

Chapter 2

The Systematics of the Trematoda

Aneta Kostadinova and Ana Pérez-del-Olmo

2.1 Introduction

The Trematoda Rudolphi, 1808 are a class of the phylum Platyhelminthes that comprises two subclasses, the Aspidogastrea Faust & Tang, 1936 and the Digenea Carus, 1863. The subclass Aspidogastrea is a small group (4 families, 12 genera considered valid, *c.* 80 species) parasitic in molluscs, fishes and chelonians [1, 2]. Aspidogastreans like the digeneans use molluscs as first obligate hosts but are characterised by being external rather than internal parasites of these hosts, and by having a single-generation life-cycles lacking asexual reproduction and a stage comparable to the cercaria [2–4]. Key information on the aspects of morphology, life-cycles, taxonomy, systematics and phylogeny of the aspidogastreans can be found in Rohde [1, 2, 5, 6], Gibson [3], Gibson and Chinabut [7] and Zamparo and Brooks [8].

The subclass Digenea comprises a large and diverse group (*c.* 2,500 nominal genera, *c.* 18,000 nominal species; see [9]) of cosmopolitan platyhelminths that are obligatory parasitic in invertebrate intermediate and vertebrate definitive hosts. Digeneans are found in all vertebrate classes but are less diverse in agnathans and chondrichthyans [10, 11]. The subclass is characterised by a number of autapomorphies, associated with the unique complex digenean life-cycle: (i) acquisition of a

A. Kostadinova (✉)

Institute of Parasitology, Biology Centre of the Academy of Sciences of the Czech Republic,
Branišovská 31, 370 05 České Budějovice, Czech Republic
e-mail: aneta.kostadinova@uv.es; kostadinova@paru.cas.cz

A. Pérez-del-Olmo

Unitat de Zoologia Marina, Institut Cavanilles de Biodiversitat i Biologia Evolutiva,
Parc Científic, Universitat de València, Aptdo 22085, 46071 Valencia, Spain
e-mail: ana.perez-olmo@uv.es

vertebrate host as a terminal addition to the life history; (ii) alternation of sexual and asexual reproductive generations; (iii) a series of asexual generations within the first intermediate host (typically mollusc); (iv) free-swimming cercaria with a tail; (v) tiers of ectodermal cells on the miracidium; (vi) lack of digestive system in the miracidium and mother sporocyst [3, 4, 12]. For details and apomorphies at lower taxonomic levels see the review by Cribb et al. [4]. Although the complexity of digenean life-cycles may have influenced the expansion of the Digenea rendering it the most speciose group among Platyhelminthes [12], the mainstay of digenean systematics has been the information obtained from examination of the sexual generation, i.e. the adults from vertebrates [13].

The classification of the Digenea has long been a challenge especially because of the difficulties in establishing relationships and finding diagnostic characters for identification keys of the higher taxa [3, 14, 15]. Thus whereas most groupings established at lower taxonomic levels using adult morphology have been widely accepted, the search of apparent non-homoplasious morphological characters at the higher taxonomic levels has been the subject of debate and (sometimes heated) discussions (for details, see Gibson [3], Pearson [16], Gibson and Bray [14]).

The early attempts for classification of the digeneans relied upon sucker arrangements initially at the generic level, i.e. *Monostoma* Zeder, 1800, *Distoma* Retzius, 1786, *Amphistoma* Rudolphi, 1801 and *Gasterostomum* von Siebold, 1848, were unsatisfactory [15] whereas later treatments have incorporated more morphological characters including features of the daughter sporocyst/redia and/or cercaria, and life history patterns [17–23]; see Gibson [3] for a detailed discussion on the aspects of the evolution of the Trematoda.

2.2 Keys to the Trematoda

Perhaps one of the most important endeavours of this century in the field of digenean taxonomy is the publication of the *Keys to the Trematoda*, a series on the systematics and identification of the platyhelminth class Trematoda [24–26]. The three volumes provide detailed historical background and novel concepts for the systematics and taxonomy at the generic and suprageneric levels and a reappraisal of the generic diagnoses via re-examination of type- and/or other representative species. Considering just these two aspects makes the series an essential unique source of information on the Trematoda well into the twenty-first century. Furthermore, although the superfamily was treated as the basic unit of classification, the editors have made a substantial effort towards a classification reflecting a natural system of the Digenea considering morphological evidence in conjunction with phylogenies inferred from molecular data. This provides a sound basis for future molecular studies addressing phylogenetic relationships at the suprageneric level.

There are 148 families with 1,577 genera considered valid in the *Keys to the Trematoda*. An examination of the distribution of generic diversity (estimated as the number of valid genera) across digenean superfamilies related to their complexity

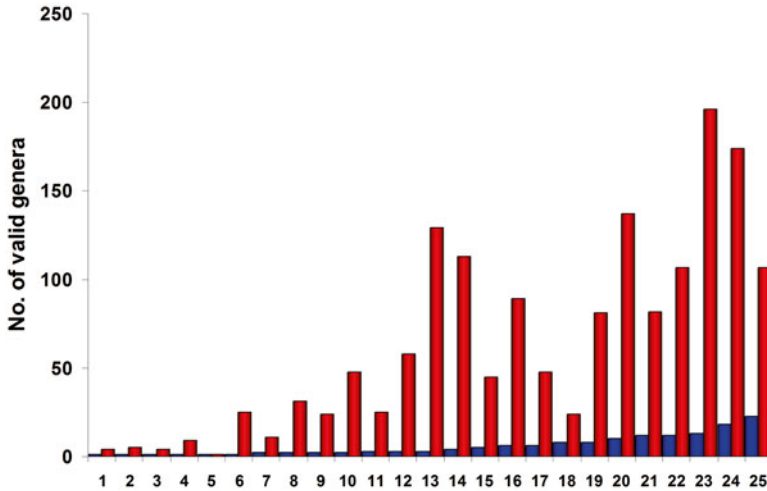


Fig. 2.1 The distribution of digenean generic diversity (assessed as the number of the genera considered valid, *red bars*) along a gradient of increasing superfamily complexity (assessed as the number of constituent families, *blue bars*). Data extracted from the *Keys to the Trematoda* [24–26]. *Order of superfamilies*: 1, Azygioidea; 2, Bivesiculoidea; 3, Transversotrematoidea; 4, Haploplanchnoidea; 5, Heronimoidea; 6, Bucephaloidea; 7, Clinostomoidea; 8, Haploporoidea; 9, Microscaphidioidea; 10, Monorchioidea; 11, Cyclocoeloidea; 12, Schistosomatoidea; 13, Opisthorchioidea; 14, Allocreadioidea; 15, Gymnophalloidea; 16, Diplostomoidea; 17, Pronocephaloidea; 18, Brachylaimoidea; 19, Echinostomatoidea; 20, Lepocreadioidea; 21, Gorgoderioidea; 22, Paramphistomoidea; 23, Hemiurioidea; 24, Microphalloidea; 25, Plagiorchioidea

(estimated as the number of families) illustrates a general trend of association between the two estimates (Fig. 2.1). The lower extreme of the complexity gradient is represented by six monotypic superfamilies [1–6, characterised by poor generic richness (1–9 genera with only superfamily Bucephaloidea Poche, 1907 containing 25 genera)]. The upper extreme comprises the most complex superfamilies, i.e. the Hemiurioidea Looss, 1899, Microphalloidea Ward, 1901 and Plagiorchioidea Lühe, 1901 (comprising 13–23 families), with generic richness varying between 107 and 196 genera. The variability in the middle range is due to two patterns indicating the need of further systematic work. Thus two superfamilies, the Opisthorchioidea Looss, 1899 and the Allocreadioidea Looss, 1902, are characterised by a large number of genera (129 and 113, respectively) whose familial affiliations require further scrutiny. Notably, these are among the superfamilies recovered to contain paraphyletic taxa, i.e. Heterophyidae Leiper, 1909 + Opisthorchiidae Looss, 1899 (see Olson et al. [27], Thaenkhram et al. [28]) and Opecoelidae Ozaki, 1925 + Opistholebetidae Fukui, 1929 [27], respectively, and this supports our suggestion (also see below).

The second pattern observed in Fig. 2.1 is associated with a relatively low generic richness that is unequally distributed among the families: Gymnophalloidea Odhner, 1905 (42 genera among 5 families); Pronocephaloidea Looss, 1899 (48 genera among 6 families); and Brachylaimoidea Joyeux & Foley, 1930 (24 genera

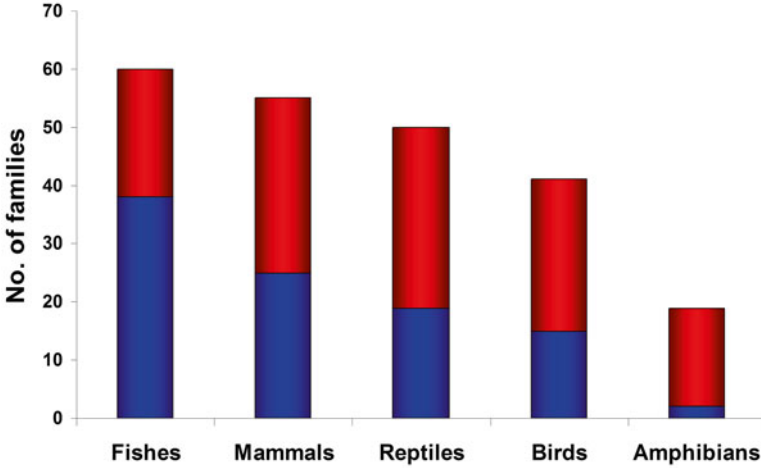


Fig. 2.2 The distribution of digenean diversity (assessed as the number of families) among the major definitive host groups (data from Bray [9]). Highlighted in *blue* are the digenean families found exclusively in a given host group, the remaining (highlighted in *red*) are found in more than one group

among 8 families). The large number (relative to content) of the families within these higher taxa indicates higher rates of “splitting” and the molecular phylogeny of Olson et al. [27] has demonstrated that this is the case on at least one occasion, i.e. the Brachylaimidae Joyeux & Foley, 1930 was recovered as paraphyletic, with the Leucochloridiidae Poche, 1907 nested. However, a molecular-based hypothesis based on denser taxon sampling within these superfamilies is required to test this suggestion.

Regarding the distribution of digenean taxonomic diversity among the major definitive host groups, 99 (67 %) of the digenean families are parasitic in a single vertebrate group (highlighted in blue in Fig. 2.2) whereas the remaining are found in more than one host group (highlighted in red in Fig. 2.2). Data from Bray [9] plotted in Fig. 2.2 illustrate that the highest number of the digenean families that occur in fishes are exclusively fish parasites whereas the number of families found only in amphibians is the lowest; the remaining vertebrate groups occupy intermediate position with respect to their exclusive associations with the digenean families.

2.3 Molecular Approaches to Digenean Phylogeny: Higher Taxa

Molecular data from nucleic acid sequences represent a useful source of independent data for phylogenetic inference. As predicted by Gibson and Bray [14], our understanding of the diversity, systematics and evolutionary relationships of the Digenea

has substantially advanced as a result of the expansion of molecular-based studies in the past 15 years. Ribosomal RNA (rRNA) genes (and their spacer regions) and mitochondrial (mt) genes have been the most popular markers used in the systematic studies of digeneans at several nested taxonomic scales; this is associated with the varying rates of evolution of the gene regions. Whereas rRNA genes have been used for inferring relationships at higher taxonomic levels, the internal transcribed spacer (ITS1 and ITS2) separated by the 5.8S rRNA gene has been utilised for exploring species boundaries in a range of studies related to 155 species of 19 digenean families (see Nolan and Cribb [13] for an exhaustive review). Another relatively recent review on the advances and trends in the molecular systematics of the parasitic platyhelminths covers comprehensively studies on the Digenea at several taxonomic scales [29]. Here we highlight selected examples with significant contribution towards the development of the molecular phylogeny of the Digenea at the higher taxonomic levels rather than provide an account of the investigations at the lower levels.

The first studies of the relationships within the Digenea at the suprageneric scale used the most conserved of the nuclear rRNA genes, the gene encoding the 18S subunit [30–33]. Soon focus has been placed on the 5' variable domains (D1–D3) of the 28S rRNA gene as suitable data source for inferring relationships at several levels, i.e. among species, genera and closely related families [34], and the first studies at the suprageneric level proved to be influential.

Tkach et al. [35] developed a molecular phylogeny of one of the most diverse digenean groups, the formerly recognised suborder Plagiorchiata, based on partial 28S rDNA sequences for 28 species of 13 families. They demonstrated the derived position of the Plagiorchiata in relation to all major digenean lineages considered in their analysis. Tkach et al. [35] also found that Plagiorchiata of the hypotheses based on morphological and life-cycle characters [22, 36–38] is paraphyletic and suggested as a solution the exclusion of the superfamilies Opecoeloidea, Dicrocoelioidea and Gorgoderioidea. These authors considered the Plagiorchiata *sensu stricto* to comprise the superfamilies Plagiorchioidea (including the Plagiorchiidae Lühe, 1901, Haematoloechidae Freitas & Lent, 1939, Telorchiidae Looss, 1899, Brachycoeliidae Looss, 1899 and Leptophallidae Dayal, 1938) and Microphalloidea (including the Microphallidae Ward, 1901, Prosthogonimidae Lühe, 1909, Lecithodendriidae Lühe, 1901 and Pleurogenidae Looss, 1899). Their analyses also indicated close relationships between the genera *Macrodera* Looss, 1899 and *Paralepoderma* Dollfus, 1950, *Leptophallus* Lühe, 1909 and *Metaleptophallus* Yamaguti, 1958, and *Opisthioglyphe* Looss, 1899 and *Telorchis* Lühe, 1899. The first four genera were later (in the *Keys to the Trematoda*) placed in the family Leptophallidae [39] and the latter two were accommodated within the family Telorchiidae [40].

In an updated analysis of partial 28S rDNA sequences using a larger number of diverse taxa (51 species belonging to 27 families), Tkach et al. [41] assessed the relationships of Plagiorchiata with 14 digenean families. The results of their study confirmed the main groupings (and their content), i.e. the Plagiorchioidea and Microphalloidea, found in Tkach et al. [35] and revealed a basal position of the families Schistosomatidae Stiles & Hassall, 1898, Diplostomidae Poirier, 1886, Strigeidae Railliet, 1919, Brachylaimidae and Leucochloridiidae and a sister-group

relationship between the Rencolidae Dollfus, 1939 and Eucotylidae Cohn, 1904, both associated with the superfamily Microphalloidea.

Tkach et al. [42] used partial 28S rDNA sequences to explore the phylogenetic interrelationships of 32 species belonging to 18 genera and four families of the superfamily Microphalloidea with members of the Plagiorchiidea (eight species of six genera) as outgroups. They demonstrated that the representatives of the Microphalloidea form three main lineages corresponding to the families Lecithodendriidae, Microphallidae and Pleurogenidae + Prosthogonimidae and suggested synonymies at the generic level (*Floridatrema* Kinsella & Deblock, 1994 with *Maritrema* Nicoll, 1907, *Candidotrema* Dollfus, 1951 with *Pleurogenes* Looss, 1896, and *Schistogonimus* Lühe, 1909 with *Prosthogonimus* Lühe, 1899). Whereas the first synonymy was not accepted by Deblock [43], the latter two were considered in the *Keys to the Trematoda* [44, 45].

All of the above mentioned studies concern solving pieces of the puzzle of digenean relationships at higher taxonomic levels. The first step to a more inclusive analysis of digenean phylogeny is that of Cribb et al. [10] who attempted a combined evidence approach using morphological characters for all stages of the digenean life-cycle and complete 18S rDNA sequences for 75 digenean species of 55 families. Analyses of this first morphological dataset with a published character matrix identified the Bivesiculidae Yamaguti, 1934 + Transversotrematidae Witenberg, 1944 as the sister group to the remainder of the Digenea and the Diplostomoidea Poirier, 1886 + Schistosomatoidea Stiles & Hassall, 1898 as the next most basal taxon. The combined evidence solution of Cribb et al. [10] was found to exhibit greater resolution than morphology alone with the predominant effect of the molecular data on tree topology. Analyses of the combined data found no support for a basal position of the Heronimidae Ward, 1917 and revealed that the earliest divergent digeneans include the Diplostomoidea (Diplostomidae and Strigeidae) and Schistosomatoidea (Sanguinicolidae and Schistosomatidae) with the Transversotrematidae and Bivesiculidae progressively less basal.

Although Cribb et al. [10] found poor resolution of higher digenean taxa, the relationships at the superfamily level were well resolved. These include the superfamilies:

- The Hemiuroidea, with the Azygiidae Lühe, 1909 as basal, the Sclerodistomidae Odhner, 1927, Accacoeliidae Odhner, 1911, Syncoeliidae Looss, 1899, Derogenidae Nicoll, 1910 and Didymozoidae Monticelli, 1888 in one clade, and the Hemiuridae Looss, 1899 (recovered as paraphyletic) and Lecithasteridae Odhner, 1905 in the other.
- The Paramphistomoidea Fiscoeder, 1901 [including the Paramphistomidae Fiscoeder, 1901, Diplodiscidae Cohn, 1904, Microscaphidiidae Looss, 1900 (as Angiodictyidae Looss, 1902) and Mesometridae Poche, 1926].
- The Opisthorchiidea (including the Cryptogonimidae Ward, 1917, Heterophyidae and Opisthorchiidae).
- The Echinostomatoidea Looss, 1899 (including the Echinostomatidae Looss, 1899, Fasciolidae Railliet, 1895, Philophthalmidae Looss, 1899 and Cyclocoelidae Stossich, 1902).

- The Acanthocolpoidea Nahhas & Cable, 1964 (including the Acanthocolpidae Lühe, 1906, Campulidae Odhner, 1926 and Nasitrematidae Ozaki, 1935).
- The Lepocreadioidea Odhner, 1905 [with the Lepocreadiidae Odhner, 1905 (recovered as paraphyletic), Enenteridae Yamaguti, 1958 and Gyliauchenidae Fukui, 1929 but not the Apocreadiidae Skrjabin, 1942 which grouped with the Haploporoidea Nicoll, 1914 and Monorchioidea]; there was no support for a close relationship between the superfamily Haploporoidea and the Haplospilanchnidae Poche, 1926.
- The Haploporoidea (the Haploporidae Nicoll, 1914 and Atractotrematidae Yamaguti, 1939).

Cribb et al. [10] found weak support for Fellodistomoidea (containing the Tandanicolidae Johnston, 1927 and Fellodistomidae Nicoll, 1909) and the Plagiorchiidea (containing a subgroup formed by the Plagiorchiidae, Brachycoeliidae and Cephalogonimidae Looss, 1899; and Microphallidae, Pachypsolidae Yamaguti, 1958, Zoogonidae Odhner, 1902 and Faustulidae Poche, 1926). On the other hand, the Opecoelidae and Opistholebetheidae Fukui, 1929 were strongly related as well as there was a strong sister relationship between the Monorchioidea Odhner, 1911 and the enigmatic genus *Cableia* Sogandares-Bernal, 1959 which has variously been placed in the Lepocreadiidae, Opecoelidae, Enenteridae and the Acanthocolpidae.

The most comprehensive phylogeny of the Digenea to date is that of Olson et al. [27]; it is also the first re-evaluation of relationships at higher taxonomic levels that has affected digenean classification. These authors estimated digenean relationships after adding a substantial number of novel sequences for complete 18S and partial (variable domains D1–D3) 28S rRNA genes (80 and 124, respectively). Their combined dataset which was found to yield the most strongly supported results thus comprised a rich and diverse array of taxa representing all major digenean groups (163 species of 77 families) (see Table 2.1). One important outcome of this study is the first molecular-based classification proposed based on the results from Bayesian analysis of the combined dataset; the authors went further by considering in association of putative synapomorphies that add morphological or ontological support to the molecular data.

Generally the molecular phylogenetic analyses of Olson et al. [27] supported the most recent classification of the Digenea provided in the *Keys to the Trematoda* at the familial and superfamilial levels (but see differences in superfamilial placements highlighted in Table 2.1) but provided strong evidence for a different subdivision (and membership in some cases) at the higher taxonomic levels. This has led to the recognition of a number of new taxa at the ordinal and subordinal levels (one order and nine suborders; see Table 2.1).

Important in the new classification is the reflection that the molecular phylogeny of the Digenea does not support its traditional division into three groups at the ordinal level, i.e. the Strigeida La Rue, 1957, the Echinostomida La Rue, 1957 and the Plagiorchiida La Rue, 1957 [14, 18, 46]. Olson et al. [27] split the subclass Digenea into two major groups, the order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003 and the order Plagiorchiida La Rue, 1957 (these were referred to

Table 2.1 Classification of the Digenea of Olson et al. [27] and of the *Keys to the Trematoda* (Gibson et al. [24], Jones et al. [25] and Bray et al. [26])

Olson et al. [27]		Keys to the Trematoda
Superfamily	Family	Different superfamilial placements and/or additional families
Order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Suborder Diplostomata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Brachylaimoidea Joyeux & Foley, 1930	Brachylaimidae Joyeux & Foley, 1930+Leucochloridiidae Poche, 1907 ^a	Hasstielsiidae Hall, 1916; Leucochloridiomorphidae Yamaguti, 1958; Moreauiidae Johnston, 1915; Ovariopteridae Leonov, Spasskii & Kulikov, 1963; Panopistidae Yamaguti, 1958; Thapariellidae Srivastava, 1953
Diplostomoidea Poirier, 1886	Diplostomidae Poirier, 1886+Strigeidae Railliet, 1919	Bolbocephaloideidae Strand, 1935; Brauninidae Wolf, 1903; Cyathocotylidae Mühling, 1898; Proterodiplostomidae Dubois, 1936
Schistosomatoidea Stiles & Hassall, 1898	Schistosomatidae Stiles & Hassall, 1898 Clinostomidae Lühe, 1901 Sanguinicolidae von Graff, 1907 Spirorchidae Stunkard, 1921	Clinostomoidea Lühe, 1901 (also including <u>Liolopidae Odhner, 1912</u>)
Order Plagiorchiida La Rue, 1957		
Suborder Apocreadiata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Apocreadiotea Skrjabin, 1942	Apocreadiidae Skrjabin, 1942	Lepocreadioidea Odhner, 1905
Suborder Bivesiculata Olson, Cribb, Tkach, Bray & Littlewood, 2003		
Bivesiculotea Yamaguti, 1934	Bivesiculidae Yamaguti, 1934	
Suborder Bucephalata La Rue, 1926		
Bucephaloidea Poche, 1907	Bucephalidae Poche, 1907	
Gymnophalloidea Odhner, 1905	Fellodistomidae Nicoll, 1909 Tandanicolidae Johnston, 1927	<u>Gymnophallidae Odhner, 1905</u> ; <u>Botulisaccidae Yamaguti, 1971</u>

Suborder Echinostomata La Rue, 1926

- Echinostomatoidea Looss, 1899 Echinostomatidae Looss,
 1899 + Fasciolidae Railliet, 1895
 Philophthalmidae Looss, 1899
 Psilostomidae Looss, 1900
 Cyclocoelidae Stossich, 1902

Calycodidae Dollfus, 1929; Cathaemasidae Fuhrmann, 1928; Rhopalidae Looss, 1899; Rhytidodidae Odhner, 1926

Suborder Haploplanchnata Olson, Cribb, Tkach, Bray & Littlewood, 2003

- Haploplanchnoidea Poche, 1926 Haploplanchnidae Poche, 1926

Suborder Hemiurata Skrjabin & Guschanskaja, 1954

- Azygioidea Lühe, 1909 Azygiidae Lühe, 1909
 Hemiuroidea Looss, 1899 Hemiuridae Looss, 1899 + Lecithasteridae
 Odhner, 1905

Bathycotyliidae Dollfus, 1932; Dictysarcidae Skrjabin & Guschanskaja, 1955; Hirudinellidae Dollfus, 1932; Isoparorethidae Travassos, 1922; Ptychogonimidae Dollfus, 1937; Sclerodistomoididae Gibson & Bray, 1979

- Accacoeiliidae Odhner, 1911

- Derogenidae Nicoll, 1910 (*Hemiperina*
 Manter, 1934; *Derogenes* Lühe, 1900)^b

- Didymozoidae Monticelli, 1888

- Sclerodistomidae Odhner, 1927

- Syncoeliidae Looss, 1899

Suborder Heronimata Skrjabin & Schulz, 1937

- Heronimoidea Ward, 1917

- Heronimidae Ward, 1917

Suborder Lepocreadiata Olson, Cribb, Tkach, Bray & Littlewood, 2003

- Lepocreadioidea Odhner, 1905 Lepocreadiidae Odhner, 1905

Deropristidae Cable & Humninen, 1942; Liliatrematidae Gubanov, 1953; Megaperidae Manter, 1934

- Enenteridae Yamaguti, 1958

- Gorgocephalidae Manter, 1966

- Gyliauchenidae Fukui, 1929

(continued)

Suborder Transversotremata Olson, Cribb, Tkach, Bray & Littlewood, 2003	
Transversotrematoidea	Transversotrematidae Witenberg, 1944
Suborder Xiphiidiata Olson, Cribb, Tkach, Bray & Littlewood, 2003	
Allocreadioidea Looss, 1902	Opoeoeliidae Ozaki, 1925 + Opistholebetidae Fukui, 1929
	Acanthocolpidae Lühe, 1906 ^b
	Brachycladiidae Odhner, 1905
Gorgoderoidea Looss, 1899	Gorgoderidae Looss, 1899
	Callodistomidae Odhner, 1910
	Dicrocoeliidae Looss, 1899
	Encyclometridae Mehra, 1931
	Haploporidae Nicoll, 1914 + Atractotrematidae Yamaguti, 1939
	Orchipedidae Skrzjabin, 1913
	Paragonimidae Dollfus, 1939
	Troglorematidae Odhner, 1914
Microphalloidea Ward, 1901	Microphallidae Ward, 1901 (<i>Maritrema</i> Nicoll, 1907, <i>Microphallus</i> Ward, 1901)
	Anerotreematidae Yamaguti, 1958; Diplangidae Yamaguti, 1971; Eumegacetidae Travassos, 1922; Exotidnridae Mehra, 1935; Gyraeascidae Macy, 1935; Leyogonimidae Dollfus, 1951; Phaneropsolidae Mehra, 1935; Renschetrematidae Yamaguti, 1971; Stomylotrematidae Poche, 1926; Taiwanrematidae Fischthal & Kuntz, 1981
	Cyclocoeloidea Stossich, 1902
	Eucotylidae Cohn, 1904
	(continued)

Table 2.1 (continued)

Olson et al. [27]		Keys to the Trematoda
Superfamily	Family	Different superfamilial placements and/or additional families
Plagiorchioidea Lühe, 1901	Lecithodendriidae Lühe, 1901	Dolichoperoididae Johnston & Angel, 1940; Echinoporidae Krasnolobova & Timofeeva, 1965; Gekkonotrematidae Yamaguti, 1971; Glypthelminthidae Cheng, 1959; Haematoloecidae Freitas & Lent, 1939; Leptophallidae Dayal, 1938; Meristocotylidae Fischthal & Kuntz, 1964; Mesotretidae Poche, 1926; Ocadiatrematidae Fischthal & Kuntz, 1981; Opisthogonimidae Travassos, 1928; Orientocreadiidae Yamaguti, 1958; Remiferidae Pratt, 1902; Styphlotrematidae Baer, 1924; <u>Thrinascotrematidae Jue Sue & Platt, 1999; Urotrematidae Poche, 1926</u>
	Pachypsolidae Yamaguti, 1958	
	Pleurogenidae Looss, 1899	
	Prosthogonimidae Lühe, 1909	
	Renicolidae Dollfus, 1939	
	Zoogonidae Odhner, 1902+Faustulidae Poche, 1926	
	Plagiorchiidae Lühe, 1901 (<i>Glypthelmins</i> Stafford, 1905, <i>Skryabinocces</i> Sudarikov, 1950, <i>Haematoloechus</i> Looss, 1899)	
Gorgoderioidea Looss, 1899	Auridistomidae Stunkard, 1924	
	Brachycoelidae Looss, 1899	
	Cephalogonimidae Looss, 1899	
	Choanocotylidae Jue Sue & Platt, 1998	
	Macroderoididae McMullen, 1937	
	Omphalometridae Looss, 1899	
	Telorchidae Looss, 1899	

Different superfamilial placements are indicated in bold; underlined are families for which molecular data are required

^aParaphyletic relationships in the analysis of Olson et al. [27] indicated with a +

^bPolyphyletic in Olson et al. [27]

as superorders by Cribb et al. [4]) thus confirming the prediction of Gibson and Bray [14] and the results of Cribb et al. [10]. The Diplostomida comprises three superfamilies whereas the Plagiorchiida has a more complex structure with 13 suborders (referred to as orders by Cribb et al. [4] and Littlewood [12]) containing a total of 19 superfamilies (see Table 2.1 for details). The four more inclusive suborders in the phylogeny of Olson et al. [27] are:

- The Hemiurata Skrjabin & Guschanskaja, 1954 represented by two superfamilies, the Azygioidea Lühe, 1909 (monotypic) and the Hemiuroidea (seven families as in Cribb et al. [10], see above).
- The Bucephalata La Rue, 1926 represented by two superfamilies, the Bucephaloidea (monotypic) and the Gymnophalloidea (including two families).
- The Pronocephalata Olson, Cribb, Tkach, Bray & Littlewood, 2003 represented by two superfamilies, the Paramphistomoidea (including four families) and the Pronocephaloidea (including five families).
- The Xiphidiata Olson, Cribb, Tkach, Bray & Littlewood, 2003 represented by four superfamilies, the Gorgoderoidea (monotypic), the Microphalloidea (including nine families), the Allocreadioidea (including four families) and the Plagiorchioidea (including eight families; Table 2.1).

An important outcome of the development of a molecular phylogeny of the Digenea is that inferences can be made on the origins and evolution of the digenean life-cycle. Cribb et al. [4] used the hypothesis and the classification of Olson et al. [27] and life-cycle traits derived from a large database (c. 1,350 species) of information on the life-cycles for the Digenea to explore the evolution of the digenean life-cycle. Cribb et al. [4] inferred that gastropods were the basal host group for the Digenea, parasitism of bivalves being a result of host-switching that has occurred multiple times, and found no convincing evidence for a deep level coevolution between the major digenean clades and their molluscan hosts. Regarding the second intermediate hosts, these authors illustrated a great diversity with discontinuous distributions on the phylogeny of different host types and concluded that three-host life-cycles have been derived from two-host life-cycles and adopted repeatedly. With respect to definitive hosts, these authors suggested an origin for the Digenea in association with teleosts followed by host-switching into chondrichthyans and provided alternative explanations for parasitism in tetrapods based on the topologies of relationships within the Xiphidiata and Diplostomida.

2.4 Towards Greater and Focused Representation of Digenean Diversity in Phylogenies

The development of the molecular phylogeny of the Digenea coincided with that of the *Keys to the Trematoda* so that a full consensus with the classification based on the molecular results of Cribb et al. [10], Olson et al. [27] and Tkach et al. [35, 41, 42]

has been reached in the treatment of the five superfamilies in the third volume [9]. Overall, a comparison between the two classifications of the Digenea summarised in Table 2.1 shows a considerable congruence. The superfamilial placement of 12 families (8 %, highlighted in bold in Table 2.1) in the *Keys to the Trematoda* departed from that inferred from the molecular phylogeny of Olson et al. [27].

Although the analysis of Olson et al. [27] represents the broadest sampling of the Digenea to date (52 % of a total of 148 currently recognised digenean families), a number of omissions (families highlighted in Table 2.1) were depicted [27, 29]. We here comment upon the taxa involved in the molecular phylogeny of the Digenea in association with the content of the *Keys of the Trematoda* focusing on additional important omissions rather than on those previously identified in an attempt to outline the suprageneric taxa that require further exploration in a molecular phylogeny.

Olson et al. [27] did not include in their analysis representatives of the type-families of the Allocreadioidea (the Allocreadiidae Looss, 1902), Gymnophalloidea (the Gymnophallidae Odhner, 1905) and Paramphistomoidea (the Paramphistomidae). Therefore, the basis of each of these superfamilies was not actually established and should not be considered definitive; also see [47]. At the lower taxonomic level, the same problem exists, i.e. lack of data from the type-genera of the families Cryptogonimidae, Opisthorchiidae, Strigeidae and Plagiorchiidae. Notably, the first three taxa were recovered in clades in which paraphyly was detected [27]. The Plagiorchioidea represents a special case. Formally, the type-family has been sampled at the time of the study of Olson et al. [27]. However, the three genera whose representatives have been sequenced (*Glypthelmins* Stafford, 1905, *Skrjabinoeces* Sudarikov, 1950 and *Haematoloechus* Looss, 1899) were transferred to different families, recognised in 2008, i.e. the Glypthelminthidae Cheng, 1959 and the Haematoloechidae [48, 49]. Therefore, the Plagiorchioidea also needs re-establishment preferably based on molecular data from representatives of the type-genus *Plagiorchis* Lühe, 1899 of the type-family Plagiorchiidae.

A number of superfamilies characterised by high taxonomic diversity at the generic and suprageneric levels have been underrepresented in the broad phylogeny of the Digenea by Olson et al. [27]. These are (in order of increasing generic richness, data from the *Keys to the Trematoda*; see also Fig. 2.1): Echinostomatoidea, Diplostomoidea, Paramphistomoidea, Plagiorchioidea, Allocreadioidea, Opisthorchioidea, Lepocreadioidea and Microphalloidea.

Using only a small fraction of the actual generic/familial diversity in the phylogeny of the Digenea by Olson et al. [27] has typically led to problems in resolving relationships. Thus the family Echinostomatidae (correct name given in the taxonomic listing of the taxa studied and the trees but referred to as “Echinostomidae”(sic) elsewhere in the text; see [27]) was represented by just two genera, *Echinostoma* Rudolphi, 1809 and *Euparyphium* Dietz, 1909 (sequence for *Euparyphium melis*, a synonym of *Isthmiophora melis* (Schränk, 1788), see [50, 51]) and found to be paraphyletic. The family represents a diverse and complex group comprising 43 genera belonging to 10 subfamilies [51] and it is likely that denser sampling would lead to better resolution of the relationships within the superfamily Echinostomatoidea

(molecular data for 7 out of 81 genera currently available [27]); effort should also be focused on representation of the four families not sampled to date (Table 2.1).

The superfamily Diplostomoidea was represented by five out of 89 genera, two diplostomid (*Alaria* Schrank, 1788 and *Diplostomum* Nordmann, 1832) and three strigeid genera (*Apharyngostrigea* Ciurea, 1927, *Cardiocephaloides* Sudarikov, 1959 and *Ichthyocotylurus* Odening, 1969) and the members of these genera were found intermingled in the clade of Diplostomoidea; the type-genus of the Strigeidae was not sampled [27]. The assessment of the relationships within the superfamily therefore, requires further exploration based on a wider array of taxa including the type-genus of the family Strigeidae, *Strigea* Abildgaard, 1790; we also mark as important omissions the families Cyathocotylidae Mühling, 1898 and Proterodiplostomidae Dubois, 1936 (Table 2.1).

Although the Opisthorchioidea and one of its constituent families, the Cryptogonimidae, were resolved in the phylogeny of Olson et al. [27], the remaining two families were not since the Heterophyidae was recovered as paraphyletic with the Opisthorchiidae nested within it. Seven out of a total of 129 genera of the superfamily were sampled in their study but none of the type-genera of the three families; their re-establishment in a molecular phylogeny is therefore still in the pending state. Recently, Thaenkham et al. [28] added 18S rDNA sequences for species of three genera, *Haplorchis* Looss, 1899, *Procerovum* Onji & Nishio, 1916 and *Metagonimus* Katsurada, 1912, and examined the relationships within the Opisthorchioidea based on a wider generic representation (including a sequence for a species of *Opisthorchis* Blanchard, 1895, the type-genus of the Opisthorchiidae) but their analysis also recovered a paraphyletic relationship between the Heterophyidae and Opisthorchiidae, the latter nested within the former.

The diversity of the family Microphallidae, the type of the Microphalloidea, was underrepresented (2 out of 47 genera, i.e. *Microphallus* Ward, 1901 and *Maritrema*) in the study of Olson et al. [27]. These authors found that in some analyses the representatives of the two subfamilies, the Microphallinae Ward, 1901 and the Maritrematinae Nicoll, 1907, were split among different clades. Tkach et al. [42] added sequences for more representatives of the two genera and recovered the Microphallidae as monophyletic. Nevertheless, the complex structure and diverse content of the family still awaits exploration of relationships based on a much wider taxon sampling.

2.5 Integrated Approaches to Digenean Diversity, Taxonomy and Systematics

A review of the history of the development of studies on the most comprehensively studied digenean superfamilies (Hemiuroidea, Lepocreadioidea, Plagiiorchioidea and Schistosomatoidea) indicates a framework that would lead to robust estimates of phylogeny: (i) systematic inventory of the group; (ii) detailed understanding of morphology; (iii) taxonomic revision; (iv) classification system; (v) molecular

phylogeny; (vi) revised classification. In this section, we shall illustrate the progress within this framework focusing on the superfamilial level.

Historically, the most extensively studied digenean higher-level taxon appears to be the Hemiuroidea, a highly diverse group of parasites found predominantly in marine teleosts but also in freshwater teleosts, elasmobranchs and occasionally in amphibians and reptiles [52]. The knowledge on species diversity accumulated over two centuries and focused sampling and revisionary work based on material from the North-East Atlantic have largely contributed to an improved classification of this large and heterogeneous group (13 families and 196 genera recognised in the *Keys to the Trematoda*). In a series of monographs Gibson and Bray provided original descriptions, detailed comments on the morphology and life-cycles, host-parasite records (including larval stages) and identification keys for all of the hemiuroid species recorded from the North-East Atlantic; these included representatives of the families Accacoeliidae, Azygiidae, Hemiuridae, Hirudinellidae Dollfus, 1932, Ptychogonimidae Dollfus, 1937, Sclerodistomidae and Syncoeliidae [53–55]. Gibson and Bray [56] revised the superfamily and proposed a classification and a hypothesis for the evolution of the Hemiuroidea based on the functional morphology of the adults; these authors also provided detailed definitions of hemiuroid structures and analysis on their systematic value and possible function based on original data. According to Gibson and Bray's [56] classification the Hemiuroidea is divided into 14 families: Accacoeliidae (with two subfamilies), Azygiidae (with two subfamilies), Bathycotylidae Dollfus, 1932, Bunocotylidae Dollfus, 1950 (with four subfamilies), Derogenidae (with three subfamilies), Dictysarcidae Skrjabin & Guschanskaja, 1955 (with three subfamilies), Hemiuridae (with nine subfamilies), Hirudinellidae, Isoparorchidae Travassos, 1922, Lecithasteridae (with six subfamilies), Ptychogonimidae, Sclerodistomidae (with three subfamilies), Sclerodistomoididae Gibson & Bray, 1979 and Syncoeliidae (with two subfamilies). The studies of Gibson and Bray thus provided a much needed systematic framework to be evaluated with the aid of molecular evidence.

The first molecular phylogeny of the Hemiuroidea was based on the V4 variable domain of the 18S rRNA gene for 33 species representative of ten hemiuroidean families after the concept of Gibson and Bray [56] plus the Didymozoidae [31]. Analyses of Blair et al. [31] supported the monophyly of the Hemiuroidea as represented by the taxa sampled and revealed two main groups, one containing all members of the Hemiuridae and the lecithasterinae lecithasterids and one comprises the members of Derogenidae, Didymozoidae, Hirudinellidae, Sclerodistomidae, Syncoeliidae and Accacoeliidae whereas the Isoparorchidae and the hysterolecithinae lecithasterids appeared separately close to the base of the hemiuroid tree and the Azygiidae fell outside the hemiuroid clade. Hemiuroids were well represented although with a lower number of taxa (18 species belonging to 7 families) in the phylogeny of the Digenea of Olson et al. [27]. Their analyses strongly supported the distinct status of the Hemiurata with Hemiuroidea and Azygioidea as separate superfamilies. Within the Hemiuroidea, the Derogenidae was recovered as polyphyletic and a paraphyletic relationship of the Hemiuridae and the Lecithasteridae was depicted (as in [10, 31]). Consequently the results of

the molecular phylogenies were considered in the *Keys to the Trematoda*: the Azygiidae was recognised at the superfamily level [57] and the Didymozoidae was included within the Hemiuroidea [52].

Recently, Pankov et al. [58] described a new bunocotyline genus *Robinia* Pankov, Webster, Blasco-Costa, Gibson, Littlewood, Balbuena & Kostadinova, 2006 and presented a phylogenetic hypothesis for the Bunocotylinae Dollfus, 1950 and the Hemiuroidea based on sequence data analyses of an increased number of taxa (from 22 species for complete 18S and partial 28S rRNA genes and from 37 species for the V4 domain of the 18S rRNA gene). Both molecular analyses confirmed the monophyly of the Hemiuroidea, its division into two major clades and the polyphyly of the Derogenidae, as in previous studies [10, 27, 31], and suggested that the Gonocercinae Skrjabin & Guschanskaja, 1955 (with two genera, *Gonocerca* Manter, 1925 and *Hemipera* Nicoll, 1913), may require a distinct familial status. The authors found poor support for the distinct status of the Lecithasteridae and Hemiuridae, following previous suggestions based on different sequence data sets [10, 27, 31]. The results of this study also indicated that increased taxon sampling for and analysing the V4 domain of the 18S rRNA gene separately, failed to resolve many monophyletic hemiurid subfamilies thus adding little to the study of Balir et al. [31]. Pankov et al. [58] suggested that much greater taxon sampling for both 18S and 28S genes is needed in order to test the consistency of the present classification system of the Hemiuroidea with the evolutionary relationships of its members.

The Lepocreadioidae is one of the complex and problematic digenean superfamilies. Ten families and 137 genera are recognised in the *Keys to the Trematoda* but molecular studies have demonstrated that three of these families (Acanthocolpidae, Apocreadiidae and Brachycladiidae Odhner, 1905) are not closely related to the Lepocreadiidae ([10, 27, 59]; see Table 2.1). Bray, Cribb and colleagues devoted a comprehensive series of studies (c. 50 papers) on the diversity of the Lepocreadioidae in marine teleosts, predominantly in the Indo-West Pacific and the North-East Atlantic, which resulted in detailed descriptions of a vast number of species (including many new), erection of new and/or reassessment of the existing genera and construction of identification keys to species and parasite-host and host-parasite lists (see Bray et al. [60] for a list of the most inclusive references). These data provided a sound basis for revisory work [61–66]. On the other hand, extensive sampling for molecular studies carried out in parallel with morphological assessments has supplied an admirable number of sequences for species from a wide range of genera. Bray et al. [60] assessed the phylogenetic relationships of representative species of the superfamily Lepocreadioidae using partial 28S rDNA and *nad1* sequences for members of the families Lepocreadiidae (42 species), Enenteridae (6 species), Gyliuchenidae (6 species) and Gorgocephalidae Manter, 1966 (1 species), along with 22 species representing eight other digenean families. The study recovered the Lepocreadioidae as monophyletic, comprising six groups: three well-recognised families (Enenteridae, Gorgocephalidae and Gyliuchenidae) and three groups resulting from the partitioning of the Lepocreadiidae in the phylogenetic tree. The latter were recognised as families by Bray & Cribb [67] who also provided amended family diagnoses.

A similar increased effort to collect and characterise morphologically and/or molecularly representatives of the members of the Plagiorchioidea and Microphalloidea by Tkach and colleagues [68–71] has contributed significantly to our understanding of the relationships and family structures of these large taxa (see above). The results of the molecular phylogenies [35, 41, 42] are partially reflected in the family level classifications in the *Keys to the Trematoda* [39, 48, 49, 72]. However, the two superfamilies are far too large and still require sustained systematic research.

Augmented representation of the species/genera of blood flukes has also resulted in advancing the knowledge on the relationships within the superfamily Schistosomatoidea. Snyder & Locker [73] examined phylogenetic relationships among ten genera (*Austrobilharzia* Johnston, 1917, *Bilharziella* Looss, 1899, *Dendrobilharzia* Skrjabin & Zakharow, 1920, *Gigantobilharzia* Odhner, 1910, *Heterobilharzia* Price, 1929, *Orientobilharzia* Dutt & Srivastava, 1955, *Ornithobilharzia* Odhner, 1912, *Schistosoma* Weinland, 1858, *Schistosomatium* Tanabe, 1923 and *Trichobilharzia* Skrjabin & Zakharow, 1920) of the family Schistosomatidae using 28S rDNA sequences (variable domains D1–D2) and found two major clades, one comprising the genera *Schistosoma* and *Orientobilharzia* parasitic in mammals and one consisting of predominantly bird parasites. These authors suggested an Asian origin of *Schistosoma*. Snyder [74] expanded the data on the Schistosomatoidea by generating 18S and 28S rDNA sequences for species belonging to eight genera of the Spirorchiidae Stunkard, 1921. Phylogenetic analyses involving representatives of the order Diplostomida recovered Spirorchiidae as paraphyletic with three genera from marine turtles exhibiting a sister-group relationship with the Schistosomatidae whereas five genera from freshwater turtles were found to occupy basal positions in the phylogeny of the tetrapod blood flukes. This coupled with the basal position within the schistosomatid clade of the genera *Austrobilharzia* and *Ornithobilharzia*, both comprising species with marine life-cycles, led to a suggestion that schistosomatids arose after a marine turtle blood fluke ancestor successfully colonised birds [74]. Lockyer et al. [75] presented the most comprehensive phylogeny of the Schistosomatidae to date, based on the sequences of three genes, complete 18S and 28S rRNA and mitochondrial cytochrome *c* oxydase subunit 1 (COI), for 30 species representing ten of the 13 known genera and almost all species of *Schistosoma*. The phylogeny provided evidence for the validity of two of the four currently accepted subfamilies [76], the Gigantobilharziinae Mehra, 1940 (comprising the genera *Dendrobilharzia* and *Gigantobilharzia*) and the Schistosomatinae Stiles & Hassall, 1898 (including *Austrobilharzia*, *Heterobilharzia*, *Orientobilharzia*, *Ornithobilharzia*, *Schistosoma* and *Schistosomatium*) but not for the subfamily Bilharzeillinae Price, 1929 since the representatives of the *Bilharziella* and *Trichobilharzia* did not form a monophyletic clade. The study of Lockyer et al. [75] confirmed an Asian origin for *Schistosoma* and the position of *Orientobilharzia* within the *Schistosoma*. The nomenclatural change has recently been formally justified by Aldhoun and Littlewood [77] who transferred to *Schistosoma* the four species of *Orientobilharzia* they considered valid [as *Schistosoma bomfordi* Montgomery, 1906, *S. turkestanicum* Skrjabin,

1913, *S. dattai* (Dutt & Srivastava, 1952), *S. harinasutai* (Kruatrachue, Bhaibulaya & Harinasuta, 1965)] and provided an amended generic diagnosis of *Schistosoma* and a revised key to the subfamily Schistosomatinae.

2.6 Future Research Prospects

In conclusion, molecular phylogenetics appears key to understanding the evolution of the Digenea. Although there is an agreement that further effort is needed towards achieving an improved representation of digenean taxonomic diversity in molecular phylogenies [27, 29], challenges in selection of gene loci exist and a direction of efforts appear to have been clarified recently. It is apparent that molecular analyses of digenean relationships at higher taxonomic levels will continue to rely upon the 18S and 28S rDNA sequences because a rich database has already been acquired.

However, evidence has been accumulating recently that promotes the utility of complete 28S rRNA gene as phylogenetic marker and illustrates the benefits of improved phylogenetic signal when used in combination with 18S rRNA gene at different levels within and between metazoan taxa including platyhelminths, e.g. [75, 78, 79]. Lockyer et al. [79] examined the utility of this approach in resolving the interrelationships between the major flatworm clades and stressed that Bayesian inference and maximum likelihood appear to give more congruent trees than maximum parsimony with respect to traditional concepts [75]. Mallatt and colleagues [80, 81] have evaluated the phylogenetic relationships in Ecdysozoa (molting animals) using likelihood-based Bayesian inference on nearly complete 18S+28S rDNA sequences and suggested that this may prove to be a combination of best genes and a tree-building method for reconstruction of ecdysozoan phylogenies. Waeschenbach et al. [82] used nearly complete 28S rDNA sequences (4,047–4,593 nt) in combination with complete 18S rDNA sequences (1,940–2,228 nt) and Bayesian analyses, to resolve cestode interrelationships at the ordinal level. They demonstrated that the addition of domains D4–D12 of 28S rRNA gene contributes to a substantial improvement of phylogenetic signal resulting in overall better nodal support, topology stability and greater resolution compared with previous molecular estimates of cestode interrelationships based on 18S+partial (domains D1–D3) 28S rRNA genes. With regard to digenean interrelationships, the pioneer study of Lockyer et al. [79] is a promising start especially because it is the first phylogeny inferred from a combination of three independent datasets (i.e. for 18S, 28S and COI).

Mitochondrial genomes may offer a wealth of homologous markers for both systematics and diagnostics, but in contrast to nuclear ribosomal genes, few mitochondrial genes have been tested because of the limited availability of PCR primers and the higher rates of evolution thus rendering them more suitable for resolving more recent radiations; see, e.g. [83] for a review. However, whole mitochondrial genome sequences have been shown to resolve deep-level relationships in many metazoan groups [84] and the use of mtDNA spanning multiple genes has been

considered promising [83]; also see Philippe et al. [85] for an in-depth focus on the use of genome-scale data in phylogenies. At the less inclusive taxonomic levels, modern genomic approaches may also provide an in-depth understanding of the patterns of speciation and construction of robust phylogenies as illustrated by the recent developments in the genetic research on species of the genus *Schistosoma*; see, e.g. [86–90].

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