, 2000; Giribet et al., 2004).

Conclusively, there are currently four competing theories concerning the sister taxon of the Acanthocephala. One hypothesis suggests that Acanthocephala is a sister group to Bdelloidea (Lorenzen, 1985), based on supposed homology between the acanthocephalan proboscis and lemnisci (Fig. 4, le, pr), and the bdelloid rostrum and sac-like syncytial organs, respectively, but the presence of such bdelloid structures in Acanthocephala has been questioned (Melone et al., 1998; Ricci, 1998). Nevertheless, several molecular studies actually support the hypothesis (Garey et al., 1996; Near et al., 1998; Garey et al., 1998; Near, 2002), but the genes and taxa chosen in these studies were stated to be problematic due to long-branch attraction (Garey et al., 1998; Near, 2002). A sister group relationship between Acanthocephala and Bdelloidea was also recovered in a study of triploblastic animals using 18S rDNA combined with morphology (Giribet et al., 2000) and further supported in a recent study using four molecular loci (Giribet et al., 2004). Although *Seison* is a key taxon to understand the phylogeny of the Syndermata it was not included in any of the molecular studies mentioned above.

In the study by Near (2002) only the maximum-parsimony analysis placed bdelloid rotifers as the sister group to Acanthocephala. When the maximum-likelihood optimality criterion was used with the same 18S rDNA data; it resulted in another hypothesis, namely monophyletic Rotifera as sister group to Acanthocephala. In a cladistic study that assumed monophyletic Rotifera such a relationship was inferred from morphological characters (Nielsen et al., 1996) and supported in another molecular study using 18S rDNA (Garcia-Varela et al., 2000). However, Near (2002) criticized the latter study for using dendrogram-based similarity alignments that were adjusted by eye.

In a study that included *Seison*, Mark Welsch (2000) found strong support for a third possible sister group relationship between Acanthocephala and Eurotatoria using the nuclear coding gene for the heat shock protein hsp82. This also was

supported by phylogenetic analyses using 18S rDNA (Miquelis et al., 2000). A cladistic study using morphological characters gave some additional support for this relationship (Sørensen et al., 2000), since spermatozoa of both monogononts and acanthocephalans lack an acrosome.

The fourth hypothesis suggests that Acanthocephala and *Seison* are sister groups supported by the presence of similar filament bundles in the epidermis and dense bodies in the spermatozoon (Ahlrichs, 1997, 1998). Though, Ahlrichs (1998) also showed the presence of an acrosome in *Seison*, a feature absent in the acanthocephalan spermatozoon (Dunagan & Miller, 1991). The Acanthocephala – *Seison* relationship got further support in a total evidence study by Zrzavý (2001) and a recent study based on 18S rDNA sequences with the inclusion of *Seison* (Herlyn et al., 2003).

In conclusion the support for monophyletic Syndermata is immense, while the sister group to the Acanthocephala is still not identified with certainty (Fig. 6).

Myzostomida – an annelid or a highly specialized gnathiferan?

Myzostomida is an enigmatic group of small marine worms that contains about 150 described species. Most myzostomids live in close association, as commensals or parasites, with asteroid and especially crinoid echinoderms. The animals can reach a size of several millimetres with a usually dorsoventral flattened body that can be elongated, oval, or irregular in shape (Fig. 5). Certain parts of the body are repeated along the anterior–posterior axis (Fig. 5, ci, lo, pa), which has traditionally been regarded as an indication of metamery. Five pairs of unarticulated appendages (parapodia) are present (Fig. 5, pa), normally containing a supportive rod and a protrusible hook. Usually myzostomids have pit-like lateral organs (Fig. 5, lo) that may vary in number and location, and have been interpreted as chemo- and mechanoreceptors (Eeckhaut & Jangoux, 1993). Most myzostomes have their mouth opening situated on a retractable proboscis (Fig. 5, mo, pr). The stomach extends into one or several

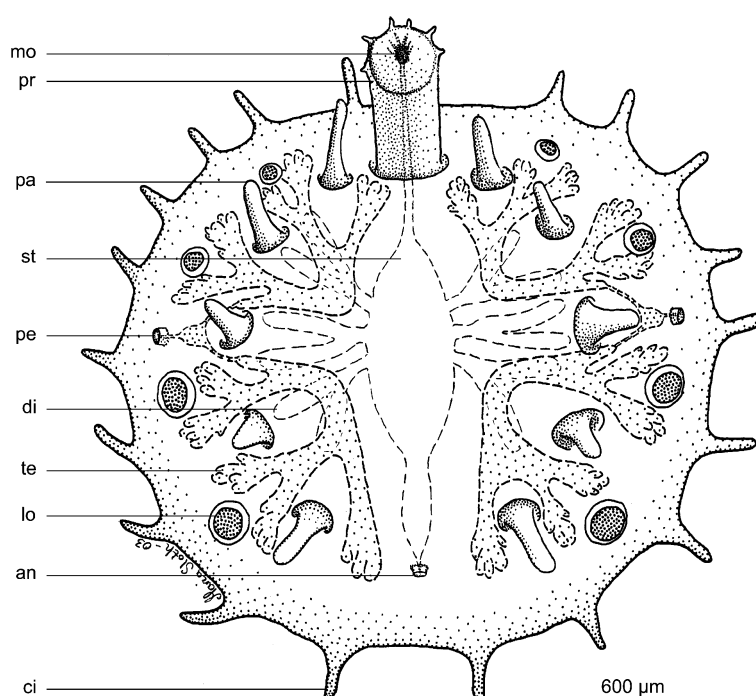


Figure 5. Drawing of *Myzostoma cirriferum* (Myzostomida), ventral view. an – anus; ci – cirrus; di – diverticulum; lo – lateral organ; mo – mouth opening; pa – parapodium; pe – penis; pr – proboscis; st – stomach; te – testis.

diverticula and a short intestine leads to a ventral anus (Fig. 5, an, di, st). Five pairs of protonephridia have been described from *Myzostoma cirriferum* (Pietsch & Westheide, 1987). The adult nervous system consists of a circumesophageal ring with two small cerebral ganglia, two nerve rings in the proboscis, and a ventral nerve cord. From here five pairs of nerves extend from the latter to the parapodia (Grygier, 2000; Müller & Westheide, 2000). The larval nervous system consists of five ventral longitudinal nerve cords (Eeckhaut et al., 2003). Most myzostomids are protandric hermaphrodites with paired testis diverticula that develop ventral to the digestive system (Fig. 5, te) and unpaired uterus diverticula that develop dorsally. The lumen of the ducts and branches of the reproductive system has been interpreted as the only remnants of a coelom (Jägersten, 1940). Some species have a pair of protrusible penises (Fig. 5, pe). In mature spermatozoa, the flagellum passes aside the main cell body and extends anteriorly (Afzelius, 1983; Grygier, 2000), a centriolar derivative is situated at their free end, and a nuclear membrane is absent. The chromatin is condensed into one or more rows of bead-like bodies. Sperms are transferred in a spermatophore during hypodermic impregnation (Eeckhaut & Jangoux, 1991). The development takes place via a free-swimming trochophore larva that temporarily develops chaetae as appendages.

Evaluation of phylogenetic affinity between Syndermata and Myzostomida

Myzostomida is one of the more problematic bilaterian taxa to place phylogenetically. The first scientist to describe a myzostomid regarded them as trematodes (Leuckart, 1827), but this idea is no longer considered valid. For a century Myzostomida have traditionally been placed within or close to Annelida (see Rouse & Fauchald, 1995), based on the presence of parapodia with chaetae, a trochophore larva and traces of segmentation (Fauchald & Rouse, 1997; Rouse & Fauchald, 1997). Recently, the group attracted special attention, and many authors have addressed the question of myzostomidan phylogenetic affinity.

Mattei & Marchand (1987, 1988) suggested a sister group relationship between Myzostomida and Acanthocephala based on similarities in spermatozoan ultrastructure and spermiogenesis.

However, similar sperm morphology is also known from other taxa such as monogonont and seisonid rotifers (Melone & Ferraguti, 1994; Ahlrichs, 1998; Ferraguti & Melone, 1999). More support for a myzostomid affinity to Syndermata emerged when Zrzavý et al. (2001) analyzed combined morphological and molecular data (18S and 28S rDNA) and found evidence for a monophyletic clade including Myzostomida, Cycliophora, and Syndermata. Presumably the group was supported by synapomorphic presence of spermatozoa with anteriorly directed flagellum and no accessory centriole, although cycliophoran sperm is insufficiently known (Funch & Kristensen, 1997).

More evidence for a non-annelid relationship emerged in a cladistic analysis by Eeckhaut et al. (2000) who presented molecular evidence that myzostomids are closer related to Platyhelminthes than to Annelida. Zrzavý et al. (2001) argued that the characters supporting an annelid origin of myzostomids are usually either symplesiomorphic or convergent. Furthermore, the putative teloblastic growth of myzostomids needs to be confirmed, and the absence of an obvious coelom as well as the observed variation in the number of lateral organs suggests that 'segmented' patterns are only superficial.

On the contrary other studies have confirmed a strong annelid affinity. Müller & Westheide (2000) showed that the nervous system of *Myzostoma* is polychaete-like with obvious signs of segmentation and suggested to place them within Annelida. In a cladistic analysis by Rouse & Fauchald (1997) myzostomids are placed as a family nested within the polychaetes.

In conclusion, myzostomid relationships are unresolved (Zrzavý, 2003; Zrzavý & Hypša, 2003), but some evidence suggests that they are not within annelids (Haszprunar, 1996b; Eeckhaut et al., 2000). A recent study by Eeckhaut et al. (2003) showed several homologies between myzostomid and polychaete trochophores. However, the authors argued that these might be plesiomorphic and appeared early during the evolution of Spiralia.

The gnathiferan ground pattern and phylogenetic position

The phylogenetic position of Gnathifera is far from being resolved and different hypotheses

about the metazoan phylogeny are being published instantly. The problem becomes even more puzzling because of the uncertainties about the gnathiferan ground pattern. Even though the possession of jaws appears to be a strong gnathiferan synapomorphy (Fig. 6), the basic body plan of, e.g., rotifers and gnathostomulids differs greatly. Rotifera possesses typical larval traits, such as the arrangement of the ciliary bands that resembles the trochophoran pattern with cilia forming a prototroch, metatroch, gastrotroch and telotroch (Nielsen, 1987), and this leads to the idea that the Gnathifera evolved by progenesis from an annelid-like ancestor, and hence were closely related with the trochozoans. However, it is difficult to explain how the gnathostomulids with their simple, monociliated epithelium and lack of distinct ciliary bands could be descendants of such an ancestor. Alternatively, the ancestor could have had a biphasic life cycle with a gnathostomulid-like, acoelomate adult and a trochophore-like larva. In this case one could imagine that the recent Gnathostomulida resemble the adult gnathiferan ancestor, but have undergone some modifications, such as, loss of larval stage and reversal to monociliated epidermis, whereas Micrognathozoa and Rotifera evolved from the ancestral larva by progenesis. However, this solution is highly speculative, and it has both advantages and disadvantages. If the gnathiferan evolution has followed this schedule it would support earlier proposed relationships with Platyhelminthes, Gastrotricha or both, and it would also explain the similarities in the platyhelminth and gnathostomulid body plans. The theory is, however, weakened by the fact that neither Platyhelminthes nor Gastrotricha appear to have a biphasic life cycle as a basal trait. Nielsen (1987) discussed some structural similarities between trochophores and the polyclad Müller's larva, but it is important to note that no platyhelminth phylogeny places Polycladia as a basal taxon, so it is most likely that Müller's larva is unique, and has evolved within the Platyhelminthes.

Gnathifera has been placed as a sister group to the Platyhelminthes (Ahlrichs, 1995b; Garey et al., 1998; Melone et al., 1998) or as a member of a clade Platyzoa also containing Platyhelminthes and Gastrotricha (see Giribet et al., 2000). An

alternative position of Gnathifera as the most basal spiralian group was suggested by Sørensen et al. (2000) and later supported by some of the analyses presented by Zrzavý (2003).

The position of Gnathifera as a basal spiralian group is not unlikely, but it should be noted that to this point it is poorly supported. In the analysis of Sørensen et al. (2000) the Spiralia (*viz* Euspiralia + Gnathifera) are supported by the presence of egg cleavage with a spiralian cleavage pattern and ciliary bands formed by multiciliate cells that use a downstream system for suspension feeding (for further explanation see Nielsen, 1987, 2001; Riisgård et al., 2000). However, both characters may be problematic for Gnathifera. The cleavage pattern for Syndermata is far from being unambiguous spiralian and the assumed spiral cleavage in Gnathostomulida is based on a single observation (see Riedl, 1969) and needs to be confirmed with modern methods. Downstream collecting ciliary bands are certainly present in Rotifera (Nielsen, 1987), but if this is a basal trait for Gnathifera, it would require that radical modifications have happened on the branch that leads to Gnathostomulida. Also, the splits that take place in Euspiralia after deviation of Gnathifera are weakly supported. Euspiralia are solely supported by the shift from monociliated to multiciliated protonephridial terminal cells, and the following clade that comprises Teloblastica and Nemertea + Platyhelminthes simply lacks unambiguous support.

A sister group relationship between Platyhelminthes and Gnathifera has been supported by many authors and most recently by Ahlrichs (1995a, b, 1997) and Melone et al. (1998). However, Jenner (2004) recently reviewed the proposed synapomorphies for this clade and concluded that all of them were highly homoplastic characters that only could support Platyhelminthes + Gnathifera in pruned trees with a highly selective taxon choice.

The clade Platyzoa has been supported by molecular data (Winnepeninckx et al., 1995; Giribet, 2002), but the morphological support for this clade is scarce. Rieger (1976) showed that the epidermis of some macrodasyid gastrotrichs shares some striking similarities with the monociliated epidermis found in Gnathostomulida. He considered this as a plesiomorphic condition

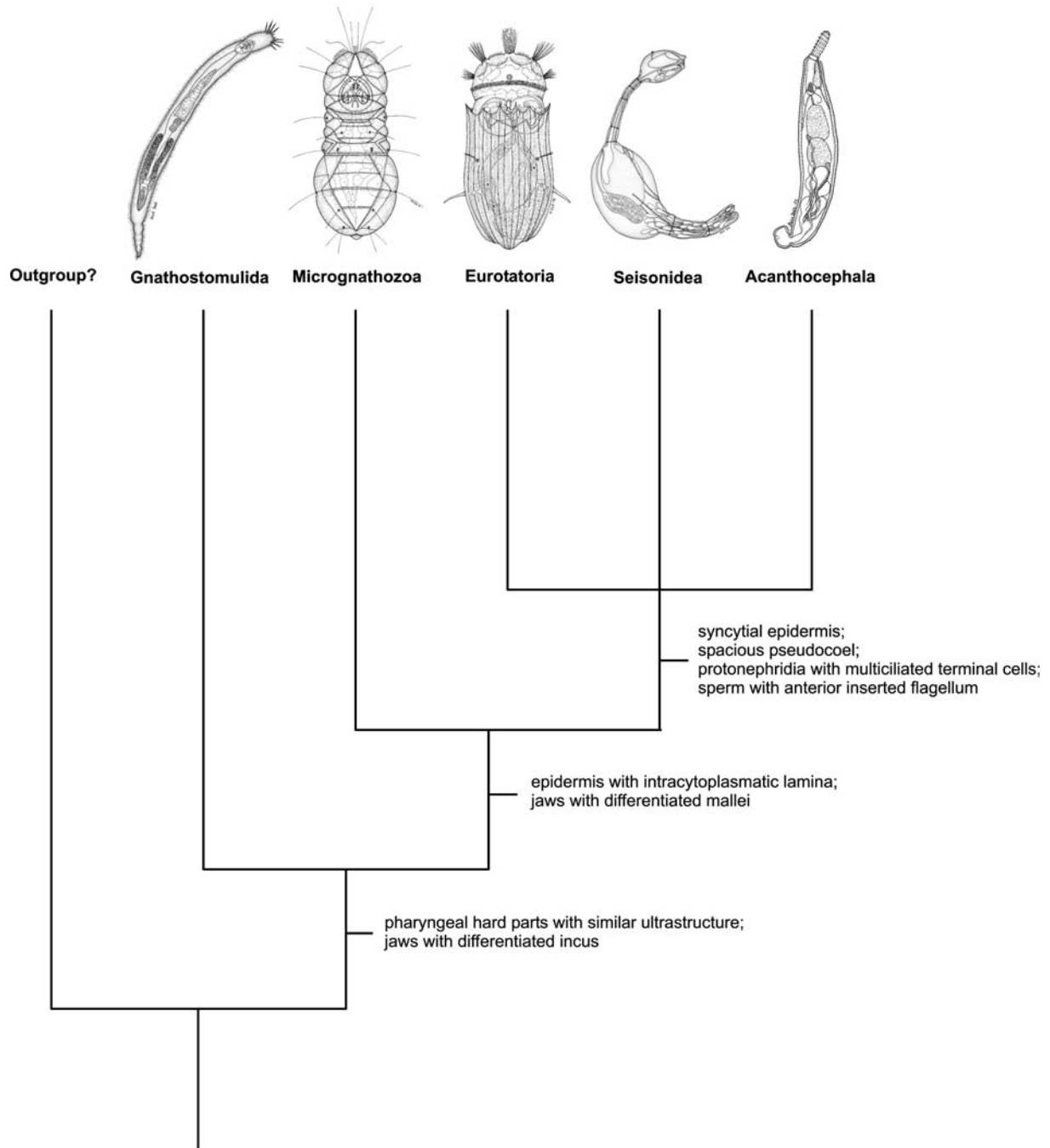


Figure 6. Phylogeny of the Gnathifera.

that indicated a basal position in Bilateria for both taxa. However, there are still conflicting opinions about the basal ciliary pattern in Gastrotricha (see Hochberg and Litvaitis, 2000;

Zrzavý, 2003) and the question is at present unresolved (M. A. Todaro personal communication). Platyzoan monophyly also is challenged by the strong affinities between Gastrotricha and

Ecdysozoa (see Schmidt-Rhaesa et al., 1998; Zrzavý, 2003).

Thus the phylogenetic position of Gnathifera remains uncertain. Further data are needed to determine if any of the three possibilities discussed above should be favoured. New information and interpretations of the cleavage pattern in the gnathiferan groups could turn out to be extremely valuable, and a re-evaluation of the morphology in the platyzoan taxa could be interesting in light of the results that have been generated from molecular evidence.

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References

- Afzelius, B. A., 1983. The spermatozoon of *Myzostomum cirriferum* (Annelida, Myzostomida). *Journal of Ultrastructure Research* 83: 58–68.
- Ahlrichs, W. H., 1995a. *Seison annulatus* und *Seison nebaliae*. Ultrastruktur und Phylogenie. *Verhandlungen der Deutschen Zoologischen Gesellschaft* 88: 155.
- Ahlrichs, W. H., 1995b. Ultrastruktur und Phylogenie von *Seison nebaliae* (Grube 1859) und *Seison annulatus* (Claus 1876). Cuvillier Verlag, Göttingen, 310 pp.
- Ahlrichs, W. H., 1997. Epidermal ultrastructure of *Seison nebaliae* and *Seison annulatus*, and a comparison of epidermal structures within the Gnathifera. *Zoomorphology* 117: 41–48.
- Ahlrichs, W. H., 1998. Spermatogenesis and ultrastructure of the spermatozoa of *Seison nebaliae* (Syndermata). *Zoomorphology* 118: 255–261.
- Aloia, R. C. & R. L. Moretti, 1973. Ultrastructural analysis of the functional copulatory organ of the male rotifer, *Asplanchna brightwelli*. *Journal of Morphology* 140: 285–306.
- Amin O. M., 1984. Variability and redescription of *Acanthocephalus dirus* (Acanthocephala: Echinorhynchidae) from freshwater fishes in North America. *Proceedings of the Helminthological Society of Washington* 51: 225–237.
- Ax, P., 1956. Die Gnathostomulida, eine rätselhafte Wurmgruppe aus dem Meeressand. *Abhandlungen der Akademie der Wissenschaften und der Literatur in Mainz, mathematisch-naturwissenschaftliche Klasse* 8: 1–32.
- Bartolomaeus, T. & P. Ax, 1992. Protonephridia and metanephridia - their relation within the Bilateria. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 30: 21–45.
- Beauchamp, P. d., 1909. Recherches sur les Rotifères: Les formations tégumentaires et l'appareil digestif. *Archives De Zoologie Experimentale Et Generale* 10: 1–410.
- Beauchamp, P. d., 1965. Classe des Rotifère. In Grassé, P. P. (ed.), *Traité de Zoologie, Anatomie, Systématique, Biologie*. Masson, Paris: 1225–1379.
- Bender, K. & W. Kleinow, 1988. Chemical properties of the lorica and related parts from the integument of *Brachionus plicatilis*. *Comparative Biochemistry and Physiology B-Biochemistry and Molecular Biology* 89: 483–487.
- Cain, G. D., 1970. Collagen from the giant acanthocephalan *Macracanthorhynchus hirudinaceus*. *Archives of Biochemistry and Biophysics* 141: 264–270.
- Cavalier-Smith, T., 1998. A revised six-kingdom system of life. *Biological Reviews* 73: 203–266.
- Clément, P., 1985. The relationships of rotifers. In Conway-Morris, S., J. D. George, R. Gibson, & H. M. Platt (eds), *The Origins and Relationships of Lower Invertebrates*. Systematic Association, Clarendon Press, Oxford: 224–247.
- Clément, P. & E. Wurdak, 1991. Rotifera. In Harrison, F. W. & E. Ruppert (eds), *Microscopic Anatomy of Invertebrates*, 4 Wiley-Liss, New York: 219–298.
- Clément, P., E. Wurdak & J. Amsellem, 1983. Behavior and ultrastructure of sensory organs in rotifers. *Hydrobiologia* 104: 89–129.
- De Smet, W. H., 2002. A new record of *Linnognathia maerski* Kristensen and Funch, 2000 (Micrognathozoa) from the subantarctic Crozet Islands, with redescription of the trophi. *Journal of Zoology* 258: 381–393.
- Dunagan, T. & D. M. Miller, 1991. Acanthocephala. In Harrison, F. W. & E. Ruppert (eds), *Microscopic Anatomy of Invertebrates* 4. Wiley-Liss, New York: 299–332.
- Eeckhaut, I., L. Fievez & M. C. Müller, 2003. Larval development of *Myzostoma cirriferum* (Myzostomida). *Journal of Morphology* 258: 269–283.
- Eeckhaut, I. & M. Jangoux, 1991. Fine-structure of the spermatophore and intradermic penetration of sperm cells in *Myzostoma cirriferum* (Annelida, Myzostomida). *Zoomorphology* 111: 49–58.
- Eeckhaut, I. & M. Jangoux, 1993. Integument and epidermal sensory structures of *Myzostoma cirriferum* (Myzostomida). *Zoomorphology* 113: 33–45.
- Eeckhaut I., D. McHugh, P. Mardulyn, R. Tiedemann, D. Monteyne, M. Jangoux & M. C. Milinkovitch, 2000. Myzostomida: a link between trochozoans and flatworms? *Proceedings of the Royal Society of London Series B-Biological Sciences* 267: 1383–1392.
- Ehlers, U., W. H. Ahlrichs, C. Lemburg & A. Schmidt-Rhaesa, 1996. Phylogenetic systematization of the Nematelminthes (Aschelminthes). *Verhandlungen der Deutschen Zoologischen Gesellschaft* 89: 8.

- Fauchald, K. & G. Rouse, 1997. Polychaete systematics: Past and present. *Zoologica Scripta* 26: 71–138.
- Ferraguti, M. & M. Balsamo, 1994. Sperm morphology and anatomy of the genital organs in *Mesodasys laticaudatus* Remane, 1951 (Gastrotricha, Macrotrichida). *Journal of Submicroscopic Cytology and Pathology* 26: 21–28.
- Ferraguti, M. & G. Melone, 1999. Spermiogenesis in *Seison nebaliae* (Rotifera, Seisonidea): further evidence of a rotifer-acanthocephalan relationship. *Tissue and Cell* 31: 428–440.
- Funch, P., 1996. The chordoid larva of *Symbion pandora* (Cycliophora) is a modified trochophore. *Journal of Morphology* 230: 231–263.
- Funch, P. & R. M. Kristensen, 1995. Cycliophora is a new phylum with affinities to Entoprocta and Ectoprocta. *Nature* 378: 711–714.
- Funch, P. & R. M. Kristensen, 1997. Cycliophora. In Harrison, F. W. & R. M. Woollacott (eds), *Microscopic Anatomy of Invertebrates* 13. Wiley-Liss, New York: 409–474.
- García-Varela, M., G. P. P. Leon, P. la Torre, M. P. Cummings, S. Sarma & J. P. Lacleste, 2000. Phylogenetic relationships of Acanthocephala based on analysis of 18S ribosomal RNA gene sequences. *Journal of Molecular Evolution* 50: 532–540.
- Garey, J. R., T. J. Near, M. R. Nonnemacher & S. A. Nadler, 1996. Molecular evidence for Acanthocephala as a subtaxon of Rotifera. *Journal of Molecular Evolution* 43: 287–292.
- Garey, J. R., A. Schmidt-Rhaesa, T. J. Near & S. A. Nadler, 1998. The evolutionary relationships of rotifers and acanthocephalans. *Hydrobiologia* 387/388: 83–91.
- Gilbert, J. J., 1983. Rotifera. In Adiyodi, K. G. & R. G. Adiyodi (eds), *Reproductive Biology of Invertebrates* 2. John Wiley and Sons, New York: 181–193.
- Giribet, G., 2002. Current advances in the phylogenetic reconstruction of metazoan evolution. A new paradigm for the Cambrian explosion? *Molecular Phylogenetics and Evolution* 24: 345–357.
- Giribet, G., D. L. Distel, M. Polz, W. Sterrer & W. C. Wheeler, 2000. 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. *Systematic Biology* 49: 539–562.
- Giribet, G., M. V. Sørensen, P. Funch, R. M. Kristensen & W. Sterrer, 2004. Investigations into the phylogenetic position of Micrognathozoa using four molecular loci. *Cladistics* 20: 1–13.
- Grygier, M. J., 2000. Class Myzostomida. In Beesley, P. L., G. J. B. Ross, & C. J. Glasby (eds), *Polychaetes and Allies: The Southern Synthesis* 4A. CSIRO, Melbourne: 297–329.
- Haffner, K. v., 1950. Organisation und systematische Stellung der Acanthocephalen. *Zoologischer Anzeiger (Supplement)* 145: 243–274.
- Haszprunar, G., 1996a. Platyhelminthes and Platyhelminthomorpha - paraphyletic taxa. *Journal of Zoological Systematics and Evolutionary Research* 34: 41–48.
- Haszprunar, G., 1996b. The Mollusca: coelomate turbellarians or mesenchymate annelids? In Taylor, J. (ed.), *Origin and Evolutionary Radiation of the Mollusca*. Oxford University Press, Oxford: 3–28.
- Herlyn, H., O. Piskurek, J. Schmitz, U. Ehlers & H. Zischler, 2003. The syndermatan phylogeny and the evolution of acanthocephalan endoparasitism as inferred from 18S rDNA sequences. *Molecular Phylogenetics and Evolution* 26: 155–164.
- Hermes, G., 1932. Studien über die Konstanz histologischer Elemente. IV. Die Männchen von *Hydatina senta* Ehrenberg, *Rhinops vitrea* Hudson und *Asplanchna priodonta* Gosse. *Zeitschrift für Wissenschaftliche Zoologie* 141: 581–725.
- Hochberg, R. & M. K. Litvaitis, 2000. Phylogeny of Gastrotricha: a morphology-based framework of gastrotrich relationships. *Biological Bulletin* 198: 299–305.
- Hyman, L. H., 1951. *The Invertebrates. Acanthocephala, Aschelminthes and Entoprocta. The Pseudocoelomate Bilateria*, McGraw-Hill, New York, 572 pp.
- Jägersten, G., 1940. Zur Kenntnis der Morphologie, Entwicklung und Taxonomie der Myzostomida. *Nova Acta Regiae Societatis Scientiarum Upsaliensis* 11: 1–84.
- Jenner, R. A., 2004. Towards a phylogeny of the Metazoa: evaluating alternative phylogenetic positions of Platyhelminthes, Nemertea, and Gnathostomulida, with a critical reappraisal of cladistic characters. *Contributions to Zoology* 73: 3–163.
- Jondelius, U., I. Ruiz-Trillo, J. Baguna & M. Riutort, 2002. The Nemertodermatida are basal bilaterians and not members of the Platyhelminthes. *Zoologica Scripta* 31: 201–215.
- Knauss, E. B. & R. M. Rieger, 1979. Fine structure of the male reproductive system in two species of *Haplognathia* Sterrer (Gnathostomulida, Filospermoidea). *Zoomorphologie* 94: 33–48.
- Kristensen, R. M. & P. Funch, 2000. Micrognathozoa: a new class with complicated jaws like those of Rotifera and Gnathostomulida. *Journal of Morphology* 246: 1–49.
- Lammert, V., 1991. Gnathostomulida. In Harrison, F. W. & E. Ruppert (eds) *Microscopic Anatomy of Invertebrates*. 4. Wiley-Liss, New York: 19–39.
- Leuckart, F. S., 1827. Versuch einer naturgemässen Einteilung der Helminthen nebst den Entwurf einer Verwandtschafts- und Stufenfolge der Thiere ueberhaupt. *Neue Akademische Buchhandlung von Karl Gross, Heidelberg und Leipzig*, 88 pp.
- Littlewood, D. T. J., M. J. Telford, K. A. Clough & K. Rohde, 1998. Gnathostomulida – An enigmatic metazoan phylum from both morphological and molecular perspectives. *Molecular Phylogenetics and Evolution* 9: 72–79.
- Lorenzen, S., 1985. Phylogenetic aspects of pseudocoelomate evolution. In Conway-Morris, S., J. D. George, R. Gibson & H. M. Platt (eds), *The Origins and Relationships of Lower Invertebrates*. Systematic Association, Clarendon Press, Oxford: 210–223.
- Mainitz, M., 1989. Gnathostomulida. In Adiyodi, K. G. & R. G. Adiyodi (eds), *Reproductive Biology of Invertebrates* John Wiley and Sons, Chichester: 167–177.
- Mark Welch, D. B., 2000. Evidence from a protein-coding gene that acanthocephalans are rotifers. *Invertebrate Biology* 119: 17–26.
- Mattei, X. & B. Marchand, 1987. Les spermatozoïdes des Acanthocéphales et des Myzostomides. Ressemblances et conséquences phylétiques. *Comptes Rendus de l'Académie des Sciences Serie III Sciences de la Vie* 305: 525–529.

- Mattei, X. & B. Marchand, 1988. La spermiogenese de *Myzostomum* sp. (Procoelomata, Myzostomida). *Journal of Ultrastructure and Molecular Structure Research* 100: 75–85.
- Melone, G. & M. Ferraguti, 1994. The spermatozoon of *Brachionus plicatilis* (Rotifera, Monogononta) with some notes on sperm ultrastructure in rotifera. *Acta Zoologica* 75: 81–88.
- Melone, G., C. Ricci, H. Segers & R. L. Wallace, 1998. Phylogenetic relationships of phylum Rotifera with emphasis on the families of Bdelloidea. *Hydrobiologia* 387/388: 101–107.
- Miquelis, A., J. F. Martin, E. W. Carson, G. Brun & A. Gilles, 2000. Performance of 18S rDNA helix E23 for phylogenetic relationships within and between the Rotifera–Acanthocephala clades. *Comptes Rendus de l'Academie Des Sciences. Serie III, Sciences de la Vie* 323: 925–941.
- Monks, S., 2001. Phylogeny of the Acanthocephala based on morphological characters. *Systematic Parasitology* 48: 81–116.
- Müller, M. C. & W. Westheide, 2000. Structure of the nervous system of *Myzostoma cirriferum* (Annelida) as revealed by immunohistochemistry and cLSM analyses. *Journal of Morphology* 245: 87–98.
- Near, T. J., 2002. Acanthocephalan phylogeny and the evolution of parasitism. *Integrative and Comparative Biology* 42: 668–677.
- Near, T. J., J. R. Garey & S. A. Nadler, 1998. Phylogenetic relationships of the Acanthocephala inferred from 18S ribosomal DNA sequences. *Molecular Phylogenetics and Evolution* 10: 287–298.
- Nielsen, C., 1987. Structure and function of metazoan ciliary bands and their phylogenetic significance. *Acta Zoologica* 68: 205–262.
- Nielsen, C., 2001. *Animal Evolution: Interrelationships of the Living Phyla*. Oxford University Press, Oxford, 563 pp.
- Nielsen, C., N. Scharff & D. Eiby-Jacobsen, 1996. Cladistic analyses of the animal kingdom. *Biological Journal of the Linnean Society* 57: 385–410.
- Nogrady, T., R. L. Wallace & T. W. Snell, 1993. *Rotifera: Biology, Ecology and Systematics*. SPB Academic Publishing, Amsterdam, 142 pp.
- Obst, M. & P. Funch, 2003. The dwarf male of *Symbion pandora* (Cycliophora). *Journal of Morphology* 255: 261–278.
- Pennak, R. W., 1989. *Fresh water invertebrates of the United States*. John Wiley, New York.
- Peterson, K. J. & D. J. Eernisse, 2001. Animal phylogeny and the ancestry of bilaterians: inferences from morphology and 18S rDNA gene sequences. *Evolution and Development* 3: 170–205.
- Pietsch, A. & W. Westheide, 1987. Protonephridial organs in *Myzostoma cirriferum* (Myzostomida). *Acta Zoologica* 68: 195–203.
- Remane, A., 1929–1933. *Rotatoria*. Akademische Verlagsgesellschaft mbH, Leipzig, 576 pp.
- Reisinger, E., 1961. *Morphologie der Coelenteraten, acoelomaten und pseudocoelomaten Würmer*. *Fortschritte der Zoologie* 13: 1–82.
- Ricci, C., 1998. Are lemnisci and proboscis present in the Bdelloidea? *Hydrobiologia* 387/388: 93–96.
- Ricci, C., G. Melone & C. Sotgia, 1993. Old and new data on Seisonidea (Rotifera). *Hydrobiologia* 255/256: 495–511.
- Riedl, R. J., 1969. Gnathostomulida from America. *Science* 163: 445–452.
- Rieger, R. M., 1976. Monociliated epidermal cells in Gastrotricha: significance for concepts of early metazoan evolution. *Zeitschrift für Zoologische Systematik und Evolutionsforschung* 14: 198–226.
- Rieger, R. M. & S. Tyler, 1995. Sister-group relationship of Gnathostomulida and Rotifera–Acanthocephala. *Invertebrate Biology* 114: 186–188.
- Riisgård, H. U., C. Nielsen & P. S. Larsen, 2000. Downstream collecting in ciliary suspension feeders: the catch-up principle. *Marine Ecology-Progress Series* 207: 33–51.
- Rouse, G. W. & K. Fauchald, 1995. The articulation of annelids. *Zoologica Scripta* 24: 269–301.
- Rouse, G. W. & K. Fauchald, 1997. Cladistics and polychaetes. *Zoologica Scripta* 26: 139–204.
- Ruppert E. E. & R. D. Barnes, 1994. *Invertebrate Zoology*. Saunders College Publishing, New York, 1056 pp.
- Schmidt-Rhaesa, A., T. Bartolomaeus, C. Lemburg, U. Ehlers & J. R. Garey, 1998. The position of the Arthropoda in the phylogenetic system. *Journal of Morphology* 238: 263–285.
- Storch, V. & U. Welsch, 1969. Über den Aufbau des Rotatorienintegumentes. *Zeitschrift für Zellforschung und Mikroskopische Anatomie* 95: 405–414.
- Storch, V. & U. Welsch, 1970. Über den Aufbau resorbierender Epithelien darmloser Endoparasiten. *Zoologischer Anzeiger (Supplement)* 33: 617–621.
- Sørensen, M. V., 2000. An SEM study of the jaws of *Haplognathia rosea* and *Rastrognathia macrostoma* (Gnathostomulida), with a preliminary comparison with rotiferan trophi. *Acta Zoologica* 81: 9–16.
- Sørensen, M. V., 2002. On the evolution and morphology of the rotiferan trophi, with a cladistic analysis of Rotifera. *Journal of Zoological Systematics and Evolutionary Research* 40: 129–154.
- Sørensen, M. V., 2003. Further structures in the jaw apparatus of *Limnognathia maerski* (Micrognathozoa), with notes on the phylogeny of the Gnathifera. *Journal of Morphology* 255: 131–145.
- Sørensen, M. V., P. Funch, E. Willerslev, A. J. Hansen & J. Olesen, 2000. On the phylogeny of the metazoa in the light of Cycliophora and Micrognathozoa. *Zoologischer Anzeiger* 239: 297–318.
- Sørensen, M. V. & W. Sterrer, 2002. New characters in the gnathostomulid mouth parts revealed by scanning electron microscopy. *Journal of Morphology* 253: 310–334.
- Wallace, R. L., 1999. Rotifera. In Knobil, E. & J. D. Neil (eds), *Encyclopaedia of Reproduction*. Academic Press, New York: 290–301.
- Wallace, R. L. & R. A. Colburn, 1989. Phylogenetic relationships within phylum Rotifera - orders and genus *Notholca*. *Hydrobiologia* 186: 311–318.
- Wallace, R. L., C. Ricci & G. Melone, 1996. A cladistic analysis of pseudocoelomate (aschelminth) morphology. *Invertebrate Biology* 115: 104–112.
- Wesenberg-Lund, C., 1923. Contributions to the biology of the Rotifera. I. The males of the Rotifera. *Det Kongelige*

- Danske Videnskabernes Selskabs Skrifter, Naturvidenskabelig og Matematisk Afdeling. 8. Række 4: 189–345.
- Winnepenninckx, B. M. H., T. Backeljau & R. M. Kristensen, 1998. Relations of the new phylum Cycliophora. *Nature* 393: 636–638.
- Winnepenninckx, B. M. H., T. Backeljau, L. Y. Mackey, J. M. Brooks, R. DeWachter, S. Kumar & J. R. Garey, 1995. 18S rRNA data indicate that Aschelminthes are polyphyletic in origin and consist of at least three distinct clades. *Molecular Biology and Evolution* 12: 1132–1137.
- Zrzavý, J., 2001. The interrelationships of metazoan parasites: a review of phylum- and higher-level hypotheses from recent morphological and molecular phylogenetic analyses. *Folia Parasitologica* 48: 81–103.
- Zrzavý, J., 2003. Gastrotricha and metazoan phylogeny. *Zoologica Scripta* 32: 61–81.
- Zrzavý, J. & V. Hypša, 2003. Myxozoa, *Polypodium*, and the origin of the Bilateria: The phylogenetic position of “Endocnidozoa” in the light of the rediscovery of *Buddenbrockia*. *Cladistics* 19: 164–169.
- Zrzavý, J., V. Hypša & D. F. Tietz, 2001. Myzostomida are not annelids: Molecular and morphological support for a clade of animals with anterior sperm flagella. *Cladistics* 17: 170–198.
- Zrzavý, J., S. Mihulka, P. Kepka, A. Bezdek & D. Tietz, 1998. Phylogeny of the Metazoa based on morphological and 18S ribosomal DNA evidence. *Cladistics* 14: 249–285.