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Invited review

Phylogenetics of the Monogenea – evidence from a medley of molecules ☆

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Abstract

Nuclear ribosomal DNA sequences of Monogenea from both complete small and partial large (D1–D2) subunits were determined and added to previously published sequences in order to best estimate the molecular phylogeny of the group. A total of 35 ssrDNA, 100 D1 lsrDNA and 51 D2 lsrDNA monogenean sequences were used, representing a total of 27 families. From these sequences different data sets were assembled and analysed to make the best use of all available molecular phylogenetic information from the taxa. Maximum parsimony and minimum evolution trees for each data partition were rooted against published sequences from the Cestoda, forcing the Monogenea to appear monophyletic. There was broad agreement between tree topologies estimated by both methods and between genes. Well-supported nodes were restricted to deeply diverging major groupings and more derived taxa with the lsrDNA data but were at most nodes with ssrDNA. The Polyonchoinea showed the greatest resolution with a general pattern of ((Monocotylidae(Capsalidae(Udonellidae + Gyrodactylidea)))((Anoplodiscidae + Sundanonchidae)(Pseudomurraytrematidae + Dactylogyridae))). The Heteronchoinea readily split into the Polystomatoinea + Oligonchoinea, and Chimaericolidae and Hexabothriidae were successively the most basal of oligonchoinean taxa. Relationships within the Mazocraeidea, comprising 27 families of which 15 were sampled here, were largely unresolved and appear to reflect a rapid radiation of this group that is reflected in very short internal branches for ssrDNA and D1 lsrDNA, and highly divergent D2 lsrDNA. A reduced morphological matrix, employing only those families represented by molecules, contrasted sharply with respect to polyonchoinean interrelationships. Deep branches of the Heteronchoinea were similar for both classes of data but also showed that the interrelationships of the mazocraeidean families are labile and susceptible to sampling.

Keywords: Monogenea; Platyhelminthes; lsrDNA; ssrDNA

1. Introduction

Hypotheses of the interrelationships of the Monogenea based on molecular data include works by Mollaret et al. (1997, 2000a) for the class, Jovelin and Justine (2001) for the Heteronchoinea (= Polyopisthocotylea), Chisholm et al. (2001b) for the Monocotylidae, and a small number of papers addressing more specific systematic and evolutionary questions (e.g. Chisholm et al., 2001a; Cunningham et al., 1995; Littlewood et al., 1997, 1998; Mollaret et al., 2000b; Sinnappah et al., 2001; Bentz et al., 2001). A majority of these studies have been based on limited regions of the lsrDNA gene (variable domains D1 or D2, or both), whereas ssrDNA data have come primarily from studies addressing broader relationships within the phylum Platyhelminthes (e.g. Littlewood et al., 1999a; Littlewood and Olson, 2001), giving less emphasis to the interrelationships of the constituent groups themselves. Non-ribosomal data for the monogeneans are lacking, with the exception of a few cytochrome oxidase-1 sequences found to be too saturated to resolve higher-level relationships within the class (Jovelin and Justine, 2001). Taken together, these studies make available genetic data of taxa representing more than half of the 53 currently recognised families in the class (Boeger and Kritsky, 2001), but unfortunately, data from any single gene region are far more limited. This latter fact provides a less than ideal situation for researchers wishing to expand upon previous works through more comprehensive analyses and thus to some extent, isolates these works to the specific questions they address.

The present study brings together all published monogenean rDNA data, adds to this new sequences from additional taxa, and evaluates the state of affairs regarding the contribution of molecular data to the study of monogenean systematics in light of published morphologically based hypotheses (i.e. Boeger and Kritsky, 1993, 1997, 2001). In

☆ Nucleotide sequence data reported in this paper are available in the GenBank™, EMBL and DDBJ databases under the accession numbers AF382035-63 and AF382069-70.

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doing so, we have been forced to generate a number of independent estimates of phylogeny based on subsets of taxa for which either lsrDNA or ssrDNA data were available, whilst making inferences based on the overall picture created by the combination of these results. This approach makes use of the greatest amount of available data possible, but lacks the rigour of having fully complementary data sets with respect to the exemplar taxa upon which the phylogenetic estimates are based.

Evaluating the extent to which results stemming from molecular analyses support or reject traditional classifications of the Monogenea is made difficult by the fact that the few proposed classifications of the Monogenea above the familial level (e.g. Bychowsky, 1961; Yamaguti, 1963; Lebedev, 1988; Boeger and Kritsky, 1993) have been controversial, as has the usage of various names for the constituent groups (e.g. Monogenea vs. Monogenoidea, Monopisthocotylea vs. Polyonchoinea, etc.; see Wheeler and Chisholm (1995) and Boeger and Kritsky (2001) for two sides of this debate and Table 1 in Mollaret et al. (2000a) for a listing of equivalent taxon names). Much of this controversy is purely nomenclatural and as such has no bearing on hypotheses of phylogeny. In our evaluation of the molecular data, we have chosen to compare our results to the phylogenetically based classification of Boeger and Kritsky (1993, 2001) (see Appendix A herein) as their system is explicit and based on testable hypotheses stemming from character analyses. Other than our use of the name Monogenea, we also follow their naming system (Boeger and Kritsky, 1993, 2001) in order to be consistent in our discussion; we do not, however, wish to endorse nor take a position on the widespread adoption of their nomenclature.

1.1. A note on the monophyly of the Monogenea

Recent molecular analyses of the Platyhelminthes and Neodermata have failed to support monophyly of the class, being found to be either paraphyletic (Mollaret et al., 1997; Littlewood et al., 1999a,b) or unresolved (Littlewood and Olson, 2001); see also Justine (1998) and Littlewood et al. (2001). Our goal here was to examine the interrelationships of the Monogenea, and although our results strongly support monophyly of the class, the inclusion of only gyrocotylidean and eucestode outgroup taxa hardly makes for a rigorous test in comparison to the studies listed above. Whether the class is indeed paraphyletic, or if previous studies relying on rDNA have produced erroneous results due to the disparity in the divergence rates of the two major divisions (see Section 4.4), is difficult to determine. Studies are now underway to examine this question using sequence data from a number of non-ribosomal genes characterised from a wide diversity of platyhelminths in the hope that a more consistent pattern may emerge.

2. Materials and methods

2.1. Sources of data

Previously published lsr- and ssrDNA sequences provided the foundation of the data analysed herein, including lsrDNA D1 region (Mollaret et al., 2000a,b), D2 region (Jovelin and Justine, 2001), D1–D2 regions (Chisholm et al., 2001a,b; Littlewood et al., 1997; Mollaret et al., 1997), and complete sequences of the ssrDNA gene (Littlewood et al., 1998; Littlewood and Olson, 2001; Cunningham et al., 1995). To these, 29 new lsrDNA (D1–D2 or D1–D3 regions) sequences representing 17 monogenean families, and two new complete ssrDNA sequences representing the Diclidophoridae have been added. In total, 27 monogenean families were represented by at least one gene or gene region. A complete taxonomic listing of the species analysed, including sequence accession numbers and host and localities for taxa from which new sequences are derived is shown in Appendix A. All new sequences were generated from freshly collected, ethanol preserved specimens according to methods described in Olson et al. (2001).

2.2. Sequence alignment and data partitions

Separate data partitions were constructed to best utilise the different gene regions available for different subsets of taxa. The largest comprised lsrDNA D1 sequences (390 aligned positions) of 100 monogenean, two gyrocotylidean and

Table 1
Data partitions and tree statistics

Data partition	No. taxa	Number of characters (%)				Tree statistics				
		Aligned	Included	Constant	Parsimony informative	No. equally parsimonious trees	Length (steps)	Consistency index	Retention index	Rescaled consistency index
lsrDNA (D1)	100	390	282 (72)	100 (26)	155 (40)	> 37,500	1524	0.26	0.75	0.19
lsrDNA (D1–D2)	44	1061	595 (56)	242 (23)	288 (27)	3	1313	0.46	0.71	0.33
lsrDNA (D1)	34	390	282 (72)	123 (32)	144 (37)	35	878	0.36	0.65	0.24
ssrDNA	35	2468	1550 (63)	980 (40)	440 (18)	14	1600	0.51	0.8	0.41
Morphology[a]	27	N/A	90	6	70	4	156	0.66	0.82	0.54

[a] Data modified from Boeger and Kritsky (2001) (see text).

seven basal (Olson et al., 2001) eucestode taxa (see Appendix A). Because the lsrDNA D2 region was largely unalignable among the polyonchoinean taxa (see Section 4), a separate lsrDNA D1–D2 data set (1061 aligned positions) of 51 taxa was constructed only for the heteronchoinean taxa, using members of the Polystomatoinea (seven taxa) as a functional outgroup. Unlike the D1 alignment, this alignment included representatives of the Chimaericolidae and Pyragraphoridae for which only D2 sequences were available. Similarly, 19 taxa in this alignment were represented only by either D1 or D2 lsrDNA sequences. An alignment of all available monogenean ssrDNA sequences (35 taxa), as well as those of the gyrocotylidean and eucestode taxa was constructed, and an alignment of lsrDNA D1 sequences of the complementary subset of monogenean (except for *Gyrodactylus salaris* for which no lsrDNA data were available) and cestode taxa was constructed for more direct comparison with the results from ssrDNA. See Appendix A for the genes and gene regions available for each taxon (including citations of the original publications in which they appear) and Table 1 for a summary of the data partitions. All alignments were done by eye using MacClade (Maddison, D.R., Maddison, W.P., 2000. MacClade 4: Analysis of Phylogeny and Character Evolution. Version 4.0. Sinauer Associates, Sunderland, MA, USA) and saved as NEXUS-formatted files. Regions that could not be aligned unambiguously were excluded from the analyses (see Table 1). Separate alignment files, that may be adapted as NEXUS files are available by anonymous FTP from ftp.ebi.ac.uk in directory/pub/databases/embl/align or via the EMBLALIGN database via SRS at http://www.srs.ebi.ac.uk, under the following accessions: ssrDNA (all taxa): ALIGN_000146; lsrDNA (D1, all taxa): ALIGN_000149; lsrDNA (D1–D2, Heteronchoinea): ALIGN_000150. Exclusion sets are added as notes.

2.3. Phylogenetic analyses

All phylogenetic analyses were conducted using PAUP* (Swofford, D.L., 2001. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, MA, USA). Each molecular data partition was analysed by both maximum parsimony and minimum evolution. Maximum parsimony analyses were run using a heuristic search strategy (100 search replicates, except for the full lsrDNA D1 partition; see below) with random-addition taxon sampling, tree bisection–reconnection branch swapping, equally weighted characters and gaps treated as missing data. Bootstrap analysis was performed using a fast heuristic search strategy (100,000 replicates) for the full lsrDNA D1 data set, and using full heuristic searches for the other molecular data partitions (1000 replicates). Minimum evolution analyses were based on genetic distances estimated by maximum likelihood using a general time-reversible model of nucleotide evolution incorporating estimates of invariant sites and among-site rate variation as this model was found to provide the best fit to the data (using methods outlined in Olson and Caira, 1999).

Due to the effects of the large number of taxa and high level of homoplasy of the full lsrDNA D1 data partition, the maximum parsimony analysis was stopped prior to completion of the initial heuristic search and a consensus was based on the equally parsimonious trees found after 24 h of branch swapping on a dual processor 450 MHz G4 Macintosh™ computer. Another heuristic method termed the Parsimony Ratchet (Nixon, 1999; as implemented in PAUP* by Sikes and Lewis, 2001) was employed to examine the possibility that the previously terminated search may have been 'stuck' on a local optima (tree island) and that shorter or equal length trees on different island(s) could be recovered.

2.4. Morphological analysis

In order to avoid confounding the comparisons of familial interrelationships inferred from molecules vs. morphology by differences in taxon representation, the familial-level character matrix of Boeger and Kritsky (2001) was reduced to include only the 27 families listed in Appendix A for which molecular data were available for at least one or more exemplar taxa. Also differing from their analysis (Boeger and Kritsky, 2001), only the Gyrocotylidea was used as an outgroup. No characters or character states were altered from their original definitions (Boeger and Kritsky, 2001). The reduced matrix was then subjected to maximum parsimony and bootstrap analysis as described above.

3. Results

3.1. lsrDNA D1 region (Monogenea)

Fig. 1 shows the results of maximum parsimony and minimum evolution analyses of the lsrDNA D1 data partition including 100 monogenean taxa, two gyrocotylidean and seven eucestode outgroup taxa. Analyses using the heuristic search algorithm implemented in PAUP* (Swofford, 2001) as well as the Parsimony Ratchet (Nixon, 1999) produced trees of equal length (1524 steps; Table 1) and those trees found by the Ratchet were a subset of the >37,500 trees found by PAUP*. Because the PAUP* heuristic search was stopped after 24 h of branch swapping, additional equally parsimonious trees may still be found, although topological differences are likely to be restricted to minor rearrangements within terminal clades and would be expected to have little impact on familial interrelationships in so far as they were resolved herein.

Monophyly was supported by strict consensus of the Monogenea and its major subdivisions (Table 2; Fig. 1a), the Heteronchoinea and its constituent groups Oligonchoinea and Polystomatoinea, and the Polyonchoinea. Bootstrap support for the interrelationships within basal families of the Heteronchoinea and Polyonchoinea, the Polystomatidae and Capsalidea, respectively, was high, whereas the interrela-

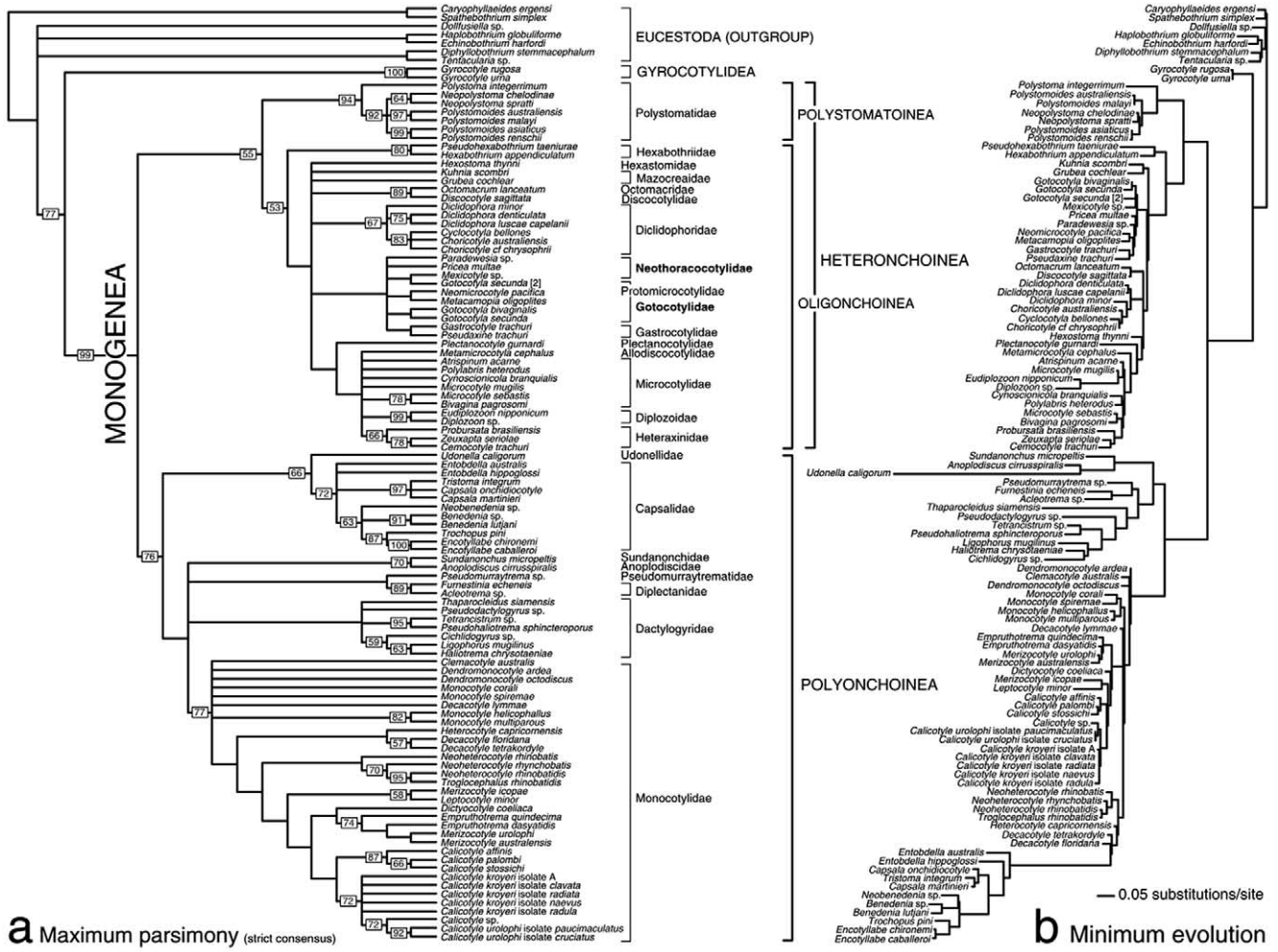

Fig. 1. Phylogenetic analyses of the Monogenea taxa based on the D1 region of lsrDNA. (a) Strict consensus of 37,500 equally parsimonious trees showing bootstrap support where ≥50%. (b) Phenogram based on minimum evolution analysis. Families found to be paraphyletic are shown in bold.

tionships of most other families and especially deeper nodes generally showed little or no support above 50% (Fig. 1a). Tree statistics were similarly poor (Table 1), indicating high levels of homoplasy. The oligonchoinean families Gotocotylidae and Neothoracocotylidae were found to be paraphyletic and the interrelationships of many families were left unresolved. The Hexabothriidae was found to be the sister to the remaining oligonchoinean families represented, whereas little resolution was obtained among basal members of the Polyonchoinea. Notably, the positions of the Chimaericolidae and Gyrodactylidae were not tested due to the lack of lsrDNA D1 data (see Appendix A). The minimum evolution based topology (Fig. 1b) was largely consistent with that of maximum parsimony except in the placement of the Capsalidae, which appeared highly derived, rather than basal, within the Polyonchoinea and was far removed from the long-branching Udonellidae. Branch lengths of most internal nodes were extremely small except within the Capsalidae, Dactylogyridae and a few other polyonchoinean families.

3.2. lsrDNA D1–D2 region (Heteronchoinea)

Fig. 2 shows the results of maximum parsimony and minimum evolution analyses of the lsrDNA D1–D2 data partition including 44 heteronchoinean and seven polystomatoinean taxa. Of these, 11 taxa were represented only by D1 sequences and eight only by D2 sequences (see Appendix A). This resulted in a significant number of unknown character states (i.e. gaps) in the alignment for these taxa. The effect of this was examined by comparing analyses based on the subset of taxa for which complete D1–D2 sequences were available (results not shown) with those obtained through the analysis of all taxa, including the gapped positions (treated as missing data). With regard to the estimation of familial interrelationships, the results were identical and we therefore present those including the larger number of exemplar taxa (Fig. 2). Monophyly of most families was supported by both minimum evolution and maximum parsimony analyses, although whereas maximum parsimony analysis supported monophyly of the Hexabo-

Table 2
Support for monophyly of higher monogenean taxa[a]

Taxon	Data partition[b]		
	lsrDNA D1	lsrDNA D1–D2	ssrDNA
Monogenea	MP (99)/ME	N/A	MP (100)/ME
Polyonchoinea	MP (76)/ME	N/A	MP (100)/ME
Capsalidea	MP (66)/ME	N/A	MP (100)/ME
Dactylogyridea	~ /ME	N/A	MP (91)/ME
Gyrodactylidea	–/–	N/A	MP (93)/ME
Monocotylidea	MP (77)/–	N/A	MP (100)/ME
Heteronchoinea	MP (55)/ME	N/A	MP (100)/ME
Oligonchoinea	MP (53)/ME	~ /ME	MP (96)/ME
Mazocraeidea	MP/ME	MP (55)/ME	MP (100)/ME
Discocotylinea	–/–	MP/ME	–/–
Gastrocotylinea	–/–	MP/ME	–/–
Mazocraeinea	–/–	–/–	~ /–
Microcotylinea	–/–	–/–	–/–

[a] As defined by Boeger and Kritsky (2001).
[b] Monophyly supported by maximum parsimony (MP) analysis (with bootstrap support where ≥50%), minimum evolution (ME), not supported (–), or equivocal (~).

thriidae (Fig. 2a), minimum evolution analysis did not (Fig. 2b), and the reverse was true for the Microcotylidae. Monophyly of mazocraeidean suborders Gastrocotylinea and Discotylinea was supported, whereas the Mazocraeinea and Microcotylinea were both polyphyletic. Minimum evolution analysis supported the Chimaericolidae as the sister group to the remaining oligonchoinean families, followed by the 'Hexabothriidae', whereas maximum parsimony left the position of the Chimaericolidae unresolved in a dichotomy together with the Polystomatidae whilst supporting the basal position of the Hexabothriidae. Bootstrap support was high for terminal clades but generally <50% for nodes separating families, and this lack of character support probably resulted in the discrepancies between the minimum evolution and maximum parsimony analyses. Although better resolved, results were similar to those of the lsrDNA D1 analysis above.

3.3. ssrDNA and lsrDNA (Monogenea-reduced taxon set)

Fig. 3 shows the results of maximum parsimony and minimum evolution analyses of the ssrDNA data partition (Fig. 3a), as well as those of the lsrDNA D1 data partition (Fig. 3b)

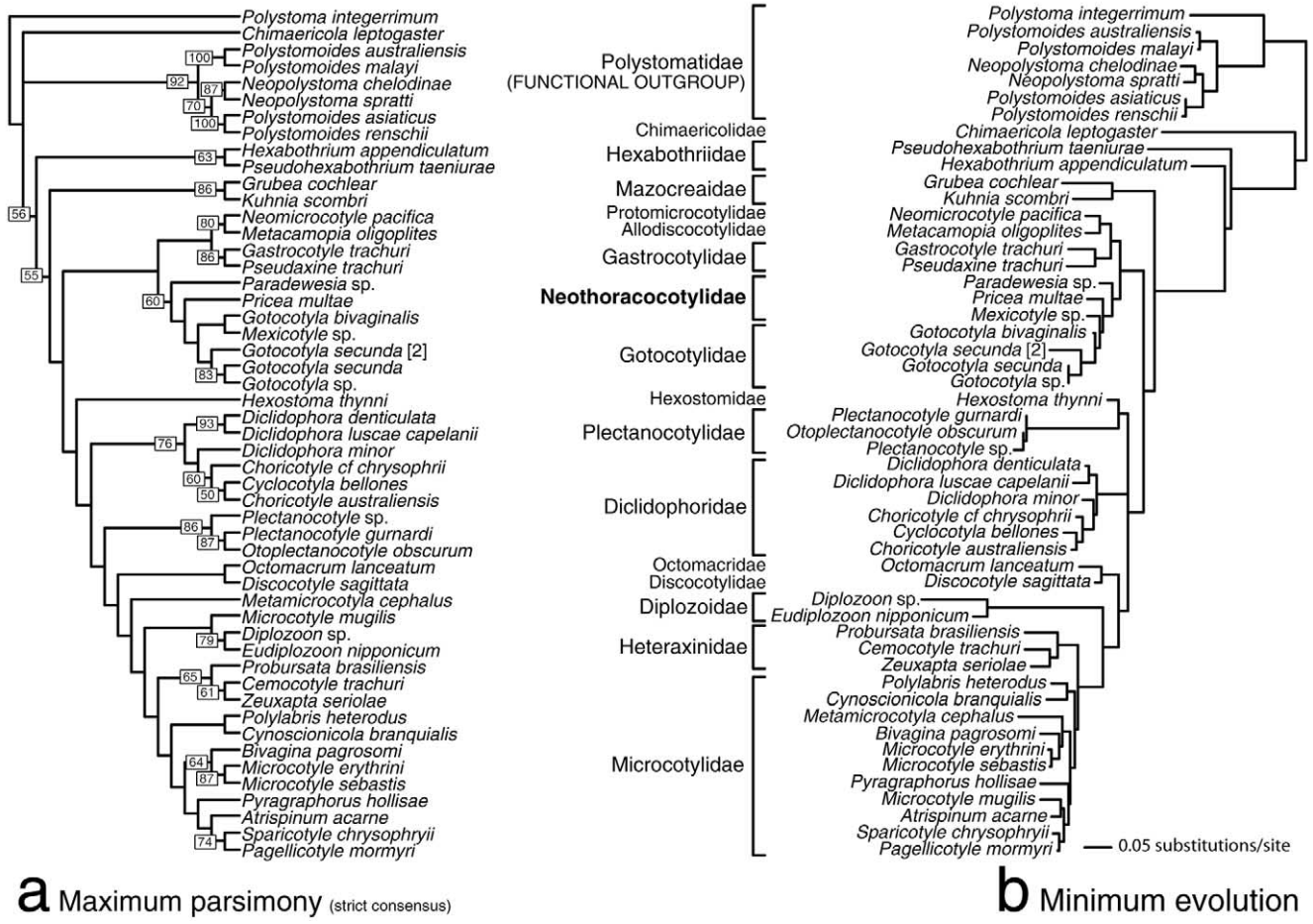

Fig. 2. Phylogenetic analyses of the Heteronchoinea based on D1–D2 regions of lsrDNA. (a) Strict consensus of three equally parsimonious trees showing bootstrap support where ≥ 50%. (b) Phenogram based on minimum evolution analysis. Families found to be paraphyletic are shown in bold.

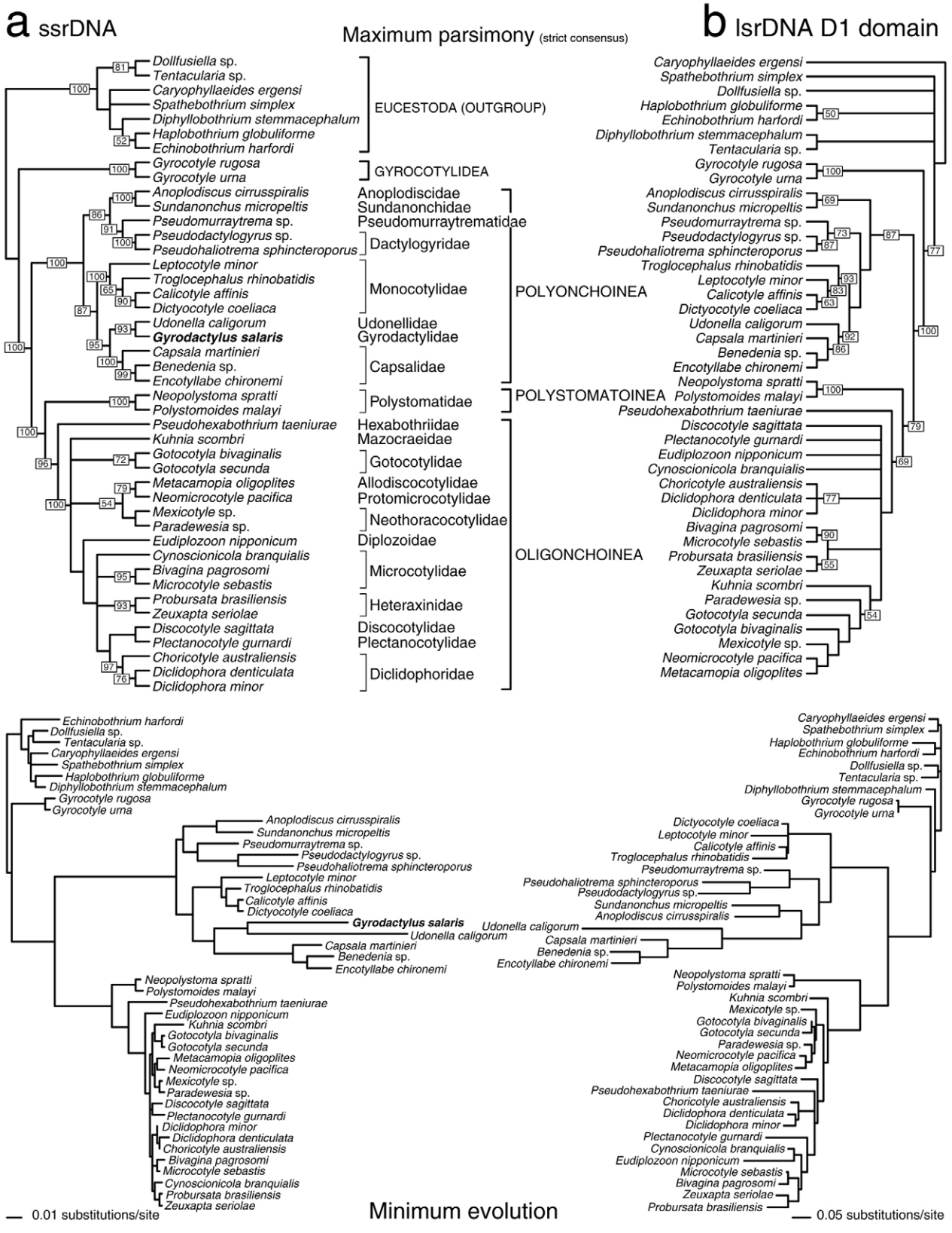

Fig. 3. Comparison of phylogenetic analyses of the Monogenea based on a subset of taxa for which ssrDNA data were available. (a) Strict consensus of 14 equally parsimonious trees showing bootstrap support where ≥ 50% based on complete sequences of ssrDNA. (b) Strict consensus of 35 equally parsimonious trees showing bootstrap support where ≥ 50% based on the D1 region of lsrDNA. Phenograms resulting from analyses by minimum evolution shown below. Sequence data for *Gyrodactylus salaris* (shown in bold) were available only for ssrDNA.

including only those taxa for which ssrDNA sequences were available (save *Gyrodactylus salaris*). All analyses supported the monophyly of the Monogenea, Polyonchoinea, Heteronchoinea, Polystomatoinea and Oligonchoinea. Familial interrelationships within the Heteronchoinea and Polyonchoinea were similar between the ssrDNA and lsrDNA partitions, although the ssrDNA provided greater resolution and showed stronger bootstrap support across all nodes. Pronounced differences in the rates of evolution between the Polyonchoinea and Heteronchoinea were apparent in the analyses by minimum evolution, with the Polyonchoinea exhibiting a much higher degree of divergence both within and among families, corresponding, in this case, to higher nodal support. The Gyrodactylidae, not represented in any of the lsrDNA analyses, grouped strongly with the Udonellidae, which together formed the sister group of the Capsalidae. Although the ssrDNA and reduced lsrDNA (D1) data sets are combinable under the criterion of conditional combination (incongruence length difference test passed using phylogenetically informative sites only; $P = 1.00$), we do not provide a combined molecular evidence solution here on the grounds that such an estimate would be premature given the few taxa sampled for both gene fragments.

3.4. Morphology

Maximum parsimony analysis of the morphological data matrix of Boeger and Kritsky (2001) reduced to the 27 monogenean families represented herein by molecular data resulted in four equally parsimonious trees, compared with the 2899 equally parsimonious trees reported by Boeger and Kritsky (2001) in their analysis including all 53 monogenean families. A strict consensus of the four equally parsimonious trees is shown in Fig. 4. Monophyly of all of the higher taxa defined by Boeger and Kritsky (2001), was supported with the exception of the Microcotylinea due to the placement of the Diclidophoridae outside of the clade including the Heteraxinidae, Microcotylidae and Pyragraphoridae. Interfamilial relationships within the Polyonchoinea were nearly identical to those in Boeger and Kritsky (2001; their figure 10.2) whilst there were a number of discrepancies within the large heteronchoinean order Mazocraeidea.

4. Discussion

4.1. Overview

Our study consolidates published and newly available nuclear ribosomal sequence data in order to present the most comprehensive molecular based estimates of monogenean interrelationships. Whilst the fragmentary nature of the molecular data sets and uneven sampling do not allow strict comparability or combinability with morphological assessments (e.g. Boeger and Kritsky, 2001), or indeed a single estimate of adequate taxonomic breadth, a number of important conclusions can be drawn. Here we restrict our comments to topologies and nodes that are well supported by high bootstrap values or through congruence between independent estimates. The over-interpretation or uncritical acceptance of tree topologies can undermine the value of key nodes and taxonomic affiliations. By pooling all available data we believe we now understand how an optimal molecular data set that is fully complementary to morphological data, might best be achieved.

4.2. The limits of resolution

A review of nodal (bootstrap) support across our molecular solutions indicates the present and future utility of the genes and gene fragments utilised. Few deep branches are well supported for the D1 lsrDNA data set alone and for such a relatively small fragment, whilst it has provided a good proportion of phylogenetically informative sites, its future use will likely be restricted to higher-level problems, e.g. for the resolution of species within families. Prime candidate families for study with this gene fragment include the Polystomatidae, Capsalidae and perhaps the Dactylogyridae.

The addition of D2 lsrDNA provided little extra resolution to interpreting the phylogeny of the Heteronchoinea. Again, only the more distal nodes were better supported suggesting that both D1 and D2 might best be used for resolving relationships within families. The D2 domain was unalignable across the Polyonchoinea but positional homology is readily detectable within families suggesting that at these higher taxonomic levels, the benefit of sequencing D2 in addition to D1 easily matches the relatively low cost. As the lsrDNA is a large molecule with a total of 12 variable domains (Hassouna et al., 1984) it is worth pursuing the resolving powers of the remainder of the molecule in determining wider monogenean interrelationships.

Complete ssrDNA provided the most robust estimates of phylogeny, as established by bootstrap resampling, of all gene partitions. Notwithstanding the fact that many fewer taxa were sampled for this gene, all deeper level nodes were well supported. The performance of ssrDNA at higher levels cannot be judged satisfactorily due to relatively poor sampling within families. Denser sampling of ssrDNA both within and between families appears to be the most profitable way forward towards estimating a full phylogeny of the Monogenea.

Whilst taxonomic sampling clearly affects the molecular data, it is worth noting that our reduced morphological dataset, gleaned from Boeger and Kritsky (2001) and illustrated in Fig. 4 also shows that current morphological solutions appear relatively labile. Interrelationships of the more derived heteronchoineans, the Mazocraeidea, are as poorly supported by morphology as they are by any of the molecular partitions. Indeed, among all the data partitions it is the Mazocraeidea that are the least well resolved. In our

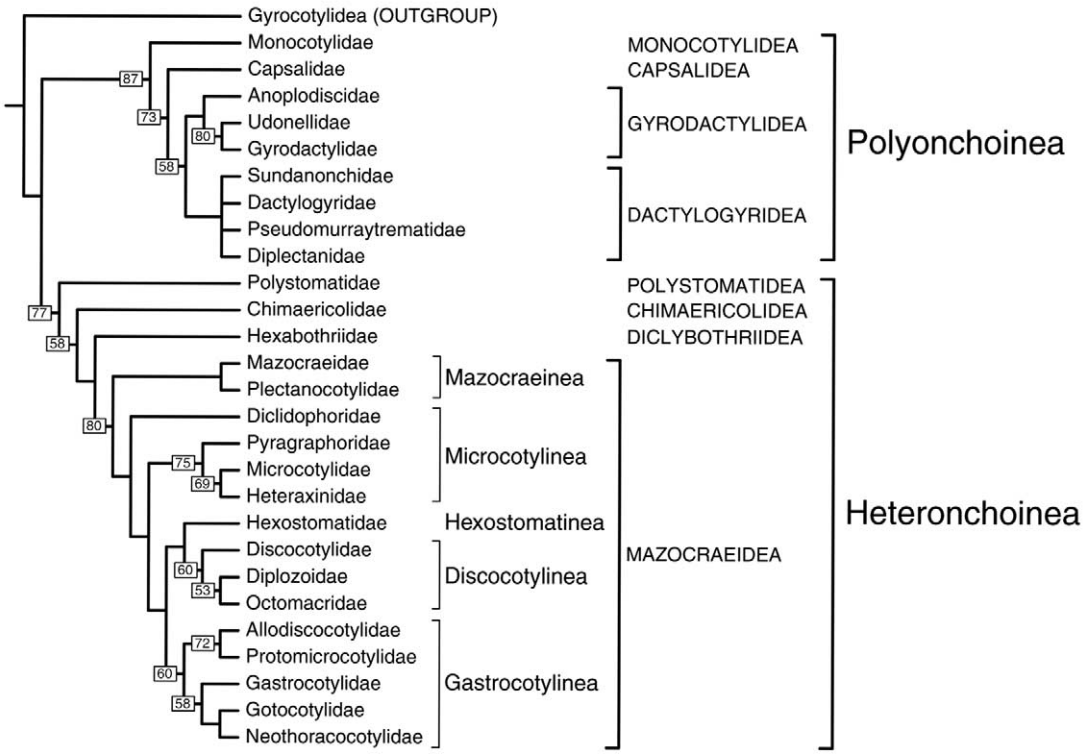

Fig. 4. Strict consensus of four equally parsimonious trees resulting from maximum parsimony analysis of morphological characters. Note: The Microcotylinea are paraphyletic.

analyses only 15 of the 27 mazocraeidean families were sampled.

Whilst we do not wish to disparage the systematic suggestions of previous molecular studies concerning broader monogenean groups based on D1 and/or D2 lsrDNA (e.g. Mollaret et al., 1997, 2000a; Jovelin and Justine, 2001), our study shows that these gene fragments have lost a great deal of resolving power as more taxa are included. Bearing in mind that many monogenean families remain unsampled for these loci, it may be premature in judging their utility or adjusting the systematics of the group to conform to what appear to be largely labile phylogenetic estimates.

4.3. Total evidence

We have chosen not to estimate a total evidence solution incorporating all available molecular and morphological data as the morphological and molecular data sets are not readily combinable. The matrix of Boeger and Kritsky (2001) is coded at the family level and not at the species level, such that combination would unduly weight multiple representatives of families. Additionally, the non-monophyly suggested by some molecular solutions would be swamped by this method of coding. Incongruence between morphological and molecular solutions would best be explored by a fully complementary approach and molecular sampling of representatives of missing taxa.

4.4. Phylogenetic inferences and evolutionary implications

From our disjunct molecular data sets we feel it is premature to fully infer monogenean phylogeny. However, based on nodes congruent between our independent estimates, it is clear that the major divisions espoused by Boeger and Kritsky (2001) are supported. Namely, Polystomatoinea and Oligonchoinea are indeed sister taxa comprising the Heteronchoinea and among the Oligonchoinea chimaericolids are the most basal followed by hexabothriids and a monophyletic but poorly resolved Mazocraeidea. However, among the Polyonchoinea the relative placement of families sampled by us show marked differences from previously published morphological assessments. Monocotylids and capsalids were not the most basal taxa. Instead, there was a well-supported split between (Monocotylidae(Capsalidae(Udonellidae+Gyrodactylidea))) and (Anoplodiscidae+‴ Sundanonchidae)(Pseudomurraytrematidae+Dactylogyridae) (Fig. 3a). The topology from the reduced lsrDNA data set (Fig. 3b) was more in agreement with ssrDNA and from our analysis ssrDNA may provide a very different, yet robust phylogenetic solution compared with either lsrDNA or morphology when more broadly sampled.

Despite these important differences between molecular and morphological estimates of polyonchoinean relationships, few if any evolutionary implications so far derived from monogenean phylogenies are affected. Host-specificity among Monogenea is well recognised (e.g. Whittington et

al., 2000). In Boeger and Kritsky's (1997) analysis of coevolution of Monogenea with fish hosts, none of their conclusions regarding early speciation or dispersal events with Gnathostomata, Chondrichthyes and Osteichthyes are altered by our molecular topologies. This seems to be partly because our sampling prevents a full assessment and because of the taxa sampled in this study we can infer only deep level associations with some confidence. Nevertheless higher-level resolution of monogenean families is not necessary to test Boeger and Kritsky's hypotheses as they too restricted themselves to deeper divergence events. Additionally, the hypotheses of host–parasite association and evolution inferred by Jovelin and Justine (2001) based on D2 lsrDNA also remain intact. However, as both host (and in particular teleost) and monogenean phylogeny is refined it will be worth revisiting these issues.

4.5. Rates of molecular divergence

One of the most striking features of the molecular data is the disparity in the relative rates of divergence between the oligonchoinean and polyonchoinean lineages, particularly evident in analyses of the complete ssrDNA sequences (see minimum evolution analyses in Fig. 3). For example, the average uncorrected genetic distance for all pairwise comparisons of the polyonchoinean taxa was more than four times greater than among the oligonchoinean taxa (0.094 ± 0.029 vs. 0.021 ± 0.01). This is reflected in the lsrDNA data in that the more variable D2 region was found to be unalignable among all but the most closely related polyonchoineans, whereas the same region was readily alignable (with exceptions) among the broad sampling of heteronchoinean taxa. These two major groups should be therefore treated independently when examined using molecular data as different genes or gene regions will be needed to accommodate their different rates of divergence. The ssrDNA gene (Fig. 3a), which has been largely ignored by monogenean systematists, appears extremely well suited to the study of polyonchoinean interrelationships, exhibiting relatively long, highly supported internodes subtending terminal clades. In the Oligonchoinea, however, the internodes as well as terminal branches were exceedingly short, poorly supported and many relationships were left unresolved. This situation is found with the lsrDNA gene as well, but the higher level of homoplasy in the lsrDNA (Table 1) makes it less desirable even when considering only the polyonchoinean taxa (at least with respect to the D1 region of the gene). Greater resolution of oligonchoinean interrelationships will probably require data from non-ribosomal genes, or perhaps a combination of sources.

4.6. The way forward

Our study demonstrates the problems associated with fragmentary data sets and the need for full complementarity between independent data sets. For the most part, complementarity should now be achievable whereby genes are sequenced for both ssrDNA and lsrDNA. However, there would appear to be severe limitations in the value of sequencing D2 lsrDNA for the Polyonchoinea as a whole and an additional, more slowly evolving gene is required to resolve the interrelationships of the group. Continued sequencing of ssrDNA would appear to be most profitable as longer internal branches and higher nodal support suggest the gene can accommodate additional sampling without the loss of too much resolution. Missing families need to be sampled for both ribosomal and additional genes. To date, morphologists dealing with the wider phylogenetics of the Monogenea have focused on coding at the familial level. For full complementarity and combinability future total evidence studies will require that characters are coded for the species being sampled for molecular work. Through a combined evidence solution this will more easily allow non-monophyletic families to reveal themselves, will assist in any taxonomic revisions deemed necessary, and through reciprocal illumination will allow accurate inference of character change and assessment of character homology.

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Appendix A. Taxonomic listing of species analysed

Classification follows Boeger and Kritsky, 1993, to the level of suborder, with updates from Boeger and Kritsky, 2001. Sequence accession numbers are shown parenthetically with those of lsrDNA first followed by ssrDNA in bold). The lsrDNA domain(s) represented by the sequence accessions are indicated, whereas all ssrDNA accessions represent complete sequences, or are nearly so. Superscripts indicate the original publications of the sequences ([1]Littlewood and Olson, 2001; [2]Mollaret et al., 1997; [3]Littlewood et al., 1998; [4]Mollaret et al., 2000a; [5]Mollaret et al., 2000b; [6]Cunningham et al., 1995; [7]Chisholm et al., 2001a; [8]Chisholm et al., 2001b; [9]Jovelin and Justine, 2001; [10]Little-

wood et al., 1997). Sequences new to the present study are indicated by '§' and are followed by the hosts and collection localities of the taxa. Cestode classification based on Khalil et al., 1994, with the original publication of the sequences appearing in [11]Olson et al., 2001, [12]Olson and Caira, 1999 and [13]Littlewood et al., 1999a,b.

A.1. HETERONCHOINEA

A.1.1. OLIGONCHOINEA

CHIMAERICOLIDEA
Chimaericolidae
Chimaericola leptogaster (AF311706, D2)[9]
DICLYBOTHRIIDEA
Hexabothriidae
Hexabothrium appendiculatum (AF131724, D1)[4]
Pseudohexabothrium taeniurae (AF382035§, D1–D3; **AJ228791**[3]) Ex. *Taeniura lymma* (Ribbontail stingray), Shark Bay, Heron Island, Australia
MAZOCRAEIDEA
DISCOCOTYLINEA
Discocotylidae
Discocotyle sagittata (AF382036§, D1–D3; **AJ287504**[1]) Ex. *Salmo trutta* (Sea trout), Isle of Man, UK
Diplozoidae
Diplozoon sp. (AF131717, D1)[4]
Eudiplozoon nipponicum[a] (AF382037§, D1–D3; **AJ287510**[1]) Ex. *Cyprinus carpio* (Common carp), Czech Republic
Octomacridae
Octomacrum lanceatum (AF131723, D1)[4]
GASTROCOTYLINEA
Allodiscocotylidae
Metacamopia oligoplites (AF382038§, D1–D6; **AJ287538**[1]) Ex. *Oligoplites* sp. (leatherjack), Paraná, Brazil
Gastrocotylidae
Gastrocotyle trachuri (AF131727, D1[4]; AF311708, D2[9])
Pseudaxine trachuri (AF131728, D1[4]; AF311715, D2[9])
Gotocotylidae
Gotocotyla bivaginalis (AF382039§, D1–D3; **AJ276424**[1]) Ex. *Scomberomorus commerson* (Spanish mackerel), Heron Island, Australia
Gotocotyla secunda (AF382040§, D1–D3; **AJ276425**[1]) Ex. *Scomberomorus commerson* (Spanish mackerel), Heron Island, Australia
Gotocotyla secunda [2] (AF026109, D1)[5]
Gotocotyla sp. (AF311711, D2)[9]
Neothoracocotylidae
Mexicotyle sp. (AF382041§, D1–D6; **AJ287539**[1]) Ex. *Scomberomorus* sp. (mackerel), Paraná, Brazil
Paradawesia sp. (AF382042§, D1–D6; **AJ287555**[1]) Ex. *Scomberomorus* sp. (mackerel), Paraná, Brazil
Pricea multae (AF026111, D1–D2)[2]
Protomicrocotylidae
Neomicrocotyle pacifica (AF382043§, D1–D3; **AJ228787**[3]) Ex. *Caranx hippos* (Black jack), Chamela Bay, Mexico
HEXOSTOMATINEA
Hexostomatidae
Hexostoma thynni (AF131721, D1)[4]
MAZOCRAEINEA
Mazocraeidae
Kuhnia scombri[b] (AF382044§, D1–D3; **AJ228783**[3]) Ex. *Scomber scombrus* (Atlantic mackerel), North Sea, UK
Grubea cochlear (AF131730, D1[4]; AF311710, D2[9])
Plectanocotylidae
Plectanocotyle gurnardi (AF382045§, D1–D3; **AJ228790**[3]) Ex. *Eutrigla gurnardus* (Grey gurnard), North Sea, UK
Plectanocotyle sp. (AF311716, D2)[9]
Octoplectanoctyle obscurum (AF311718, D2)[9]
MICROCOTYLINEA
Diclidophoridae
Choricotyle australiensis (AF382046§, D1–D3; **AF382069**§) Ex. *Rhabdosargus sarba* (Goldlined seabream), Coffs Harbour, NSW, Australia
Choricotyle cf. *chrysophrii* (AF131729, D1[4]; AF311705, D2[9])
Cyclocotyla bellones (AF131731, D1)[4]
Diclidophora luscae capelanii (AF131732, D1[4]; AF311704, D2[9])
Diclidophora denticulata (AF382047§, D1–D3; **AJ228779**[3]) Ex. *Pollachius virens* (Saithe), North Sea, UK
Diclidophora minor (AF382048§, D1–D3; **AF382070**§) Ex. *Micromesistius poutassou* (Blue whiting) North Sea, UK
Heteraxinidae
Cemocotyle trachuri (AF131726, D1)[4]
Probursata brasiliensis (AF382049§, D1–D6; **AJ276426**[1]) Ex. *Oligoplites* sp. (leatherjack), Paraná, Brazil
Zeuxapta seriolae (AF026103, D1–D2[2]; **AJ228797**[3])
Microcotylidae
Atrispinum acarne (AF131713, D1[4]; AF311702, D2[9])
Bivagina pagrosomi (Z83002, D1–D2[10]; **AJ228775**[3])
Cynoscionicola branquialis (AF382050§, D1–D3; **AJ287495**[1]) Ex. *Umbrina xanti* (Polla drum) Chamela, Mexico
Metamicrocotyla cephalus (AF131720, D1)[4]
Microcotyle erythrini (AF311712, D2)[9]
Microcotyle mugilis (AF131722, D1)[4]
Microcotyle sebastis (AF382051§, D1–D3; **AJ287540**[1]) Ex. *Sebastes* sp., North Sea, UK
Pagellicotyle mormyri (AF311713; D2)[9]
Polylabris heterodus (AF131716, D1)[4]
Sparicotyle chrysophryii (AF311719, D2)[9]
Pyragraphoridae
Pyragrophorus hollisae (AF311714, D2)[9]

A.1.2. POLYSTOMATOINEA

POLYSTOMATIDEA
Polystomatidae
Polystoma integerrimum (AF131719, D1)[4]
Polystomoides asiaticus (Z83008, D1–D2)[10]
Polystomoides australiensis (Z83012, D1–D2)[10]
Polystomoides malayi (Z83010, D1–D2[10]; **AJ228792**[3])
Polystomoides renschii (Z83014; D1–D2)[10]
Neopolystoma chelodinae (Z83004; D1–D2)[10]
Neopolystoma spratti (Z83006; D1–D2[10]; **AJ228788**[3])

A.1.3. POLYONCHOINEA

CAPSALIDEA
Capsalidae
Benedenia lutjani (AF026106, D1–D2)[2]
Benedenia sp. (AF382052§, D1–D3; **AJ228774**[3]) host information

[a] The lsrDNA D2 sequence (AF311703) is listed as *Diplozoon nipponicum* in Jovelin and Justine (2001).

[b] The lsrDNA D2 sequence (AF311709) of *Kuhnia* sp. (Jovelin and Justine, 2001) was found to differ by only a single base from that of *Grubea cochlear*, whilst differing considerably from our sequence of *Kuhnia scombri*; we thus considered Jovelin and Justine's D2 sequence of *Kuhnia* sp. to be potentially erroneous and did not include it in the analyses.

unavailable
Capsala martinieri (AF382053§, D1–D3; **AJ276423**[1]) Ex. *Mola mola* (Ocean sunfish), Skegness, UK
Capsala onchidiocotyle (AF131712; D1)[4]
Encotyllabe caballeroi (AF026112; D1–D2)[2]
Encotyllabe chironemi (AF382054§, D1–D3; **AJ228780**[3]) Ex. *Chironemus marmoratus* (Kelpfish), Coffs Harbour, NSW, Australia
Entobdella australis (AF026108; D1–D2)[2]
Entobdella hippoglossi (AF382055§, D1–D3) Ex. *Hippoglossus hippoglossus* (Atlantic halibut), North Sea, UK
Neobenedenia sp. (AF382056§, D1–D3) host information unavailable
Tristoma integrum (AF131715; D1)[4]
Trochopus pini (AF131714, D1)[4]
DACTYLOGYRIDEA
DACTYLOGYRINEA
Dactylogyridae
Cichlidogyrus sp. (AF218124, D1)[5]
Haliotrema chrysotaeniae (AF026115, D1–D2)[2]
Ligophorus mugilinus (AF131710, D1)[4]
Pseudodactylogyrus sp. (AF382057§, D1–D3; **AJ287567**[1]) Ex. *Anguilla anguilla* (eel), Harlech, N. Wales, UK
Pseudohaliotrema sphincteroporus (AF382058§; D1–D3; **AJ287568**[1]) Ex. *Siganus doliatus* (Barred spinefoot), Green Island, Australia
Tetrancistrum sp. (AF026114, D1–D2)[2]
Thaparocleidus siamensis (AF218123, D1)[5]
Diplectanidae
Acleotrema sp. (AF026118, D1–D2)[2]
Furnestinia echenesis (AF131711, D1)[4]
Pseudomurraytrematidae
Pseudomurraytrema sp. (AF382059§, D1–D3; **AJ228793**[3]) Ex. *Catostomus ardens* (Utah sucker), Snake River, Idaho, USA
TETRAONCHINEA
Sundanonchidae
Sundanochus micropeltis (AF218122, D1[5]; **AJ287579**[1])
GYRODACTYLIDEA
Gyrodactylidae
Gyrodactylus salaris (**Z26942**)[6]
Anoplodiscidae
Anoplodiscus cirrusspiralis (AF382060§, D1–D3; **AJ287475**[1]) Ex. *Sparus auratus* (Gilthead seabream), Sydney, Australia
Udonellidae
Udonella caligorum (AJ228803, D1–D2[3]; **AJ228796**[3])
MONOCOTYLIDEA
Monocotylidae
Calicotyle affinis (AF382061§, D1–D3; **AJ228777**[3]) Ex. *Chimaera monstrosa* (Rabbit fish), unknown fjord, Norway
Calicotyle kroyeri isolate A [1] (AF279748, D1–D2)[7]
Calicotyle kroyeri isolate *radula* [2] (AF279747, D1–D2)[7]
Calicotyle kroyeri isolate *radiata* [3] (AF279746, D1–D2)[7]
Calicotyle kroyeri isolate *naevus* [4] (AF279745, D1–D2)[7]
Calicotyle kroyeri isolate *clavata* [5] (AF279744, D1–D2)[7]
Calicotyle palombi (AF279749, D1–D2)[7]
Calicotyle sp. (AF279750, D1–D2)[7]
Calicotyle stossichi (AF279751, D1–D2)[7]
Calicotyle urolophi isolate *paucimaculatus* [1] (AF279753, D1–D2)[7]
Calicotyle urolophi isolate *cruciatus* [2] (AF279752, D1–D2)[7]
Clemacotyle australis (AF348350, D1–D2)[8]
Decacotyle floridana (AF348357, D1–D2)[8]
Decacotyle lymmae (AF348359, D1–D2)[8]
Decacotyle tetrakordyle (AF348358, D1–D2)[8]
Dendromonocotyle ardea (AF348351, D1–D2)[8]
Dendromonocotyle octodiscus (AF348352 (D1–D2)[8]
Dictyocotyle coeliaca (AF382062§, D1–D3; **AJ228778**[3]) Ex. *Raja radiata* (Starry skate), North Sea, UK
Empruthotrema dasyatidis (AF348345, D1–D2)[8]
Empruthotrema quindecima (AF348346, D1–D2)[8]
Heterocotyle capricornensis (AF348360, D1–D2)[8]
Leptocotyle minor[c] (AF382063§, D1–D3; **AJ228784**[3]) Ex. *Scyliorhinus canicula* (Small-spotted catshark), North Sea, UK
Merizocotyle australensis (AF348348, D1–D2)[8]
Merizocotyle icopae (AF026113, D1–D2)[2]
Merizocotyle urolophi (AF348347, D1–D2)[8]
Monocotyle corali (AF348353, D1–D2)[8]
Monocotyle helicophallus (AF348355, D1–D2)[8]
Monocotyle multiparous (AF348356, D1–D2)[8]
Monocotyle spiremae (AF348354, D1–D2)[8]
Neoheterocotyle rhinobatidis (AF026107, D1–D2)[2]
Neoheterocotyle rhinobatis (AF348361, D1–D2)[8]
Neoheterocotyle rhynchobatis (AF348363, D1–D2)[8]
Troglocephalus rhinobatidis (AF026110, D1–D2[2]; **AJ228795**[3])

A.2. CESTODA

A.2.1. GYROCOTYLIDEA

Gyrocotylidae
Gyrocotyle rugosa (AF286925, D1–D3[11]; **AF124455**[12])
Gyrocotyle urna (AF286924, D1–D3[11]; **AF228782**[13])

A.2.2. EUCESTODA

CARYOPHYLLIDEA
Lytocestidae
Caryophyllaeides ergensi (AF286913, D1–D3; **AF286979**)[11]
DIPHYLLIDEA
Echinobothriidae
Echinobothrium harfordi (AF286921, D1–D3; **AF286985**)[11]
HAPLOBOTHRIIDEA
Haplobothriidae
Haplobothrium globuliforme (AF286926, D1–D3[11]; **AF124458**[12])
PSEUDOPHYLLIDEA
Diphyllobothriidae
Diphyllobothrium stemmacephalum (AF286943, D1–D3[11]; **AF124459**[12])
SPATHEBOTHRIIDEA
Spathebothriidae
Spathebothrium simplex (AF286949, D1–D3[11]; AF124456[12])
TRYPANORHYNCHA
Eutetrarhynchidae
Dollfusiella sp. (AF286965, D1–D3; **AF287002**)[11]
Tentacularidae
Tentacularia sp. (AF286976, D1–D3[11]; **AF124461**[12])

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