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To Be or Not to Be a Tapeworm Parasite: That Is the Post-Genomic Question in *Taenia solium* Cysticercosis

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Abstract

Cestode parasites rely on their host to obtain their nutrients. Elucidation of tapeworm genomes has shown a remarkable reduction in the coding of multiple enzymes, particularly those of anabolic pathways. Previous findings showed that 10–13% of the proteins found in the vesicular fluid of *Taenia solium* cysticerci are of host origin. Further proteomic characterization allowed identification of 4,259 different proteins including 891 of host origin in the parasite's protein lysates. One explanation for this high abundance and diversity of host proteins in the parasite lysates is related to the functional exploitation of host proteins by cysticerci. Supporting this concept is the uptake of host haptoglobin and hemoglobin by the parasite, as a way to acquire iron. Surprisingly, internalized host proteins are minimally degraded by the parasite physiological machinery. Additional proteomic analysis demonstrated that these host proteins become part of the organic matrix of calcareous corpuscles; as 60–70% of the protein content are host proteins. In this review, a collection of available genomic and proteomic data for taeniid cestodes is assembled, the subject of the use and processing of host proteins is particularly addressed; a sketchy and unique cell physiological profile starts to emerge for these parasitic organisms.

Keywords: *Taenia solium*, Cestoda, Genome, Proteome, Host proteins, Calcareous Corpuscles

1. Introduction

Tapeworms are invertebrate metazoans producing zoonotic parasite diseases in animals and humans. These parasites have a worldwide distribution, but they especially affect human populations in developing countries and are considered neglected diseases [1]. Their larvae, known as metacestodes (including forms such as cysticercus in *Taenia solium* or hydatid or alveolar cysts in *Echinococcus* sp.) cause the highest morbidities due to tapeworms [2, 3], since they can produce generalized organ failure or seizures and can even result in patient's death [4–7].

Tapeworms produce long-term infections, being able to survive within its host for several years [8], maintaining a dynamic and complex host-parasite relationship [9]. Their lifecycles involve two host (intermediate and final) and include several developmental stages: embryo, larvae and adult stage [10] that can lodge in different tissues of their hosts producing diseases with a wide range of clinical presentations [11].

After description of the genomes of four tapeworms in 2013 [12], molecular studies of these organism have entered an integrative era; including approaches involving genomics, transcriptomics and proteomics [13]. These approaches are presented as promising avenues for the discovery of new pathways to improve our understanding of parasite diseases caused by cestodes, in the hope of developing better surveillance, treatment and control guidelines.

This chapter reviews current perspectives in the study of flatworms; special emphasis is placed on the genomics and proteomics of cestodes and taeniid parasites. The conspicuous and abundant presence of host proteins is particularly considered for taeniid larval forms.

2. The Platyhelminth genome

Access to massive sequencing technologies allowed characterization of entire genomes for some of the most relevant flatworms; being the free living *Schmidtea mediterranea* the first trematode to be reported in 2008 [14]. The timeline of all flatworms genome projects that have been published to date clearly shows the advent of the post-genomic era of flatworms (**Table 1**). A rapid characterization of the genomes of parasites with medical importance, such as *Schistosoma japonicum, S. mansoni, Clonorchis sinensis*, among others, followed by four cestodes: *Hymenolepis microstoma, Echinococcus granulosus, E. multilocularis* and *Taenia solium* [12]. Subsequently, the International Helminth Genomes Consortium carried out a project with a goal of 50 helminth genomes. These genomes are currently deposited in the WormBase Parasite database, where users can access 197 genomes [15], including 44 Platyhelminthes: 4 free-living flatworms, 20 trematodes, 19 cestodes and 2 monogeneans (**Figure 1**). This platform also allows searching protein domains and Gene Ontology terms,

	Genome size (Mb)	No. of Genes	Longest scaffold size (Mb)	N50 length (Mb)	N90 length (kb)	GC content (%)
T. solium	122.3	12490	0.7	0.07	5.3	43
T. multiceps	240	12890	10.5	44.8	8500	43.7
T. saginata	169	13,161	7.3	0.58	29.4	43.2
T. asiatica	168	13,323	4.2	0.34	14.3	43.1
E. multilocularis	115	10345	20.1	13.8	2900	42.2
E. granulosus	114.9	10231	16	5.2	200	42
E. canadensis	115	11449	0.574	0.075	3.8	42
E. oligarthrus	86	8756	16	10.2	11.6	41
H. microstoma	141.1	10241	2.4	0.5	82	35.9
H. diminuta	177	15169	6.9	2.3	412.2	35.3

Table 1.
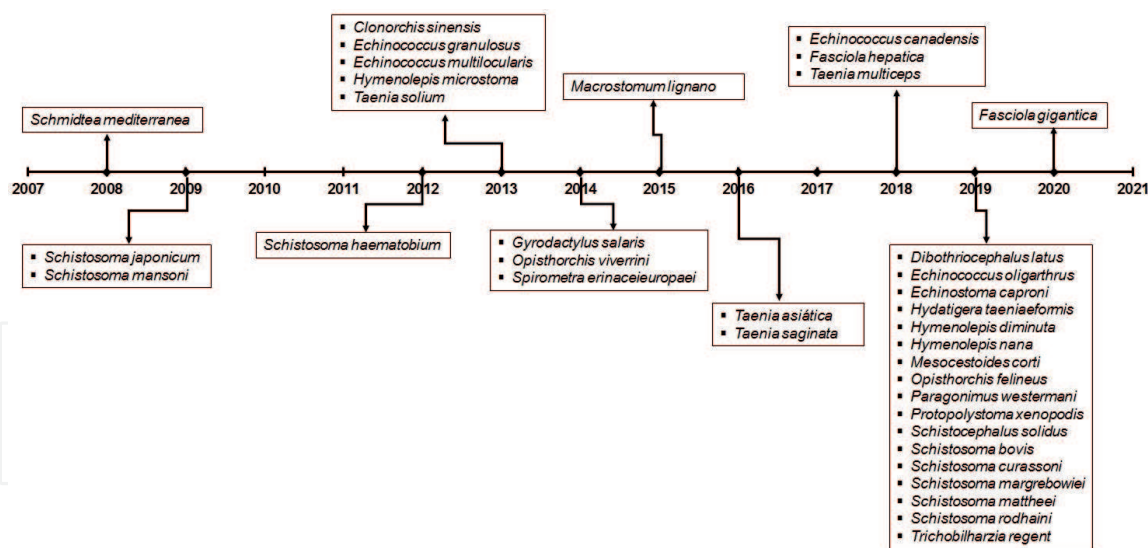*Statistics of completed genome sequencing for several tapeworms.*

Figure 1.
Timeline of flatworms genome characterization [12, 14–39].

as well as performing comparative analysis of genes and alignments of RNA-Seq data sets, specific to the life stage genomes, among other useful functions [40, 41].

Other taeniid genomes have been reported outside the International Helminth Genomes Consortium during the past five years: *T. asiatica*, *T. saginata* [31] and *T. multiceps* [34], *E. canadensis* [32], *E. oligarthrus* [35], as well as *Hymenolepis diminuta* [36]; circumstances appear prone to greatly improve our understanding of the biology and evolution in those organisms, as well as to solve old unanswered questions on their host-parasite relationships. Availability of this genomic information allows integrative studies on this ancient lineage of organisms. **Table 1** includes the basic statistics of reported assemblies for several tapeworms of medical or veterinary importance, being *E. oligarthrus* the smallest assembly (86 Mb) and *T. multiceps* the largest one (240 Mb). The average GC content of these genomes is 35-43.7%, similar to trematode genomes [23] but different to bacterial genomes whose GC range content is 13.5%-74.9% [42]. As a reference, GC average content of vertebrates is 46% [43]; mice 41.7% [44] whereas human genome is 40.9% [45].

3. Gene gain/reduction along tapeworm evolution

The genomic data of the first four tapeworm genomes sequenced [12] permitted identification of reduction events for groups of genes such as Wnt, which corroborated some data that suggested the loss of these genes in trematodes [46]. Moreover, other genes as Nek kinases, peroxisomal genes and ParaHox members, as well as neuropeptides and G-protein coupled receptors (GPCRs) [15].

The loss of approximately 10 Hox gene families in tapeworms during their evolutionary pathway apparently affected the morphology of those organisms, i.e., the lack of eye-cups and gut [12]; Hox genes such as pax3/7, gbx, hbn and rax are mainly involved in neuronal development or eye development [47–50], as well as ParaHox genes in the formation of the digestive tract [51]. Another type of proteins absent in cestodes are those related to germ cells such as piwi, tudor and Vasa, although the latter have been found possible orthologues in the PL10 family [12].

Tapeworms have developed a specialized detoxification system that includes a single cytochrome p450 gene [12, 52], as well as a redox homeostatic system based on thioredoxin glutathione reductase and the expansion of glutathione
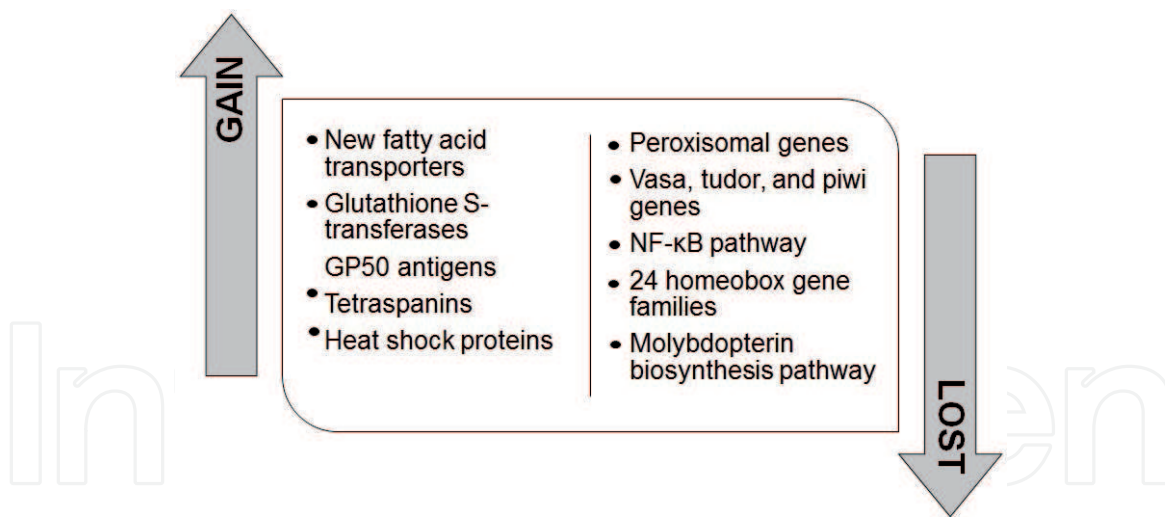
Figure 2.
Gains and losses of genes in taeniids. Phylogenetic study carried out with the genomes of the cestodes allowed finding important aspects about how these organisms acquired or lost some of their genomic traits to adapt to the conditions of their current environments.

S-transferases [53–55]. In addition, there was an expansion of some very specific protein families such as non-canonical heat shock proteins, with *Echinococcus* and *T. solium* having the highest number of genetic expansions in the cytosolic clade Hsp70 [12] suggesting that tapeworms have different mechanisms from nematodes to overcome stress [16]. In addition, taeniids have an expansion in some families of antigens such as GP50 [12, 15]. These antigens are useful for diagnostics; for example, coenurosis in goats [56] or cysticercosis in pigs [57]. For diagnosis of human cysticercosis, the use of GP50 as a diagnostic target allows a 100% specificity and 90% sensitivity using serum samples of patients [58, 59]. Some of the main gains and losses of genes in taeniids are summarized in **Figure 2**.

Our current knowledge on cestode's and taeniid's genomes is still limited but the speed of genomic data acquisition can advance significantly in this new era. We envisage a better understanding of these host–parasite interactions, at a molecular/ evolutionary level that can help us unravel events that have permitted the adaptations of these platyhelminths to the host environment.

4. Metabolic adaptations of tapeworms

A great impact of having available complete tapeworm genomes is the characterization of the metabolic pathways in these organisms. Now we know that taeniid tapeworms cannot synthesize fatty acids and cholesterol de novo [25, 60]. For example, KEGG analysis for fatty acid biosynthesis in *T. solium* clearly shows that most of the components of the pathway are absent (**Figure 3**). Therefore, these parasites cannot carry out biosynthesis of fatty acids and are obligated to acquire host fatty acids through specific transporters [63]. Moreover, no genes related to the β-oxidation pathway were found in *Echinococcus* and *Hymenolepis,* although experimental data suggest that other flatworms do carry out this metabolic process [64] for utilization of lipids as a source of energy. It is clear that their major energy source are carbohydrates such as glucose and glycogen. This is supported by the fact that most enzymes participating in carbohydrate catabolism are expressed.

The synthesis of pyrimidines is also absent for taeniids [65], indicating that they acquire pyrimidines from their hosts. The biosynthesis of purines shows a similar landscape [15]. Parasitic flatworms are considered auxotrophic for eight
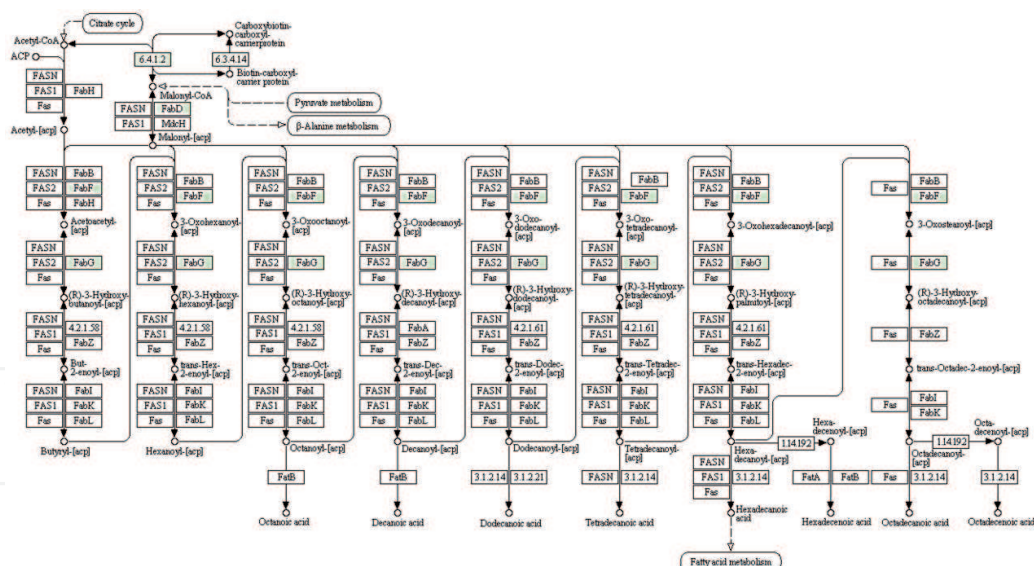
Figure 3.
KEGG analysis of the fatty acid biosynthesis in T. solium. *Enzymes available for this pathway are acetyl-CoA carboxylase (6.4.1.2), S-malonyltranferase (FabF), 3-Oxoacyl-[acyl-carrier-protein] synthetase II (FabF) and Ketoacyl-acyl carrier protein (FabG) [green squares] [61, 62].*

of the nine amino acids that are essential for humans (Phe, His, Lys, Leu, Met, Thr, Trp, and Val). Cestodes have a limited ability to synthesize amino acids, as an example, serine and proline are absent in *E. multilocularis* [16]; biosynthesis of lysine and the aromatic amino acids (Phe, Trp and Tyr) are also absent in most cestodes (**Figure 4**). Arginine is also an essential amino acid in helminths including flatworms, as they do not have all the necessary enzymes of the urea cycle to process

Figure 4.
KEGG analysis of phenylalanine, tyrosine and tryptophan biosynthesis in T. solium. *The only enzymes that are present in the* T. solium *genome are indicated in green within boxes [61, 62].*

ornithine, which is the precursor of arginine [15]. In summary, these parasitic organisms rely on their host for the acquisition of fatty acids, nucleosides and most amino acids. Metabolically speaking, they show highly simplified genomes.

5. Taeniid larval tissues contain large amounts of host proteins

The presence of host proteins in the tissues of the cystic larval forms of taeniids has been known for a long time [66–70]. It has been proposed that the mechanism for the uptake of these proteins is fluid pinocytosis in the cysticerci of *T. crassiceps* [69]. Moreover, in addition of entering the host proteins, these parasites can also secrete them [70, 71]. The biological role of those uptaken host proteins remains elusive, however, uptake of host albumin has been proposed to be involved in the maintenance of host-parasite osmotic pressure [68] and uptake of host immunoglobulins has been proposed as a mechanism of immune evasion and even as a source of amino acids [72].

Recent quantitative estimates indicated that host proteins might represent 11–13% of the protein content in the vesicular fluid of *T. solium* cysticerci, with albumin and immunoglobulins being the most abundant proteins. The use of high-throughput proteomics, allowed identifying 891 proteins of host origin from a total of 4,259 in a *T. solium* cysticerci whole protein extract [73]; thus, host proteins might represent up to 19% of the total protein species in the larval tissue lysates. Moreover, a fraction of these uptaken host proteins are intact and perhaps functionally active in the tissues of taeniid larvae [71].

6. Utilization of host proteins by cysticerci; iron chaperons and IgG

A known trait of parasitism is the use of the host as a provider of resources; sugars, amino acids, nucleosides, vitamins, coenzymes and/or microelements are good examples of resources that a parasite can acquire from its host. However, considering the abundance and diversity of host proteins present in the tissues of taeniid larvae, a pertinent question would be: are these parasites benefited by the accumulation of host proteins, beyond simply serving as a source of amino acids or as osmotic regulators? We have explored a couple of prospects: the use of host iron chaperones for the management of the parasite's iron necessities, as well as the use of host immunoglobulins as a source of amino acids [70, 71, 74].

Iron is an essential element for virtually all living organisms. Pathogens have evolved mechanisms to uptake iron from their hosts. Usually, iron is uptaken from plasma proteins: hemoglobin (heme prosthetic group) or haptoglobin-hemoglobin complexes, hemopexin (heme prosthetic group), transferrin or lactoferrin (iron), ferritin (iron), etc. In fact, the constant battle between host and pathogens for this element is well-studied [75, 76]. Hepcidin, the hormone that control iron levels in mammals, was first discovered as an antimicrobial peptide [76, 77]. In this light, it is expected that cestodes would acquire iron from their host, however, the mechanism remains elusive. Some evidence have suggested that hemoglobin or the haptoglobin-hemoglobin complexes could serve as an iron source for the cysts [78]. To support this notion, we have documented the immunolocalization of haptoglobin, hemoglobin, hepcidin and ferritin in the cyst; immunoblotting using crude larval extracts confirmed the finding [73]. We also showed that haptoglobin-hemoglobin complexes were detected in crude larval extracts in their expected molecular weight, indicating that those complexes are only marginally degraded. In fact, free haptoglobin purified from cysts protein lysates has been shown to retain

their hemoglobin binding activity, suggesting that the cyst are acquiring iron from those sources. However, future studies are needed to understand how the uptake is performed (is there a specific receptor?), how the heme prosthetic group or iron is removed from those complexes? and which parasite proteins are performing those roles.

Another aspect related to the host's protein uptake by tapeworm's larvae, is the utilization of these proteins as a source of amino acids. Internalization of IgG has been traced using a metabolically labeled (Leu-3H) IgG produced *in vitro* using a mice hybridoma [71]. Through *in vitro* culture of *T. crassiceps* (a closely related species of *T. solium*) cysts in the presence of (Leu-3H) mice IgG, uptake of the immunoglobulin can be monitored. Metabolic labelling also allowed tracking incorporation of Leu-3H into newly synthesized cyst proteins. The biochemical analyses revealed that within the tissue extracts, no other radiolabeled proteins were found. The two bands corresponding to the heavy (50 kDa) and light (25 kDa) chains remained intact after 3 days of culture. This would imply that these proteins are negligible used as a source of amino acids for the biosynthesis of the larvae's own proteins. Furthermore, the integrity and functionality of the Igs was conserved, as shown by SDS-PAGE and western blots marked with the Igs purified from tissue extracts. This finding led the research into a new direction: If immunoglobulins (and perhaps other uptaken host proteins) are only a minor source of amino acids [71], what is the fate of uptaken host proteins?

7. The calcareous corpuscles as a final deposit for host proteins

The tracking of metabolically radiolabeled IgG demonstrated that cysticerci do not significantly use these proteins as a major source of amino acids [71]. A possibility was that these proteins could end in the calcareous corpuscles (CC), that are known as a waste of toxic metabolites and other materials. These CC are microscopic calcifications occurring in the lumen of protonephridial canals, resulting after accretion of mineral salts (calcium carbonate and calcium phosphate) on an organic matrix composed by polysaccharides and other macromolecules [79, 80]. The CC
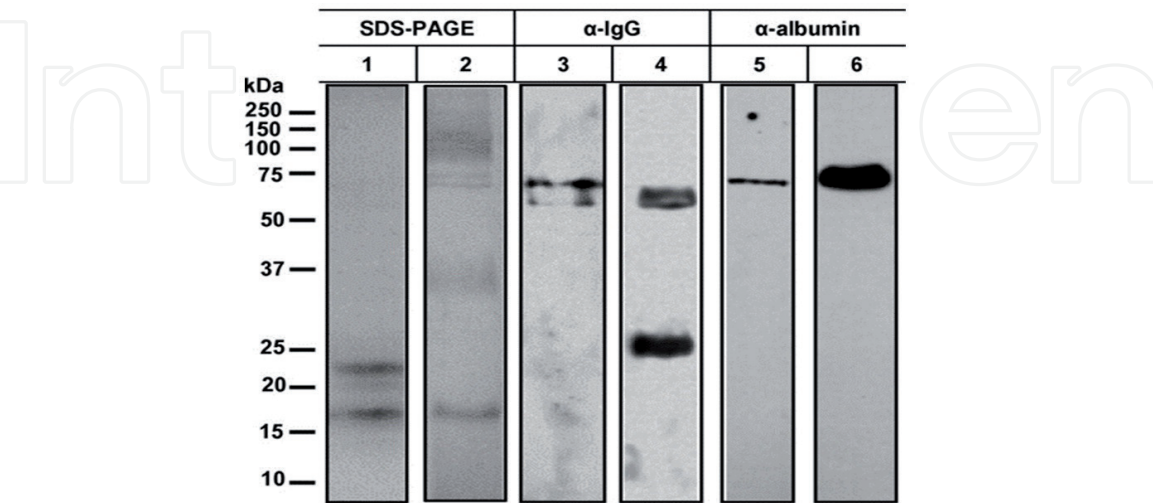

Figure 5.
Immunological identification of host IgG and albumin recovered from the protein matrix of calcareous corpuscles of T. solium *cysticerci. Lanes 1 and 2 correspond to Coomassie blue staining of protein extract from CC and silver staining of porcine serum respectively. Lanes 3 and 5 are western blots of protein extracts obtained from CC, lanes 4 and 6 are gels run with porcine serum, these blots were revealed with an α-IgG coupled to HRP (3 and 4) or sheep α-albumin and then with a rabbit α-sheep IgG coupled to HRP (5 and 6). This figure was originally published in [70].*

are involved in the removal of toxic solutes and regulation of mineral trafficking [81]. Formation of CC has been proposed as a mechanism for protecting cysticerci from calcification [79]. The CC represent about 10% of the dry weight of total larval tissue [82]. It has been estimated that in aged *T. solium* cysticerci, calcareous corpuscles can represent up to 41% of the dry weight [81, 82].

Searching for host proteins in the organic matrix of CC from *T. solium* cysticerci, a mass spectrometry analysis was carried out. A total of 636-760 proteins were identified and quantified, from which 412-508 (60-70%) corresponded to host proteins. *T. solium* proteins in the organic matrix of CC were only 224-252 (30-40%). The remarkable finding that the major protein component in the organic matrix are host proteins, suggests that CC act as a final destination for host proteins. We also showed that intact host proteins can be recovered even after dissolution of CC in a weak acid solution (**Figure 5**). Therefore, these proteins are incorporated into the organic matrix without being degraded [71]. If host antibodies are incorporated into the organic matrix of CC in the form of immune complexes, it is conceivable that cysticerci developed this strategy as a way to diminish exposure of relevant parasite antigens, which could result in a sophisticated mechanism to evade the adaptive humoral immunity of the host.

8. Conclusions

- Genomic and proteomic information on flatworms and particularly on cestode parasites is growing rapidly during the last decade, allowing new approaches to a number of questions.

- Taeniid metacestodes uptake large amounts of host proteins, some of which may be used to meet physiological needs of these parasites.

- Host proteins appear to be marginally degraded; it's importance as a source of amino acids appears to be negligible.

- Proteomic analyses of the organic matrix of the calcareous corpuscles evidenced that a majority of proteins in the organic matrix are of host origin, suggesting that these proteins are sent to the CC as a final destination.

- A consequence of the incremental uptake of host proteins during the lifetime of cysts that terminate being part of the organic matrix of calcareous corpuscles, would be a parallel increment in amount of calcareous corpuscles. Accumulation of corpuscles in larval tissue might represent a biological timer that limits the life span of cysticerci in the host.

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Conflict of interest

The authors declare that there are no conflict of interest associated with the manuscript.

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