Int. J. Morphol.,
41(2):646-653, 2023.

Morphology of *Echinococcus granulosus* Protoscolex

Morfología de los Protoescolex de *Echinococcus granulosus*

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MANTEROLA, C.; RIVADENEIRA, J.; POGUE, S. D. & ROJAS, C. Morphology of *Echinococcus granulosus* protoscolex. *Int. J. Morphol., 41(2)*:646-653, 2023.

SUMMARY: *Echinococcus granulosus* (*E. granulosus*), is a tapeworm that spreads between intermediate and definitive hosts through the ingestion of fecal matter contaminated with the parasite's eggs. The life cycle consists of differentiation from eggs to oncospheres to embryos, which eventually form cysts in organs like the liver, lungs and others. Within these cysts are protoscolices, an intermediate stage of the parasite which develop into adult tapeworms once they infect their definitive hosts. When these hydatid cysts form in humans, it is known as Cystic Echinococcosis (CE). This disease is treated through surgical excision of the cysts and or chemotherapy with benzimidazole compounds. Understanding the morphology of the intermediate developmental stage of *E. granulosus*, protoscolex stage, can allow researchers to identify defining structural changes and protein functions that could be used to develop treatment modalities for CE. Unique characteristics in the tegumental surface during the protoescolex stage and proteins associated with cyst fertility have all been identified in previous research studies and bring researchers closer to understanding the underlying mechanisms of *E. granulosus* development, and consequently, means to disrupt it to achieve better control of the disease.

KEY WORD: "*Echinococcus granulosus*"[Mesh]; "Echinococcosis"[Mesh]; Cystic Echinococcosis; Hydatid Cyst; Protoscolex; Morphology.

INTRODUCTION

Echinococcus granulosus (*E. granulosus*), is a *Cyclophyllidean* cestode, a type of tapeworm that is found in canids as adults. They are typically between two and seven millimeters in length and their morphology is characterized by a segmented body that has a head or scolex, containing hooks and suckers, at one end (Eckert & Desplazes, 2004) (Fig. 1).

The life cycle of *E. granulosus* involves the use of both intermediate and definitive hosts for the facilitation of species proliferation. The adult parasites lay their eggs, each approximately 40 mm in diameter, who then shed in their fecal matter. Intermediate hosts (these include sheep, cattle, and humans, among others) ingest the fecal matter through various means (i.e. consumption of contaminated food or water or hand-to-mouth contamination after interaction with an infected host). Once ingested, the eggs differentiate into oncospheres, tapeworm embryos, which are then hatched in the upper gastrointestinal tract and penetrate the intestinal wall. From there, the oncospheres enter the portal vein and travel to various organs. The resulting cysts that form usually occur in the liver or lungs but there is evidence of multiple organ parasitation (Buttenschoen & Buttenschoen, 2003; Manterola *et al*., 2003; Manterola & Claros, 2021). Approximately in 80 %, a solitary cyst forms in just one organ. Within these cysts, multiple protoscoleces, juvenile stages of the tapeworm, form. The definitive hosts, usually canids like dogs, jackals, and hyenas, become infected when they consume infected organs of intermediate hosts. The protoscoleces from the intermediate host will then invaginate, attach to the intestinal lining of the definitive host, and mature into adult flatworms (Centers for Disease Control and Prevention, 2019).

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Funding: Partially financed by Project MEC 80190026.

Received: 2022-08-31 Accepted: 2023-02-17

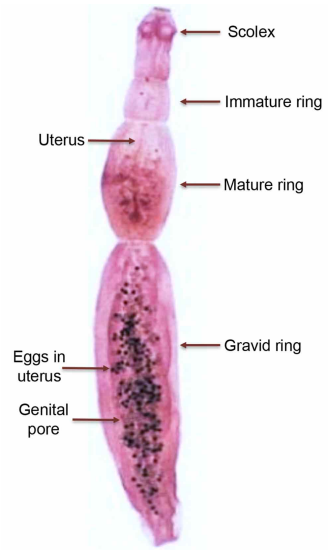

Fig. 1. Adult form in a canid intestine.

E. granulosus is found in most parts of the world but human infections rarely occur in high-income countries. Human infections typically occur in poor, pastoral, and rural regions that have sheep and cattle, and where dogs are commonly in close proximity to households. Areas where human infection with *Echinococcus granulosus* is common are Central Asia, Western China, and certain parts of South America, such as Argentina, Chile, and Peru (Agudelo Higuita *et al*., 2016). In these regions, the prevalence of human echinococcosis has been estimated to be as high as 5 % to 10 % (Moro *et al*., 2005; Tamarozzi *et al*., 2017).

The aim of this manuscript was to describe the morphological development of protoscolex of *Echinococcus granulosus*, to as a strategy to improve knowledge about it and eventually interrupt its cycle.

Cystic Echinococcosis: Pathology, Diagnosis, Treatment, and Prevention.

Cystic echinococcosis (CE) or hydatidosis, correspond to human infection by *E. granulosus* characterized by cysts formation in different organs. Cases are often asymptomatic, but the cysts can cause compression and displacement of organs and bodily structures, leading to abdominal pain and discomfort, nausea and vomiting. In rare occurrences , hydatid cysts can rupture, which can lead to dissemination by implantation of scolexes in parietal and visceral serosa of other organs, and cause anaphylaxis as a result of the release of the fluid from the ruptured cyst, and the contact between parasitic antigens and the immune respond of the host (Tinsley *et al*., 2013).

CE is typically treated using surgical methods or chemotherapy and is diagnosed using imaging techniques like ultrasonography (US), computed tomography (CT) and magnetic resonance imaging (MRI). The diagnosis of CE often is incidental. Since many cases are asymptomatic, and the cyst can first appear on imaging for an unrelated medical issue. While cysts can be seen on US, CT, or MRI, the World Health Organization (WHO) suggest the use of a classification system for CE based on US findings (Table I) (WHO Informal Working Group, 2003). Imaging findings can be confirmed serologically using indirect hemagglutination (IHA) and enzyme-linked immunosorbent assays (ELISA), and the antigens most commonly used for serological diagnosis are

Table I. Classification system for CE.

WHO stage	Ultrasound morphology	Status
CE1	Unilocular, simple cyst with uniform anechoic content. May exhibit fine echoes due to shifting of brood capsules which is often called hydatid sand ("snow flake sign"). Cyst wall is visible, and normally are round or ovals.	Usually fertile
CE2	Multivesicular, multiseptated cysts in which the daughter cyst may partly or completely fill the unilocular mother cyst. Cyst septation may produce "wheel-like structures, or the contained daughter cysts may produce a "rosette-like" or "honeycomb pattern" structure. Cyst wall is habitually visible, and normally are round or ovals.	Usually fertile
CE3a	Anechoic content with detachment of laminated membrane from the cyst wall visible as floating membrane or as "water-lily" sign, which is indicative of wavy membranes floating on top remaining cyst fluid. Cyst form may be less rounded due to decrease of intra-cystic fluid pressure.	Transitional stage (starting to degenerate)
CE3b	Unilocular cyst which may contain daughter cysts (anechoic appearance and echoic areas (disrupted membranes degenerating daughter cysts). May appear at ultrasonography as a "complex mass". Cyst form may be less rounded due to decrease of intra-cystic fluid pressure.	Transitional stage (starting to degenerate)
CE4	Heterogeneous hypoechoic or dyshomogeneous degenerative contents. No daughter cysts (4a). May show a "ball of wool" sign which is indicative of degenerating membranes (4b).	Inactive. Most cyst of this type are not fertile
CE5	Solid matrix with calcified wall, which is shaped, producing a cone shaped shadow. Degree of calcification varies from partial to complete.	Inactive. Not fertile in the majority of cases

WHO: World Health Organization.

E. granulosus Antigen B and Antigen 5 (Silva-Álvarez *et al*., 2015). These diagnostic methods work best in patients with leaking or ruptured cysts (Zhang & McManus, 2006).

For surgical interventions in the treatment of CE, there are a few treatment options that can be evaluated based on the patient risk factors, the characteristics of the case, and the hospital resources that are available where the patient is being treated. One of these conservative approaches used in limited-resource clinical settings and CE endemic regions is the PAIR method, consists of puncturing the cyst wall, aspirating the fluid inside of the cyst, injecting a scolicidal agent (i.e. hypertonic saline) in the cyst, then re-aspirating and evacuating the cyst. The PAIR procedure can be preferred in low-resource clinical settings because it has a lower cost and requires a lower level of surgical skill than other treatment options, but the risk of the procedure is that it carries high rates of recurrence and morbidity (Sozuer *et al*., 2014). To reduce the risk of recurrence, centers with hepatobiliary surgery teams prefer more aggressive approach (specially in large cysts, or with evolutionary complications), which involves the total resection of the intact cyst through a procedure known as a pericystectomy or even hepatectomies (Manterola *et al*., 2017) (Fig. 2).

In addition to surgical interventions, smaller cysts CE1 and CE3a of WHO proposal (WHO Informal Working Group, 2003), can also be treated using benzimidazole compounds, most commonly, albendazole (Nazligul *et al*., 2015). Chemotherapy is typically used as an adjuvant treatment for CE because it is useful in preventing secondary cyst formation during surgical or laparoscopic interventions, but when used on its own, is associated with a high rate of relapse in the cyst activity (Stojkovic *et al*., 2009).

Potential methods of CE prevention include livestock and dog vaccines, public education, and ultrasound surveys. A recombinant vaccine called EG95 has already been developed and showed 95 % immunity for at least 12 months when field trials were conducted on vaccinated sheep in Australia, New Zealand, Argentina, Italy, and China (Lightowlers *et al*., 1999). Efforts have also been made to administer deworming medication, praziquantel, to dogs in areas where CE is endemic, but it has only been marginally successful because those regions' present infrastructure aren't able to support the widespread administration of praziquantel for the recommended eight times per year (Larrieu & Zanini, 2012). Public education and ultrasound surveys have been performed in conjunction, with volunteer participation in US screenings, lessons on the parasite life cycle, its clinical presentation, and prevention methods (Kachani *et al*., 2003; Salviti *et al*., 2014; Acosta-Jamett *et al*., 2022).

Echinococcus granulosus development and anatomy.

The adult stage of *E. granulosus* has a segmented anatomy that can be described by two parts: the scolex (head) and the strobila (body). At the tip of the scolex are the rostellar hooks, each consisting of a base and a curved tooth. These hooks are arranged in a ring pattern on a knob-like protrusion, forming a structure known as the rostellum (Holcman & Heath, 1997).

The suckers are found below the rostelum, and the remaining neck connects the scolex to the strobila. The strobila consists of three segments which proceed in the following order when starting at the neck: the immature proglottid, the mature proglottid, and the gravid proglottid. The gravid proglottid contains the cestode's sex organs: the egg-filled uterus and the cirrus sac (Fig. 1).

Typically, definitive hosts eliminate the taeniid eggs in their feces, contaminating waterways and vegetables that

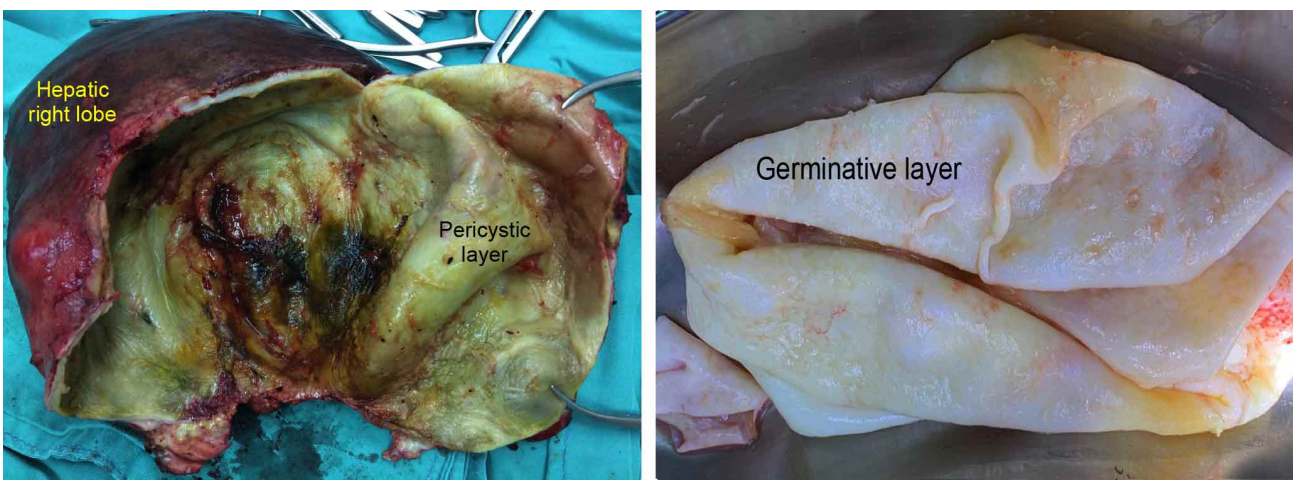

Fig. 2. Macroscopy of a hepatic hydatid cyst structure.

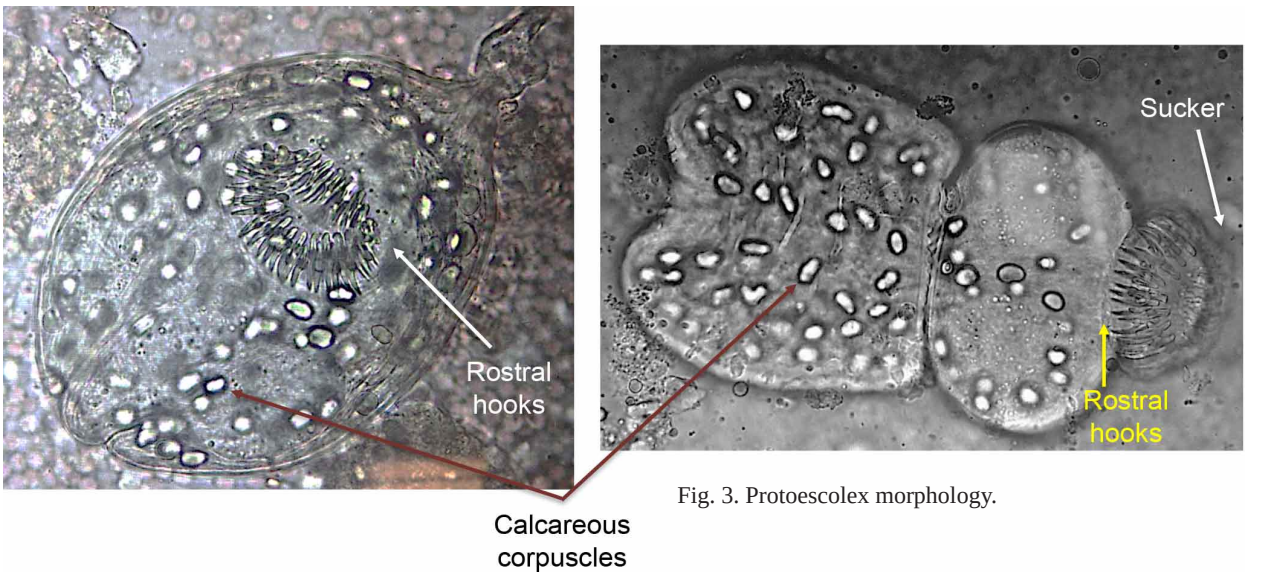

Fig. 3. Protoescolex morphology.

grow in the ground; so being available so that the intermediate hosts (including humans), which will develop hydatid cysts (Thompson, 2017). The life cycle of the *E. granulosus* parasite is shown in Figure 4.

When an egg is ingested by an intermediate host, mechanical digestive action and digestive juices release the oncosphere. Through the evagination of their hooks, penetration glands that lyse the tissues and protect from the digestive glands, penetrating the crypts and villi of jejunum and upper ileum; to later reach an intestinal venule or a lymphatic vessel; and then to migrate to a target organ such as liver, lungs, etc. At these sites, the oncospheres may die spontaneously, be destroyed by the cellular reaction or initiate the vesicular evolution (Cordero del Campillo & Rojo Vázquez, 2000).

Then, the hexacanth embryo will go through a series of transformations to become an hydatid cyst. Initially it will lose its hooks and the tissues without major utility will

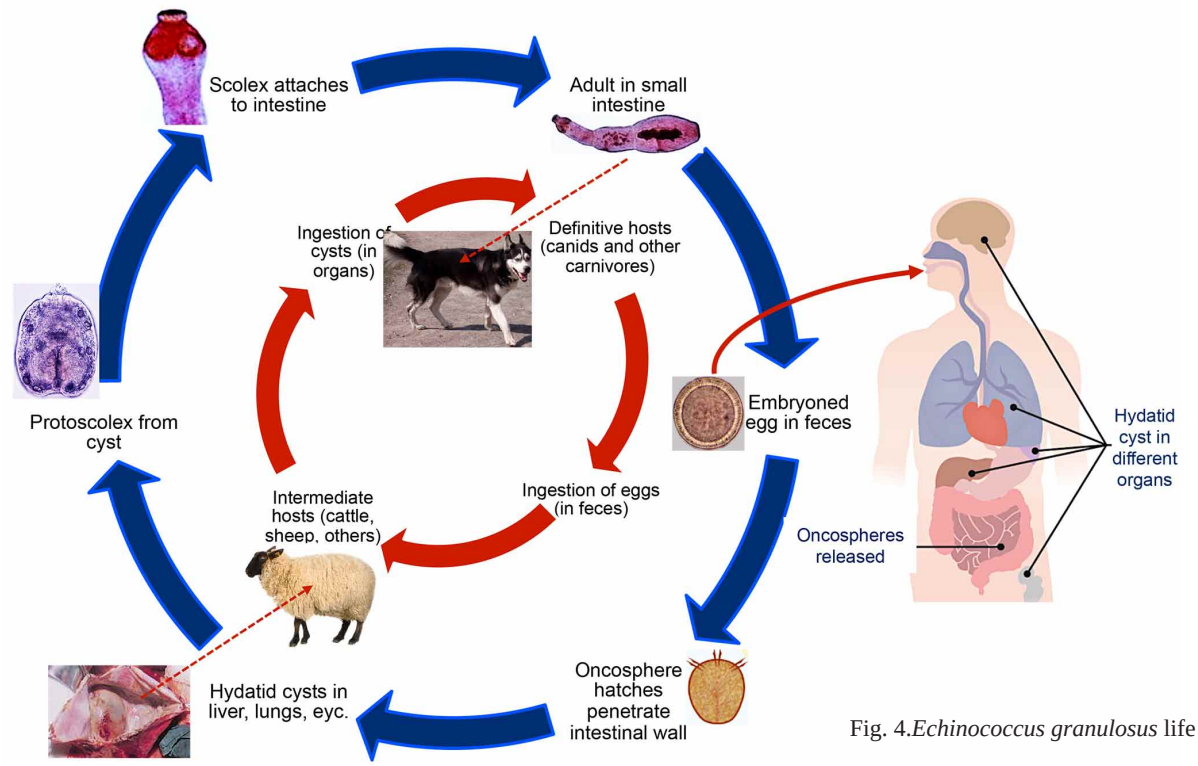

Fig. 4.*Echinococcus granulosus* life cycle.

undergo degeneration in order to initiate cell proliferation. Cysts can grow at an average of 1 to 30 mm per year and go several years without transformation, depending, among others, on the parasitized organ. The final size of a cyst in humans can reach up to 30 cm in diameter (even more) (Sapunar, 2013; Martínez *et al*., 2016).

At 3 weeks the hydatid is about 250 mm in diameter and has a central cavity. By the 5th month it measures one cm, and its wall is made up of two layers: a thick laminated outer layer, 10um in charge of protecting the hydatid cyst from the host's immune response, and a granular germinal inner layer, 15 to 20 um in charge of asexual reproduction (Acha & Szyfres, 2003; Sapunar, 2013; Martínez *et al*., 2016).

Protoscolex morphology.

From the 5th month post-infection, it is possible to see the prolifera capsules, which correspond to an internal vacuolization of the germinal to which it remains attached. Habitually 30 to 40 protoescolex develop (Fig. 3); which appear about 16 months post-infection in sheep cysts and at 10 to 12 months in pig cysts (Cordero del Campillo & Rojo Vázquez, 2000; Sapunar, 2013; Martínez *et al*., 2016).

There are 7 developmental stages in the formation of a protoscolex from a bud (Galindo *et al*., 2002) (Fig. 5).

The 1st state is a spheric bud. They are hollow and born from the germinative layer, but don't emerge from any one cell type specific.

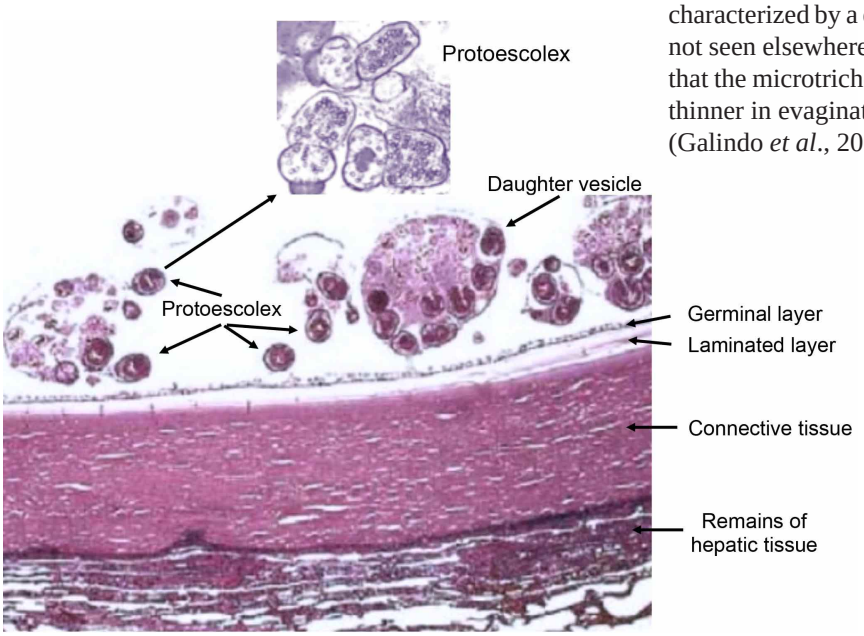

Fig. 5. Microscopy of hydatid cyst structure.

The 2nd state is an early elongated bud the base attached to the germ membrane narrows forming a stem, which make it possible to identify an anterior and a posterior region. The cells cluster in the apical and apical-lateral regions, as shown in Figure 6 and enclose a cell free space known as the foramen, which becomes the location of invagination in later stages of development (Martínez *et al*., 2005).

The 3rd state is a Late elongated bud with presumptive scolex and body. Cell nuclei form around the margin of the bud and some nuclei cluster in a central anterior region where the hooks and suckers develop from. As the bud matures, calcium deposits called calcareous corpuscles form around the base of the protoscolex but it remains unclear what the function of the corpuscles are (Galindo, *et al*. 2008). As the bud matures, calcium deposits called calcareous corpuscles form around the base of the protoscolex but it remains unclear what are their function (Martínez *et al*., 2005).

In the 4th state, nuclei are concentrated in the rostelar pad and the saplings of the protoscolex. After the elongated yolk has developed, the rostellum begins to form in the apical region of the scolex around the invagination region, so that there is a distinguishable body and scolex. Hooks develop in two rows around the foramen, alternating between large and small sizes, in number of 32 to 37 in total, with variations related to the host (Singh, *et al*., 2012). Then, hooks are attached to the rostelar pad, which contains muscle fibers that can allow extension and retraction of the hooks when attached to the intestinal mucosa. Hooks are also characterized by a denser, branched network of microtriches not seen elsewhere in the protoscolex. It has been observed that the microtriches are smaller (approximately 6 mm) and thinner in evaginated than invaginated protoscoces (7 mm) (Galindo *et al*., 2002).

The 5th state shows a developing protoscolex with suckers' formation, a stage called "protoscolex in differentiation". At this stage, there are circular projections and indentations that demonstrate where the saplings will develop, but the saplings are only fully formed when the protoscolex has finished its development.

In the 6th state, protoscolexis fully developed with 4 fully-formed suckers, each along the midline, distal to the rostellar hooks, but attached to the germinal layer.

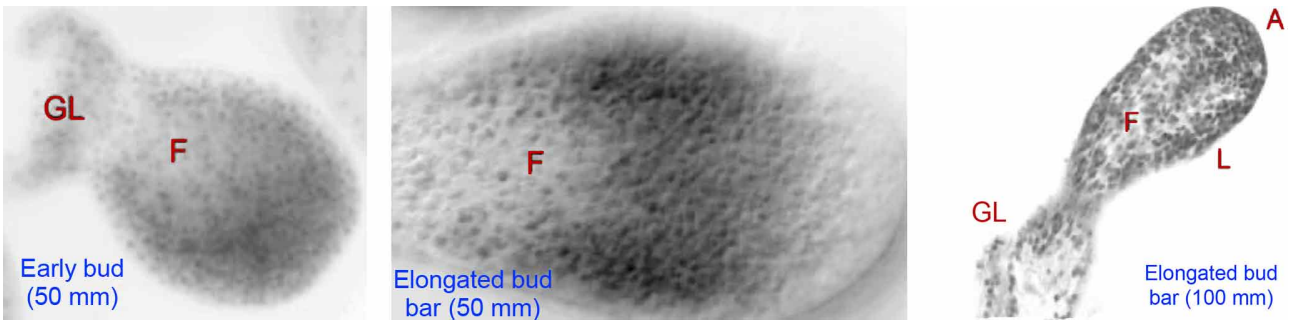

Fig. 6. Different stages of buds attached to the hydatid cyst germinal layer. A: apical region. L: lateral region. F: foramen. GL: germinal layer. Adapted from Martínez *et al*. (2005).

Finally, free protoscolex in cyst represent the 7th state (Galindo *et al*., 2002).

A correlation has been observed in the amount of newly synthesized protein and the formation of the suckers on the protoscoleces, which could indicate there is increased protein production during protoscolex development in the region that the suckers originate from. As for structures in the body of the protoscolex, the protoscolex utilizes flame cells for osmoregulation, and serve as a means for filtering waste, and these flame cells are concentrated in the parenchymal tissue (Galindo *et al*., 2008).

The tegumental surface of the protoscolex is covered in spines that are contained within an external membrane. This is a trait shared among tapeworms of the Taeniidae family. These spines could also serve as an important morphological indicator to determine the life cycle stage of *E. granulosus* because in protoscoleces, the regions posterior to the suckers have been shown to be covered in undeveloped spines, that are topped by caps rather than spikes. In adult worms, the spikes are fully developed across all aspects of the tegumental surface, and the membrane coating that covers the protoscolex is lost (ibid).

Additionally, through proteomic analysis, researchers have uncovered candidate protoscolex proteins that could be involved in the molecular mechanisms of the hydatid cyst fertility (Hidalgo *et al*., 2016). These proteins are Prostaglandin-H2 D-isomerase, Endophilin B1, Protein DJ 1, and Nuclear DBF2-related kinase. The latter protein is proposed as possibly being involved in the host immune response in infertile cysts. In another study, SNW domain-containing proteins were found when strobilation was induced in protoscoleces, which indicates that Vitamin D and retinoid receptors play a role in stimulating protoscoleces to grow and become adult worms. In the same study, proteins that are a part of the Endosomal Sorting Complex Required For Transport III (ESCRT-III) were present in strobilating protoscoleces, which indicates that there is potential for cell-cell communication pertaining to sexual reproduction and survival (Debarba *et al*., 2015).

Significance and Next Steps

The morphology of the *E. granulosus* protoscolex offers many clues into the mechanisms of the organism's development and the pathological mechanisms of CE. With a better understanding of the interrelation of the parasite's structures in this developmental stage, it is possible to determine potential alternatives of interrupting the worm's life cycle, which could be further developed into novel treatment modalities for CE.

Potential next steps would be to try and determine the importance of some of the morphological characteristics of the protoscolex stage (underdeveloped tegumental spikes, the presence of particular fertility proteins, etc.) and to run further studies analyzing the proteins of strobilating protoscoleces so that the proteins that aid in hydatid cyst fertility can be more definitively identified (Debarba *et al*., 2015; Silva-Álvarez *et al*., 2015; La-Rocca *et al*., 2019; Faridi *et al*., 2021).

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RESUMEN: El *Echinococcus granulosus* (*E. granulosus*), es un cestodo que se propaga entre huéspedes intermedios y definitivos a través de la ingestión de materia fecal contaminada con los huevos del parásito. El ciclo de vida consiste en la diferenciación de huevos a oncosferas y embriones, que finalmente forman quistes en órganos como el hígado, los pulmones y otros. Dentro de estos quistes hay protoescólices, una etapa intermedia del parásito que se convierte en su forma adulta (tenia), una

vez que infectan a sus huéspedes definitivos. Cuando estos quistes hidatídicos se desarrollan en seres humanos, se les conoce como equinococosis quística (EC). Esta enfermedad se trata mediante la extirpación quirúrgica de los quistes o la quimioterapia con compuestos benzimidazólicos. La comprensión de la morfología de la etapa de desarrollo intermedia del *E. granulosus* y la etapa de protosclex, puede permitir a los investigadores identificar cambios estructurales definidos y funciones de proteínas que podrían usarse para desarrollar modalidades de tratamiento para la CE. Las características únicas en la superficie tegumentaria durante la etapa de protoescolex y las proteínas asociadas con la fertilidad del quiste se han identificado en estudios de investigación anteriores y acercan a los investigadores a la comprensión de los mecanismos subyacentes del desarrollo del *E. granulosus* y, en consecuencia, los medios para interrumpirlo para lograr un mejor control de la enfermedad.

PALABRS CLAVE: *Echinococcus granulosus*; Echinococcosis; Echinococcosis quística; Quiste hidatídico; Protoescolex; Morfología.

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