Novel implications for the basal internal relationships of Gastrotricha revealed by an analysis of morphological characters

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A cladistic analysis of Gastrotricha based on morphological characters is presented. Unlike previous morphological analyses, our study uses species rather than higher level taxa, for which the ground pattern is often unknown. The analysis comprises 79 ingroup taxa, 4 outgroup taxa and 135 binary and multistate characters in total. Character coding is based on a careful assessment of original species descriptions. Characters included cover general body organization, internal and external features as, for example, data on the adhesive tubes, digestive tract or cuticle armament. Character systems such as many ultrastructural findings, for which it was problematic to obtain data for a large set of the included taxa, were not considered. To minimize *a priori* assumptions, all characters were treated with equal weight and left unordered. The four outgroup representatives were chosen in accordance with the current sister group hypotheses for Gastrotricha. Two search strategies, a heuristic search (maximum parsimony) and a parsimony ratchet search, reveal a comparable scenario. Gastrotricha split into two sister taxa. One group comprises genus *Neodasys* only, the sister group N.N.1 (Eutubulata nom. nov.) consists of all remaining Gastrotricha. Within Eutubulata, monophyletic Macrodasyida *s. str.* and N.N.2 (Abursata nom. nov.) are sister taxa of highest rank. Abursata consists of the 'freshwater macrodasyids' *Marinellina* and *Redudasys* as sister group of monophyletic Paucitubulatina. Some traditional families are supported by this analysis. We evaluate possible apomorphies for the most basal stem lineages and track the evolution of selected organs. Our findings reveal that secondary character loss may play an important role in the stem lineage of Abursata and further in Paucitubulatina. Moreover, according to this analysis there might have been a single invasion of the freshwater environment in the stem lineage of Abursata followed by several independent returns to marine habitats within the monophylum Paucitubulatina.

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Introduction

The exclusively aquatic and meiobenthic taxon Gastrotricha is a well-supported monophyletic group of the Bilateria for which a very basal position within the phylogenetic system of Bilateria is assumed. The study of species of Gastrotricha at the ultrastructural level and considering their basal position has shed light on the early evolution of the Bilateria, for example, in conjunction with the ciliated epidermis (e.g. Rieger 1976) or the protonephridial system (Bartolomaeus & Ax 1992). However, there is still controversy about the exact phylogenetic position of Gastrotricha (see Schmidt-Rhaesa 2002, 2007).

According to different systematic hypotheses, they may be the sister group of Plathelminthes (Winnepenninckx *et al.* 1995; Giribet *et al.* 2000; Giribet 2002), Cycloneuralia (Ehlers *et al.* 1996; Sørensen *et al.* 2000; Ax 2003), Ecdysozoa (Schmidt-Rhaesa *et al.* 1998; Peterson & Eernisse 2001) or Gnathostomulida (Zrzavý *et al.* 1998). In a recent study on molecular phylogeny of Gastrotricha (Petrov *et al.* 2007), all common sister group hypotheses for Gastrotricha are summarized and evaluated. The 18S rRNA gene data of that study, however, strongly favours a sister group relationship of Gastrotricha and a group comprising Plathelminthes, Syndermata, Nemertea and Lophotrochozoa.
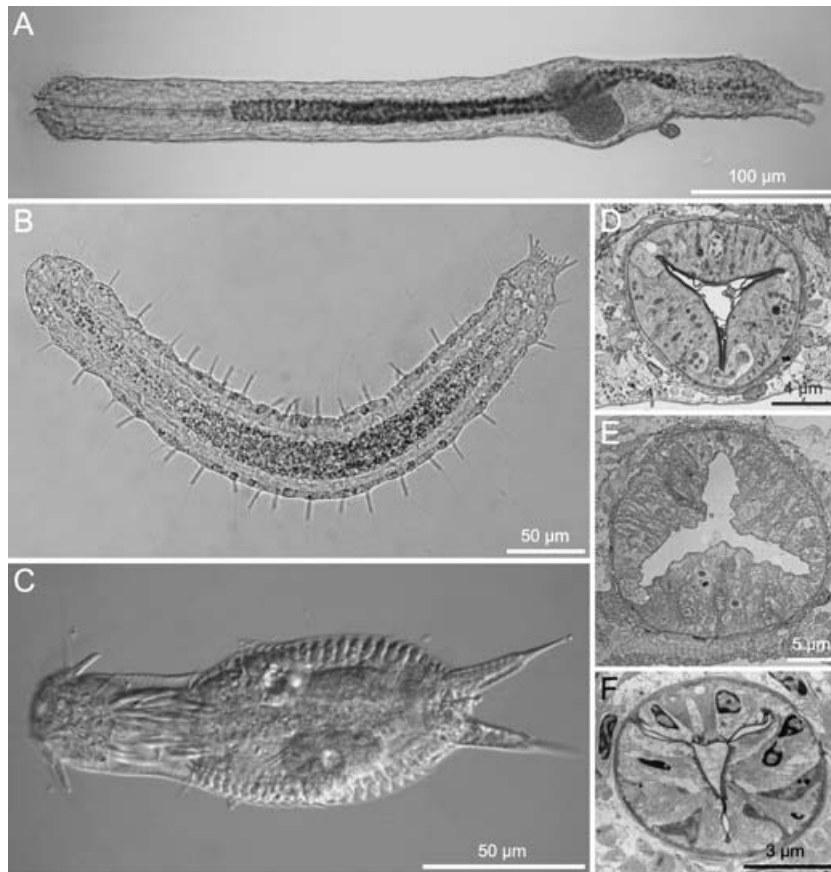
We suggest that a more comprehensive understanding of the basal relationships within Bilateria depends upon a better knowledge of the character patterns of the stem species (ground pattern) of its monophyletic subtaxa. We have started this reconstruction for some organ systems (protonephridia and reproductive organs) of the Gastrotricha based on our own ultrastructural investigations in combination with former results and recent hypotheses on the internal phylogeny of Gastrotricha (Kieneke *et al.* 2007, 2008a,b). The results of such a ground pattern reconstruction by mapping characters onto a given phylogenetic scenario may, in some cases, strongly depend on the underlying topology. Comparing recent phylogenetic analyses of the Gastrotricha shows that there is still controversy about the internal relationships of this taxon. However, these internal relationships need to be well known for a reliable reconstruction of the ground pattern of Gastrotricha.

Starting with the monographic work of all Gastrotricha, marine as well as freshwater groups, carried out by Remane in the first half of the 20th century (Remane 1929, 1936), this group has traditionally been subdivided into two orders: Macrodasyida (Fig. 1B) with eight families and Chaetonotida (Fig. 1A,C) with seven families. Within Chaetonotida, genus *Neodasys* (forming monogeneric suborder Multitubulatina, Fig. 1A) is the sister group of all remaining species in the six families (united as suborder Paucitubulatina, Fig. 1C) (see d'Hondt 1971). These basal internal relationships of Gastrotricha were supported either by a phylogenetic assessment of the ultrastructure of the gastrotrich pharynx (Ruppert 1982) and of the gastrotrich body wall (Travis 1983). Additionally, the first comprehensive phylogenetic analysis of numerous morphological characters strongly confirms this scenario (Hochberg & Litvaitis 2000). Furthermore, the results of a recent cladistic analysis based on ultrastructural data of spermatozoa from 28 gastrotrich species (Marotta *et al.* 2005) are congruent with the traditional systematic classification of Gastrotricha.

More recently, some phylogenetic analyses dealing with the internal relationships of Gastrotricha have been published for which sequences of the 18S rRNA gene have been used (or a combination of sequences and morphological characters, respectively). These studies are based on a fairly comprehensive taxon sampling among the gastrotrich families (Todaro *et al.* 2003, 2006a; Zrzavý 2003; Manylov *et al.* 2004; Petrov

et al. 2007). Monophyly was supported for the subtaxon Paucitubulatina in all mentioned molecular studies as it was supported for several traditional families. However, the different studies reveal different signals for the basal relationships of Gastrotricha. The analysis of Todaro *et al.* (2003) gives evidence for a paraphyletic Macrodasyida with monophyletic Paucitubulatina being the most derived group within Gastrotricha. While the study of Manylov *et al.* (2004) confirms monophyly of Macrodasyida and Paucitubulatina, both subtaxa cluster with different bilaterian taxa, thus suggesting Gastrotricha to be a polyphyletic group. The most recent molecular approaches (Todaro *et al.* 2006a; Petrov *et al.* 2007), both including sequences of the important taxon *Neodasys*, reveal Gastrotricha to consist of paraphyletic Macrodasyida with *Neodasys* being a macrodasyidan ingroup taxon, and the monophyletic Paucitubulatina in a derived position. The combined analysis of Zrzavý (2003) results in a paraphyletic Macrodasyida as well, but reveals *Neodasys* to be the sister taxon of Paucitubulatina and thus forming monophyletic Chaetonotida.

The novel hypothesis that Macrodasyida may be a paraphyletic assemblage, based exclusively on sequence data, inspired us to generate a new data matrix comprising morphological structures to test this hypothesis and to get a better idea of the possible evolution of Gastrotricha. Rather than finishing this study when the consensus tree is computed, we concentrate on a reconstruction and evaluation of the basal internal relationships of Gastrotricha. Furthermore, we herewith obtain putative apomorphies for the resulting clades and provide a hypothesis for the character pattern of the stem species of Gastrotricha.

Materials and methods

The data matrix

The species–character matrix, generated in the nexus format (Maddison *et al.* 1997) using the Nexus Data Editor 0.5.0 (Page 2001a), has the dimension of 83 taxa, 79 gastrotrich species and 4 outgroup taxa, and 135 characters in total (see Appendices A and B). To minimize *a priori* assumptions, all characters, almost half of which are multistate characters with up to 13 different character states (included ones), are unordered and unweighted. Multistate characters (composite coding) are preferred instead of non-additive binary coding in order to reduce the risk of 'artificial grouping' of taxa, which coincide in numerous 'absent' states that the program falsely treats as homologous absence when, in fact, they are non-homologous absence (Strong & Lipscomb 1999). For obtaining a maximum amount of information from a single character, we have transformed the information content into a kind of 'character cascade'. These partitioned pieces of information cover, for example, existence, number, arrangement and shape of a certain structure. In the case of structures that contain substructures, such a cascade of characters may consist of even more subunits. Coding characters this way leads to many instances of character dependence. Nevertheless, this is considered the best method to extract information from hierarchically structured characters that reflect the hierarchy of the phylogenetic relationships between organisms. Fusing the whole cascade of characters into one single multistate character involves a loss of phylogenetic information (Lee & Bryant 1999). In taxa that do not contain a certain structure, all dependent characters are coded as 'inapplicable'.

In the character and character state descriptions, we have used a kind of 'morphological type concept'. The majority of characters and character states is linked to a certain species by a '— type species xy'. All species in the matrix that are coded to have an identical character state are linked to the same 'morphological type species' (the mentioned concept shall be formally introduced and refined in a future contribution). Since no living individuals in nature exactly resemble each other, even within a common species or population, we had to use abstractions to decide whether a species has a certain character state or not. However, we tried to use an optimal degree of abstraction (e.g. the transformation from a variety of distinct numbers of adhesive tubes into few groups like 'few' or 'many' tubes, see Appendix A) for obtaining a maximum of phylogenetic information.

Ingroup taxa

A novelty for phylogenetic analysis of all Gastrotricha based on morphology is the use of species as terminal taxa instead of higher level taxa as, for example, genera. When using higher level taxa as terminals, one would have to code the character pattern of the stem species of these taxa. However, these character patterns are not known in the majority of cases. As representatives of the taxon Gastrotricha, we have chosen 79 species of almost all recent gastrotrich genera (Table 1). For some genera more than one species were included because of the taxonomic heterogeneity of the given group. For example, as the huge genus *Chaetonotus* is separated into numerous subgenera (Schwank 1990), we have coded species of each of these subgenera. The source for the species vectors in the matrix are the original species descriptions or monographic works as it is, for example, the case in the majority of freshwater gastrotrichs (coded according to Schwank 1990, see Table 1).

Outgroup taxa

Four different outgroup taxa were chosen (Table 1) to cover most of the common sister group hypotheses for Gastrotricha (summarized, e.g. in Schmidt-Rhaesa 2002; Petrov *et al.* 2007). The 'Nematode' outgroup (coded according to common school books, see, e.g. Table 1) is the representative for either the Nemathelminthes hypothesis (Gastrotricha as sister taxon

Table 1 List of all taxa (species) implemented in the matrix. Monographic works comprising three or more species descriptions used for the matrix and descriptions of the outgroup taxa are provided in the reference list (indicated by an asterisk). Studies dealing with the reproductive system which were used for coding the taxa are also specified in the references section.

Ingroup taxa	Literature used for coding the species	Additional remarks
Acanthodasys lineatus Clausen, 2000	Clausen (2000)	
Anacanthoderma paucisetosum Marcolongo, 1910	Schwank (1990)*	
Arenotus strixinoi Kisielewski, 1987	Kisielewski (1987)	
Aspidiophorus paradoxus Voigt, 1902	Schwank (1990)*	
Aspidiophorus silvaticus Varga, 1963	Schwank (1990)*	
Aspidiophorus polystictos Balsamo and Todaro, 1987	Balsamo and Todaro (1987)	
Cephalodasys maximus Remane, 1926	Remane (1926)*	
Chaetonotus (*Brevipedichaeta*) *uncinus* Voigt, 1902	Schwank (1990)*	
Chaetonotus (*Bifasciculatella*) *linguaeformis* Voigt, 1902	Schwank (1990)*	
Chaetonotus condensus Mock, 1979	Mock (1979)*	
Chaetonotus (*Diversichaetatella*) *acanthocephalus* Valkanov, 1937	Schwank (1990)*	
Chaetonotus (*Euchaetonotus*) *maximus* Ehrenberg, 1831	Schwank (1990)*	
Chaetonotus (*Hystricochaetonotus*) *hystrix* Metschnikoff, 1865	Schwank (1990)*	
Chaetonotus (*Schizochaetonotus*) *schultzei* Metschnikoff, 1851	Schwank (1990)*	
Chaetonotus (*Zonochaeta*) *succinctus* Voigt, 1902	Schwank (1990)*	
Chitonodytes collini Remane, 1927	Schwank (1990)*	
Chordodasiopsis antennatus Rieger *et al.* 1974	Rieger *et al.* (1974)	
Crasiella diplura Clausen, 1968	Clausen (1968)	
Dactylopodola baltica Remane, 1926	Remane (1926)*	
Dasydytes (*Dasydytes*) *ornatus* Voigt, 1909	Schwank (1990)*	
Dasydytes (*Prodasydytes*) *papaveroi* Kisielewski, 1991	Kisielewski (1991)*	
Dasydytes (*Setodytes*) *tongiorgii* Balsamo, 1982	Schwank (1990)*	
Dasydytes (*Setopus*) *bisetosus* Thompson, 1891	Schwank (1990)*	
Dendrodasys gracilis Wilke, 1954	Wilke (1954)*	
Dendropodola transitionalis Hummon *et al.* 1993	Hummon, Todaro & Tongiorgi (1993)	
Desmodasys phocoides Clausen, 1965	Clausen (1965)	
Dichaetura capricornia Metschnikoff, 1865	Schwank (1990)*	
Dinodasys mirabilis Remane, 1927	Remane (1927)*	
Diplodasys ankeli Wilke, 1954	Wilke (1954)*	Data on reproductive system according to Ruppert (1978)
Diuronotus aspetos Todaro *et al.*, 2005	Todaro *et al.* (2005)	
Dolichodasys elongatus Gagné, 1977	Gagné (1977)	
Draculiceteria tesselata Renaud-Mornant, 1968	Ruppert (1979)*	
Fluxiderma verrucosum Roszczak, 1935	Schwank (1990)*	
Halichaetonotus spinosus Mock, 1979	Mock (1979)*	
Haltidytes crassus Greuter, 1917	Schwank (1990)*	
Hemidasys agaso Claparède, 1867	Claparède (1867)	
Heterolepidoderma grandiculum Mock, 1979	Mock (1979)*	
Heterolepidoderma ocellatum Metschnikoff, 1865	Schwank (1990)*	
Heteroxenotrichula transatlantica Ruppert, 1979	Ruppert (1979)*	
Ichthydium (*Euichthydium*) *podura* Müller, 1773	Schwank (1990)*	
Ichthydium (*Forficulichthys*) *forficula* Remane, 1927	Schwank (1990)*	
Ichthydium hummoni Ruppert, 1977	Mock (1979)*	
Ichthydium (*Pseudichthydium*) *balatonicum* Varga, 1949	Schwank (1990)*	
Kijanebalola canina Kisielewski, 1991	Kisielewski (1991)*	
Lepidochaetus brasilense Kisielewski, 1991	Kisielewski (1991)*	
Lepidodasys platyurus Remane, 1927	Remane (1927)*	Data on reproductive system according to *Lepidodasys* sp. and *L. unicarenatus* Balsamo, Fregni & Tongiorgi, 1994 (Guidi *et al.* 2004)
Lepidodermella squamata Dujardin, 1841	Schwank (1990)*	
Macrodasys buddenbrocki Remane, 1924	Remane (1924)	
Marinellina flagellata Ruttner-Kolisko, 1955	Schwank (1990)*	
Megadasys pacificus Schmidt, 1974	Schmidt (1974)	
Mesodasys laticaudatus Remane, 1951	Remane (1951)	Data on reproductive system combined from original description and Ferraguti & Balsamo (1994)

Table 1 *Continued.*

Ingroup taxa	Literature used for coding the species	Additional remarks
Musellifer sublitoralis Hummon, 1969	Hummon (1969)	
Neodasys chaetonotoideus Remane, 1927	Remane (1927)*	Data on reproductive system combined from Remane (1936) and Guidi *et al.* (2003). Number of lateral adhesive tubes from Remane (1936)
Neogossea antennigera Gosse, 1851	Schwank (1990)*	
Ornamentula paraensis Kisielewski, 1991	Kisielewski (1991)*	
Paradasys littoralis Rao & Ganapati, 1968	Rao & Ganapati (1968)	
Paraturbanella scanica Clausen, 1996	Clausen (1996)	
Planodasys marginalis Rao and Clausen, 1970	Rao & Clausen (1970)	
Platydasys maximus Remane, 1927	Remane (1927)*	
Pleurodasys helgolandicus Remane, 1927	Remane (1927)*	Data on reproductive system according to *Pleurodasys megasoma* Boaden, 1963
Polymerurus nodicaudus Voigt, 1901	Schwank (1990)*	
Proichthydioides remanei Sudzuki, 1971	Sudzuki (1971)	
Proichthydium coronatum Cordero, 1918	Sudzuki (1971)	Coded according to a single illustration in Sudzuki (1971)
Prostobuccantia brocha Evans and Hummon, 1991	Evans & Hummon (1991)	
Pseudostomella cataphracta Ruppert, 1970	Ruppert (1970)*	
Pseudostomella roscovita Swedmark, 1956	Ruppert (1970)*	
Pseudoturbanella stylifera d'Hondt, 1968	d'Hondt (1968)	
Ptychostomella mediterranea Remane, 1927	Remane (1927)*	
Redudasys fornerise Kisielewski, 1987	Kisielewski (1987)	
Rhomballichthys punctatus Greuter, 1917	Schwank (1990)*	
Stylochaeta scirtetica Brunson, 1950	Schwank (1990)*	
Tetranchyroderma arcticum Clausen, 2000	Clausen (2000)	Data on reproductive system according to *Tetranchyroderma bunti* Thane-Fenchel, 1970 (Ruppert 1978)
Tetranchyroderma megastoma Remane, 1927	Remane (1927)*	Data on reproductive system according to *Tetranchyroderma bunti* Thane-Fenchel, 1970 (Ruppert 1978)
Thaumastoderma heideri Remane, 1926	Remane (1926)*	
Turbanella cornuta Remane, 1925	Remane (1925)	
Undula paraensis Kisielewski, 1991	Kisielewski (1991)*	
Urodasys viviparus Wilke, 1954	Wilke (1954)*	
Xenodasys sanctigoulveni Swedmark, 1967	Swedmark (1967)	Data on frontal adhesive tubes and reproductive system according to *Xenodasys eknomios* Todaro *et al.*, 2006 (Todaro *et al.* 2006b)
Xenotrichula punctata Wilke, 1954	Wilke (1954)* and Mock (1979)*	
Outgroup taxa:		
Gnathostomula paradoxa Ax, 1956	Ax (1956)*	
Praeconvoluta minor Faubel, 1974	Faubel (1974a)*	
Macrostomum pusillum Ax, 1951	Faubel (1974b)*	
'Nematode'	Ruppert *et al.* (2004)*	Compiled from data of the Nematoda chapter

of Cycloneuralia; Ehlers *et al.* 1996; Sørensen *et al.* 2000; Ax 2003) or for Gastrotricha as sister taxon of Ecdysozoa (see Schmidt-Rhaesa *et al.* 1998; Peterson & Eernisse 2001). *Gnathostomula paradoxa* represents Gnathostomulida (or Gnathifera as a whole) and *Macrostomum pusillum* taxon Plathelminthes, each hypothesized to be sister taxon of Gastrotricha (Gnathostomulida + Gastrotricha: Zrzavý *et al.* 1998, Plathelminthes + Gastrotricha: Giribet *et al.* 2000; Giribet 2002). Alternatively, Gastrotricha, Plathelminthes and Gnathifera are suggested to form the taxon Platyzoa (Cavalier-Smith 1998; Giribet 2002). As a representative of the taxon Acoela, we have coded *Praeconvoluta minor*. While the taxon Acoela was formerly regarded to be a basal branch of the Plathelminthes (Ehlers 1985), more recently Acoela consistently turns out to be an isolated, early bilaterian lineage (e.g. Peterson & Eernisse 2001; Wallberg *et al.* 2007). Therefore, we have chosen to root the calculated networks against *P. minor*. The remaining outgroup taxa were not constrained as fixed outgroup taxa.

Search strategy

In order to find the optimal trees, we have carried out a search using the parsimony ratchet (Nixon 1999), which samples trees from many tree islands thus evaluating the tree space more widely in a fraction of time used for usual search strategies. The commands for the parsimony ratchet were created using the program PRAP (Müller 2004) with a parameter setting of 200 ratchet iterations and 25% of randomly chosen characters weighted with '2'. The calculation was performed with the program PAUP* v4.0b10 (Swofford 2002) using the commands generated in PRAP. To estimate possible effects of mere chance for the basal relationships of the ingroup, we have repeated the parsimony ratchet 20 times, each time generating new commands for PAUP*.

Additionally, we have run a conventional heuristic search using PAUP* to compare the results with those of the parsimony ratchet. The h-search was performed with 1000 replicates with a re-arrangement limit of 10 000 000 for each replicate. Starting trees were obtained by stepwise addition (randomly) and branch swapping was performed with the TBR algorithm. The number of maximum trees to be saved during the search was set to be automatically increased. Branches with a length of '0' were eliminated.

For both search strategies (parsimony ratchet and h-search), branch lengths of the equally parsimonious trees were saved. A 50% majority rule consensus tree was computed of the equally parsimonious trees.

Tree evaluation

To estimate the robustness of the nodes, we have calculated bootstrap support values (Felsenstein 1985) as well as bremer support indices (Bremer 1988, 1994). Bootstrap values were obtained running a bootstrap search in PAUP* with 2500 bootstrap replicates. The search settings were the same as in the h-search but with five replicates per bootstrap replicate only.

Decay indices where calculated with PAUP* using the parsimony ratchet (Müller 2004). Again, commands for PAUP* where generated with the program PRAP using the original data matrix and the 50% majority rule consensus tree to be evaluated. The number of ratchet iterations used for evaluating each node was reduced to 10. For assessing the results from bootstrapping and the calculation of decay indices, the program TREEVIEW 1.6.5 (Page 2001b) has been applied.

For character optimization, we have either generated an apomorphy list with PAUP* ('ACCTRAN' optimization) or used the 'trace all changes' tool of the program MACCLADE 4.0 (Maddison & Maddison 1989, 2000). To reconstruct the character pattern of the stem species of Gastrotricha, the 'trace character' function ('ACCTRAN' optimization) of MACCLADE was used.

Preparations for micrographs

We provide examples of selected gastrotrich species to illustrate most of the suggested autapomorphies within the basal stem lineages of Gastrotricha (see Figs 1 and 5). These examples comprise light microscopic images (bright field and differential interference contrast optics), scanning as well as transmission electron microscopic images and a projection of some confocal images of the phalloidin-labelled muscular system of a marine gastrotrich. Preparation techniques and details of the used microscopic systems can be found in Kieneke *et al*. (2008b,c).

Results

General results

The data matrix consists of 86 parsimony informative characters (+8 excluded characters, 36 uninformative characters and 5 constant characters). Many characters and character states are distributed homoplastic along the equally parsimonious trees found (CI: 0.47, RI: 0.73, Fig. 3). The parsimony ratchet provided 76 equally parsimonious trees of 497 steps length in about 1 min, the heuristic search, which took 1 h 51 min with the given settings, did not find trees shorter than 497 steps (7532 equally parsimonious trees were retained). Moreover,

Fig. 2 Consensus tree (50% majority rule) of 76 equally parsimonious trees found in the parsimony ratchet search carried out with the programs PRAP and PAUP*. Putative autapomorphies of monophyletic groups of high rank within Gastrotricha are indicated by numbered black squares. Only for some selected derived taxa apomorphies are shown. Decay values are mapped on the left side and bootstrap values (in italics) are mapped on the right side of a branch. Bootstrap support below 50% is not indicated. Autapomorphies: 1, bilobed caudal end, *existence of lateral adhesive tubes*, cilia covered with cuticle, visceral helicoidal muscles; 2, blunt and triangular frontal end, *caudal and lateral adhesive tubes as non-duo-gland organs* (*mastoid openings*), club-shaped mouth tube, loss of vasa deferentia; 3, existence of frontal adhesive tubes, *inverted Y-shaped pharyngeal lumen*, caudo-frontally directed maturation of oocytes, existence of duo gland adhesive organs*; 4, existence of epidermal glands, existence of pharyngeal pores; 5, neck constriction at the level of frontal adhesive tubes, blunt frontal end, caudal end with two simple lobes; 6, prominent inflexion of seminal ducts; 7, additional caudal adhesive tubes (exclusive of furcal tubes), v-shaped buccal cavity; 8, only few lateral adhesive tubes, only one additional caudal adhesive tube per side; 9, discoidal caudal end; 10, bilobed caudal end, existence of ventral adhesive tubes, *funnel-shaped buccal cavity*, head- and trunk scales as 'ancres'; 11, *slightly convex frontal end*, *body covered with tetrancres*, unpaired right testis, single vas deferens discharges into the caudal organ; 12, depression of the pharynx region, *loss of lateral adhesive tubes*, loss of fully developed testes, loss of frontal- and caudal organ; 13, bilobed caudal end as short appendages at the angles of the blunt margin; 14, compact, sole-shaped habitus, only one adhesive tube per furcal branch, *loss of frontal adhesive tubes, Y-shaped pharyngeal lumen*; 15, neck constriction at caudal end of pharynx, sickle-shaped caudal adhesive tubes; 16, *existence of basked-like supportive structures at the mouth*, existence of head- and trunk scales; 17, existence of fully developed testes; 18, subterminal mouth opening, *occurrence of rudimentary testes, occurrence of an X-organ*; 19,
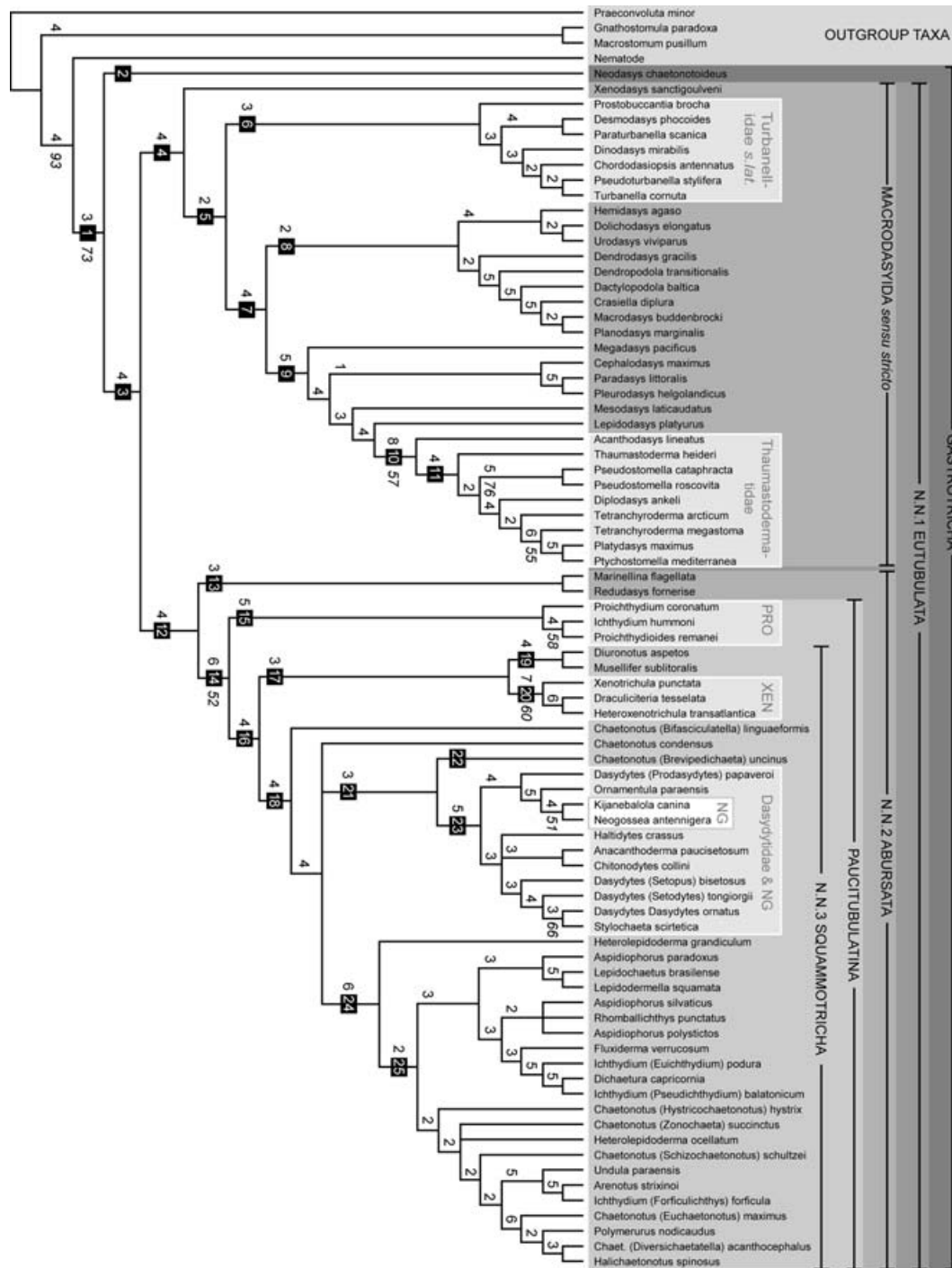
conical buccal cavity, loss of vasa deferentia; 20, base of furca covered with stalked scales, sensory cilia of the head composed as cirri, locomotory cilia composed as cirri, head and trunk scales as stalked scales, existence of ventrolateral hydrofoil scales, *ring-shaped testes*, existence of a testicular anastomosis; 21, *rudimentary caudal adhesive tubes* (*direct opening of adhesive glands*), *terminal anus*, existence of terminal spines; 22, claw-shaped terminal spines; 23, non-bilobed caudal end, existence of an unpaired, fronto-dorsal cuticular plate at the head, existence of motile spines, existence of conspicuous ciliary girdle(s) at the head; 24, existence of an unpaired, fronto-dorsal cuticular plate at the head; 25, fivefold lobate frontal end (head). Apomorphies in italics indicate ambiguous changes. Abbreviations: N.N., nomen nominandum; NG, Neogosseidae; PRO, Proichthydiidae; XEN, Xenotrichulidae; *, the character 'duo glands' is not included in the matrix and taken from the literature (for references see text).

Praeconvoluta minor
Gnathostomula paradoxa
Macrostomum pusillum
Nematode
Neodasys chaetonotoideus
Xenodasys sanctigoulveni
Prostobuccantia brocha
Desmodasys phocoides
Paraturbanella scanica
Dinodasys mirabilis
Chordodasiopsis antennatus
Pseudoturbanella stylifera
Turbanella cornuta
Hemidasys agaso
Dolichodasys elongatus
Urodasys viviparus
Dendrodasys gracilis
Dendropodola transitionalis
Dactylopodola baltica
Crasiella diplura
Macrodasys buddenbrocki
Planodasys marginalis
Megadasys pacificus
Mesodasys laticaudatus
Cephalodasys maximus
Paradasys littoralis
Pleurodasys helgolandicus
Lepidodasys platyurus
Acanthodasys lineatus
Thaumastoderma heideri
Pseudostomella cataphracta
Pseudostomella roscovita
Diplodasys ankeli
Tetranchyroderma arcticum
Tetranchyroderma megastoma
Platydasys maximus
Ptychostomella mediterranea

Marinellina flagellata
Redudasys fornerise
Proichthydium coronatum
Ichthydium hummoni
Proichthydioides remanei
Diuronotus aspetos
Musellifer sublitoralis
Xenotrichula punctata
Draculiciteria tesselata
Heteroxenotrichula transatlantica
Chaetonotus (Bifasciculatella) linguaeformis
Chaetonotus condensus
Chaetonotus (Brevipedichaeta) uncinus
Dasydytes (Prodasydytes) papaveroi
Ornamentula paraensis
Kijanebalola canina
Neogossea antennigera
Dasydytes (Dasydytes) ornatus
Stylochaeta scirtetica
Anacanthoderma paucisetosum
Chitonodytes collini
Haltidytes crassus
Dasydytes (Setodytes) tongiorgii
Dasydytes (Setopus) bisetosus
Heterolepidoderma grandiculum
Aspidiophorus paradoxus
Lepidochaetus brasilense
Lepidodermella squamata
Aspidiophorus silvaticus
Rhomballichthys punctatus
Aspidiophorus polystictos
Fluxiderma verrucosum
Ichthydium (Euichthydium) podura
Dichaetura capricornia
Ichthydium (Pseudichthydium) balatonicum
Chaetonotus (Hystricochaetonotus) hystrix
Chaetonotus (Zonochaeta) succinctus
Heterolepidoderma ocellatum
Chaetonotus (Schizochaetonotus) schultzei
Undula paraensis
Arenotus strixinoi
Ichthydium (Forficulichthys) forficula
Chaetonotus (Euchaetonotus) maximus
Polymerurus nodicaudus
Chaetonotus (Diversichaetatella) acanthocephalus
Halichaetonotus spinosus

— 2 steps

Fig. 3 One of the 76 equally parsimonious trees found by the parsimony ratchet search showing branch lengths. The parsimony ratchet ran with 200 iterations and weighting of 25% of included characters. Tree length is 497 (included characters only), CI = 0.47, RI = 0.73.

the topology for the basal relationships revealed by both strategies was exactly the same (compare Figs 2 and 4). To estimate the risk of possible coincidence effects, we have repeated the parsimony ratchet 20 times and compared the resulting topologies (consensus trees were compared).

Again, the resulting basal relationships of each of the 'ratchet replicates' were the same. However, relationships within and between more derived groups varied among the different searches. For analysing character evolution and evaluating possible internal relationships of Gastrotricha, we have chosen
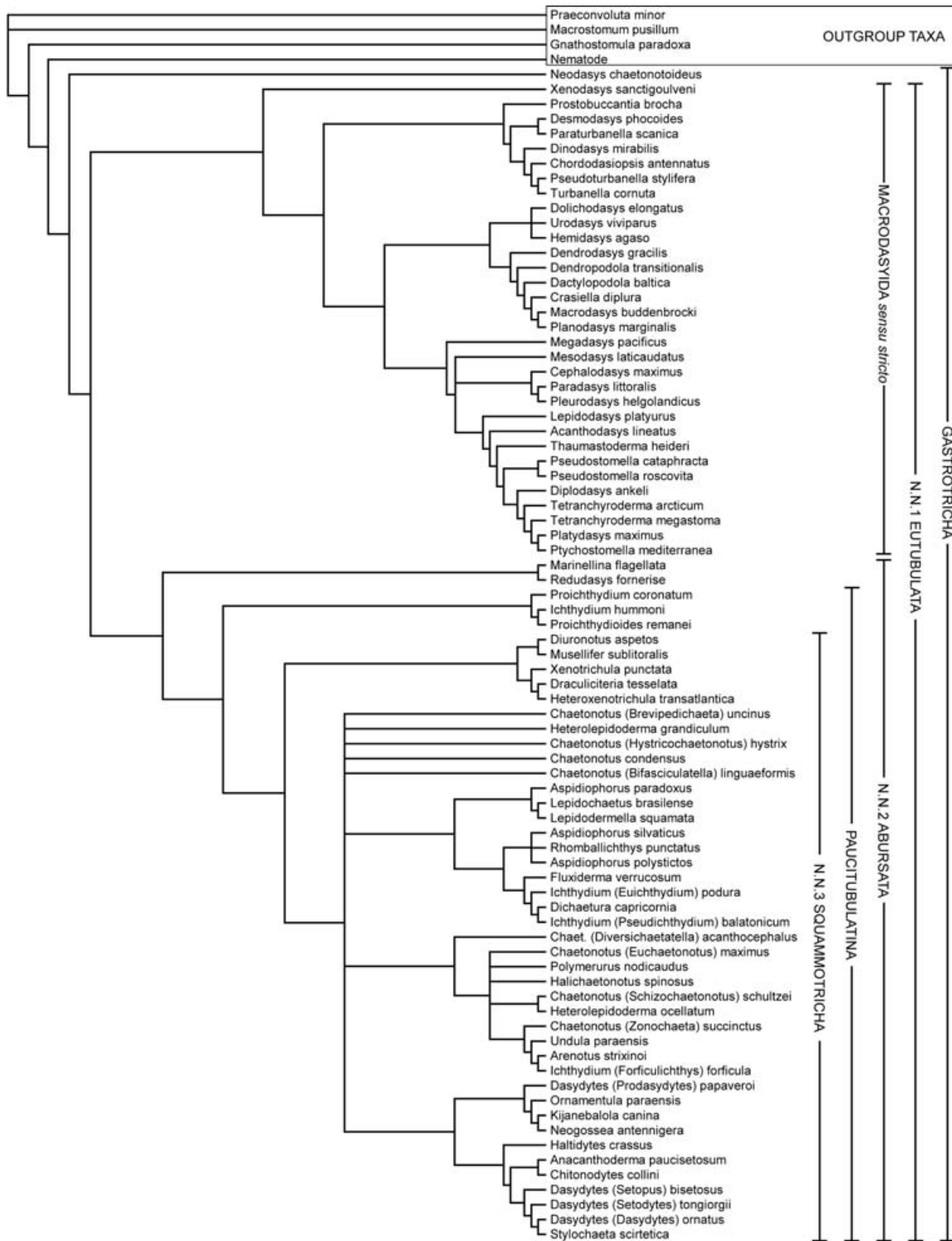
Fig. 4 Consensus tree (50% majority rule) of 7532 equally parsimonious trees found by the heuristic search carried out with PAUP*. Tree length is 497 steps (included characters only), CI = 0.48, RI = 0.73. Identical major clades are found as in the parsimony ratchet (compare with Fig. 2).

a search that provides resolution in nearly all higher taxa of Gastrotricha.

Relationships within Gastrotricha

In the following descriptions, we use, in the majority of cases, the names of the genera instead of the distinct species names for terminal taxa. For ease of communication, we introduce provisional names for some higher level taxa that, according to this analysis, turned out to be monophyla of high rank (Figs 2 and 4). The names are suggestions but, if these putative monophyletic groups are supported in the future, they are intended for use as valid taxon names.

All Gastrotricha form a monophyletic group with the 'Nematode' outgroup as the closest related bilaterian taxon (see Fig. 2). The initial speciation separates Gastrotricha into *Neodasys* and all remaining gastrotrich species as sister groups of highest rank. The monophyletic group exclusive of *Neodasys* (N.N.1) is called Eutubulata *nomen novum* according to the general construction of their adhesive organs or tubes (see below). The Taxon Eutubulata splits into Macrodasyida *s. str.* and a taxon (N.N.2) called Abursata *nomen novum*. Macrodasyida *s. str.* comprises all traditional macrodasyidan genera exclusive of the freshwater taxa *Redudasys* and *Marinellina*, which are, united in a common clade, the sister group of traditional Paucitubulatina that are confirmed to be a monophyletic group in this analysis.

Within Macrodasyida *s. str.*, *Xenodasys* is the sister taxon to al remaining macrodasyids. These split into monophyletic Turbanellidae *sensu lato* (traditional Turbanellidae + *Chordodasiopsis*) and a monophylum that consists of the majority of macrodasyidan genera. Within that clade, a group containing the genera of traditional Lepidodasyidae (partial), Dactylopodolidae, Macrodasyidae and Planodasyidae is the sister taxon of a group comprising the genera of traditional Lepidodasyidae (partial) and monophyletic Thaumastodermatidae (Figs 2 and 4). However, monophyly of Dactylopodolidae, Macrodasyidae, Lepidodasyidae and Planodasyidae was not confirmed.

Within Paucitubulatina, sister taxa of highest rank are a clade comprising traditional Proichthydiidae, *Proichthydium* and *Proichthydioides*, + *Ichthydium hummoni*, and all remaining paucitubulatan genera (N.N.3) forming a monophyletic group Squammotricha *nomen novum*. Within Squammotricha, a clade comprising monophyletic Xenotrichulidae and *Musellifer*

+ *Diuronotus* is the sister group of all remaining paucitubulatan genera. Within that clade, there are three major monophyletic subtaxa (Figs 2 and 4), one of which is a monophylum that consists of traditional Dasydytidae with Neogosseidae as an ingroup taxon.

Autapomorphies of selected monophyla

The analysis has revealed a number of well-defined putative autapomorphies for the basal monophyla of Gastrotricha and for many of the more derived taxa (see Fig. 2). Unambiguous evolutionary transformations within the stem lineage of Gastrotricha were the development of a bilobed caudal end (Fig. 5A–F), the covering of cilia with exo-cuticle (Fig. 5H), and the evolution of a visceral muscle helix (Fig. 2: 1, 5J). The existence of lateral adhesive tubes (Fig. 5G), however, is an ambiguous transformation. Novelties in the stem lineage of Eutubulata are the existence of frontal adhesive tubes (Fig. 5K–M) and a caudo-frontally directed maturation of the oocytes (Fig. 2: 3, 5N). The character 'existence of duo gland adhesive organs' which gives the whole group the name 'Eutubulata' was not included in the data matrix. However, since species of *Neodasys* are, as far as we presently know, the only gastrotrichs possessing non-duo-gland adhesive organs or tubes, respectively (see Tyler *et al.* 1980), we suggest that the duo gland adhesive tubes evolved only once within Gastrotricha in the stem lineage of Eutubulata (Fig. 2: 3). However, Hochberg & Litvaitis (2000) stress that the universal occurrence of duo-gland adhesive tubes in all gastrotrichs exclusive of *Neodasys* still has not been confirmed.

Macrodasyida *s. str.* are characterized well by the possession of epidermal glands (Fig. 5O) and pharyngeal pores (Fig. 2: 4, 5J, P). In the stem lineage of the sister group, Abursata, several reductions (secondary losses) took place (Fig. 2: 12). The testes were completely lost or modified (Fig. 5Q,R) to 'rudimentary testes', which represent an ambiguous evolutionary novelty for a monophyletic subgroup within Paucitubulatina (Fig. 2: 18). Additionally, both accessory reproductive organs known from Macrodasyida *s. str.* and *Neodasys*, the frontal and caudal organ, are lost within Abursata (Fig. 5Q,R).

Traditional monophyletic Paucitubulatina are characterized by the compact, sole-shaped habitus (Fig. 5Q,R), the reduction of the caudal adhesive tubes to a single pair (one tube per furcal branch, Figs 1C and 5B,F,Q,R), and, ambiguously, a

Fig. 5 A–R. Examples of valid gastrotrich species illustrating characters assumed to be apomorphic for the gastrotrich monophyla of highest rank. A–F. Different types of the bilobed caudal end (existence of bilobed caudal end suggested as autapomorphy of Gastrotricha). —A. *Dactylopodola typhle*. —B. *Lepidodermella squamata*. —C. *Neodasys chaetonotoideus* (juvenile). —D. *Polymerurus nodicaudus*. —E. *Turbanella ambronensis*. —F. *Xenotrichula carolinensis*. G–J. Further apomorphies of Gastrotricha. —G. Lateral adhesive tubes (*Turbanella hyalina*). —H. Locomotory cilia of *Neodasys chaetonotoideus* covered by epicuticle. —J. Projection of five confocal images of phalloidin-stained F-actin (pharyngeal region) of *Turbanella hyalina* illustrating the visceral muscle helix (mh) and the muscular bulbs of pharynx leading to pharyngeal pores (pp, autapomorphy of Macrodasyida *s. str.*). K–M. Different types of arrangement of frontal adhesive tubes (existence of frontal adhesive tubes suggested as
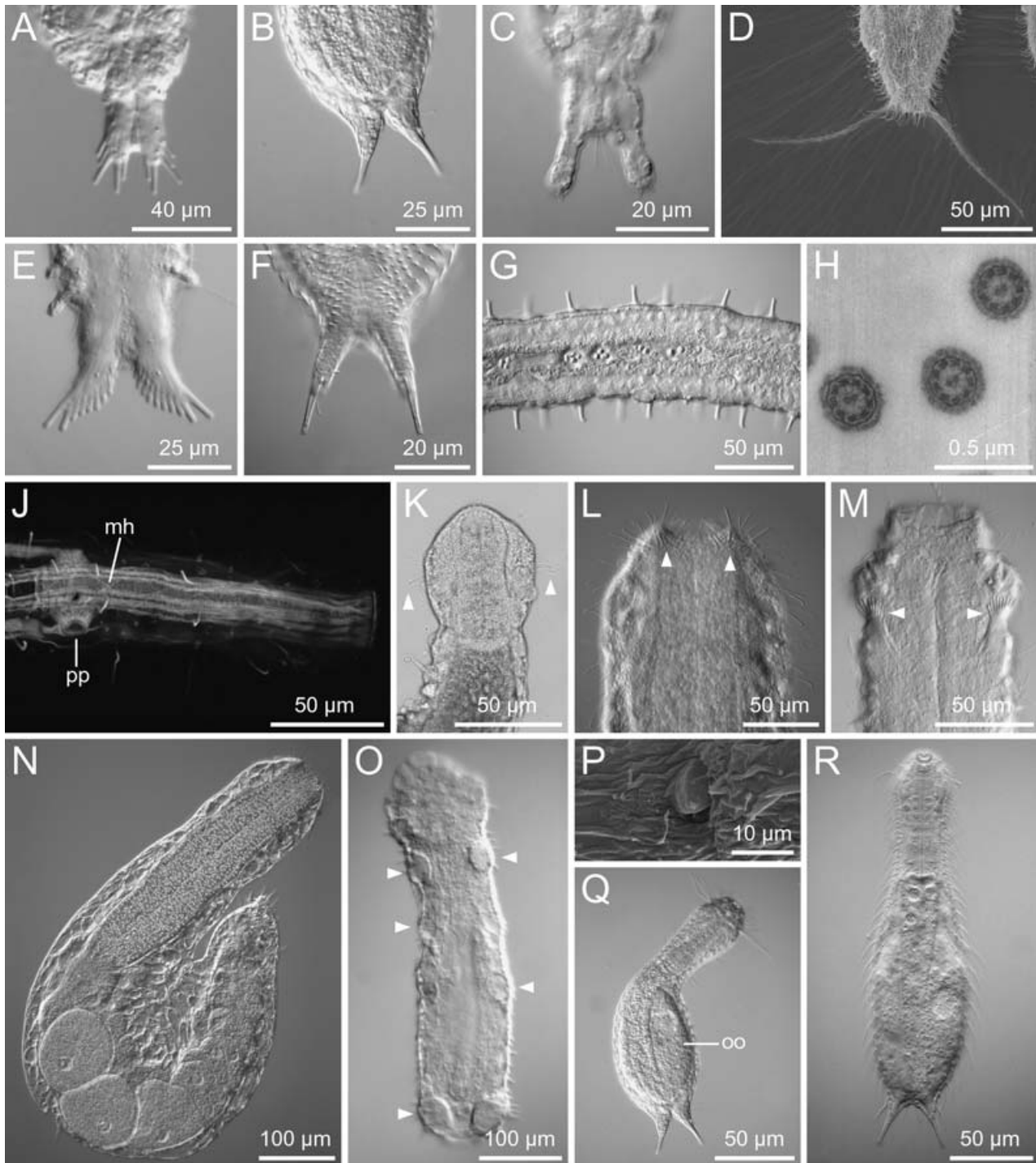
autapomorphy of Eutubulata), indicated by arrowheads. —K. *Dactylopodola typhle*. —L. *Macrodasys caudatus*. —M. *Turbanella ambronensis*. —N. Further apomorphy of Eutubulata: caudo-frontal directed maturation of oocytes (*Macrodasys caudatus*). O–P. Suggested apomorphies of Macrodasyida *s. str.* —O. Epidermal glands (*Diplodasys ankeli*). —P. Pharyngeal pores (*Desmodasys abyssalis*). Q–R. Suggested apomorphies of Abursata (loss of testes, frontal and caudal organ) and Paucitubulatina (sole-shaped habitus, only one adhesive tube per furcal branch, loss of frontal adhesive tubes) illustrated by two members of traditional Chaetonotidae. —Q. *Lepidodermella squamata*. —R. *Chaetonotus maximus*. A–C, E–G, K–O, Q–R: light microscopic images; D, P: scanning electron microscopic images; H: transmission electron microscopic image; J: z-projection of five confocal images.

complete loss of the frontal adhesive tubes (Fig. 2: 14, 5Q,R). The species of paucitubulatan subtaxon Squammotricha are united by a shared possession of cuticular scales on the head and trunk region (Fig. 2: 16, see stalked scales in Fig. 1C and spined scales in Fig. 5R). The absence of such cuticular formations in the putative sister group, the Proichthydiidae (including *I. hummoni*), is a primary absence as it is in all scale-less Macrodasyida, *Redudasys*, *Marinellina* and *Neodasys*.

Within both major gastrotrich clades, Macrodasyida *s. str.* and Paucitubulatina, there are some well-characterized monophyla as well. For example, in the stem lineage of monophyletic Thaumastodermatidae, there are several transformations (Fig. 2: 10): Two columns of ventral adhesive tubes appear, the mouth is developed as a funnel-like buccal field (ambiguous transformation), and cuticular formations (as 'ancres') cover the body. Within Thamastodermatidae, there are further transformations (Fig. 2: 11): The unpaired right testis discharges directly into the caudal organ. Monophyletic paucitubulatans Dasydytidae + Neogosseidae are characterized by the secondary loss of the caudal furca with its adhesive tubes and the possession of ciliary girdles at the head and motile spines at the trunk (Fig. 2: 23).

Statistic support

Bootstrap support for most basal nodes and for many of the more terminal ones was always very low. Values higher than 70% were only obtained for the common node of Nematode and Gastrotricha and for the common node of all Gastrotricha (see Fig. 2). However, robustness of nodes obtained by calculating decay indices was supported for many clades within Gastrotricha. Especially many of the basal nodes are supported by decay values of four or higher (see Fig. 2).

Ground pattern of Gastrotricha

The reconstruction of the character states of the ground pattern of Gastrotricha carried out by parsimonious character optimization yields the following hypothesis (Fig. 6A–D, see also Appendix B): The stem species of Gastrotricha is an elongate, dorso-ventrally flattened hermaphroditic worm that lived in a marine, benthic environment. The organism is provided with a cuticle that covers all external cilia (locomotory cilia are possibly restricted to the ventral side). It has a slightly convex frontal end, a bilobed caudal end and possesses a neck constriction (this is possibly formed by a depression of the whole trunk region as it is present in mature *Neodasys*). There are no distinct sensory structures like palps, auricles or pigmented eye spots but individual sensory cilia at the frontal end.

The gastric tract (consisting of a myoepithelial sucking pharynx and a straight midgut) starts with the terminal, round mouth opening followed by the cylindric buccal cavity, straight pharynx and unbranched intestine, and opens into the permanent, ventral anus. There are no pharyngeal pores.

The shape of the cross section of the pharyngeal lumen (Y-shaped vs. inverted Y-shaped) could not be reconstructed.

The stem species of Gastrotricha possess adhesive tubes in two lateral columns and in two groups at the branches of the bilobed caudal end. There are few tubes (up to five) at each furcal branch and many tubes (8–30) in each lateral group. Frontal-, ventral-, and dorso-lateral adhesive tubes evolved later within certain lineages of Gastrotricha. We could not reconstruct whether the cytological construction of the adhesive tubes of the stem species of Gastrotricha was a non-duo-gland or a duo-gland.

There are paired gonads, testes as well as ovaries, which mature in a fronto-caudal direction. The testes are long and tube-shaped and continue in caudally directed seminal ducts. The seminal ducts possibly open into paired ventral gonopores. The gastrotrich stem species has a frontal organ (as a sperm storing device) and a caudal organ (as a sperm transferring device).

Discussion

Comparison of alternative phylogenetic scenarios

Our study offers a novel phylogenetic scenario for the early evolution of Gastrotricha and highlights the important position of *Neodasys* as the sister group to all remaining Gastrotricha, that is, Eutubulata. For a structured comparison of alternative hypotheses for the internal phylogeny of Gastrotricha, it is appropriate to 'reduce' the diversity of the whole monophylum to the traditional major groups (Macrodasyida, Chaetonotida, Paucitubulatina and *Neodays*), and to refer to their possible mono-, para-, or polyphyly. A graphical comparison of the most common hypotheses is given in Fig. 7.

A major congruence between most phylogenetic assessments and analyses is (i) the monophyly of Gastrotricha as a whole, and (ii) the monophyly of its sub taxon Paucitubulatina (Fig. 7A,C,E,F). However, the initial analysis of gastrotrich DNA sequence data (Wirz *et al.* 1999) reveals a paraphyletic Paucitubulatina with several offshoots from the direct stem lineage of Macrodasyida (Fig. 7B). Another molecular approach carried out by Manylov *et al.* (2004) indicates monophyly of both, Paucitubulatina and Macrodasyida but suggests Gastrotricha to be a polyphyletic group with sequences of Plathelminthes, Gnathifera and *Symbion pandora* to be more closely related to Paucitubulatina than are the macrodasyid sequences (Fig. 7D). Manylov *et al.* (2004) further suggest that the macrodasyid sequences used in the analysis of Wirz *et al.* (1999) actually are sequences of misidentified paucitubulatan species. It has to be stressed that in both analyses mentioned above (Wirz *et al.* 1999; Manylov *et al.* 2004), sequences of the important taxon *Neodasys*, which possesses a character pattern intermediate between species of Macrodasyida and Paucitubulatina (see Ruppert 1991), were not included.
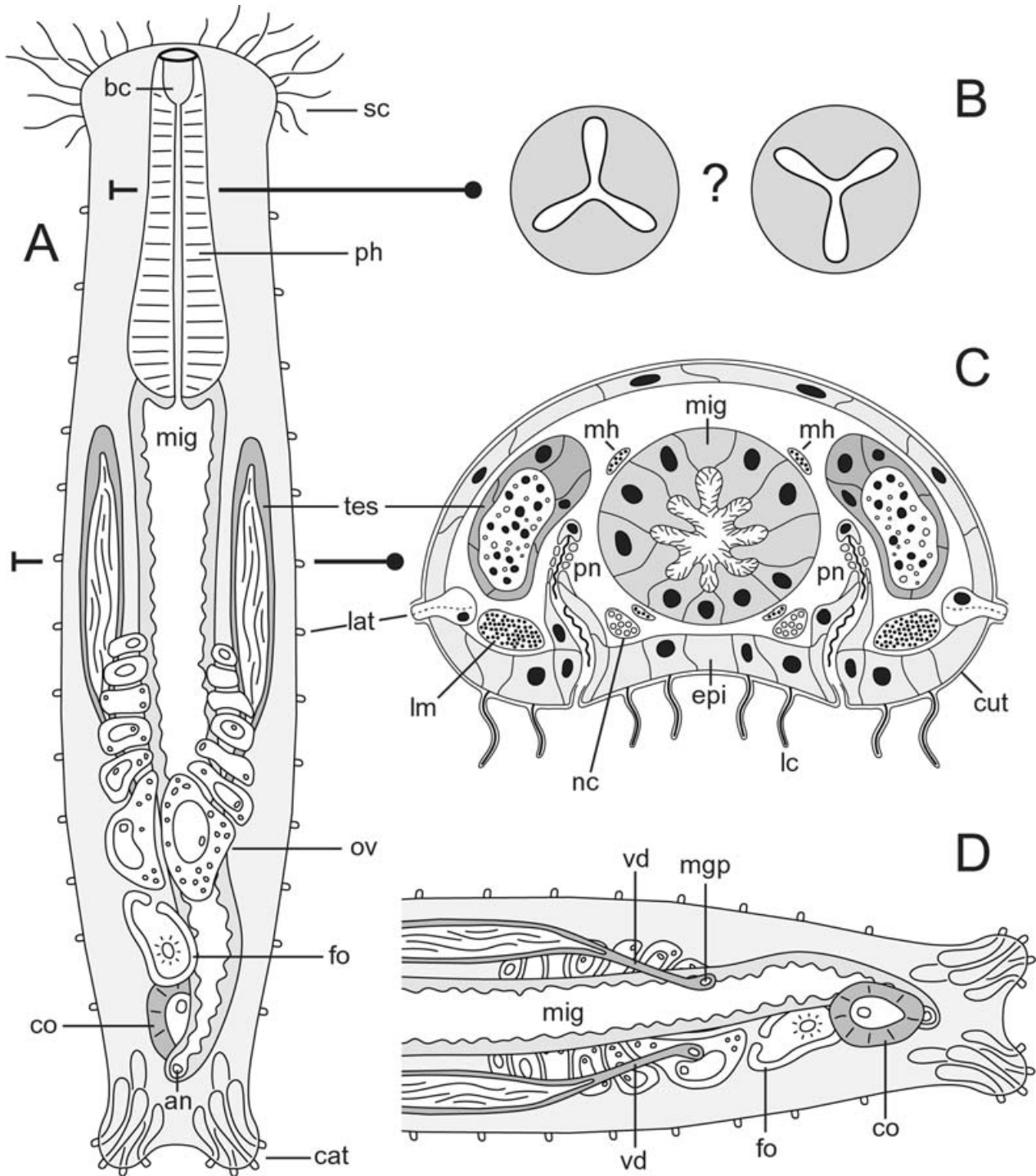
Fig. 6 A–D. Reconstruction of the stem species of Gastrotricha. —A. Habitus and gross body organization, seen from dorsal. —B. Possible cross section of the pharyngeal lumen. —C. Schematic cross section of the trunk at the level of testes. —D. Ventral view of the posterior trunk region with seminal ducts and male gonopores. Abbreviations: an, anus; bc, buccal cavity; cat, caudal adhesive tubes; co, caudal organ; cut, cuticle; epi, epidermis; fo, frontal organ; lat, lateral adhesive tubes; lc, locomotory cilia; lm, longitudinal muscles; mgp, male gonopore; mh, muscular helix; mig, midgut; nc, ventral nerve chords; ov, ovaries; ph, pharynx; pn, protonephridium; sc, sensory cilia; tes, testes; vd, vas deferens.
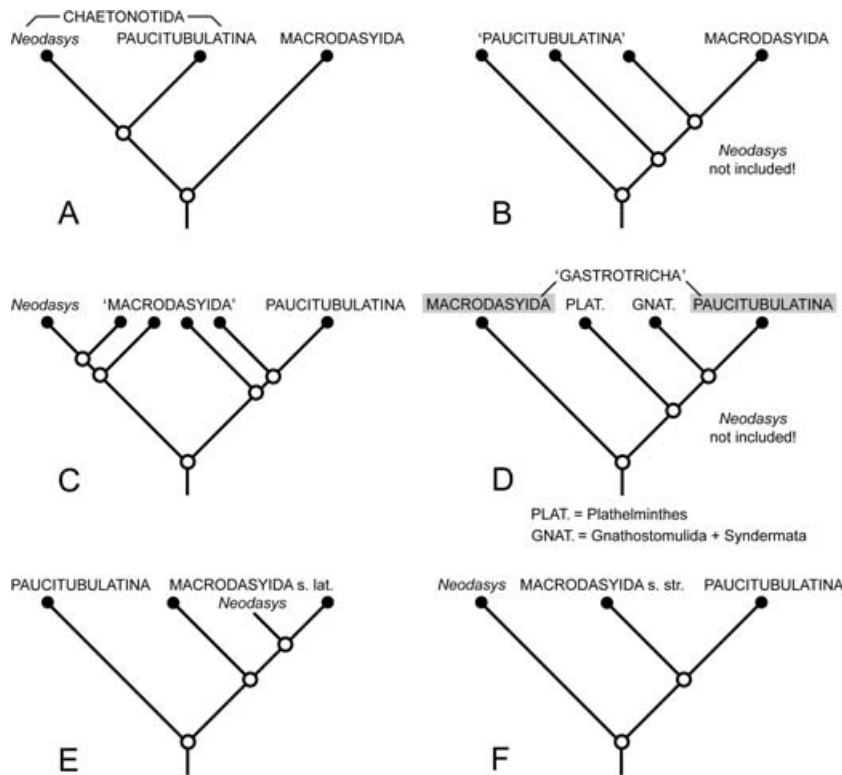
Fig. 7 A–F. Most basal relationships within Gastrotricha (simplified) according to different phylogenetic suggestions and analyses. Taxa in quotes indicate para- or polyphyletic groups. —A. Traditional systematics supported by the studies of Ruppert (1982), Travis (1983) and Hochberg & Litvaitis (2000). —B. Paraphyly of Paucitubulatina according to Wirz *et al*. (1999). —C. Polyphletic Macrodasyida with *Neodasys* as an ingroup of partial Macrodasyida (Todaro *et al*. 2003, 2006a) or paraphyletic Macrodasyida with *Neodasys* as sister taxon of Paucitubulatina (Zrzavý 2003). —D. Polyphyletic Gastrotricha with monophyletic Macrodasyida and Paucitubulatina at different positions within Bilateria (Manylov *et al*. 2004). —E. Monophyletic Macrodasyida and Paucitubulatina with *Neodasys* as an ingroup taxon of Macrodasyida (Petrov *et al*. 2007). —F. *Neodasys* as the sister group of all remaining Gastrotricha (present analysis).

The traditional Macrodasyida are revealed to be monophyletic in several studies (Ruppert 1982; Travis 1983; Wirz *et al*. 1999; Hochberg & Litvaitis 2000; Manylov *et al*. 2004; Petrov *et al*. 2007) as they are, with the exclusion of *Redudasys* and *Marinellina*, in our analysis (Fig. 7A,B,D–F). Members of traditional Macrodasyida form a para- or polyphyletic assemblage according to analyses of partial 18S rRNA gene sequence data (Todaro *et al*. 2003, 2006a) and according to a combined approach (Zrzavý 2003; see Fig. 7C). The traditional taxon Chaetonotida consisting of Paucitubulatina and *Neodasys* as sister taxa is revealed by morphological (and a combination of morphology and sequences, respectively) data only (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 2000; Zrzavý 2003). However, monophyly of Chaetonotida was not consistently supported by morphological data (this study, compare Fig. 7A,C,F).

The important taxon *Neodasys*, having a key role for gastrotrich phylogeny, has occupied different positions within Gastrotricha in different studies. When the first species was described, *Neodasys chaetonotoideus*, it was originally placed into the taxon Macrodasyida (Remane 1927, 1929). In his later monographic work of Gastrotricha, Remane (1936) revised the systematic position of *Neodasys* on the basis of some histological findings and moved it to the taxon Chaetonotida. This position within Chaetonotida was later supported by different authors,

mainly based on ultrastructural data (Ruppert 1982; Travis 1983; Hochberg & Litvaitis 2000; Zrzavý 2003, Fig. 7A,C). On the basis of DNA sequence data, *Neodasys* turns out to be an ingroup taxon of Macrodasyida, independent of the possible mono- or paraphyly of Macrodasyida (Todaro *et al*. 2003, 2006a; Petrov *et al*. 2007, Fig. 7C,E). The present analysis of morphological structures, predominantly external and internal features that do not go beyond the lightmicroscopic level, reveals *Neodasys* to be the most basal group within Gastrotricha (Fig. 7F).

Statistic support for the basal relationships within Gastrotricha mentioned before, bootstrap values, bremer support indices, and posterior probabilities, vary between the different phylogenetic analyses. However, many of the basal internal nodes are more or less well supported (see Wirz *et al*. 1999; Hochberg & Litvaitis 2000; Todaro *et al*. 2003, 2006a; Manylov *et al*. 2004; Petrov *et al*. 2007). In our analysis, the bootstrap support is generally very low (Fig. 2). These low values must be correlated with the fact that in our analysis most clades are characterized by few unambiguous transformations only (Fig. 3). The chance to get a high bootstrap value is generally low for nodes supported by one or two unambiguous transformations only (see Rieppel 1999). But most of the basal as well as the more terminal nodes in our analysis are satisfactorily supported by relatively high decay indices (Fig. 2). However, Wägele (2005) stresses that any

kind of statistic test associated with cladistic methods generally do not provide any likelihood for a historic evolutionary process. 'A good test value at best proves that the topology reflects well the information used by the method and contained in the data set' (Wägele 2005).

Evolution of Gastrotricha

Our new phylogenetic scenario in combination with the reconstructed ground pattern and record of apomorphic character transformations allows us to redefine evolutionary novelties within key lineages of Gastrotricha and further elucidate some potential evolutionary pathways. Because such evolutionary scenarios are a chapter of it own, we here focus on some striking processes such as the invasion of freshwater habitats.

As stated in previous studies (e.g. Rieger & Rieger 1977, Tyler & Rieger 1980), key innovations that define Gastrotricha include the evolution of caudal and lateral adhesive tubes (whether duo- or mono-glandular remains ambiguous), an exocuticular covering of all external cilia, and a dorso-ventrally flattened and elongate body. Such structural features can be interpreted as adaptations to the benthic, interstitial life, as it is assumed for many bilaterian taxa inhabiting the mesopsammon (Giere 1993). Within Gastrotricha (stem lineage of Eutubulata, see Fig. 2), the evolution of duo gland adhesive tubes is most probable (see also Tyler *et al.* 1980) and adhesive tubes in a frontal group occur. In the stem lineage of Abursata and later on in that of Paucitubulatina, the lateral and the frontal adhesive tubes are successively reduced (completely lost, see Fig. 2). Also in the stem lineage of Abursata, the accessory reproductive organs (frontal- and caudal organ) are completely lost and the testes are reduced (either transformed to packets of simple, rod-like sperms in many freshwater gastrotrichs (see Weiss 2001), or completely lost). The adaptive value of all these losses still remains obscure. Possibly, they conferred saving of energy and matter, which make accessible new degrees of freedom for the development of other organ systems.

It has been assumed that an undiscovered loss of characters can cause problems in phylogenetic reconstructions on the basis of morphological data and that this may be a reason for incongruence between morphological and molecular analyses (Bleidorn 2007). With the example of the internal relationships of Annelida, Bleidorn (2007) suggests identifying these secondary losses prior to the cladistic analysis for improving it, but stresses that the hypothesis of a character loss has to be verified with independent data (e.g. analyses of DNA sequences). However, in many cases it is hard to decide whether the absence of a structure is a primary absence or a secondary loss. As we have distinguished between these two types of absence in few characters only (see Appendix A), many of the secondary losses, especially in the stem lineage of Abursata and Paucitubulatina (Fig. 2), are revealed by overall parsimony *a posteriori*.

One developmental evolutionary process that contributes to character loss is heterochrony (see Bleidorn 2007 and references therein). Most common types of heterochrony are neoteny (attainment of sexual maturity in a larval stage) and paedogenesis (attainment of sexual maturity in a juvenile stage). As the number of lateral adhesive tubes in macrodasyid gastrotrichs, for example, in the eulittoral species *Turbanella mustela*, increases nearly three times during postembryonic development (Hochberg 1998), it is possible that a heterochronic evolution within Gastrotricha may have lead to species with few and, later on, to species with no lateral tubes. Such a process can be deduced from our analysis and happened at least three times: in the stem lineage of Abursata and in the stem lineages of two genera, *Desmodasys* and *Paradasys*.

Heterochronic evolution aside, character loss may also be the result of regressive evolution (i.e. character degeneration due to an evolutionary change in lifestyle) or reversion (i.e. when a derived character state evolves to a state present in the ancestor). Within Gastrotricha, regressive evolution may account for the loss of adhesive tubes and the apparent degeneration of the testes in the stem lineage of Abursata. Given this, the occurrence of fully developed testes within the stem linage of *Musellifer* + *Diuronotus* + Xenotrichulidae represents a reversion. Alternatively, a convergent testicular degeneration within Abursata (correlated with a convergent invasion of freshwater habitats, see below) and a plesiomorphic existence of testes within *Musellifer* + *Diuronotus* + Xenotrichulidae might be possible. However, this is not the most parsimonious scenario.

Since we cannot reconstruct the ancestral orientation of the pharyngeal lumen — Y-shaped or inverted Y-shaped — it is possible that either orientation is the plesiomorphic character state. If the inverted Y-shaped lumen characteristic of Macrodasyida *s. str.* is plesiomorphic, then the orientation of the lumen in *Neodasys* and Paucitubulatina evolved independently of one another (as opposed to previous scenarios that hypothesized homology of lumen orientation in Chaetonotida (see Travis 1983; Hochberg & Litvaitis 2000). Alternatively, the Y-shaped lumen may be plesiomorphic (and therefore evidence of nematode relations, see Ruppert 1982), and so the inverted Y-shape of most macrodasyidans is derived. Of importance may also be Ruppert's (1982) hypothesis that a circular lumenal shape is also a possibility for the plesiomorphic character state in Gastrotricha, making both Y-shaped and inverted Y-shaped lumen the derived character states (this was based on observations of juvenile *Lepidodasys*). It has to be stressed that the pharyngeal lumen is a single character only and that overall parsimony in our analysis determines the scenario regarding its orientation, even if the character 'pharyngeal lumen' is an important one for traditional systematic of Gastrotricha (see d'Hondt 1971).

Possible scenarios for the origin of Gastrotricha, limnetic or marine, or the invasion of freshwater or marine habitats have been discussed before (e.g. Kisielewski 1987, 1990; Wirz *et al.* 1999; Balsamo *et al.* 2008). On the basis of our results, the stem species of Gastrotricha unambiguously was a benthic, marine organism. There was a single invasion of freshwater habitats in the stem lineage of Abursata. Within monophyletic Paucitubulatina, for which the freshwater environment represents the ancestral habitat, there repeatedly occurred independently a re-invasion of the marine environment. This is also essential for the monophyletic group consisting of marine Xenotrichulidae, *Diuronotus* and *Musellifer*. Here, the (secondary) marine lifestyle correlates with a fully developed hermaphroditic life cycle. However, it is also possible but not necessarily parsimonious, that the marine environment is the plesiomorphic habitat of Abursata and that there was a convergent invasion of the freshwater in at least the stem lineages of *Redudasys* + *Marinellina*, of Proichthydiidae, and of Squammotricha exclusive of *Diuronotus* + *Musellifer* + Xenotrichulidae. Marine macrodasyid species such as *T. cornuta* and *T. lutheri* are known to inhabit oligohaline, estuary sediments (Riemann 1966) or even freshwater springs of marine beaches (Kisielewski 1987). Such records highlight a possible convergent colonization of the freshwater by marine species.

Extending the ground pattern of Gastrotricha
Several characters of the stem species of Gastrotricha provided by Zrzavý (2003) are confirmed by the reconstructions carried out in this study. However, there are some differences as well depending on the different tree topologies used for the ground pattern reconstructions (compare Fig. 7C and F). Differences in both ground pattern hypotheses are, for example, absence of pharyngeal pores and epidermal glands vs. their presence.

Future analyses of morphological characters in Gastrotricha will provide additional structures which were not treated here. This will complete the hypothesized stem species of Gastrotrich, for example, with data concerning the patterns of locomotory and sensory cilia. Most ultrastructural data were excluded from this analysis in order to obtain a data matrix as complete as possible. But such data, that are normally obtainable for a small number of species only, can be mapped onto tree topologies in order to infer the possible evolution of different organ systems and their ancestral states. Such approaches have been applied, for example, for the muscular system (Hochberg & Litvaitis 2001b) and for the protonephridial system (Kieneke *et al.* 2007, 2008a). These phylogenetic assessments of certain organ systems will expand our idea of the gastrotrich ancestor and will help find the actual phylogenetic position of Gastrotricha. Ground pattern reconstructions still have to be carried out for the reproductive organs (including life cycle strategies), the nervous system, and ecological requirements (habitat, life style, feeding, etc.).

Conclusions
We here provide a novel hypothesis for early evolution of Gastrotricha with the conspicuous taxon *Neodasys* being the sister group of all other gastrotrich species, and with the 'freshwater macrodasyids' *Redudasys* and *Marinellina* being the sister group of Paucitubulatina. According to parsimonious character optimization, the stem species of Paucitubulatina turns out to be a freshwater inhabitant, contrary to the former view, which regards the paucitubulatan ancestor to be a marine organism. But all these estimations have to be tested with independent data, preferably DNA sequences.

The possible paraphyly of Macrodasyida (*Redudasys* and *Marinellina* not considered) implied by most molecular studies was not confirmed with the data set at hand. Paraphyly of Macrodasyida would translate to a sister group relationship between Paucitubulatina and partial Macrodasyida (in the absence of *Redudasys* and *Marinellina*), but there is no evidence of any synapomorphies that might support this hypothesis. Future morphological cladistic analyses will be expanded and should include the pattern of the ciliation (see above) and the complex scale morphology in Paucitubulatina (and that of ancres and scales in Thaumastodermatidae, respectively) to get a more stable resolution within more derived groups. Furthermore, the gross anatomy of the reproductive system, which has always been important for taxonomy in some groups, is only poorly understood for many genera and will provide more informative characters. Also, future studies should consider a refined choice of terminal taxa. There are certainly species that have, relating to the genus they are representing, derived character states and are therefore not optimal.

Wägele (2005) recommends to estimate the probability of homology of characters prior to the cladistic analysis and to deduce a suitable weighting scheme on the base of this estimation. The results of the present and prior phylogenetic analyses should be used to evaluate the probability of homology of different organs and structures of the Gastrotricha.

The hypothesis for the stem species of Gastrotricha is intended to be a step toward elucidating the phylogenetic position of Gastrotricha within Bilateria. According to our analysis, the 'Nematode' outgroup (representative of Cycloneuralia) turns out to be the sister group of Gastrotricha (see Figs 2 and 4), but a recent molecular study (Petrov *et al.* 2007) rejects a close relation of Nematoda (or Cycloneuralia) with Gastrotricha. We think, an association of 'platyzoan taxa' (Plathelminthes and/or Gnathostomulida) and Gastrotricha is possible (e.g. Winnepenninckx *et al.* 1995; Zrzavý *et al.* 1998; Giribet *et al.* 2000; Giribet 2002). But such estimations on the basis of the present study are little more than speculations and a broad comparison and evaluation of

the different ground pattern hypotheses of bilaterian taxa is needed.

Acknowledgements

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Appendix A Characters and character state descriptions

The following commented list of characters contains some characters excluded from the search (see characters 1, 14, 22, 27, 31, 37 and 43). Most of these excluded characters comprise numerical data of the different groups of adhesive tubes. We here provide these original numbers because they are the basis for the establishment of groups as character states (e.g. few, many and very many tubes) in each following character (see characters 15, 23, 28, 32, 38 and 44).

1 *Habitat — salinity*: (0) seawater; (1) seawater to brackish water; (2) only brackish water; (3) brackish to freshwater; (4) freshwater. This character is excluded from the search.

2 *Habitus — neck constriction — existence*: (0) absent; (1) present.

3 *Habitus — neck constriction — shape*: (0) depression of the pharynx region — type *Euchaetonotus maximus*; (1) strong depression of the pharynx region — type *Dasydytes ornatus*; (2) constriction at the level of the frontal adhesive tubes — type *Cephalodasys maximus*; (3) constriction at the level of the pharyngeal pores — type *Dactylopodola baltica*; (4) constriction at the caudal end of the pharynx — type *Ichthydium hummoni*; (5) depression of the whole trunk — type *Neodasys chaetonotoideus*; (6) constriction at the level of the mouth — type *Gnathostomula paradoxa*; (7) constriction in the middle of pharynx — type *Dendropodola transitionalis*.

4 *Habitus — frontal end — shape*: (0) slightly convex — type *Planodasys marginalis*; (1) highly convex — type *Turbanella cornuta*; (2) convex with notched margin — type *Tetranchyroderma megastoma*; (3) blunt — type *Acanthodasys lineatus*; (4) trapezoid — type *Thaumastoderma heideri*; (5) triangular, acuminate — type *Pleurodasys helgolandicus*; (6) triangular, blunt — type *Neodasys chaetonotoideus*; (7) hammerhead shark-like — type *Dendrodasys gracilis*; (8) stag beetle-like — type *Pseudostomella roscovita*; (9) threefold lobate — type *Neogossea antennigera*; (10) fivefold lobate — type *Euchaetonotus maximus*; (11) tapering — type Nematode.

5 *Habitus — prebuccal apparatus — existence*: (0) absent; (1) present — type *Pseudostomella cataphracta*.

6 *Habitus — trunk section — compression*: (0) dorso-ventrally flattened — type *Neodasys chaetonotoideus*; (1) rotationally symmetric (more or less) — type *Chitonodytes collini*. This character refers to the body shape in cross section.

7 *Habitus — shape and proportions*: (0) long, vermiform (length-width-ratio higher than 6 to 1) — type *Mesodasys laticaudatus*; (1) tongue-shaped (length-width-ratio between 4 : 1 and 5 : 1) — type *Platydasys maximus*; (2) sole-shaped, elongated — type *Dactylopodola baltica*; (3) sole-shaped, compact — type *Euchaetonous maximus*; (4) tenpin-shaped — type *Chitonodytes collini*; (5) Nectochaeta-like — type *Metadasydytes quadrimaculatus*.

8 *Habitus — caudal end — shape*: (0) not bilobed — type *Macrodasys buddenbrocki*; (1) bilobed — type *Neodasys chaetonotoideus*. Any kind of paired, solid appendages are considered to represent a bilobed caudal end — exclusive of the styli of *Stylochaeta scirtetica*.

9 *Habitus — non-bilobed end — shape*: (0) tapering — type *Macrodasys buddenbrocki*; (1) rounded — type *Pleurodasys helgolandicus*; (2) blunt — type *Platydasys maximus*; (3) discoidal (Schwanzplatte) — type *Cephalodasys maximus*; (4) elongated pedicle equipped with adhesive tubes — type *Urodasys vivparus*; (5) spherical (no adhesive tubes) — type *Chitonodytes collini*; (6) with two short styli (no adhesive tubes) — type *Stylochaeta scirtetica*; (7) with two small bulges (no adhesive tubes) — type *Dasydytes ornatus*. This character is only applied if character 8 is coded with state 0 (none bilobed caudal end).

10 *Habitus — bilobed end — shape*: (0) fin-shaped — type *Turbanella cornuta*; (1) blunt pedicles — type *Neodasys chaetonotoideus*; (2) simple lobes — type *Dactylopodola baltica*; (3) inverted Y-shaped fork — type *Dendrodasys gracilis*;

(4) simple dichotomic fork — type *Euchaetonotus maximus*; (5) elongated dichotomic fork — type *Polymerurus nodicaudus*; (6) closely arranged, parallel furca — type *Pseudichthydium balatonicum*; (7) 'furcette' — type *Dendropodola transitionalis*; (8) oval lobes — type *Planodasys marginalis*; (9) short appendages at the angles of the blunt caudal end — type *Ptychostomella mediterranea*; (10) elongated lobes — type *Pseudostomella roscovita*; (11) 'Xenotrichulidae-furca' (furcal base covered with stalked scales) — type *Draculiciteria tesselata*; (12) short appendages at the angles of the blunt caudal end — type *Redudasys fornerise*. This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

11 *Habitus — bilobed end — foot pseudosegment — existence*: (0) absent; (1) present — type *Ichthydium hummoni*. This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

12 *Habitus — bilobed end — adhesive tubes — existence*: (0) absent — type *Undula paraensis*; (1) present — type *Turbanella cornuta*. This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

13 *Habitus — bilobed end — adhesive tubes — shape*: (0) raised cuticularized tubes — type *Dactylopodola baltica*; (1) mastoid uprisings — type *Neodasys chaetonotoideus*; (2) bristle-like, elongated tubes — type *Forficulichthys forficula*; (3) sickle-shaped, bicuspid tubes — type *Pseudichthydium balatonicum*; (4) sickle-shaped tubes — type *Ichthydium hummoni*; (5) adhesive pads — type *Chordodasiopsis antennatus*; (6) elongated, annulated pedicles — type *Polymerurus nodicaudus* (function as an adhesive structure is improbable); (7) rudimentary tubes, simple opening of adhesive glands — type *Brevipedichaeta uncinus*. This character is only applied if character 8 is coded with state 1 (bilobed caudal end). The states 0 and 5 (raised cuticularized tubes, adhesive pads) and probably states 2, 3, 4 and 7 correspond to the duo-gland adhesive organs, which are reported for several species of traditional Macrodasyida and Paucitubulatina (Tyler & Rieger 1980). The state 1 (mastoid uprisings) corresponds to the non-duo-gland adhesive organs, which are only known from *Neodasys* (Tyler *et al.* 1980).

14 *Habitus — bilobed end — adhesive tubes — number per branch*: (0) one — type *Euchaetonotus maximus*; (1) two — type *Marinellina flagellata*; (2) three — type *Ptychostomella mediterranea*; (3) four — type *Acanthodasys lineatus*; (4) five — type *Dinodasys mirabilis*; (5) eight — type *Dactylopodola baltica*; (6) nine — type *Turbanella cornuta*; (7) 13 — type *Paraturbanella scanica*; (8) 15 — type *Desmodasys phocoides*; (9) 16 — type *Planodasys marginalis*. The given numbers are maximum data of the original descriptions. This character is excluded from the search since different numbers of adhesive tubes are fused to three classes (see character 15). This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

15 *Habitus — bilobed end — adhesive tubes — number per branch*: (0) one — type *Euchaetonotus maximus*; (1) few tubes (up to five); (2) many tubes (more than five). This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

16 *Habitus — bilobed end — adhesive tubes — alternating length — existence*: (0) absent; (1) present — type *Desmodasys phocoides*. Species of *Desmodasys* have two columns of adhesive tubes on the caudal margin of the furca differing conspicuously in length. This gives the appearance of short and longer tubes regularly alternating. This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

17 *Habitus — bilobed end — secondary adhesive tubes — existence*: (0) absent; (1) present — type *Diuronotus aspetos*. The secondary adhesive tubes of *Diuronotus* species are treated as not belonging to the furcal tubes. This character is only applied if character 8 is coded with state 1 (bilobed caudal end).

18 *Habitus — median tail (cone) — existence*: (0) absent; (1) present — type *Turbanella cornuta*.

19 *Epidermal glands — existence*: (0) absent; (1) present. Unicellular epidermal glands occur in almost all species of traditional Macrodasyida. Their ultrastructure seems to be uniform among different species (Ruppert 1991). Therefore, we propose homology of epidermal glands.

20 *Frontal adhesive tubes — existence*: (0) absent (primary); (1) absent (secondary loss) — type *Chaetonotus maximus*; (2) present. We have distinguished two different types of absence because in earlier searches all Paucitubulatina (monophyletic) always grouped with *Redudasys fornerise* + *Marinellina flagellata* (both containing frontal adhesive tubes) as sister taxon, thus indicating a secondary loss of frontal adhesive tubes in Paucitubulatina.

21 *Frontal adhesive tubes — shape*: (0) raised cuticularized tubes — type *Dactylopodola baltica*. This character is constant and only applied if character 20 is coded with state 2 (frontal adhesive tubes present).

22 *Frontal adhesive tubes — number*: (0) one tube on each side — type *Marinellina flagellata*; (1) two tubes on each side — type *Dactylopodola baltica*; (2) three tubes on each side — type *Urodasys viviparus*; (3) four tubes on each side — type *Pleurodasys helgolandicus*; (4) five tubes on each side — type *Dinodasys mirabilis*; (5) six tubes on each side — type *Pseudoturbanella stylifera*; (6) seven tubes on each side — type *Paradasys turbanelloides*; (7) nine tubes on each side — type *Paraturbanella scanica*; (8) 10 tubes on each side — type *Macrodasys buddenbrocki*; (9) 14 tubes on each side — type *Desmodasys phocoides*; (10) 16 tubes on each side — type *Planodasys marginalis*. The given numbers are maximum data of the original descriptions. This character is excluded from the search since different numbers of adhesive tubes are fused to two classes (see character 23). This character is only applied if character 20 is coded with state 2 (frontal adhesive tubes present).

23 *Frontal adhesive tubes — number*: (0) many tubes per side (6 and more); (1) few tubes per side (1–5). This character is

only applied if character 20 is coded with state 2 (frontal adhesive tubes present).

24 *Frontal adhesive tubes — arrangement*: (0) tubes arranged in a convex arch — type *Macrodasys buddenbrocki*; (1) tubes arranged in a concave arch (along the ventral margin of the mouth) — type *Diplodasys ankeli*; (2) tubes arranged in a tuft — type *Desmodasys phocoides*; (3) tubes arranged in a hand-like organ — type *Turbanella cornuta*; (4) tubes arranged in two diagonal rows — type *Planodasys marginalis*; (5) tubes arranged in a 'W' — type *Urodasys viviparus*; (6) tubes arranged in two ventrolateral, longitudinal fields — type *Crasiella diplura*; (7) tubes arranged as a 'T' — type *Acanthodasys lineatus*. This character is only applied if character 20 is coded with state 2 (frontal adhesive tubes present).

25 *Ventral adhesive tubes — existence*: (0) absent (primary); (1) absent (secondary loss); (2) present — type *Diplodasys ankeli*. We have distinguished two different types of absence because in earlier searches all Thaumastodermatidae always clustered together with *Ptychostomella mediterranea* and *Tetranchyroderma megastoma* in a more derived position within Thamastodermatidae, thus indicating a secondary loss of ventral tubes in these two species.

26 *Ventral adhesive tubes — shape*: (0) raised cuticularized tubes — type *Platydasys maximus*. This character is constant and only applied if character 25 is coded with state 2 (ventral adhesive tubes present).

27 *Ventral adhesive tubes — number*: (0) seven tubes on each side — type *Pseudostomella roscovita*; (1) 12 tubes on each side — type *Pseudostomella cataphracta*; (2) 16 tubes on each side — type *Acanthodasys lineatus*; (3) 24 tubes on each side — type *Tetranchyroderma arcticum*; (4) 30 tubes on each side — type *Platydasys maximus*. The given numbers are maximum data of the original descriptions. This character is excluded from the search since different numbers of adhesive tubes are fused to two classes (see character 28). This character is only applied if character 25 is coded with state 2 (ventral adhesive tubes present).

28 *Ventral adhesive tubes — number*: (0) up to 20 tubes; (1) more than 20 tubes. This character is only applied if character 25 is coded with state 2 (ventral adhesive tubes present).

29 *Lateral adhesive tubes — existence*: (0) absent; (1) present — type *Turbanella cornuta*.

30 *Lateral adhesive tubes — shape*: (0) raised cuticularized tubes — type *Dactylopodola baltica*; (1) mastoid uprisings — type *Neodasys chaetonotoideus*; (2) 'double tubes' — type *Dinodasys mirabilis*. Character 30 is only applied if character 29 is coded with state 1 (lateral adhesive tubes present). The states 0 and 2 (raised cuticularized tubes, double tubes) correspond to the duo-gland adhesive organs, which are reported for several species of traditional Macrodasyida (Tyler & Rieger 1980). The state 1 (mastoid uprisings)

corresponds to the non-duo-gland adhesive organs, which are only known from *Neodasys* (Tyler *et al.* 1980).

31 *Lateral adhesive tubes — number*: (0) one tube — type *Pseudoturbanella stylifera*; (1) two tubes on each side — type *Ptychostomella mediterranea*; (2) three tubes on each side — type *Dendropodola transitionalis*; (3) four tubes on each side — type *Chordodasiopsis antennatus*; (4) six tubes on each side — type *Dactylopodola baltica*; (5) eight tubes on each side — type *Macrodasys buddenbrocki*; (6) nine tubes on each side — type *Paraturbanella scanica*; (7) 10 tubes on each side — type *Dinodasys mirabilis*; (8) 12 tubes on each side — type *Lepidodasys platyurus*; (9) 13 tubes on each side — type *Cephalodasys maximus*; (10) 15 tubes on each side — type *Neodasys chaetonotoideus*; (11) 20 tubes on each side — type *Paradasys turbanelloides*; (12) 23 tubes on each side — type *Prostobuccantia brocha*; (13) 30 tubes on each side — type *Mesodasys laticaudatus*; (14) up to 40 tubes on each side — type *Turbanella cornuta*; (15) up to 60 tubes on each side — type *Megadasys pacificus*; (16) up to 140 tubes on each side — type *Planodasys marginalis*. The given numbers are maximum data of the original descriptions. This character is excluded from the search since different numbers of adhesive tubes are fused to four classes (see character 32). This character is only applied if character 29 is coded with state 1 (lateral adhesive tubes present).

32 *Lateral adhesive tubes — number*: (0) many tubes per side (8–30); (1) one tube per side — type *Pseudoturbanella mediterranea*; (2) few tubes per side (2–6); (3) very many tubes per side (more than 30). This character is only applied if character 29 is coded with state 1 (lateral adhesive tubes present).

33 *Lateral adhesive organ (Seitenfüßchen) — existence*: (0) absent; (1) present — type *Pleurodasys helgolandicus*.

34 *Lateral adhesive organ (Seitenfüßchen) — number of tubes per organ*: (0) two adhesive tubes per organ — type *Prostobuccantia brocha*; (1) three adhesive tubes per organ — type *Pleurodasys helgolandicus*. This character is only applied if character 33 is coded with state 1 (lateral adhesive organ present).

35 *Dorso-lateral adhesive tubes — existence*: (0) absent; (1) present — type *Cephalodasys maximus*.

36 *Dorso-lateral adhesive tubes — shape*: (0) raised cuticularized tubes — type *Cephalodasys maximus*; (1) rudimentary tubes ('Stäbchen') — type *Ptychostomella mediterranea*. This character is only applied if character 35 is coded with state 1 (dorso-lateral adhesive tubes present).

37 *Dorso-lateral adhesive tubes — number*: (0) five tubes on each side — type *Paraturbanella scanica*; (1) six tubes on each side — type *Lepidodasys platyurus*; (2) seven tubes on each side — type *Ptychostomella mediterranea*; (3) eight tubes on each side — type *Macrodasys buddenbrocki*; (4) more than 10 tubes — type *Cephalodasys maximus*; (5) 16 tubes on each side — type *Acanthodasys lineatus*. The given numbers are maximum data of the original descriptions. This character is excluded from the search since different numbers of adhesive tubes are

fused to two classes (see character 38). This character is only applied if character 35 is coded with state 1 (dorso-lateral adhesive tubes present).

38 *Dorso-lateral adhesive tubes — number*: (0) up to 10 tubes per side; (1) more than 10 tubes per side. This character is only applied if character 35 is coded with state 1 (dorso-lateral adhesive tubes present).

39 *Posterior adhesive 'foot' — existence*: (0) absent; (1) present — type *Pseudostomella cataphracta*.

40 *Posterior adhesive 'foot' — number of tubes per organ*: (0) four tubes per organ — type *Pseudostomella cataphracta*. This character is constant and only applied if character 39 is coded with state 1 (posterior adhesive foot present).

41 *Caudal adhesive tubes (exclusive of furcal tubes) — existence*: (0) absent; (1) present — type *Macrodasys buddenbrocki*; (2) present — type *Macrostomum pusillum*. We have distinguished two types of presence of caudal adhesive tubes because plathelminth and gastrotrich adhesive organs are not considered to be homologous (see also character 42).

42 *Caudal adhesive tubes (exclusive of furcal tubes) — shape*: (0) raised cuticularized tubes — type *Dendrodasys gracilis*; (1) *Macrostomum*-type. This character is only applied if character 41 is coded with states 1 or 2 (caudal adhesive tubes present). The state 0 (raised cuticularized tubes) corresponds to the duo-gland adhesive organs, which are reported for several species of traditional Macrodasyida (Tyler & Rieger 1980). Although interstitial representatives of Plathelminthes bear duo-gland adhesive organs, state 1 (*Macrostomum*-type) is considered a different one, because duo-glands in Plathelminthes and Gastrotricha are probably not homologous (Tyler & Rieger 1980).

43 *Caudal adhesive tubes (exclusive of furcal tubes) — number*: (0) a single median tube — type *Dolichodasys elongatus*; (1) one tube on each side — type *Dendrodasys gracilis*; (2) three tubes on each side — type *Tetranchyroderma megastoma*; (3) four tubes on each side — type *Diplodasys ankeli*; (4) five tubes on each side — type *Pleurodasys helgolandicus*; (5) seven tubes on each side — type *Platydasys maximus*; (6) eight tubes on each side — type *Paradasys turbanelloides*; (7) 10 tubes on each side — type *Cephalodasys maximus*; (8) 12 tubes on each side — type *Mesodasys laticaudatus*; (9) 30 tubes on each side — type *Urodasys viviparus*; (10) 40 tubes on each side — type *Macrodasys buddenbrocki*. The given numbers are maximum data of the original descriptions. This character is excluded from the search since different numbers of adhesive tubes are fused to four classes (see character 44). This character is only applied if character 41 is coded with states 1 or 2 (caudal adhesive tubes present). Character state 0 refers to adult specimens of *Dolichodasys* elongates. However, subadult specimens possess up to four caudal adhesive tubes (Gagné 1977).

44 *Caudal adhesive tubes (exclusive of furcal tubes) — number*: (0) many tubes per side (6–20); (1) one tube per side or a single median tube — type *Dolichodasys elongatus* and *Dendrodasys gracilis*; (2) few tubes per side (2–5); (3) very many tubes per side (more than 20). This character is only applied if character 41 is coded with states 1 or 2 (caudal adhesive tubes present).

45 *Head — mouth opening — position*: (0) terminal — type *Ichthydium hummoni*; (1) subterminal — type *Euchaetonotus maximus*; (2) ventral — type *Macrostomum pusillum*.

46 *Head — mouth opening — shape*: (0) narrow pore — type *Praeconvoluta minor*; (1) rotund pore — type *Neodasys chaetonotoideus*; (2) triangular pore — type *Proichthydioides remanei*; (3) funnel-like buccal field — type *Platydasys maximus*; (4) longitudinal slit — type *Marinellina flagellata*.

47 *Head — mouth opening — supportive structures*: (0) no supportive structures — type *Macrostomum pusillum*; (1) cuticularized club — type *Neodasys chaetonotoideus*; (2) mouth basked — type *Euchaetomotus maximus*; (3) longitudinal ridges — type *Halichaetonotus spinosus*; (4) cuticularized mouth ring — type *Euichthydium podura*; (5) cuticular pedicles — type *Musellifer sublitoralis*; (6) small hooks — type *Crasiella diplura*; (7) triangular, pointed projections — type *Prostobuccantia brocha*; (8) mouth equipped with lips — type Nematode.

48 *Head — mouth opening — buccal cavity — existence*: (0) absent; (1) present.

49 *Head — mouth opening — buccal cavity — shape*: (0) V-shaped — type *Megadasys pacificus*; (1) cylindric — type *Paraturbanella scanica*; (2) conical — type *Urodasys vivparus*; (3) wide funnel — type *Tetranchyroderma arcticum*; (4) 'mastax lumen' — type *Gnathostomula paradoxa*; (5) slit-like — type *Marinellina flagellata*. This character is only applied if character 48 is coded with state 1 (buccal cavity present).

50 *Head — cerebral eyes — existence*: (0) absent; (1) present — type *Heteroxenotrichula transatlantica*.

51 *Head — cerebral eyes — number of pairs*: (0) one pair — type *Heteroxenotrichula transatlantica*; (1) two pairs — type *Metadasydytes quadrimaculatus*. This character is only applied if character 50 is coded with state 1 (cerebral eyes present).

52 *Head — pseudocelli — existence*: (0) absent; (1) present — type *Heterolepidoderma ocellatum*.

53 *Head — statocyst — existence*: (0) absent; (1) present — type *Praeconvoluta minor*.

54 *Head — pestle organs — existence*: (0) absent; (1) present — type *Planodasys marginalis*.

55 *Head — sensory appendages — existence*: (0) absent; (1) present. Sensory ciliary tufts or sensory cirri are not included in character 55.

56 *Head — sensory appendages — shape*: (0) ciliated auricles — type *Turbanella cornuta*; (1) ciliated tentacles — type *Heteroxenotrichula transatlantica*; (2) sensory palps — type *Neogossea antennigera*; (3) articulated cephalic tentacles — type *Chordodasiopsis antennatus*; (4) ciliated palps — type *Dinodasys mirabilis*; (5) smooth cephalic tentacles — type *Xenodasys*

sancti-goulveni; (6) anterodorsal papillae — type *Undula paraensis*. This character is only applied if character 55 is coded with state 1 (sensory appendages present).

57 *Head — sensory appendages — number of pairs*: (0) one pair; (1) two pairs — type *Xenodasys sancti-goulveni*; (2) three pairs — type *Chordodasiopsis antennatus*. This character is only applied if character 55 is coded with state 1 (sensory appendages present).

58 *Head — sensory cilia — existence*: (0) absent; (1) present.

59 *Head — sensory cilia — composition*: (0) composed as individual cilia — type *Macrodasys buddenbrocki*; (1) composed as sensory bristles — type *Gnathostomula paradoxa*; (2) composed as sensory cirri — type *Draculiciteria tesselata*. This character is only applied if character 58 is coded with state 1 (sensory cilia present).

60 *Head — circles of sensory setae — existence*: (0) absent; (1) present — type Nematode.

61 *Head — amphids — existence*: (0) absent; (1) present — type Nematode.

62 *Head — ventrolateral fins — existence*: (0) absent; (1) present — type *Dendrodasys gracilis*.

63 *Head — muzzle — existence*: (0) absent; (1) present — type *Musellifer sublitoralis*.

64 *Head — mouth cone — existence*: (0) absent; (1) present — type *Dinodasys mirabilis*.

65 *Pharynx — lumen — shape*: (0) roundish — type *Macrostomum pusillum*; (1) Y-shaped I — type *Neodasys chaetonotoideus*; (2) inverted-Y-shaped — type *Dactylopodola baltica*; (3) Y-shaped II — type Nematode. Although there are several accordances between the ultrastructure of nematode and gastrotrich pharynx that gave rise to a hypothesized sister group relationship between Nematoda and Gastrotricha (Neuhaus 1994), we have treated the shape of the pharyngeal lumen of nematodes and chaetonotidan gastrotrichs as two different states. We have done this to avoid an artificial nesting of the 'Nematode' outgroup within Paucitubulatina.

66 *Pharynx — pharyngeal pores — existence*: (0) absent; (1) present — type *Dactylopodola baltica*.

67 *Pharynx — pharyngeal pores — position*: (0) near the end of the pharynx — type *Chordodasiopsis antennatus*; (1) in the caudal third of the pharynx — type *Megadasys pacificus*; (2) around the middle of the pharynx — type *Pleurodasys helgolandicus*. This character is only applied if character 66 is coded with state 1 (pharyngeal pores present).

68 *Pharynx — pharyngeal jaws — existence*: (0) absent; (1) present — type *Gnathostomula paradoxa*.

69 *Pharynx — pharyngeal basal plate — existence*: (0) absent; (1) present — type *Gnathostomula paradoxa*.

70 *Persistent anus — existence*: (0) absent, primary — type *Macrostomum pusillum*; (1) absent, secondary loss — *Urodasys viviparus*; (2) present. We have distinguished two different types of absence (primary absence vs. secondary loss) because

in earlier phylogenetic analyses (e.g. Hochberg & Litvaitis 2000; Todaro *et al.* 2006a) genus *Urodasys* has a more derived position within Gastrotricha than other taxa, so a secondary loss of the anus in Urodasys is probable.

71 *Persistent anus — position*: (0) ventral — type *Ichthydium hummoni*; (1) terminal — type *Stylochaeta scirtetica*; (2) dorsal — type *Euchaetonotus maximus*. This character is only applied if character 70 is coded with state 2 (persistent anus present).

72 *Body cuticle — existence*: (0) absent — type *Gnathostomula paradoxa*; (1) present — type *Neodasys chaetonotoideus*.

73 *Body cuticle — head — frontodorsal plate — existence*: (0) absent; (1) present. Characters 73–94 are only applied if character 72 is coded with state 1 (body cuticle present).

74 *Body cuticle — head — frontodorsal plate — shape*: (0) solid cuticular cap — type *Xenodasys sancti-goulveni*; (1) cephalion — type *Euchaetonotus maximus*. We have considered the cuticular formations at the head region of *Xenodasys sancti-goulveni* and that of paucitubulatan species (characters 73–81) as non-homologous structures because *Xenodasys* is obviously not an ingroup taxon of Paucitubulatina. Therefore, it is probable that the plates at the head region evolved convergently in both groups.

75 *Body cuticle — head — dorsolateral plates (I) — existence*: (0) absent; (1) present. Plate (I) is the most anterior paired cuticular plate at the head, caudal to the unpaired frontodorsal one.

76 *Body cuticle — head — dorsolateral plates (I) — shape*: (0) solid cuticular plates — type *Xenodasys sancti-goulveni*; (1) epipleuri — type *Euchaetonotus maximus*.

77 *Body cuticle — head — epipleuri — fusion with cephalion — existence*: (0) absent (epipleuri isolated) — type *Euchaetonotus maximus*; (1) present — type *Aspidiophorus polystictos*. Character 77 is only applied if character 76 is coded with state 1 (dorsolateral plates (I) as epipleuri).

78 *Body cuticle — head — dorsolateral plates (II) — existence*: (0) absent; (1) present. Plate II is the most posterior paired cuticular plate at the head, caudal to the paired plate I.

79 *Body cuticle — head — dorsolateral plates (II) — shape*: (0) hypopleuri — type *Euchaetonotus maximus*. This is a constant character.

80 *Body cuticle — head — ventral, postoral cuticular formation — existence*: (0) absent; (1) present.

81 *Body cuticle — head — ventral, postoral cuticular formation — shape*: (0) robust cuticular plate with polygonal subplates — type *Xenodasys sancti-goulveni*; (1) hypostome — type *Euchaetonotus maximus*. Character 81 is only applied if character 80 is coded with state 1 (poststomal cuticular formation present).

82 *Body cuticle — headscales — existence*: (0) absent; (1) present.

83 *Body cuticle — headscales — category*: (0) 'scales'; (1) 'ancres'. Characters 83–85 are only applied if character 82 is coded with state 1 (headscales present). We have decided to

code the pure existence of scales or ancres (character 82) as a primary homology in order to test if the possession of these structures might be a potential synapomorphy of Thaumastodermatidae and Paucitubulatina.

84 *Body cuticle — headscales — 'scales' — shape*: (0) claw-shaped scales — type *Diversichaetatella acanthocephalus*; (1) placoid scales — type *Diplodasys ankeli*; (2) polygonal cuticular plates — type *Xenodasys sancti-goulveni*; (3) long and oval scales with two longitudinal keels — type *Lepidodasys platyurus*; (4) spined scales — type *Euchaetonotus maximus*; (5) keeled scales — type *Heterolepidoderma ocellatum*; (6) circular scales — type *Fluxiderma verrucosum*; (7) polygonal scales — type *Lepidodermella squamata*; (8) rhombic scales — type *Rhomballichthys punctatus*; (9) stalked skales — type *Aspidiophorus paradoxus*; (10) compound scales — type *Draculiciteria tesselata*. Character 84 is only applied if character 83 is coded with state 0 (headscales as 'scales').

85 *Body cuticle — headscales — 'ancres' — shape*: (0) uniancres — type *Acanthodasys lineatus*; (1) tetrancres ('Vierhaker') — type *Pseudostomella roscovita*; (2) pentancres ('Fünfhaker') — type *Tetranchyroderma megastoma*. Character 85 is only applied if character 83 is coded with state 1 (headscales as 'ancres').

86 *Body cuticle — truncscales (dorsal and dorsolateral) — existence*: (0) absent; (1) present.

87 *Body cuticle — trunkscales — category*: (0) 'scales'; (1) 'ancres'. Characters 87–89 are only applied if character 86 is coded with state 1 (trunkscales present). We have decided to code the pure existence of scales or ancres (character 86) as a primary homology in order to test if the possession of these structures might be a potential synapomorphy of Thaumastodermatidae and Paucitubulatina.

88 *Body cuticle — truncscales 'scales' (dorsal and dorsolateral) — shape*: (0) placoid scales — type *Diplodasys ankeli*; (1) long and oval scales with two longitudinal keels — type *Lepidodasys platyurus*; (2) spined scales — type *Euchaetonotus maximus*; (3) circular scales — type *Fluxiderma verrucosum*; (4) keeled scales — type *Heterolepidoderma ocellatum*; (5) polygonal scales — type *Lepidodermella squamata*; (6) rhombic scales — type *Rhomballichthys punctatus*; (7) stalked skales — type *Aspidiophorus paradoxus*; (8) compound scales — type *Draculiciteria tesselata*; (9) ornamented plates — type *Ornamentula paraensis*. Character 88 is only applied if character 87 is coded with state 0 (trunkscales as 'scales').

89 *Body cuticle — truncscales 'ancres' (dorsal and dorsolateral) — shape*: (0) uniancres — type *Acanthodasys lineatus*; (1) tetrancres ('Vierhaker') — type *Pseudostomella roscovita*; (2) pentancres ('Fünfhaker') — type *Tetranchyroderma megastoma*. Character 89 is only applied if character 87 is coded with state 1 (trunkscales as 'ancres').

90 *Body cuticle — ventrolateral hydrofoil scales — existence*: (0) absent; (1) present — type *Draculiciteria tesselata*. The

hydrofoil scales are two parallel, ventrolateral columns of specialized stalked scales present in almost all Xenotrichulidae. Species of Aspidiophorus, however, lack hydrofoil scales although possessing stalked scales.

91 *Body cuticle — lateral thorns — existence*: (0) absent; (1) present — type *Diplodasys ankeli*.

92 *Body cuticle — lateral teethlets — existence*: (0) absent; (1) present — type *Platydasys maximus*.

93 *Body cuticle — medio-dorsal formation — existence*: (0) absent; (1) present — type *Pseudichthydium balatonicum*.

94 *Body cuticle — medio-dorsal formation — shape*: (0) dorsal ridge — type *Pseudichthydium balatonicum*; (1) dorsal 'Schienenstrang' — type *Ichthydium hummoni*. Character 94 is only applied if character 93 is coded with state 1 (medio-dorsal formation present).

95 *Motile spines — existence*: (0) absent; (1) present — type *Stylochaeta scirtetica*.

96 *Motile spines — prominent crossing — existence*: (0) absent; (1) present — type *Haltidytes crassus*. Character 96 is only applied if character 95 is coded with state 1 (motile spines present).

97 *Terminal spines — existence*: (0) absent; (1) present — type *Dasydytes (Setopus) bisetosus*.

98 *Terminal spines — shape*: (0) long spines — type *Dasydytes (Setopus) bisetosus*; (1) short claws — type *Chaetonotus (Brevipedichaeta) uncinus*; (2) short spines — type *Kijanebalola canina*. Character 98 is only applied if character 97 is coded with state 1 (terminal spines present).

99 *Terminal spines — symmetry*: (0) spines equal in length — type *Dasydytes (Dasydytes) ornaus*; (1) spines unequal in length — type *Dasydytes (Setodytes) tongiorgii*. Character 99 is only applied if character 97 is coded with state 1 (terminal spines present).

100 *Caudal spines (Schleppenstacheln) — existence*: (0) absent; (1) present — type *Neogossea antennigera*.

101 *Belt spines — existence*: (0) absent; (1) present — type *Chaetonotus (Zonochaeta) succinctus*.

102 *Belt spines — number of rings*: (0) one ring of belt spines — type *Chaetonotus (Zonochaeta) succinctus*; (1) two rings of belt spines — type *Chaetonotus (Diversichaetatella) acanthocephalus*. Character 102 is only applied if character 101 is coded with state 1 (belt spines present).

103 *Pseudo-annulation of the trunk — existence*: (0) absent; (1) present — type *Dichaetura capricornia*.

104 *Locomotory ciliation (trunk)*: (0) individual cilia — type *Neodasys chaetonotoideus*; (1) composite cirri — type *Draculiciteria tesselata*. Character 104 is only applied if character 105 is coded with states 0 or 1 (locomotory cilia present).

105 *Locomotory ciliation (trunk) — occurence of cilia*: (0) on the whole surface — type *Gnathostomula paradoxa*; (1) restricted to the ventral surface — type *Neodasys chaetonotoideus*; (2) completely absent — type Nematode.

106 *Locomotory ciliation (head) — conspicuous ciliary girdles — existence*: (0) absent; (1) present — type *Dasydytes (Dasydytes) ornatus*.

107 *Lateral ciliated cones — existence*: (0) absent; (1) present — type *Dinodasys mirabilis*.

108 *Dorso-lateral ciliated cones — existence*: (0) absent; (1) present — type *Dinodasys mirabilis*.

109 *Chordoid organ — existence*: (0) absent; (1) present — type *Chordodasiopsis antennatus*.

110 *Articulated sensory processes, lateral row — existence*: (0) absent; (1) present — type *Chordodasiopsis antennatus*.

111 *Articulated sensory processes, dorsolateral row — existence*: (0) absent; (1) present — type *Chordodasiopsis antennatus*.

112 *Lateral coniform outgrowths — existence*: (0) absent; (1) present — type *Xenodasys sancti-goulveni*.

113 *Granular cuticle — existence*: (0) absent; (1) present — type *Platydasys maximus*.

114 *Dorso-lateral 'cirrata tubes' — existence*: (0) absent; (1) present — type *Thaumastoderma heideri*. The term 'cirrata tubes' is used in accordance with Hummon *et al*. (1996). The original term 'cirri' (pl.), introduced for Thamastoderma heideri (see Remane 1926), can be confused with the cirri composed of numerous cilia as they occur in the Xenotrichulidae.

115 *Caudal 'cirrata tubes' — existence*: (0) absent; (1) present — type *Thaumastoderma heideri*. The term 'cirrata tubes' is used in accordance with Hummon *et al*. (1996). The original term 'cirri' (pl.), introduced for Thamastoderma heideri (Remane 1926), can be confused with the cirri composed of numerous cilia as they occur in the Xenotrichulidae.

116 *Meandering membranes on the surface of body — existence*: (0) absent; (1) present — type *Proichthydioides remanei*.

117 *Ovary — existence*: (0) absent; (1) present.

118 *Ovary — arrangement*: (0) paired, bilateral — type *Macrodasys buddenbrocki*; (1) unpaired — type *Cephalodasys maximus*; (2) paired, opposed — type Nematode. Characters 118–119 are only applied if character 117 is coded with state 1 (ovary present).

119 *Ovary — direction of maturation*: (0) caudo-frontally — type *Macrodasys buddenbrocki*; (1) fronto-caudally — type *Cephalodasys maximus*; (2) proximo-distally — type Nematode. Of course the oocytes in Gastrotricha mature proximo-distally, but here it is important if distal is directed frontally or caudally. In Nematoda, both parts of the paired ovary are opposed.

120 *Testes — existence*: (0) absent (or only rudimentary); (1) present.

121 *Testes — arrangement*: (0) paired, bilateral — type *Cephalodasys maximus*; (1) unpaired, right — type *Thaumastoderma heideri*; (2) paired, opposed — type Nematode. Characters 121–127 are only applied if character 120 is coded with state 1 (testes present).

122 *Testes — shape*: (0) long, tube-shaped — type *Macrodasys buddenbrocki*; (1) longitudinal columns of follicles — type *Gnathostomula paradoxa*; (2) sac-like — type *Diuronotus aspetos*; (3) ring-shaped — type *Heteroxenotrichula transatlantica*.

123 *Testes — anastomosis — existence*: (0) absent; (1) present — type *Heteroxenotrichula transatlantica*. An anastomosis is reported for the testes of Xenotrichulidae (Ruppert 1979).

124 *Testes — vasa deferentia — existence*: (0) absent; (1) present. If character 124 is coded with state 0, it is likely that testes open directly via male gonopores.

125 *Testes — vasa deferentia — shape*: (0) slender ducts — type *Macrodasys buddenbrocki*; (1) short ducts — type *Gnathostomula paradoxa*; (2) solid, unpaired duct — type Nematode.

126 *Testes — vasa deferentia — direction*: (0) v.d. directed caudally — type *Macrodasys buddenbrocki*; (1) v.d. directed ventro-medially — *Gnathostomula paradoxa*; (2) v.d. directed frontally — type *Heteroxenotrichula transatlantica*.

127 *Testes — vasa deferentia — prominent inflection — existence*: (0) absent; (1) present — type *Desmodasys phocoides*; (2) present — type *Dactylopodola baltica*. The existence of seminal ducts in species of Dactylopodola is improbable (see Kieneke *et al*. 2008b). To avoid an artificial grouping of Dactylopodola with other species (all of traditional family Turbanellidae), we have coded a different character state for Dactylopodola baltica.

128 *Rudimentary testes — existence*: (0) absent; (1) present — type *Arenotus strixinoi*. Character 128 is only applied if character 120 is coded with state 0 (testes absent or rudimentary). 'Rudimentary testes' means one or more pairs of packets of rod-like spermatozoa or spermatogenic cysts which occur in many species of Paucitubulatina that never have fully developed testes (see Weiss 2001).

129 *Frontal organ — existence*: (0) absent; (1) present. We have treated the 'bursa seminalis' (Faubel 1974a) of Praeconvoluta minor and the 'bursal organ' (Ax 1956) of Gnathostomula paradoxa as primary homologue to the frontal organ of species of Gastrotricha. However, there are several structural variations of, for example, the bursal organ among different species of Gnathostomulida (see Lammert 1991). Therefore, a putative homology of these sperm storing organs in lower bilaterian groups has to be tested intensely.

130 *Caudal organ — existence*: (0) absent; (1) present. We have treated the 'penis sack' (Faubel 1974a) of Praeconvoluta minor, the 'vesicula granulorum with stylett' (see Faubel 1974b) of Macrostomum pusillum and the 'copulatory organ' (Ax 1956) of Gnathostomula paradoxa as primary homologue to the caudal organ of species of Gastrotricha. However, there are several structural variations of, for example, the male copulatory organs in species of Plathelminthes (see Ehlers 1985) or the copulatory organs among different species of Gnathostomulida (see Lammert 1991). Therefore, a putative homology of these sperm transferring organs in lower bilaterian taxa has to be tested intensely.

131 *Caudal organ — vasa deferentia discharge into the caudal organ*: (0) absent; (1) present — type *Platydasys maximus*; (2) present — type *Mesodasys laticaudatus*; (3) present — type *Praeconvoluta minor*. We have treated three different types of direct connections of the seminal duct(s) with the caudal (copulatory-) organ to be convergently evolved. For instance, the seminal ducts of Mesodasys laticaudatus open into the caudal organ wall lining its lumen (see Ferraguti & Balsamo 1994), whereas the single duct in species of Thaumastodermatidae open next to the position of the ventral caudal organ pore (see Ruppert 1970). The situation in Acoela is not quite clear. Character 131 is only applied if character 130 is coded with state 1 (caudal organ present).

132 *X-organ — existence*: (0) absent; (1) present — type *Arenotus strixinoi*.

133 *Protonephridia — existence*: (0) absent; (1) present. The pure existence of one or several pairs of protonephridia is known for many species of Gastrotricha (see Kieneke *et al.* 2007, 2008a and references therein).

134 *External cilia covered with cuticle — existence*: (0) absent — type *Praeconvoluta minor*; (1) present — type *Neodasys chaetonotoideus*. It is a unique feature of Gastrotricha that the epicuticle covers all cilia, locomotory as well as sensory (see Ruppert 1991). Some authors consider this character an autapomorphy of Gastrotricha (see Lorenzen 1996). In contrast, Ax (2003) argues for the cuticular covering of cilia to be an apomorphy of Nemathelminthes.

135 *Visceral muscular helix — existence*: (0) absent; (1) present — type *Dactylopodola baltica*. The visceral muscular helix, discovered for the first time in 9 gastrotrich species from all major subgroups by means of phalloidin staining and fluorescence microscopy (Hochberg & Litvaitis 2001a), is now confirmed for many species of Gastrotricha (see Kieneke *et al.* 2008c and references therein).

Appendix B Species-character-matrix, characters 1–45. A dash (–) indicates inapplicable character states.

Taxa										10										20										30										40					
Acanthodasys lineatus	0	0	–	3	0	0	0	1	–	2	0	1	0	3	1	0	0	0	1	2	0	3	1	7	2	0	2	0	0	–	–	–	0	–	1	0	5	1	0	–	0	–	–	–	0
Anacanthoderma paucisetosum	4	1	0	0	0	1	4	0	5	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	0
Arenotus strixinoi	4	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Aspidiophorus paradoxus	4	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Aspidiophorus sylvaticus	4	1	0	0	0	0	3	1	–	6	1	1	4	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	2
Aspidiophorus polystictos	0	1	0	0	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Cephalodasys maximus	0	1	2	5	0	0	0	3	–	–	–	–	–	–	–	–	–	0	1	2	0	4	1	3	0	–	–	–	1	0	9	0	0	–	1	0	4	1	0	–	1	0	7	0	0
Chaetonotus (Brevipedichaeta) uncinus	4	1	0	0	0	0	3	1	–	4	0	1	7	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus (Bifasciculatella) linguaeformis	4	1	0	1	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus condensus	0	1	0	0	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus (Diversichaetatella) acanthocephalus	4	1	0	1	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus (Euchaetonotus) maximus	3	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus (Hystricochaetonotus) hystrix	3	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus (Schizochaetonotus) schultzei	3	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chaetonotus (Zonochaeta) succinctus	4	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Chitonodytes collini	4	1	0	0	0	1	4	0	5	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	0
Chordodasiopsis antennatus	0	1	2	1	0	0	0	1	–	3	0	1	5	0	0	0	0	0	1	2	0	4	1	2	0	–	–	–	1	0	3	2	1	0	0	–	–	–	0	–	0	–	–	–	0
Crasiella diplura	0	0	–	0	0	0	0	1	–	2	0	1	0	5	2	0	0	0	1	2	0	9	0	6	0	–	–	–	1	0	14	3	0	–	0	–	–	–	0	–	0	–	–	–	0
Dactylopodola baltica	0	1	3	0	0	0	2	1	–	2	0	1	0	5	2	0	0	0	1	2	0	1	1	2	0	–	–	–	1	0	4	2	0	–	0	–	–	–	0	–	0	–	–	–	1
Dasydytes (Dasydytes) ornatus	4	1	1	0	0	0	3	0	7	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Dasydytes (Prodasydytes) papaveroi	4	1	0	10	0	0	3	0	5	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Dasydytes (Setodytes) tongiorgii	4	1	1	9	0	1	4	0	7	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Dasydytes (Setopus) bisetosus	4	1	1	10	0	1	4	0	7	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Dendrodasys gracilis	0	1	3	7	0	0	2	1	–	3	0	1	0	1	1	0	0	0	1	2	0	0	1	3	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	1	0	1	1	0
Dendropodola transitionalis	0	1	3	0	0	0	2	1	–	7	0	1	0	0	0	0	0	0	1	2	0	0	1	2	0	–	–	–	1	0	2	2	0	–	0	–	–	–	0	–	1	0	1	1	0
Desmodasys phocoides	0	0	–	3	0	0	2	1	–	0	0	1	0	8	2	1	0	0	1	2	0	9	0	2	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	0
Dichaetura capricornia	4	0	–	0	0	0	3	1	–	4	0	1	3	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Dinodasys mirabilis	0	0	–	1	0	0	0	1	–	0	0	1	0	4	1	0	0	0	1	2	0	4	1	3	0	–	–	–	1	2	7	0	0	–	0	–	–	–	0	–	0	–	–	–	0
Diplodasys ankeli	0	1	0	0	0	0	1	1	–	2	0	1	0	1	1	0	0	0	?	2	0	?	?	1	2	0	?	?	1	0	0	1	0	–	0	–	–	–	0	–	1	0	3	2	1
Diuronotus aspetos	0	1	0	0	0	0	3	1	–	4	0	1	0	0	0	1	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	0
Dolichodasys elongatus	0	0	–	1	0	0	0	0	–	–	–	–	–	–	–	–	–	0	1	2	0	1	1	3	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	1	0	0	1	0
Draculiciteria tesselata	0	1	0	6	0	0	3	1	–	11	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	0
Fluxiderma verrucosum	4	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Halichaetonotus spinosus	0	1	0	0	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Haltidytes crassus	4	1	0	10	0	1	4	0	5	–	–	–	–	–	–	–	–	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Hemidasys agaso	0	0	–	3	0	0	1	0	1	–	–	–	–	–	–	–	–	0	1	2	0	2	1	1	0	–	–	–	1	0	2	2	0	–	0	–	–	–	0	–	1	0	1	1	0
Heterolepidoderma grandiculum	0	1	0	0	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Heterolepidoderma ocellatum	3	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Heteroxenotrichula transatlantica	0	1	0	0	0	0	3	1	–	11	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Ichthydium (Euichthydium) podura	4	1	0	10	0	0	3	1	–	4	0	1	0	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Ichthydium (Forficulichthys) forficula	4	1	0	10	0	0	3	1	–	4	0	1	2	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	1
Ichthydium hummoni	0	1	4	0	0	0	3	1	–	6	1	1	4	0	0	0	0	0	0	1	–	–	–	–	0	–	–	–	0	–	–	–	0	–	0	–	–	–	0	–	0	–	–	–	0

Appendix B *Continued.*

	Characters
Ichthydium (Pseudichthydium) balatonicum	4 1 0 10 0 0 3 1 – 6 0 1 3 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Kijanebalola canina	4 1 0 0 0 0 3 0 5 – – – – – – – – – 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Lepidochaetus brasilense	4 1 0 0 0 0 3 1 – 4 0 1 0 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Lepidodasys platyurus	0 0 – 3 0 0 0 0 3 – – – – – – – – – 0 0 2 0 9 0 6 0 – – – 1 0 8 0 0 – 1 0 1 0 0 – 1 0 7 0 0
Lepidodermella squamata	3 1 0 10 0 0 3 1 – 4 0 1 0 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Macrodasys buddenbrocki	0 0 – 0 0 0 0 0 0 – – – – – – – – – 0 1 2 0 8 0 0 0 – – – 1 0 5 0 0 – 1 0 3 0 0 – 1 0 10 3 0
Marinellina flagellata	4 1 0 0 0 0 1 1 – 12 0 1 0 1 1 0 0 0 2 0 0 1 2 0 – – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Megadasys pacificus	0 0 – 3 0 0 0 0 3 – – – – – – – – – 0 1 0 – – – – 0 – – – 1 0 15 3 0 – 0 – – – 0 – 1 0 8 0 0
Mesodasys laticaudatus	0 0 – 3 0 0 0 0 3 – – – – – – – – – 0 1 2 0 4 1 0 0 – – – 1 0 13 0 0 – 0 – – – 0 – 1 0 8 0 0
Musellifer sublitoralis	0 1 0 1 0 0 3 1 – 5 0 1 0 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 0
Neodasys chaetonotoideus	0 1 5 6 0 0 0 1 – 1 0 1 1 3 1 0 0 0 0 0 – – – – 0 – – – 1 1 10 0 0 – 0 – – – 0 – 0 – – – 0
Neogossea antennigera	4 1 0 9 0 0 3 0 5 – – – – – – – – – 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 0
Ornamentula paraensis	4 1 1 0 0 1 4 0 5 – – – – – – – – – 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 0
Paradasys littoralis	0 0 – 1 0 0 0 0 1 – – – – – – – – – 0 1 2 0 1 1 2 0 – – – 0 – 0 – – – 0 – 1 0 3 2 0
Paraturbanella scanica	0 0 – 3 0 0 0 1 – 0 0 1 0 7 2 1 0 1 1 2 0 7 0 3 0 – – – 1 0 6 0 1 0 1 0 0 0 0 – 0 – – – 0
Planodasys marginalis	0 0 – 0 0 0 0 1 – 8 0 1 0 9 2 0 0 0 1 2 0 10 0 4 0 – – – 1 0 16 3 0 – 0 – – – 0 – 0 – – – 0
Platydasys maximus	0 0 – 2 0 0 1 0 2 – – – – – – – – – 0 1 2 0 5 0 0 2 0 4 1 1 0 3 2 0 – 0 – – – 0 – 1 0 5 0 1
Pleurodasys helgolandicus	0 1 2 5 0 0 0 0 1 – – – – – – – – – 0 1 2 0 3 1 3 0 – – – 1 0 6 0 1 1 0 – 0 – – – 0 – 1 0 4 2 0
Polymerurus nodicaudus	4 1 0 0 0 0 3 1 – 5 0 1 6 0 0 – 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Proichthydioides remanei	4 1 4 0 0 0 3 1 – 6 1 1 4 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Proichthydium coronatum	4 1 4 0 0 0 3 1 – 4 0 1 4 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 0
Prostobuccantia brocha	0 0 – 3 0 0 0 1 – 2 0 1 0 4 1 0 0 0 1 2 0 3 1 3 0 – – – 1 0 12 0 1 0 0 – 0 – – – 0 – 0 – – – 0
Pseudostomella cataphracta	0 1 4 8 1 0 1 1 – 2 0 1 0 4 1 0 0 0 1 2 0 2 1 4 2 0 1 0 1 0 2 2 0 – 0 – – – 1 0 0 – – – 0
Pseudostomella roscovita	0 1 4 8 1 0 1 1 – 10 0 1 0 3 1 0 0 0 1 2 0 1 1 4 2 0 0 0 0 – 0 – – – 0 – 0 – – – 0
Pseudoturbanella stylifera	0 1 2 1 0 0 0 1 – 0 0 1 0 3 1 0 0 1 1 2 0 5 0 3 0 – – – 1 0 0 1 0 – 0 – – – 0 – 0 – – – 0
Ptychostomella mediterranea	0 0 – 2 0 0 0 1 – 9 0 1 0 2 1 0 0 0 1 2 0 3 1 0 1 – – – 1 0 1 2 0 – 1 1 2 0 0 – 1 0 4 2 1
Redudasys fornerise	4 0 – 0 0 0 0 1 – 12 0 1 0 1 1 0 0 0 0 2 0 1 1 2 0 – – – 0 – 0 – – – 0 – 0 – – – 0
Rhomballichthys punctatus	3 1 0 0 0 0 3 1 – 4 0 1 0 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Stylochaeta scirtetica	4 1 1 9 0 0 3 0 6 – – – – – – – – – 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 1
Tetranchyroderma arcticum	0 0 – 0 0 0 0 1 – 2 0 1 0 2 1 0 0 0 1 2 0 8 0 1 2 0 3 1 1 0 8 0 0 – 0 – – – 0 – 1 0 2 2 1
Tetranchyroderma megastoma	0 0 – 2 0 0 1 1 – 9 0 1 0 2 1 0 0 0 1 2 0 4 1 0 1 – – – 1 0 13 0 0 – 0 – – – 0 – 1 0 2 2 1
Thaumastoderma heideri	0 0 – 4 0 0 0 1 – 9 0 1 0 1 1 0 0 0 1 2 0 3 1 6 2 0 2 0 0 – – 0 – 0 – – – 0 – 1 0 3 2 0
Turbanella cornuta	0 1 2 1 0 0 0 1 – 0 0 1 0 6 2 0 0 1 1 2 0 4 1 3 0 – – – 1 0 14 3 0 – 1 0 4 1 0 – 0 – – – 0
Undula paraensis	4 1 1 10 0 0 3 1 – 4 0 0 – – – – – 0 0 0 1 – – – – 0 – – – – 0 – – – – 0 – 0 – – – 1
Urodasys viviparus	0 0 – 3 0 0 0 0 4 – – – – – – – – – 0 1 2 0 2 1 5 0 – – – 1 0 4 2 0 – 0 – – – 0 – 1 0 9 3 0
Xenodasys sanctigoulveni	0 1 5 0 0 0 1 1 – 4 0 1 0 2 1 0 0 0 1 1 0 2 1 3 0 – – – 1 0 8 0 0 – 0 – – – 0 – 0 – – – 1
Xenotrichula punctata	0 1 0 0 0 0 3 1 – 11 0 1 0 0 0 0 0 0 0 1 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 0
Gnathostomula paradoxa	0 1 6 1 0 0 0 0 0 – – – – – – – – – 0 0 0 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 2
Praeconvoluta minor	0 0 – 0 0 0 1 0 1 – – – – – – – – – 0 0 0 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 2
Macrostomum pusillum	0 0 – 0 0 ? 0 0 3 – – – – – – – – – 0 0 0 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 2 1 7 0 2
Nematode	0 0 – 11 0 1 0 0 0 – – – – – – – – – 0 0 0 – – – – 0 – – – 0 – – 0 – 0 – – – 0 – 0 – – – 0
Ground pattern of Gastrotricha	0 1 5 0 0 0 0 1 – ? 0 1 ? ? 1 0 0 0 0 0 – – – – 0 – – – 1 ? ? 0 0 – 0 – – – 0 – 0 – – – 0

Appendix B Species-character-matrix, characters 46–90.

Taxa	Characters 46–90
	...50...............60...............70...............80...............90
Acanthodasys lineatus	1 0 1 3 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 ? ? ? 0 0 2 0 1 0 – 0 – – 0 – 0 – 1 1 – 0 1 1 – 0 0
Anacanthoderma paucisetosum	1 0 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Arenotus strixinoi	1 2 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 1 1 0 – – – 0 – – – 0
Aspidiophorus paradoxus	1 3 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – – 0 – 1 1 1 0 9 – 1 0 7 – 0
Aspidiophorus sylvaticus	2 4 0 – 0 – 1 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 0 1 1 1 0 – – 0 – 0 – 1 0 9 – 1 0 7 – 0
Aspidiophorus polystictos	1 4 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 0 1 1 1 1 1 0 – 1 1 1 0 9 – 1 0 7 – 0
Cephalodasys maximus	1 0 1 0 1 0 0 0 0 0 – – 1 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – – 0
Chaetonotus (Brevipedichaeta) uncinus	1 3 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 0 – 0 – – 0 – 0 – 1 0 4 – 1 0 2 – 0
Chaetonotus (Bifasciculatella) linguaeformis	1 ? 0 – 0 – 1 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 0 – 0 – – 0 – ? ? 1 0 4 – 1 0 2 – 0
Chaetonotus condensus	1 3 ? ? 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 0 1 0 – 0 – – 0 – ? ? 1 0 4 – 1 0 2 – 0
Chaetonotus (Diversichaetatella) acanthocephalus	1 3 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 0 – 1 1 1 0 4 – 1 0 2 – 0
Chaetonotus (Euchaetonotus) maximus	1 2 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 1 1 1 0 4 – 1 0 2 – 0
Chaetonotus (Hystricochaetonotus) hystrix	1 3 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 2 1 1 1 0 – 0 – – 0 – 1 0 4 – 1 0 2 – 0
Chaetonotus (Schizochaetonotus) schultzei	1 3 1 1 0 – 1 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 1 1 1 0 4 – 1 0 2 – 0
Chaetonotus (Zonochaeta) succinctus	1 4 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 0 – 1 0 4 – 1 0 2 – 0
Chitonodytes collini	1 3 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 2 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Chordodasiopsis antennatus	1 0 0 – 0 – 0 0 0 1 3 2 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Crasiella diplura	1 6 1 2 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Dactylopodola baltica	1 6 0 – 1 0 0 0 0 0 – – 1 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Dasydytes (Dasydytes) ornatus	1 2 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 1 1 1 1 0 – 0 – – 0 – 0 – – – 0 – – – 0
Dasydytes (Prodasydytes) papaveroi	1 3 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 1 1 1 0 4 – 1 0 2 – 0
Dasydytes (Setodytes) tongiorgii	1 3 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 1 0 4 – 0 – – – 0
Dasydytes (Setopus) bisetosus	1 3 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 0 – – – 0 – – – 0
Dendrodasys gracilis	1 0 1 0 0 – 0 0 0 0 – – 1 0 0 1 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Dendropodola transitionalis	1 0 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Desmodasys phocoides	1 0 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 2 1 0 0 0 2 ? 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Dichaetura capricornia	1 4 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 ? 0 – 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Dinodasys mirabilis	1 0 1 1 0 – 0 0 0 1 4 0 1 0 0 0 0 0 1 2 ? ? 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Diplodasys ankeli	3 0 1 3 ? ? 0 0 0 0 – – 1 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 1 0 1 – 1 0 0 – 0
Diuronotus aspetos	1 2 1 2 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 0 – 0 – – 0 – 0 – 1 0 4 – 1 0 2 – 0
Dolichodasys elongatus	1 0 1 2 0 – 0 0 0 0 – – 1 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Draculiciteria tesselata	1 2 1 0 0 – 0 0 0 0 – – 1 2 0 0 0 0 0 1 0 – 0 0 2 0 1 1 1 1 1 0 1 0 1 1 1 0 10 – 1 0 8 – 1
Fluxiderma verrucosum	1 4 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – ? ? 1 0 6 – 1 0 3 – 0
Halichaetonotus spinosus	1 3 ? ? 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 ? ? 1 0 4 – 1 0 2 – 0
Haltidytes crassus	1 0 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 0 – – – 0 – – – 0
Hemidasys agaso	1 0 1 2 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 ? ? ? 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0
Heterolepidoderma grandiculum	1 3 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 1 0 5 – 1 0 4 – 0
Heterolepidoderma ocellatum	1 3 1 0 0 – 1 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 0 – 1 0 5 – 1 0 4 – 0
Heteroxenotrichula transatlantica	2 ? 1 0 1 0 0 0 0 1 1 0 1 2 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 0 – 1 0 9 – 1 0 7 – 1
Ichthydium (Euichthydium) podura	1 0 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 0 – – – 0 – – – 0
Ichthydium (Forficulichthys) forficula	1 3 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 2 1 1 1 1 1 0 1 0 ? ? 0 – – – 0 – – – 0
Ichthydium hummoni	1 3 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – 0

Appendix B *Continued.*

Characters

Taxon	Character states
Ichthydium (Pseudichthydium) balatonicum	1 4 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – – 0 – ? ? 0 – – – 0 – – – – 0
Kijanebalola canina	1 2 0 – 0 – 0 0 0 1 2 0 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – – 0 – 1 1 0 – – – 1 0 2 – 0
Lepidochaetus brasilense	1 2 ? ? 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 1 0 – 1 1 1 0 7 – 1 0 5 – 0
Lepidodasys platyurus	1 0 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 1 0 3 – 1 0 1 – 0
Lepidodermella squamata	1 2 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 0 – 0 – 0 – 1 1 1 0 7 – 1 0 5 – 0
Macrodasys buddenbrocki	1 6 1 2 0 – 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Marinellina flagellata	4 0 1 5 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 ? 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Megadasys pacificus	1 6 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 1 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Mesodasys laticaudatus	1 3 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Musellifer sublitoralis	1 5 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 1 0 1 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 1 0 4 – 1 0 2 – 0
Neodasys chaetonotoideus	1 1 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Neogossea antennigera	1 2 1 0 1 0 0 0 0 1 2 0 1 0 0 0 0 0 0 1 0 – 0 0 2 1 1 0 – 0 – 0 – 0 – 1 0 4 – 1 0 2 – 0
Ornamentula paraensis	1 2 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 1 1 0 1 0 1 1 1 0 4 – 1 0 9 – 0
Paradasys littoralis	1 0 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Paraturbanella scanica	1 0 1 1 0 – 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Planodasys marginalis	1 6 1 1 ? ? 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Platydasys maximus	3 0 1 3 0 – 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Pleurodasys helgolandicus	1 0 1 0 0 – 0 0 0 0 – – 1 0 0 1 0 2 1 2 0 0 0 ? ? 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Polymerurus nodicaudus	1 2 1 0 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 2 1 1 1 1 1 1 0 1 1 1 0 4 – 1 0 2 – 0
Proichthydioides remanei	2 0 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 2 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Proichthydium coronatum	1 0 ? ? 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Prostobuccantia brocha	1 7 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 1 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Pseudostomella cataphracta	3 0 1 3 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 1 1 – 2 1 1 – 2 0
Pseudostomella roscovita	3 0 1 3 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 1 1 – 1 1 1 – 1 0
Pseudoturbanella stylifera	1 0 0 – 0 – 0 0 0 1 4 0 1 0 0 0 0 0 0 2 1 0 0 0 ? ? 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Ptychostomella mediterranea	3 0 1 3 0 – 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Redudasys fornerise	1 0 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Rhomballichthys punctatus	1 0 0 – 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 ? ? 1 1 1 0 – 0 – 0 – 1 0 8 – 1 0 6 – 0
Stylochaeta scirtetica	1 2 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 1 0 – 0 0 2 1 1 1 1 0 – 0 – 0 – 0 – – – 0 – – – – 0
Tetranchyroderma arcticum	3 0 1 3 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 1 1 – 1 1 1 – 1 0
Tetranchyroderma megastoma	3 0 1 3 0 – 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 ? ? 1 0 – 0 – 0 – 0 – 1 1 – 2 1 1 – 2 0
Thaumastoderma heideri	1 3 0 – 1 1 0 0 0 1 2 1 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 1 1 – 1 1 1 – 1 0
Turbanella cornuta	1 0 0 – 1 0 0 0 0 1 0 0 1 0 0 0 0 0 0 2 1 0 0 0 2 0 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Undula paraensis	1 2 1 1 0 – 0 0 0 1 6 0 1 0 0 0 0 0 0 1 0 – 0 0 2 ? ? 1 1 1 1 1 0 1 0 1 1 0 – – – 1 0 2 – 0
Urodasys viviparus	1 0 1 2 0 – 0 0 1 0 – – 1 0 0 0 0 0 0 2 1 0 0 0 1 – 1 0 – 0 – 0 – 0 – 0 – – – 0 – – – – 0
Xenodasys sanctigoulveni	1 0 0 – 0 – 0 0 0 1 5 1 1 0 0 0 0 0 0 2 1 1 0 0 2 0 1 1 0 1 0 – 0 – 1 0 1 0 2 – 0 – – – – 0
Xenotrichula punctata	1 2 1 1 0 – 0 0 0 0 – – 1 2 0 0 0 0 0 1 0 – 0 0 2 0 1 0 – 0 – 0 – 0 – 1 0 9 – 1 0 7 – 1
Gnathostomula paradoxa	0 0 1 4 0 – 0 0 0 0 – – 1 1 0 0 0 0 0 0 0 – 1 1 0 – 0 – – – – – – – – – – – – – – – – – –
Praeconvoluta minor	0 0 0 – 0 – 0 1 0 0 – – ? ? 0 0 0 0 0 0 0 – 0 0 0 – 0 – – – – – – – – – – – – – – – – – –
Macrostomum pusillum	0 0 0 – 1 0 0 0 0 0 – – 1 0 0 0 0 0 0 0 0 – 0 0 0 – 0 – – – – – – – – – – – – – – – – – –
Nematode	1 8 1 0 0 – 0 0 0 0 – – 0 – 1 1 0 0 0 3 0 – 0 0 2 0 1 0 – 0 – – 0 – 0 – 0 – – – 0 – – – – 0
Ground pattern of Gastrotricha	1 0 1 1 0 – 0 0 0 0 – – 1 0 0 0 0 0 0 ? 0 – 0 0 2 0 1 0 – 0 – – – 0 – 0 – – – 0 – – – – 0

Appendix B Species-character-matrix, characters 91–135.

Taxa:										100										110										120										130					
Acanthodasys lineatus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	1
Anacanthoderma paucisetosum	0	0	0	–	0	–	1	0	0	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1
Arenotus strixinoi	0	0	0	–	0	–	0	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Aspidiophorus paradoxus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1
Aspidiophorus sylvaticus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1
Aspidiophorus polystictos	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	–	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1
Cephalodasys maximus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	1
Chaetonotus (Brevipedichaeta) uncinus	0	0	0	–	0	–	1	1	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Chaetonotus (Bifasciculatella) linguaeformis	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Chaetonotus condensus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Chaetonotus (Diversichaetatella) acanthocephalus	0	0	0	–	0	–	0	–	–	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Chaetonotus (Euchaetonotus) maximus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Chaetonotus (Hystricochaetonotus) hystrix	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Chaetonotus (Schizochaetonotus) schultzei	0	0	0	–	0	–	0	–	–	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Chaetonotus (Zonochaeta) succinctus	0	0	0	–	0	–	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1		
Chitonodytes collini	0	0	0	–	1	0	0	–	–	0	0	–	0	0	1	1	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Chordodasiopsis antennatus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	1	1	1	0	0	0	0	1	?	?	1	0	0	0	1	0	0	0	0	1	?	?	0	1	1	1
Crasiella diplura	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	?	?	?	1	0	0	1	1	0	0	0	1	1	0	0	1	1	1
Dactylopodola baltica	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	2	0	?	1	0	0	1	1	1
Dasydytes (Dasydytes) ornatus	0	0	0	–	1	0	1	0	0	0	0	–	0	0	1	1	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Dasydytes (Prodasydytes) papaveroi	0	0	0	–	1	0	1	0	0	0	0	–	0	0	1	1	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	0	0	?	?	1	1	1	
Dasydytes (Setodytes) tongiorgii	0	0	0	–	1	0	1	0	1	0	0	–	0	0	1	1	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Dasydytes (Setopus) bisetosus	0	0	0	–	0	–	1	0	0	0	0	–	0	0	1	1	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Dendrodasys gracilis	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	1
Dendropodola transitionalis	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	1
Desmodasys phocoides	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	?	1	0	0	1	1	1
Dichaetura capricornia	0	0	0	–	0	–	0	–	–	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	1
Dinodasys mirabilis	0	0	0	–	0	–	0	–	–	0	0	–	0	1	0	1	1	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	1	1	1
Diplodasys ankeli	1	?	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1	1	0	0	1	1	1
Diuronotus aspetos	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	2	0	0	–	–	–	0	0	0	–	?	1	1	1
Dolichodasys elongatus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	1	1	0	0	1	0	0	0	0	1	0	0	1	1	1			
Draculiciteria tesselata	0	0	0	–	0	–	0	–	–	0	0	–	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1
Fluxiderma verrucosum	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Halichaetonotus spinosus	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Haltidytes crassus	0	0	0	–	1	1	0	–	–	0	0	–	0	0	1	1	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Hemidasys agaso	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	1	1	0	0	1	0	0	0	0	?	1	0	0	1	1	1
Heterolepidoderma grandiculum	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Heterolepidoderma ocellatum	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Heteroxenotrichula transatlantica	0	0	0	–	0	–	0	–	–	0	0	–	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	3	1	1	0	2	0	0	0	0	–	?	1	1	1
Ichthydium (Euichthydium) podura	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	1	0	0	–	1	1	1	1	
Ichthydium (Forficulichthys) forficula	0	0	0	–	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	
Ichthydium hummoni	0	0	1	1	0	–	0	–	–	0	0	–	0	0	1	0	0	0	0	0	0	0	0	0	0	1	?	?	0	–	–	–	–	–	–	–	?	0	0	–	?	1	1	1	

Appendix B *Continued.*

Characters

Taxon	Characters
Ichthydium (Pseudichthydium) balatonicum	0 0 1 0 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 0 – – – – – – – – ? 0 0 – ? 1 1 1
Kijanebalola canina	0 0 0 – 0 – 1 2 0 1 0 – 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 0 – – – – – – – – ? 0 0 – ? 1 1 1
Lepidochaetus brasilense	0 0 0 – 0 – 1 0 0 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 0 – – – – – – – – 1 0 0 – 1 1 1 1
Lepidodasys platyurus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 1 0 0 0 1 0 0 0 0 ? ? ? ? 0 1 1 1
Lepidodermella squamata	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 – – – – – – – – – 1 0 0 – 1 1 1 1
Macrodasys buddenbrocki	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 0 0 0 0 1 1 0 0 1 1 1
Marinellina flagellata	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 ? ? ? 0 – – – – – – – – ? 0 0 – ? 1 1 1
Megadasys pacificus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 0 0 0 0 0 1 0 0 1 1 1
Mesodasys laticaudatus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 0 1 0 0 0 0 ? 1 2 0 1 1 1
Musellifer sublitoralis	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 2 0 0 – – – 0 0 1 0 0 1 1 1
Neodasys chaetonotoideus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 1 0 0 0 0 – – – 0 1 1 – ? 1 1 1
Neogossea antennigera	0 0 0 – 0 – 1 1 0 1 0 – 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 0 0 0 – – – – – – – – ? 0 0 – ? 1 1 1
Ornamentula paraensis	0 0 0 – 1 0 1 0 0 0 0 – 0 0 1 1 0 0 0 0 0 0 0 0 0 0 ? ? ? ? ? ? ? ? ? ? ? ? 0 0 – ? 1 1 1
Paradasys littoralis	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 1 1 0 0 0 ? ? ? ? 0 1 ? ? 0 1 1 1
Paraturbanella scanica	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 0 0 1 0 ? 1 0 0 1 1 1
Planodasys marginalis	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 2 0 0 0 1 0 0 0 1 0 0 0 0 ? 1 ? 0 1 1 1
Platydasys maximus	0 1 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 1 0 0 1 0 0 1 0 0 1 1 1 0 1 1 1
Pleurodasys helgolandicus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 0 0 0 1 0 0 0 0 ? 1 0 0 1 1 1
Polymerurus nodicaudus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 – – – – – – – – 1 0 0 – 1 1 1 1
Proichthydioides remanei	0 0 1 0 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 ? ? 0 – – – – – – – – ? 0 0 – ? 1 1 1
Proichthydium coronatum	0 0 0 – 0 0 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 ? ? ? ? ? ? ? ? ? ? ? ? 0 0 – ? 1 1 1
Prostobuccantia brocha	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? 1 0 0 0 1 0 0 1 0 1 ? ? 0 1 1 1
Pseudostomella cataphracta	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 0 1 0 0 0 0 1 1 1 ? 1 1 1
Pseudostomella roscovita	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 0 1 0 0 0 0 1 1 1 ? 1 1 1
Pseudoturbanella stylifera	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 ? ? ? 1 ? ? ? ? ? ? ? ? 0 ? ? ? ? 1 1 1
Ptychostomella mediterranea	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 0 1 0 0 0 0 1 1 1 0 1 1 1
Redudasys fornerise	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 0 – – – – – – – – ? 0 0 – 0 1 1 1
Rhomballichthys punctatus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 0 – – – – – – – – ? 0 0 – ? 1 1 1
Stylochaeta scirtetica	0 0 0 – 1 0 0 – – 0 0 – 0 0 1 1 0 0 0 0 0 0 0 0 0 0 1 ? ? ? 0 – – – – – – – – 1 0 0 – 1 1 1 1
Tetranchyroderma arcticum	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 0 1 0 0 0 0 1 1 1 0 1 1 1
Tetranchyroderma megastoma	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 0 1 0 0 0 0 1 1 1 0 1 1 1
Thaumastoderma heideri	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 1 1 0 1 1 0 0 1 0 0 0 0 1 1 1 0 1 1 1
Turbanella cornuta	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 0 1 0 0 0 1 0 0 1 0 ? ? ? ? 1 1 1
Undula paraensis	0 0 0 – 0 – 1 0 0 1 0 – 0 0 1 1 0 0 0 0 0 0 0 0 0 0 ? ? ? 0 – – – – – – – – ? 0 0 – ? 1 1 1
Urodasys viviparus	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 0 – – – – – – – – 0 0 0 – 0 1 1 1
Xenodasys sanctigoulveni	0 ? 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 1 0 0 0 1 0 0 1 0 0 0 1 0 0 0 0 ? ? ? 0 1 1 1
Xenotrichula punctata	0 0 0 – 0 – 0 – – 0 0 – 0 1 1 0 0 0 0 0 0 0 0 0 0 0 1 ? ? 1 0 3 1 1 0 2 0 0 0 0 – ? 1 1 1
Gnathostomula paradoxa	– – – – 0 – 0 – – 0 0 – 0 0 0 0 0 0 0 0 0 0 0 – 0 0 0 1 1 1 1 0 1 0 1 1 1 1 0 0 1 1 0 0 1 0 0
Praeconvoluta minor	– – – – 0 – 0 – – 0 0 – 0 0 0 0 0 0 0 0 0 0 0 – 0 0 0 1 0 1 1 0 0 0 1 0 0 0 0 1 1 3 0 0 0 0
Macrostomum pusillum	– – – – 0 – 0 – – 0 0 – 0 0 0 0 0 0 0 0 0 0 0 – 0 0 0 1 1 1 1 0 0 0 ? ? ? ? 0 0 1 ? 0 1 0 0
Nematode	0 0 0 – 0 – 0 – – 0 0 – 0 – 2 0 0 0 0 0 0 0 0 0 0 0 1 2 2 1 2 0 0 1 2 0 0 0 0 0 – 0 0 0 0 0
Ground pattern of Gastrotricha	0 0 0 – 0 – 0 – – 0 0 – 0 0 1 0 0 0 0 0 0 0 0 0 0 0 1 0 1 1 0 0 0 1 0 0 0 0 1 1 0 0 1 1 1