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Taxonomic position and glochidia from the freshwater mussel, *Anodonta luteola* (Unionoida: Unionidae), in Costa Rica

Rafael A. Cruz & Rebeca Quesada

Estación de Biología Marina, Escuela de Ciencias Biológicas, Universidad Nacional, Puntarenas, Costa Rica; rcruzoto47@gmail.com, rebeqc@gmail.com

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ABSTRACT: Glochidia larvae from *Anodonta luteola* were observed in gills of *Oreochromis niloticus* from a farm in Cañas, Guanacaste. Samples were taken and analyzed by electron microscopy (SEM) and light microscopy. Glochidia were 311, 8µm total length (TL), 331,7µm total height (TH), and 251,1µm hinge dorsal length (HDL). *Anodonta* has 21 species reported for the Panamíca province, and *A. luteola* has five synonyms, often used in different reports.

Key words: *Anodonta luteola*, taxonomic position, glochidium morphology, Costa Rica.

RESUMEN: Posición taxonómica y el gloquidio del mejillón dulceacuícola *Anodonta luteola* (Unionoida: Unionidae), en Costa Rica.

Las larvas glochidio de mejillón *Anodonta luteola* se observaron en las branquias de los especímenes de *Oreochromis niloticus* de una granja en Cañas, Guanacaste, Costa Rica. Se tomaron muestras y se analizaron mediante microscopía electrónica de barrido (SEM) y microscopía de luz. Los glochidios midieron en promedio 311,8µm de longitud total (LT), 331,7µm de altura total (TH), y 251,1µm de longitud de la charnela (HDL). *Anodonta* cuenta con 21 especies reportadas para la Provincia Panamíca, además, *A. luteola* cuenta con cinco sinónimos, usados a menudo en diferentes informes científicos.

Palabras clave: *Anodonta luteola*, posición taxonómica, morfología del gloquidio, Costa Rica.

Anodonta luteola is distributed from Tierra Blanca and Malacotoya river in Nicaragua to the Isthmus of Darien in Panamá (von Martens 1980-1981). The glochidia larvae of *Anodonta luteola* were observed as ectoparasites on fish, attached fixed on the fins, gills, mouths and eye edges mainly. Several reports are found in Costa Rica for this species. In a fish culture system some researches were made on the reproduction of this species (Ruiz, 1982; Ruiz & Bonilla 1982); the reproductive behavior and biometrics was studied by Cruz & Villalobos, (1984); Cruz & Villalobos, (1985) related sexual maturity with total size; and Cruz & Villalobos, (1984) and Villalobos et al. (1985) reported the size distribution, growth and reproduction. Recently, Castillo-Martinez (2000) included this species on the impact of pesticides on banana production in aquatic ecosystems, and Arias-Andrés et al. (2014) used them in eco toxicological evaluation of aquaculture and agriculture as biomarkers.

The glochidia larvae from Unionidae Family are classified into three types, based in morphology and presence

or absence of hooks and spinules: subcircular unhooked, subtriangular hooked with spinules, subtriangular hooked without spinules (Graf & Cumming, 2006). The aim of this study was to report the taxonomic position of *A. luteola* and describe its glochidia larvae.

MATERIALS & METHODS

To determine the number of species of the genus *Anodonta* reported for the Panamíca province and to determine its taxonomy position the work of Von Martens, (1890-1901); Simpson, (1900); Pilsbry, (1920); Parodiz & Bonetto (1963), and MUSSELp. (2013) were revised.

Between September and October 2014, 80 specimens of *A. luteola* were collected. The mussels were taken from an *Oreochromis niloticus* culture system of the Universidad Técnica Nacional (UTN), located in Cañas, Guanacaste (10°20'8" N, 85°9'19" W) with 0,5 m deep. The mussels were transported alive on a cool box to Estación

de Biología Marina (EBM), Universidad Nacional (UNA), located in Puntarenas (9°58'43''N, 84°50'25''W) to be analyzed at once. Gills from mussels were examined for the presence of glochidia. Samples of glochidia were fixed on ME solution (2.0% of paraformaldehyde, 2.5% of glutaraldehyde and 5% of sucrose in 0.1 M sodium cacodylate buffer at pH 7.4) and were stored at 7 °C before scanning electron microscopy (SEM) analysis at Universidad de Costa Rica (UCR) (Hoggart, 1999). Samples were rinsed in the same buffer three times during 10 minutes, centrifugated at 2000 rpm to separate glochidia larvae from the tissue. Postfixation was done with 1% osmium tetroxide for one hour and the samples were rinsed 4 times with buffer before ethanol dehydration series. Samples were coated with 30 nm platinum and observed on a Hitachi S-2360 N scanning electron microscope.

Glochidia larvae were identified in a light microscope (Engel & Wächtler, 1989) with camera. Glochidia pictures were taken at natural and eocin staining was done to distinguish organs. The glochidia (n = 102) were measured for total length (TL), height (H) and hinge dorsal length (HDL), and mean values were calculated (mean ± standard deviation (SD), variation coefficient (VC)). To determinate the glochidia type based on Graf & Cumming (2006), a sample was examined with light microscopy (Engel & Wächtler, 1989).

RESULTS

The Holotype of *Anodonta luteola* is located in Smithsonian Institute, U. S. National Museum of Natural History, Department of Invertebrate Zoology, IZ MOL 86766. (MUSSEL p., 2013).

Species of the genus *Anodonta* present to Panamic Province: von Martens (1896-1900):

- *A. banbusearum* von Martens, 1900
- *A. chalcensis* Crosse & Fischer, 1893
- *A. cylindracea* Lea, 1838
- *A. ciconia* Carpenter, 1857
- *A. coarctata* Potiez & Michaud, 1844
- *A. depexa* von Martens, 1900
- *A. exilior* Lea, 1871
- *A. glauca* Humboldt & Bonpland, 1827

- *A. globosa* Lea, 1841
- *A. grijalvae* Morelet, 1884
- *A. henryana* Lea, 1857
- *A. inaequalis* Simpson, 1900
- *A. jewettiana* Lea, 1868
- *A. lurulenta* Morelet, 1849
- *A. luteola* Lea, 1858
- *A. montezuma* Lea, 1841
- *A. nicaraguae* Tate, 1870
- *A. nopalatensis* Sowerby, 1867
- *A. tabascoensis* Simpson, 1900
- *A. tehuantepecensis* Crosse Finsch, 1893
- *A. richardsoni* von Martens, 1900
- Synonymy: (MUSSEL p., 2013)
- *Anodontites luteolus* (Lea, 1858); Pilsbry, (1920).
- *Anodonta (Pachyanodon) luteola* Lea, 1858. – von Martens (1890-1901).
- *Glabaris (s.s.) luteolus* (Lea, 1858); Simpson, (1900).
- *Anodon luteolus* (Sowerby, (1870); Simpson, (1900)
- *Margaron (Anodonta) luteola* (Lea, 1870); Simpson, (1900)

Anodonta luteola has elliptical shell, thin, inflated, and often slightly winged posteriorly and rounded leading edge. The posterior edge of the shell was slightly winged, corrugated, and the umbo is smooth, with concentric ridges. Smooth, shiny and greenish-yellow surface with fine lines of growth were characteristics in the juveniles shells. In adult specimens the shell color turns dark green with strong growth lines. Periostracum of the umbo region spent in adults. Hinge toothless, reduced a single line regularly curved. Adductor muscles hardened, shiny nacre and mildly iridescent (Simpson, 1900 modified) (Fig. 1).

Details from the morphology the shell of the glochidia of *A. luteola* can be seen in Fig. 2.

The shape of the glochidia *Anodonta luteola* is subtriangular with hook and spinules (Fig. 3).

Table 1 shows the measurements taken to glochidia. The mean dimensions of the glochidia as follows: total length: 311.8µm, height: 331.7µm. The lowest coefficient of variation (3.42%) corresponded to the total length.

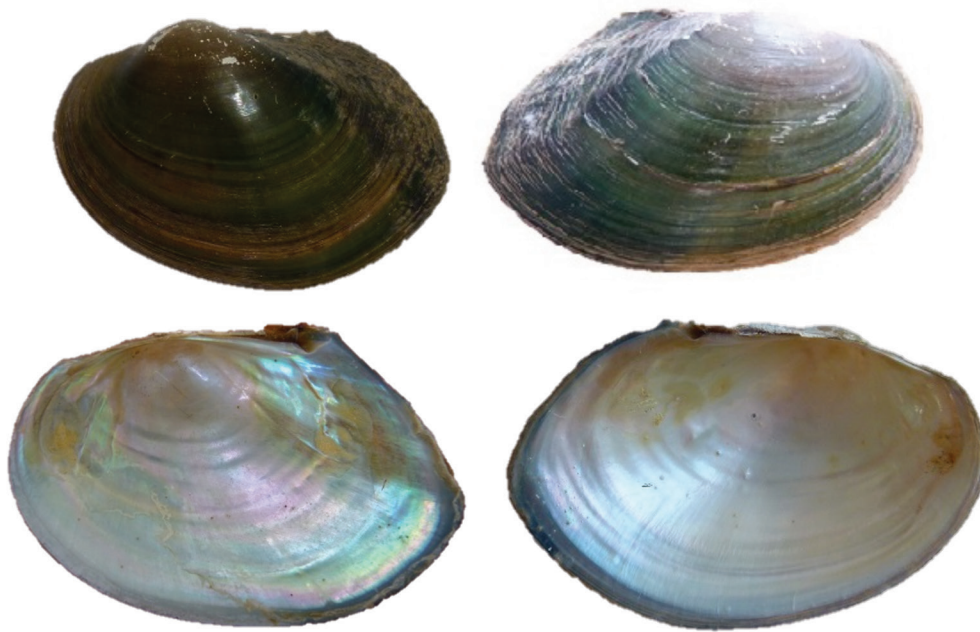


Fig. 1. Dorsal and ventral view of *Anodonta luteola*.

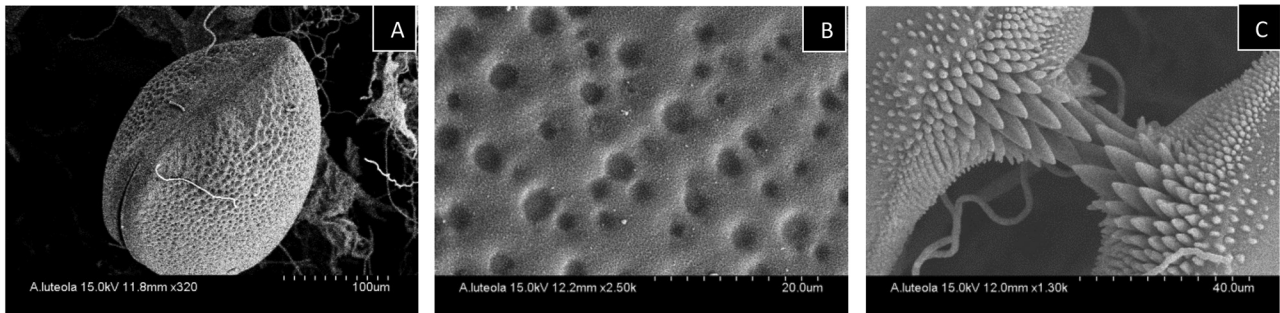


Fig. 2. Details of the morphology the shell of the glochidia at SEM of *Anodonta luteola*. A) Dorsal view B) Outer surface of valve showing the porosity of the shell C) Detail of the spinules.

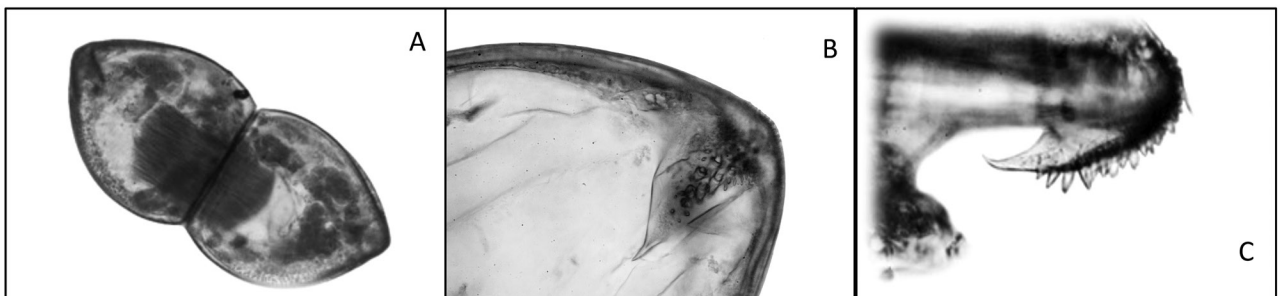


Fig. 3. Details of the shell of the glochidia of *Anodonta luteola* at light microscopy A) View of the subtriangular shape and the adductor muscle of the glochidia (10X). B) Frontal view of the hook (40X). C) Lateral view of the hook with spinules (40X).

TABLE 1
Statistical data on glochidia of *Anodonta luteola*

	Range (µm)	Mean (µm)	CV %
Total Length	262,5-337,5	311,8 ± 10,67	3,42
Height	287,5-375,0	331,7 ± 14,25	4,30
Hinge Dorsal Length	200,0-312,0	251,1 ± 12,94	5,15

DISCUSSION

According to the results of this study there is no doubt that in the works published by Cruz & Villalobos (1984), Cruz & Villalobos (1985), Villalobos & Cruz (1984) & Villalobos, Cruz & Báez (1984) under the name of *Glabaris luteolus* (Mycetopodidae), and those of Ruiz (1982), Ruiz & Bonilla (1982), Sandin, (1995), Castillo-Martínez (2000), Arias-Andrés, Mena & Pinnoch (2014) published under the name *Anodontites luteola* (Mycetopodidae) the species were misidentified. In all cases, the species listed above refers to *Anodonta luteola* (Unionidae). Species from Unionidae family produce glochidia on its larval stage unlike species of the family Mycetopodidae that produce lasidium larvae (Parodiz & Bonetto, 1963).

The glochidia of *A. luteola* showed the following measures, TL = 311,8µm, H = 331,7µm. These measures are bigger than the measurements shown by several species of the genus *Unio*, like *U. marcus* (TL = 216,8µm, H = 193,7µm), *U. delphinus* (TL = 229,0µm, H = 221,0µm) and *U. tumiformis* (TL = 202,0µm, H = 221,0 µm) (Araujo et al., 2009). Species of the genus *Anodonta* shows bigger measurements than those found in *A. luteola*, such as *A. cygnea* (TL= 350,0µm, H = 350,0µm) and *A. anatina* (TL = 355,0µm, H = 350,0µm) reported by Araujo et al. (1999) and *A. pseudodopsis* (TL = 328,0µm, H = 303,0µm) (except for the height), in Serefişan et al. (2009).

Moreover, in all the above cited species, including *A. luteola*, the glochidia only stay in the external demibranchia. However, this condition is not characteristic of all members of the family Unionidae; for example, the glochidia in *Potamida littoralis* uses the four branquias as marsupia for the incubation (Araujo et al., 2009).

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