

Diversification, dioecy and dimorphism in schistosomes

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In addition to causing one of the great neglected diseases of the world, schistosomes have unusual biological features that further command attention, including their habit of living in the blood of their hosts and the presence of separate, dimorphic sexes. Phylogenetic studies including a more complete sampling of pivotal and rare schistosomes and their relatives, provide an improved framework for interpreting schistosome biology. From such studies, it is inferred that schistosomes are exclusively parasites of endotherms. It is argued that a commitment to life in the endothermic hepatic portal system favored a filiform body form for egg deposition and led to the emergence of dioecy. Schistosome sexual dimorphism and mating systems have subsequently been influenced by the duration of opportunities for transmission and by the nature of the vascular habitats in which they live. A comparative perspective provides valuable insights for interpreting the biology of schistosomes, including the species that cause disease in humans.

Schistosome basics and peculiarities

Several features make the ~100 species of schistosomes (Schistosomatidae) [1] among the most unusual of the ~18,000 nominal species [2] of digenetic trematodes. They have a two-host life cycle instead of the more typical three-host fluke cycle. Forked-tail cercariae emerge from snails and penetrate their definitive hosts directly, so there is no encysted metacercaria stage in a second intermediate host. Adult schistosomes live in the vascular system of their definitive hosts, and are typically slender and elongate in form. Their predilection for a life in the blood stream has been associated with the presence of distinctive double or even triple unit membranes covering the tegumental surface [3]. Schistosomes share this peculiarity with two other prominent fluke families that are also usually found in the vascular system and that are also called 'blood flukes', namely the Sanguinicolidae of fishes and the Spirorchiidae of turtles [3]. An additional noteworthy attribute of schistosomes, unlike the other blood flukes, is that they are dioecious, often with a marked degree of dimorphism between male and female worms.

A desire to understand the unusual biology of schistosomes is further justified by the fact that schistosomiasis remains common in the developing world and has

been significantly underestimated in its impact on causing human morbidity, in what King *et al.* [4] have referred to as a 'silent pandemic'. Some of the salient features of schistosome biology are examined here, starting with a hypothesis of phylogenetic relationships among 13 of the named 15 [1,5] genera of schistosomes. Specimens of several genera have become available for such analysis only within the past few years. The phylogeny is then used to explore the origins of dioecy and the diversification of the schistosome body form, including the development of sexual dimorphism.

The emerging schistosome family tree

Box 1 provides a brief chronology of events in deciphering the evolutionary relationships among schistosomes. The phylogenetic tree presented in Figure 1 emphasizes diversity across the Schistosomatidae by including representatives of all named schistosome genera (except *Jilobilharzia* and the recently described *Allobilharzia* [5]), three as yet undescribed but genetically distinct schistosomes known only from cercariae, and freshwater and marine spirorchiid and sanguinicolid outgroups. This tree, as with any other, is a hypothesis, with addition of more species and more sequence data always desirable goals.

The topography of this tree indicates that the crocodile blood fluke *Griphobilharzia amoena*, originally described as the sole representative of the schistosome subfamily Griphobilharzinae [6], nests with strong support within the freshwater spirorchiids [7], so it is likely to be an aberrant member of the Spirorchiidae that has colonized a crocodylian. The remaining schistosomes comprise a monophyletic group, exclusively parasitic in endothermic vertebrates (birds and mammals) [7], with spirorchiids from marine turtles forming the sister group to the schistosomes [8]. The failure to retrieve *G. amoena* as a basal schistosome is important because: (i) the hypothesis that extant schistosomes, including those in modern archosaurs (birds), arose directly from blood flukes in ancestral archosaurs (crocodylians are extant representatives of an ancient archosaur lineage) is not supported; and (ii) it alters previous interpretations [9] of several aspects of schistosome evolution, including the emergence of dioecy.

As with previous studies [7,8,10–12], the tree provides strong support for four major schistosome clades: the AO clade (*Austroilharzia* and *Ornithobilharzia*), the BSO clade (*Bivitellobilharzia*, *Schistosoma* and *Orientobilharzia*), the

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Box 1. Unraveling evolutionary relationships in the Schistosomatidae – a chronology

This effort, very much 'a work in progress', will be further enlightened by additional information forthcoming from schistosome genome projects, by more extensive comparative sequence and morphological databases, and by further collecting efforts to reveal schistosome diversity before it disappears forever. An overview of the currently recognized taxonomic scheme of the Schistosomatidae, and a history of its development, is discussed by Khalil [18]. Below are represented some of the major developments that have been helpful in revealing relationships across the family, particularly among genera of schistosomes.

1983: Short [22] provided a synthetic overview of the sex chromosomes among species of *Schistosoma* and several other genera of schistosomes.

1984: Carmichael (A.C. Carmichael, PhD thesis, Michigan State University, 1984) provided the first detailed morphologically based phylogenetic analysis that incorporated all genera of schistosomes recognized at that time, and, in agreement with several previous authors, named spirorchiids as sister group to the schistosomes.

1986: Walker *et al.* [49] ushered in the molecular era by using restriction fragment length polymorphisms with the rRNA gene complex to assess relationships among *Schistosoma* species.

1991: Platt *et al.* [6] described *G. amoena* from the Australian freshwater crocodile – the first schistosomes described from other than a bird or mammal – and erected a new subfamily, the Griphobilharzinae, for it. Basch [1] provided an insightful review of schistosome diversity, with an emphasis on schistosome reproductive biology and its various modifications among different species.

1992: The use of sequence-based studies of relationships among schistosomes was introduced by Després *et al.* [50], who examined ITS2 rDNA and mitochondrial 16S rDNA sequences in *Schistosoma*.

1995: Després *et al.* [47] provided ITS2 sequence data for *S. hippopotami*, and with it new insights into the evolution of *Schistosoma*, highlighting the need for more inclusive sampling.

1997: Rollinson *et al.* [30], with an emphasis on *Schistosoma*, provided an extensive overview of the first applications of molecular approaches to schistosome phylogenetics, which affirmed the close relationships among members of the four species groups available for study up to that time. Platt and Brooks [14] provide an overview of schistosome diversity, including various hypotheses for relationships among genera, and a valuable discussion of the origin of dioecy and colonization of the venous system.

2000: The morphological characters used by Carmichael, along with additional available molecular information, were combined by Morand and Müller-Graf [9] to develop a supertree for the schistosomes. Important insights were provided regarding the origins of dioecy and trade-offs between the development of the gynecophoric canal and numbers of testes in male schistosomes. Snyder and Loker [10] provided the first sequence-based schistosome phylogeny to incorporate a broad representation of schistosome genera (ten of 14),

based on 1100 bases of 28S rDNA, and used both sanguinicolid and spirorchiid outgroups. They proposed an Asian origin for *Schistosoma*, a view independently developed by Hirai *et al.* [31] based on chromosome data and supported by mitochondrial gene order changes within *Schistosoma* noted by Le *et al.* [51].

2001: Zhang *et al.* [52] provided an additional specimen of *Orientobilharzia* and concluded that more study was needed regarding the geographical origin of *Schistosoma*. Blair *et al.* [16] provided an overview of schistosome evolution with a detailed discussion of the ability of schistosomes to add new hosts, emphasizing the ability of schistosomes to extend to new snail hosts, or to undergo long-range switches into different gastropod lineages.

2002: Attwood *et al.* [53] provided specimens of rare Asian mammalian schistosomes, permitting new insights on *Schistosoma* species groups and the phylogeography of Asian mammalian schistosomes. Agatsuma *et al.* [54] provided specimens of *S. incognitum* and showed that the traditionally recognized *S. indicum* species group is not monophyletic.

2003: Olson *et al.* [2] presented an overview of digenean phylogenetics and classification, again verifying spirorchiids as sister to schistosomes. Lockyer *et al.* [11] provided a more extensive sequence database using complete 18S rDNA, partial 28S rDNA and cytochrome oxidase sequences (~7000 bases) for the same ten schistosome genera and for 17 of 21 known species of *Schistosoma*, using a sanguinicolid outgroup. This phylogeny was also examined with respect to morphological characters, host associations and biogeography, and an Asian origin for *Schistosoma* was supported. Morgan *et al.* [15] provided specimens and sequence data for rare African *Schistosoma*, identified a distinct *Schistosoma* lineage with cercaria with long tail-stems, showed that the traditionally recognized *Schistosoma mansoni* species group is not monophyletic, and discussed the role of hippos in schistosome evolution.

2004: Snyder [8] developed a molecular phylogeny based on ~3000 bases of both 18S and 28S rDNA that incorporates three spirorchiid genera from marine turtles and five genera from freshwater turtles, as well as other diplostomid outgroups, and that indicated that spirorchiids from marine turtles form a group basal and sister to the schistosomes. Agatsuma *et al.* [55] provided sequence information for the first time regarding the elephant schistosome genus *Bivitellobilharzia*.

2005: Brant and Loker [7] obtained specimens of *G. amoena* and showed that it is probably a spirorchiid, and not a schistosome, with implications for schistosome evolution and systematics.

2006: Additional specimens of rare schistosomes, such as *Macrobilharzia* and as yet undescribed schistosomes, were provided by Brant *et al.* [12], and more extensive mitochondrial gene order studies published by Littlewood *et al.* [56] confirmed a major gene order change within *Schistosoma* and supported the 'out of Asia' hypothesis for the origin of the genus.

SH clade (*Schistosomatium* and *Heterobilharzia*) and the BTGD clade (*Bilharziella*, *Trichobilharzia*, *Gigantobilharzia* and *Dendritobilharzia*). The position of *Macrobilharzia*, and the AO and HS clades, has been problematic, although the application of Bayesian methods using mixed models (accommodating data heterogeneity) for each gene used provides good support for the placement shown and for improved resolution among deeper branches.

In agreement with Olson *et al.* [2] and Snyder [8], the basal schistosomes in this tree are *Austrobilharzia* and *Ornithobilharzia*, parasites of marine birds and snails, compatible with the suggestion that schistosomes arose after a marine turtle blood fluke colonized marine birds, and that schistosomes later transitioned to freshwater life cycles and colonized mammalian hosts [8]. Because birds and mammals have been separate lineages for at least 350 million years [13], schistosomes have

colonized members of two distantly related endothermic host groups. Adult schistosomes have several features that justify their description as 'vascular system specialists' [14], yet neither their evident specialization nor the intimate contact they endure with the vertebrate immune system have prevented them from colonizing new hosts [7]. Consequently, the overall pattern of diversification of the family does not mirror the diversification of a particular vertebrate host group. However, some schistosome lineages do show evidence of having diversified within particular host lineages, such as *Bivitellobilharzia* with elephants or a related group of two or three putative *Schistosoma* species with the hippopotamus [15]. A more complete understanding of speciose genera such as *Trichobilharzia* might eventually provide stronger evidence for a role of cospeciation in schistosome diversification.

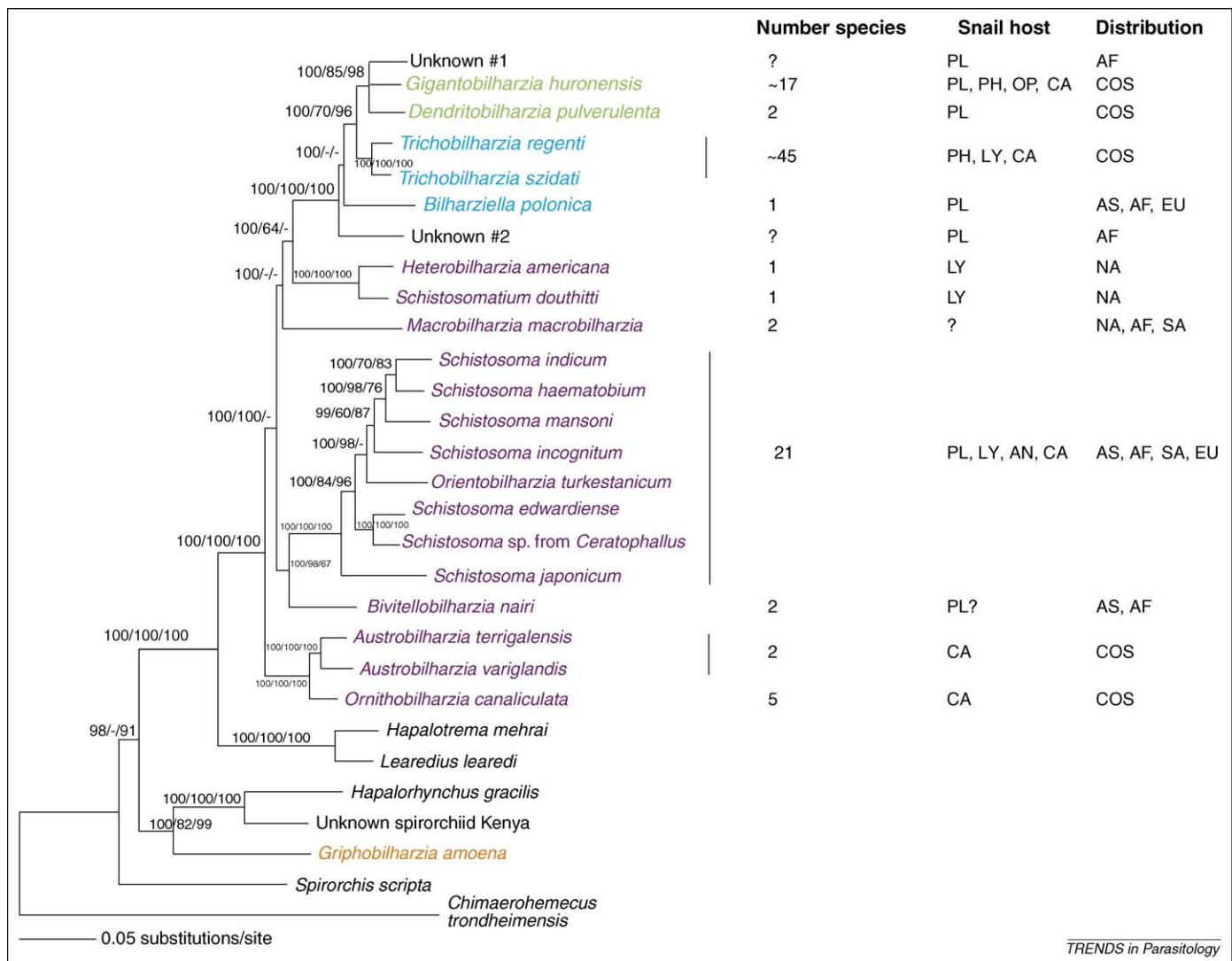


Figure 1. Phylogenetic reconstruction of the family Schistosomatidae [7,12]. Members of this family are highlighted based on traditional taxonomy of the subfamilies (green: Gigantobilharziinae Mehra, 1940; blue: Bilharziellinae Price, 1929; purple: Schistosomatinae Stiles and Hassall, 1898; orange: Griphobilharziinae Platt, Blair, Purdie and Melville, 1991). Following the tree are three columns: number of species in each genus, the snail intermediate hosts (PL = planorbid, PH = physid, LY = lymnaeid, AN = ancyliid, OP = opisthobranch, CA = caenogastropod) and geographical distribution (NA, North America; SA, South America; AS, Asia; AF, Africa; EU, Europe; COS, cosmopolitan), respectively. Analyses were computed with maximum likelihood (ML), minimum evolution (ME) and maximum parsimony (MP) implemented in PAUP [70], and Bayesian algorithms were applied with MrBayes 3.1 [71] for a combined dataset of 18S [1782 base pairs (bp)], 28S (3813 bp) rDNA and cytochrome oxidase 1 (728 bp) mitochondrial DNA. Outgroups were members of Sanguinicolidae (*Chimaerohemecus trondheimensis*) and Spirorchidae (marine *H. mehrai*, *L. learedi* and freshwater *H. gracilis*, *G. amoena*, *S. scripta*) [2,8,12] (also see A.C. Carmichael, PhD thesis, Michigan State University, 1984). For the MP analysis (stepwise addition, 500 reps, TBR branch swapping). For the ML and MP analyses in PAUP, the model [GTR + I + G] was selected by Modeltest [72], and a heuristic analysis was performed (stepwise addition, 500 reps for ME, 5 reps for ML, TBR branch swapping). For the Bayesian analyses, the dataset was divided into five partitions: 18S, 28S (Nst = 6 rates = gamma ngammacat = 4) CO1 codon1, CO1 codon2, CO1 codon3 (Nst = 6 rates = invgamma ngammacat = 4) followed by unlink shape = (all) pinvar = (all) statfreq = (all) revmat = (all); prset ratepr = variable. Four chains were run simultaneously for 5×10^5 generations, trees sampled every 100 cycles, the first 2000 trees with preasymptotic likelihood scores were discarded as 'burnin'. The remaining trees were used to compute Bayesian posterior probabilities for each clade of the consensus tree. Bootstrap (200 reps) was computed for the ME analysis but not for ML because the computing time was unrealistic. Tree topologies were the same for the ML, ME and Bayesian reconstructions. The tree shown is from ML analysis. Three measures of branch support are indicated by Bayesian posterior probabilities, ME and MP bootstrap values, respectively (see Refs [7] and [12] for additional information on taxa accession numbers). A dash '-' indicates lack of nodal support. Note: recently, worms representing a new genus of schistosome, *Allobilharzia*, were found in a swan. This genus is aligned, based on internal transcribed spacer (ITS) sequence, to the BTGD clade as basal to *Trichobilharzia* [5].

With respect to snail hosts, there are some schistosome lineages that are confined to a particular snail genus or family, such as members of the *Schistosoma haematobium* species group to *Bulinus*, or of North American mammalian schistosomes to lymnaeids (Figure 1). Nonetheless, the tree suggests that there has been considerable host switching with respect to snails. Schistosomes have colonized at least three major gastropod lineages, the Opisthobranchia, the Caenogastropoda and the Pulmonata, all of which have been distinct for at least 300 million years [16]. On more than one occasion, even within a schistosome

genus (Figure 1), switches appear to have occurred from one major gastropod lineage to another. In *Gigantobilharzia*, a genus that is otherwise transmitted by freshwater snails, one member has even secondarily colonized marine snails [17].

Another implication of the tree in Figure 1 is that there is more to be revealed regarding the global diversity of schistosomes: some cercariae recovered from African snails have sequences that are not closely matched by any of the 14 genera for which sequences are available [5,11,12]. As suggested previously [10,11], the emerging picture of

phylogenetic relationships among schistosomes suggests that several revisions in more traditional taxonomic schemes [18] are in order.

Dioecy and the connection to endothermy

The vast majority of all platyhelminth species are hermaphrodites, yet dioecy has arisen rarely among 'turbellarians' (*Kronborgia*), cestodes (mostly Dioecocestidae) and digenetic trematodes (some didymozoids, and schistosomes) [19,20]. Here, the focus is on schistosomes and why they are dioecious.

That schistosomes spring from hermaphroditic ancestors is illustrated by the tendency of schistosome adults occasionally to exhibit secondary hermaphroditism [1]. Such worms are usually functionally male with some female characteristics, although the female organs are not fully functional. Also, the potential for dioecy is noted in the closest relatives of the schistosomes, the spirorchiids, most notably with *Griphobilharzia* [6]. Further study is needed to determine if this peculiar worm is a true gonochorist or a hermaphrodite with male and female function not fully partitioned into separate individuals. Other spirorchiids might be androdioecious [21], potentially a step on a path leading to dioecy. Nonetheless, spirorchiids are otherwise hermaphrodites, and schistosomes are always gonochorists, a dichotomy that has stimulated considerable discussion [1,9,14,22–27].

The realization that schistosomes are exclusively parasites of endotherms, as suggested by Figure 1, helps to understand why this dichotomy exists. Endotherms have much higher metabolic rates than ectotherms and consequently have higher rates of nutrient intake [13]. These nutrients are largely conveyed to the liver via the hepatic portal system, so there are obvious benefits of colonizing this habitat. Yet, this environment poses some considerable problems, including the frequently noted problem of moving against the flow of portal blood to reach oviposition sites in the mesenteric veins. A plausible explanation is that there was, on the one hand, a need for a filiform body shape to enable insinuation into the tiniest venules in the intestinal wall, to minimize the distance a released egg would have to travel before reaching the gut lumen [1,28], referred to by Platt and Brooks [14] as 'precision egg laying'. On the other hand, there was an apparent need for a muscular body, to enable the worms to move against the flow of portal blood to oviposition sites [1]. Because it would be difficult to incorporate both functions into a single body, a 'division of labor' between the sexes would be a strong force favoring dioecy [27]. Female schistosomes are significantly more slender, on average, than either male schistosomes (Figure 2) or adults of other blood flukes [14], and extreme filiform body shapes among digeneans are otherwise rare [29].

How was the transition from hermaphroditism to dioecy achieved in schistosomes? A hypothesis to explain how the heteromorphic sex chromosomes of schistosomes were derived from the chromosomes of spirorchiids has been proposed [22]. Although a chromosomal mechanism for sex determination is evident, and chromosome morphology offers unique insights into schistosome origins and

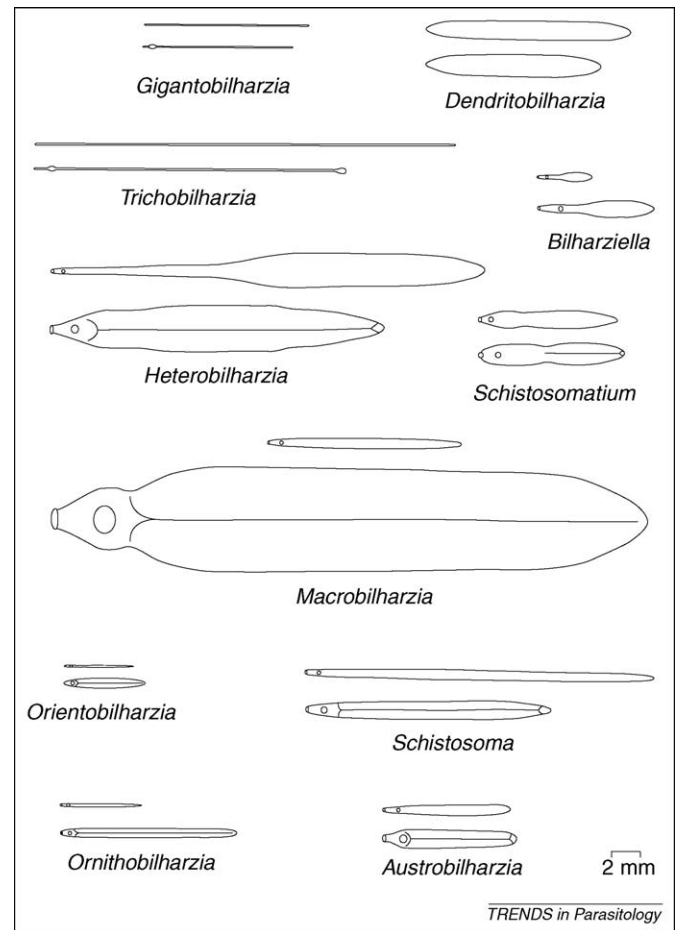


Figure 2. Outlines of adult female (top) and male (bottom) worms among the different genera of schistosomes. Worms were drawn to the same scale.

diversification [22,30,31], as noted by Basch [1], 'It is likely that gender differentiation in chromosomal morphology accompanied, but did not induce, sexual differentiation in ancestral schistosomes'.

As noted above, constraints with respect to oviposition would have been a strong impetus for sexual differentiation, possibly involving androdioecious intermediates [14]. Thus, a slender hermaphroditic form would have first been present, and as this slender form aged, it would have become thicker, backed out of oviposition sites, suppressed female function and then functioned as a male. Later, potentially via the action of antimale cytoplasmic factors, female worms might have developed [14].

Given the overriding importance of a slender body form for oviposition, an alternative explanation is the initial emergence of worms that suppressed male function (gynodioecy) [32]. The elimination of the male reproductive system would enable the worms to be more slender and more efficient in oviposition, thus giving them a reproductive advantage. Such females might coexist with hermaphroditic individuals for a while but the advantage of hermaphrodites persisting becomes less clear, given the superior egg-laying abilities of females, and exclusively male forms would then have been favored. The greater likelihood of secondary hermaphroditism appearing in male than female worms [1] is consistent with the idea that distinct females have existed for longer than males,

in support of a 'female first' scenario for the origins of schistosome dioecy.

Why are some schistosomes dimorphic, and some not?

The genera that occupy the more basal positions in the tree in Figure 1 conform to the standard view of adult schistosome morphology: a relatively stout male with a well-developed gynecophoric canal that embraces a far more slender female worm (Figure 2). Normally, one female is found with one male [1], although polygamy has been observed, as with *Austrobilharzia variglandis*, where a male embraces from 3–8 females [33]. Although such dimorphic schistosomes can and do change partners [34,35], the formation of pairs is indicative of the need for intimate and ongoing associations between males and females. The male provides the female with one or more stimuli, probably including nutrients but still not fundamentally understood, that favor her full reproductive development and ultimately her ability to produce eggs [36]. Other important functions of muscular males are to assist the nonmuscular females in feeding on blood, and in reaching oviposition sites, particularly important in larger-bodied hosts, where the distance from the liver to the oviposition site is considerable [1].

By contrast, in other schistosomes, the gynecophoric canal is much reduced or absent (Figure 2). The North American species *Schistosomatium douthitti* is unique among the mammalian schistosomes in having a reduced, flaplike gynecophoric canal that occupies only ~60% of the length of the body of the male. Particularly in the avian BTGD clade, not only is the gynecophoric canal reduced or completely absent, but the extent of dimorphism is also dramatically reduced, and a much greater range in adult body shape is seen. Furthermore, what can be gleaned from the literature indicates that worms of this clade do not exhibit long-term associations between the sexes: the males and females are not recovered from hosts as intimate pairs [37–39]. There is no indication that BTGD females need prolonged stimulation from males to maintain their reproductive organs in a functional state, nor is it likely they are assisted by males in moving against the flow of blood to their oviposition sites: the males are as slender as the females.

Why are some schistosomes markedly dimorphic, with heavy-set males with well-developed gynecophoric canals in which male-dependent females are found, whereas other species are less dimorphic, with small or absent gynecophoric canals and more independent females? The former situation is consistent with circumstances in which males potentially compete for, acquire and then guard females in a gynecophoric canal, and so prevent them from copulating with other males [9]. Larger males can use their size advantage to take possession of females from smaller males [34,35], although the details of how this fascinating process is accomplished remain to be determined. Across schistosome genera, an inverse correlation between development of the gynecophoric canal and the number of testes has been noted [9], and although the impact of testes numbers on the actual capacity to produce sperm remains to be investigated, the idea is that males of species with well-developed gynecophoric canals invest heavily in

sequestering females and thus avoid the need for extensive sperm production that would be required if all males had ready access to females and copulations. The genital pore of a female held by a male with a well-developed gynecophoric canal would at times be exposed, such as when she oviposits. Mating opportunities for other males might thus exist at or around this time but they would seem to be minimal, given the positional advantage of the male actually embracing the female. Given the lack of direct *in vivo* observations, a role for females in selecting males cannot, as yet, be ruled out but it seems unlikely that they could resist males, given their relative lack of muscle.

Three kinds of circumstances would seem to favor sexual dimorphism with heavy-bodied males with well-developed gynecophoric canals forming intimate pairs with females: (i) conditions of male-biased sex ratios, which are typical of many schistosomes [1,9,40], where males would compete for females; (ii) occupation of stable, long-lived habitats, where female sequestration with associated exclusive rights to fertilization would be favored because the prospects for continued egg production would be good; and (iii) colonization of large-bodied hosts, where the difficulties of a slender female moving against the flow of hepatic portal blood are great.

In the case of the BTGD clade, where sexual dimorphism and gynecophoric canals are reduced or absent, there is no evidence of guarding of females through the formation of lasting pairs, and the large numbers of testes are believed to be indicative of extensive sperm production [9]. The relative lack of dimorphism in the members of this clade might, in part, be explained by the following quote: 'If a parasite has as hosts a migratory duck and a snail that occurs on the duck's breeding ground but not on its wintering ground, it is clearly advantageous to the parasite to complete its maturation quickly so that viable eggs will leave the birds while they are still in the habitat of suitable snails'. [37] The implication is that the period of useful egg production is temporally limited. For *Trichobilharzia*, which is better known than other members of this clade, maturation occurs very quickly, generally within two weeks [37,41–44], most of the eggs are produced over a period of about a month [43,44], and then most of the adult worms die. Some egg production might continue after the initial pulse of egg production but it is sporadic and accounts for no more than 5% of the total egg output [43]. Under these circumstances, most females might head directly for oviposition sites deep in the intestinal mucosa. In such positions, they cease to feed on blood and, because their anterior ends lie within a few cells of the intestinal lumen, they are at risk of being expelled from the intestinal wall with mucosal sloughing [43]. Also, they might not have additional opportunities to mate. Thus, the presumed large sperm-producing capacity of *Trichobilharzia* males might reflect the need to inseminate adequately females they might never again encounter. Also, the advantage to a male of guarding a female under such temporal constraints is not obvious.

A final comment about *Trichobilharzia* and *Gigantobilharzia* is that for worms of either sex, despite having no or poorly developed suckers and slender

anatomy, they are capable of moving against the flow of portal blood and entering the mesenteric venules. The implication is that the presence of heavy-bodied muscular males *per se* might not have been an essential prerequisite for the emergence of dioecy. They might be able to achieve this because their avian hosts are relatively small. A similar explanation might apply to *S. douthitti*, a parasite of rodents. Females of *S. douthitti* can and do migrate from the liver to the mesenteric venules without males present,

although males also pair with females and assist them to this location [45].

Unlike most other mammalian schistosomes, *S. douthitti* females are also able to mature without males present, and this is the only schistosome known to date that can produce eggs parthenogenetically that will hatch and yield miracidia infective to snails [45,46]. Here again is evidence of adult reproductive strategies that are modified by environmental constraints. Because their rodent hosts

Box 2. Abiding schistosome mysteries

There is much about schistosomes yet to discover, and by contemplating the full breadth of schistosome diversity, we gain perspective and insight into both basic and applied aspects of their biology. Some examples of the fundamental questions that they continue to pose are listed below:

- What is the full extent of schistosomes diversity? Based on classical descriptions, the number of known schistosome species ranges from ~85–100 [1,57,58]. Because of their medical relevance, the schistosomes as a group are much better known than most flatworms but recent collecting efforts indicate there is more schistosome diversity, both in mammals [15] and especially in birds [12], yet to discover. Some huge geographical areas, such as South America and southern Asia, have not been adequately surveyed, and major surprises probably remain. It seems probable that the majority of schistosome species and genera will eventually be recovered from birds, with significant additions to the BTGD clade. The continued application of molecular approaches will help to refine and revise our current understanding of schistosome diversity, with a major challenge being to relate the results of modern investigations to classical descriptions from the literature. Further study of spirorchiiids and sanguinicolidis is also needed to help to provide a more complete picture of blood fluke evolution in general.
- Why do some species, such as *S. japonicum*, have a broad range of epidemiologically relevant hosts, whereas others, such as *S. haematobium*, have adopted transmission cycles that more heavily favor a single definitive host, humans in this case? This is a restatement of Combes' [28] original conjecture as to why schistosomes in Africa that have colonized humans are represented as distinct species, whereas their Asian counterparts have not formed distinct species more dependent solely on humans. Is it indicative of different immune evasive capacities among African and Asian schistosomes, of different transmission ecology, or does it reflect a longer association between humans and schistosomes in Africa [28]? As genome information becomes available for *S. japonicum* [59], an important comparison to be made is between the Taiwanese strain of *S. japonicum*, which does not readily colonize humans [60], and other strains of this species, which do. Such an analysis might reveal particular genetic factors essential for colonizing humans. It will then be of interest to determine if members of the *S. indicum* group which, perplexingly, have not colonized humans on the Indian subcontinent [61] lack such factors.
- Why have some schistosomes abandoned the usual habitat within the hepatic portal system for more exotic locales? Most schistosome species inhabit the hepatic portal system and exploit the mesenteric veins surrounding the intestine as their primary oviposition site, although conspicuous deviations from this pattern appear in various lineages in the schistosome tree. *S. haematobium* is well known for its predilection to oviposit in the walls of the bladder and ureters, and *Schistosoma nasale*, along with several species of *Trichobilharzia* [42,62], has adopted the nasal mucosae as their primary oviposition sites. Also, as discussed in the text, two schistosomes have colonized the arterial system, *D. pulverulenta* occupies the arterial system of inland diving and sea ducks (F. Vande Vusse, PhD thesis, Iowa State University, 1967), and *S. hippopotami* is frequently recovered from the right heart and pulmonary arteries of the hippopotamus [48]. It seems likely that interspecific competition has favored such habitat shifts. For example, the ancestors of *S. haematobium* coexisted with several

other schistosome species in Africa, including both close relatives and more-distantly-related species such as *S. mansoni*. There would be a strong selective pressure to avoid pairings that would not result in production of viable offspring [1,63]. This selection could result in a means to distinguish between productive and unproductive mates [63] and/or the avoidance of unproductive partners by occupying different oviposition habitats. Similar considerations could apply to both *S. nasale* with potential competitors *S. indicum* and *S. spindale* in India, and with species like *Trichobilharzia regenti*, a nasal schistosome that might have to contend with several other *Trichobilharzia* species in European birds [64]. Another possibility is that the acquisition of novel oviposition sites might avoid problems of gaining access to good intestinal oviposition sites, or assures access to sites that have not already been compromised by prior oviposition activities [1].

- Why are avian hosts seemingly more able to develop strong immunity to schistosomes than are mammals? It has been noted that once a duck has been infected with *Trichobilharzia ocellata*, the infection runs a short course of a few months, and thereafter the animal is solidly immune to challenge infection [43,65]. What is different about the nature of the immune response provoked by these schistosomes from what is engendered by mammalian schistosomes? One possibility is that the species of ducks used are not completely adequate hosts, and worm reproduction consequently declines, comparable to what is observed with *S. mansoni* in rhesus monkeys [66]. Or does the answer involve the fundamentally different mating systems or life histories exemplified by these two groups of schistosomes? For example, do short-lived adult schistosomes lack essential immune evasive capacities of their more long-lived counterparts? Use of microarrays to compare the responses of long-lived and short-lived species to the immune responses of their respective hosts would be instructive, as would be attempts to determine if components of the successful avian response, particularly antibodies, have any activity against mammalian worms. It would be gratifying to have an immunological study of an obscure schistosome–avian host system point the way to developing a more cogent immune response to worms in humans.
- How can historical patterns suggestive of abundant host switching with respect to snail hosts be reconciled with the results of many unsuccessful experimental attempts [67] to extend the normal host range of schistosomes into snails of different families, or even into confamilial genera? Do phylogenetic studies inform us of host-switching events that are simply exceedingly rare but that, given enough time, will occur, or has the capacity of schistosomes to switch snail hosts diminished over time? Given that switches with respect to the snail host are prominent even in a putatively derived genus such as *Gigantobilharzia* and within a single lineage of *Schistosoma* [15], it would seem that the capacity to make such switches has not been lost. Mechanisms for how such switches occur, given the host specificity of particular schistosome species, are obscure at best but might involve situations where infection with digenetic species adapted to the host to be colonized are immunosuppressive and thus facilitate transfer of schistosome colonists to the new host [68,69]. An example of how this might occur is provided by *A. terrigalensis*, which is apparently only able to colonize snails already harboring digenetic trematodes, and is specialized for suppressing the development of the prior colonists [69].

are relatively short lived, it might be expected that *S. douthitti* worms mature quickly, and they do, having the shortest prepatent period (as short as 28 days) of any known mammalian schistosome. The potential for long-term sequestration of females by males would seem to be less because of the short life span of the host, a factor that would be expected to favor reduction of the gynecophoric canal. Short host life spans would also favor the capacity of females to move independently to oviposition sites and to be able to produce eggs parthenogenetically, if needed. This is because some females might find themselves in hosts lacking males, hosts for which there is little prospect of acquiring male worms before the short life span of the host is spent.

Exceptions to prove the rule?

One of the most peculiar of schistosomes is *Dendrobilharzia pulverulenta*, a relatively derived member of the BTGD clade, and an inhabitant of the arterial system of ducks. Eggs of this species are carried *with* the flow of blood to capillary beds around the large intestine (F. Vande Vusse, PhD thesis, Iowa State University, 1967). They have no need to oviposit in the most slender of venules – in fact, they live and oviposit in relatively large arteries. Thus, there is no premium on having a slender female in this species. Interestingly, both sexes of *D. pulverulenta* are similar in size and have more typical fluke body shapes, suggestive of a relaxation of the need for filiform body shape seen in other schistosomes.

The other schistosome reported from the arterial system is *Schistosoma hippopotami*. This distinct and unusual species [47] is found in major arteries and the heart, as well as in major veins and the hepatic portal system, of the hippopotamus [48]. The normal oviposition sites and the mode of egress of eggs from the body are not known for this species. Although adult morphology might be somewhat plastic in response to different environmental conditions, males of *S. hippopotami* recovered from major arteries are stout, with extraordinarily large suckers. They have a well-developed gynecophoric canal in which the female is held. Females are unusual for being shorter than the male, again suggesting that the unusual high blood velocity environment of this species has selected for divergent adult morphology [48].

The value of a comparative perspective

The ongoing effort to reveal the full spectrum of schistosome diversity, and then to integrate this information into improved hypotheses for relatedness among the members of the family, is an intrinsically interesting process but also provides a valuable perspective from which to understand basic schistosome biology, including the species that are of medical or veterinary importance. For example, by knowing that schistosomes are parasites of endotherms and not crocodiles, we can better understand why schistosome evolution took a different course from that followed by their spirorchiid forebears, a course that can be characterized as a 'dioecious radiation'. It is also helpful to realize that not all schistosomes are the same with respect to their degree of sexual dimorphism, proclivity to form pairs and their mating systems, and that the constraints imposed by

unique transmission cycles or habitats will strongly affect their biology. As noted in Box 2, a comparative perspective also helps us to appreciate how much we have yet to learn about these fascinating worms and how knowledge about some of the oddballs in the schistosome world can illuminate our attempts to understand and control schistosomiasis.

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