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Prospective enzymes for omega-3 PUFA biosynthesis found in endoparasitic classes within the phylum Platyhelminthes

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Abstract

The free-living infectious stages of macroparasites, specifically, the cercariae of trematodes (flatworms), are likely to be significant (albeit underappreciated) vectors of nutritionally important polyunsaturated fatty acids (PUFA) to consumers within aquatic food webs, and other macroparasites could serve similar roles. In the context of *de novo* omega-3 (n-3) PUFA biosynthesis, it was thought that most animals lack the fatty acid (FA) desaturase enzymes that convert stearic acid (18:0) into α-linolenic acid (ALA; 18:3n-3), the main FA precursor for n-3 long-chain PUFA. Recently, novel sequences of these enzymes were recovered from 80 species from six invertebrate phyla, with experimental confirmation of gene function in five phyla. Given this wide distribution, and the unusual attributes of flatworm genomes, we conducted an additional search for genes for *de novo* n-3 PUFA in the phylum Platyhelminthes. Searches with experimentally confirmed sequences from Rotifera recovered nine relevant FA desaturase sequences from eight species in four genera in the two exclusively endoparasite classes (Trematoda and Cestoda). These results could indicate adaptations of these particular parasite species, or may reflect the uneven taxonomic coverage of sequence databases. Although additional genomic data and, particularly, experimental study of gene functionality are important future validation steps, our results indicate endoparasitic platyhelminths may have enzymes for *de novo* n-3 PUFA biosynthesis, thereby contributing to global PUFA production, but also representing a potential target for clinical antihelmintic applications.

Introduction

Lipids, including fatty acids (FA, especially saturated FA) are key drivers in the flow of energy in many food webs as they are the densest form of energy available to consumers (Karasov & Martinez del Rio, 2007). They also play key roles in organismal function, health and survival. Polyunsaturated FA (PUFA), for instance, are important constituents of biological cell membranes, and contribute to their stability and function (Brett & Müller-Navarra, 1997). Moreover, some PUFA are precursors of bioactive eicosanoid signalling molecules that affect hormonal and neural pathways (Twining *et al.*, 2016). This is especially true for essential omega-3 (n-3) long-chain PUFA (LC-PUFA; ≥20 carbons), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). These two LC-PUFA can be biosynthesized in animals from relevant FA precursors (e.g. α-linolenic acid or ALA, 18:3n-3), but generally only at low rates (Das, 2006). Furthermore, it was previously thought that animals were unable to synthesize 18C PUFA precursors (e.g. ALA and linoleic acid; LNA, 18:2n-6). The conventional wisdom has therefore been that animals preferentially obtain ALA and LNA and their downstream products (EPA, DHA, and arachidonic acid (ARA) 20:4n-6) from their diets as pre-formed molecules (Twining *et al.*, 2016). In most food webs, PUFA are transferred from primary producers to higher trophic levels through consumption and accumulation in the biomass of consumers; as such, these PUFA are selectively retained by consumers based on their physiological requirements and food preferences (Kainz *et al.*, 2004; Gladyshev *et al.*, 2009).

Several key reactions in n-3 PUFA biosynthesis require specific FA desaturases, i.e. enzymes that facilitate the insertion of double bonds in saturated FA molecules. The delta-9 (Δ9) desaturase inserts the first double bond in the saturated stearic acid (18:0), thereby forming oleic acid (18:1n-9). Methyl-end (ωx) desaturases then insert double bonds between this first double bond and the methyl-end (-CH3) of the FA molecule (Sperling *et al.*, 2003; Castro *et al.*, 2016). Specifically, the omega-6 (ω6) desaturase (a.k.a. Δ12 desaturase) converts oleic acid to linoleic acid (LNA, 18:2n-6), and the omega-3 (ω3) desaturase (a.k.a. Δ15 desaturase) converts LNA to ALA, which is the main FA precursor for n-3 LC-PUFA (Nichols, 2003; Sperling *et al.*, 2003). Most animals carry the necessary Δ9 desaturase, but lack either the ω6 or ω3 desaturase,

thereby limiting their capacity to complete the further reactions in this chain (Sperling *et al.*, 2003; Kabeya *et al.*, 2018). In contrast, microalgae, protists and bacteria (mainly from marine ecosystems) contain various enzymes related to n-3 PUFA biosynthesis. These include the previously mentioned Δ9 and ωx desaturases, 'front-end desaturases' that insert double bonds at the carboxyl (-COOH) end of the FA molecule, and 'elongase' enzymes that facilitate chain lengthening (e.g. ALA to EPA and DHA) (Monroig & Kabeya, 2018). Owing to their substantial enzymatic capacity for *de novo* n-3 PUFA biosynthesis, and their biomass, microorganisms are responsible for most of the natural production of n-3 PUFA globally (Nichols, 2003; Pereira *et al.*, 2003; Khozin-Goldberg *et al.*, 2011).

Until recently, the main exceptions for the existence of ωx desaturases in animals were documented only in the free-living nematode *Caenorhabditis elegans* (Spychalla *et al.*, 1997; Peyou-Ndi *et al.*, 2000) and the arthropod *Bemisia argentifolii* (Buckner & Hagen, 2003). These enzymes have conserved motifs in their protein sequences (as explained in Kabeya *et al.*, 2018). Thus, they may be used to search for potential ωx desaturases in other animal taxa. By doing so, Kabeya *et al.* (2018) recently found 121 novel sequences of these enzymes in the genomes of 80 species from six invertebrate phyla. These sequences were associated with three distinct clades: Clade 1 was dominated by Nematoda; Clade 2 was dominated by Cnidaria; and Clade 3 comprised Rotifera, Mollusca, Annelida and Arthropoda. Kabeya *et al.* (2018) also tested the functionality of several putative desaturases from taxa in Clades 2 and 3 using a heterologous yeast expression system and subsequent FA analysis. Since all species exhibited ω6 and ω3 desaturase activity, these invertebrates were deemed to be capable of playing a significant role in *de novo* n-3 PUFA production in the ecosystems where they reside.

The ground-breaking study by Kabeya *et al.* (2018) uncovered genes for *de novo* n-3 PUFA production in a wide variety of animals from six phyla, including several endoparasitic nematodes and an ectoparasitic crustacean arthropod. However, higher taxa composed exclusively of parasites, such as the Neodermata (a clade comprising the classes Trematoda, Cestoda and Monogenea within the phylum Platyhelminthes), were conspicuously absent. We propose three explanations for this. First, *de novo* synthesis of n-3 PUFA may be truly absent in these groups. This is plausible because neodermatans such as cestodes and digenean trematodes live within the bodies of other animals, often in nutrient-rich tissues, and synthesis of PUFA may thus be unnecessary. Second, the lack of ωx desaturase genes in neodermatans in Kabeya *et al.* (2018) could result from a lack of sequence data, but some unsequenced taxa may have related genes. Finally, it is possible that already sequenced n-3 PUFA genes in neodermatans were not detected by Kabeya *et al.* (2018). The latter scenario is plausible because the parasitic lifestyle is associated with genetic variations that may affect the success of gene sequence search strategies. Parasitic genomes are often smaller and compacted compared with those of free-living relatives (reviewed by Poulin & Randawa, 2015). For example, the genomes of nine parasitic neodermatans (one aspidogastrean, six digeneans, two cestodes) average 0.77 (SE 0.14) pg of DNA, while those of 48 free-living Rhabditophora (*sensu* Egger *et al.*, 2015) have a mean of 2.39 (SE 0.51) pg of DNA (Kruskal–Wallis $H(1) = 6.0077$, $P = 0.01424$, data from the Animal Genome Size Database, Gregory, 2020; see supplementary table. The mitochondrial genomes of neodermatans also display distinctive nucleotide bias and skew, and codon usage (Min & Hickey, 2007; Bernt *et al.*, 2013). While neither genome reduction nor mitochondrial mutation have direct bearing on nuclear genes such as FA desaturases, these tendencies do suggest unique and substantive evolutionary trends in neodermatan genomes. Consequently, it seems plausible that sequence-searching strategies tailored to the neodermatans and their relatives may uncover genes for *de novo* n-3 PUFA biosynthesis that might be missed by more general approaches.

It is also important to consider the evolutionary history of parasitism within different taxa. While parasitism has been estimated to have independently evolved within the phylum Nematoda up to 18 different times (Viney, 2017), there is overwhelming support for the Neodermata to be considered a monophyletic clade with a single ancestral origin for parasitism, and for trematodes (flukes) and cestodes (tapeworms) to comprise a single clade of endoparasites (Park *et al.*, 2007; Riutort *et al.*, 2012). Related to this, the considerable genetic and functional variation within phylum Platyhelminthes, reported by various studies, supports selection for traits which benefit either a free-living or parasitic lifestyle (e.g. Chen & Wen, 2011). Considering the very different evolutionary histories for parasites within the phylum Nematoda or Platyhelminthes, it is thus quite possible that putative sequences for ω6 and ω3 desaturase in nematodes are easier to identify as orthologues to those in free-living invertebrates. Less restrictive search criteria than those used by Kabeya *et al.* (2018) may thus be helpful in determining whether members of the Platyhelminthes have the capacity for *de novo* n-3 PUFA biosynthesis.

Establishing whether non-nematode helminths potentially have this ability is important because macroparasites are found in all ecosystems, and can influence organisms at multiple trophic levels due to their often complex life cycles (reviewed in Marcogliese, 2005; Lafferty *et al.*, 2008). Importantly, many macroparasites produce large numbers of their free-living infectious stages, particularly the aquatic cercariae of trematodes, thus contributing substantial biomass in certain ecosystems (e.g. Kuris *et al.*, 2008; Preston *et al.*, 2013; Rosenkranz *et al.*, 2018). Consequently, better understanding the nutritional contributions and role(s) of macroparasite infectious stages within ecosystems is critical for explaining the nuances of food web structure and function (reviewed in Marcogliese & Cone, 1997; Marcogliese, 2005; Lafferty *et al.*, 2008; Hatcher *et al.*,, 2012). For instance, McKee *et al.* (2020) recently determined that cercariae from the freshwater trematode *Ribeiroia ondatrae* have appreciable amounts of EPA and DHA, making them a viable dietary source of n-3 PUFA, also finding that dragonfly larvae fed either *R. ondatrae* cercariae or *Daphnia* spp. had no significant differences in their FA profiles (e.g. PUFA content).

Trematodes have complex multi-host life cycles, including asexually reproducing parthenitae situated in the digestive gland–gonad (DGG) complex of their molluscan first intermediate hosts (commonly aquatic snails) that either directly absorb nutrients or consume DGG tissue (Kuris, 1990; Esch *et al.*, 2002). Cercariae are clonally produced within parthenitae, emerging as a free-living motile stage that seeks out the next host (Esch *et al.*, 2002). Owing to their short lifespans and low odds of host encounter, most cercariae are unsuccessful at transmission and have other fates (Morley, 2012). It is estimated that ~50–80% of total cercariae biomass is consumed by non-host organisms, or contributes to detritus (Johnson *et al.*, 2010). Cercariae consumption has been noted in various aquatic taxa, such as copepods (Mironova *et al.*, 2019), and insects and fish (e.g. Orlofske

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et al., 2015). Given their size and traits, the ecological roles of trematode cercariae within food webs are thus arguably equivalent to that of other zooplankton (Morley, 2012). In terms of nutritional value, trematodes may contain significant quantities of PUFA considering that their parthenitae (and cercariae developing within) obtain nutrients from the DGG complex of snails that often feed on PUFA-rich periphyton. These hosts may also be capable of *de novo* n-3 PUFA biosynthesis (Kabeya *et al.*, 2018), which would increase their inherent value as a parasite nutrient source.

Despite their presence, the intrinsic function(s) of PUFA in platyhelminths is not well understood. Researchers have linked cercarial FA to buoyancy, energy reserves and metabolic waste in various trematode species (Schariter *et al.*, 2002; Fried & Toledo, 2009), as well as the ability of *Schistosoma mansoni* cercariae to penetrate into host tissues (Furlong, 1991). In addition, adult cestodes and trematodes are capable of using PUFA to synthesize a variety of eicosanoids for within-host immunomodulation and signalling, as are nematodes (Belley & Chadee, 1995). Select FA also appear to have important functions during larval development for both trematodes and cestodes (e.g. Minematsu *et al.*, 1990; Bexkens *et al.*, 2019). Thus, PUFA may play critical roles in helminth transmission success and nutrient contributions within food webs, making it important to understand the origin of these compounds. While certain platyhelminths are able to modify FA precursor molecules (ALA and LNA) obtained from hosts (to produce EPA, DHA and ARA), thus engaging in some degree of PUFA biosynthesis (Smyth & McManus, 1989; Furlong, 1991; Tielens, 1997), *de novo* synthesis capability for 18C PUFA has not been documented in this phylum.

Kabeya *et al.* (2018) determined that ωx desaturase sequences were present in various invertebrates, including phylum Rotifera. Based on a recent animal phylogenetic tree, phylum Platyhelminthes is closely related to Rotifera; the latter is found within the clade Gnathifera, which is nested within the larger clade Platyzoa, along with Platyhelminthes and Gastrotricha (see Edgecombe *et al.*, 2011). Given their common ancestry, it is possible that ωx desaturases are shared among these phyla; however, Kabeya *et al.* (2018) did not find putative sequences for these enzymes within Platyhelminthes. We suggest that a revised molecular approach, with less-restrictive sequence search criteria, may be appropriate to determine whether this phylum has ωx desaturases given that the identification of remote orthologues is likely more challenging for Platyhelminthes (Martín-Durán *et al.*, 2017; International Helminth Genomes Consortium, 2019) than Nematoda owing to their respective evolutionary histories (see above).

As noted earlier, ωx desaturases have conserved motifs in their protein sequences (see Kabeya *et al.*, 2018). It is also assumed that protein sequences (or protein-coding genes) with significant functional importance are under strong selective constraints (Assis & Kondrashov, 2014), i.e. they are not lost, and remain functional throughout the evolutionary history of the taxa being considered. These protein sequences should therefore be highly conserved within a phylum and less vulnerable to random mutations. With this in mind, we used protein sequences (for ωx desaturases) from Rotifera to conduct genomic data searches for homologous sequences in Platyhelminthes (both phyla are in the clade Platyzoa), hypothesizing that sequences required for ωx desaturase synthesis are actually present in the latter, and could be identified using less-restrictive search criteria than in Kabeya *et al.* (2018). However, we also predicted that only a few taxa within Platyhelminthes would have these sequences given that their distribution is cryptic in Rotifera (Kabeya *et al.*, 2018), and because the exclusively endoparasitic classes of flatworm could show divergence owing to adaptations associated with this lifestyle.

Materials and methods

We closely followed the approach employed by Kabeya *et al.* (2018), with a few exceptions noted below. Confirmed ωx desaturase sequences from Rotifera were chosen specifically as 'template' sequences for genomic data searches (conducted from December 2018 to September 2020) on homologous sequences in Platyhelminthes (see supplementary table). To start, the relevant FASTA sequences from Rotifera were acquired from NCBI (also known as GenBank®; Clark *et al.*, 2016), using sequence identifiers from Kabeya *et al.* (2018) (*Adineta vaga*, ATV93533.1 and ATV93531.1). These FASTA sequences were inputted into NCBI's Protein BLAST (Protein Basic Local Alignment Search Tool; Altschul *et al.*, 1997) for 'protein to protein' comparison using the following parameters: Database (non-redundant protein sequences), Organism (Platyhelminthes, Trematoda, Cestoda) and Algorithm (Position-Specific Iterated BLAST, expect threshold = 0.05). Trematoda and Cestoda were of particular interest in our searches, as these are the classes within the Platyhelminthes with the most parasitic taxa relative to the free-living 'turbellarians' and Monogenea (Olson & Tkach, 2005).

To meet our search specifications (see below), sequences found in Platyhelminthes had to be at least 250 AA long. Sequences with greater values for per cent identities (defined as the score for similarity between a pair of aligned sequences; Clark *et al.*, 2016) were prioritized, as there would be a higher likelihood for sequence conservation. Further, sequences identified generically as 'fatty acid desaturases' were prioritized, while sequences identified as 'delta-4', 'delta-9' or 'sphingolipid' desaturases were exempted. Essentially, these considerations allowed us to narrow the search focus on ωx desaturases (as per Kabeya *et al.*, 2018). 'Hypothetical' and 'unnamed' protein sequences (i.e. proteins whose functions have not yet been formally assigned, due to a lack of experimental evidence), as well as general 'fatty acid' desaturases were still considered, as they could have been misclassified in the databases. If there were promising hits from the Protein BLAST searches (i.e. protein sequences that matched the given requirements), then these sequences were inputted back into Protein BLAST to find related sequences, that may not have been captured in initial searches. Additional sequences were also obtained from Protein BLAST searches on WormBase© (Howe *et al.*, 2017). The FASTA sequences from Rotifera and the Protein BLAST searches were then inputted to NCBI's Translated BLAST to look for additional hits in translated nucleotide databases, using the following parameters: Database (whole genome shotgun contigs, transcriptome shotgun assembly), Organism (Platyhelminthes, Trematoda, Cestoda), Algorithm (expect threshold = 0.05). In total, we considered 31 of the same platyhelminth species as Kabeya *et al.* (2018), as well as an additional 33 species for which potentially informative protein sequences were available, for a total of 64 flatworm species (see supplementary table).

All search hits from NCBI and WormBase© were then scanned for the presence of three specific histidine box motifs (H-box), to characterize the sequences as putative ωx desaturases (as in Kabeya *et al.*, 2018). Our search specifications were that (1) H-box1 should have 5 AA in the format HXXXH (where X

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represents any other amino acid), to exclude the possibility for Δ9 desaturases; (2) H-box2 should have 5 AA in the format HXXHH, to exclude the possibility for other front-end desaturases; (3) the distance between H-box1 and the start of H-box2 should also be ~30–32 AA long; (4) H-box3 should have 5 AA in the format HXXHH; and (5) the distance between H-box2 and the start of H-box3 should be ~120–250 AA long (Kabeya *et al.*, 2018). The sequences were then inputted to SMART (Simple Modular Architecture Research Tool; Letunic *et al.*, 2015) to filter out sphingolipid desaturases from our results based on the presence of the Pfam protein domain Lipid_DES (PF08557.8) within the sequences (which is typically associated to this desaturase subfamily – see Kabeya *et al.*, 2018). However, sequences with the general Pfam protein domain FA_desaturase (PF00487.22) were still considered as putative ωx sequences. By employing SMART, we thus used an alternative procedure to that by Kabeya *et al.* (2018) to distinguish between putative ωx and sphingolipid desaturase sequences, i.e. this was not based upon the empirical frequency of amino acid occurrence within the histidine boxes of known eukaryotic sequences (Hashimoto *et al.*, 2008) so as to calculate the probability of a desaturase sequence being assigned to one of these two subfamilies. By doing so, we more widely considered sequences that might represent highly diverged ωx desaturases within Platyhelminthes, i.e. if these depart from the usual expectations for amino acid frequencies at certain positions.

Finally, the confirmed and putative sequences were inputted as FASTA sequences into CLUSTAL Omega© (version 1.2.4; Sievers *et al.*, 2011) to conduct a multiple sequence alignment (MSA) analysis. Here, specific characters were used to indicate sequence positions where there were fully conserved amino acids (i.e. with an asterisk), or the conservation of amin acids with strongly or weakly similar properties (i.e. with a colon and a period, respectively) (Sievers *et al.*, 2011). Using the LG + G + I model of amino acid evolution (selected using the Bayesian Information Criterion in MEGAX, Kumar *et al.* 2018), or its nearest equivalent, phylogenetic reconstructions among the confirmed rotifer and putative platyhelminth ωx desaturase genes were generated using maximum likelihood with 500 booststrapped pseudoreplicates using RAXML (Silvestro & Michalak, 2012; Stamatakis, 2014) and Bayesian inference (Ronquist *et al.* 2012).

Results

In total, nine sequences with the characteristic H-boxes were found in eight species representing two of the four traditional classes of Platyhelminthes (table 1; also see supplementary table). Specifically, we found these sequences in four cestode species (representing two genera), as well as four trematode species (also representing two genera). The amino acid sequences of the putative ωx desaturases in *Microphallus* were phylogenetically closer (fig. 1), and more similar, to confirmed ωx desaturases of the rotifer *A. vaga* (mean 29.0, range 24.9–32.1% identity with rotifer sequences) than those of other platyhelminths (mean 13.5, range 12.2–14.7% identity with rotifer sequences). Inspection of aligned sequences revealed suggestive patterns in variable amino acids inside the three H-boxes (fig. 1). Variable sites in all three H-boxes of two sequences from the trematode *Microphallus* sp. 2 LB. 2017 were highly similar to the confirmed ωx desaturases of the rotifer *A. vaga* (fig. 1; also see supplementary table).

In H-box 1 of both the rotifer and all nine platyhelminth sequences, the second amino acid was glutamic acid (E) or aspartic acid (D), both of which are acidic and charged, and the third amino acid was invariant (G). In H-box2, the second position was occupied by an amino acid with a side chain that was either polar and neutral (glutamine, Q; asparagine, N), or basic and charged (histidine, H; arginine, R). The two potentially variable amino acids within H-box 3 were identical within *Schistosoma*, *Taenia* and *Echinococcus*, and the first position was additionally conserved in both *Microphallus* sequences, one of which shared an amino acid (I) with the rotifer *A. vaga* in the second variable position.

Amino acids in the variable positions of H-boxes in the nine putative ωx desaturase sequences found in platyhelminths also shared similarities to those found in 43 eukaryotes by Hashimoto *et al.* (2008). In H-box 1, we observed D or E at the first and C, I or V at the second of these positions, and G at the third, while Hashimoto *et al.* (2008) found D/A and C/A and almost uniformly G in these three respective positions. The presence of G in the third position, notably, excludes the H-box 1 of sphingolipid desaturases, in which Hashimoto *et al.* (2008) recorded only S or T at this position. In H-box 3 of ωx desaturases, Hashimoto *et al.* (2008) found V to be common in the first variable position, and roughly equal frequencies of L, V, A and I in the second. Similarly, V occupied first variable position in the H-box 3 sequences of all nine platyhelminths, and I occurred in one.

Generally, variable positions in the three H-boxes in the nine putative platyhelminth ωx desaturases showed similar degrees of conservation as in the H-boxes of 43 ωx desaturases in Hashimoto *et al.* (2008). For example, in H-box 2, the two variable positions were more heterogeneous across the alignment than the variable positions in H-boxes 1 and 3, as also seen in Hashimoto *et al.* (2008). The most conserved positions (in order of decreasing conservation: H-box 3, variable position 1, H-box 1, variable positions 2 and 3) were the same in our data and in the 43 ωx desaturases in Hashimoto *et al.* (2008), in which this ranking was not observed in other types of desaturases. Thus, on the whole, the H-box motifs of the nine sequences recovered from platyhelminths shared numerous similarities with ωx desaturases that Hashimoto *et al.* (2008) found using other methods, including attributes that distinguish them from other types of desaturases.

Discussion

We found evidence of FA desaturase genes within the genomes of eight species within the phylum Platyhelminthes, although experimental characterization of the products of these genes is needed to confirm and further characterize gene functions, and the reasons for the presence of these genes in these particular platyhelminths are unclear. Putative ωx desaturase sequences were only found in trematodes (flukes) and cestodes (tapeworms), mainly in well-studied species of significance for human and livestock health (Hotez *et al.*, 2008; Pottinger & Jong, 2017). In trematodes, these sequences were found in the genus *Schistosoma*, including *S. mansoni*, a key species linked to human schistosomiasis, as well as in *Microphallus* sp. In cestodes, sequences were found in *Echinococcus* spp. and *Taenia* spp. – species that commonly infect livestock and humans (Machado-Pinto & Laborne, 2016). The absence of putative sequences in the monogeneans and 'turbellarians' considered here may indicate that these genes are not found in all platyhelminths, although the sparse genomic coverage of platyhelminths precludes a definitive statement.

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Table 1. Putative ωx desaturase sequences found by genomic data searches for four classes within the phylum Platyhelminthes.[a,b]

Class and species	Sequence identifiers (NCBI)	Sequence identifiers (WormBase©)
Trematoda		
Microphallus sp. 2 LB-2017	GFFL01026184.1:90-1050, GFFL01027767.1:226-1095	
Schistosoma mattheei	VDP41578.1	–
Schistosoma curassoni	VDP72892.1	–
Schistosoma mansoni	–	Smp_242890.1
Cestoda		
Echinococcus multilocularis	CDI96495.1	EmuJ_000357100.1
Echinococcus granulosus	CDS24921.1	–
Taenia solium	–	TsM_000914800
Taenia multiceps	–	Tm2G006491

See supplementary material for list of all species searched.
[a]Sequences acquired from NCBI and WormBase© are unique, i.e. there are no exact duplicates in the results from both databases (see ESM for full FASTA sequences). If duplicates were found in the genomic data searches (i.e. same sequence uploaded on both NCBI and WormBase©), then only the sequence identifiers from NCBI were reported (to avoid double counting).
[b]If multiple sequences are reported for one species, these represent protein sequences from different individuals that may have slight variations, or different sections of a protein sequence from the same individual. Further information can be acquired from the databases using the given sequence identifiers.

Intriguingly, while the three classes of obligate parasite (Trematoda, Cestoda and Monogenea) within this phylum form a monophyletic clade (Neodermata) with a single ancestral origin for parasitism, there is also strong support for the trematodes and cestodes to comprise a single clade of endoparasites (Park *et al.*, 2007; Riutort *et al.*, 2012). Our results thus suggest that the capacity for *de novo* biosynthesis of PUFA may be limited to a few species in this endoparasitic clade. Kabeya *et al.* (2018) did not identify putative ωx desaturase sequences from the eight species in which we recovered them, which we attribute to the less-restrictive search criteria we applied based on two considerations. First, unique genomic trends in platyhelminths, particularly neodermatans, suggest that evolutionary divergence could mask the presence of genes in some cases (see Introduction). Second, the wide distribution of ωx desaturase genes established by Kabeya *et al.* (2018) indicates that alternative search methods are appropriate for large lineages in which these genes were absent, such as Platyhelminthes.

We recovered putative ωx desaturase sequences from only a few endoparasitic platyhelminths, which may reflect a lack sequences in other taxa in which homologous genes are present, or may approximate the actual distribution of these genes. PUFA are critical for within-host interactions for both trematodes and cestodes, and the ability to synthesize these compounds is therefore likely to be a particular advantage for endoparasites, as opposed to ectoparasitic (monogenean) or free-living ('turbellarian') platyhelminths. As noted earlier, cestodes, trematodes and parasitic nematodes are all capable of using various PUFA to synthesize immunomodulatory eicosanoids (Belley & Chadee, 1995), and ARA is the precursor for various within-host signalling molecules used by trematodes (Tielens, 1997). Given this role in endohelminth establishment and the putative ωx desaturase sequences found in parasitic nematodes (Kabeya *et al.*, 2018), their presence in endoparasitic platyhelminths makes intuitive sense. In addition, FA appear to play important roles in larval development for both trematodes and cestodes (e.g. Minematsu *et al.*, 1990; Bexkens *et al.*, 2019). With these critical functions of PUFA for endoparasitic platyhelminths, it is possible that their biosynthesis abilities extend beyond modifying host-derived precursor molecules (Smyth & McManus, 1989; Furlong, 1991) to *de novo* production.

Although putative ωx desaturase sequences were found in the Platyhelminthes, they were uncommon and concentrated in just four genera, and two occurred in a single species. Some of this apparent absence may represent true absence. For example, other than the eight platyhelminth species from which putative ωx desaturase sequences were recovered here, the genomes of 25 other Platyhelminthes are assembled on WormBase© (Howe *et al.*, 2017). In this respect, our results parallel the findings of Kabeya *et al.* (2018), who documented ωx desaturase sequences in few genera or species within much larger phyla. For instance, within Rotifera, relevant sequences were only found in five species (*A. vaga* and *Rotario* spp.), and >1 ωx desaturase was recovered in several species (Kabeya *et al.*, 2018). Below, we speculate how this distribution of putative genes, and the relationships among them, may either reflect particular life history features in the eight species potentially possessing *de novo* PUFA biosynthesis ability, or results from missing or incomplete sequence data in this phylum.

The two trematode genera for which putative ωx desaturase sequences were identified are not closely related (belong to different orders) but share a conspicuous life history feature unusual among trematodes, in that all *Schistosoma* spp. have a two-host (dixenous) life cycle, as do the majority of *Microphallus* spp. (Brant & Loker, 2005; Galaktionov & Skirnisson, 2007). Schistosome cercariae penetrate directly into their vertebrate definitive hosts, and seem to be stimulated by essential PUFA found in host skin (mainly LNA and ARA) to produce immunomodulatory eicosanoids (reviewed by Furlong, 1991; Daugschies & Joachim, 2000). The capacity for *de novo* PUFA biosynthesis could thus be advantageous, although this does not explain why ωx desaturase sequences would be absent in other schistosomatids, such as *Trichobilharzia* spp. In addition, the utility of *de novo* PUFA biosynthesis is unclear for cercariae of *Microphallus* spp., which encyst in their first intermediate molluscan host. *Taenia* and *Echinococcus* are the only two genera in the family Taeniidae (Lavikainen *et al.*, 2008), which is unique among cestodes in requiring two obligate mammalian hosts (Knapp *et al.*, 2011). Similar to trematodes, cestodes can convert PUFA into immunomodulatory eicosanoids (Belley & Chadee, 1995), thus *de novo* PUFA biosynthesis could prove highly useful when combatting the defences of two hosts with relatively complex immune responses. However, further work to confirm gene functionality will be critical to confirm the capacity for *de novo* PUFA synthesis in these species before testing hypotheses regarding their distribution among platyhelminth taxa.

The alignment and phylogenetic analysis of the amino acid sequences of the putative ωx desaturase genes yielded two apparent groups: (1) rotifers + *Microphallus* sp.; and (2) *Schistosoma* spp. + Taeniidae. The overall topology is inconsistent with relationships among these taxa, in that the ωx desaturase genes of

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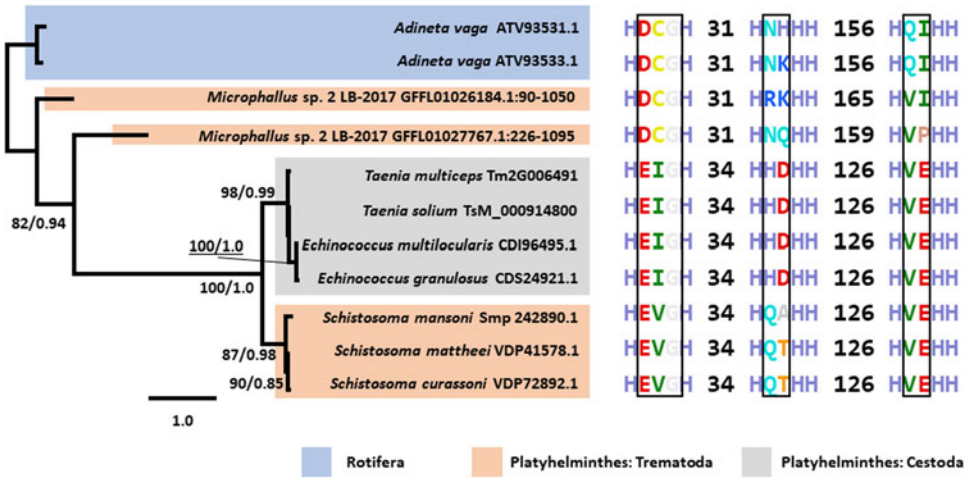

Fig. 1. Maximum likelihood gene tree of ωx desaturases in the Rotifera and Platyhelminthes (two confirmed sequences from Rotifer, from Kabeya et al., 2018, and nine putative homologues from Platyhelminths). Nodes are annotated with per cent support in 500 bootstrap replicates in maximum likelihood and Bayesian posterior probability (before and after slash, respectively), based on an alignment of 179 amino acids stripped of gaps. Histidine box motifs (H-boxes) are colour coded to highlight amino acid functional group similarity, and separated by the number of amino acids between each H-box. The outlined rectangles spanning the H-boxes show alignment sites free to vary according to search criteria.

Microphallus (Trematoda) are distant and apparently early divergent from the remaining sequences from both cestodes and trematodes. This might reflect the presence of two genes for two different, but related, proteins within these species, and it may indicate highly uneven rates of protein evolution among these two groups of species. Kabeya *et al.* (2018) also found cases of distinct putative ωx desaturase genes within species, and evidence of horizontal transfer in some cases, with an overall gene tree presenting marked inconsistency with relationships at high levels (e.g. ωx desaturase genes of nematodes not grouping with those of arthropods, which were allied with one of two paraphyletic annelid clades) and low levels (e.g. divergent ωx desaturase genes within *Lepeophtheirus salmonis*). Additional genomic (particularly nucleotide level) sequence data and broader taxonomic coverage are needed to clarify duplication, loss and transfer of these genes within Platyhelminthes.

Another factor which undoubtedly affects our results is missing sequence data. Out of the 774, 353 protein sequences available for Platyhelminthes on NCBI (as of October 2020) (National Center for Biotechnology Information, 2020), only nine putative sequences were found. This can be attributed to a lack of genomic coverage in NCBI (and equally, WormBase©) for the vast majority of platyhelminths vs. the alternative possibility that ωx desaturase genes are truly absent from most platyhelminths. Sequencing coverage inevitably overrepresents certain species (e.g. 28 of 31 platyhelminth genomes on WormBase© are from species infecting humans, their companion animals, or host species with importance in agriculture or aquaculture). Recent estimates suggest that NCBI only contains sequences from 3% of the identified species on Earth, with an inevitable bias towards larger and more 'charismatic' vertebrates (Mora *et al.*, 2011), and even biodiversity-oriented sequencing efforts lag behind the number of species even in the relatively small number of medically important parasites (Ondrejicka *et al.*, 2014). This situation greatly impedes estimation of the distribution and evolution of less frequently studied genes such as FA desaturases.

From the MSA analysis, we detected several patterns in amino acids found inside and surrounding the H-box of the two confirmed and nine putative sequences. Based on these patterns, it is plausible that functional aspects of the confirmed sequences are also present in the putative sequences. These H-box amino acids are most likely part of the active sites of the enzymes, which would explain why they are highly conserved in their protein sequence; however, the function of the putative sequences should also be further validated by additional biochemical data, such as that obtained using a heterologous yeast expression system and subsequent FA analysis (as was done in Kabeya *et al.*, 2018). Future studies could attempt to detect relevant ω6 and ω3 desaturase activity (i.e. LNA and ALA production) from transformed yeast cells expressing the putative genes, and also supplement the transformed yeast cells with relevant FA precursors to potentially induce other reactions related to n-3 PUFA biosynthesis. For instance, Kabeya *et al.* (2018) supplemented the transformed yeast cells with n-6 PUFA precursors (e.g. LNA and ARA) and detected additional Δ17 desaturase activity (i.e. the conversion of ARA to EPA). Similar protocols have also been used by Ferraz *et al.* (2019) to verify the full enzymatic capacity of the Amazonian fish *Colossoma macropomum* for LC-PUFA biosynthesis. These methods could therefore confirm the total enzymatic capacity for the taxa in which we identified n-3 PUFA biosynthesis capability.

As mentioned previously, the intrinsic function(s) of PUFA (and by association, n-3 PUFA biosynthesis) in platyhelminths are still not well understood. Beyond modifying host-derived FA to produce immunomodulatory eicosanoids as discussed above, it is not clear whether PUFA contribute to parasite energy resources, as is suspected in certain trematode cercariae (Schariter *et al.*, 2002; Fried & Toledo, 2009). In addition, host–parasite interactions may affect n-3 PUFA biosynthesis in platyhelminths considering that infected hosts often have considerable lipid resources, and their parasites likely have strategies to obtain these. For instance, trematode parthenitae (sporocysts and rediae) are completely dependent on their snail intermediate host and can manipulate its energy reserves (e.g. lipids) to support their own survival, development and reproductive output (Arakelova *et al.*, 2004). Related to this, Fried & Sherma (1990) and Tunholi-Alves *et al.* (2011) found that the neutral lipid content of snail hosts (*Helisoma trivolvis* and *Biomphalaria glabrata*,

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respectively) increased after trematode infection (by *Echinostoma trivolvis* and *E. paraensi*, respectively). In particular, neutral lipid content increased within the DGG complex of snails where these larval parasites are localized (Esch *et al.*, 2002). In both cases, trematodes may have actually contributed to the lipid reserves of snails if they are capable of *de novo* n-3 PUFA synthesis and transferred some of these lipids to their hosts. If true, then larval trematodes could be considered as 'conditionally helpful parasites' (Fellous & Salvaudon, 2008), as such actions would reduce the costs of infection while simultaneously supporting parasitism in their hosts.

It has been established that trematode cercariae play a role in transferring nutritional PUFA to pelagic consumers within aquatic food webs (McKee *et al.*, 2020) and a wide range of cercariae consumers have been reported (e.g. Orlofske *et al.*, 2015; Mironova *et al.*, 2019). Although there are no reports of predation upon cestode larvae within the environment (such as aquatic coracidia) that we are aware of, it is likely that a wide variety of free-living parasite infectious stages are consumed (Johnson *et al.*, 2010). Confirming the possibility for *de novo* n-3 PUFA biosynthesis in trematodes and cestodes may therefore adjust our understanding of the nutritional contributions of free-living parasite infectious stages within food webs. Such findings have considerable value given that dietary PUFA are vital for maintaining consumer populations. For instance, many terrestrial animals cannot biosynthesize essential PUFA, and are also unable to obtain sufficient PUFA from their primary terrestrial-based food sources (Gladyshev *et al.*, 2009). Thus, they rely heavily on PUFA exports from aquatic ecosystems (e.g. shore drift, aquatic insect and amphibian emergence, preying on fish in adjacent water bodies); these aquatic-based PUFA are then transferred to higher trophic levels and are highly conserved within terrestrial food webs (Gladyshev *et al.*, 2009). The possibility of *de novo* PUFA biosynthesis in taeniids is particularly interesting, because these worms have terrestrial life cycles.

In aquatic systems, primary PUFA sources are being depleted by a variety of causes, including climate warming (Colombo *et al.*, 2020) and it is unclear whether alternative PUFA producers can fill the void (Arts *et al.*, 2001). If supported by further studies indicating gene functionality, our findings indicate that at least some trematodes and terrestrial cestodes may subsidize the natural production of PUFA in ecosystems, in addition to established roles of cercariae transferring host-derived PUFA within aquatic food webs (McKee *et al.*, 2020). These findings are especially promising given the ubiquity of infectious stages (e.g. Johnson *et al.*, 2010). For example, Preston *et al.* (2013) found that trematodes in pond ecosystems could produce between 14 and 1660 free-living cercariae snail^{-1} 24 h^{-1} throughout the summer, resulting in a total of 70–220 mg m^{-2} y^{-1} of dry cercarial biomass. McKee *et al.* (2020) then used these results to estimate the annual FA contributions of *R. ondatrae* in such ecosystems and determined that their cercariae may represent up to 337 µg m^{-2} y^{-1} of EPA and up to 6.2 µg m^{-2} y^{-1} of DHA.

Here we report putative sequences for ωx desaturases from endoparasites within the phylum Platyhelminthes. Although future work is needed to confirm the function of these genes, our results suggest at least some trematodes and cestodes are capable of *de novo* n-3 PUFA synthesis. Given that the free-living infectious stages of these helminths may be a good source of PUFA for aquatic consumers, it will be important to examine key genetic sequences in a wider range of platyhelminths in order to determine the extent to which macroparasites are capable of *de novo* synthesis. In addition, the disruption of desaturase activity in trematodes and cestodes may lead to possible therapeutic compounds, as suggested for FA in other parasites (Gratraud *et al.*, 2009). Notably, while many antihelmintics target parasite membrane ion channels, some also disrupt enzyme pathways, including those critical for metabolic activity (Martin *et al.*, 1997). It is thus necessary to understand the extent to which *de novo* n-3 PUFA biosynthesis is possible by macroparasites, such as those found in the phylum Platyhelminthes.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X20000954

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References

Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W and Lipman DJ (1997) Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402.

Arakelova KS, Chebotareva MA and Zabelinskii SA (2004) Physiology and lipid metabolism of *Littorina saxatilis* infected with trematodes. *Diseases of Aquatic Organisms* **60**, 223–231.

Arts MT, Ackman RG and Holub BJ (2001) 'Essential fatty acid' in aquatic ecosystems: A crucial link between diet and human health and evolution. *Canadian Journal of Fisheries and Aquatic Sciences* **58**, 122–137.

Assis R and Kondrashov AS (2014) Conserved proteins are fragile. *Molecular Biology and Evolution* **31**, 419–424.

Belley A and Chadee K (1995) Eicosanoid production by parasites: From pathogenesis to immunomodulation? *Parasitology Today* **11**, 327–334.

Bernt M, Bleidorn C, Braband A, *et al.* (2013) A comprehensive analysis of bilaterian mitochondrial genomes and phylogeny. *Molecular Phylogenetics and Evolution* **69**, 352–364.

Bexkens ML, Mebius MM, Houweling M, Brouwers JF, Tielens AG and van Hellemond JJ (2019) *Schistosoma mansoni* does not and cannot oxidise fatty acids, but these are used for biosynthetic purposes instead. *International Journal for Parasitology* **49**, 647–656.

Brant SV and Loker ES (2005) Can specialized pathogens colonize distantly related hosts? Schistosome evolution as a case study. *PLoS Pathogens* **1**, e38.

Brett M and Müller-Navarra D (1997) The role of highly unsaturated fatty acids in aquatic food web processes. *Freshwater Biology* **38**, 483–499.

Buckner J and Hagen M (2003) Triacylglycerol and phospholipid fatty acids of the silverleaf whitefly: Composition and biosynthesis. *Archives of Insect Biochemistry and Physiology* **53**, 66–79.

Castro LFC, Tocher DR and Monroig Ó (2016) Long-chain polyunsaturated fatty acid biosynthesis in chordates: Insights into the evolution of FADS and ELOVL gene repertoire. *Progress in Lipid Research* **62**, 25–40.

Chen B and Wen JF (2011) The adaptive evolution divergence of triosephosphate isomerases between parasitic and free-living flatworms and the discovery of a potential universal target against flatworm parasites. *Parasitology Research* **109**, 283–289.

Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J and Sayers EW (2016) Genbank. *Nucleic Acids Research* **44**, D67–D72.

Colombo SM, Rodgers TFM, Diamond ML, Bazinet RP and Arts MT (2020) Projected declines in global DHA availability for human consumption as a result of global warming. *Ambio* **49**, 865–880.

Das UN (2006) Essential fatty acids: Biochemistry, physiology and pathology. *Biotechnology Journal* **1**, 420–439.

Daugschies A and Joachim A (2000) Eicosanoids in parasites and parasitic infections. *Advances in Parasitology* **46**, 182–240.

Edgecombe GD, Giribet G, Dunn CW, Hejnol A, Kristensen RM, Neves RC, Rouse GW, Worsaae K and Sørensen MV (2011) Higher-level metazoan relationships: Recent progress and remaining questions. *Organisms Diversity & Evolution* **11**, 151–172.

Egger B, Lapraz F, Tomiczek B, *et al.* (2015) A transcriptomic-phylogenomic analysis of the evolutionary relationships of flatworms. *Current Biology* **25**, 1347–1353.

Esch GW, Barger MA and Fellis KJ (2002) The transmission of digenetic trematodes: Style, elegance, complexity. *Integrative and Comparative Biology* **42**, 304–312.

Fellous S and Salvaudon L (2008) How can your parasites become your allies? *Trends in Parasitology* **25**, 62–66.

Ferraz RB, Kabeya N, Lopes-Marques M, Machado AM, Ribeiro RA, Salaro AL, Ozório R, Castro LFC and Monroig Ó (2019) A complete enzymatic capacity for long-chain polyunsaturated fatty acid biosynthesis is present in the Amazonian teleost tambaqui, *Colossoma Macropomum*. *Comparative Biochemistry and Physiology, Part B* **227**, 90–97.

Fried B and Sherma J (1990) Thin layer chromatography of lipids found in snails (Gastropoda: Mollusca). *Journal of Planar Chromatography* **3**, 290–299.

Fried B and Toledo R (2009) *The biology of echinostomes: From the molecule to the community*. 333 pp. Springer Science and Business Media. New York.

Furlong ST (1991) Unique roles for lipids in *Schistosoma mansoni*. *Parasitology Today* **7**, 59–62.

Galaktionov KV and Skirnisson K (2007) New data on *Microphallus breviatus* Deblock & Maillard, 1975 (Microphallidae: Digenea) with emphasis on the evolution of dixenous life cycles of microphallids. *Parasitology Research* **100**, 963–971.

Gladyshev M, Arts M and Sushchik N (2009) Preliminary estimates of the export of omega-3 highly unsaturated fatty acids (EPA + DHA) from aquatic to terrestrial ecosystems. pp. 179–209 in Kainz M, Brett M, Arts M (Eds) *Lipids in aquatic ecosystems*. New York, Springer.

Gratraud P, Huws E, Falkard B, *et al.* (2009) Oleic acid biosynthesis in *Plasmodium falciparum*: Characterization of the stearoyl-CoA desaturase and investigation as a potential therapeutic target. *PLoS One* **4**, e6889.

Gregory TR (2020) Animal Genome Size Database. In: Genome Size http://www.genomesize.com. Accessed 18 October 2020

Hashimoto K, Yoshizawa AC, Okuda S, Kuma K, Goto S and Kanehisa M (2008) The repertoire of desaturases and elongases reveals fatty acid variations in 56 eukaryotic genomes. *Journal of Lipid Research* **49**, 183–191.

Hatcher MJ, Dick JT and Dunn AM (2012) Diverse effects of parasites in ecosystems: Linking interdependent processes. *Frontiers in Ecology and the Environment* **10**, 186–194.

Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ and Jacobson J (2008) Helminth infections: The great neglected tropical diseases. *Journal of Clinical Investigations* **118**, 1311–1321.

Howe KL, Bolt BJ, Shafie M, Kersey P and Berriman M (2017) Wormbase ParaSite – a comprehensive resource for helminth genomics. *Molecular and Biochemical Parasitology* **215**, 2–10.

International Helminth Genomes Consortium (2019) Comparative genomics of the major parasitic worms. *Nature Genetics* **51**, 163.

Johnson PTJ, Dobson A, Lafferty KD, Marcogliese DJ, Memmott J, Orlofske SA, Poulin R and Thieltges DW (2010) When parasites become prey: Ecological and epidemiological significance of eating parasites. *Trends in Ecology & Evolution* **25**, 362–371.

Kabeya N, Fonseca MM, Ferrier DEK, Navarro JC, Bay LK, Francis DS, Tocher DR, Castro FC and Monroig Ó (2018) Genes for *de novo* biosynthesis of omega-3 polyunsaturated fatty acids are widespread in animals. *Science Advances* **4**, eaar6849.

Kainz M, Arts MT and Mazumder A (2004) Essential fatty acids in the planktonic food web and their ecological role for higher trophic levels. *Limnology and Oceanography* **49**, 1784–1793.

Karasov WH and Martinez del Rio C (2007) *Physiological ecology: How animals process energy, nutrients, and toxins*. 741 pp. Princeton, New Jersey, Princeton University Press.

Khozin-Goldberg I, Iskandarov U and Cohen Z (2011) LC-PUFA from photosynthetic microalgae: Occurrence, biosynthesis, and prospects in biotechnology. *Applied Microbiology and Biotechnology* **91**, 905–915.

Knapp J, Nakao M, Yanagida T, Okamoto M, Saarma U, Lavikainen A and Ito A (2011) Phylogenetic relationships within *Echinococcus* and *Taenia* tapeworms (Cestoda: Taeniidae): An inference from nuclear protein-coding genes. *Molecular Phylogenetics and Evolution* **61**, 628–638.

Kumar S, Stecher G, Li M, Knyaz C and Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution* **35**, 1547–1549.

Kuris A (1990) Guild structure of larval trematodes in molluscan hosts: Prevalence, dominance and significance of competition. pp. 69–100 in Esch GW, Bush AO, Aho JM (Eds) *Parasite communities: Patterns and process*. London, Chapman and Hall.

Kuris AM, Hechinger RF, Shaw JC, *et al.* (2008) Ecosystem energetic implications of parasite and free-living biomass in three estuaries. *Nature* **454**, 515–518.

Lafferty KD, Allesina S, Arim M, *et al.* (2008) Parasites in food webs: The ultimate missing links. *Ecology Letters* **11**, 533–546.

Lavikainen A, Haukisalmi V, Lehtinen MJ, Henttonen H, Oksanen A and Meri S (2008) A phylogeny of members of the family Taeniidae based on the mitochondrial cox1 and nad1 gene data. *Parasitology* **135**, 1457–1467.

Letunic I, Doerks T and Bork P (2015) SMART: Recent updates, new developments and status in 2015. *Nucleic Acids Research* **43**, D257–D260.

Machado-Pinto J and Laborne L (2016) Cestodes. pp. 421 in Tyring SK, Lupi O, Hengge UR (Eds) *Tropical dermatology*. Edinburgh, Elsevier.

Marcogliese DJ (2005) Parasites of the superorganism: Are they indicators of ecosystem health? *International Journal for Parasitology* **35**, 705–716.

Marcogliese DJ and Cone DK (1997) Food webs: A plea for parasites. *Trends in Ecology & Evolution* **12**, 320–325.

Martin RJ, Robertson AP and Bjorn H (1997) Target sites of anthelmintics. *Parasitology* **114**, 111–124.

Martín-Durán JM, Ryan JF, Vellutini BC, Pang K and Hejnol A (2017) Increased taxon sampling reveals thousands of hidden orthologs in flatworms. *Genome Research* **27**, 1263–1272.

McKee KM, Koprivnikar J, Johnson PTJ and Arts MT (2020) Parasite infectious stages provide essential fatty acids and lipid-rich resources to freshwater consumers. *Oecologia* **192**, 477–488.

Min XJ and Hickey DA (2007) DNA asymmetric strand bias affects the amino acid composition of mitochondrial proteins. *DNA Research* **14**, 201–206.

Minematsu T, Yamazaki S, Uji Y, Okabe H, Korenaga M and Tada I (1990) Analysis of polyunsaturated fatty acid composition of *Strongyloides ratti* in relation to development. *Journal of Helminthology* **64**, 303–309.

Mironova E, Gopko M, Pasternak A, Mikheev V and Taskinen J (2019) Trematode cercariae as prey for zooplankton: Effect on fitness traits of predators. *Parasitology* **146**, 105–111.

Monroig Ó and Kabeya N (2018) Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: A comprehensive review. *Fisheries Science* **84**, 911–928.

Mora C, Tittensor DP, Adl S, Simpson AGB and Worm B (2011) How many species are there on earth and in the ocean? *PLoS Biology* **9**, e1001127.

Morley NJ (2012) Cercariae (Platyhelminthes: Trematoda) as neglected components of zooplankton communities in freshwater habitats. *Hydrobiologia* **691**, 7–19.

National Center for Biotechnology Information (2020) Taxonomy statistics: txid6157. In: NCBI. https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=6157&lvl=3&p=has_linkout&p=blast_url&p=genome_blast&p=mapview&lin=f&keep=1&srchmode=1&unlock. Accessed 18 October 2020

Nichols DS (2003) Prokaryotes and the input of polyunsaturated fatty acids to the marine food web. *FEMS Microbiology Letters* **219**, 1–7.

Olson PD and Tkach VV (2005) Advances and trends in the molecular systematics of the parasitic Platyhelminthes. *Advances in Parasitology* **60**, 165–243.

Ondrejicka DA, Locke SA, Morey K, Borisenko AV and Hanner RH (2014) Status and prospects of DNA barcoding in medically important parasites and vectors. *Trends in Parasitology* **30**, 582–591.

Orlofske SA, Jadin RC and Johnson PTJ (2015) It's a predator–eat–parasite world: How characteristics of predator, parasite and environment affect consumption. *Oecologia* **178**, 537–547.

Park JK, Kim KH, Kang S, Kim W, Eom KS and Littlewood DTJ (2007) A common origin of complex life cycles in parasitic flatworms: Evidence from the complete mitochondrial genome of *Microcotyle sebastis* (Monogenea: Platyhelminthes). *BMC Evolutionary Biology* **7**, 11.

Pereira SL, Leonard AE and Mukerji P (2003) Recent advances in the study of fatty acid desaturases from animals and lower eukaryotes. *Prostaglandins, Leukotrienes & Essential Fatty Acids* **68**, 97–106.

Peyou-Ndi MM, Watts JL and Browse J (2000) Identification and characterization of an animal delta (12) fatty acid desaturase gene by heterologous expression in *Saccharomyces cerevisiae*. *Archives of Biochemistry and Biophysics* **376**, 399–408.

Pottinger PS and Jong EC (2017) Trematodes. pp. 664 in Sanford CA, Jong EC, Pottinger PS (Eds) *The travel and tropical medicine manual*. Edinburgh, Elsevier Health Sciences.

Poulin R and Randhawa HS (2015) Evolution of parasitism along convergent lines: From ecology to genomics. *Parasitology* **142**, S6–S15.

Preston DL, Orlofske SA, Lambden JP and Johnson PTJ (2013) Biomass and productivity of trematode parasites in pond ecosystems. *Journal of Animal Ecology* **82**, 509–517.

Riutort M, Álvarez-Presas M, Lázaro E, Solà E and Paps J (2012) Evolutionary history of the Tricladida and the Platyhelminthes: An up-to-date phylogenetic and systematic account. *International Journal of Developmental Biology* **56**, 5–17.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

Rosenkranz M, Lagrue C, Poulin R and Selbach C (2018) Small snails, high productivity? Larval output of parasites from an abundant host. *Freshwater Biology* **63**, 1602–1609.

Schariter JA, Pachuski J, Fried B and Sherma J (2002) Determination of neutral lipids and phospholipids in the cercariae of *Schistosoma mansoni* by high performance thin layer chromatography. *Journal of Liquid Chromatography & Related Technologies* **25**, 1615–1622.

Sievers F, Wilm A, Dineen D, *et al.* (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using clustal Omega. *Molecular Systems Biology* **7**, 539.

Silvestro D and Michalak I (2012) raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity and Evolution* **12**, 335–337.

Smyth JD and McManus DP (1989) *The physiology and biochemistry of Cestodes*. 2nd. Ed. Cambridge, Cambridge Univ. Pr.

Sperling P, Ternes P, Zank TK and Heinz E (2003) The evolution of desaturases. *Prostaglandins, Leukotrienes & Essential Fatty Acids* **68**, 73–95.

Spychalla JP, Kinney AJ and Browse J (1997) Identification of an animal omega-3 fatty acid desaturase by heterologous expression in *Arabidopsis*. *Proceedings of the National Academy of Sciences* **94**, 1142–1147.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Tielens AG (1997) Biochemistry of trematodes. pp. 309–343 in Fried B, Graczyk TK (Eds) *Advances in trematode biology*. CRC Press. Boca Raton.

Tunholi-Alves VM, Tunholi VM, Gôlo P, Lustrino D, Maldonado A, Bittencourt VREP, Maria de Lurdes A and Pinheiro J (2011) Lipid levels in *Biomphalaria glabrata* infected with different doses of *Echinostoma paraensei* miracidia. *Experimental Parasitology* **128**, 212–216.

Twining CW, Brenna JT, Hairston NG and Flecker AS (2016) Highly unsaturated fatty acids in nature: What we know and what we need to learn. *Oikos* **125**, 749–760.

Viney M (2017) How can we understand the genomic basis of nematode parasitism? *Trends in Parasitology* **33**, 444–452.

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