Embryonic development of *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia: Mycetopodidae)

Silva-Souza, AT.[a]*, *Guardia-Felipi, P.*[b] *and Arrebola, NR.*[c]

[a]Laboratório de Ecologia de Parasitos de Organismos Aquáticos – LEPOA, Departamento de Biologia Animal e Vegetal, Centro de Ciências Biológicas, Universidade Estadual de Londrina – UEL, CEP 86051-990, Londrina, PR, Brazil

[b]Programa de Pós-graduação em Ciências Biológicas, Departamento de Biologia Animal e Vegetal, Universidade Estadual de Londrina – UEL, CEP 86051-990, Londrina, PR, Brazil

[c]Departamento de Histologia, Universidade Estadual de Londrina – UEL, CEP 86051-990, Londrina, PR, Brazil

*e-mail: ateresa@uel.br

Received April 1, 2010 – Accepted June 7, 2010 – Distributed February 28, 2011

(With 3 figures)

Abstract

The phases of embryonic development of *Anodontites trapesialis lasidia* are described for the first time. Adult specimens were obtained from two fish farms located in Londrina, Paraná, Brazil. The internal demibranchs of 120 individuals were studied using a routine histological technique; 70 of these carried eggs and/or larvae in the marsupium and were utilized for the description of the phases of embryonic development. The demibranchs of five specimens were evaluated by scanning electron microscopy to detail the morphology of the larvae. Five phases of development were established: phase I, corresponding to the initial stage of cleavage with the formation of apical cells; phase II, including the stages of the morula and blastula; phase III, where the gastrula forms; phase IV, where the larva formed is still inside the egg envelope; and phase V, where the *lasidium* can still be identified immediately after eclosion.

Keywords: fish farming, *Anodontites trapesialis*, morphology, embryogenesis, *lasidium*.

Desenvolvimento embrionário de *Anodontites trapesialis* (Lamarck, 1819) (Bivalvia: Mycetopodidae)

Resumo

As fases do desenvolvimento embrionário das *lasídias* de *Anodontites trapesialis* são descritas pela primeira vez. Espécimes adultos foram obtidos de duas pisciculturas localizadas no município de Londrina, Paraná, Brasil. As demibrânquias internas de 120 indivíduos foram estudadas por técnicas histológicas rotineiras; 70 apresentavam ovos e/ou larvas no marsúpio e foram utilizadas para a descrição das fases do desenvolvimento embrionário. As demibrânquias de cinco espécimes foram avaliadas por microscopia eletrônica de varredura para detalhar a morfologia da larva. Cinco fases do desenvolvimento foram estabelecidas: fase I, correspondente ao estágio inicial da clivagem com formação das células apicais; fase II, incluindo os estágios de mórula e blástula; fase III, na qual se forma a gástrula; fase IV, na qual a larva formada ainda encontra-se dentro do ovo; e fase V, na qual a *lasidium* pode ser identificada imediatamente após a eclosão.

Palavras-chave: piscicultura, *Anodontites trapesialis*, morfologia, embriogenia, *lasidium.*

1. Introduction

The bivalves of the family Mycetopodidae incubate their eggs in internal demibranchs, in a so-called marsupium (Castellanos and Landoni, 1990). The egg gives rise to the *lasidium*, a temporary ectoparasite of fish and present only in the families Mutelidae in Africa and Mycetopodidae in South America (Parodiz and Bonetto, 1963; Wächtler et al., 2001). When mature, the larvae are passed from the marsupium to the branchial canal and eliminated to the exterior through the exhalant opening (Castellanos and Landoni, 1990). In the environment, it needs to find a host fish to complete its development, to which it adheres by means of an adhesive organ (Bonetto and Ezcurra, 1962; Silva-Souza and Eiras, 2002).

Descriptions of the general characteristics of the family Mycetopodidae, for the majority, are based on studies with species of the genus *Anodontites* Bruguière, 1792 (Castellanos and Landoni, 1990).

According to Simone (1994), the first studies on *Anodontites trapesialis* were carried out by Simpson (1900). Later, its anatomy was detailed by Veiteinheimer (1973) and Hebling (1976). The species has been utilised in environmental monitoring studies (Callil and Junk, 1999;

Jacomini, 2002; Tomazelli et al., 2003) and, more recently, in aquaculture works (Tello-Panduro et al., 2003, 2004; Vivanco et al., 2003).

Studies on the reproduction of *A. trapesialis* are rare. The only one on the gametogenesis and dynamics of reproduction was conducted by Callil and Mansur (2007), but nothing was recorded on the embryonic development of the *lasidia*.

Thus, due to the scarcity of information, the aim of the present work was to describe the development of the eggs of *A. trapesialis* in the marsupium up to the eclosion of the *lasidia.*

2. Material and Methods

Monthly collections of specimens of *Anodontites trapesialis* were performed in the period of May 2006 to April 2007, in fish culture tanks on the properties of "Toca do Jacaré" (23° 24' 46.8" S, 51° 08' 33.5" W) and "Paraíso da Tilápia" (23º 25' 16.7" S, 51° 09' 13.6" W), located in the Distrito Espírito Santo, Londrina, PR, Brazil.

The specimens were located by touching the bottom of the tanks with the feet, collecting them manually and transporting them to the Laboratory of Ecology of Parasites of Aquatic Organisms (LEPOA), Department of Animal and Vegetal Biology, State University of Londrina, Londrina, PR, in plastic containers with water from the same tank, without mechanical aeration. In the laboratory, the animals were kept alive in a dry container for the elimination of the water contained in the paleal cavity, weighed (total weight in grams) and measured (total length in millimeters). Next, the gills were examined under a stereomicroscope for the verification of the presence of eggs and for morphological characterisation.

Samples of 1.0 cm^2 of both internal demibranchs of 120 specimens of *A. trapesialis* were fixed in Bouin's solution for 24 hours, preserved in 70% alcohol, dehydrated in the an ethanol series, cleared in xylol and embedded in Paraplast for histological analyses. Serial sections of 0.7 µm thickness were prepared with a Leica RM 2145 rotary microtome, mounted on slides and stained with hematoxylin and eosin (HE). The sections were analysed with a Zeiss Axiophot photomicroscope, and the images were obtained with a Leica DM 4500B microscope equipped with a DFC 300FX camera and with the Leica IM50 4.0 program.

For scanning electron microscopy (SEM), samples of demibranchs of five individuals selected randomly were fixed in a solution of 2% glutaraldehyde, 2% paraformaldehyde and 0.1 M cacodylate buffer, washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide, washed again in 0.1 M cacodylate buffer, and dehydrated in an increasing ethanol series. They were then critical-point dried, coated with a film of gold with thickness of 25 nm and analysed with a FEI Quanta 200 scanning electron microscope in the Electron Microscopy and Microanalysis Laboratory from PROPPG/UEL.

3. Results

A total of 120 specimens of *A. trapesialis* with a mean total weight of 159.24 ± 70.85 g (range: 24.69 g to 322.71 g) and mean total length of 119.44 ± 18.58 mm (range: 62.59 mm to 153.17 mm) were examined. Of these, only 70 showed eggs and larvae inside the marsupium of the internal demibranchs. When examined with a stereomicroscope, the marsupia with eggs in the initial phase had a beige colour, and during development they showed intensification in colouration.

The marsupium is lined by a simple cuboidal epithelial tissue that rests on loose connective tissue where blood cells are identified. This connective tissue fills the spaces between the lacunae, which are lined by an epithelial tissue similar to that of the marsupium (Figure 1a).

Histologically, it was possible to establish five phases of egg development in *A. trapesialis,* as described below.

- *Phase I.* The eggs observed in the initial phase are characterised by intense acidophilia. They are identified by their spherical and regular structure, covered by an acellular envelope appearing slightly basophilic. Initially, the contents of the egg are intensely acidophilic, and a thin layer of cell clusters appear at one of the poles (Figure 1b). At the end, there is an increase in the number of cells, which remain aggregated at one of the poles, accompanied by a reduction in the quantity and acidophilia of the remaining contents (Figure 1c). This phase appears to be the initiation of cleavage, where there appear to be a few apical cells.
- *Phase II.* When compared to the preceding phase, the eggs appear less acidophilic, and their envelope has an irregular surface. They are practically filled by an aggregate of cells whose nuclei are well visible and show little variation in size and coloration, which appears to correspond to the morula (Figure 1d). At the end, the cells are grouped and displaced, where part of the egg appears to be empty, corresponding to the blastula (Figure 1e).
- *Phase III.* This can be characterised as a joining of various layers of cells, which are grouped at one of the poles as an open ring in the shape of the letter C, constituting the gastrula. There are small, rounded nuclei with dense chromatin or loose chromatin and slightly basophilic cytoplasm (Figure 1f).
- *Phase IV*. The envelope is very irregular. It is possible to identify four constituents of the larva that localize at one of the poles: the anterior region consisting of two acidophilic lobes, with a homogeneous appearance and surface covered by cilia; wider medial region, constituted by a group of cells that have small, spherical slightly basophilic nuclei and slightly acidophilic cytoplasm; and a shorter posterior region which is less stained than the others. The space around the larva is filled with

a structure that is irregular, acellular and slightly basophilic, suggesting that it could be the adhesive organ (Figure 1g).

- *Phase V.* The larva is indentified just after eclosion, generally close to the empty envelope. The anterior region, predominantly acidophilic in relation to the others, comprises two types of cells, one containing large, spherical nuclei and the other with small, elliptical nuclei. This whole region is covered by a large amount of cilia. The medial and posterior regions are more basophilic. In the medial region, the nuclei are spherical and have loose chromatin. The posterior region is shorter, bilobed, formed by cells with elliptical nuclei and dense chromatin. In a small portion of the anterior region, there is a structure similar to that seen

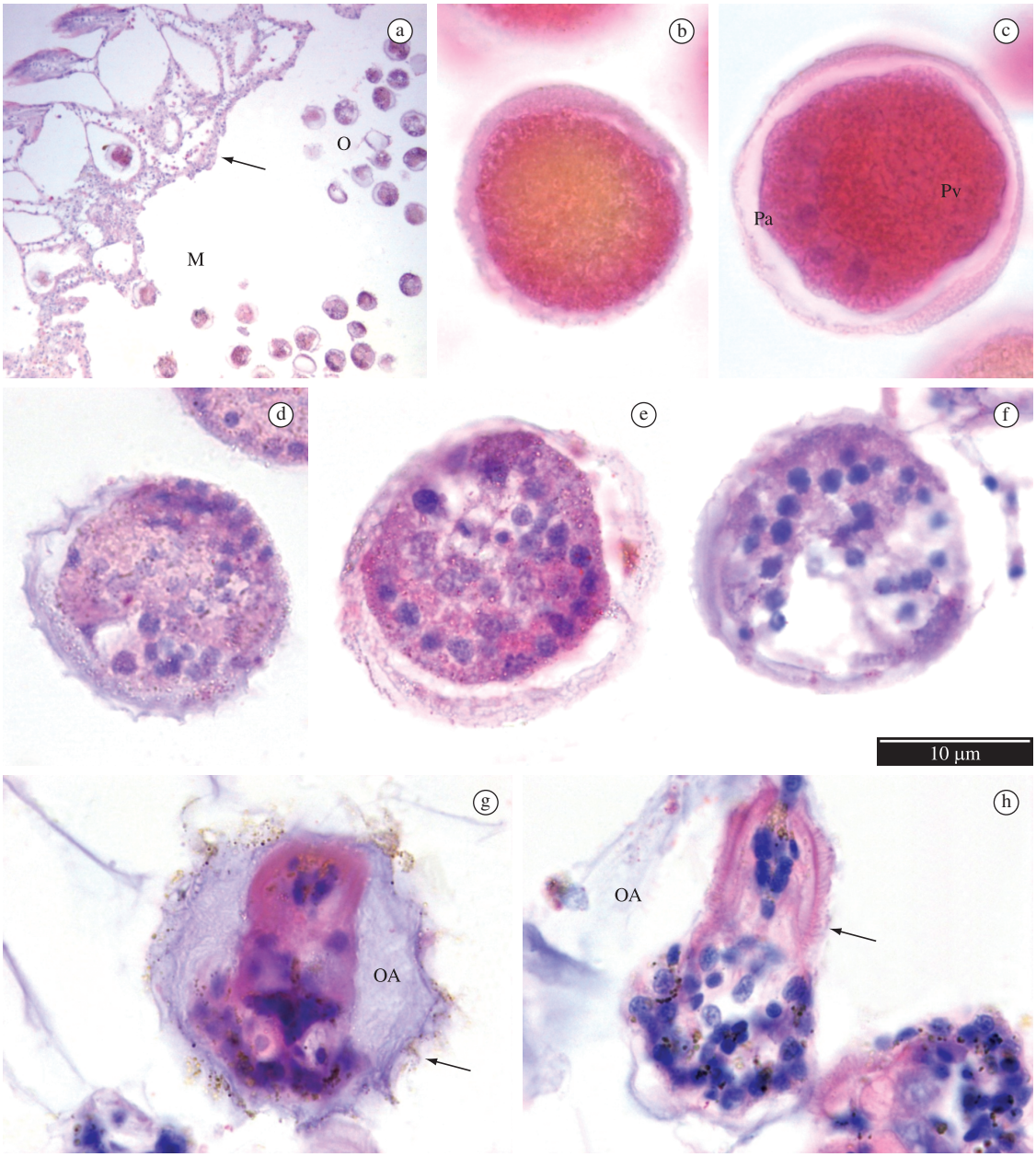

Figure 1. Photomicrographs of the development phases of the eggs in the marsupium of specimens of *Anodontites trapesialis* collected in "Toca do Jacaré" and "Paraíso da Tilápia," Londrina, Paraná, Brasil. a) Eggs (O) in the marsupium (M) with details of the wall of the marsupium (arrow), HE. 100X, b) phase I, beginning; c) phase I, end, with details of the nuclei (arrow) in the animal pole (Pa) and vitellum in the vegetative pole (Pv); d) phase II, beginning - morula; e) phase II, end - blastula; f) phase III - gastrula; g) phase IV, larva formed inside of egg with adhesive organ (OA) and details of the irregular envelope (arrow); and h) Phase V - larva recently ecloded with adhesive organ (OA) and cilia in the anterior lobes (arrow), HE. 1000X (bar).

inside the egg in phase IV, which confirms that it is the adhesive organ. The envelope of the eggs is identified as a thin structure of irregular shape and generally surrounded by pigmented cells (Figure 1h).

The samples of internal demibranchs analysed by scanning electron microscopy showed details of the eggs on the wall of the marsupium (Figure 2a) and confirmed the modifications of the envelope during development (Figure 2b-d).

Figure 3 shows the details of the *lasidium*: ciliated anterior lobes (a and b), middle region with larval shell (c) and posterior lobes with hooks curved downward and inward (d). It is noted that the adhesive arises from the anterior region in the dorsal portion, close to an opening that appears to be the mouth (Figure 3a, b). It is noted that the hooks of the right posterior lobe are not present.

4. Discussion

The examination of the specimens of *Anodontites trapesialis* in the present study revealed the presence of eggs only in the internal demibranchs, as found by Bonetto and Ezcurra (1962) for *Anodontites trapesialis forbesianus* (Lea, 1860), Mansur (1974) for *Monocondylaea minuana* d'Orbigny, 1835 and Veiteinheimer-Mendes and Mansur (1978) for *Mycetopoda legumen* (Martens, 1888).

In *A. trapesialis*, as reported by Mansur (1974) for *M. minuana*, the marsupium occupies almost all of the internal demibranch, leaving only a narrow ventral strip and some aquifer tubes in the anterior and posterior regions. On the other hand, in *Mycetopoda legumen*, the marsupium occupies only the anterior half of the demibranch (Veiteinheimer-Mendes and Mansur, 1978).

Contrary to that described by Maldonado et al. (1990), who observed only small whitish masses corresponding to mature larvae, in the present study, the presence of the *lasidia* in the marsupium conferred a brown coloration.

Larvae with a body divided into three regions, observed for *Anodontites trapesialis* studied here, constituted a peculiar characteristic of the *lasidium* cited by Bonetto and Ezcurra (1962, 1965), Parodiz and Bonetto (1963), Veiteinheimer-Mendes and Mansur (1978), Castellanos and Landoni (1990), Maldonado et al. (1990) and Wächtler et al. (2001).

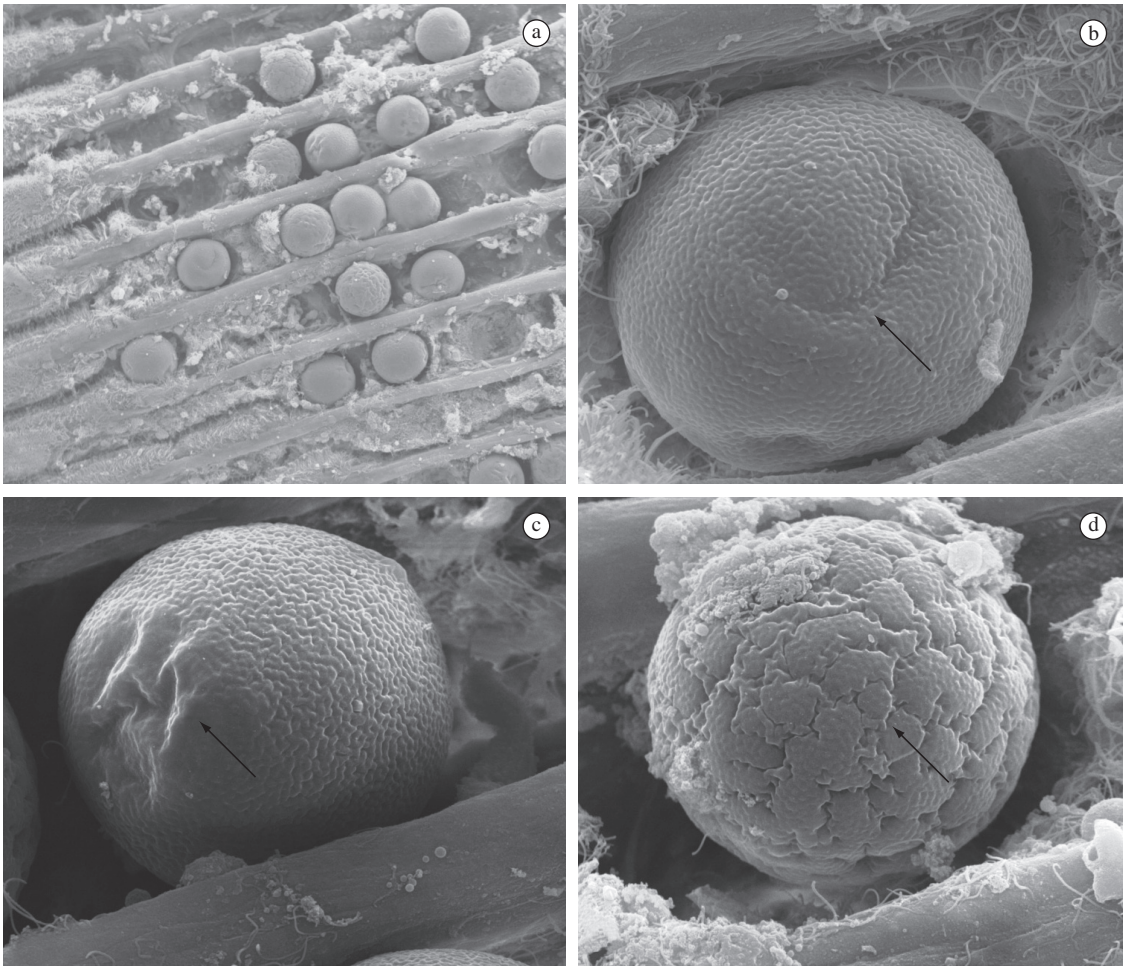

Figure 2. Electron micrographs of the eggs of *Anodontites trapesialis* in the marsupium a) 560x with details of the modifications of the envelope of the eggs at different phases of development (arrows); and b–d) 4,000x.

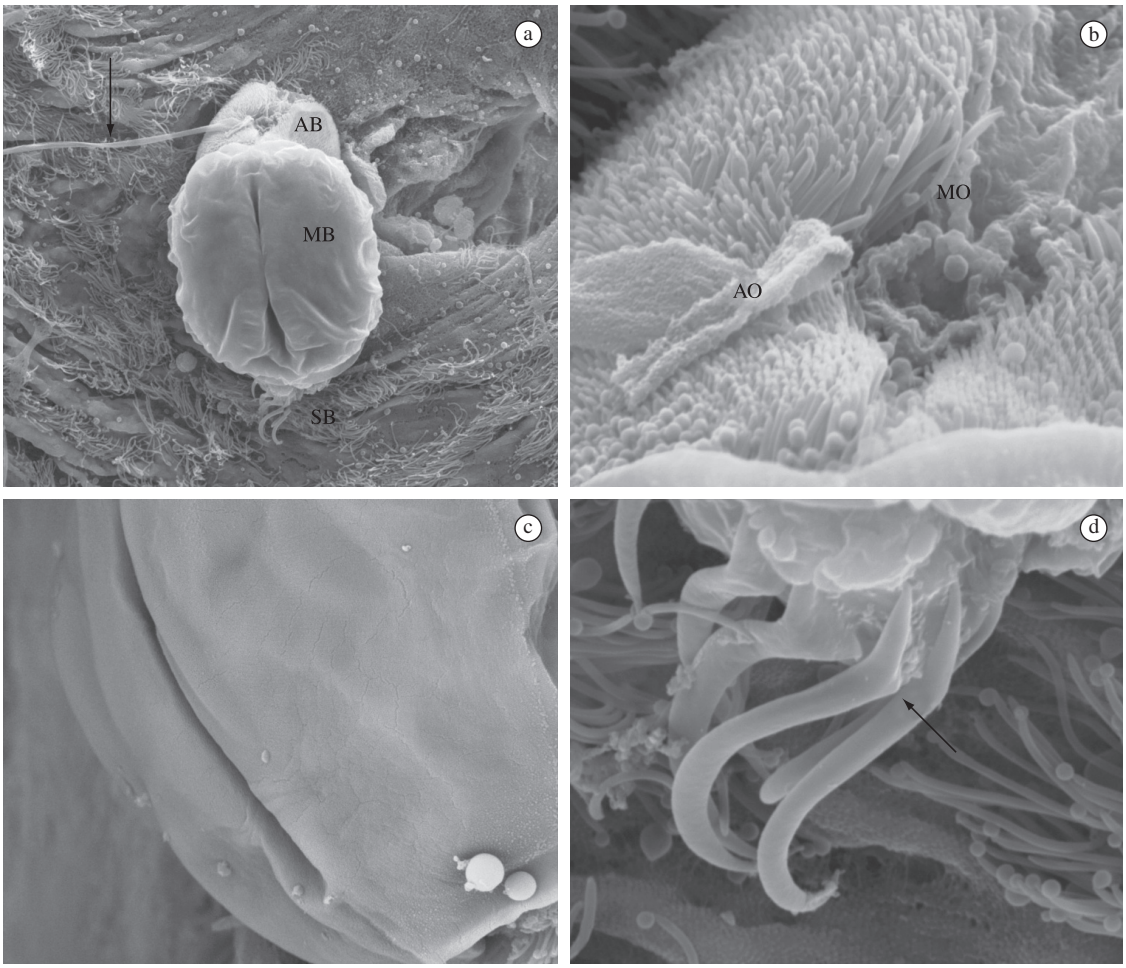

Figure 3. Electron micrographs of the larvae in the marsupium of *Anodontites trapesialis*. a) Anterior (AB) with adhesive organ (arrow), medial (MB) and posterior (SB) regions, 1,500x; b) details of the anterior region with part of the adhesive organ (AO) and mouth (Mo), 12,000x; c) details of larval shell, 5,000x; and d) posterior region with details of the hooks of the left lobe (arrow). 12,000x.

Bonetto and Ezcurra (1962) and Castellanos and Landoni (1990) reported that the adhesive organ arises from the medial region in the ventral position of the larva. However, scanning electron microscopy showed that in the larvae of *Anodontites trapesialis*, it originates from the dorsal portion of the anterior region, close to an opening that resembles a mouth. In histological sections, an accumulation of cells in a circular arrangement can be seen in the region corresponding to the mouth. However, no digestive tract appears to be contiguous with it, and no reference to the presence of a mouth in *lasidia* was found in the available literature. Therefore, further studies are necessary for such speculations to be clarified.

The medial and posterior regions concur with those described in the literature, also with respect to the larval shell (Bonetto and Ezcurra, 1962; Castellanos and Landoni, 1990).

With respect to the hooks in the posterior region, a group of six to seven was found present in each lobe, similar to that described by Bonetto and Ezcurra (1962) in *A. trapesialis forbesianus*. On the other hand, *Anodontites trapezeus* (Spix, 1827) shows approximately 11 short hooks in each of the lobes, and *Mycetopoda siliquosa* (Spix, 1827) has seven or eight hooks (Bonetto and Ezcurra, 1965). In *Mycetopoda legumen,* the number of hooks varies from four to seven (Veiteinheimer-Mendes and Mansur, 1978). The hooks are responsible for establishing the first firm contact with the body of the host, in which the larva will complete its metamorphosis and reach the juvenile stage (Wächtler et al., 2001).

It is known that mollusks show holoblastic spiral cleavage. However, characteristics of the egg during development generally are not described for the majority of species. Most studies of this nature refer to marine species of economic importance (Mouëza et al., 1999).

With respect to freshwater species, Maldonado et al. (1990) reported the occurrence of three phases of egg development in the marsupium of *Anodontites (Anodontites) soleniformes* Orbigny, 1835 of Bolivia, without providing any description. In the present study, it was possible to

describe, for the first time, five distinct phases of egg development in the marsupium of *Anodontites trapesialis*, where the last corresponded to the *lasidia* immediately after eclosion.

Acknowledgements – Thanks go to Mr. Helio, owner of Pesque-Pague "Toca do Jacaré", and to Mr. Nelson, owner of the fish farm "Paraíso da Tilápia," for permitting the collections in the tanks, and to Aparecido de Souza, Edson Santana da Silva and Wanner Galves for their help with the collections. Dr. Celia G. T. de Jesus Andrade, Juca A. Barrera San Martin and Osvaldo Capello from EMML / PROPPG / UEL assisted with the SEM analyses. Renata Baldo Rodrigues Carvalho from the Histology Laboratory, UEL, helped with the histological procedures. Dr. Claudia Tasso Callil from Universidade Federal do Mato Grosso confirmed the identification of the species. Dr. A. Leyva helped with the translation of the manuscript. CAPES provided financial support.

References

BONETTO, AA. and EZCURRA, ID., 1962. El desarrolo del lasidium de *Anodontites trapesialis forbesianus* (Lea) (Mollusca, Lamellibranchiata). *Physis*, vol. 23, no. 65, p. 195-203.

-, 1965. Notas malacológicas III. 5) La escultura del periostraco en el genero *Anodontites* 6) el *lasidium* de *Anodontites trapezeus* (Spix) 7) el *lasidium* de *Mycetopoda siliquosa* (Spix). *Physis*, vol. 25, no. 69, p. 197-204.

CALLIL, CT. and JUNK, WJ., 1999. Concentração e incorporação de mercúrio por moluscos bivalves *Anodontites trapesialis* (Lamarck, 1819) e *Castalia ambigua* (Lamarck, 1819) do Pantanal de Poconé - MT, Brasil. *Biociências*, vol. 7, no. 1, p. 3-28.

CALLIL, CT. and MANSUR, MCD., 2007. Gametogênese e dinâmica da reprodução de *Anodontites trapesialis* (Lamarck) (Unionoida; Mycetopodidae) no lago Baía do Poço, planície de inundação do rio Cuiabá, Mato Grosso, Brasil. *Revista Brasileira de Zoologia*, vol. 24, no. 3, p. 825-840.

CASTELLANOS, ZJA. and LANDONI, N., 1990. La familia Mycetopodidae Gray, 1840 em La Republica Argentina. In RINGUELET, RA. (Ed.). *Fauna de água dulce de la Republica Argentina*. FECIC. vol. 16, p. 1-86.

HEBLING, NJ., 1976. The functional morphology of *Anodontites trapezeus* (Spix) and *Anodontites trapesialis* (Lamarck). (Bivalvia: Mycetopodidae). *Boletim de Zoologia da Universidade de São Paulo*, vol. 1, p. 265-298.

JACOMINI, AE., 2002. *Bioacumulação do herbicida atrazina pelas espécies de bivalves limnicos Anodontites trapesialis (Lamarck, 1819) e Corbicula fluminea (Muller, 1774)*. Ribeirão Preto: Universidade de São Paulo. 89 p. Dissertação de Mestrado em Biologia Comparada.

MALDONADO, M., ACOSTA, F., ISURZA, LJ. and BARRA, C., 1990. Aspectos biológicos de *Anodontites (Anodontites) soleniformes* (Mollusca-Bivalvia) en el Arroyo Chipiriri (Cochabamba-Bolivia). *Acta Limnologica Brasiliensia*, vol. 3, p. 847-863.

MANSUR, MCD., 1974. *Monocondylaea minuana* Orbigny, 1835: variabilidade da concha e morfologia do sistema digestivo (Bivalvia, Mycetopodidae). *Iheringia, Série Zoologia*, vol. 45. p 3-25.

MOUËZA, M., GROS, O. and FRENKIEL, L., 1999. Embryonic, larval and postlarval development of the tropical clam, *Anomalocardia brasiliana* (Bivalvia, Veneridae). *Journal of Molluscan Studies*, vol. 65, p. 73-88.

PARODIZ, JJ. and BONETTO, AA., 1963. Taxonomy and zoogeographic relationships of the South American naiades (Pelecypoda:Unionacea and Mutelacea). *Malacologia*, vol. 1, p. 179-213.

SILVA-SOUZA, AT. and EIRAS, JC., 2002. The histopathology of the infection of *Tilapia rendalli* and *Hypostomus regani* (Osteichthyes) by Lasidium larvae of *Anodontites trapesialis* (Mollusca, Bivalvia). *Memórias do Instituto Oswaldo Cruz*, vol. 97, no. 3, p. 431-433.

SIMONE, LRL., 1994. Anatomical characters and systematics of *Anodontites tapesialis* (Lamarck, 1819) from South America (Mollusca, Bivalvia, Uionoida, Muteloidea). *Studies on neotropical Fauna and Environment*, vol. 29, no. 3, p. 69-185.

TELLO-PANDURO, B., GARCÍA-VASQUEZ, Y., VIVANCO-MEDINA, M. and TABOADA, OM., 2004. Air drying of clams (*Anodontites trapesilais*) and tilapia (*Oreochromis niloticus*) fillet. In *Proceedings of the Fourteenth International Drying Symposium*, 2004. São Paulo. vol. C, p. 1977-1983. Available from: <http://www.feq.unicamp.br/~ids2004/volC/pp%201977-1983.pdf>. Access in: 6 Feb. 2006.

TELLO-PANDURO, B., GARCÍA-VASQUEZ, Y., VIVANCO-MEDINA, M., VITERI, JD., BAÇA, JH. and TABOADA, OM., 2003. Secado de tilpaia (*Oreochromis* sp.), almejas (*Anodontites trapesialis*) y camarón gigante (*Macrobrachium rosenbergii*). In *Proceedings of the Segundo Congreso Iberoamericano Virtual de Acuicultura*, 2003. p. 775-783. Available from: <http://www.revistaaquatic.com/civa2003/coms/listado.asp?cod=56>. Access in: 19 July 2006.

TOMAZELLI, AC., MARTINELLI, LA., AVELAR, WEA., CAMARGO, PB., FOSTIER, AH., FERRAZ, ESB., KRUG, FJ. and SANTOS JÚNIOR, D., 2003. Biomonitoring of Pb and Cd in two impacted watersheds in Southeast Brazil, using the freshwater mussel *Anodontites trapesilais* (Lamarck, 1819) (Bivalvia: Mycetopodidadae) as a biological monitor. *Brazilian Archives of Biology and Technology*, vol. 46, no. 4, p. 673-684. Available from: <http://www.scielo.br/pdf/babt/v46n4/a22v46n4.pdf>. Access in: 19 Mar. 2006.

VEITEINHEIMER, IL., 1973. *Anodontites* Bruguière, 1972 no Guaíba-RS (Bivalvia:Mycetopodidae) I. *Anodontites trapesialis forbesianus* (Lea, 1860). *Iheringia, Série Zoologia*, vol. 44, p. 32-49.

VEITEINHEIMER-MENDES, IL. and MANSUR, MCD., 1978. *Mycetopoda legumen* (Martens, 1888): lasídio e desenvolvimento parasitário (Bivalvia: Mycetopodidae). *Revista Brasileira de Biologia = Brazilian Journal of Biology,* vol 38, no. 3, p. 531-536.

VIVANCO, MM., ORDINOLA, AA. and TABOADA, OM., 2003. Secado natural y solar de almejas de agua dulce (*Anodontites trapesialis*). In *Proceedings of the Segundo Congreso Iberoamericano Virtual de Acuicultura*, 2003. p. 797-807. Available from: <http://www.revistaaquatic.com/civa2003/coms/listado.asp?cod=43>. Access in: 19 Jul. 2006.

WÄCHTLER, K., DREHER-MANSUR, MC. and RICHTER, T., 2001. Larval types and early postlarval biology in Naiads (Unionoida). In: BAUER, G. and WÄCHTLER, K. (Eds.). *Ecology and evolution of the freshwater mussels unionoida*. Ecological Studies 145. Berlin: Springer. p. 93-125.