Parasite life-cycle studies: a plea to resurrect an old parasitological tradition

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Abstract

Many helminth taxa have complex life cycles, involving different life stages infecting different host species in a particular order to complete a single generation. Although the broad outlines of these cycles are known for any higher taxon, the details (morphology and biology of juvenile stages, specific identity of intermediate hosts) are generally unknown for particular species. In this review, we first provide quantitative evidence that although new helminth species are described annually at an increasing rate, the parallel effort to elucidate life cycles has become disproportionately smaller over time. We then review the use of morphological matching, experimental infections and genetic matching as approaches to elucidate helminth life cycles. Next we discuss the various research areas or disciplines that could benefit from a solid knowledge of particular life cycles, including integrative taxonomy, the study of parasite evolution, food-web ecology, and the management and control of parasitic diseases. Finally, we end by proposing changes to the requirements for new species descriptions and further large-scale attempts to genetically match adult and juvenile helminth stages in regional faunas, as part of a plea to parasitologists to bring parasite life-cycle studies back into mainstream research.

Introduction

Tens of thousands, if not hundreds of thousands, of helminth parasites have complex life cycles, in which different life stages must infect different host species in a particular sequence in order to complete a single generation. This is also true of large numbers of other parasites, from unicellular eukaryotes (notably apicomplexans such as *Plasmodium* and *Toxoplasma*) to arthropods (pentastomids, pennellid copepods and several tick species). These life cycles may include two, three or even four host species, and they are defining features of parasite species. Thus, a juvenile worm inside an intermediate host is to its adult in the definitive host what an aquatic tadpole is to a terrestrial toad, or a drab caterpillar to a strikingly beautiful butterfly. One life stage cannot be fully understood without knowledge of previous or subsequent stages, and of their respective host habitats.

Some well-studied species provide good examples of the intricacies of helminth life cycles and the connections between their life stages. For instance, the cestode *Schistocephalus solidus* has a three-host life cycle involving piscivorous birds, such as the grey heron *Ardea cinerea*, as definitive hosts (Clarke, 1954). Adult worms release their eggs in water with host faeces, where they hatch as coracidium larvae before being accidentally ingested by the parasite's first intermediate host, i.e. planktonic copepods of the genera *Cyclops* or *Macrocyclops*. Once inside, the coracidium settles in the haemocoel of the copepod, develops into a procercoid and induces the copepod to swim erratically (Urdal *et al.*, 1995; Wedekind & Milinski, 1996). The copepod's aberrant behaviour may affect its susceptibility to predation by three-spined sticklebacks, *Gasterosteus aculeatus*, which act as the cestode's second intermediate host. Inside the stickleback, the parasite develops into a plerocercoid juvenile within the host's body cavity, grows

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rapidly to a mass approaching that of the fish host (Barber, 2005) and induces behavioural changes in the fish that seem to increase its risk of predation by bird definitive hosts (Barber et al., 2004), thus favouring the completion of the cycle. As a second example, consider the trematode *Diplostomum spathaceum*. It also has a three-host life cycle, with adult worms living in the digestive tract of piscivorous birds, mainly gulls, *Larus* spp. (Chappell et al., 1994). Their eggs are released in host faeces and hatch in water into free-swimming ciliated miracidia, which seek and infect their first intermediate host, the snail *Lymnaea stagnalis*. In the snail, the miracidium develops into a mother sporocyst, which then produces second-generation daughter sporocysts through asexual multiplication, which in turn will produce large numbers of fork-tailed cercariae for most of the remaining life of the snail host. Cercariae exit the snail to seek the second intermediate host, which can be any of a range of small freshwater fishes. They use a specific set of chemical cues from fish mucus and skin to recognize and penetrate suitable hosts (Haas et al., 2002). During penetration, they ditch their tail and then proceed to migrate toward the eyes of the fish host by following a series of tissue-specific cues (Haas et al., 2007). Once in the eye, they settle in the lens, become metacercariae, and gradually induce cataracts through excretory products and direct damage to the lens (Karvonen et al., 2004), impairing the host's vision and presumably enhancing the parasites' chances of reaching their bird definitive hosts via predation on the fish host.

The above examples illustrate how strongly interconnected the various life stages truly are, and how a parasite species can only be understood as the whole life cycle. Although visualized as the adult form, a species includes all stages of life, each representing a different phenotypic expression of the same genome and separated from each other by sudden ontogenetic transitions. Comparative studies have highlighted the linkages among life stages. For instance, across different species, the body size achieved by one life stage in one host is proportional to that achieved by the preceding life stage in a different host, among both trematodes (Loker, 1983; Poulin & Latham, 2003) and acanthocephalans (Poulin et al., 2003). Also, among trematodes, the characteristics of cercariae, such as relative tail length, produced in one host depend on the identity of the next host in the life cycle (Koehler et al., 2012). Natural selection cannot act separately on different life stages, and the life cycle as a whole should be considered as the unit of selection rather than adult individuals. Although this is not a new idea (see Bonner, 1993), its application to parasites with complex life cycles is rather new (Benesh, 2016).

Much of the recent interest in complex life cycles of parasites has centred on their evolution, that is, under what conditions the insertion of a distinct new life stage passing through an additional host is favoured by selection. Mathematical models have revealed that increases in life-cycle complexity that enhance the probability of transmission to the definitive host, opportunities for sexual outcrossing in that host, or lifetime fecundity should be under strong selection in many plausible situations (Brown et al., 2001; Choisy et al., 2003; Parker et al., 2003, 2015). Predictions from these models generally fit well

with empirical observations. For instance, genetic investigations have confirmed that adding a second intermediate host to the trematode life cycle can increase the genetic diversity of sexually reproducing adults in the definitive host (Rauch et al., 2005; Keeney et al., 2007), and phylogenetic reconstructions of life-cycle evolution indicate that this addition has been a recurring theme among independent trematode lineages (Cribb et al., 2003). Therefore, our conceptual understanding of how complex life cycles have evolved has recently taken a giant step forward.

In contrast, recent years have seen relatively few studies attempting to resolve the particular life cycles of the vast majority of helminth species that are known only from their adult stage, and for which juvenile stages have never been described and intermediate host species are still unknown. This sort of research on parasite life cycles peaked in the middle of the 20th century, especially the 1950s, 1960s and 1970s in Europe (Faltýnková et al., 2016) and North America (Scholz & Choudhury, 2014), which represented a golden age of research on life cycles. This period culminated in the publication of comprehensive synthetic monographs on helminth juvenile stages and/or partial life cycles (e.g. Voge, 1967; Šlais, 1973; Yamaguti, 1975; Combes et al., 1980), which are now out of print and not easily accessible. This research effort did not merely consist of studies identifying intermediate hosts of helminths, but also included many studies on parasite development, i.e. the length of time that eggs survive in the external environment, the time required for juvenile stages in intermediate hosts to become infective to the definitive host, or how long juvenile stages persist in intermediate hosts. Although basic research on life cycles and parasite natural history has persisted longer in some places, such as Russia (e.g. Galaktionov et al., 2006; Galaktionov, 2009; Prokofiev et al., 2011; Regel et al., 2003), it no longer seems to occupy a central place in the research programmes of most parasitologists.

The objectives of this brief review are: (1) to look back at recent historical trends in the study of parasite life cycles, specifically at efforts to identify all hosts used by particular parasites at their various life stages; (2) to discuss the various ways in which parasitologists may attempt to elucidate life cycles; and (3) to explore the many reasons why knowledge of full life cycles can benefit science. We focus mostly on helminths, but the general issues we address apply broadly to other parasite taxa.

Historical trends in life-cycle studies

Publication data can be used to provide a quantitative assessment of the relative effort allocated to elucidating parasite life cycles, and whether or not it has decreased over the past several decades. Here, we use two slightly different datasets to address these issues. Neither of them is totally perfect for this purpose, but together they paint a clear picture of how the study of life cycles is not keeping pace with the discovery and description of new parasite species.

The first dataset is that from Poulin & Presswell (2016), and comprises all 2366 species descriptions of trematodes, cestodes and nematodes published in either *Journal of Parasitology* or *Systematic Parasitology* in the 35-year period

from 1980 to 2014. A little over a third of these were not new species descriptions but re-descriptions of previously discovered species. In addition to recording various measures of taxonomic quality, the authors also noted whether or not at least one larval or juvenile stage (other than eggs) was also described morphologically, with at least one intermediate host identified. By convention, helminth species are named and described based solely on adult worms, therefore descriptions also characterizing larval and juvenile stages and the intermediate hosts in which they are found go beyond the minimum and make an effort to elucidate the species' life cycle, or at least part of it. The data show quite clearly that life-cycle information has long been, and continues to be, rarely included in species descriptions, particularly for cestodes (fig. 1). Although the number of species descriptions published annually is generally increasing in all three helminth groups, the proportion providing some life-cycle information remains small.

The second dataset was compiled via a search of the Web of Science™ for all papers describing helminth species published each year from 1970 to 2015. To quantify the effort put into finding and describing (based on adults only) new species, we used the keywords: 'new species' or 'n. sp.' or 'sp. n.' or 'new gen*' or 'n. gen' or 'gen. n.'. This was done separately for each of the four major taxa of parasitic helminths by adding search terms for trematodes (digene* or trematod* not monogen*), cestodes (cestod* or tapeworm*), nematodes (nematod* and parasit* not plant, to exclude free-living species and plant-parasitic species), or acanthocephalans (acanthocephal*). Many of the articles retrieved provided descriptions of more than one species, but here each article is given the same weight. We then performed a second search to reveal how many articles per year provided information on the life cycle of helminths, by searching the Web of Science™ with the taxonomic terms above and the additional term 'the life cycle of' in the title of articles only. This approach may lead to the erroneous inclusion of studies that mention life cycles without really elucidating them; however, a detailed examination of about 20% of all records returned by the searches indicates that this procedure is over 90% accurate at identifying true life-cycle studies. The patterns emerging from graphical analysis of this dataset are very similar to those obtained with the first dataset (fig. 2). The number of articles published annually that describe helminth species has risen sharply in recent decades for all helminth groups, whereas the number of articles characterizing helminth life cycles has remained invariably low (fig. 2). In other words, as parasite taxonomists have greatly increased their output of species descriptions, the parallel effort devoted to elucidating life cycles has become disproportionately smaller over time.

A similar but less precise survey of the amphibian taxonomic literature indicates that the inclusion of tadpole descriptions in published descriptions of new frog species is also relatively rare, though increasing in frequency in recent years (results not shown). This problem is therefore not unique to helminths with complex life cycles. With so little effort directed at resolving the life cycles of the huge (and ever growing) number of known helminth species, it is no wonder that so few life cycles are fully known. For example, of the almost 1000 known cestode species
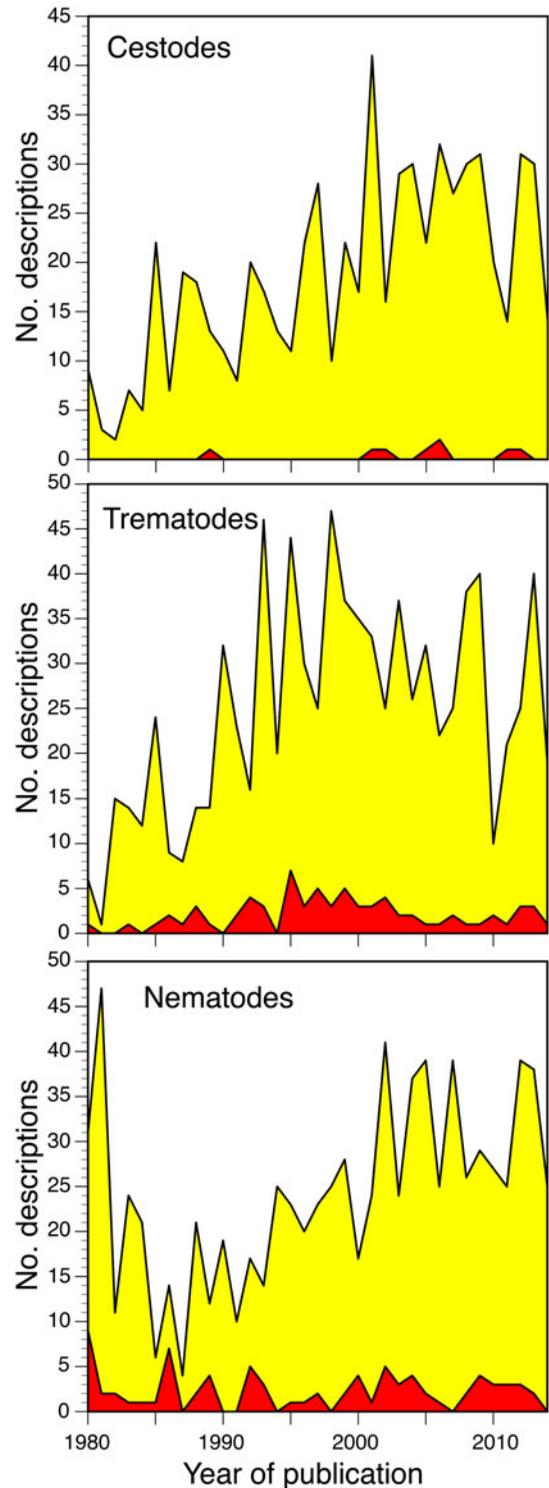

Fig. 1. Number of species descriptions per year (yellow area), and subset of those that characterize morphologically at least one larval or juvenile stage other than the egg and identify at least one intermediate host (red area). Data are shown separately for cestodes ($N = 651$ descriptions), trematodes ($N = 865$) and nematodes ($N = 850$). Data from Poulin & Presswell (2016).
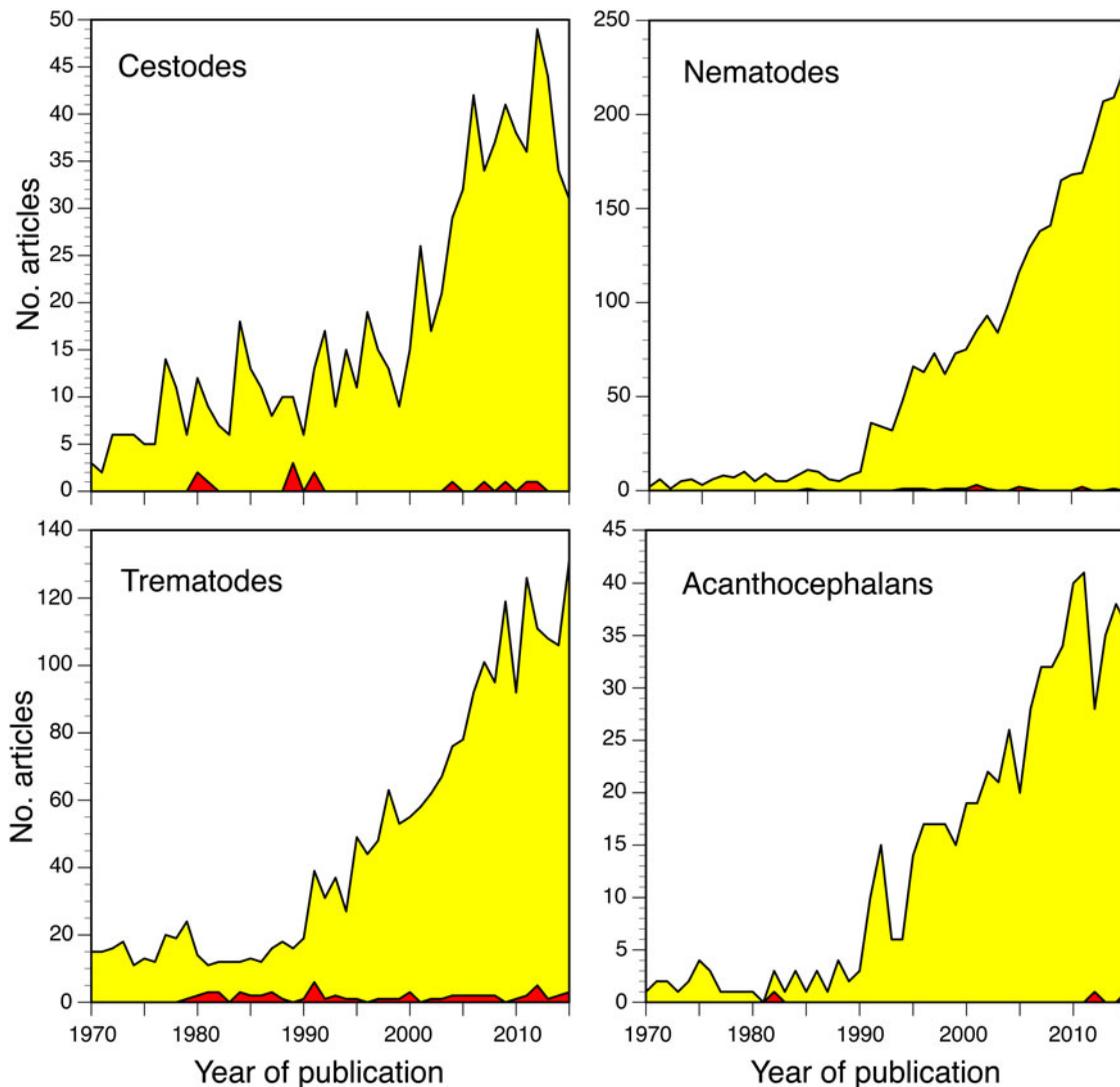
Fig. 2. Number of articles describing helminth species per year (yellow area) and number of articles per year investigating helminth life cycles (red area). Data are shown separately for cestodes, trematodes, nematodes and acanthocephalans, and are from a search of the Web of Science™ (see text for details).

parasitizing elasmobranchs (sharks, rays and skates) worldwide, complete life cycles have been established for fewer than five species (Sakanari & Moser, 1989; Caira & Jensen, 2014). Similarly, out of approximately 326 trematode species described and possibly another 1000+ to be discovered from fishes of the Great Barrier Reef (Cribb et al., 2014, 2016), full life cycles have been resolved for only four species (Pearson, 1968; Rohde, 1973; Downie & Cribb, 2011; Huston et al., 2016). Overall, it is estimated that full life cycles have been elucidated for fewer than 5% of all marine helminth parasites (Poulin et al., 2016). There are exceptions, of course. Due to a long history of research by many devoted parasitologists, our current knowledge of partial or full life cycles is relatively good for trematodes parasitic in European freshwater fish (Faltýnková et al., 2016). A single prolific

researcher can also make a difference locally, with an in-depth investigation of juvenile stages in intermediate hosts of a restricted geographical area (e.g. Cable, 1963). However, by and large, we know the full life cycle of only a tiny fraction of the global fauna of helminth parasites, and our efforts to improve this are at an all-time low.

How to elucidate life cycles

Life cycles can be resolved in many ways, which should be thought of as complementary approaches rather than mutually exclusive alternatives. Ideally, the end point would be the characterization of the morphology and basic biology (within-host migration and site selection, life span, etc.) of each life stage, the identification of all

intermediate, paratenic and definitive hosts, and the determination of the modes of transmission and infection connecting the different life stages. Rarely are all these accomplished, yet even partial life-cycle information is better than none.

The first, and simplest, way to elucidate life cycles consists of morphologically matching the juvenile and adult forms that are found in different hosts occurring in sympatry, i.e. in the same geographical location. This approach used to be widely employed in the early days of ecological parasitology, when the field had a stronger natural history component. It was then routinely integrated with the search for new parasite species from wildlife. For example, Van Cleave (1920) found cystacanths in amphipods, and was able to match them morphologically with adult acanthocephalans already described and known to infect local freshwater fish species. This approach is still in use (e.g. Dezfuli et al., 2012). However, except for taxa such as acanthocephalans and trypanorhynch cestodes, where diagnostic features remain unchanged from the juvenile to the adult stage, this method does not provide a very rigorous demonstration that juvenile and adult are one and the same species. Morphological similarity can also exist between different life stages belonging to related but different species. For this reason, this method of resolving life cycles has been largely abandoned.

The second approach to elucidate life cycles is through experimental infection of one or more hosts. This can provide crucial information when different life stages suspected of belonging to the same species are found in one locality, or when certain life stages cannot be found in nature and must be produced in the laboratory. Typically, juveniles found in an intermediate host are fed to the suspected definitive hosts or to members of a known definitive host species in which adult forms have been found. Alternatively, putative hosts are exposed to free-living infective stages. When naturally occurring host species cannot be used for experimental infections, they can be substituted by a laboratory model (such as a rat, chick or guppy), often immunosuppressed to facilitate infection and development of the parasite. Full or partial resolution of the life cycle can be achieved in this way. Morphological matching between specimens obtained through experimental infection and those recovered from naturally infected hosts then serves to confirm the conspecificity of life stages. Thus, if the adults experimentally grown from juveniles match the morphology of adults found in wild hosts, the two stages are shown to belong to the same species and the life cycle is assumed to be resolved. This has been a common approach in parasitological studies from the mid-20th century (e.g. Rendtorff, 1948; DeGiusti, 1949; Cable, 1953; Hoffman & Hundley, 1957) to the present (e.g. Ostrowski de Núñez, 2007; Alda et al., 2013; Al-Jahdali et al., 2015). One possible problem with this approach comes from the fact that conspecific helminths grown in different host species can show intraspecific, host-induced variation in morphology (e.g. Pérez-Ponce de León, 1995). Thus, adult worms grown in an experimental host may not quite match those recovered from naturally infected hosts. Nevertheless, experimental infections can generally achieve a higher level of proof than morphological matching of life stages recovered from naturally infected hosts, and are an important tool for life-cycle resolution.

The experimental approach may sometimes be impossible because of the logistical or ethical difficulties associated with infecting vertebrates with parasites in captivity. Growing adults in vitro using a culture medium that simulates conditions within the definitive host is one way to overcome these impediments to resolve life cycles (for a review, see Smyth, 1990). For instance, culture media that re-create conditions in the gut of birds have been used to grow in vitro adults of cestodes (Presswell et al., 2012) and trematodes (Presswell et al., 2014) to confirm the conspecificity of juvenile and adult forms and the transmission pathways linking them.

The third approach to resolve life cycles involves the genetic, usually combined with morphological, matching of juvenile and adult forms that occur in different hosts but in sympatry. This is the modern approach, made possible by the decreasing cost of DNA sequencing. Simply put, this approach consists of obtaining and comparing sequences of suitable genes, such as the mitochondrial cytochrome c oxidase subunit I (COI) or the internal transcribed spacers (ITS1 and ITS2), from two or more life stages. Perfect, or almost perfect, similarity of sequences indicates that the different life stages belong to the same species. Molecular data allow one to avoid the pitfalls associated with cryptic species, i.e. genetically distinct species of helminths that are morphologically indistinguishable (Nadler & Pérez-Ponce de León, 2011; Poulin, 2011a), which could otherwise confound attempts to solve life cycles. This approach has been used frequently to either elucidate life cycles, or confirm suspected life cycles, in particular helminth species (e.g. Bartoli et al., 2000; Pina et al., 2009; Randhawa, 2011; Li et al., 2012; Alcántar-Escalera et al., 2013; Jezewski et al., 2013; Selbach et al., 2015; Huston et al., 2016; Gonchar & Galaktionov, 2017). In fact, Blasco-Costa et al. (2016) have shown that from 2011 to 2015, the use of genetic data in trematode life-cycle studies has been the most common approach (approximately 65% of all trematode life-cycle studies), making this the method of choice to elucidate and/or confirm helminth life cycles.

Genetic matching can also be used on a larger scale, by obtaining and comparing gene sequences from multiple larvae and adults of a particular taxon, found in multiple host species within an area (Leung et al., 2009; Hernández-Orts et al., 2013; Leiva et al., 2016). This allows life-cycle resolution at the level of a particular food web or ecosystem. Jensen & Bullard's (2010) comprehensive study of elasmobranch cestodes provides a good example of this approach. These authors obtained sequence data from 25 species of adult cestodes from elasmobranch hosts in the Gulf of Mexico, as well as sequences from 27 larval cestode species recovered from teleosts, molluscs and crustaceans of the same area. Matches between the two subsets of sequences allowed some life cycles to be resolved; the large number of 'orphan' sequences also means that further host sampling will be necessary to complete the remaining life cycles (Jensen & Bullard, 2010). A similar effort is under way to match sequences from metacercariae and adult trematodes of fishes from the Great Barrier Reef (Miller et al., 2009; Downie & Cribb, 2011; T. Cribb, pers. comm.), an area boasting a hyper-diverse fauna of trematode species (Cribb et al., 2014, 2016) currently known almost exclusively from

their adult stage. Notwithstanding the effort of linking parasite life stages in their respective hosts via molecular data, simultaneous morphological characterization and collection of biological data associated with each parasite stage should not be neglected when using such approaches.

DNA sequencing can also allow matching of parasite stages from faeces of candidate definitive hosts in a geographical area; for instance, when a DNA sequence database is already available for local larval stages. Examination of faecal samples is carried out regularly in veterinary sciences to detect infection through observation of parasite eggs (e.g. Cringoli et al., 2010; Presswell & Lagrue, 2016). Nowadays, DNA isolated from eggs present in fresh stool samples can be sequenced and compared to a reference database of local parasites or to GenBank sequences. Alternatively, metabarcoding and metagenomic techniques, like those applied to study host diet from faecal pellets, can also be used to detect and identify parasites present in host stool samples (e.g. Srivathsan et al., 2015). To the best of our knowledge, these techniques are still rarely applied to helminths despite representing non-invasive means to obtain information on host–parasite associations. On the downside, information on morphology and biology of the parasites would likely be missed, and biases towards the most common parasites present in the gut may arise, as happens with prey items in diet studies using these techniques.

Put together, the experimental and genetic approaches represent a powerful toolkit for the elucidation of helminth life cycles and narrowing down possible host–parasite associations in a particular region. In the next section, we provide several reasons why these tools need to be applied more consistently to more parasite taxa.

Why resolving life cycles matters

There are many reasons why greater efforts should be aimed at elucidating parasite life cycles. The examples of possible benefits for science we offer here are far from representing an exhaustive list.

First, full resolution of life cycles can inform taxonomy. A species of helminth is more than just the adult form. For traditional reasons, practically all formal species descriptions of helminth parasites are solely based on the adult stage, and currently a species only receives a Latin binomial name if the adult is described. Generally speaking, the morphological characterization of juvenile stages and the identity of the intermediate host species they use represent unnecessary additional information for taxonomic purposes. However, at least in some cases, elucidating life cycles can be of huge importance for parasite taxonomy, as in some groups morphological features of juvenile stages are more useful to classification than those of adult worms (e.g. Niewiadomska, 2002). Nevertheless, in most groups only adults are considered for species characterization and delimitation, and this creates a knowledge gap that must be bridged if the full value and power of taxonomy are to be of use to ecologists and conservation biologists. Remedying this general absence of data on juvenile stages from species descriptions would also capture the spirit of the new 'integrative

taxonomy' (Dayrat, 2005). Therefore, the full characterization of a species should include all life stages and their respective hosts, if we are to understand the ecology and evolution of any particular parasite species.

Second, detailed knowledge of life cycles can cast new light on the evolution and basic biology of helminth parasites. Different life stages of the same parasite are different manifestations of the same genome, occurring in a particular ontogenetic sequence. Fundamental life-history traits are correlated across life stages (Loker, 1983; Poulin & Latham, 2003; Poulin et al., 2003; Benesh, 2016), and fully understanding the biology of the adult stage is practically impossible without knowledge of juvenile stages and their living environment, i.e. their intermediate hosts. In addition, identifying some of the driving forces behind the evolution of complex life cycles requires comparative studies among multiple well-resolved cycles. For example, Pearson (1972) concluded from the examination of the resolved life cycles of multiple trematodes that the metacercarial stage was a late addition in the evolution of trematode life cycles, serving to prolong the infective life of the cercaria and boost its chances of ingestion by the definitive host. Knowledge of the life cycle may also explain why certain helminth taxa have evolved to use particular types of definitive hosts and not others (Tkach et al., 2016). Although life cycles in different higher taxa have converged toward a similar level of complexity (Poulin, 2011b), the underlying evolutionary drivers may differ, and can only be identified through detailed investigations of well-resolved life cycles.

Third, there is renewed interest among ecologists in using food-web structure and dynamics to explore ecosystem stability, and the inclusion of parasites into food webs has become a key factor in efforts to obtain complete and fully integrated webs (Lafferty et al., 2008; Dunne et al., 2013). This requires detailed knowledge of parasite life cycles to allow these to be mapped on to food webs (Sukhdeo, 2010), and to quantify how parasite transmission depends on trophic links and energy flow through a food web (Thompson et al., 2013). At present, incomplete knowledge of parasite life cycles means that the identity of intermediate hosts is often based on educated guesswork, and many host–parasite links are left out of food webs due to lack of knowledge (Lafferty et al., 2008; Rossiter, 2013). Definitive conclusions regarding the impact of parasites on the structure and stability of trophic interaction networks will need to be revisited if and when we ever achieve the inclusion of fully resolved parasite life cycles in large, natural food webs.

Fourth, applied ecology can also benefit from greater resolution of parasite life cycles. For instance, predicting and mitigating the impact of species introductions on parasite transmission requires knowledge of the hosts needed by a parasite (whether introduced or native) at all life stages (e.g. Rauque et al., 2003; Paterson et al., 2011). Similarly, management of zoonotic diseases requires that we first identify all host species acting as reservoirs of juvenile as well as adult parasite stages and contributing to the epidemiology of the disease (Thompson, 2013). Finally, knowledge of parasite life cycles is also essential for parasite control. Each stage in the life cycle of a parasite earmarked for eradication is a potential target for control measures. Elucidation of the

life cycle of human pathogens such as *Plasmodium* spp. and *Schistosoma* spp. has opened the door for blocking the vectoring potential of *Anopheles* mosquitoes (e.g. Collins *et al.*, 1986; Killeen *et al.*, 2002) and for control methods against *Biomphalaria* and other intermediate snail hosts (King & Bertsch, 2015; Sokolow *et al.*, 2016), respectively, that were not even considered previously. Who knows which helminth parasite of wildlife, known today from its adult only, will tomorrow become a disease agent of concern to conservation biologists?

Looking ahead

We hope this short review will serve as a call to arms to parasitologists not to abandon life-cycle studies, but instead to bring them back to the fore. We have highlighted some of the many benefits arising from a knowledge of full parasite life cycles. The necessary tools to obtain that knowledge are there to be used. All that seems to be missing is the motivation to chase that knowledge in an age when purely descriptive research is hardly ever funded. Here, we offer two avenues to remedy our general ignorance of particular life cycles that are compatible with current research trends.

First, parasite taxonomists should strive to include full or partial life-cycle information (description of juvenile stages, identification of intermediate hosts) whenever they describe a new species. Although this suggestion is likely to be controversial, the 'adult only' approach clashes with the philosophy of the modern integrative taxonomy approach (Dayrat, 2005). The content and quality of species descriptions have evolved over time (Poulin & Presswell, 2016). In the early 20th century, verbal descriptions of new helminth species were sufficient. Later, the inclusion of line drawings became *de rigueur,* followed by the addition of light microscopy and scanning electron microscopy images. In recent years the inclusion of genetic data has rapidly become the norm, if not yet a requirement. So why not information on juvenile stages and intermediate hosts? Providing this extra information would require more effort per description of new species, and therefore possibly slow down the rate at which new species are described. For this reason, a compromise may be necessary when data on juvenile stages are simply too difficult to obtain. We suggest that providing this information should be added to the guidelines for describing new parasite species (Slapeta, 2013). No doubt it would require much extra effort to sample likely intermediate hosts and find juvenile stages. However, if this became part of the standard content of new species descriptions, it would be a major step in the right direction.

Second, in ecosystems where multiple helminth species have been identified previously based on adult forms only, large-scale genetic matching of unidentified helminth juveniles with known adults is the most promising way to resolve multiple life cycles simultaneously. Recent and ongoing studies on the elasmobranch cestodes of the Gulf of Mexico (Jensen & Bullard, 2010) and the trematodes of fishes from the Great Barrier Reef (T. Cribb, pers. comm.) are excellent examples of the power of this approach. Sampling multiple species of potential intermediate hosts and the subsequent recovery of helminth

juvenile stages from these samples can be a massive undertaking. This is why greater collaboration between ecologists and taxonomists, as well as between taxonomists specializing in different types of hosts or parasite life stages, is necessary. For instance, simultaneous applications for funding can be coordinated for ecological studies paired with taxonomic or barcoding surveys, with the success of one not totally dependent on that of the other, but their joint success leading to multiple synergistic re-use of the same samples. The decreasing cost of using next-generation sequencing technology to obtain genetic data makes this approach feasible, and its ecosystem-wide scale should make it more competitive for funding.

The two research directions we propose above are not without problems, and they will not resolve our inadequate knowledge of life cycles overnight. Nevertheless, they are achievable and may represent our best chance of unlocking the benefits that follow from fully resolved parasite life cycles.

References

Alcántar-Escalera, F.J., Garcia-Varela, M., Vázquez-Domínguez, E. & Pérez-Ponce de León, G. (2013) Using DNA barcoding to link cystacanths and adults of the acanthocephalan *Polymorphus brevis* in central Mexico. *Molecular Ecology Resources* **13**, 1116–1124.

Alda, P., Bonel, N., Hechinger, R.F. & Martorelli, S.R. (2013) *Maritrema orensense* and *Maritrema bonaerense* (Digenea: Microphallidae): descriptions, life cycles, and comparative morphometric analyses. *Journal of Parasitology* **99**, 218–228.

Al-Jahdali, M.O., El-Said Hassanine, R.M. & El-Said Touliabah, H. (2015) The life cycle of *Sclerocollum saudii* Al-Jahdali, 2010 (Acanthocephala: Palaeacanthocephala: Rhadinorhynchidae) in amphipod and fish hosts from the Red Sea. *Journal of Helminthology* **89**, 277–287.

Barber, I. (2005) Parasites grow larger in faster growing fish hosts. *International Journal for Parasitology* **35**, 137–143.

Barber, I., Walker, P. & Svensson, P.A. (2004) Behavioural responses to simulated avian predation in female three spined sticklebacks: the effect of experimental *Schistocephalus solidus* infections. *Behaviour* **141**, 1425–1440.

Bartoli, P., Jousson, O. & Russell-Pinto, F. (2000) The life cycle of *Monorchis parvus* (Digenea: Monorchiidae) demonstrated by developmental and molecular data. *Journal of Parasitology* **86**, 479–489.

Benesh, D.P. (2016) Autonomy and integration in complex parasite life cycles. *Parasitology* **143**, 1824–1846.

Blasco-Costa, I., Cutmore, S.C., Miller, T.L. & Nolan, M. J. (2016) Molecular approaches to trematode systematics: 'best practice' and implications for future study. *Systematic Parasitology* **93**, 295–306.

Bonner, J.T. (1993) *Life cycles: Reflections of an evolutionary biologist*. Princeton, New Jersey, Princeton University Press.

Brown, S.P., Renaud, F., Guégan, J.-F. & Thomas, F. (2001) Evolution of trophic transmission in parasites:

the need to reach a mating place? *Journal of Evolutionary Biology* **14**, 815–820.

Cable, R.M. (1953) The life cycle of *Parvatrema borinquenae* gen. et sp. nov. (Trematoda: Digenea) and the systematic position of the subfamily Gymnophallinae. *Journal of Parasitology* **39**, 408–421.

Cable, R.M. (1963) Marine cercariae from Curaçao and Jamaica. *Zeitschrift für Parasitenkunde* **23**, 429–469.

Caira, J.N. & Jensen, K. (2014) A digest of elasmobranch tapeworms. *Journal of Parasitology* **100**, 373–391.

Chappell, L.H., Hardie, L.J & Secombes, C.J. (1994) Diplostomiasis: the disease and host–parasite interactions. pp. 59–86 in Pike, A.W. & Lewis, J.W. (*Eds*) *Parasitic diseases of fish*. Dyfed, UK, Samara Publishing.

Choisy, M., Brown, S.P., Lafferty, K.D. & Thomas, F. (2003) Evolution of trophic transmission in parasites: why add intermediate hosts? *American Naturalist* **162**, 172–181.

Clarke, A.S. (1954) Studies on the life cycle of the pseudophyllidean cestode *Schistocephalus solidus*. *Proceedings of the Zoological Society of London* **124**, 257–302.

Collins, F.H., Sakai, R.K., Vernick, K.D., Paskewitz, S., Seeley, D.C., Miller, L.H., Collins, W.E., Campbell, C.C. & Gwadz, R.W. (1986) Genetic selection of a *Plasmodium*-refractory strain of the malaria vector *Anopheles gambiae*. *Science* **234**, 607–610.

Combes, C., Albaret, J.L., Arvy, L., Bartoli, P., Bayssade-Dufour, C., Deblock, S., Durrette-Desset, M.C., Gabrion, C., Jourdane, J., Lambert, A., Léger, N., Maillard, C., Matricon, M., Nassi, H., Prévost, G., Richard, J. & Théron, A. (1980) Atlas mondial des cercaires. *Mémoire du Muséum National d'Histoire Naturelle, Série A Zoologie* **115**, 1–235.

Cribb, T.H., Bray, R.A., Olson, P.D. & Littlewood, D.T.J. (2003) Life cycle evolution in the Digenea: a new perspective from phylogeny. *Advances in Parasitology* **54**, 197–254.

Cribb, T.H., Bott, N.J., Bray, R.A., McNamara, M.K.A., Miller, T.L., Nolan, M.J. & Cutmore, S.C. (2014) Trematodes of the Great Barrier Reef: emerging patterns of diversity and richness in coral reef fishes. *International Journal for Parasitology* **44**, 929–939.

Cribb, T.H., Bray, R.A., Diaz, P.E., Huston, D.C., Kudlai, O., Martin, S.B., Yong, R.Q.-Y. & Cutmore, S.C. (2016) Trematodes of fishes of the Indo-west Pacific: told and untold richness. *Systematic Parasitology* **93**, 237–247.

Cringoli, G., Rinaldi, L., Maurelli, M.P. & Utzinger, J. (2010) FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nature Protocols* **5**, 503–515.

Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society* **85**, 407–415.

DeGiusti, D.L. (1949) The life cycle of *Leptorhynchoides thecatus* (Linton), an acanthocephalan of fish. *Journal of Parasitology* **35**, 437–460.

Dezfuli, B.S., Lui, A., Squerzanti, S., Lorenzoni, M. & Shinn, A.P. (2012) Confirmation of the hosts involved in the life cycle of an acanthocephalan parasite of *Anguilla anguilla* (L.) from Lake Piediluco and its effect on the reproductive potential of its amphipod intermediate host. *Parasitology Research* **110**, 2137–2143.

Downie, A.J. & Cribb, T.H. (2011) Phylogenetic studies explain the discrepant host distribution of *Allopodocotyle heronensis* sp. nov. (Digenea, Opecoelidae) in Great Barrier Reef serranids. *Acta Parasitologica* **56**, 296–300.

Dunne, J.A., Lafferty, K.D., Dobson, A.P., Hechinger, R.F., Kuris, A.M., Martinez, N.D., McLaughlin, J.P., Mouritsen, K.N., Poulin, R., Reise, K., Stouffer, D.B., Thieltges, D.W., Williams, R.J. & Zander, C.D. (2013) Parasites affect food web structure primarily through increased diversity and complexity. *PLoS Biology* **11**, e1001579.

Faltýnková, A., Sures, B. & Kostadinova, A. (2016) Biodiversity of trematodes in their intermediate mollusc and fish hosts in the freshwater ecosystems of Europe. *Systematic Parasitology* **93**, 283–293.

Galaktionov, K.V. (2009) Description of the maritae and determination of the species status of *Microphallus pseudopygmaeus* sp. nov. (Trematoda: Microphallidae). *Parazitologiya* **43**, 288–298.

Galaktionov, K.V., Irwin, S.W.B. & Saville, D.H. (2006) One of the most complex life-cycles among trematodes: a description of *Parvatrema margaritense* (Ching, 1982) n. comb. (Gymnophallidae) possessing parthenogenetic metacercariae. *Parasitology* **132**, 733–746.

Gonchar, A. & Galaktionov, K.V. (2017) Life cycle and biology of *Tristriata anatis* (Digenea: Notocotylidae): morphological and molecular approaches. *Parasitology Research* **116**, 45–59.

Haas, W., Stiegeler, P., Keating, A., Kullmann, B., Rabenau, H., Schönamsgruber, E. & Haberl, B. (2002) *Diplostomum spathaceum* cercariae respond to a unique profile of cues during recognition of their fish host. *International Journal for Parasitology* **32**, 1145–1154.

Haas, W., Wulff, C., Grabe, K., Meyer, V. & Haeberlein, S. (2007) Navigation within host tissues: cues for orientation of *Diplostomum spathaceum* (Trematoda) in fish towards veins, head and eye. *Parasitology* **134**, 1013–1023.

Hernández-Orts, J.S., Aznar, F.J., Blasco-Costa, I., Garcia, N.A., Villora-Montero, M., Crespo, E.A., Raga, J.A. & Montero, F.E. (2013) Description, microhabitat selection and infection patterns of sealworm larvae (*Pseudoterranova decipiens* species complex, Nematoda: Ascaridoidea) in fishes from Patagonia, Argentina. *Parasites & Vectors* **6**, 252.

Hoffman, G.L. & Hundley, J.B. (1957) The life-cycle of *Diplostomum baeri eucaliae* n. subsp. (Trematoda: Strigeida). *Journal of Parasitology* **43**, 613–627.

Huston, D.C., Cutmore, S.C. & Cribb, T.H. (2016) The life-cycle of *Gorgocephalus yaaji* Bray & Cribb, 2005 (Digenea: Gorgocephalidae) with a review of the first intermediate hosts for the superfamily Lepocreadioidea Odhner, 1905. *Systematic Parasitology* **93**, 653–665.

Jensen, K. & Bullard, S.A. (2010) Characterization of a diversity of tetraphyllidean and rhinebothriidean cestode larval types, with comments on host associations and life-cycles. *International Journal for Parasitology* **40**, 889–910.

Jezewski, W., Bunkowska-Gawlik, K., Hildebrand, J., Perec-Matysiak, A. & Laskowski, Z. (2013) Intermediate and paratenic hosts in the life cycle of *Aelurostrongylus abstrusus* in natural environment. *Veterinary Parasitology* **198**, 401–405.

Karvonen, A., Seppälä, O. & Valtonen, E.T. (2004) Eye fluke-induced cataract formation in fish: quantitative analysis using an ophthalmological microscope. *Parasitology* **129**, 473–478.

Keeney, D.B., Waters, J.M. & Poulin, R. (2007) Clonal diversity of the marine trematode *Maritrema novaezealandensis* within intermediate hosts: the molecular ecology of parasite life cycles. *Molecular Ecology* **16**, 431–439.

Killeen, G.F., Fillinger, U., Kiche, I., Gouagna, L.C. & Knols, B.G.J. (2002) Eradication of *Anopheles gambiae* from Brazil: lessons for malaria control in Africa? *Lancet Infectious Diseases* **2**, 618–627.

King, C.H. & Bertsch, D. (2015) Historical perspective: snail control to prevent schistosomiasis. *PLoS Neglected Tropical Diseases* **9**, e0003657.

Koehler, A.V., Brown, B., Poulin, R., Thieltges, D.W. & Fredensborg, B.L. (2012) Disentangling phylogenetic constraints from selective forces in the evolution of trematode transmission stages. *Evolutionary Ecology* **26**, 1497–1512.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.A., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.A., McLaughlin, J.P., Mordecai, E.A., Pascual, M., Poulin, R. & Thieltges, D.W. (2008) Parasites in food webs: the ultimate missing links. *Ecology Letters* **11**, 533–546.

Leiva, N.V., López, Z., González, M.T. & Muñoz, G. (2016) Determining intermediate hosts for Opecoelidae and Microphallidae species (Platyhelminthes: Trematoda) in the Southeastern Pacific Coast, using molecular markers. *Journal of Parasitology*, in press.

Leung, T.L.F., Donald, K.M., Keeney, D.B., Koehler, A.V., Peoples, R.C. & Poulin, R. (2009) Trematode parasites of Otago Harbour (New Zealand) soft-sediment intertidal ecosystems: life cycles, ecological roles and DNA barcodes. *New Zealand Journal of Marine and Freshwater Research* **43**, 857–865.

Li, L., Liu, Y.-Y. & Zhang, L.-P. (2012) Morphological and molecular identification of *Hysterothylacium longilabrum* sp. nov. (Nematoda: Anisakidae) and larvae of different stages from marine fishes in the South China Sea. *Parasitology Research* **111**, 767–777.

Loker, E.S. (1983) A comparative study of the life-histories of mammalian schistosomes. *Parasitology* **87**, 343–369.

Miller, T.L., Downie, A.J. & Cribb, T.H. (2009) Morphological disparity despite genetic similarity; new species of *Lobosorchis* Miller & Cribb, 2005 (Digenea: Cryptogonimidae) from the Great Barrier Reef and the Maldives. *Zootaxa* **1992**, 37–52.

Nadler, S.A. & Pérez-Ponce de León, G. (2011) Integrating molecular and morphological approaches for characterizing parasite cryptic species: implications for parasitology. *Parasitology* **138**, 1688–1709.

Niewiadomska, K. (2002) The Diplostomoidea Poirier, 1886. pp. 150–166 *in* Gibson, D.I., Jones, A. & Bray, R.A. (*Eds*) *Keys to the Trematoda*. Wallingford, Oxon., UK, CAB International and The Natural History Museum,

Ostrowski de Núñez, M. (2007) Life cycle of *Stephanoprora uruguayense* (Digenea: Echinostomatidae) in Argentina. *Journal of Parasitology* **93**, 1090–1096.

Parker, G.A., Chubb, J.C., Ball, M.A. & Roberts, G.N. (2003) Evolution of complex life cycles in helminth parasites. *Nature* **425**, 480–484.

Parker, G.A., Ball, M.A. & Chubb, J.C. (2015) Evolution of complex life cycles in trophically transmitted helminths. I. Host incorporation and trophic ascent. *Journal of Evolutionary Biology* **28**, 267–291.

Paterson, R.A., Townsend, C.R., Poulin, R. & Tompkins, D.M. (2011) Introduced brown trout alter native acanthocephalan infections in native fish. *Journal of Animal Ecology* **80**, 990–998.

Pearson, J.C. (1968) Observations on the morphology and life-cycle of *Paucivitellosus fragilis* Coil, Reid & Kuntz, 1965 (Trematoda: Bivesiculidae). *Parasitology* **58**, 769–788.

Pearson, J.C. (1972) A phylogeny of life cycle patterns of the Digenea. *Advances in Parasitology* **10**, 153–189.

Pérez-Ponce de León, G. (1995) Host-induced morphological variability in adult *Posthodiplostomum minimum* (Digenea: Neodiplostomidae). *Journal of Parasitology* **81**, 818–820.

Pina, S., Barandela, T., Santos, M.J., Russell-Pinto, F. & Rodrigues, P. (2009) Identification and description of *Bucephalus minimus* (Digenea: Bucephalidae) life cycle in Portugal: morphological, histopathological, and molecular data. *Journal of Parasitology* **95**, 353–359.

Poulin, R. (2011a) Uneven distribution of cryptic diversity among higher taxa of parasitic worms. *Biology Letters* **7**, 241–244.

Poulin, R. (2011b) The many roads to parasitism: a tale of convergence. *Advances in Parasitology* **74**, 1–40.

Poulin, R. & Latham, A.D.M. (2003) Effects of initial (larval) size and host body temperature on growth in trematodes. *Canadian Journal of Zoology* **81**, 574–581.

Poulin, R. & Presswell, B. (2016) Taxonomic quality of species descriptions varies over time and with the number of authors, but unevenly among parasite taxa. *Systematic Biology* **65**, 1107–1116.

Poulin, R., Wise, M. & Moore, J. (2003) A comparative analysis of adult body size and its correlates in acanthocephalan parasites. *International Journal for Parasitology* **33**, 799–805.

Poulin, R., Blasco-Costa, I. & Randhawa, H.S. (2016) Integrating parasitology and marine ecology: seven challenges towards greater synergy. *Journal of Sea Research* **113**, 3–10.

Presswell, B. & Lagrue, C. (2016) Assessing parasite infections from avian faecal samples: the old methods are still the best. *Notornis* **63**, 32–36.

Presswell, B., Poulin, R. & Randhawa, H.S. (2012) First report of a gryporhynchid tapeworm (Cestoda: Cyclophyllidea) from New Zealand and from an eleotrid fish, described from metacestodes and *in vitro*-grown worms. *Journal of Helminthology* **86**, 453–464.

Presswell, B., Blasco-Costa, I. & Kostadinova, A. (2014) Two new species of *Maritrema* Nicoll, 1907 (Digenea: Microphallidae) from New Zealand: morphological and molecular characterisation. *Parasitology Research* **113**, 1641–1656.

Prokofiev, V.V., Levakin, I.A., Losev, E.A., Zavirsky, Y.V. & Galaktionov, K.V. (2011) Clonal variability in expression of geo- and photoorientation in cercariae of

Himasthla elongata (Trematoda: Echinostomatidae). *Parazitologiya* **45**, 345–357.

Randhawa, H.S. (2011) Insights using a molecular approach into the life cycle of a tapeworm infecting great white sharks. *Journal of Parasitology* **97**, 275–280.

Rauch, G., Kalbe, M. & Reusch, T.B.H. (2005) How a complex life cycle can improve a parasite's sex life. *Journal of Evolutionary Biology* **18**, 1069–1075.

Rauque, C.A., Viozzi, G.P. & Semenas, L.G. (2003) Component population study of *Acanthocephalus tumescens* (Acanthocephala) in fishes from Lake Moreno, Argentina. *Folia Parasitologica* **50**, 72–78.

Regel, K.V., Guliaev, V.D. & Pospekhova, N.A. (2013) On the life cycle and morphology of metacestodes *Dioecocestus asper* (Cyclophyllidea: Dioecocestidae). *Parazitologiia* **47**, 3–22.

Rendtorff, R.C. (1948) Investigations on the life cycle of *Oöchoristica ratti*, a cestode from rats and mice. *Journal of Parasitology* **34**, 243–252.

Rohde, K. (1973) Structure and development of *Lobatostoma manteri* sp. nov. (Trematoda: Aspidogastrea) from the Great Barrier Reef, Australia. *Parasitology* **66**, 63–83.

Rossiter, W. (2013) Zeros in host–parasite food webs: are they real? *International Journal for Parasitology – Parasites and Wildlife* **2**, 228–234.

Sakanari, J.A. & Moser, M. (1989) Complete life cycle of the elasmobranch cestode *Lacistorhynchus dollfusi* Beveridge and Sakanari, 1987 (Trypanorhyncha). *Journal of Parasitology* **75**, 806–808.

Scholz, T. & Choudhury, A. (2014) Parasites of freshwater fishes in North America: why so neglected? *Journal of Parasitology* **100**, 26–45.

Selbach, C., Soldánová, M., Georgieva, S., Kostadinova, A. & Sures, B. (2015) Integrative taxonomic approach to the cryptic diversity of *Diplostomum* spp. in lymnaeid snails from Europe with a focus on the 'Diplostomum mergi' species complex. *Parasites & Vectors* **8**, 300.

Šlais, J. (1973) Functional morphology of the cestode larvae. *Advances in Parasitology* **11**, 395–480.

Slapeta, J. (2013) Ten simple rules for describing a new (parasite) species. *International Journal for Parasitology – Parasites and Wildlife* **2**, 152–154.

Smyth, J.D. (1990) *In vitro cultivation of parasitic helminths.* Florida, USA, CRC Press.

Sokolow, S.S., Wood, C.L., Jones, I.J., Swartz, S., Lopez, M., Hsieh, M., Lafferty, K.D., Kuris, A.M. & DeLeo, G.A. (2016) Global assessment of schistosomiasis control over the past century shows targeting the snail intermediate host works best. *PLoS Neglected Tropical Diseases* **10**, e0004794.

Srivathsan, A., Sha, J.C., Vogler, A.P. & Meier, R. (2015) Comparing the effectiveness of metagenomics and metabarcoding for diet analysis of a leaf-feeding monkey (*Pygathrix nemaeus*). *Molecular Ecology Resources* **15**, 250–261.

Sukhdeo, M.V.K. (2010) Food webs for parasitologists: a review. *Journal of Parasitology* **96**, 273–284.

Thompson, R.C.A. (2013) Parasite zoonoses and wildlife: One Health, spillover and human activity. *International Journal for Parasitology* **43**, 1079–1088.

Thompson, R.M., Poulin, R., Mouritsen, K.N. & Thieltges, D.W. (2013) Resource tracking in marine parasites: going with the flow? *Oikos* **122**, 1187–1194.

Tkach, V.V., Kudlai, O. & Kostadinova, A. (2016) Molecular phylogeny and systematics of the Echinostomatoidea Looss, 1899 (Platyhelminthes: Digenea). *International Journal for Parasitology* **46**, 171–185.

Urdal, K., Tierney, J.F. & Jakobsen, P.J. (1995) The tapeworm *Schistocephalus solidus* alters the activity and response, but not the predation susceptibility of infected copepods. *Journal of Parasitology* **81**, 330–333.

Van Cleave, H.J. (1920) Notes on the life cycle of two species of Acanthocephala from fresh water fishes. *Journal of Parasitology* **6**, 167–172.

Voge, M. (1967) The post-embryonic developmental stages of cestodes. *Advances in Parasitology* **11**, 707–730.

Wedekind, C. & Milinski, M. (1996) Do three-spined sticklebacks avoid consuming copepods, the first intermediate host of *Schistocephalus solidus*? An experimental analysis of behavioural resistance. *Parasitology* **112**, 371–383.

Yamaguti, S. (1975) *A synoptical review of life histories of digenetic trematodes of vertebrates.* Tokyo, Japan, Keigaku Publishers.