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In silico identification of tetraspanins in monopisthocotylean (Platyhelminthes: Monogenea) parasites of fish

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Abstract

Tetraspanins are a superfamily of transmembrane proteins that in flatworms have structural roles in the development, maturation or stability of the tegument. Several tetraspanins are considered as potential candidates for vaccines or drugs against helminths. Monopisthocotylean monogeneans are ectoparasites of fish that are health hazards for farmed fish. The aim of this study was to identify *in silico* putative tetraspanins in the genomic datasets of four monopisthocotylean species. The analysis predicted and classified 40 tetraspanins in *Rhabdosynochus viridisi*, 39 in *Scutogyrus longicornis*, 22 in *Gyrodactylus salaris* and 13 in *Neobenedenia melleni*, belonging to 13 orthologous groups. The high divergence of tetraspanins made it difficult to annotate their function. However, a conserved group was identified in different metazoan taxa. According to this study, metazoan tetraspanins can be divided into 17 monophyletic groups. Of the 114 monogenean tetraspanins, only seven were phylogenetically close to tetraspanins from non-platyhelminth metazoans, which suggests that this group of proteins shows rapid sequence divergence. The similarity of the monopisthocotylean tetraspanins was highest with trematodes, followed by cestodes and then free-living platyhelminths. In total, 27 monopisthocotylean-specific and 34 flatworm-specific tetraspanins were identified. Four monogenean tetraspanins were orthologous to TSP-1, which is a candidate for the development of vaccines and a potential pharmacological target in trematodes and cestodes. Although studies of tetraspanins in parasitic flatworms are scarce, this is an interesting group of proteins for the development of new methods to control monogeneans.

Introduction

Tetraspanins are a superfamily of transmembrane proteins characterized by having four transmembrane domains that form a small and a large extracellular loop (Maecker *et al.*, 1997). Metazoan members of this superfamily are classified into four major monophyletic families, named CD, CD63, uroplakin and RDS (Garcia-España *et al.*, 2008). Tetraspanins participate in several aspects of the regulation of cellular development, proliferation, activation and motility. These roles are thought to be associated with the capacity of tetraspanins to interact with other proteins such as adhesion molecules, receptor and co-receptor molecules, major histocompatibility complex antigens, cytoplasmic kinases and also other tetraspanins (Hemler, 2003; Seigneuret *et al.*, 2013).

In parasitic platyhelminths, particularly of the classes Cestoda and Trematoda, tetraspanins have structural roles in the development, maturation and stability of the tegument (Tran *et al.*, 2010; Mousavi *et al.*, 2020), and possibly in modulating the host immune system (Dang *et al.*, 2012). Platyhelminths have tetraspanins that are abundantly expressed in the tegument, where they are exposed to the host's immune system (Dang *et al.*, 2012; Piratae *et al.*, 2012). Some of these tetraspanins have been proposed as potential vaccine and/or drug target candidates against platyhelminth infections (Dang *et al.*, 2009; Piratae *et al.*, 2012). To date, the tetraspanins have not been studied in platyhelminths of the class Monogenea.

Monogeneans are mainly fish ectoparasites, classified into two subclasses: Monopisthocotylea and Polyopisthocotylea. Infection with these parasites can cause haemorrhaging, inflammation, epithelial hyperplasia and fused lamellae (Andree *et al.*, 2015), and leads to mortality, reduced growth and stress in cultured fish (Ogawa, 2015), with economic loss as a consequence (Shinn *et al.*, 2015). *In silico* analysis of tetraspanin sequences in monogeneans can be used to predict their roles and to find candidates for vaccine development and as drug targets.

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Here, we identify and classify for the first time the tetraspanin proteins in the genome, transcriptome or expressed sequence tags (ESTs) of the monopisthocotylean monogeneans *Rhabdosynochus viridisi*, *Scutogyrus longicornis*, *Gyrodactylus salaris* and *Neobenedenia melleni*. This allowed us to assign orthologs of tetraspanins both inside and outside monogenean lineage. Among these proteins was a potential drug/vaccine target for the control of monogenean parasitic infections.

Materials and methods

Tetraspanin identification

Predicted tetraspanins were identified from the *de novo* transcriptome assemblies of the monopisthocotylean monogeneans *R. viridisi* and *S. longicornis* (Mendeley Data repository doi: 10.17632/2wvnwn4d7p.1), the genome of *G. salaris* (Hahn *et al.*, 2014; Howe *et al.*, 2017) and EST sequences of *N. melleni* that are available in the National Center for Biotechnology Information (NCBI) database (accession numbers GW917985.1–GW924710.1). Predicted proteins were aligned against the Pfam protein domain database version 34 (Finn *et al.*, 2016) using HMMSCAN 3.1.b2 (Potter *et al.*, 2018) with default parameters. Only the sequences with the tetraspanin domain (PF00335) were retained as the best hits. The transmembrane domain was identified using the Phobius web server (Käll *et al.*, 2007). To detect potential contaminant and non-tetraspanin sequences, the proteins were aligned against the NCBI non-redundant protein database using NCBI BLASTp with default parameters (e-values <0.05). To avoid overrepresentation of genes, the longest isoform for each gene was extracted using the Trinity helper script 'get_longest_isoform_seq_per_trinity_gene.pl' and CD-HIT 4.8.1 (Li & Godzik, 2006) with 100% identity.

In addition, for comparative purposes, tetraspanin proteins were identified in the polyopisthocotyleans *Protopolystoma xenopodis* and *Eudiplozoon nipponicum*; in the cestodes *Echinococcus multilocularis*, *Hymenolepis microstoma* and *Taenia asiatica*; in the trematodes *Fasciola hepatica* and *Schistosoma mansoni*; and in the free-living platyhelminths *Bothrioplana semperi* and *Schmidtea mediterranea* (Laumer *et al.*, 2015; Howe *et al.*, 2017; Vorel *et al.*, 2021).

Phylogenetic analysis

The tetraspanin superfamily in metazoans was first classified using the reviewed UniProt proteins database. To this end, a phylogenetic analysis was performed using the protein domains of *Homo sapiens* and *Mus musculus* (supplementary fig. S1). A second phylogenetic analysis was performed using the domains of 159 tetraspanin proteins belonging to 24 metazoan species (supplementary fig. S2). To retrieve the region of the tetraspanin Pfam domain, the sequence was searched in MOTIF (https://www.genome.jp/tools/motif/MOTIF.html).

The sequences were aligned using MAFFT 7.31 (Katoh & Standley, 2013) using default parameters for phylogenetic analysis, and the gaps were removed with Trimal (Capella-Gutiérrez *et al.*, 2009) using the automated mode (-gappyout). The best evolutionary models were obtained with the ModelFinder program (Kalyaanamoorthy *et al.*, 2017), and the tree was constructed in IQ-TREE 1.6.12 (Nguyen *et al.*, 2015) using the approximate likelihood ratio test, which is similar to the Shimodaira–Hasegawa test (1000 replicates). The tree was visualized and annotated with FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and the ITOL web server (Letunic & Bork, 2021). To support functional prediction, we considered only the nodes with bootstrap values greater than or equal to 80%.

To verify the consistency of the classification, a third phylogenetic analysis was performed with the sequences previously used and additional tetraspanin domains from the plant *Arabidopsis thaliana* and the amoeba *Dictyostelium discoideum* (supplementary fig. S3); a fourth used the complete sequence of the 159 metazoan proteins already analysed (supplementary fig. S4). According to our analysis, the metazoan tetraspanins can be divided into 17 groups (supplementary table S1 and supplementary figs S1–S4), which were used to classify the monogenean proteins. It was not possible to cluster metazoan proteins into more specific groups.

Three phylogenetic analyses were performed for the monopisthocotylean tetraspanins using the methodology described above. The first of these analyses was performed to predict proteins orthologous to metazoan tetraspanins using monopisthocotylean protein domains, with reference to *Homo sapiens* and *Mus musculus*. The second phylogenetic analysis was performed using protein domains from monopisthocotyleans and *S. mansoni*, to determine orthologous and paralogous groups between monogenean species and to identify proteins orthologous to Sm-TSP-1, Sm-TSP-2 and Sm23 (GenBank accession numbers AAN17278.2, AAN17276.1 and P19331.1, respectively), which are potential vaccine candidates from *S. mansoni* (Loukas *et al.*, 2007). We considered orthologous groups with similar topology in the phylogenetic analysis.

The proteins were aligned to the following NCBI non-redundant protein databases to identify lineage-specificity of the monogenean tetraspanins and their similarity with other taxa: Cestoda (taxid: 6199), Trematoda (taxid: 6178), Rhabditophora (taxid: 147100), Lophotrochozoa (taxid: 1206795; exclude: Platyhelminthes), Spiralia (taxid: 2697495; exclude: Lophotrochozoa), Protostomia (taxid: 33317; exclude: Spiralia) and Bilateria (taxid: 33213; exclude: Protostomia). The e-values were visualized in a heatmap using the ITOL web server.

Results

Tetraspanin superfamily in monogeneans

We identified 473 putative tetraspanins in 13 species of Platyhelminthes (fig. 1 and supplementary table S2). The sequences in fasta format are available in supplementary file S1. The most abundant tetraspanins were those with four domains (supplementary fig. S5). Monopisthocotylean monogeneans contributed 114 putative tetraspanins (40 in *R. viridisi*, 39 in *S. longicornis*, 22 in *G. salaris* and 13 in *N. melleni*), containing mainly either three or four domains (supplementary table S2).

Phylogenetic analysis

The phylogenetic analysis yielded 13 orthologous groups and nine paralogous groups of monopisthocotylean tetraspanins (fig. 2 and supplementary table S3). We identified putative monopisthocotylean tetraspanins closely related to Sm-TSP-1 ('ortholog group 8': snap_masked-scf7180006948348-processed-gene-0.12-mRNA-1 of *G. salaris*, TRINITY_DN20_c0_g2_i4.p1 and TRINITY_DN693_c0_g1_i9.p1 of *R. viridisi*, and TRINITY_DN1839_c0_g1_i4__g.6175 of *S. longicornis*), a tetraspanin from the trematode *S. mansoni* considered to be a potential candidate for use in a

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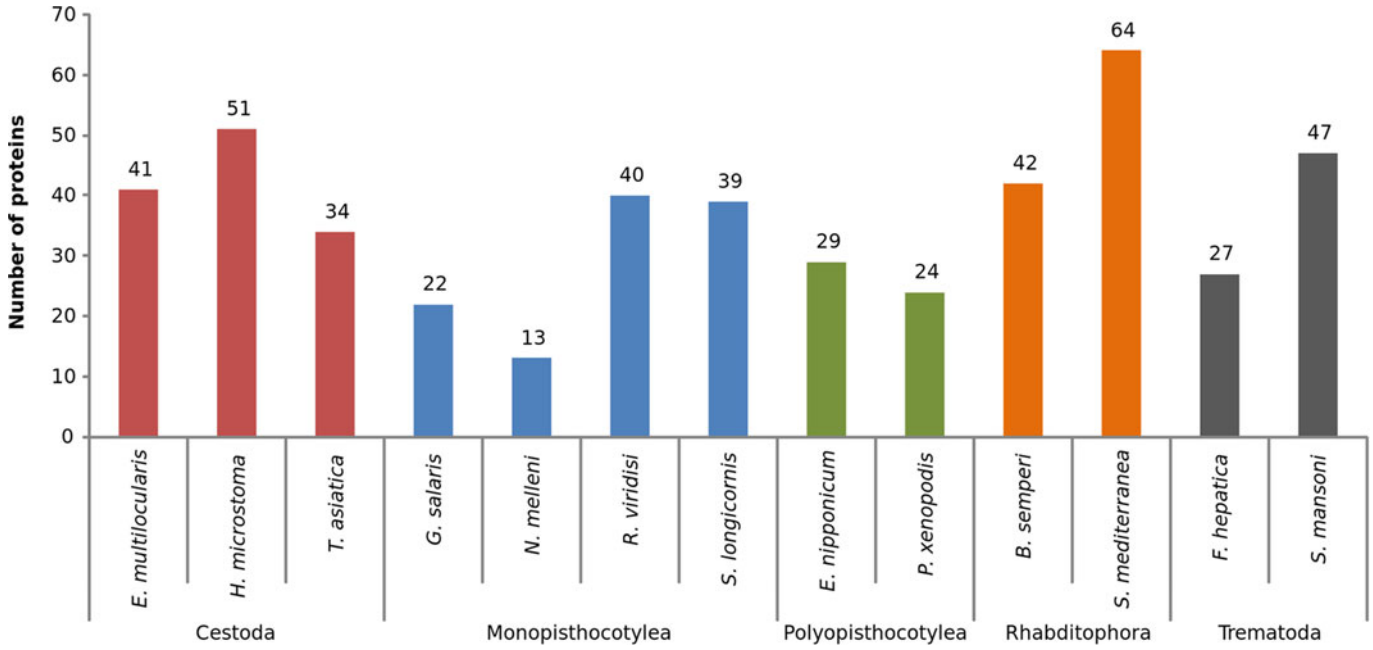

Fig. 1. The numbers of tetraspanin proteins identified in various platyhelminths.

vaccine (fig. 3). Orthologous relationships between monogenean proteins and Sm-TSP-2 and Sm23 were not detected.

Seven of the 114 monopisthocotylean tetraspanins had phylogenetic closeness to four monophyletic groups of metazoan tetraspanins (fig. 3, supplementary fig. S6 and supplementary table S3). Five proteins from *R. viridisi*, *S. longicornis* and *G. salaris* ('ortholog group 4' and 'ortholog group 13') were close to the proteins TSP-5, TSP-10, TSP-12, TSP-14, TSP-15, TSP-17, TSP-33, PRPH2, RDS and ROM from other metazoans. A protein from *S. longicornis* (TRINITY_DN10990_c2_g2_i1__g.19781) was phylogenetically close to TSP-11 and CD-151, whereas another (TRINITY_DN1652_c0_g2_i2__g.9741) was close to TSP-13 and TSP-31.

Twenty-seven monopisthocotylean-specific and 34 flatworm-specific tetraspanins were identified (e-value >$1e^{-5}$) (supplementary table S4). The similarity of the monopisthocotylean tetraspanins was highest with trematodes, followed by cestodes and then free-living platyhelminths. The ortholog group 13 proteins of monopisthocotyleans were the most conserved tetraspanins, having phylogenetic closeness to proteins of trematodes, cestodes, free-living platyhelminths and distant taxa (fig. 2). Orthologous proteins of this group were identified in platyhelminths through phylogenetic analysis (supplementary fig. S7). With the exception of ortholog group 13, monogenean tetraspanins showed little similarity to bilaterian proteins (see heatmap in fig. 2).

Discussion

In the present study, the identification and classification of the tetraspanin superfamily in monopisthocotylean monogeneans was performed through a bioinformatic pipeline. The number of tetraspanins found in monopisthocotyleans was similar to those found in other parasitic flatworms. Orthologous relationships between monopisthocotylean tetraspanins were scarce. The monopisthocotylean tetraspanins present large sequence divergence, as observed in other metazoans (Garcia-España *et al.*, 2008). This could be related to the ability of tetraspanins to undergo functional adaptation, because they serve important, overlapping and non-essential functions (Huang *et al.*, 2010). For example, tetraspanins are involved in diverse biological processes such as motility, cell adhesion, signalling, protein trafficking and cell–cell interaction (Levy & Shoham, 2005; Andreu & Yáñez-Mó, 2014). This suggests that in some taxa, including monogeneans, tetraspanins are the result of rapid sequence divergence.

The 17 monophyletic groups of metazoan tetraspanins identified herein contrast with the classification proposed by Garcia-España *et al.* (2008), who suggested four major monophyletic subfamilies. This difference is probably due to the classification methods used. We used the approximate likelihood ratio test, similar to the Shimodaira–Hasegawa test, for the phylogenetic analysis, whereas Garcia-España *et al.* (2008) used the maximum parsimony approach, in which the amino acid characters are weighted according to the genetic analysis used.

It was difficult to predict functionality in the monopisthocotylean tetraspanins because they were clustered with multiple reference tetraspanins having different functions. For highly divergent protein groups, their orthologous relationships with proteins from related species can infer and support the prediction of functionality. However, in helminth parasites, the functions of only a few tetraspanins are known (Mousavi *et al.*, 2020). The high divergence of monopisthocotylean tetraspanins is similar to that observed in other worms (Huang *et al.*, 2005). Nonetheless, our results suggest that monopisthocotylean tetraspanins of ortholog group 13 are conserved. It is possible that the current repertoire of monopisthocotylean tetraspanins diverged from ortholog group 13 through duplication events and rapid divergence, as suggested for other taxa (Huang *et al.*, 2010). This group includes essential proteins that in vertebrates participate in the regulation of photoreceptor architecture and maturation of tissues (fig. 3). The parasitic platyhelminths also have photoreceptors, which participate in directional responses to light and in the process of

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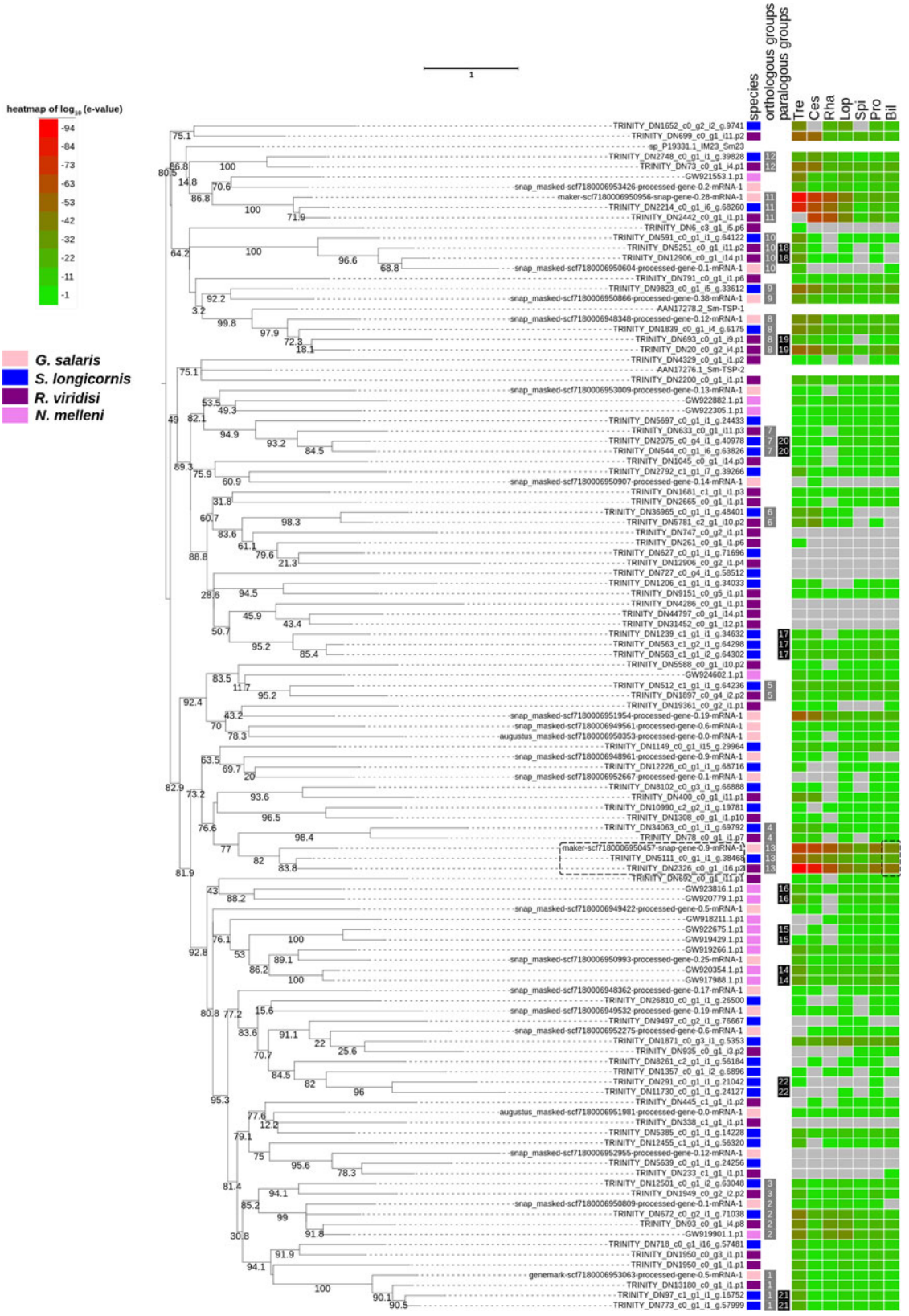

Fig. 2. Phylogenetic analysis performed using the protein domains of 114 tetraspanins belonging to monopisthocotyleans and three from *S. mansoni*. The midpoint-rooted phylogenetic tree was constructed using 1000 replicates of the approximate likelihood ratio test (similar to the Shimodaira–Hasegawa test). The WAG + F+R5 model was implemented. The coloured boxes indicate the $\log_{10}$-transformed e-values obtained from the alignment of the monogenean sequences against sequences from different taxa using the NCBI database. Abbreviations: Tre, Trematoda; Ces, Cestoda; Rha, Rhabditophora; Lop, Lophotrochozoa; Spi, Spiralia; Pro, Protostomia; Bil, Bilateria. The proteins Sm23, Sm-TSP-1 and Sm-TSP-1 of *S. mansoni* were used as reference sequences. Dashed lines show ortholog group 8 and information of the similarity with bilaterian tetraspanins. Colors in the species column represent: purple, *R. viridisi*; blue, *S. longicornis*; skin, *G. salaris*; pink, *N. melleni*.

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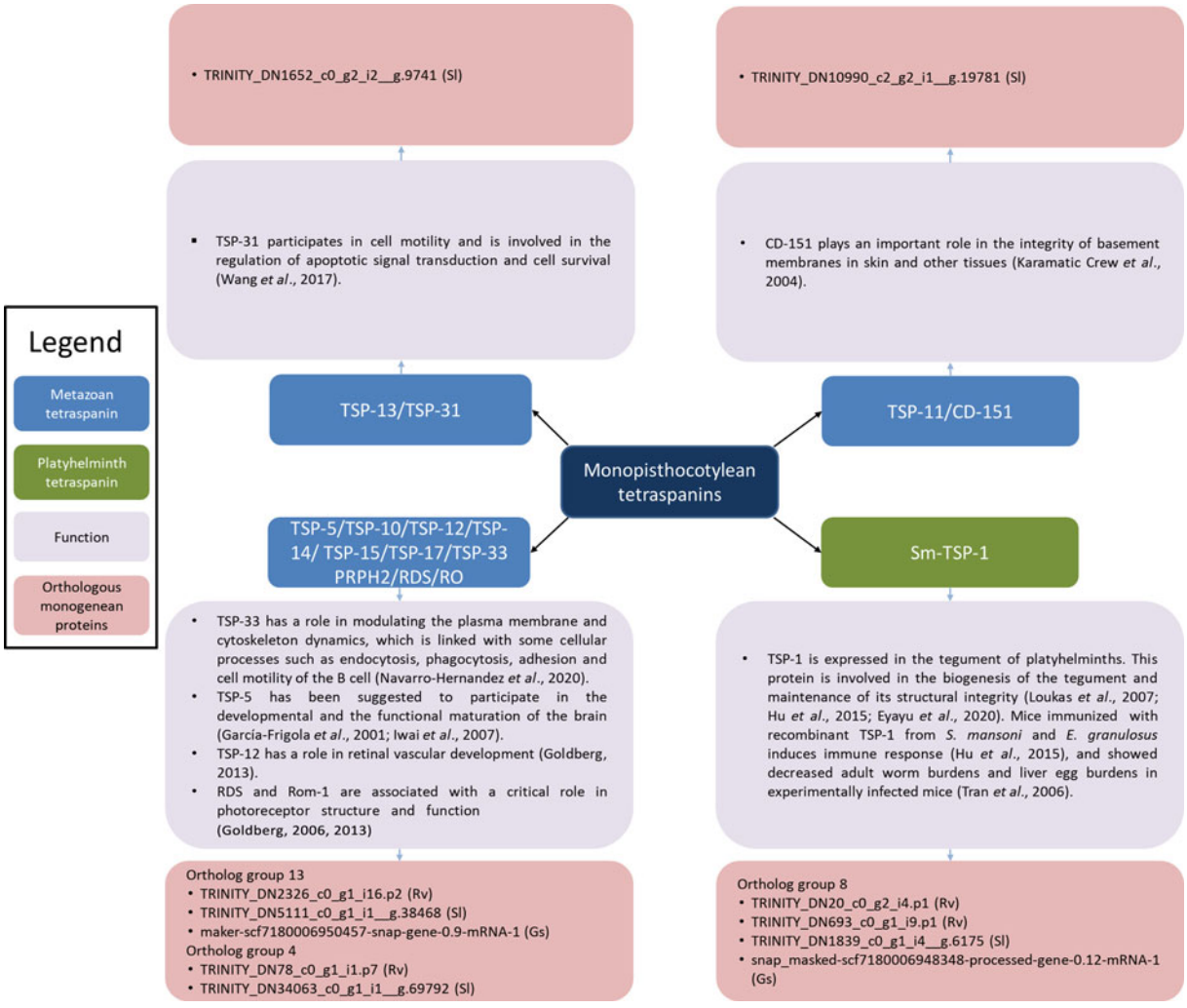

Fig. 3. Monopisthocotylean tetraspanins that are phylogenetically closer to other metazoan tetraspanins. Information on the functions of the reference proteins and the identifiers of the monopisthocotylean tetraspanin are shown. Abbreviations: Rv, *R. viridisi*; Sl, *S. longicornis*; Gs, *G. salaris*.

locating the larva to a host (Pike & Wink, 1986). Given the essential functions of this protein group in diverse taxa, these tetraspanins might have a similar role in monogeneans.

Special attention was given to proteins with potential for improving strategies to control parasites. Thus, the orthologous relationship between some monopisthocotylean tetraspanins (ortholog group 8) and Sm-TSP-1 represents an interesting finding. Sm-TSP-1 and Sm-TSP-2 are proteins expressed in the tegument of *S. mansoni*, and represent potential vaccine candidates (Loukas *et al.*, 2007). For example, mice inoculated with recombinant Sm-TSP-1 and Sm-TSP-2 from *S. mansoni* showed decreased adult worm burdens and liver egg burdens after being infected (Tran *et al.*, 2006). Similarly, mice immunized with recombinant TSP1 from *Echinococcus granulosus* showed induction of IgG1 and IgG2a antibodies, IFN-γ and IL-12 (Hu *et al.*, 2015). Therefore, TSP-1 in monogeneans might be a potential vaccine candidate for controlling these parasites in fish.

According to the present study, TSP-1 is absent in non-platyhelminth metazoans. In *E. granulosus*, Eg-TSP-1 is expressed in the tegument and is involved in its biogenesis and maintenance of its structural integrity (Hu *et al.*, 2015; Eyayu *et al.*, 2020). The important function of this protein and its possible absence in hosts suggest that TSP-1 might be a potential drug target for controlling platyhelminth infections.

One of the main shortcomings of this study was in the assignment of putative functionality because few monogenean tetraspanins showed orthology to functionally characterized proteins. The limited amount of genomic information available in monopisthocotyleans might have prevented a larger number of orthologous and paralogous groups being found in the phylogenetic analysis. This is important considering that proteins conserved in different species can represent pharmacological targets for an antiparasitic drug. In this study, given the type of data acquired, some tetraspanins were not identified, perhaps because the arrangement of introns and exons was not analysed, which might be important for the detection of these proteins (Huang *et al.*, 2005).

Because the transcriptome information was developed from adult monopisthocotylean organisms, it was not possible to detect proteins at specific stages of growth, which might be useful for understanding how tetraspanins participate in the development of monogeneans. It is important to validate the *in silico*-predicted tetraspanins using other omic technologies and experimental approaches. For example, for *G. salaris*, transcriptomic information could validate that the tetraspanins predicted from the

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genome are expressed. Similarly, proteomic information would help validate tetraspanins predicted from the *R. viridisi* and *S. longicornis* transcriptome and *N. melleni* ESTs. Experimental approaches such as RNA interference (RNAi), transgenesis, CRISPR–Cas9 gene editing and immunofluorescence assays could help determine the function and localization of tetraspanins from monogeneans. In non-model organisms in which genetic manipulations are difficult, RNAi is an important tool for determining phenotypes (McVeigh *et al.*, 2018). Immunofluorescence assays have been used with other platyhelminths to determine the localization of tetraspanins, which is important for identifying the proteins of the tegument that can represent potential target vaccines, as was done for Sm-TSP-1 (Loukas *et al.*, 2007). CRISPR–Cas9 has shown potential for experimental silencing or enhancement of transcription for inferring function in genes of interest in helminths (Lok *et al.*, 2017).

In summary, the phylogeny of monogenean tetraspanins was reconstructed and a possible evolutionary scenario proposed. One can speculate that the proteins in ortholog group 13, which are phylogenetically close to TSP-5, TSP-10, TSP-12, TSP-14, TSP-15, TSP-17, TSP-33, PRPH2, RDS and ROM, represent an ancestral state of monopisthocotylean tetraspanins. The present study identified vaccine candidates and other potential drug-target proteins in monogeneans. The tetraspanin superfamily represents an important group of proteins to which more attention should be given as potential targets in the development of new control methods against monogenean parasites.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0022149X22000098.

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Conflicts of interest. None.

Ethical standards. The research did not involve human and/or animal experimentation.

References

Andree KB, Roque A, Duncan N, Gisbert E, Estevez A, Tsertou MI and Katharios P (2015) *Diplectanum sciaenae* (Van Beneden & Hesse, 1863) (Monogenea) infecting meagre, *Argyrosomus regius* (Asso, 1801) Broodstock in Catalonia, Spain. A case report. *Veterinary Parasitology: Regional Studies and Reports* **1**, 75–79.

Andreu Z and Yáñez-Mó M (2014) Tetraspanins in extracellular vesicle formation and function. *Frontiers in Immunology* **5**, 442.

Capella-Gutiérrez S, Silla-Martínez JM and Gabaldón T (2009) Trimal: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973.

Dang Z, Yagi K, Oku Y, *et al.* (2009) Evaluation of *Echinococcus multilocularis* tetraspanins as vaccine candidates against primary alveolar echinococcosis. *Vaccine* **27**, 7339–7345.

Dang Z, Yagi K, Oku Y, *et al.* (2012) A pilot study on developing mucosal vaccine against alveolar echinococcosis (AE) using recombinant tetraspanin 3: vaccine efficacy and immunology. *PLoS Neglected Tropical Diseases* **6**, e1570.

Eyayu T, Zeleke AJ and Worku L (2020) Current status and future prospects of protein vaccine candidates against *Schistosoma mansoni* infection. *Parasite Epidemiology and Control* **11**, e00176.

Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J and Mitchell AL (2016) The Pfam protein families database: towards a more sustainable future. *Nucleic Acids Research* **44**, D279–D285.

García-Frigola C, Burgaya F, de Lecea L and Soriano E (2001) Pattern of expression of the tetraspanin Tspan-5 during brain development in the mouse. *Mechanisms of Development* **106**, 207–212.

Garcia-España A, Chung PJ, Sarkar IN, Stiner E, Sun TT and DeSalle R (2008) Appearance of new tetraspanin genes during vertebrate evolution. *Genomics* **91**, 326–334.

Goldberg AF (2006) Role of peripherin/rds in vertebrate photoreceptor architecture and inherited retinal degenerations. *International Review of Cytology* **253**, 131–175.

Goldberg AF (2013) Essential tetraspanin functions in the vertebrate retina. pp. 321–343 *in* Berditchevski F and Rubinstein E (*Eds*) *Tetraspanins*. Dordrecht, Springer.

Hahn C, Fromm B and Bachmann L (2014) Comparative genomics of flatworms (Platyhelminthes) reveals shared genomic features of ecto-and endoparastic neodermata. *Genome Biology and Evolution* **6**, 1105–1117.

Hemler ME (2003) Tetraspanin proteins mediate cellular penetration, invasion, and fusion events and define a novel type of membrane microdomain. *Annual Review of Cell and Developmental Biology* **19**, 397–422.

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.

Huang S, Tian H, Chen Z, Yu T and Xu A (2010) The evolution of vertebrate tetraspanins: Gene loss, retention, and massive positive selection after whole genome duplications. *BMC Evolutionary Biology* **10**, 1–17.

Huang S, Yuan S, Dong M, *et al.* (2005) The phylogenetic analysis of tetraspanins projects the evolution of cell–cell interactions from unicellular to multicellular organisms. *Genomics* **86**, 674–684.

Hu D, Song X, Xie Y, *et al.* (2015) Molecular insights into a tetraspanin in the hydatid tapeworm *Echinococcus granulosus*. *Parasites & Vectors* **8**, 1–10.

Iwai K, Ishii M, Ohshima S, Miyatake K and Saeki Y (2007) Expression and function of transmembrane-4 superfamily (tetraspanin) proteins in osteoclasts: reciprocal roles of Tspan-5 and NET-6 during osteoclastogenesis. *Allergology International* **56**, 457–463.

Käll L, Krogh A and Sonnhammer EL (2007) Advantages of combined transmembrane topology and signal peptide prediction—the Phobius web server. *Nucleic Acids Research* **35**, W429–W432.

Kalyaanamoorthy S, Minh BQ, Wong TK, Von Haeseler A and Jermiin LS (2017) Modelfinder: fast model selection for accurate phylogenetic estimates. *Nature Methods* **14**, 587–589.

Karamatic Crew V, Burton N, Kagan A, *et al.* (2004) CD151, the first member of the tetraspanin (TM4) superfamily detected on erythrocytes, is essential for the correct assembly of human basement membranes in kidney and skin. *Blood* **104**, 2217–2223.

Katoh K and Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* **30**, 772–780.

Laumer CE, Hejnol A and Giribet G (2015) Nuclear genomic signals of the 'microturbellarian' roots of platyhelminth evolutionary innovation. *eLife* **4**, e05503.

Letunic I and Bork P (2021) Interactive tree of life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Research* **49**, W293–W296.

Levy S and Shoham T (2005) The tetraspanin web modulates immune-signaling complexes. *Nature Reviews Immunology* **5**, 136–148.

Li W and Godzik A (2006) Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. *Bioinformatics* **22**, 1658–1659.

Lok JB, Shao H, Massey HC and Li X (2017) Transgenesis in *Strongyloides* and related parasitic nematodes: historical perspectives, current functional genomic applications, and progress towards gene disruption and editing. *Parasitology* **144**, 327–342.

Loukas A, Tran M and Pearson MS (2007) Schistosome membrane proteins as vaccines. *International Journal for Parasitology* **37**, 257–263.

Maecker HT, Todd SC and Levy S (1997) The tetraspanin superfamily: molecular facilitators. *The FASEB Journal* **11**, 428–442.

McVeigh P, McCusker P, Robb E, *et al.* (2018) Reasons to be nervous about flukicide discovery. *Trends in Parasitology* **34**, 184–196.

Mousavi SM, Afgar A, Mohammadi MA, Mortezaei S, Faridi A, Sadeghi B and Fasihi Harandi M (2020) Biological and morphological consequences

of dsRNA-induced suppression of tetraspanin mRNA in developmental stages of *Echinococcus granulosus*. *Parasites & Vectors* **13**, 1–10.

Navarro-Hernandez IC, López-Ortega O, Acevedo-Ochoa E, *et al.* (2020) Tetraspanin 33 (TSPAN33) regulates endocytosis and migration of human B lymphocytes by affecting the tension of the plasma membrane. *The FEBS Journal* **287**, 3449–3471.

Nguyen LT, Schmidt HA, Von Haeseler A and Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* **32**, 268–274.

Ogawa K (2015) Diseases of cultured marine fishes caused by Platyhelminthes (Monogenea, Digenea, Cestoda). *Parasitology* **142**, 178–195.

Pike AW and Wink R (1986) Aspects of photoreceptor structure and phototactic behavior in Platyhelminthes, with particular reference to the symbiotic turbellarian Paravortex. *Hydrobiologia* **132**, 101–104.

Piratae S, Tesana S, Jones MK, *et al.* (2012) Molecular characterization of a tetraspanin from the human liver fluke, *Opisthorchis viverrini*. *PLoS Neglected Tropical Diseases* **6**, e1939.

Potter SC, Luciani A, Eddy SR, Park Y, Lopez R and Finn RD (2018) HMMER web server: 2018 update. *Nucleic Acids Research* **46**, W200–W204.

Seigneuret M, Conjeaud H, Zhang HT and Kong XP (2013) Structural bases for tetraspanin functions. pp. 1–29 *in* Berditchevski F and Rubinstein E (*Eds*) *Tetraspanins*. Dordrecht, Springer.

Shinn A, Pratoomyot J, Bron J, Paladini G, Brooker E and Brooker A (2015) Economic impacts of aquatic parasites on global finfish production. *Global Aquaculture Advocate* **2015**, 58–61.

Tran MH, Pearson MS, Bethony JM, *et al.* (2006) Tetraspanins on the surface of *Schistosoma mansoni* are protective antigens against schistosomiasis. *Nature Medicine* **12**, 835–840.

Tran MH, Freitas TC, Cooper L, *et al.* (2010) Suppression of mRNAs encoding tegument tetraspanins from *Schistosoma mansoni* results in impaired tegument turnover. *PLoS Pathogens* **6**, e1000840.

Vorel J, Cwiklinski K, Roudnický P, *et al.* (2021) *Eudiplozoon nipponicum* (Monogenea, Diplozoidae) and its adaptation to haematophagy as revealed by transcriptome and secretome profiling. *BMC Genomics* **22**, 1–17.

Wang J, Zhou Y, Li D, Sun X, Deng Y and Zhao Q (2017) TSPAN 31 is a critical regulator on transduction of survival and apoptotic signals in hepatocellular carcinoma cells. *FEBS Letters* **591**, 2905–2918.

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