Phylogeny of Gastrotricha: a Morphology-Based Framework of Gastrotrich Relationships

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Abstract. Currently, the phylum Gastrotricha is divided into the orders Macrodasyida and Chaetonotida, with the structure of the myoepithelial pharynx being an important distinguishing feature. Macrodasyida currently has six recognized families, and Chaetonotida comprises seven families. However, within-group relationships are poorly understood. To arrive at a better understanding of gastrotrich systematics and phylogeny, we performed the first cladistic analysis of nearly all known gastrotrich genera using 71 morphological characters. Results suggest that the Gastrotricha is a monophyletic group (supported by 82% of bootstrap replications) with its most primitive taxa distributed among the families Dactylopodolidae and Neodasyidae. Monophyly of Macrodasyida and Chaetonotida was supported by 90% and 52% bootstrap replications, respectively. Within the Macrodasyida, the families Dactylopodolidae, Turbanellidae, Macrodasyidae, and Thaumastodermatidae all formed monophyletic clades. The families Planodasyidae and Lepidodasyidae were paraphyletic. Among the Chaetonotida, the marine family Xenotrichulidae was monophyletic, supported by 51% of bootstrap replications. A second clade containing all freshwater families was supported by 62% bootstrap values. However, Chaetonotidae were paraphyletic. Using this analysis as a framework, we now can explore possible patterns of evolution within it, and arrive at a consensus of the gastrotrich ground pattern. Moreover, in future molecular studies of metazoan phylogeny, we will be able to select gastrotrich species that are more appropriate representatives of the phylum.

Introduction

Gastrotrichs figure prominently in metazoan phylogeny because they share a suite of complex morphological characteristics with several other members of the Bilateria, including the Gnathostomulida and the Nematoda. But their microscopic size, their cryptic interstitial habitat, and the lack of fossil record have exacerbated the usual barriers to phylogenetic analysis. As a consequence, gastrotrichs are one of the least understood groups of aquatic invertebrate animals. Furthermore, the relationship of Gastrotricha to other extant metazoans is uncertain; results from recent molecular and morphological studies are in conflict (Winnepenninckx et al., 1995; Littlewood et al., 1996; and Wallace et al., 1996). The currently accepted classification of the phylum Gastrotricha is based on morphological features (Hummon, 1982; Ruppert, 1988) and has two monophyletic orders, the Macrodasyida and Chaetonotida. Both orders are defined primarily by the structure of the myoepithelial pharynx (Ruppert, 1991), yet several other characters are important in the systematic classification of genera (Hummon, 1982; Ruppert, 1988; Ruppert, 1991) (Fig. 1).

Accepted morphological homologies supporting gastrotrich monophyly include the presence of unique, cuticle-covered duo-gland adhesive organs (Tyler and Rieger, 1980), a multilayered epicuticle (Rieger and Rieger, 1977), and cuticle-covered locomotory and sensory cilia (Rieger and Rieger, 1977). Other features often used to categorize gastrotrichs—cross-striated muscles (Travis, 1983), monociliated epidermis (Rieger, 1976), triradiate myoepithelial pharynx (Schmidt-Rhaesa et al., 1998), and bilayered nature of the cuticle (Schmidt-Rhaesa et al., 1998)—are plesiomorphies and, therefore, of limited systematic value. Of particular significance is the presence of a monociliated epidermis; this condition is considered a primitive trait among Metazoa (Rieger, 1976). A monociliated epidermis is found in several gastrotrich taxa (Rieger, 1976), including the Dactylopodolidae and Neodasyidae, arguing further for their basal positions within their respective orders. That the
order Chaetonotida may be paraphyletic with respect to the phylum Nematoda, as suggested previously (Ruppert, 1982), is indicated by similarities in cuticular and pharyngeal ultrastructure (upright Y-shaped lumen, pharyngeal-intestinal valve), and pharyngeal innervation. Because of the complete absence of locomotory cilia in Nematoda, in conjunction with several other morphological characters, we agree with Lorenzen (1985) that the Nematoda is, at best, a sister taxon to the Gastrotricha.

**Materials and Methods**

We have analyzed relationships of nearly all known gastrotrich genera and evaluated them using parsimony analysis (PAUP 4.0*; Swofford, 1999). Eighty-one characters were used in the analysis, all unordered and unweighted (Table 1). For maximum parsimony, default settings included ACCTRAN, and multistate taxa were treated as uncertainties. Bootstrap options included 1000 replicates of 81 characters. Full heuristic searches were performed with starting trees obtained by stepwise random addition (10 replicates with two trees held at each step). Tree bisection-reconnection (TBR) branch swapping was performed with the MULTREES option to save all minimum-length trees. Two genera, *Marinellina* and *Undula*, were excluded from the analysis due to a lack of information. The Gnathostomulida and Nematoda were used as outgroups. The data matrix is available upon request from the first author.

**Results**

Our analysis resulted in a monophyletic Gastrotricha (82% bootstrap values). Within the phylum, Chaetonotida (supported by 52% of bootstrap replications) and Macrodasyida (90% of bootstrap replications) formed two monophyletic clades (Fig. 2). Although the Macrodasyida is a
### Table 1

**Characters and character states used in parsimony analysis of Gastrotricha (a = absent, p = present)**

**Adhesive Tubes**
- 1. Cuticularized adhesive organs (tubes) (0 = a, 1 = p)
- 2. Lateral adhesive tubes (0 = a, 1 = p)
- 3. Lateral adhesive organs indistinguishable, present as minute papillae (0 = a, 1 = p)
- 4. Ventrally placed anterior adhesive tubes (0 = a, 1 = p)
- 5. Anterior adhesive tubes in arc-like orientation (0 = a, 1 = p)
- 6. Anterior adhesive tubes on a mobile elevated base (0 = a, 1 = p)
- 7. Anterior adhesive tubes arranged together—as hands or clumps (0 = a, 1 = p)
- 8. Posterior adhesive tubes (0 = a, 1 = p)
- 9. Rounded caudal end bearing numerous adhesive tubes (0 = a, 1 = p)
- 10. Caudal end with tail-like extension bearing adhesive tubes (0 = a, 1 = p)
- 11. Caudal end with elongate peduncle bearing adhesive tubes (0 = a, 1 = p)
- 12. Caudal end is biramous/forked (0 = a, 1 = p)
- 13. Caudal end with only 2 adhesive tubules (0 = a, 1 = p)
- 14. Proximal/distal separation of caudal adhesive tubules on elongate peduncle (0 = a, 1 = p)
- 15. Median caudal cone (0 = a, 1 = p)
- 16. Caudal furca reduced to bilateral protuberances without adhesive tubes (0 = otherwise, 1 = p)
- 17. Ventrally placed extraordinary adhesive tubes as Seitenflüssehen (0 = a, 1 = p)
- 18. Seitenflüssehen as paired “brocha” tubules (0 = a, 1 = p)
- 19. Seitenflüssehen as single “cirrata” tubules (0 = a, 1 = p)

**Alimentary System**
- 20. Condition of buccal capsule (0 = simple opening, 1 = extended/folded/etc.)
- 21. Wide flaring buccal region (0 = a, 1 = p)
- 22. Shape of myoepithelial pharyngeal lumen (0 = a, 1 = Y-shape, 2 = upside-down Y-shape)
- 23. Pharyngeal clefts (0 = a, 1 = p)
- 24. Myoepithelial pharynx with cilia (0 = a, 1 = p)
- 25. Microvilli penetrate pharynx exocuticle (0 = a, 1 = p)
- 26. Pharyngeal foldings/plug (0 = a, 1 = p)
- 27. Pharyngeal intrusions “teeth” (0 = a, 1 = p)
- 28. Small pharyngeal pores (0 = a, 1 = p)
- 29. Ciliated gut epithelium (0 = a, 1 = p)
- 30. Distinct head enclosing most of the pharynx (0 = a, 1 = p)

**Body Wall**
- 31. Y-cells derived from longitudinal muscle block (0 = a, 1 = p)
- 32. Muscle striation pattern (0 = X-Striated, 1 = Oblique)
- 33. Y-cell with myofilaments (0 = a, 1 = p)
- 34. Epidermal glands with band-like contents (0 = a, 1 = p)
- 35. Circular muscles (0 = p, 1 = a)
- 36. Circular muscles absent from lateral body regions (0 = p, 1 = a)
- 37. Dorsal epidermal glands (0 = absent, 1 = present)
- 38. Body shape (0 = elongate, 1 = ten-pin)

**Ciliation**
- 39. Epidermal ciliation (0 = monociliation, 1 = multiciliation, 2 = no ciliation)
- 40. One pair of short “cirri” (0 = a, 1 = p)
- 41. Ventral cilia and head sensoria modified as cirri (0 = a, 1 = p)
- 42. Construction of ventral and head cirri (0 = a, 1 = constructed as a singular unit, 2 = each cilium with own cuticle)
- 43. Stylochaeta-type ciliation (0 = a, 1 = p)
- 44. Ventral locomotory cilia restricted to head region as a distinct patch (0 = a, 1 = p)
- 45. Ventral locomotory cilia present in 2 longitudinal rows (0 = a, 1 = p)
- 46. Longitudinal rows of locomotory cilia abbreviated at mid-body region (0 = otherwise, 1 = p)
- 47. Cilia covered with cuticle (0 = a, 1 = p)

**Cuticle**
- 48. Scales constructed from basal cuticular layer—form solid endocuticular thickenings (0 = a, 1 = p)
- 49. Cuticular hooks/ancres (triancres, tetranctres, pentancres) (0 = a, 1 = p)
- 50. Spines sculpted from surface zone of basal cuticular layer (not originating from flat scales) (0 = a, 1 = p)
- 51. Keeled scales derived from surface-zone of basal cuticular layer (0 = a, 1 = p)
- 52. Ventrolateral hydrofoil scales derived from surface-zone of basal cuticular layer (0 = a, 1 = p)
highly heterogeneous group defined primarily by pharyngeal structure (Ruppert, 1991), the presence of groups of adhesive tubules (Hummon, 1982; Ruppert, 1988), and curious reproductive organs (Ruppert and Shaw, 1977; Ruppert, 1978), our analysis confirmed several monophyletic families within the order (Fig. 2). In fact, characters of the latter two organ systems substantiate monophyly of the two ecologically most diverse families, the Turbanellidae and Thaumastodermatidae (Fig. 2). These two families occur in nearly all marine environments at all depths, inhabit a wide array of sand types, and are probably among the most ubiquitous and successful groups of interstitial animals. As noted previously (Ruppert, 1988), the Lepidodasyidae is an unnatural taxon difficult to define on current morphological criteria. Our analysis found a paraphyletic Lepidodasyidae with some genera clustering with other families, although with low bootstrap values (Fig. 2). The monophyletic Dactylopodolidae (95% bootstrap values) is affirmed as the most primitive macrodasyidan family, with retention of several plesiomorphic traits including epidermal monociliation, alimentary canal ciliation, cross-striated muscles, and separate multiple nerve cords in some genera (Fig. 2). Additional research on this family, in particular the relatively unknown Dendrodasys and Dendropodola, should further elucidate the ground pattern for the Gastrotricha.

Bootstrap values advocating a monophyletic Chaetonotida are relatively strong (52%), and values for the monophyly of the suborder Paucitubulatina are even more robust (93%) (Fig. 2). All chaetonotidans are largely defined by the configuration of the pharyngeal lumen (upright Y-shaped) and the absence of pharyngeal pores. But often, body-shape (“ten-pin”) and the absence of anterior or lateral adhesive tubes are the most easily seen diagnostic characters. The monogeneric Multitubulatina, however, contains an anomalous vermiform Neodasys that shares traits with both Macrodasys and Paucitubulatina. However, unlike all other members of the Chaetonotida, Neodasys is highly elongate and in possession of some potentially plesiomorphic characteristics: hermaphroditic gonads, complex reproductive organs, monociliated epidermis, smooth cuticle, and numerous adhesive tubules (Ruppert, 1991). Furthermore, the adhesive organ of Neodasys lacks a releaser gland (Tyler et
The presence of only one gland type in the adhesive organ is interpreted as a plesiomorphy when viewed in combination with the retention of other primitive traits (Tyler et al., 1980). Still, the presence of duogland adhesive organs in all Gastrotricha has not been confirmed, and it remains to be seen whether the presence of only a single gland is actually the "primitive" condition or is secondarily derived.

Within the Paucitubulatina, the largest and structurally most diverse family—the Chaetonotidae—appears to be paraphyletic (Fig. 2). This highly speciose taxon (>250 spp.) is extremely heterogeneous with respect to cuticular
armature, habitat type, and reproductive anatomy. The family is presumed to have evolved in the freshwater benthos, radiating into the marine environment secondarily (Kisielewski, 1990). Yet, the existence of a very basal marine genus, Musellifer, might also argue for a marine origin. Resolution of this vastly diverse taxon will undoubtedly improve with greater attention to its reproductive anatomy (presence of hermaphroditic organs is unknown for many genera; the homology of the X-organ is in question) and the complex microstructure of both scales and spines.

Still, several important monophyletic groupings were confirmed within the Chaetonotida, including the entirely freshwater Dasydytidae, Neogosseidae, and Proichthydidae (Fig. 2). Our analysis thus confirmed a clade of freshwater families with 62% of bootstraps. In addition, the marine family Xenotrichulidae and the subfamily Xenotrichulinae were also monophyletic (Fig. 2).

Discussion

The current analysis goes beyond classification; it is aimed at an elucidation of phylogenetic trends. In particular, trends in nervous-system structure in the Gastrotricha are revealed within the tree topology of the Macrodasida. Basal genera often have multiple, separate nerve cords (4 in Dactylopodola), whereas more derived genera show partial fusion of nerve cords (4 fuse into 2 in Turbanella) or presumably complete fusion (2 in Thaumastodermatidae). The functional significance of this transition series is unknown; moreover, the trend is more ambiguous than supposed because other “intermediate” forms like Cephalodasyss maximus show fusion (“schmelzen”) of multiple (12) nerve cords, followed by subsequent bifurcation (Wiedermann, 1995). Other trends are also evident. For example, changes in reproductive biology (from hermaphroditic to parthenogenetic) seem to have occurred in chaetonotidan transitions from marine to freshwater biotopes.

This cladistic analysis also provides evidence that many characters used to define gastrotrichs in morphology-based phylogenies of extant Metazoa often are apomorphic for the phylum and, therefore, inappropriate. For example, Wallace et al. (1996) used the characters parthenogenesis, syncytial epidermis, and hypodermic impregnation in their analysis. The first two characters are known only in some chaetonotidans, and the third is unconfirmed except for the family Macrodasidae and is unknown in basal taxa such as Dactylopodolidae. Backeljau et al. (1993) incorrectly used eutely (found only in some chaetonotidans) and radial cleavage (gastrotrichs have aberrant bilateral cleavage) as characters defining gastrotrichs as a whole in their analysis. Finally, Zrzavy et al. (1998) used the character pseudocoelomate in their analysis, although all gastrotrichs are acoelomate.

Our cladistic analysis also provides a good test for molecular phylogenetic studies. Several earlier molecular studies have focused on derived taxa (Chaetonotus sp., Lepidodermella squamata) to resolve phylum-level relationships (Winneppeninckx et al., 1995; Littlewood et al., 1998; Zrzavy et al., 1998; Ruiz-Trillo et al., 1999). Such taxa are particularly easy to culture, but their utility as representative models is questionable. Lepidodermella squamata is especially controversial because it inhabits fresh water and is parthenogenetic—characteristics that are uncommon among chaetonotidans and virtually absent in macrodasidans. The use of such derived forms should be avoided until the systematics of the Chaetonotida is better resolved.

Additionally, molecular systematics place the Gastrotricha in a variety of positions within the metazoan tree: as a sister group to either the Acanthocephala (Carranza et al., 1997), the Gnathostomulida (Littlewood et al., 1998), the Nematomorpha (Carranza et al., 1997), or the Platyhelminthes (Winneppeninckx et al., 1995). Other studies place the Gastrotricha basal to most of the Bilateria (Carranza et al., 1997), or to the Lophotrochozoa (Ruiz-Trillo et al., 1999) or the Spiralia (Littlewood et al., 1998). We, however, contend that the position of the Gastrotricha remains questionable until more primitive gastrotrich species are used, namely Neodasys or species of the Dactylopodolidae. These additions to the molecular data set would be a good test of tree strength and confirmation of their branching point from the remaining phyla.

A comprehensive and congruent classification of the phylum Gastrotricha is essential if its origin and phylogenetic significance are to be fully appreciated. The currently accepted classification is in dire need of revision, especially concerning the order Chaetonotida and the family Lepidodasyidae. At the same time, though, previous work on the systematics of the phylum has successfully navigated the treacheries of convergence and parallelism and displayed good phylogenetic congruence (Remane, 1933; Hummon, 1982; Ruppert, 1988). Yet, despite the rather small size of the phylum, the species are highly variable in particular characters (e.g., adhesive tubes, ciliary patterns, cuticular sculpture, reproductive anatomy), and recognition of pleiomorphy is often difficult.

Our study presents an evolutionary hypothesis for all Gastrotricha. Within this morphological framework, we now can address specific questions of relationships within and among individual gastrotrich taxa. For example, characters of the nervous system (i.e., number of nerve cords, fusion, distribution of neurotransmitters) can now be evaluated in light of the presumed primitiveness of the Dactylopodolidae. This framework also allows for a more careful selection of gastrotrich species representing the phylum as a whole in future global molecular studies.
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