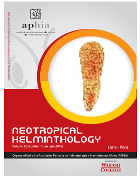
ISSN Versión impresa 2218-6425 ISSN Versión Electrónica 1995-1043

*Neotropical Helminthology, 2018, 12(1), ene-jun:63-70.*

aphia
ASOCIACIÓN PERUANA DE HELMINTOLOGÍA E INVERTEBRADOS AFINES

Neotropical Helminthology




ORIGINAL ARTICLE / ARTÍCULO ORIGINAL

# INTRAMARSUPIAL DEVELOPMENT OF BRAGA PATAGONICA SCHIODT & MEINERT, 1884 (ISOPODA: CYMOTHOIDAE)

# DESARROLLO INTRAMARSUPIAL DE BRAGA PATAGONICA SCHIODT & MEINERT, 1884 (ISOPODA: CYMOTHOIDAE)

Amanda Karen Silva de Souza*; Marília Rodriguês da Vitória; Daniel Brito Porto & José Celso de Oliveira Malta

atjsouza15@gmail.com*, mariliavmarilia@gmail.com, danielbporto81@gmail.com, jcmalta10@gmail.com
National Research Institute of the Amazon, Av. André Araújo, 2936 - Petrópolis, Manaus - AM, 69067-375, Brasil

## ABSTRACT

The Intra marsupial developmental stages of *Braga patagonica* Schiodt & Meinert, 1884 collected from fish in an Amazonian floodplain lake, were ascertained. Gravid females were screened according to their gestational stage. Their eggs, embryos and larvae were measured and images were drawn. Six intra marsupial stages have been identified, namely, eggs mass, egg, embryo differentiation I, embryo differentiation II, Pullus I larva, Pullus II larva plus one Manca III post-marsupial infective swimmer-free.

**Keywords**: Amazon – Fish Parasites – Peracarida – Phlabellifera

## RESUMEN

Las etapas de desarrollo intramarsupiales de *Braga patagonica* Schiodt & Meinert, 1884 colectadas de peces en un lago de planicie amazónica, fueron indagadas. Las hembras grávidas fueron seleccionadas de acuerdo a su etapa de gestación. Sus huevos, embriones y larvas fueron medidos y dibujados. Seis estadios intramarsupiales fueron identificados, a saber, la masa de huevo, el huevo, la diferenciación de embriones I, diferenciación de embriones II, larva Pullus I, larva Pullus II más uno Manca III post- marsupial infecciosa nadadora libre.

**Palabras clave**: Amazonas – parásitos de peces – Peracarida – Phlabellifera

## INTRODUCTION

Isopods are the most diverse and species-rich crustaceans of the superorder Peracarida (isopods, amphipods, tanaids, and their kin). Isopods are common inhabitants of nearly all environments, yet they are unusual among Crustacea due to their ubiquity. Isopoda include approximately 10,000 described species, in 10 suborders. These animals range in length from 0.5 mm to 500 mm. Phylogenetic analyses and the fossil record (limited though it is) suggest the group to date back to at least the Paleozoic Carboniferous Period of approximately 300 million years ago (Brusca *et al*., 2001).

At the development of the cymothoid the eggs show to be centrolecithal, oval and enclosed in a chorion and vitelline membrane, the latter not being clearly visible until the first few divisions have taken place. The number of eggs carried in the marsupium varies directly with body length within a given species, ranging from about 200 to 1600. In mouth/gill inhabiting species, the hatching mancas always seem to be released via the host's gill chamber and opercula opening (Holdich, 1968; Brusca, 1981).

Manca and pullus larva stages typically bear large eyes, spinose pereopods, and setose pleonal appendages to facilitate swimming. Manca and young males typically attach to almost any convenient fish for short periods. Little is known regarding these temporary attachments, although some workers have referred to juveniles and young males as “facultative parasites” on “intermediate hosts”. It is not known whether the isopod actively feeds on the fish at this time or not. As the juvenile matures, it eventually finds an acceptable “definitive” host fish upon which to attach to permanently. The natatory setae are lost upon attachment to this host and transition into a functional male (Brusca, 1981).

The genus *Braga* Schiodte & Meinert (1881) includes eight species: *B. cichlae* Schiodte & Meinert (1881); *B. nasuta* Schiodte & Meinert (1881); *B. brasiliensis* Schiodte & Meinert (1881); *B, patagonica* Schiodte & Meinert (1884); *B. fluviatilis* Richardson (1911); *B. bachmanni* Stadler (1972); *B. amapaenses* Thatcher (1996) and *B. cigarra* (Szidat & Shubart, 1960, Thatcher, 2002).

This work is the first one addressing a *Braga* species' intra marsupial development and early infectious stage.

## MATERIAL AND METHODS

Ten *B. patagonica* females were collected whilst parasitizing the gills of *Pygocentrus nattereri* (Kner, 1858), *Serrassalmus elongatus* (Kner, 1858), *Brycon amazonicus* (Spix & Agassiz, 1829), *Triportheus albus* Cope, 1872 and *Mylesinus* sp. The hosts were captured with gillnets at Catalão Lake (03$^{0}$10`04” S and 59$^{0}$54`45”W). After being captured each fish was examined in search for Isopoda specimens. The isopods found were collected, labeled and preserved in 70% ethyl alcohol.

Females were measured and dissected, had their eggs and larvae removed from the marsupium and their development stage identified (Brusca, 1984; Castro & Silva, 1985; Monteiro *et al*., 2001; Ramdane *et al.,* 2007).

Permanent preparations were made utilizing Hoyer medium. Drawings were made with the aid of a camera lucida. Measurements are in micrometers (µm) unless designated as millimeters (mm). The voucher material was deposited at the INPA Crustacea Invertebrates Collection (INPA-2193, 2194, 2195, 2196 e 2197).

## RESULTS

Ten females at different gravid stages were analyzed. Six intramarsupial stages were identified: eggs mass, egg, embryo I, embryo II pullus I larva, and pullus II larva, as well as one manca stage. At the intramarsupial development stage, the analyzed females amounted to: one with eggs mass, four with individualized eggs, one with embryos at stage I, one with embryos at stage II, one with pullus I larva stage and one with pullus II larva stage. In the post-marsupial stage one larva was found at the manca stage.

Ten specimens of each stage and adult females were measured in millimeters (Fig. 1). Measurements averaged: Eggs 1.6 ± 0.1 long by 1.3 ± 0.1 wide; embryo I 1.7 ± 0.05 by 1.1 ± 0.03; pullus I 2.7 ± 0.2 by 1.1 ± 0.04; pullus II 2.5 ± 0.2 by 1 ± 0.1; adults 17.4 ± 2 by 9.1 ± 1.5.

Intramarsupial Development

Stage 1 - Eggs mass (Fig. 2): initial stage of embryonic development, grouping of the eggs, oval in shape with no suspender filament. Each egg is individualized in the structure's ensemble.

Stage 2 - Egg (Fig. 3): all eggs are morphologically similar, circular-shaped and orange yellow colored with lots of vitellus. With no segmentation. Total number of eggs 88 (56-317). The egg size from four females showed to be 19 ± 9.5 21 ± long by 11.8 wide.

Stage 3 - Embryo I (Fig. 4): head is differentiated and initiates the differentiation of the eyes. The differentiation of what will become the yolk sac initiates in the dorsal part. The embryos are longer than wide and 317 embryos I were found.

Stage 4 - Embryo II (Fig. 5): there is a very large difference between head and body. Body is differentiated in head, pereon, Pleon. The rudiments of the telson, and gnathopod are visible. Eyes well developed and visible. Yolk sac starts to get circular-shaped, and 120 embryos II were found. Female showed to be 1.9 ± 1.1 2.1 ± long by 1.1 wide.

Stage 5 - Larva pullus I (Fig. 6): head with antennas, antennules and body segmentation quite apparent. Pereon, Pleon, uropods and Telson clearly differentiated. Six pairs of unornamented legs. Visible yolk sac in the pereonite 3 dorsal part. One exuvia externally coating the body, which had undergone a molt when going to pullus II, was observed six individuals were collected in the marsupium.

Stage 6 - pullus II (Fig. 7): larger than the previous stage, no exuvia was observed. The bristles and thorns are evident on the pereopods. It has six pairs of legs. All pullus II free in the marsupium. A total of 196 pullus II were found. This is the last stage of intramarsupial development, and is released into the environment after molt as Manca I.

Post- marsupial development

Manca III (Fig. 8): the young individual is similar to the adult male, with all the morphological characters of the species but on a smaller scale. One can already observe 7 pairs of legs and all the fully formed body segments. Only one individual was found parasitizing *Triportheus albus* Cope, 1872.

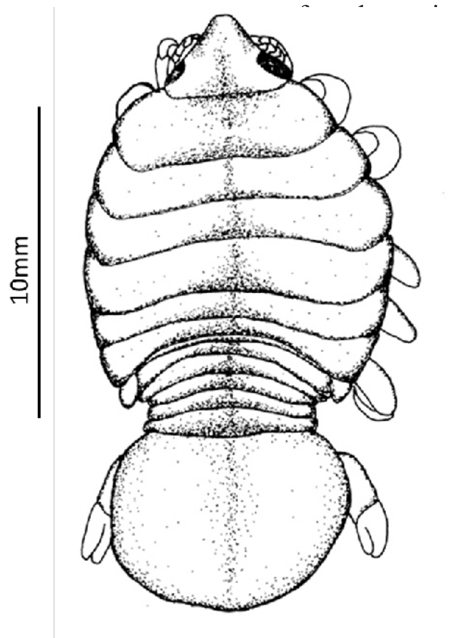
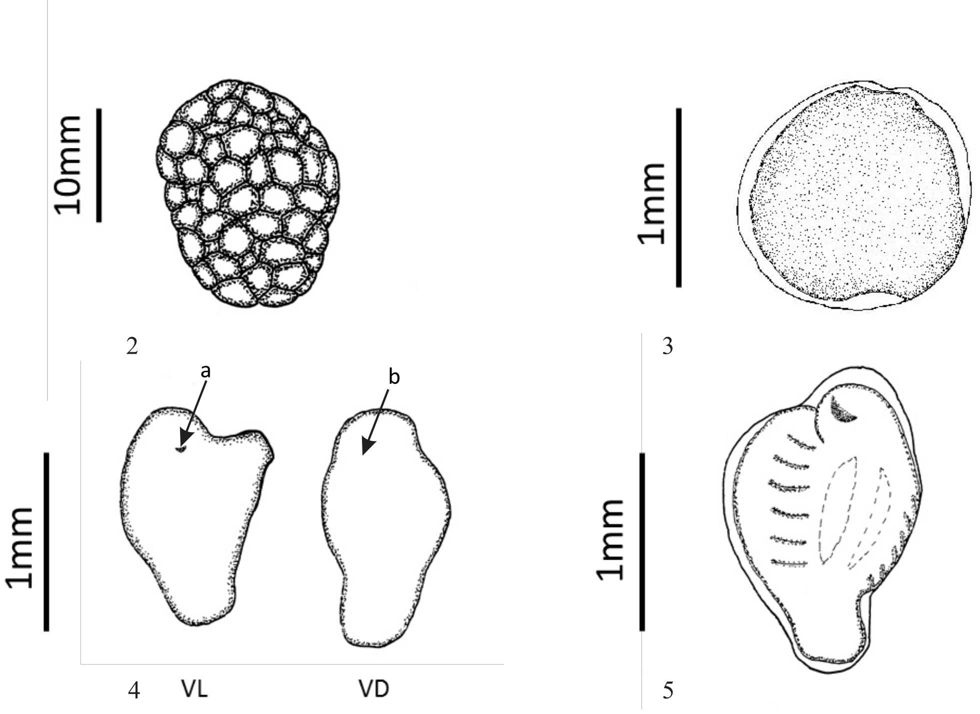



**Figure** 1. *Braga patagonica*: Adult female.




**Figures 2-5.** intramarsupial early stages of development of *B. patagonica*. 1 - Egg Mass 2 - individual egg 3 - Side and back of developing embryo I, a - eyes b- head; 4 - dorsolateral view of the developing embryo II.

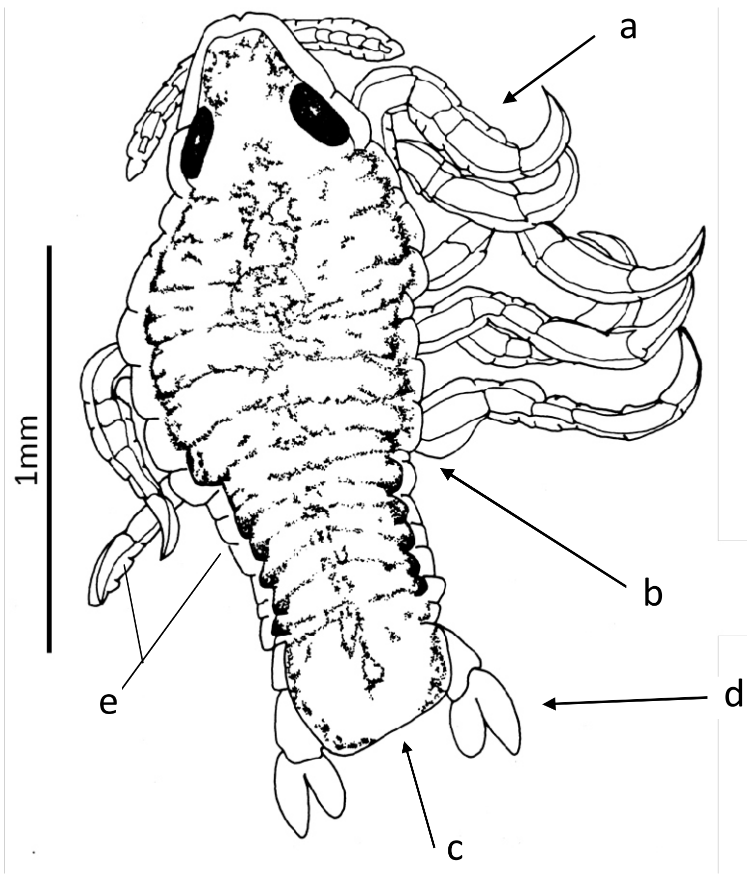



**Figure 6**. *Braga patagonica*: Pullus I larva, a – legs, b - pereon, c - pleon, d – uropods, e - exuvia.

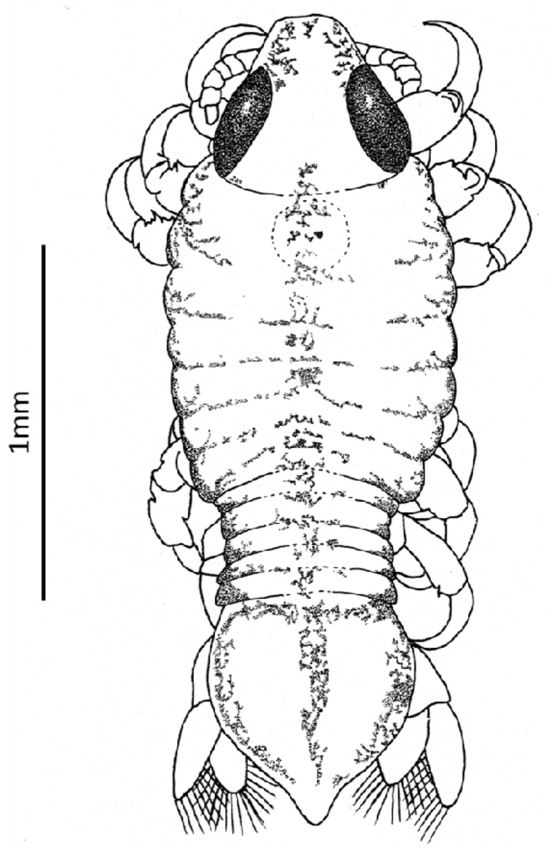



**Figure 7**. *Braga patagonica*: Pullus II larva.

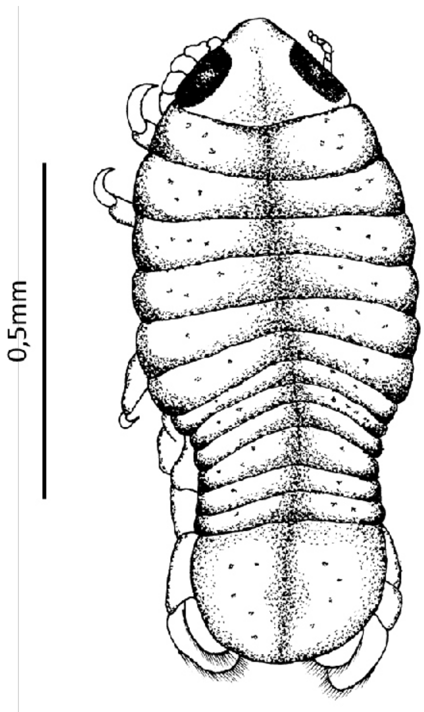



**Figure 8.** *Braga patagonica*: Manca III larva.

## DISCUSSION

The terminology used in the study of embryonic development of isopods molts varies according to the author. However, most of them point out an intra and post-marsupial development period. Inside the marsupium the eggs develop up to the formation of the larvae. They leave maternal oostegits with characteristics similar to those of the male species, but lacking the seventh pair of pereiopods that will only arise when they reach the juvenile period following a succession of molts (Alberto *et al*., 2001).

The post-larval and pubertal period is characterized by a general growth of the young individual, with the gradual emergence of the male sexual characteristics. The pubertal period is reached through a series of molts and it is characterized in females and males by the appearance of oostegits and sexual characteristics, respectively (Alberto *et al*., 2001).

In *B. patagonica* there also occurred an intra and post-marsupial development period. Stage I was considered to be the egg mass not individualized in the marsupium. Following the eggs individualization differentiation of them will initiate the development of subsequent developmental stages.

In intramarsupial development of *Telotha henselii* Von Martens, 1869 the sequence of embryo segmentation stages with differentiation of structures and the gradual reduction of yolk sac (Alberto *et al*., 2001). In this work the methodology adopted was that of Alberto *et al.* (2001). Morphological differences were observed in the development of *B. patagonica* indicating the development to be similar between Isopoda species with little variation from species to species.

In intramarsupial development stages of *Livoneca vulgares* (Stimpson, 1857) the individualized eggs developed themselves and there were five molts up to the infective larva stage (manca) (Brusca, 1978). Four intramarsupial development stages occurred in *Serolis complete* (Moreira, 1971) (Albert *et al*., 2001; Moreira, 1973; Sartor, 1987; Thatcher, 1988). In *Artystone trysibia* (Schiodte, 1866) the egg mass was considered the first development stage (Pugues *et al*., 1998).

The present work observed six intramarsupial stages and one manca stage. The egg mass was considered as the first intramarsupial development stage according to Pugues *et al.* (1998). Some researchers indicate the individualized egg as the first stage (Alberto *et al*., 2001; Moreira, 1973; Sartor & Pires, 1987).

In stage 3 the individualized eggs were more elongated and the first visible differentiation under protective membrane was the head and the visible rudimentary eyes in dorsal and ventral view (Alberto *et al*., 2001; Pugues *et al*., 1998). In this work, stage 3 exhibited the same characteristics and it was possible to observe the ocelos primordium and the developing somatic mass.

Stage 4 of the marsupial development is marked by the development of rudimentary eyes seen both in dorsal and ventral view, the early formation of appendages: antennae, pereiopods and pleopods and the beginning of differentiation of a yolk sac, which begins as a protuberance on the dorsal part (Alberto *et al*., 2001; Moreira, 1973; Pugues *et al*., 1998).

In this work we observed the same characteristics mentioned by Moreira (1972), Pugues *et al.* (1998) and Alberto *et al.* (2001). In the fourth *B. patagonica* development stage the head, pereon and pleon are well defined and there is the beginning of the formation of appendages: antennae, pereiopods and pleopods. The egg membrane remains and the differentiation of the yolk sac, which begins as a protrusion on the dorsal part.

In stage 5 the eyes are already formed and the yolk sac remains evident in *A. trysibia* (Pugue *et al*., 1998). In *T. henselii* stage 5 the thoracic, abdominal segments and six pairs of pereopods show to be differentiated. The pereopods have neither pigmentation nor ornamentation. The yolk sac is located on the third pereonite. At this stage the embryo is pullus I larva (Alberto *et al*., 2001).

The present work shows the *B. patagonica* stage 5 characteristics to be similar to those of the above mentioned species. *Braga patagonica* differs by having evident pigmentation at the ends and in the middle of pereonites and pleonites; they have no ornamentation and there is a cuticle surrounding the larva and the larva is pullus I.

Stage 6 is called pullus II and in *T. hanselii* the adornments, bristles and spines, pigmentation and the rudiments of the yolk sac in the third pereonite are quite evident; the antennas and antennules are thinner (Alberto *et al*., 2001). Pullus II in *A. trysibia* also exhibits all the ornaments and pigmentations and was considered a juvenile. It has all the adult characteristics, except for remaining open within the marsupium (Pugues *et al*., 1998).

In *B. patagonica,* pullus II exhibits the same characteristics of the above mentioned species. The species characteristic pigmentation seems to be diffused by the pereonites, pleonites and telson; the yolk sac rudiments can still be observed. Bristles of uropods are evident. The seventh pair of pereópodos is not yet formed.

In developing *Cymothoa liannae* (Sartor & Pires, 1988) pullus II was the last stage in the marsupium and was released to the environment after maturation (Sartor, 1987). In *B. patagonica* pullus II was also the last stage within the marsupium.

In *C. liannae* the manca stage is the beginning of the post-marsupial stage. Individuals look like adults but are smaller-sized. And usually before becoming sexually reproductive adults they go through six stages (two for juveniles, two for males and two for females (Sartor, 1987). In some Isopoda species there may also occur five stages (molts) up to the adult stage (Brusca, 1981).

This same pattern of six molts was also found for *Serolis completa* (Moreira, 1973) where there occurred maturation of the young and the differentiation between male and female (Moreira, 1973). This young similar to adults feature is characteristic in the development of Isopoda. The manca stage was described in the development of terrestrial Isopoda *Porcellio dilatatus* (Brand, 1833) with three development stages (Brum & Araújo, 2007).

The manca stage was not mentioned on the work addressing the *T. henselli* development but it is correct to assert that further studies will surely describe this stage (Alberto *et al*., 2001). In this work with *B. patagonica* the manca stage was attached to mouth of *T. albus* probably in stage 3 development. *T. albus* may likely exhibit the mancas' latest stages.

## BIBLIOGRAPHIC REFERENCES


Alberto, RMF, Goulart, MS, Pereira, EHL. & Bertoletti, JJ. 2001. *Desenvolvimento intramarsupial de* Telotha henselii *(Von Martens, 1869) (Crustacea: Isopoda: Cymothoidae).* Comunicação do Museu de Ciência e Tecnologia PUCRS série Zoológica*,* vol. 14*,* pp. 73–87.

Brum, PED. & Araújo, PB. 2007. *The manca stages of* Porcellio dilatatus *Brandt (Crustacea, Isopoda, Oniscidea).* Revista Brasileira de Zoologia *,* vol. 24*,* pp. 93–502.

Brusca, RC. 1978. *Studies on the cymothoid fish symbionts of the eastern Pacific (Crustacea: Isopoda: Cymothoidae), 2: Biology and systematics of* Lironeca vulgaris. Allan Hancock College Foundation, Occasional paper vol. 2, pp. 1–19.

Brusca, RC. 1981. *A monograph on the Isopoda Cymothoidae (Crustacea) of the eastern Pacific*. Zoological Journal of the Linnean Society*,* vol. 73*,* pp. 117–199.

Brusca, RC. 1984. *Phylogeny, evolution and biogeography of the marine isopod subfamily Idoteinae (Crustacea: Isopoda: Idoteidae).* Transactions of San Diego Society of Natural History, vol. 20, pp. 99–134

Castro, AL & Silva, JL. 1985. *Manual de identificação de invertebrados límnicos do Brasil, 33, Isopoda.* MCT/CNPq.

Holdich, DM. 1968. *Reproduction, growth and bionomics of* Dynamene bidentata *(Crustacea: Isopoda)*. Journal of Zoology*,* vol. 156*,* pp. 137–153.

Monteiro, NM, Almada, VC, Santos, AM & Vieira, MN. 2001. *The breeding ecology of the pipe¢sh* Nerophis lumbriciformis *and its relation to latitude and water temperature.* Journal of the Marine Biological Association of the United Kingdom, vol. 81, pp. 1031-1033.

Moreira, PS. 1973. *Biologia de* Serolis completa

*(Crustacea, Isopoda, Flabellifera). Estádios de desenvolvimento.* Boletim do Instituto Oceanográfico da Universidade de São Paulo, vol. 22, pp. 93–108.

Pugues, SM, Alberto, RMF, Pereira, EHL, Bertoletti, JJ. 1998. *Diferenciação morfológicas no desenvolvimento embrionário de* Artystone trysibia *Schioedte, 1866 (Crustacea, Isopoda, Cymothoidae).* Revista Brasileira de Biologia, vol. 58, pp. 47–53.

Ramdane, Z, Bensouilah, MA, Trilles, JP. 2007. *The Cymothoidae (Crustacea: Isopoda), parasites on marine fishes, from Algerian fauna.* Belgian Journal of Zoology, vol. 137, pp. 67–74.

Sartor, SM. 1987. *Desenvolvimento marsupial e ciclo de vida de* Cymothoa liannae. Boletim Instituto de Oceanografia, vol. 35, pp. 43–51.

Sartor & Pires 1987. *(Isopoda, Cymothoidae), parasitas de peixes.* Boletim do Instituto Oceanográfico da Universidade de São Paulo, vol. 35, pp. 43–51.

Szidat, L & Schubart, O. 1960. *Neue und seltene Parasitische Süsswasser Anselm der Familie Cymothoidae aus dem Rio Mogi-Guassú, Brasilien (Isopoda).* Anais da Academia Brasileira de Ciência, vol. 32, pp. 107-124.

Thatcher, VE. 1988. Asotana magnífica *n. sp. (Isopoda, Cymothoidae) a ununsual parasite (Commensal?) of the buccal cavities of piranhas (*Serrasalmus *sp.) from Roraima, Brazil. Amazoniana,* vol. 10, pp. 239–248.

Thatcher, VE. 2002. *The isopods of South American fishes.* Boletim do Museu Paraense Emílio Goeldi, vol.18, pp. 77-199.

Received February 23, 2018.
Accepted April 3, 2018.