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The *Schistosoma mansoni* phylome: using evolutionary genomics to gain insight into a parasite's biology

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Abstract

Background: *Schistosoma mansoni* is one of the causative agents of schistosomiasis, a neglected tropical disease that affects about 237 million people worldwide. Despite recent efforts, we still lack a general understanding of the relevant host-parasite interactions, and the possible treatments are limited by the emergence of resistant strains and the absence of a vaccine. The *S. mansoni* genome was completely sequenced and still under continuous annotation. Nevertheless, more than 45% of the encoded proteins remain without experimental characterization or even functional prediction. To improve our knowledge regarding the biology of this parasite, we conducted a proteome-wide evolutionary analysis to provide a broad view of the *S. mansoni*'s proteome evolution and to improve its functional annotation.

Results: Using a phylogenomic approach, we reconstructed the *S. mansoni* phylome, which comprises the evolutionary histories of all parasite proteins and their homologs across 12 other organisms. The analysis of a total of 7,964 phylogenies allowed a deeper understanding of genomic complexity and evolutionary adaptations to a parasitic lifestyle. In particular, the identification of lineage-specific gene duplications pointed to the diversification of several protein families that are relevant for host-parasite interaction, including proteases, tetraspanins, fucosyltransferases, venom allergen-like proteins, and tegumental-allergen-like proteins. In addition to the evolutionary knowledge, the phylome data enabled us to automatically re-annotate 3,451 proteins through a phylogenetic-based approach rather than solely sequence similarity searches. To allow further exploitation of this valuable data, all information has been made available at PhylomeDB (http://www.phylomedb.org).

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Conclusions: In this study, we used an evolutionary approach to assess *S. mansoni* parasite biology, improve genome/proteome functional annotation, and provide insights into host-parasite interactions. Taking advantage of a proteome-wide perspective rather than focusing on individual proteins, we identified that this parasite has experienced specific gene duplication events, particularly affecting genes that are potentially related to the parasitic lifestyle. These innovations may be related to the mechanisms that protect *S. mansoni* against host immune responses being important adaptations for the parasite survival in a potentially hostile environment. Continuing this work, a comparative analysis involving genomic, transcriptomic, and proteomic data from other helminth parasites, other parasites, and vectors will supply more information regarding parasite's biology as well as host-parasite interactions.

Keywords: Phylogenomics, Maximum likelihood analysis, Homology prediction, Functional annotation, Paralogous families, Parasite genomics, Schistosomiasis

Background

Schistosoma mansoni, S. haematobium, and S. japonicum (Platyhelminthes: Trematoda) are the main causative agents of human schistosomiasis, a neglected tropical disease that is endemic in 77 countries where more than 237 million people require preventive chemotherapy and other 779 million live in areas of risk of infection [1-4]. The genomes of these parasites have been recently published providing insights into parasite's development, infection, and host-parasite interactions [5-7]. However, even with the progress made over the last years, schistosomiasis control depends primarily on the treatment of infected patients with Praziquantel®, the only drug available for mass treatment (e.g. [5,8,9]). Drawbacks of this drug are that it does not prevent against reinfection and its effectiveness varies depending on several factors such as the parasite's gender, developmental stage, and the time of infection. Furthermore, Praziquantel®-resistant parasites have been found both in the laboratory and in the field, thus increasing the urgent need for new effective drugs and vaccines [10-13].

Schistosoma mansoni infects 7.1 million people in America, 95% of which in Brazil, and 54 million people in Sub-Saharan Africa causing intestinal and hepatosplenic schistosomiasis [14,15]. The *S. mansoni* genome sequencing data was published in 2009 and a new version was recently released [5,16]. The improved genome has 364.5 megabases (Mb) assembled in 885 scaffolds, half of which are represented in scaffolds greater than 2 kilobases [16]. A total of 10,852 genes were identified, encoding over 11,000 proteins, 45% of which remain without known or predicted function [5,16,17]. 81% of the genome was assembled onto the parasite's chromosomes, providing a partial genetic map [16,18]. The availability of genomic data offers new opportunities for innovation in the control of schistosomiasis, by providing information that allows for the identification of novel drug targets and vaccine candidates through a system-wide perspective [5,19,20].

Making accurate functional predictions for genes or proteins is a key step in every genome sequencing project. However, on average, 30 to 50% of the predicted proteome remains uncharacterized while for the remaining set only general predictions are made. To deal with the gap between the rapid progress in genome sequencing and experimental characterization of genes and gene products, computational methods have been developed [21-23]. Two main approaches are generally used for functional prediction of genes and their products: one based on sequence similarity searches and another on phylogenetic analysis.

Owing to the computational cost and complexity of large scale phylogenetic analysis, the accurate identification of orthology relationships remains a challenge in comparative genomics and most of the orthology prediction methods rely on similarity-based search (e.g. BLAST [24], OrthoMCL [25], InParanoid [26]). In these cases, functional prediction is obtained based on the transfer of information from the most similar sequences in the database to the gene or protein of interest (e.g. [24]). However, several limitations are associated with this method, mainly the lack of a straightforward relationship between sequence similarity and protein function [21,27-29]. Since this approach is fast, simple, and can be automated to analyze thousands of genes, it has been used frequently to predict functional products encoded by newly sequenced genomes. Over the last years this practice has generated systematic errors, the extent of which is not completely known [22,27-32].

In an attempt to improve the accuracy of functional prediction at a large scale, phylogenetic methods may be applied [33,34]. The advantage of such methods is that they focus on the evolutionary history of genes rather than merely on their sequence similarity [30,35,36]. Ideally, functional transfer in the genomic context or for specific genes/proteins should be performed only when there is any experimental evidence for those used as source of information. However, in databases as UniProt,

only 3% of proteins have experimental support for their annotations [28]. To deal with the absence of experimental support for most part of the available proteomes, transfer of functional annotation aiming to provide hints regarding the gene/protein function needs to follow strict requirements to avoid, as much as possible, misclassifications. In the last decade, the publication of a large number of genomic and proteomic data and the development of faster and powerful computers, new software, and automated pipelines have allowed for the reconstruction of phylogenetic trees of the complete set of proteins encoded in a genome – the so called phylome [37].

The phylome data may give a broad view of the evolution of an organism, since it comprises the phylogenies of all proteins encoded in its genome [37]. Most notably, a phylome can be used to detect specific evolutionary scenarios, to quantify the fraction of individual phylogenies whose topologies are consistent with a given hypothesis, and to improve functional annotation of proteins and biological systems [38,39]. Furthermore, comparing genomes or proteomes through an evolutionary perspective may provide insights to the understanding of the metabolism, physiology, pathogenicity, and the adaptation to a particular life style of organisms. In this context, the availability of *S. mansoni* genomic data provides the opportunity to study this parasite from a genome-wide perspective rather than from individual gene or protein analyses.

Taking advantage of the benefits provided by a genome-wide approach combined with an evolutionary perspective, we reconstructed the *S. mansoni* phylome with the goals of i) gaining insight into lineage-specific evolutionary events potentially related to the parasitic lifestyle, and ii) improving the functional annotation of the genome/proteome.

Phylogenetic techniques used in the present work included multiple sequence alignment [40-43] alignment trimming [44], neighbor-joining tree building [45], evolutionary model testing, and maximum likelihood analysis [46]. The resulting phylome data contains 7,964 protein phylogenetic trees, covering the analysis of 11,763 *S. mansoni* proteins and their homologs in 12 other organisms, out of which we identified evolutionary events and homology relationships. The results provided useful information about the parasite's genome evolution such as the identification of gene duplication events and expanded protein families such as proteases, tetraspanins, fucosyltransferases, venom allergen-like proteins (also called as SmVAL or SCP-like), tegumental-allergen-like proteins (SmTAL), among others. Altogether, the results obtained are likely to pave the way for a better understanding of the parasite's biology including host-parasite interactions. This, in turn will accelerate the search for new drugs and vaccine directed toward the control and eradication of schistosomiasis.

Results and discussion

Reconstruction of the *S. mansoni* phylome

The *S. mansoni* phylome reconstructed in this work was derived from the comparative analysis of all proteins encoded in the parasite genome (predicted proteome) and their homologs in 12 other eukaryotic proteomes whose genomes were completely sequenced (Table 1). The set of selected species is particularly rich in metazoans (11 species), including ten invertebrates, one tunicate, and one vertebrate. One choanoflagellate, *Monosiga brevicollis*, was included as outgroup of the phylogenetic reconstruction. The metazoan species selected represent important evolutionary innovations, e.g. the origin of the third germ layer, the development of organs, systems, complex patterns of communication, and the emergence of the adaptive immune system, making this dataset set especially suitable for addressing the evolutionary innovations in *S. mansoni* in the context of metazoan evolution.

To perform the phylogenetic analyses, we applied an automated pipeline similar to the one used for the human phylome project [39]. This pipeline is illustrated here (Figure 1). The resulting alignments, phylogenies, and orthology predictions can be accessed at PhylomeDB [47] (http://phylomedb.org).

Using this phylogenomic approach, we analyzed 11,763 *S. mansoni* proteins and obtained 7,964 phylogenetic trees covering 70% of the parasite's proteome. This coverage is remarkably similar to that of other phylome data of newly sequenced genomes such as that of the pea aphid *Acyrthosiphon pisum* (67%) [38].

The absence of trees for the remaining 3,490 proteins is either due to a possible high degree of divergence between the *S. mansoni* proteins and their homologs in the other selected species, an indication of the uniqueness of the parasite's proteome, or it reflects the presence of errors in gene models. Out of the 7,964 phylogenetic trees, 3.038 (38%) correspond to trees with "seed" proteins with a completely unknown function and without any GO [48] assignment in SchistoDB [17].

Phylogeny-based orthology prediction

In order to create a complete list of orthology and paralogy relationships among *S. mansoni* proteins and those encoded in the other eukaryotic proteomes included in this work, we analyzed the parasite's phylome using a *species-overlap* algorithm as previously described [39]. The comprehensive catalogue of phylogeny-based orthology and paralogy relationships among *S. mansoni* and other species was made publicly available at PhylomeDB [47].

Table 1 Proteomes selected for the *S. mansoni* phylome reconstruction

Scientific Name	UniProt Species Code[1]	TaxID[2]	Proteins[3]	Source[4]	Download
Monosiga brevicollis	MONBE	81824	9,170	JGI	2011-06-01
Ciona intestinalis	CIOIN	7719	14,048	UniProt Reference Proteomes	2011-07-09
Nematostella vectensis	NEMVE	45351	24,424	UniProt Reference Proteomes	2011-07-09
Schistosoma haematobium	SCHHA	6185	12,767	SchistoDB	2012-03-09
Schistosoma mansoni	SCHMA	6183	11,103	SchistoDB	2012-03-09
Schistosoma japonicum	SCHJA	6182	12,636	SchistoDB	2012-03-09
Caenorhabditis elegans	CAEEL	6239	19,758	UniProt Reference Proteomes	2011-07-09
Ascaris suum	ASCSU	6253	18,430	WormBase	2012-03-09
Brugia malayi	BRUMA	6279	19,916	WormBase	2012-03-09
Trichinella spiralis	TRISP	6334	15,878	WormBase	2012-03-09
Drosphila melanogaster	DROME	7227	11,794	FlyBase	2011-09-13
Tribolium castaneum	TRICA	7070	16,533	BeetleBASE - HGSC	2011-12-16
Homo sapiens	HUMAN	9606	20,965	UniProt Reference Proteomes	2011-07-09

1 - Code assigned to each species in the *S. mansoni* phylome. 2 - Taxonomic identifier at NCBI (TaxID). 3 - Number of proteins analyzed per species. 4 - Database from which the protein data were retrieved.

Owing to the increasing rate at which new fully sequenced genomes are released, the accumulation of genomic and proteomic data has been much higher than the rates at which genes or proteins are experimentally characterized. Aiming at producing a high confidence set of functional predictions for *S. mansoni* proteins, we used the evolutionary relationships as inferred from phylogenetic trees to obtain subsets of one-to-one (single homolog in *S. mansoni* and in other species) homology relationships among *S. mansoni* proteins and the homologs from other species included in the present study (Figure 2).

By using such phylogeny-based approach, we transferred 10,175 functional annotations (GO terms [48]) to 3,451 *S. mansoni* proteins, from which 790 (7% of the parasite's proteome) were previously annotated as "hypothetical protein", corresponding to proteins whose function had not been predicted or experimentally tested before (Additional file 1 Table S1). The transfer was performed from each ortholog with known function in the selected taxa to the *S. mansoni* "seed" protein. For the other proteins that already had any functional prediction, the annotation was confirmed or improved. Consequently, a "seed" protein could receive more than one functional description. In these cases, all functional annotations were maintained allowing the user to choose the closest related transferred functional annotation, those that came from model organisms, or even to create a consensus based on all of them.

To validate the applied methodology, we retrieved reviewed *S. mansoni* proteins from UniProt [49], including experimentally confirmed ones, to evaluate the annotation transferred by the phylogenomic approach. The functional annotations performed by PhylomeDB correspond to known functions in the aforementioned database (Additional file 1 Table S2). Even though the BLAST search may detect distant homologs with additional domains, our subsequent phylogenetic reconstruction and our selection of orthologs will select those orthologs that are likely to have similar domain architecture. This is an additional reason why an orthology-based annotation is preferred over sequence similarity searches, since orthologs as compared to paralogs have a higher tendency to share a similar domain architecture [50].

Although less reliable than those based on one-to-one orthology relationships, annotation transfer based on more complex subsets (one-to-many, many-to-one, or many-to-many) may provide important hints to predict the biological function of *S. mansoni* proteins. However, in these cases, one or more genes are co-orthologous to a set of genes in another genome due to lineage-specific duplication(s) that can be associated with functional shifts, affecting the reliability of the functional transfer [38,51]. An example of a one-to-one transfer from a *Drosophila melanogaster* protein to a *S. mansoni* protein comes from the phylogenetic reconstruction of the Phy000V14T_SCHMA (Smp_170950) protein, potentially related to the glycine cleavage system, and its homologs in the selected species (Figure 3). The analysis of this tree resulted in six transfers of functional annotation from homologous proteins to the *S. mansoni* "seed" protein. The GO terms in all six functional annotations are related to aminomethyltransferase activity and glycine catabolic process providing further support for the annotation transfer. In this example, to illustrate a case of a one-to-one transfer, we chose the functional annotation transferred from *Drosophila melanogaster* once, according to the information available in UniProt [49], it is one of the orthologs with known function and experimental validation. Tags for homologous sequences with experimental validation are not available in

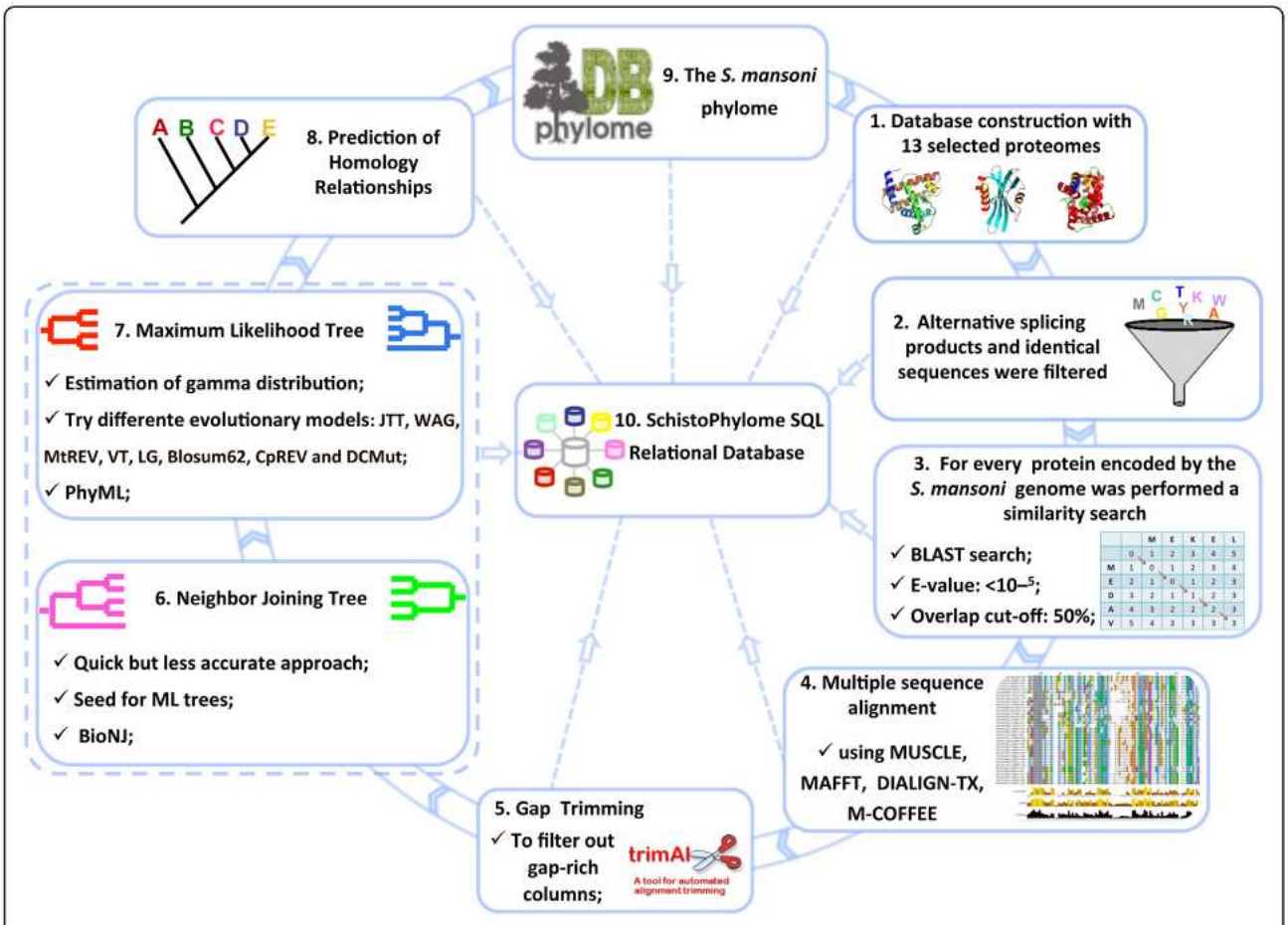

Figure 1 Pipeline used to reconstruct and analyze the *S. mansoni* phylome. Each protein sequence encoded in the parasite genome was compared against a database of proteins from other 12 fully sequenced eukaryotic proteomes (Table 1) to select putative homologous proteins. Groups of potential homologs were aligned and subsequently trimmed to remove gap-rich regions. The refined alignment was used to build a NJ tree, which was then used as a "seed" tree to perform a ML likelihood analysis as implemented in PhyML. In the ML analysis, up to five different evolutionary models were tested and the model best fitting to the data was determined by the Akaike Information Criterion (AIC). Different algorithms were used to identify homology relationships and lineage-specific duplications. To extract and interpret the large data set obtained a Structured Query Language (SQL) relational database was built. This database was the main resource for data mining in this work. Adapted from [39].

PhylomeDB [47]. However, links to UniProt [49] and other databases are provided.

To explore the benefits offered by comparative genomics in order to improve functional annotation of genes and gene products, it is also necessary to consider the limitations involved in this approach. Although it is generally accepted that functional annotation through orthology, rather than just homology relationship, constitutes one of the most promising annotation approaches, these surveys are designed to provide predictions regarding the likely protein function, but it does not substitute experimental confirmation [36,52]. Functional diversity is often associated with significant divergence at the sequence level, but high levels of identity do not ensure that two or more proteins perform the same function, since subtle changes in active sites are able to completely change the protein function [53].

As we previously mentioned, evolutionary analysis involving fully sequenced genomes/proteomes remains a challenge. Although the tools here applied were not originally designed for large scale phylogenetic analysis, we adapted them to work on a large scale, since we strongly believe that a system-wide perspective on evolutionary processes can greatly improve the understanding on how genomes came to be and what evolutionary process took them there. Functional prediction as described in the present work could be used as a starting point for future projects, prioritizing the selection of certain genes or proteins for new experimental studies.

Detection of gene duplications in *S. mansoni*

An additional advantage of the phylogeny-based approach is that it readily provides a collection of gene evolutionary histories that can be mined for particular

muscles, calcium propagation in the gut, gap junction-mediated oocyte, and sensory neuron identity [89].

In summary, we identified that approximately 45% of the *S. mansoni* predicted proteins that were covered by this phylogenomic analysis have, at least, one paralog encoded in the parasite genome that might have arisen by gene duplication events that occurred after its divergence from other selected taxa (Additional file 1 Table S3). In other eukaryotic genomes this value ranges from 30 and 65% [90], whereas in *C. elegans* this value is equal to 49% [91].

Altogether, the present results indicate that besides the exploitation of host endocrine and immune signals, the parasite genome exhibit multiple events of gene duplication which may be, at least partially, an adaptive response related to the parasitic lifestyle. These expansions probably reflect the intriguing complexity of evolutionary events that happened over time, resulting in important characteristics in schistosome's biology with consequences to the disease it causes. Taking into account the host environment and the selective forces that it imposes to a parasite, the phylogeny of host(s) and parasite(s) are probably closely related, once this coevolution will be responsible for the continuity or elimination of such an interaction. Nonetheless, previous empirical experiments involving schistosomes and the intermediate host provide further support to suggest the potential for host-schistosome coevolution [92].

In this context, it is important to analyze the evolutionary history of protein families during screening for potential targets for drug and vaccine development. Incorporating the evolutionary perspective in drug development studies can improve our understanding regarding drug resistance and effectiveness, as well as to guide new strategies of drug discovery. Gene duplication events as well as adaptive evolution should be considered during this process, since an anti-parasitic drug could bind a single protein or in all proteins encoded by a multi-gene family [93]. As a consequence, therapies which target a subset of genes that arose by duplication may not be effective at low doses. To solve this problem, the drug's effectiveness can be increased when a single-copy gene is targeted and its function is inactivated causing complete perturbation of a vital pathway [93,94].

Conclusions

Through a systemic approach, we may accelerate the advance towards the understanding of schistosomiasis, its etiologic agents, and host-parasite interactions, optimizing the discovery of therapeutic targets to the development of new drugs and vaccines. Besides promoting a significant improvement in the functional annotation of the *S. mansoni* predicted proteome, our approach provided relevant information about the parasite's genome evolution such as the identification of gene duplication events and expanded protein families, supplying important information regarding the mechanisms involved in *Schistosoma*'s genome evolution. Among the parasite paralog groups, we identified proteases, tetraspanins, fucosyltransferases, venom allergen-like proteins (also called as SmVAL or SCP-like), and tegumental-allergen-like proteins (SmTAL) that may be related to morphological or physiological specificities of this parasite. In addition, we strongly believe that the *S. mansoni* phylome data will pave the way for other, more detailed analysis, such as those that have been already performed on expanded peptidases families [65].

One of the remaining challenges is to understand which evasion strategies enable this parasite to survive for years in a potentially hostile environment, protected from the host immune system action and/or actively making the host response ineffective. Different mechanisms may be involved in these processes, including the generation of variant proteins by expression of micro-exon genes (MEG), which have been pointed as a potential strategy [94], and small non-coding RNAs which perform many essential regulatory functions [95].

Insights obtained through this phylogenomic approach will help us to guide forward genetic approaches to better understand the host-pathogen relationships toward to the elucidation of novel drug targets and vaccine candidates urgently needed to reduce the morbidity and mortality caused by schistosomiasis worldwide. Continuing this work, a comparative analysis involving genomic, transcriptomic, and proteomic data from other helminth species as *Taenia solium*, *Echinococcus multiloculares*, *Echinococcus granulosus*, *Fasciola hepatica*, other parasites, and vectors will provide valuable information from a system-wide perspective of a broad range of organisms, improving our understanding regarding the parasitic lifestyle.

Methods

Organisms and sequence data

Predicted proteomes from 13 fully sequenced eukaryotic genomes were downloaded from JGI Genome Projects, SchistoDB, Quest For Orthologs, WormBase, BeetleBASE, and FlyBase (Table 1). The taxon sampling was selected according to the availability of the predicted proteomes and based on the phylogenetic position of each species. The comprehensive taxa selected cover important evolutionary innovations making this dataset set especially suitable for addressing the evolutionary innovations in schistosomes in the context of metazoan evolution. Model organisms were also included to provide functional annotations that could be potentially transferred to *S. mansoni* homologous proteins.

Additional file

Additional file 1: Table S1: Functional prediction for the*S. mansoni* "seed" proteins. **Table S2**: Comparison between reviewed proteins with functional annotation at UniProt and GO terms transferred by phylogenomic approach. **Table S3**: Homologous proteins to the *S. mansoni* "seed" proteins.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

LLS: carried out the phylogenetic and functional annotation studies, and drafted the manuscript. MM: performed the phylome reconstruction and functional annotation transfer. LAN: participated in the coordination of this study, and co wrote this manuscript. AZ: wrote the Perl scripts for data manipulation and provided computational support for this study. TG: participated in the coordination of this study, supervised the phylome reconstruction, and co wrote this manuscript. GO: participated in the design and coordination of this study, and co wrote this manuscript. All authors read and approved the final manuscript.

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