

Two Dimensions of Biodiversity Research Exemplified by Nematomorpha and Gastrotricha¹

ANDREAS SCHMIDT-RHAESA²

Zoomorphology and Systematics, University Bielefeld, P.O. Box 100131, 33501 Bielefeld, Germany

SYNOPSIS. Biodiversity research combines two dimensions, the horizontal one that contains species diversity, patterns among this diversity and its interconnections and the vertical one that deals with the history of biodiversity, *i.e.*, its phylogeny. With these tight interconnections, the importance of so-called “lesser known groups” such as Nematomorpha and Gastrotricha can be shown. Two examples are the life cycle of Nematomorpha and the phylogenetic position of Gastrotricha. The life cycle of Nematomorpha is only partially known and almost no conclusions can be made about the impact of Nematomorpha on their hosts. For the phylogenetic position of Gastrotricha, alternative hypotheses are available, mainly due to different results of morphological and molecular (18S rDNA) analyses. It is demonstrated how these different hypotheses influence character interpretation and reconstruction among Protostomia (Gastroneuralia).

INTRODUCTION

Besides direct importance to our human species such as medical or economic uses, the inherent value of nature itself has been increasingly realized. This is summarized in the keyword *biodiversity*. We recognize a diversity of living organisms in nature as the product of a historical process and whose parts interconnect with each other and with their environment. Knowledge of this diversity, its history and interconnections is essential in understanding and anticipating the effects of disturbances in this system such as habitat destruction or the exchange of organisms between different localities.

This leads to a concept for biodiversity research. Biodiversity is characterized by two dimensions: the horizontal and the vertical one (Fig. 1). In the center of the horizontal dimension is the recognition of primary units, usually species. This includes theoretical (such as species concepts) and practical aspects (such as recognizing polymorphic and sibling species). Among the diversity of species, patterns may be recognized, such as biogeographical patterns or patterns of morphological, genetical, ecological and other similarities. Finally, interconnections between and within species as well as connections to the environment have to be understood. Current biodiversity is the result of a historical process and all extant species are connected phylogenetically through time. This is the topic of the vertical dimension of biodiversity research which covers phylogenetic relationships and other evolutionary research such as paleontology and population genetics.

To illustrate this approach to biodiversity, two examples are given from small, understudied taxa. These are the horsehair worms or Nematomorpha and the Gastrotricha. As parasites during one phase of their life cycle, nematomorphs have an impact on their hosts.

Can we estimate this impact? To answer this question it is necessary to review how much we know about the life cycle of nematomorphs. This is used as an example of the horizontal dimension of biodiversity research, because the nematomorph life cycle is a good example for parasite-host relationships and therefore for connections of one taxon with another. Gastrotrichs are likely to be very basal bilaterian animals. As such, they have an impact on the reconstruction of the bilaterian ancestor and on the interpretation of character evolution within Bilateria. To illustrate the vertical dimension of biodiversity research, I will review what we know about the position of Gastrotricha within the Bilateria. Although both Nematomorpha and Gastrotricha are “lesser known groups,” it will be shown that detailed knowledge is important in understanding more general biodiversity patterns and processes.

THE LIFE CYCLE OF NEMATOMORPHA

The Nematomorpha is a monophyletic taxon of parasitic worms that develop in their hosts but reproduce in aquatic environments. About 300 species have been described from freshwater (taxon Gordiida, Fig. 2A) while only five species are marine (genus *Nectonema*). Nematomorphs are dioecious, they reproduce with true copulation (*Nectonema*) or pseudocopulation (Gordiida) (Schmidt-Rhaesa, 1999). From eggs, tiny larvae (about 100 μm) hatch (Fig. 2C). They are equipped with two or three rings of cuticular hooks and terminal stylets with which they are able to penetrate soft epithelia of their hosts.

Knowledge about the nematomorph life cycle is crucial for a number of topics such as their impact on host populations, but still there are numerous open questions. In spite of the fact that the nematomorph life cycle is similar to that of mermithid nematodes, details of the nematomorph life cycle are distinctly less well known. One reason for the advanced study of mermithids is that some species parasitize mosquitoes and have been tested as biological parasiticide agents against mosquito-transferred diseases such as malaria (Platzer, 1981; Yu, 1986; Gafurov *et al.*, 1987; Rojas *et al.*, 1987).

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² E-mail: a.schmidt-rhaesa@biologie.uni-bielefeld.de

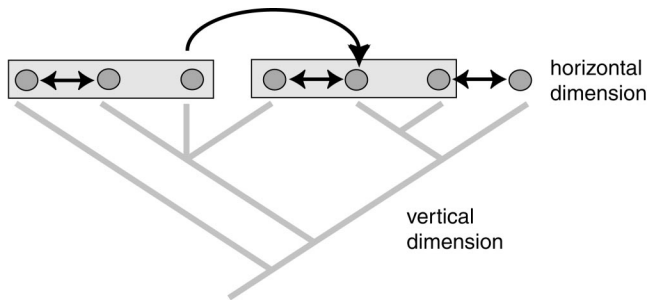


FIG. 1. The approach to biodiversity research used in the paper: species (dots) are interconnected in the horizontal and in the vertical dimension (for further explanations see text).

Observations concerning the life cycle of Nematomorpha are more or less sporadic and few systematic approaches have been made. There are numerous host reports for larval and juvenile nematomorphs (the term “juvenile” is used here for wormlike stages in the parasitic phase, the term “larva” only for the morphologically distinct earliest stage of development). Early reports, such as the lists by Siebold (1842) seem to be of limited reliability, because at that time gordiids and mermithids were often included into one taxon and not further distinguished. Hosts reported for gordiid larvae are quite different from hosts of juveniles: while almost any aquatic animal (*e.g.*, insect larvae, molluscs, annelids, fishes, frogs) may contain encysted larvae, emergence occurs mostly in insects and less often in other arthropods or (in two cases) in leeches (see Schmidt-Rhaesa, 1997, 2001a). The fascinating aspect is that gordiids copulate and develop in water but parasitize almost entirely terrestrial hosts. In the following, if results are not explicitly assigned to *Nectonema*, they refer to gordiids.

The exact mode of primary infection is unclear. The hooks and stylets of the larvae are likely boring organs as has been shown histologically (Schmidt-Rhaesa, 1997; Fig. 2D). Hosts may be infected via the integument or via the gut epithelium. Observations suggest that the exoskeleton of arthropods is too solid for an integumental infection while this may be possible in animals with a soft epidermis such as molluscs, or through unsclerotized parts of arthropods (Meissner, 1856; Poinar and Doelman, 1974; Schmidt-Rhaesa, 1997; de Villalobos and Zanca, personal communication). In all cases reported to date, gordiid larvae have been found encysted in the host tissue. This suggests a resting phase in the life cycle and the first host is therefore a paratenic (or transport) host. In *Nectonema*, larvae have been observed very rarely and the mode of infection is completely unknown.

The transition to the terrestrial host may be performed in two ways: either hosts get infected by drinking water containing gordiid larvae or they prey on the paratenic host. In the majority of cases, the terrestrial hosts are (at least partially) carnivorous animals such as carabid beetles, praying mantids (Schmidt-Rhaesa and Ehrmann, 2001) or crickets (Ensifera). In praying

mantids it has been shown that the life cycle is completed when larvae of *Chordodes japonensis* follow the metamorphosis of *Culex*-larvae and the parasitized mosquito is captured by a praying mantid (Inoue, 1960, 1962). Dorier (1930, 1935) observed that cysts with gordiid larvae are dissolved and larvae set free when they are rinsed in gastric fluids of potential hosts. Dorier (1930, 1935) also reported that larvae may encyst outside hosts, *e.g.*, on plants, but this has not been confirmed by other authors. What has been shown in praying mantids may be generalized for other carnivorous hosts but this has not been tested in detail. Some hosts are non-carnivorous, *e.g.*, grasshoppers (Caelifera). May (1919) and Thorne (1940) have successfully infected grasshoppers such as *Anabrus simplex* and others by giving them drinking water containing gordiid larvae. This indicates that a change of host may not be necessary for each gordiid species.

For *Nectonema*, only hosts containing juveniles are known and these are all decapod crustaceans—either pelagic shrimps such as *Pandalus* or benthic crabs such as *Cancer*, *Munida* or *Eupagurus*. Because nematomorph copulation takes place close to the surface, larvae may infect crustaceans while these are planktonic larvae. For populations of *Nectonema munidae* in fjords near Bergen (Norway), some of which are more than 700 m deep, infection probably takes place in the upper layers of the water and the descending crustacean larvae are already infected. However, observations are entirely lacking.

In the host, nematomorphs increase dramatically in size from about 100 μm to several centimeters (the maximum is more than two meters). Uptake of substances has been demonstrated to be through the integument (Kirjanova [1959] for *Gordius setiger*; Skaling and MacKinnon [1988] for *Nectonema agile*). Skaling and MacKinnon (1988) found additional evidence for uptake through the intestine, in contrast to Kirjanova (1959). In *Nectonema*, it appears that a single molt occurs shortly before emergence from the host (Schmidt-Rhaesa, 1996) and this is likely also the case in gordiids (Schmidt-Rhaesa, 1997). This implies that the larval cuticle is capable of enormous growth. The development of further organs, such as the nervous, muscular or reproductive systems are unknown in detail. Few histological (Vejdovsky, 1894; May, 1919; Valvassori *et al.*, 1988) and ultrastructural (Valvassori *et al.*, 1988; Lanzavecchia *et al.*, 1995) observations have been made on late juveniles.

Emergence from the host (Fig. 2B) is crucial for gordiids to complete their life cycle. An observed attraction of infected crickets and mantids towards water (Begon *et al.*, 1990; Thomas *et al.*, 2002) indicates that the parasites may induce this behavior, but the physiological stimulus is unknown.

Recently, Hanelt and Janovy (1999) were, for the first time, able to establish the life cycle of the two North American species, *Paragordius varius* and *Gordius robustus*, in the lab. They used an artificial life cycle with hosts from a region outside of where nem-

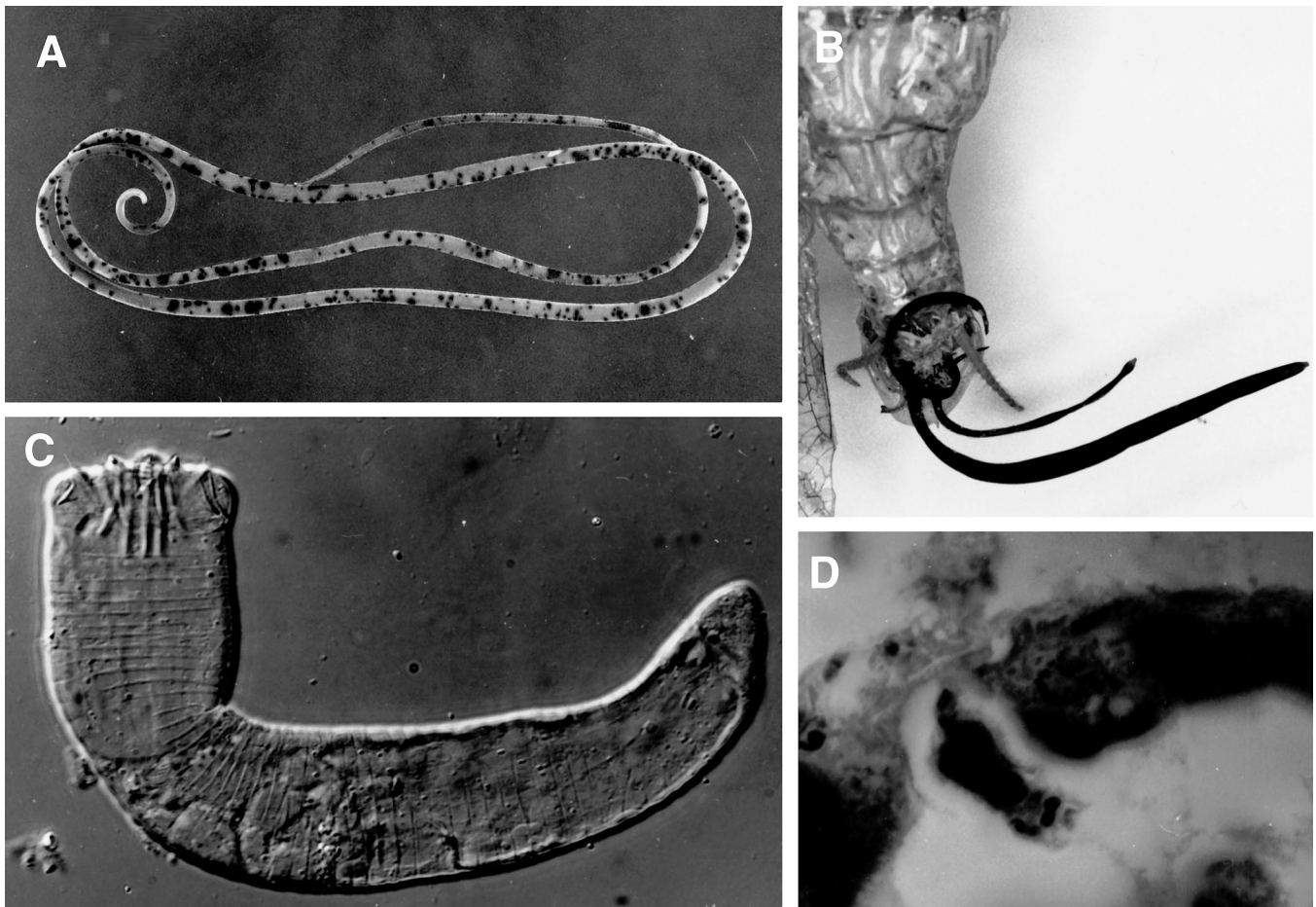


FIG. 2. A. A male of the Tanzanian gordiid *Chordodes ferox* in the free-living phase (Natural History Museum, London, # 1960.391). Width of entire figure is 6.3 cm. B. Four specimens of an undetermined gordiid emerging from the praying mantid *Hierodula dyaka*. The largest gordiid is 1.6 cm long from point of emergence to its end at right. C. Larva of *Gordius aquaticus* with inverted hooks and stylets on the anterior end. Width of entire figure is 80 μm . D. Larva of *Gordius aquaticus* penetrating the intestinal epithelium of a *Culex* larva. Maximum width of the *G. aquaticus* larva is 20 μm .

atomorphs were collected. Larvae were fed to *Tenebrio* beetles or snails (*Physa gyrina*) which were then killed and fed to crickets (*Gryllus firmus*) in which further development took place. This laboratory life cycle finally provides the tool with which further investigations can be approached in a rigorous and defined way. For example, studies on the development from the larva to the adult, the mutual influence of host and parasite, and host specificity can now be performed. It has been shown that intraspecific character variation is probably a common factor in some or several species (Schmidt-Rhaesa, 1997, 2001b). It may be suspected that such polymorphism is the product of differing host parameters (such as size and species of the host or the number of parasites in the host) or the time span that is spent within the host. These aspects can also be tested with an elaborated laboratory life cycle. Polymorphism has an important influence on the perceived species number of nematomorphs, because synonymy decreases species numbers (although new species are steadily being described). Tests according to the biological species concept, *i.e.*, experiments of

copulation and subsequent development of fertile progeny and the analysis of progeny from one clutch will be possible only when the life cycle is established.

These data demonstrate that it is impossible today to make substantive statements about the life cycle of nematomorphs or their influence on their hosts. However, the establishment of the laboratory life cycle by Hanelt and Janovy (1999) is promising sign, and interesting results on the nematomorph life cycle may be expected in the coming years.

THE PHYLOGENETIC POSITION OF GASTROTRICHA

Gastrotricha are microscopic animals that inhabit marine and freshwater environments (for summaries see Ruppert, 1991; Strayer and Hummon, 1991). About 725 species have been described to date (Weiss, 2001; Hummon, 2001). The name Gastrotricha derives from the possession of ventral locomotory cilia which are covered by cuticle, a unique feature among metazoan animals (Ruppert, 1991). Gastrotrichs have a muscular sucking pharynx composed of myoepithelial cells and a triradial lumen. There are two subtaxa which

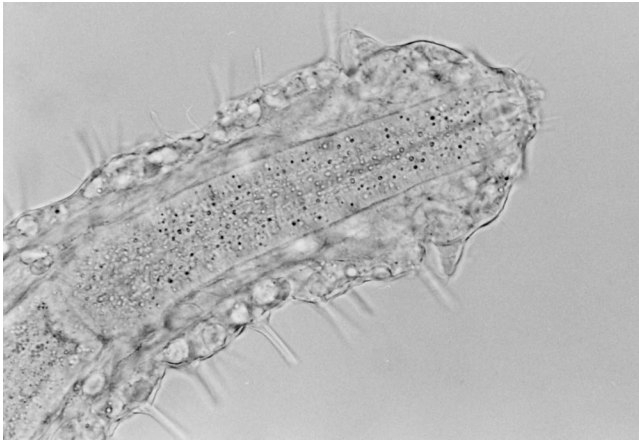


FIG. 3. Anterior end of the macrodasyid *Turbanella* sp. (Sylt, Germany) with terminal mouth opening, sucking pharynx and adhesive tubules. Its diameter behind the head is 40 μm .

are usually regarded as being monophyletic: Macro-dasyida (Fig. 3) and Chaetonotoida. All macrodasyids are marine interstitial animals that live between sand grains to which they adhere with the aid of adhesive tubules that are distributed along their whole body. The lumen of the pharynx is oriented as an inverted Y and connects to the outside by a pair of pores in the posterior part. Macro-dasyids are hermaphrodites with complex reproductive structures and copulation usually results in cross-fertilization. Chaetonotoids are marine or inhabit freshwater where they may be found among aquatic plants. Many chaetonotoids have elaborate cuticular structures such as scales, spines or bristles. The triradiate pharyngeal lumen is oriented as an upright Y, and pharyngeal pores are absent. Except in the genus *Neodasys*, adhesive tubules are found only at the posterior end. In many chaetonotoids, the male reproductive system has been reduced (although sperm can be found in some species; Weiss and Levy, 1979; Weiss, 2001) and the animals reproduce parthenogenetically.

Macro-dasyida and Chaetonotoida have been hypothesized as sister taxa (Ruppert, 1982, 1991; Travis, 1983; Fig. 4A). The genus *Neodasys* Remane, 1927 plays an important role: it resembles macrodasyids in the general shape of the body, lack of cuticular sculptures and the presence of adhesive tubules along the whole body (Remane, 1936; Ruppert, 1988, 1991), but lacks pharyngeal pores and has an orientation of the pharyngeal lumen like that in chaetonotoids. Therefore *Neodasys* has been hypothesized as sister group of all remaining chaetonotoids (named Paucitubulatina). A consequence of this is that the common ancestor of chaetonotoids and macrodasyids resembled macrodasyids in general body shape, but it is not certain whether pharyngeal pores were present and what the orientation of the pharyngeal lumen would have been. This phylogenetic hypothesis had been held valid until it was recently reinvestigated. Hochberg and Litvaitis (2000) found in a cladistic analysis of morphological

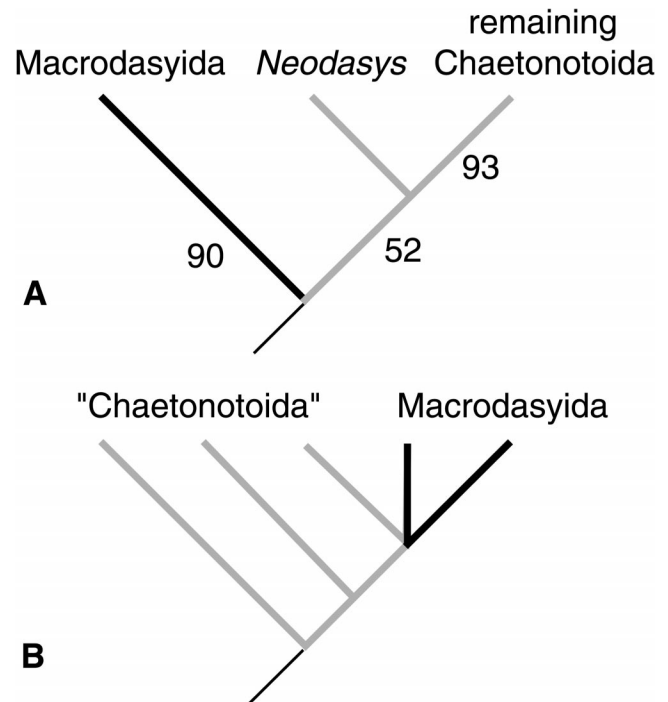


FIG. 4. Hypotheses of relationships within Gastrotricha with Macro-dasyida represented in black and Chaetonotoida in grey lines. A. Topology of Ruppert (1982) and Travis (1983), confirmed by Hochberg and Litvaitis (2000). Numbers are bootstrap values from the analysis of Hochberg and Litvaitis (2000). B. Result of the analysis of 18S rDNA sequences by Wirz *et al.* (1999): Chaetonotoids are paraphyletic. The long branches of two basal *Chaetonotus*-species are not explicitly shown here.

characters the same topology as Ruppert (1982) and Travis (1983), but with weak bootstrap support for the monophyly of Chaetonotoida, *i.e.*, the sister group relationship of *Neodasys* and Paucitubulatina (Fig. 4A). In an analysis of 18S rDNA sequences, Wirz *et al.* (1999) found that the Chaetonotoida was paraphyletic and included macrodasyids (two species analyzed) (Fig. 4B). However, the two basal *Chaetonotus*-species have extremely long branches and the topology presented by Wirz *et al.* (1999) likely includes long branch problems which might lead to an artificially paraphyletic Chaetonotoida.

Apart from these new approaches to phylogenetic relationships among gastrotrichs, the whole taxon seems to be monophyletic. Possible autapomorphies are the multilayered epicuticle (Nielsen, 2001) and the presence of helicoidal muscles along the digestive tract (Hochberg and Litvaitis, 2001). The position of Gastrotricha within the Bilateria, however, varies. Gastrotrichs have long been associated with "Aschelminthes" or pseudocoelomates. These animals were loosely grouped and could not be assigned to other taxa. In the mid-1990s, several workers and working groups reexamined phylogenetic relationships within the "Aschelminthes" (Nielsen, 1995; Ehlers *et al.*, 1996; Wallace *et al.*, 1996). They concluded that Gastrotricha is the sister-taxon of a monophyletic clade comprising

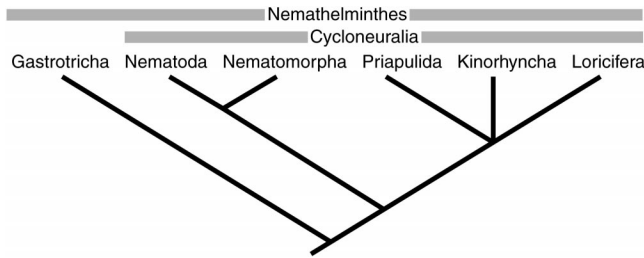


FIG. 5. Consensus of the phylogenetic analyses of the Nemathelminthes according to Ahlrichs (1995), Nielsen (1995, 2001), Ehlers *et al.* (1996) and Wallace *et al.* (1996). The names Nemathelminthes and Cycloneuralia are applied according to Ahlrichs (1995).

Nematoda, Nematomorpha, Priapulida, Kinorhyncha and Loricifera (Fig. 5). Ahlrichs (1995) named this clade Cycloneuralia, but Nielsen (1995, 2001) applied the term Introverta. For all six taxa (including gastrotrichs), Ahlrichs (1995) applied the name Nemathelminthes, while Nielsen (1995) named them Cycloneuralia. Here I use the names introduced by Ahlrichs (1995) (see Fig. 5). Possible autapomorphies of Nemathelminthes (and therefore synapomorphies of Gastrotricha and Cycloneuralia) are the possession of a cuticle composed of a fine fibrillar proteinaceous layer and an outer trilaminar epicuticle (Fig. 6) and probably also the muscular sucking pharynx. A “peripharyngeal brain with three regions” (Nielsen, 2001) is difficult to evaluate, because it is not clear whether this character is realized in Gastrotricha. The three regions appear to be present in *Turbanella cornuta* (Teuchert, 1977), but are absent in *Cephalodasys maximus* (Wiedermann, 1995). A muscular sucking pharynx is widely distributed among Nemathelminthes, but different orientations of the lumen occur which led Neuhaus (1994) to assume a non-triradiate lumen as the plesiomorphic condition. However, if one assumes that the shifting of the pharyngeal lumen of 180° is not a complicated process during development, the assumption of a triradiate lumen as an autapomorphy of the Nemathelminthes appears to be the parsimonious solution.

In the recently developed hypothesis that arthropods are the sister-group of Cycloneuralia (within a taxon named Ecdysozoa) (Aguinaldo *et al.*, 1997; Schmidt-Rhaesa *et al.*, 1998; Garey, 2001), Gastrotricha would be the sister taxon to Ecdysozoa. The interpretation of characters as discussed in the following is not influenced by this hypothesis and Ecdysozoa will therefore not be discussed or mentioned further.

The Nemathelminthes appear as the sister group of Spiralia in a taxon Protostomia (synonymous with Gastroneuralia) (Ehlers *et al.*, 1996) (Fig. 7). Nielsen (1995, 2001) and Nielsen *et al.* (1996) come to generally comparable results, although sometimes the additional taxa Rotifera, Acanthocephala and Chaetognatha were included in Aschelminthes. With the exception of Zrzavy *et al.* (1998), the published analyses of 18S rDNA data and combined molecular/morphological analyses that include sequences from

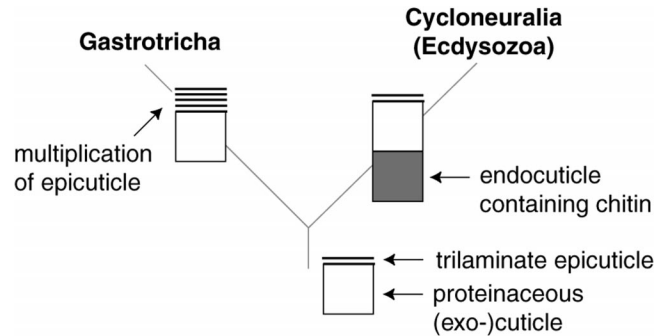


FIG. 6. Probable evolution of the cuticle within Nemathelminthes: A proteinaceous layer and a trilaminar epicuticle are the autapomorphy of Nemathelminthes. In Gastrotricha, the epicuticle was multiplied while in Cycloneuralia (in the Ecdysozoa) an additional, chitin-containing endocuticle evolved.

gastrotrichs (only chaetonotoids) do not support the monophyly of Nemathelminthes. In the analyses of Winnepeninckx *et al.* (1995) and Giribet *et al.* (2000) gastrotrichs appear closely associated with Platyhelminthes (Fig. 7). The implications of these conflicting positions are discussed for the evaluation of the characters cuticle, sucking pharynx and hermaphroditism for the Protostomia (Gastroneuralia).

A cuticle covering the body (except for a glycocalyx) and a sucking pharynx with a triradiate lumen are not present in basal spiralian taxa such as Platyhelminthes (see Rieger, 1984; Ruppert, 1982). If gastrotrichs are spiralian, the cuticle with layers as described above would either have evolved convergently or was present in the protostome ancestor and was reduced in Platyhelminthes and other spiralian taxa (Fig. 8). The same is true for the muscular sucking pharynx with a triradiate lumen (Fig. 8). If gastrotrichs are the sister group of Cycloneuralia, then it seems likely that this type of pharynx evolved once in a common stem species. If

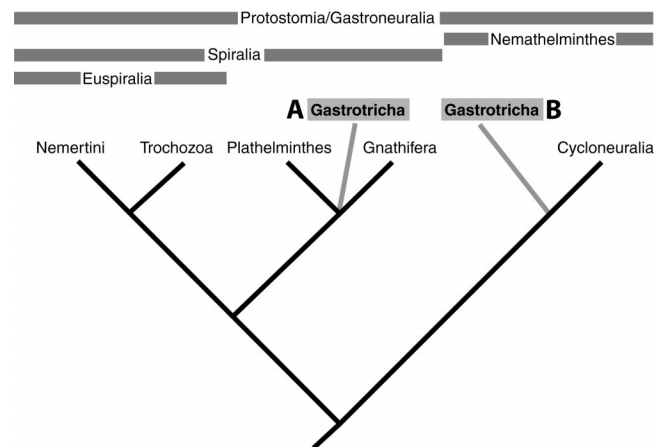


FIG. 7. Competing hypotheses of the position of Gastrotricha among Protostomia/Gastroneuralia. A represents position within Spiralia according to 18S rDNA-data (not well-resolved clustering with Platyhelminthes), B represents a sister group relationship to Cycloneuralia according to morphological data (see text for references).

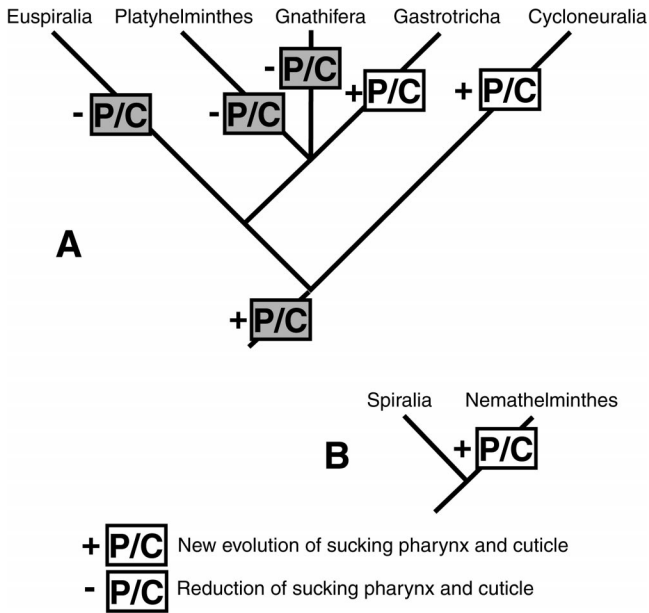


FIG. 8. Alternative hypotheses of the evolution of cuticle and pharynx according to molecular (A) or morphological (B) data. Grey and white squares in A indicate further alternatives: Either pharynx and cuticle were evolved in the protostome ancestor and subsequently reduced in several taxa (grey) or were evolved convergently in Cycloneuralia and Gastrotricha (white).

gastrotrichs are spiralian, then it was either convergently evolved or it was present in the common ancestor of Spiralia and Nematelminthes.

It is extremely hard to make hypotheses about the reproductive condition in Protostomia (Gastroneuralia). Among Nematelminthes, gastrotrichs are the only hermaphrodites (within nematodes, hermaphrodites such as *Caenorhabditis elegans* represent the derived condition). Assuming a basal branching of Spiralia into (Platyhelminthes + Gnathifera) and Euspiralia (Nemertini + Trochozoa) (Garey and Schmidt-Rhaesa, 1998), the stem species of Platyhelminthes and gnathiferans was likely a hermaphrodite but the stem species of Euspiralia was dioecious. If gastrotrichs are part of the (Platyhelminthes + Gnathifera) clade, it would be most parsimonious to assume separate sexes for the common ancestor of Protostomia (see Fig. 9). When gastrotrichs are the sister group of Cycloneuralia, we cannot decide which mode of reproduction was ancestral for Nematelminthes and therefore for Protostomia (Fig. 9).

These considerations illustrate how important the phylogenetic position of a taxon like Gastrotricha is in making hypotheses about character evolution and the reconstruction of characters of common ancestors.

CONCLUSIONS

Biodiversity research is characterized by interconnections of organisms in time and space. Therefore, several aspects are important for the understanding of a taxon, e.g., relationships to other taxa and phylogenetic history. With the two examples from Nematomorpha and Gastrotricha I aimed to exemplify these interconnections and show gaps in knowledge that are mainly due to the evaluation of nematomorphs and gastrotrichs as “lesser known groups.” However, in both examples, progress is evident and our knowledge is likely to grow in the coming years.

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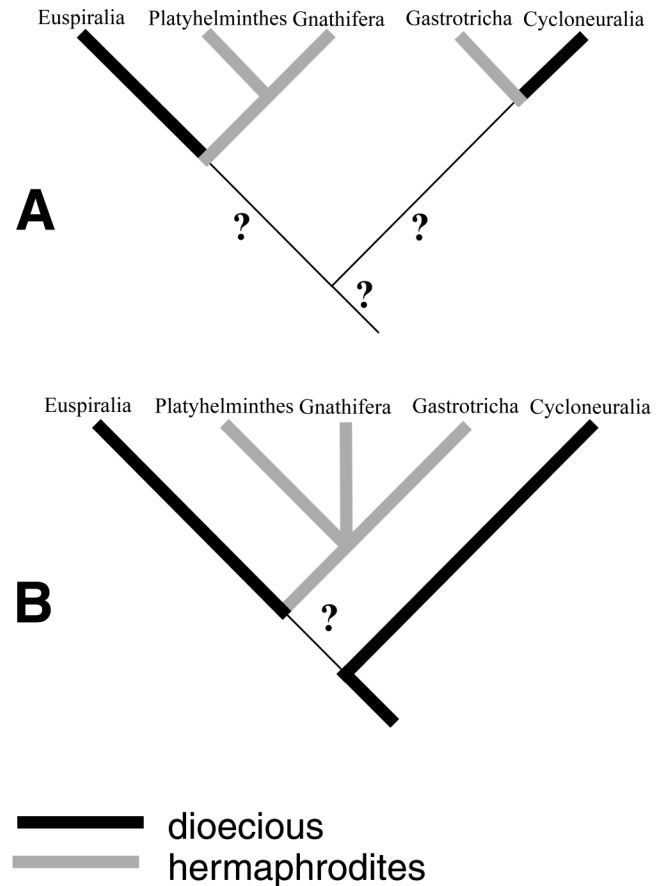


FIG. 9. Evaluation of the hermaphroditic condition according to morphological (A) and molecular (B) data. In A, no decision can be made for the ancestors of Spiralia (Euspiralia + Platyhelminthes + Gnathifera) and Nematelminthes (Gastrotricha + Cycloneuralia) and therefore also not for Protostomia (Spiralia + Nematelminthes). In B, both conditions (dioecious or hermaphroditic) are possible for the ancestor of Spiralia (Euspiralia + Platyhelminthes + Gnathifera + Gastrotricha), but according to a dioecious ancestor of Cycloneuralia a dioecious protostome ancestor is the most parsimonious hypothesis.

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