

Phylogenetic systematics of the Macrostomorpha

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In most molecular and morphological studies of flatworms, the Macrostomorpha are interpreted as the plesiomorphic sister group to all higher rhabditophoran platyhelminth taxa (Tyler 2001, this volume; Littlewood and Olson 2001, this volume). The taxon was established by Doe (1986a) to encompass the Haplopharyngida and the Macrostomida *sensu* Karling (1974). Doe used three synapomorphies to characterize this group: the duo-gland adhesive organs (Tyler 1976, 1977); the pharynx simplex coronatus (Doe 1981); and the aciliary spermatozoa (Ehlers 1984). The Macrostomorpha are therefore a very well-defined monophyletic group in the lower platyhelminths.

Paratomy has been thought likely to be a plesiomorphic feature of these animals for some time (e.g., Rieger 1971b), and the discovery of the macrostomid genus *Myomacrostomum* (Rieger 1986b) generated further evidence for this notion. Paratomy is now held to be plesiomorphic even for all the Platyhelminthes (Ehlers 1985a).

While so far only two, perhaps three, species of the marine genus *Haplopharynx* Meixner 1938, (see Ax 1971; Rieger 1977) have been described, the genus *Macrostomum* O. Schmidt 1882 contains over 100 species, and these occur widely in marine, brackish, and freshwater environments (see Ferguson 1939–1940, 1954; Papi 1950; Luther 1960; Schmidt and Sopott-Ehlers 1976; Faubel *et al.* 1994; Ax and Armonies 1987, 1990; Ax 1994b; Ladurner *et al.* 1997; and taxonomic summary of the Macrostomida by Tyler <http://www.umesci.maine.edu/biology/turb/>). The Macrostomida are divided presently into three subtaxa: the primarily marine Microstomidae, with most species in the genus *Microstomum* O. Schmidt 1848, which also have well-known freshwater species (e.g., *M. lineare* (Müller 1773)); the very heterogeneous Macrostomidae, which will need taxonomic revision; and the marine, rarely brackish-water-living species of the Dolichomacrostomidae (about one-quarter of all described species in the taxon Macrostomorpha) which are now further subdivided, mainly because of differences in the very complicated male and female genital structures, into the taxa Karlingiinae, Dolichomacrostominae, and monotypic Bathymacrostominae (see Rieger 1971b and Faubel 1977 for further discussion).

Two hypotheses of character evolution have been proposed for the Macrostomorpha: one by Tyler (1976, 1977) concerning adhesive organs, the other by Doe (1981) concerning pharyngeal ultrastructure. In most older systems of the Macrostomida – that is, Macrostomorpha without Haplopharyngida – the genus *Macrostomum* was thought to represent the plesiomorphic condition of the taxon (e.g., Graff 1882, 1904–08; Luther 1905, 1947, 1960; Bresslau 1928–33; Reisinger 1933; Papi 1953; Ax 1961, 1963, 1995, 1996). A different evolutionary interpretation was first sketched by Rieger (1971a,b,c). It was based on the assumption that asexual reproduction, in the form of paratomy, was a plesiomorphic trait in the taxon and not, as had been argued by Ax and Schulz (1959), a derived feature. This claim could be substantiated with the descriptions of *Myomacrostomum unichaeta* Rieger 1986 and *Myomacrostomum bichaeta* Rieger 1986. The discussion of phylogeny of the free-living platyhelminths by Smith *et al.* (1986) was partly based on this assumption of the plesiomorphic nature of asexual reproduction in Platyhelminthes in general.

The model for the evolution of the taxon 'Turbellaria'-Rhabditophora-Macrostomorpha proposed here tries to take into account selected characters known to be relevant, except for the protonephridial system. The latter is peculiar in being highly variable in the group (Rohde and Watson 1998; Rohde 2001, this volume) which in itself may be a primitive feature. The origin of the protonephridial canal system and cyrtocyte (terminal cell) in the lower Rhabditophora and the Catenuclida has to be dealt with separately; pulsatile bodies of the Acoelomorpha may be the key for understanding the evolution of the cyrtocyte.

In the phylogenetic hypotheses I present here, the following characters are of particular significance: the pharynx simplex coronatus, the duo-gland adhesive system, paratomy, location of shell glands and cement glands, microanatomy of the aflagellar spermatozoon, and the male copulatory structure. The reinvestigation of Westblad's macrostomid material at the Swedish Riksmuseet and my own unpublished observations on various macrostomids including some supplied by W. Sterrer and S. Tyler have played a role in formulating this hypothesis.

A key factor for constructing this phylogenetic model, however, was the discovery of a new taxon in the Haplopharyngida. The seven specimens of this new group were found in medium-coarse and coarse sand off the North Carolina coast collected in April and October 1970 and extracted in June of 1970 and in January of 1971. Serial sections of two specimens were prepared, the others were studied alive. Here, I provide a brief overview of the interesting body plan of this new group. The description of the new taxon will be published elsewhere (in honour of the late Professor Dr Tor Karling and his wife Annalisa).

A new taxon

The animals are about 1–1.5 mm long and rather sensitive to MgCl₂ anaesthetic, because of large vacuolized parenchymal cells, which are also common in the Karlingiinae, another interstitial group (Rieger 1969, 1971a). The rostrum is long and features adenal rhabdite glands throughout the epidermis and large rhammite glands which open in two distinct groups at the anterior tip of the body (Figures 4.1 and 4.2). Highly complex rod-like structures are the main kind of secretion granule in the rhammite strands (Figures 4.3, 4.8 and 4.9). The epidermis consists of large epithelial cells. Structures at the caudal end of living animals suggest the presence of a facultative anus. A large entolecithal egg is present at the end of the unpaired ovary; shell glands (Figure 4.5) occur in the epidermis in the region of the mature egg, in front, to the sides, and in back of it. Just behind the mouth, a male copulatory apparatus is situated on the ventral side, visible both in squeeze preparations and in the sectioned specimens (Figure 4.4). In sectioned specimens, so far only paired seminal vesicles were found to be linked to the copulatory apparatus.

It is mainly the location of the male pore (just behind the mouth), the penial structure (intracellular needles surrounding the male canal), and the lack of a sclerotic, tubular stylet that set this new taxon apart from all other macrostomorphan

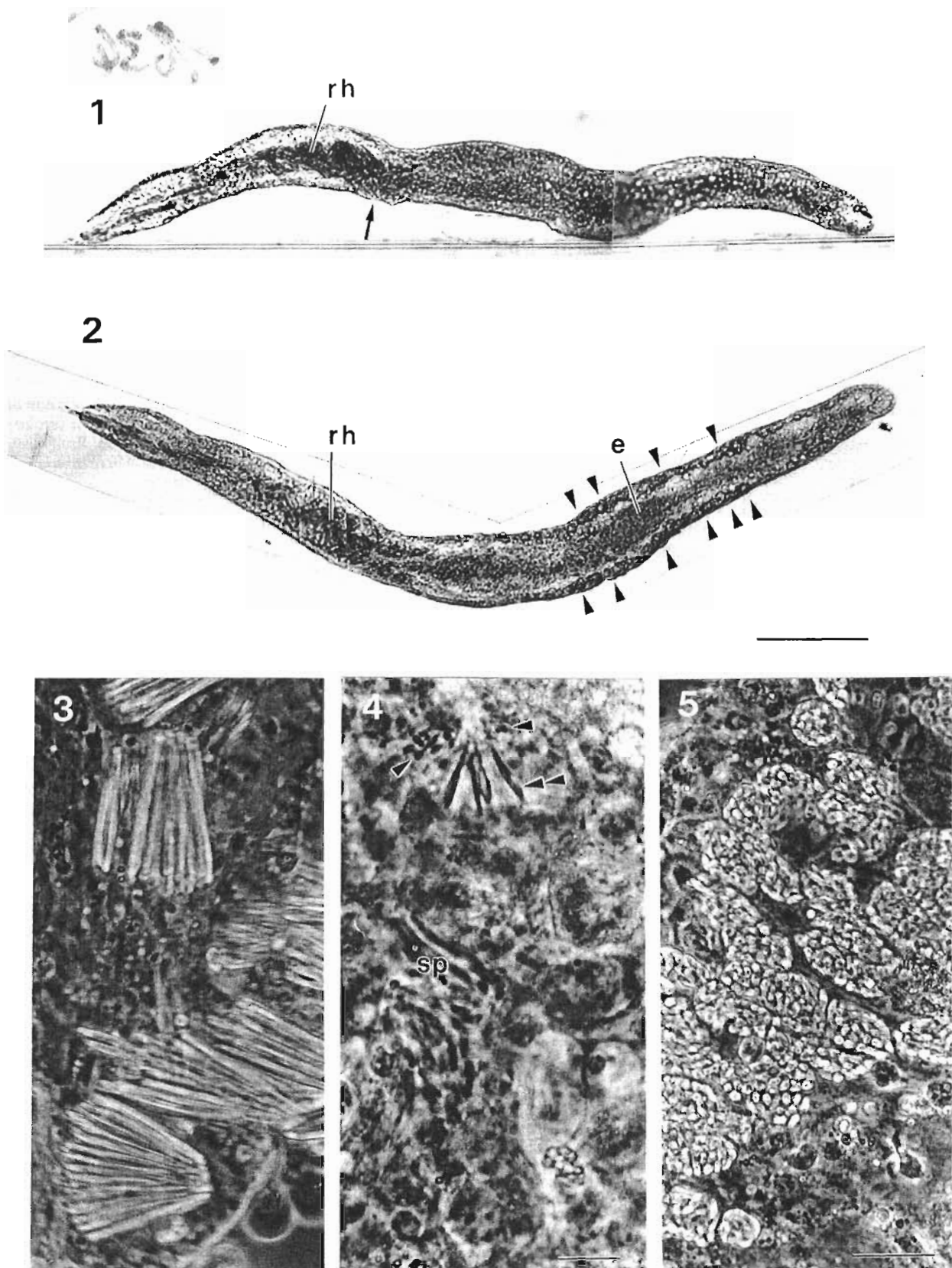


Figure 4.1-4.5 New taxon. 4.1 Lateral view, head at left, rh = rhammites, arrow = mouth region. 4.2 Dorsal view of same specimen, embedded in paraffin-celloidin, rh = rhammites, e = egg, arrowheads mark shell/cement glands. 4.3 Rhammite glands. 4.4 Male copulatory organ with stylet needles in squeeze preparation, arrowheads small penial spines, double arrow heads large penial needles; sp = spermatozoa. 4.5 Shell/cement glands in epidermis, lateral to mature egg. Scale bars: 4.1 and 4.2 200 μm ; 4.3 and 4.5 30 μm ; 4.4 10 μm .

species. Two types of needles were obvious in the live preparations (Figure 4.4). Such a copulatory organ is most similar to that of the genus *Haplopharynx*. In its basic construction it resembles that of the Paratomellidae and Hofsteniidae, both most likely representing early side branches in the phylogenetic tree of the Acoela (see literature in Steinböck 1966, 1967; Ehlers 1992a; Sopott-Ehlers and Ehlers 1999). Also species of the Polycladida and Proseriata have such a penial organ, and such a distribution leads, therefore, to the interpretation that it is a plesiomorphous trait for the Rhabditophora in general (Smith *et al.* 1986). By the ultrastructure work of Doe (1982, 1986a,b) and Brüggemann (1985) we are now able to explain the evolution of the penial structures from this new genus to *Haplopharynx* and further to the higher macrostomids.

Phylogeny based on the adhesive system, according to Tyler (1976, 1977) (see Figure 4.6)

The detailed analysis of the adhesive structures by Tyler (1976) and its phylogenetic consequence set the stage for the now

generally accepted proposal of three monophyletic groups within the 'Turbellaria' (see also Doe 1981: 181 and especially Rieger 1981b) finally leading to the new system of the Platyhelminthes by Ehlers (1984, 1985a). In Tyler's hypothesis, an adhesive organ in the plesiomorphic condition in the Macrostromorpha had several glands, as is characteristic of the Haplopharyngida. In the subtaxon Macrostromida, the genus *Myozona* was apparently primitive in having more than one of the two gland types, the viscid gland. This interpretation allows the most parsimonious explanation of the evolution of the duo-gland adhesive system for all rhabditophorans, starting with multiple viscid and releasing gland cells in the Haplopharyngida and Polycladida and leading to a reduction to one cell of each type in the Macrostromorpha and to a branching of the gland necks of both gland cell types in the Neophora (namely, those taxa investigated, the Proseriata, Tricladida, and Rhabdoceola).

It might be debatable whether the additional association of a rhabdite gland with some of the adhesive papillae, as seen in all dolichomacrostromids, *Myomacrostromum*, and *Bradynectes*, warrants the basal position of the genera *Microstromum* and

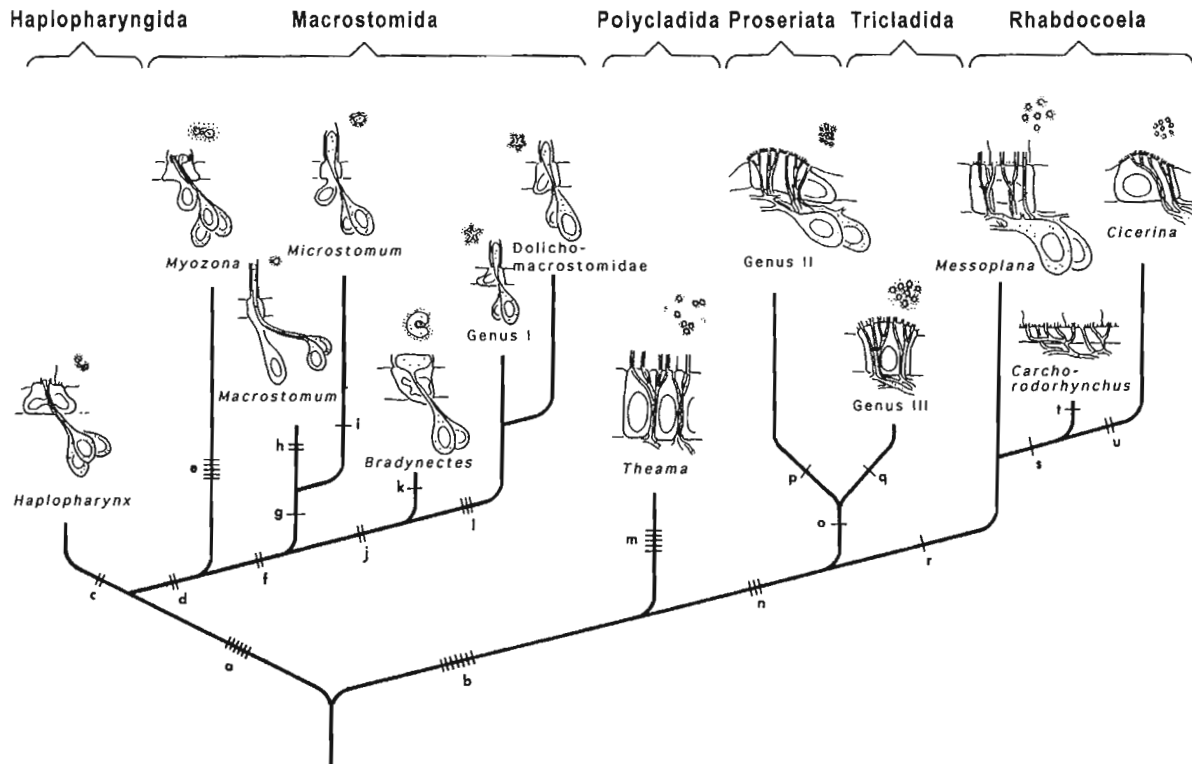


Figure 4.6 Scheme of the evolution of adhesive organs in the Turbellaria (exclusive of Acoela, Nemertodermatida, Lecithoepitheliata, Prolethophora and Catenulida). Duo-gland adhesive organs in representative genera are diagrammed. Cross bars on a branch in the scheme designate characters that are believed to have been present in the ancestor to that branch, or are characters that distinguish a branch from its sister branch. Each bar represents one character, and each set of characters is marked by a letter. **a** Viscid and releasing gland necks emerge in a common collar of microvilli; 2-5 gland cells per anchor cell; one papilla on each anchor cell; each gland has necks to only one anchor cell; tonofilament core is central in microvillus; no sensory processes in anchor cell. **b** Viscid and releasing gland necks emerge separately; gland necks branch; more than one papilla on each anchor cell; a single gland may have necks to more than one anchor cell; microvillous collar around viscid gland neck only; sensory processes and rhabdite gland necks in anchor cell; adjacent viscid gland necks share microvilli of collar; gland necks enter sides of anchor cell. **c** Two viscid and one releasing gland per anchor cell, or three viscid and two releasing glands; diffuse cell web. **d** Specialization of anchor cell surface (all microvilli and/or cilia participate in collar); elongate microvilli. **e** Insunk anchor cell; split anchor cell; two viscid and one releasing gland per anchor cell; all microvilli with core; modified cilia. **f** Only one viscid and one releasing gland; no cilia on anchor cell. **g** Insunk anchor cell. **h** Narrow papilla; single cycle of elongate microvilli in collar. **i** Releasing gland neck branches. **j** Rhabdite gland associated with some adhesive organs; all microvilli with core. **k** Special form of cell web. **l** Viscid gland neck star-shaped in papilla; releasing gland neck branches in papilla; single cycle of microvilli in collar.

Macrostromum (and *Psammomacrostromum*) next to *Myozona*. Equally, the apomorphy 'insunk anchor cell' could be interpreted also as a convergent, i.e. parallel, adaptation, in the genera *Macrostromum* and *Microstromum*. More significantly, a cluster of three linked apomorphies seems to connect the branch to *Myomacrostromum* (= Genus I) with that to the dolichomacrostromids, forming a monophyletic unit: 1) terminal viscid gland neck in papilla star-shaped; 2) releasing cell in papilla branched; and 3) single row of cored microvilli forming the papilla. The position of the genus *Bradynectes* basal to this monophylum seems the best explanation, also, for the irregularly expanded viscid neck in this genus. The apomorphy

'special form of cell web' that characterizes the lineage to *Bradynectes* and the dolichomacrostromids is sound and, therefore, marks it as a distinct phyletic branch.

Phylogeny based on construction of the pharynx, according to Doe (1981) (see Figure 4.7)

The most significant difference in the phylogenetic tree of the Macrostromorpha based on pharyngeal features (Doe 1981) is the basal position of the genus *Microstromum* in the Macrostromida. Besides a number of cytological features of

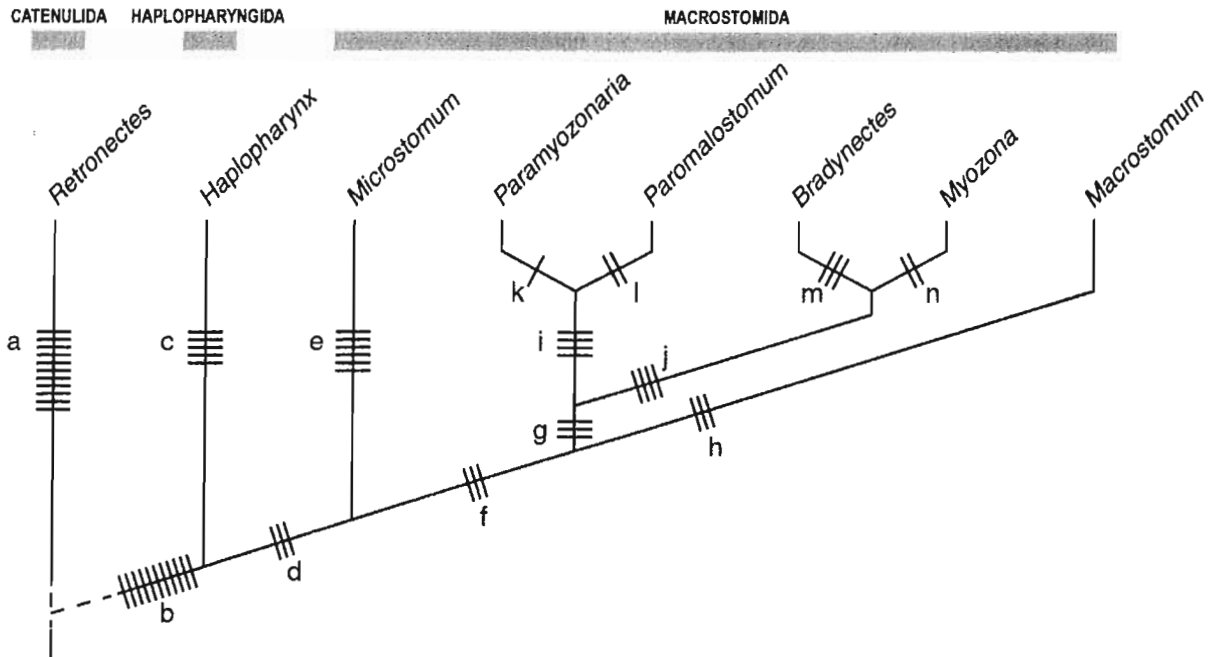


Figure 4.7 Scheme of evolution of pharynx in the Catenulida and Macrostromida. Cross bars on branch designate characters that are believed to have been present in the ancestor to that branch or are characters that separate a branch from its sister branch. Each bar represents one character, and each letter represents a set of characters. **a** No prominent commissure behind mouth; pharynx nervous system with no prominent nerve ring; recessed monociliated sensory cells; transition zone of two rings of insunk epithelial cells with epidermal-type rootlets; no or one gland cell type in pharynx; gland ring of one gland cell type at border of transition zone and pharynx proper; cytological features including microvilli with dense cores, nuclei with heterochromatin in isolated patches and mitochondria with parallel arranged cristae; muscle layers not clearly established with most of the circular muscles inside longitudinal muscles, pharynx longitudinal muscles derived from body wall longitudinal and circular muscles; mouth position between anterior third and half of body; pharynx proper with elongated caudal rootlets and shortened rostral rootlets; pharynx proper with intraepithelial to partially insunk nuclei. **b** Post-oral nerve commissure with more than 12 axons; pharynx nerve ring with more than 30 axons; unrecessed pharynx sensory cells with variable number of cilia; transition zone of 1–5 circles of insunk ciliated epithelial cells with modified rootlets and cell web or terminal web; two or more gland cell types in pharynx; gland ring of one or more gland types proximal to nerve ring in region of border of transition zone and pharynx proper; cytological features including microvilli without cores, nuclei with outer rim of heterochromatin and mitochondria with irregularly arranged cristae; with at least two layers of muscles present, circular muscles inside to longitudinal muscles and longitudinal muscles derived from regular and special body wall circular muscles; mouth occurring in first third of body and pharynx directed dorsally; pharynx proper with elongated caudal rootlets and shortened rostral rootlets; pharynx proper with intraepithelial nuclei. **c** Transition zone is single ring of unciliated cells; type I gland cell granules of protein and polysaccharide; type I gland cells restricted to gland ring; no cell web in transition zone; one gland cell type in gland ring and one type in pharynx proper. **d** Type I gland cell granules solely protein; two or more gland cell types in gland ring, pharynx proper with partially or completely insunk nuclei. **e** Pharynx oriented dorsally; transition zone and pharynx proper rostral rootlet retained and caudal rootlet absent; two gland cell types present in pharynx; no gland type restricted to gland ring; cell web absent from transition zone; longitudinal muscles inside to circular muscles. **f** Transition zone with vertical or caudal rootlet retained and rostral rootlets reduced or absent; four or more gland cell types present in pharynx, always including types I–IV; one or more gland cell types restricted to gland ring always including type I. **g** Transition zone is multiple layers of cells; no rostral rootlets in transition zone cells; gland cells in ring not in repetitive groups. **h** Transition zone is single circle of cells; rostral rootlets present in transition zone cells; gland cells in ring in repetitive groups. **i** Four to five gland cell types in pharynx; terminal web in transition zone; 50–100 axons in nerve rings; gland ring distal to proximal pharynx proper cells. **j** Six gland cell types; cell web in transition zone; less than 50 axons in nerve ring; gland ring proximal to proximal pharynx proper cells. **k** Epidermis at mouth with normal rootlets. **l** Epidermis at mouth with shortened rootlets; longitudinal muscles inside to circular muscles. **m** Longitudinal muscles inside to circular muscles; proximal ring of pharynx proper cells with cell web and modified rootlet orientation; transition zone is single circle of cells. **n** Proximal ring of pharynx proper cells typical; modified sensory cell cytology.

the pharyngeal epidermis, the diversity of pharyngeal glands was used by Doe (1981) to argue for this position, hypothesizing that the simpler condition of only two types of pharyngeal gland cells in the genus *Microstomum* is more plesiomorphic than the several pharyngeal gland types in others. Additional information about the function of the different types of pharyngeal glands might clarify this position; for example, predators may need fewer types of pharyngeal cells than diatom-eating forms. In the genus *Myozona*, for example, where diatom feeding seems to be common (e.g., Marcus 1949), a relatively high number of pharyngeal gland cell types (five) were found in both investigated species, while the Microstomidae and Dolichomacrostomidae, in which predators are particularly common (e.g., Westblad 1923, 1953; Pawlak 1969; Rieger 1971a,b; own unpublished observations on more than 30 undescribed species) have fewer. On the other hand, the genus *Macrostomum* comprises both predatory and diatom-feeding species, and of the three representatives for which we know of pharyngeal-gland fine structure, the predatory species (feeding on nematodes) has as many (seven) gland cell types as the maximum number of gland cell types found in the two diatom-feeding species (Doe 1981). Clearly, more data on feeding strategies and foregut structure are needed to evaluate the phylogenetic significance of this character.

As far as the genus *Microstomum* is concerned, it may be safe to conclude that some of the features that Doe (1981: 183) described as derived characters (e.g., orientation of pharynx, preoral gastrodermal region) are related to fission by paratomy and can, therefore, be interpreted as plesiomorphic (see below).

While the genus *Myozona* appears to be the most primitive form of the Macrostomida in terms of characters of the adhesive system, it is in a derived position in Doe's phylogenetic tree, together with *Bradynectes* and the dolichomacrostomids. In arguing for the derived position of *Myozona* not only the number of pharyngeal gland cell types (five) is significant, but also the basic similarity in structure to those of *Bradynectes* and the genus *Macrostomum*.

A new phylogenetic model for the Macrostomorpha

Using characters of the duo-gland adhesive system, pharynx simplex coronatus, and rhammites (Figures 4.8 and 4.9), as well as of the characters pertaining to parts of the female and of the male system and of reduction of fission, I propose an alternative phylogenetic tree for the Macrostomorpha, with two versions of the evolution of the female canal system

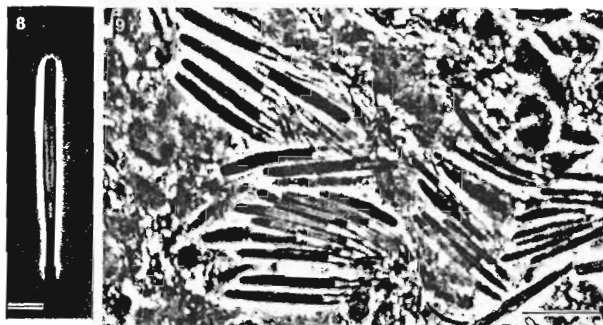


Figure 4.8, 4.9 Rhammites in the new taxon. 4.8 Single rhammite. 4.9 Rhammites, in squeeze preparation, phase contrast. Scale bars: 4.8 10 μm ; 4.9 30 μm .

(Figures 4.10 and 4.11). The most important characters are elaborated separately below.

Rhammites and the frontal glands

Following Karling (1965), Doe (1986b) specified the glandular/muscular proboscis as an apomorphy for the Haplopharyngida. New data on the truly gigantic rhammites of the new taxon (Figures 4.8 and 4.9) corroborate the speculation that the ancestor of the Haplopharyngida (and possibly all Macrostomorpha) had such a proboscis organ. This structure may be the plesiomorphic condition from which the frontal gland complex (Klauser *et al.* 1986) of higher macrostomids was derived. In *Haplopharynx* and in the new taxon, the proboscis gland necks pass by the brain dorsally and ventrally. Microstomids, the plesiomorphic sister group to the other Macrostomida, do not feature larger rhammites (own unpublished observations), but it is notable that the glands opening at the anterior tip of the animal do not penetrate the brain either. In all other macrostomids the dorsal strands of the rhammite glands clearly penetrate the neuropile of the brain, making this one of the best characters to identify all higher macrostomorphans (see Rieger 1971b).

Considering the high frequency of apical glandular-muscular organs among the Acoelomorpha (the frontal organ with frontal pore and, in *Flagellophora* of the Nemertodermatida, the proboscis; see Rieger *et al.* 1991; Sterrer 1998), it is tempting to speculate, as did Tor Karling (1965), that this character and the proboscis of nemerteans are parallel relics of an early evolutionary trend in the Bilateria.

Reduction of fission and the reversal of the genital pores

With the description of *Myomacrostomum unichaeta* and *M. bichaeta*, I have shown that the muscle ring on the gut of the genera *Myozona*, *Myozonaria*, and *Paramyozonaria* could be a derivative of the musculature in the fission plane in a paratomizing ancestor (Rieger 1986b); in particular, some specimens of *Myomacrostomum bichaeta* featured a 'normal' paratomizing division plane in the same place that other specimens had a muscle ring on the gut. Muscle rings were probably preserved for different reasons in the genus *Myozona* (for breaking of diatom frustules, see Marcus 1949) and the genera *Myozonaria* and *Paramyozonaria* (possibly for discharge of undigested food in these elongated, vermiform animals; see Karling 1965, 1966c; Rieger 1971a). Evidently in the dolichomacrostomids, as well as in the genus *Myozona* (see last summary of literature in Sopott-Ehlers and Schmidt 1974) and in probable relatives of *Myozona* constituting a species group with muscular penial organ (*Psammomacrostomum*, *Anthomacrostomum*, *Siccomacrostomum*, and *Dunwichtia*; see below), paratomy was lost, leaving a gastrodermal muscle ring in *Myozona* as a rudiment of the division plane.

This view of the evolution in the Macrostomorpha can also explain why the male genital organs are located in front of the female organs in the haplopharyngids (see Rieger 1971a for detail) but behind them in the macrostomid taxa. Both conditions could have arisen from an ancestor with two zooids that had differential development of the gonads; as paratomy was reduced in the line to the Haplopharyngida, female organs would be the only remnants of the posterior zooid; the male organs would develop from the anterior zooid (Figure 4.12). In the case of the Macrostomida both male and female organs

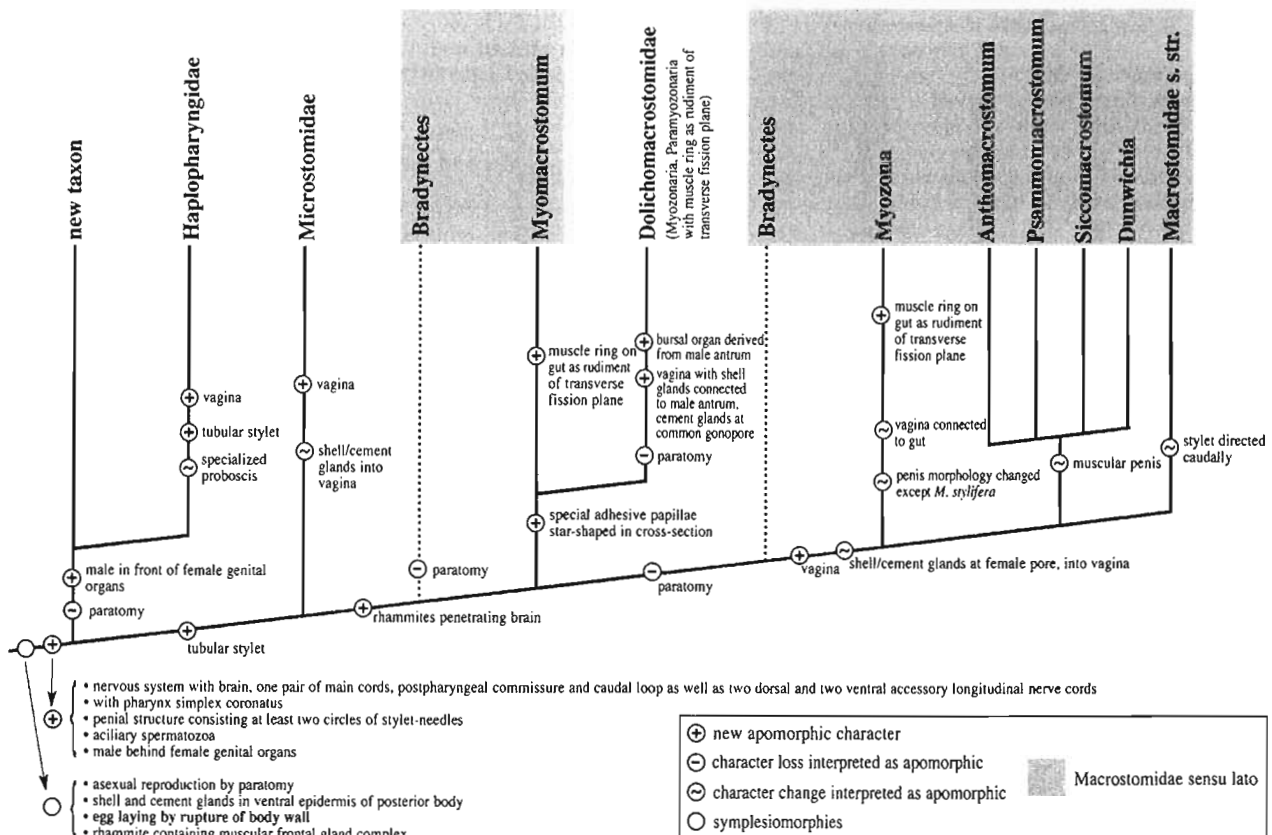


Figure 4.10 Phylogenetic tree of the Macrostomorpha, alternative 1, assuming lack of female pore and vagina in the stem species.

may be derived from sexual organs in the caudal zooid only (Figure 4.13).

With respect to paratomy, the most plesiomorphic taxon in the Macrostomida is the Microstomidae, comprising the genera *Microstomum* and *Alaurina*. This position is supported also by characters of its pharynx simplex coronatus (see above). Evidently, then, the sunken nature of the anchor cell in the adhesive organs in *Microstomum* is convergent with that of *Macrostomum*. In the proposed phylogenetic trees, paratomy is seen to have been lost more than once.

The interpretation of paratomy as a plesiomorphic trait allows actually two different placements in the phylogenetic tree for *Bradynectes* (Figures 4.10 and 4.11) and possibly *Myozonia*. In the case of *Myozonia* (and conceivably of related genera with muscular penis), one position is more in correspondence with the character phylogeny of the pharynx simplex (Figures 4.10 and 4.11). Another position, in correspondence with the adhesive organs, would place this clade between the Microstomidae and all other Macrostomida. Decisions between the possibilities will have to await further character analysis.

The female canal system, shell and cement glands

According to my observations, neither the new taxon nor other members of the Haplopharyngida have a fully developed vagina. In *Haplopharynx rostratus* and the new taxon, large gland cells containing small, often naviculated to ovoid granules, are located slightly insunken in the epidermis in the

posterior half of the animal (Figures 4.2 and 4.5). In the new taxon these gland cells occur at the level of the largest ovum (see above). In *Haplopharynx rostratus* from Rovinj, Istria they apparently form a circular 'clitellum' around the caudal end of the body just behind the largest oocyte (Figure 4.14). Meixner (1938) and Karling (1965) report a complete female canal system in *Haplopharynx rostratus*. Karling, however, mentions that the histological material he had on hand for the descriptions did not allow a 'histologische Analyse des weiblichen Apparates', and he thought that Meixner's figure (1938, his Figure 35) showing female gonad, canal, and pore was most likely adapted from live observations. Thus, a female pore in *H. rostratus* is probably known only from two live observations.

The only female part depicted by Ax (1971) for *H. quadrstimulus* is an unpaired ovary with a large egg behind the male structures and possibly shell glands behind the egg. My own unpublished, sectioned material from two North Carolina forms of *H. quadrstimulus* suggest that it also lacks a female pore. It does have epidermal glands with secretory granules similar to the shell granules in the dolichomacrostomids and *Bradynectes*.

Accordingly, it is conceivable that originally *Haplopharynx* and the new taxon lacked a female canal system, but had accumulations of epidermal glands serving as shell/cement glands located near the caudal end of an unpaired entolecithal ovary. The function of shell glands and cement glands may not yet have been distinct at this stage. Egg laying would have occurred through rupture of the body wall. A similar location of shell glands is seen in the Lecithoepitheliata-Prorhynchida,

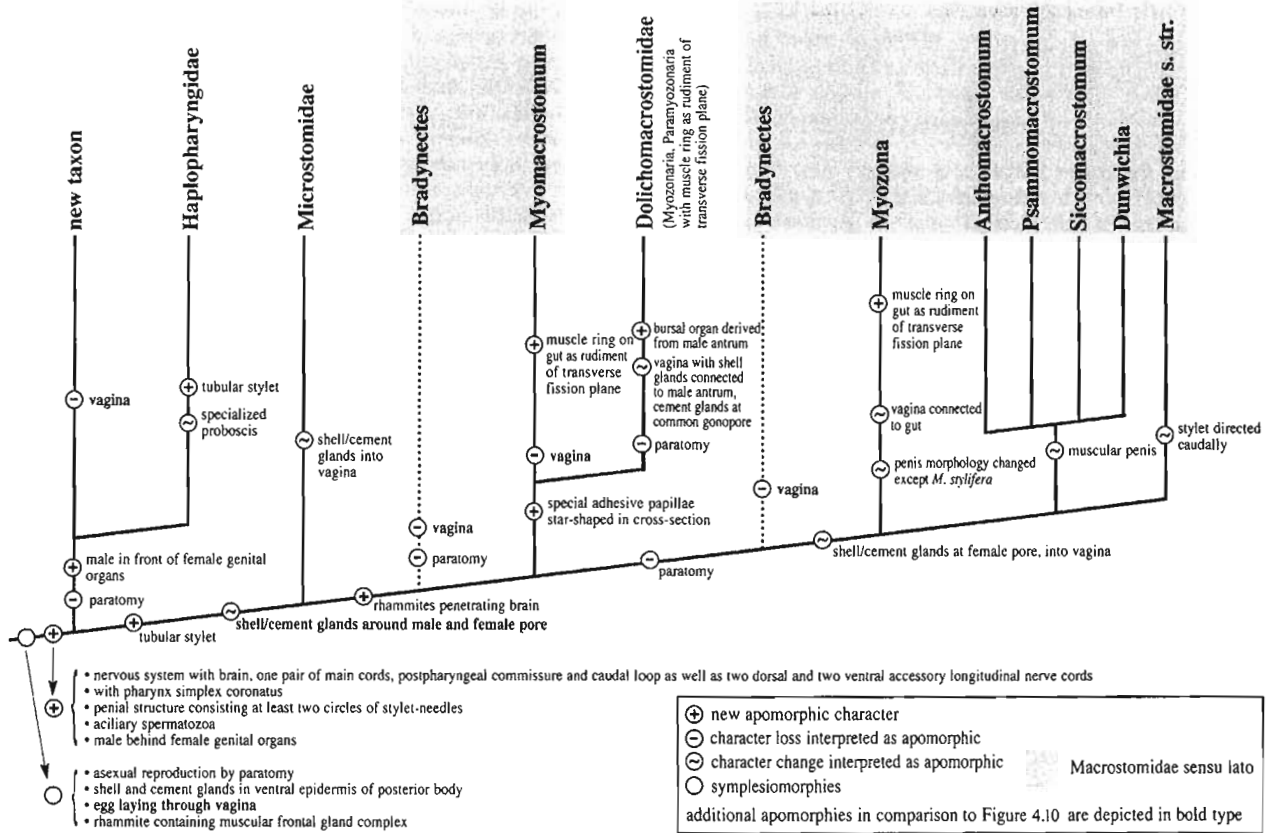


Figure 4.11 Phylogenetic tree of the Macrostromorpha, alternative 2, assuming female pore and vagina present in stem species and lost in certain descendants by virtue of tendency to progenesis.

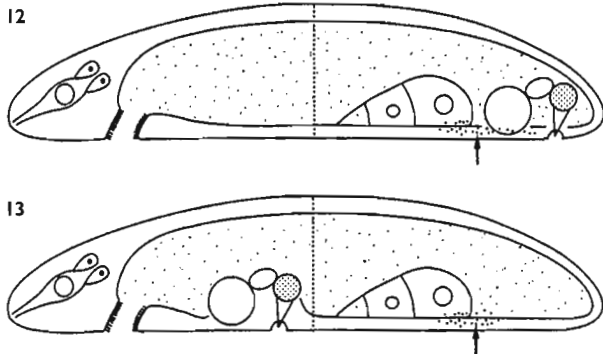


Figure 4.12, 4.13 Schemes of a two-zooid macrostromorphan ancestor similar to paratomizing *Microstromum* and *Myomacrostomum*, illustrating the possibility for reversal of sex organs during reduction of fission. 4.12 Plesiomorphic condition for the Macrostromorpha. 4.13 Reversal of that condition for the Haplopharyngida. Arrows indicate position of shell/cement glands.

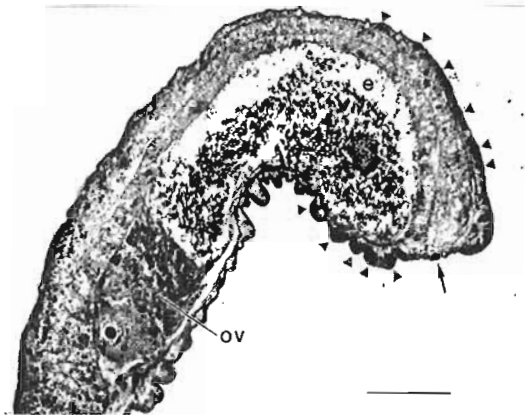


Figure 4.14 Ring of shell glands within the caudal epidermis of *Haplopharynx rostratus*, Rovinj-Istria; slightly parasagittal section through caudal end. e = egg, ov = ovary, arrowheads mark position of shell/cement glands, arrow position of anus. Scale bar: 60 μ m.

where a female canal system is present and the female pore lies closer to a midventral position (see Rieger *et al.* 1991: 102 and Figure 31A).

In one of the phylogenetic trees presented here, a female pore and a vagina are assumed to have been absent in the macrostromorphan stem species (Figure 4.10). This necessitates the

assumption of several parallel lines of evolution for the female canal system. Alternatively, the female canal system may have regressed, such that egg laying occurred secondarily through rupture of the body wall because of a strong tendency towards progenesis in at least three lines, coupled with the reduction of paratomy (Figure 4.11). More information on the postembryo-

nic development of the genital organs in the Haplopharyngida and in the basal Macrostromida (e.g., Microstromidae) might help to gauge this possibility. The further events of the evolution of the female system may have led then in parallel lines to a complete loss of a female canal (new taxon, *Bradynectes*, *Myomacrostomum*), to a secondary connection to the male antrum (dolichomacrostomids) or, in the case of *Myozona*, to a connection to the gut by a completely new structure: a developing pharynx in the caudal region of a paratomizing ancestor similar in general morphology to *Myomacrostomum*. By this interpretation, the female canal system of *Psammomacrostomum*, *Anthomacrostomum*, *Siccomacrostomum* and *Dunwichia* (see summary of literature in Faubel *et al.* 1994) may be a line of evolution parallel to that of the vagina of *Microstromum* and *Macrostomum*.

In dolichomacrostomids, cement glands are separate from shell glands. They open around the single gonopore, which, according to the interpretation advanced here, would be the original male pore and male antrum of the caudal zooid in an ancestral species.

The copulatory structure and the spermatozoa

With the discovery of the new genus, the ancestral character state of the penial organ of the Macrostromorpha is more easily surmised: that is, it would have had only intracellular needles surrounding the male canal (see also proposals by Doe 1986a,b and Smith *et al.* 1986). The tubular sclerotic stylet found in *Haplopharynx* and the plesiomorphic Macrostromida could be derived from fusion of independent sclerotic needle cells, forming a ring-shaped matrix syncytium that secretes the stylet tube, as suggested by Doe (1982, 1986a,b). The condition in *Haplopharynx quadrastimulus*, where four single stylet needles lie outside the tubular stylet, may represent a plesiomorphic condition within the Macrostromorpha. However, we still lack TEM data on accessory spines of *Haplopharynx rostratus*, in which the tubular stylet is not surrounded by accessory spines (see Doe 1986b).

Needle-like intracellular rods similar in structure to ciliary rootlets occur also to the inside of the tubular stylet in the Macrostromida (for *Macrostomum* and *Paramyozonaria* see Doe 1982; possibly also in *Myozonaria* and *Acanthomacrostomum* and all Karlingiinae; see Rieger 1971b, own unpublished observations) but seem to be absent in *Microstromum* (Doe 1982) and Dolichomacrostominae (Rieger 1971c; Brüggemann 1985). Because of their location inside the tubular stylet, these structures have been identified as non-homologous with needle-like intracellular structures located distal to the stylet (Doe 1982, 1986a,b). The intracellular rods could be another autapomorphy of the Macrostromida.

In terms of only copulatory structures, the most parsimonious solution would be to place the new group as the plesiomorphic sister taxon to all other Macrostromorpha. In considering also, however, the character 'fission by paratomy', it is more parsimonious to assume only one event of loss of paratomy for the Haplopharyngida. However, the assumption of two convergent origins for the tubular stylet, one within the Haplopharyngida and one within the Macrostromida then becomes necessary (Figures 4.10 and 4.11).

By what is evidently the plesiomorphic orientation of the male penial organ (pointing rostrally), *Microstromum* and *Myomacrostomum* are clearly set aside from the genus *Macrostomum* (copulatory stylet always points caudally). The genus *Myozona* is also plesiomorphic in this character (see *Myozona stylifera*). A gradual shift toward a caudal orientation

of the penial organ is evident in *Myozona* as well as in the four genera listed with a muscular penis.

On the other hand, the apomorphic character state of the rhammite glands, that is having certain glands penetrate the brain, clearly links *Myomacrostomum* with the dolichomacrostomids and the macrostromids *sensu lato*. Curiously, *Myomacrostomum* forms the plesiomorphic sister group to the dolichomacrostomids by virtue of the apomorphy of the adhesive organs, as noted earlier.

Spermatological evidence also supports the close relationship and thus the monophyly of the Haplopharyngida and the Macrostromida (Rohde and Faubel 1998; own unpublished observations on material supplied by S. Tyler and D. Doe). The 'blunt bristle-like structures' in the lateral cytoplasm of *Haplopharynx* spermatozoa described by Rohde and Faubel (1998) could be precursors of the bristle-like structures of spermatozoa of certain members of the genus *Macrostomum* (see e.g., Ferguson 1939–40 for light microscopy data for many species, and Rohde and Faubel 1998 for TEM literature). However, Watson (1999b) considers a homology of these structures in *Haplopharynx* and *Macrostomum* unlikely. Further information is needed to resolve this issue. I mention it here as another example of possible parallel loss within the Macrostromida: in the dolichomacrostomids (Rohde and Faubel 1998; own unpublished observations), in the genera lacking sclerotic penial structures (*Psammomacrostomum*, *Anthomacrostomum*, *Siccomacrostomum*, and *Dunwichia*; see Faubel *et al.* 1994; own unpublished observations), in various species groups in the Macrostromidae *sensu stricto* (e.g., the *M. hystricinum marinum* species group; Rohde and Faubel 1997; own unpublished observations).

Comparison with the phylogenetic system of the Macrostromorpha proposed by Sopott-Ehlers and Ehlers (1999) (see Figure 4.15)

The system proposed by these authors also places the Haplopharyngida as the plesiomorphic sister group to the Macrostromida. Autapomorphies they propose for the Macrostromorpha are specialized duo-gland adhesive system (Tyler 1976, 1977) and aciliary spermatozoa (Ehlers 1984); those for the Macrostromida are female pore in front of male pore, and special differentiation of penial structures (Doe 1982, 1986a,b; Brüggemann 1985). Except for the autapomorphy 'female pore in front of male pore' for the Macrostromida, the suggested autapomorphies correspond to the phylogenetic tree proposed in this paper. The character 'male behind female genital organs' is interpreted in this chapter as an autapomorphy for the Macrostromorpha (Figures 4.10 and 4.11). Within the Macrostromida, Sopott-Ehlers and Ehlers indicate a basal position of the Microstromidae, but not clearly separated from the Dolichomacrostomidae and *Myozona*. A monophyletic relationship is suggested between the genus *Bradynectes* and the genus *Macrostomum* on the basis of two lateral ledges in the spermatozoon. However, as mentioned above, the 'curved dense structures' (Rohde and Faubel 1998) in *Haplopharynx* might represent an early stage in the evolution of the lateral ledges and bristles in the Macrostromida.

Sopott-Ehlers and Ehlers' (1999) finding of electron-dense 'ledges' in spermatozoa of *Bradynectes* provides new support for the notion that the original spermatozoa of the Macrostromorpha featured such structures. By proposing that a 9 + 1 axoneme and intercentriolar body in the spermatid are autapomorphies of the Rhabditophora, these authors also place these features as plesiomorphies of the Macrostromorpha.

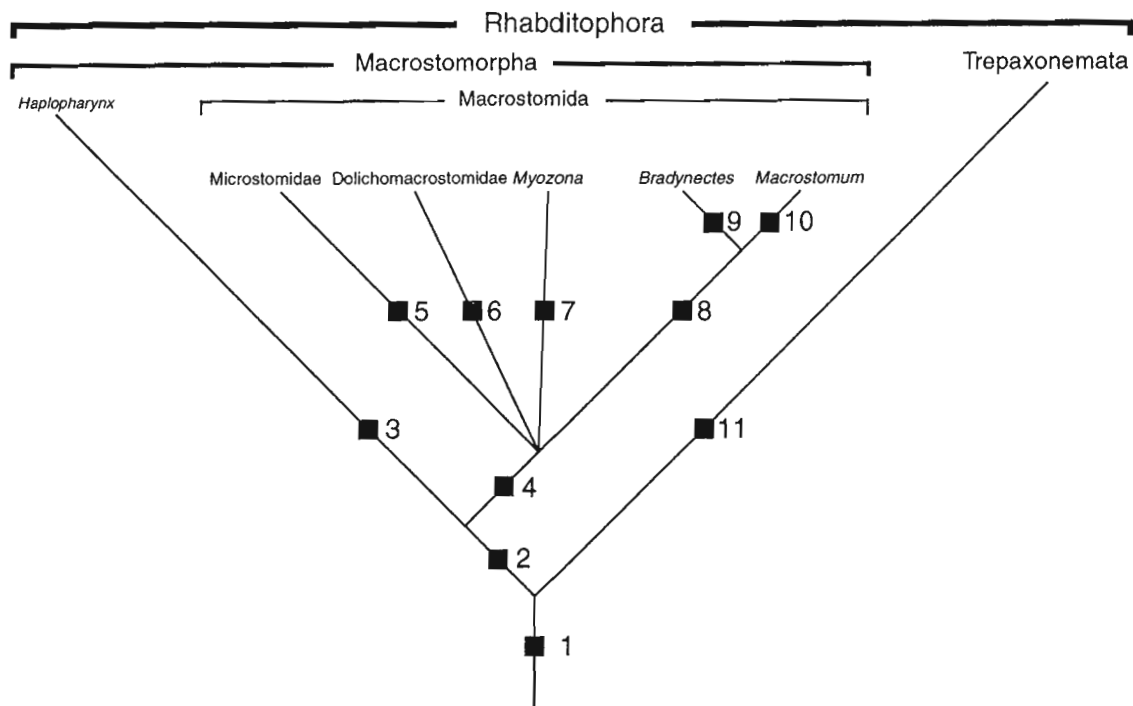


Figure 4.15 Diagram of the basic relationships within the Rhabditophora and the interrelationships of the Macrostomorpha. Black squares are sets of hypothesized autapomorphies. Spermatological characteristics are given in italics. The unique 9 + 1 pattern of ciliary axonemes in spermatozoa and an intercentriolar body in spermatids are autapomorphies of the Rhabditophora or of the Trepaxonemata. 1. Rhabditophora: lamellated rhabdites; duo-gland adhesive systems; protonephridial terminal cells with four or more cilia; pharynx simplex with a prominent nerve ring (see Doe 1981); rhabdomeric photoreceptors with few sensory cells and one mantle (pigment) cell (see Sopott-Ehlers 1996); *biciliated spermatozoon, both cilia inserting at the functional fore-end of the cell*; oocytes with polyphenol-containing eggshell forming granules; female pore. 2. Macrostomorpha: specialized duo-gland adhesive systems (see Tyler 1976, 1977); *spermatozoon without ciliary axonemes*. 3. *Haplopharynx*: cranial protrusible proboscis; specialized male organ (see Doe 1986). 4. Macrostomida: female pore in front of male pore; special differentiation of penial structures (see Brüggemann 1985; Doe 1986); *spermatozoon with two sets of cortical microtubules*. 5. Microstomidae: gut with preoral blind sac; cranial ciliated sensory pits. 6. Dolichomacrostomidae: bursal organ, accessory glandular organ, common female and male pore (see Rieger 1971b). 7. Myozona: *spermatozoon with bone-shaped rods, one cylindrical mitochondrial rod and four sets of microtubules* (see Sopott-Ehlers and Ehlers 1998b). 8. (*Bradynectes*+*Macrostomum*): *spermatozoon with two lateral ledges*. 9. *Bradynectes*: without female pore; *spermatozoon with membranous lacunae; both sets of microtubules restricted to the posterior region of the cell*. 10. *Macrostomum*: protonephridia with two-cell terminal weirs showing two rings of interdigitating microvilli (see Kunert 1988; Watson *et al.* 1991); *spermatozoon with two modified lateral ledges = bristles*. 11. Trepaxonemata: specialized duo-gland adhesive systems with branching gland necks and several papillae on each anchor cell (see Tyler 1976, 1977; Smith *et al.* 1986); with reservation (see Ehlers 1985a); protrusible muscular composite pharynx with pharyngeal cavity (see Rieger *et al.* 1991).

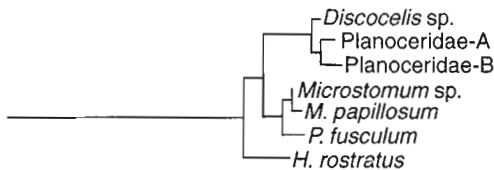
Such an assumption may be correct, but cannot yet be substantiated: no 9 + 1 axoneme has been found in any macrostomorph. One cannot discount the possibility that 9 + 2 axonemal structures could be found in spermatozoa of the new taxon. I agree with Watson (1999b), therefore, when she excludes the Macrostomorpha from the Trepaxonemata.

Evidence of recent molecular studies on the phylogeny of the Macrostomorpha (Figure 4.16a,b)

Two molecular phylogenetic studies attempted to elucidate the interrelationships of the Platyhelminthes which also included more than two species of the Macrostomorpha (Littlewood *et al.* 1999a; Litvaitis and Rohde 1999). Littlewood *et al.* (1999a) present, to date, the most representative analysis of complete 18S rDNA sequence data and comparative morphological data which are first analysed separately, and then morphological and DNA evidence in a 'total evidence' approach. These molecular-based studies suggested three alternative evolutionary

hypotheses, depending on which tree-building algorithm was used or whether morphological data were also included in the analyses. In their combined analysis of molecular and morphological characters (their Figure 5) they suggest the positioning of the Haplopharyngida between two clades of macrostomids, the first containing species of the genus *Macrostomum* only, and the second containing microstomids and dolichomacrostomids. The 'total evidence' approach for the within-group relationship of the Macrostomorpha thus would place the Haplopharyngida within the Macrostomorpha, as a monophyletic unit with a dolichomacrostomid and two microstomids; and the genus *Macrostomum* as most ancestral split. This grouping may seem to be in line with the older light histological literature, but seems rather unlikely in the light of new evidence provided in the present study, or of that discussed by Sopott-Ehlers (1999). The topology of the parsimony tree based upon 18S rDNA data (their Figure 3) is equally difficult to connect to a series of recent ultrastructural data. Here, the Haplopharyngida are resolved as a separate lineage as sister group to the Lecithoepitheliata, and outside a clade containing the macrostomids and polycladids. A separation of the

a. Litvaitis and Rohde (1999)



b. Littlewood *et al.* (1999a)

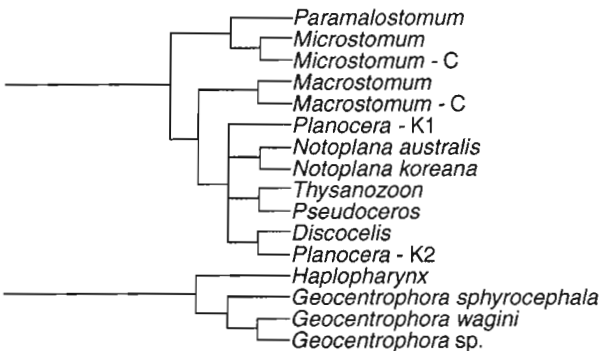


Figure 4.16 Portions of parsimony trees published for the Macrostromorpha based on **A**) partial 28S rRNA by Litvaitis and Rohde (1999), and **B**) complete 18S rRNA by Littlewood *et al.* (1999a).

Haplopharyngida and Macrostromida is indeed highly unlikely considering the evolution of various morphological characters presented in this chapter. In particular, the occurrence of the pharynx simplex coronatus in *Haplopharynx* and in all macrostromids (see Doe 1981, 1986b) speaks in favour of the monophyly of all Macrostromorpha (Haplopharyngida plus Macrostromida). However, a closer relationship of the Lecithoepitheliata and the Haplopharyngida would appear supported by the suggested evolutionary transitions of two anatomical characters only, the penial structures and the follicular surrounding of accessory cells (see above).

Littlewood *et al.* (1999a) also present the results of a neighbour-joining analysis of the complete 18S rRNA data set based upon the HKY85 maximum likelihood distance model. The neighbour-joining tree (their Figure 4) is similar to the phylogenetic hypothesis presented in this chapter. The Haplopharyngida are placed as most ancestral split and as sister group to two clades of Macrostromida, one comprising *Microstomum* and *Paromalostomum*, and the second two species of the genus *Macrostomum*. The sister group of the *Macrostomum* clade, however, was formed by several polyclads. The placement of the Haplopharyngida as most ancestral split within the Macrostromorpha, followed by two clades of Macrostromida, the first comprised of *Paromalostomum* and *Microstomum* and the second comprised of *Macrostomum*, would be in line with the phylogenetic hypothesis presented in this chapter. In our analysis the first Macrostromid clade is also formed by microstromids and dolichomacrostromids, and the second clade also contains two species of the genus *Macrostomum*. However, the inclusion of the Polycladida as sister-group to the *Macrostomum* clade suggested by the neighbour-joining tree based upon 18S rDNA would place the Macrostromida in paraphyly with respect to the Polycladida.

Litvaitis and Rohde (1999) provide two molecular trees of the Macrostromorpha based on partial 28S rDNA sequences: a neighbour-joining tree (their Figure 2) and a parsimony tree (their Figure 3). Both hypotheses agree with the topology obtained in the neighbour-joining analysis of Littlewood *et al.* (1999a) based upon 18S rDNA in that they also suggest that *Haplopharynx* represents the most ancestral split of a lineage comprising one clade of Macrostromida (*Microstomum* spp. and *Paromalostomum*) and a second comprising the Polycladida. In this analysis the genus *Macrostomum* was not included. A sister group relationship of Macrostromorpha and Polycladida, or even the suggested paraphyly of the Macrostromorpha resulting from the inclusion of the Polycladida, is highly unlikely in relation to the available morphological evidence. Pharynx construction, developmental data as well as body profile point against an equidistant relationship of *Haplopharynx* to the macrostromids and the polyclads. The Polycladida and the Macrostromorpha are – on morphological grounds – two separate, monophyletic assemblages (Karling 1974; Sopott-Ehlers and Ehlers 1999). However, a sister group relationship of the Macrostromorpha and the Polycladida may indeed be supported by our present study, if one considers the new evidence on the evolution of the male sclerotic copulatory structures of macrostromorphans (see also Smith *et al.* 1986) and new data on the organization of the ovary of *Haplopharynx rostratus* (see above). In conclusion, molecular phylogenetic analyses provide highly useful insights for the interpretation of alternative morphological transitions in Macrostromorpha. Reciprocal illumination between morphology and molecules will become extremely fruitful, as soon as more phylogenies based upon additional gene segments become available.

Conclusion and suggestions for future work

Thus, we can conclude that:

1. This study supports a basal position of the Macrostromorpha, as is evident in most phylogenetic trees of the Rhabditophora (see Sopott-Ehlers and Ehlers 1999).
2. The Haplopharyngida are the plesiomorphic sister group to the Macrostromida. Haplopharyngids, including the new taxon, may be derived from the macrostromorphan common ancestor by loss of paratomy and the apparent inversion of the male gonad and copulatory structure in relation to the ovary by virtue of that loss. Intracellular needles surrounding the male canal are very likely the plesiomorphous character state for this feature. Similar penial needles, common in the Polycladida, Lecithoepitheliata, and the acoele taxa Paratomellidae and Hofsteniidae are seen as evidence that intracellular needles may have been the original penial structure in the Rhabditophora.
3. A basal position of the taxon Microstromidae in the Macrostromida appears likely.
4. The character 'rhammites penetrating the brain' as well as the lack of pigment-cup ocelli in the new taxon, in the Haplopharyngida, and possibly in the Microstromidae is likely to be a plesiomorphic character in the Rhabditophora and not the result of loss. Such eyes are not known in the Haplopharyngida. Pigmented epithelial eye spots occur in *Microstomum lineare* (see Palmberg *et al.* 1980) and peculiar pigment cup ocelli in other Microstromidae (e.g., the ocelli in *Microstomum dermatophthalmum*

Steinböck 1933). The latter have not been studied with TEM.

5. A taxon including the genus *Myozona* and the genera with soft penial structures, such as *Psammomacrostomum*, *Acanthomacrostomum*, *Siccomacrostomum*, and *Dunwichia* might be a new monophyletic subgroup of the Macrostomida.
6. Multiple origins of characters (e.g., vagina), multiple cases of loss of characters (e.g., paratomy, vagina) as well as reversal of character trends (e.g., differentiation of sclerotic stylet needles, their transformation into a tubular sclerotic stylet, subsequent reduction and loss of the sclerotic structure and differentiation of solely muscular penial structures) appear as fundamental processes in the adaptive radiation of the Macrostomorpha.

One point which requires further study in particular is the female canal system in the genera *Haplopharynx* and *Myozona*. A search for homologies of the male penial structures with those of certain taxa of the Acoela (Paratomellidae,

Hofsteniidae) could help to identify the sister group within these acoel taxa and the Macrostomida. The absence of a female pore in *Bradynectes* should be corroborated. Finally, the shell and cement glands of the Macrostomorpha and the postembryonic development of the female canal system in the Macrostomida require further investigation.

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