

RESEARCH NOTE/NOTA CIENTÍFICA**A COMPLETE DESCRIPTION OF THE FEMALE REPRODUCTIVE SYSTEM OF
TEMNOCEPHALA CYANOGLANDULA AMATO, AMATO & DAUDT
(PLATYHELMINTHES,
TEMNOCEPHALIDA)****DESCRIÇÃO COMPLEMENTAR DO SISTEMA REPRODUTOR FEMININO DE
TEMNOCEPHALA CYANOGLANDULA AMATO, AMATO & DAUDT
(PLATYHELMINTHES, TEMNOCEPHALIDA)**Samantha A. Seixas¹, José F. R. Amato² & Suzana B. Amato³

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Neotropical Helminthology, 2015, 9(2), jul-dec: 371-376.

ABSTRACT

Temnocephala cyanoglandula Amato, Amato & Daudt, 2003 was described as epibiont of *Aegla serrana* Buckup & Rossi using the usual techniques at the time of publication, but did not include the description of the female reproductive system. A complete description of the female reproductive system was accomplished based on specimens stained with acetic carmine/fast-green for better observation of the female organs and with the aid of Nomarski's differential interference contrast (DIC). The taxonomic validation of *T. cyanoglandula*, was questioned because of the similarity between the cirrus of this species and that of *Temnocephala axenos* Monticelli, 1899 and *Temnocephala bresslaui* Pérez-González, 1949 (synonymized with *T. axenos*), has subsequently been confirmed. The cirrus in *T. cyanoglandula* is much longer than that of *T. axenos*, and, although there is similarity of the cirrus with that of *T. bresslaui*, the later has much longer female organs and is probably a valid species. The female reproductive organs of *T. cyanoglandula* and *Temnocephala mertonii* Volonteri, 2007 are similar, although both species have cirri of different size and shape.

Keywords: Aeglidae - ectosymbiont - Neotropical Region - South America - taxonomy - *Temnocephala cyanoglandula*.

RESUMO

Temnocephala cyanoglandula Amato, Amato & Daudt, 2003 foi descrita como epibionte de *Aegla serrana* Buckup & Rossi, 1977 utilizando as técnicas propostas até o momento da publicação, por essa razão não continha a descrição do sistema reprodutor feminino. A descrição complementar do sistema reprodutor feminino foi feita baseada em espécimes corados com carmin acético/ fast-green e da visualização dos órgãos femininos em contraste diferencial de interferência com prismas de Nomarski (DIC). A validade taxonômica de *T. cyanoglandula*, questionada pelas semelhanças entre os cirros desta espécie com *Temnocephala axenos* Monticelli, 1899 e *Temnocephala bresslaui* Pérez-González, 1949 (sinonimizada como *T. axenos*), foi subseqüentemente confirmada. O cirro de *T. cyanoglandula* é muito maior em comprimento do que *T. axenos* e, apesar da semelhança do cirro com *T. bresslaui*, a última apresenta as estruturas femininas muito longas e é, provavelmente, uma espécie válida. Os órgãos reprodutores femininos de *T. cyanoglandula* e *Temnocephala mertoni* Volonterio, 2007 são semelhantes, no entanto possuem cirros de tamanho e formato diferentes.

Palavras-chave: Aeglidae - América do Sul - ectosimbionte - Região Neotropical - taxonomia.

INTRODUCTION

Temnocephala cyanoglandula Amato, Amato & Daudt, 2003 has been described as epibiont of *Aegla serrana* Buckup & Rossi, 1977 following the techniques proposed by Cannon & Sewell (1995), Sewell & Cannon (1998), and Amato *et al.* (2003). The female reproductive organs found in the temnocephalan species epibiont on crustaceans are very difficult to observe. Volonterio (2007), comparing *Temnocephala mertoni* Volonterio, 2007, *Temnocephala talicei* Dioni, 1967, and *Temnocephala axenos* Monticelli, 1899 called attention to the fact that due to the difficulty in differentiating species which share the same host group or even the same host species, organs such as the vaginal sphincter have the same, or even greater importance than the description of the cirrus. Amato *et al.* (2010) when recording the presence of *Temnocephala pignalberiae* Dioni, 1967 in Brazil, proposed to use the acetic carmine/fast-green stain combination and the observation of the female reproductive system with Nomarski's differential

interference contrast (DIC) in all descriptions, mainly in the species epibiont on crustacean hosts. The proposed protocol was followed in the description of *Temnocephala longivaginata* Seixas, Amato & Amato, 2011, species which, although having a large body size, had a very small, difficult to observe female reproductive system (Seixas *et al.*, 2011). The description of *T. cyanoglandula*, correct in relation to the male organs (principally the cirrus) and the dorsolateral 'excretory' syncytial plates, did not include the description of the female reproductive system. For this reason these structures have now been stained and observed by the techniques proposed by Amato *et al.* (2010) and are described in the present note.

MATERIAL AND METHODS

The specimens described in the original manuscript (Amato *et al.* 2003), were collected at Utopia II Farm, 5 km East of State Road RS-020, locality of Tainhas, Municipality of Cambará do Sul, State of Rio Grande do Sul

(29°15'10"S, 50°15'45"W), southern Brazil, were used in the present study. These specimens were fixed in cold AFA (70% ethanol; formalin 37%; glacial acetic acid), and stained in aceto-carmine/fast-green. The female organs were extracted and mounted in Faure's mounting medium (F) (Seixas *et al.*, 2010). Photomicrographs were taken with Zeiss Axiolab and Leica DMR Hc microscopes; the Leica microscope was equipped with DIC. The photographic images were prepared using Adobe's Photoshop CC. Measurements are in micrometers (μm) and were taken from specimens killed under slight cover slip pressure and mounted in Canada balsam; ranges are followed (between parentheses) by the mean, the standard deviation values, and the number of specimens measured for a given character (when different than 10). The whole mounts of adult, as well as slides containing individual female organs mounted in F were deposited in the following scientific collections: 1. 'Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC)', Rio de Janeiro, RJ, Brazil; and 2. 'Coleção Helmintológica do Laboratório de Helminthologia, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul', Porto Alegre, RS.

RESULTS

Description. Based on 31 specimens; 10 measured. Female reproductive system. Ovary almost round (Figs 1, 2, and 4) 112.5-202.5 (144, 29) long, 100-130 (115, 10) wide; vesicula intermedia (Figs 1, 3, and 5) 20-62.5 (41, $n = 9$, 13) long; vagina (Figs 3 and 5) 35-87.5 (55, $n = 9$, 16) long, 37.5-65 (50, $n = 8$, 10) maximum width; single vaginal sphincter large and asymmetrical (Figs 3 and 5) 50-100 (78, 17) in total diameter, diameter of anterior portion 12.5-27.5 (21, 6), diameter of posterior portion 32.5-75 (47, 13); vesicula resorbens (Figs 1 and 5) 95-290 (181, $n = 6$, 64) long,

120-310 (222, $n = 6$, 64) wide.

Taxonomic summary.

Type host: *Aegla serrana* Buckup & Rossi, 1977 (Crustacea, Anomura, Aeglidae).

Type locality: Fazenda Utopia II, 5 km RS-020, Tainhas, Cambará do Sul, Rio Grande do Sul, Brazil.

Site of infestation: Branchial chambers and body surface; eggs cemented over external surfaces of exoskeleton, in different regions of the ventral side: perioral area, pleural strips, sternal plates, pereopods, and chelipods; to a lesser extent on the dorsal side of cephalothorax and dorsal side of uropods.

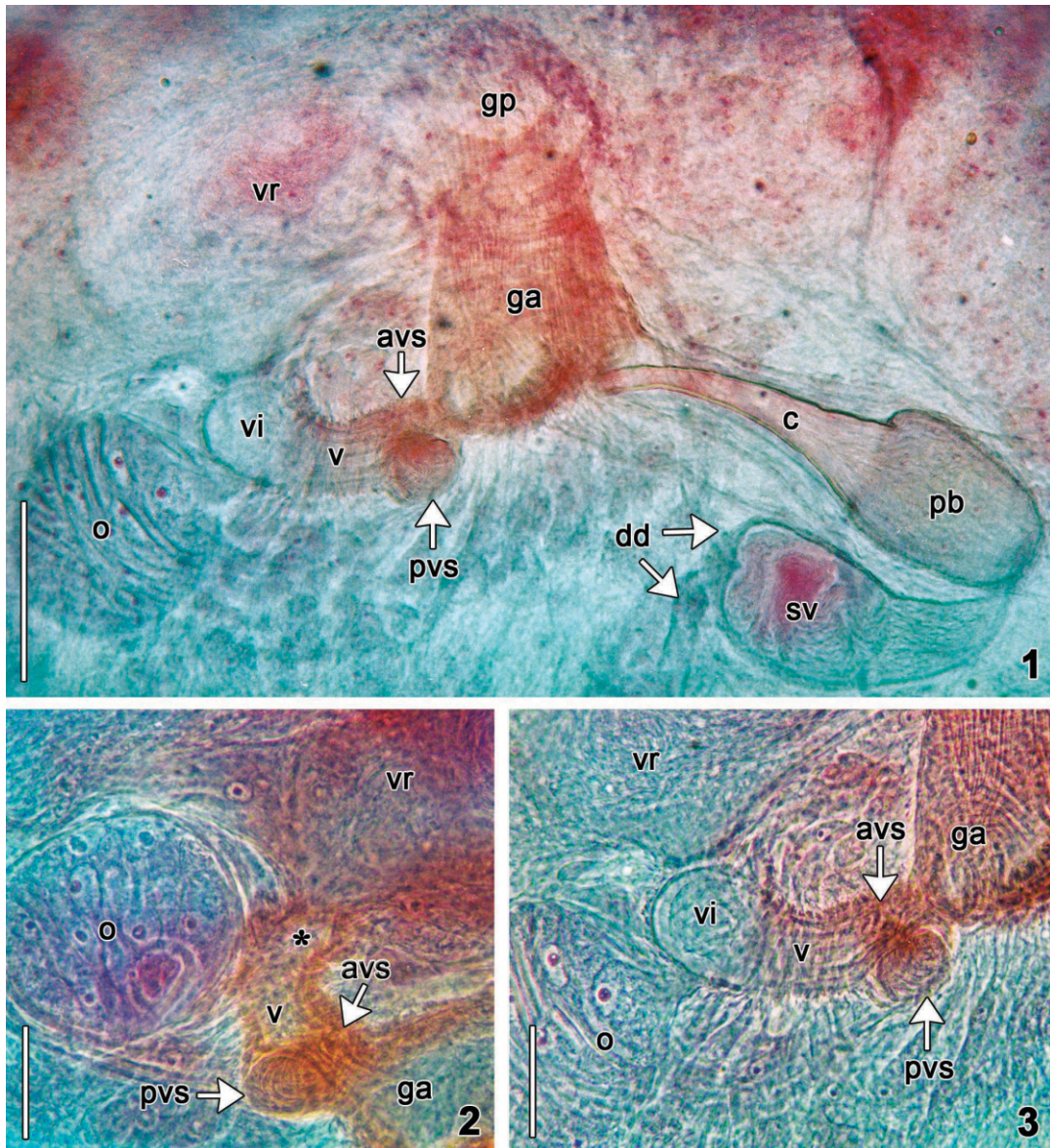
Helminth specimens deposited: 'Coleção Helmintológica do Instituto Oswaldo Cruz': CHIOC 38214 a - d.

DISCUSSION

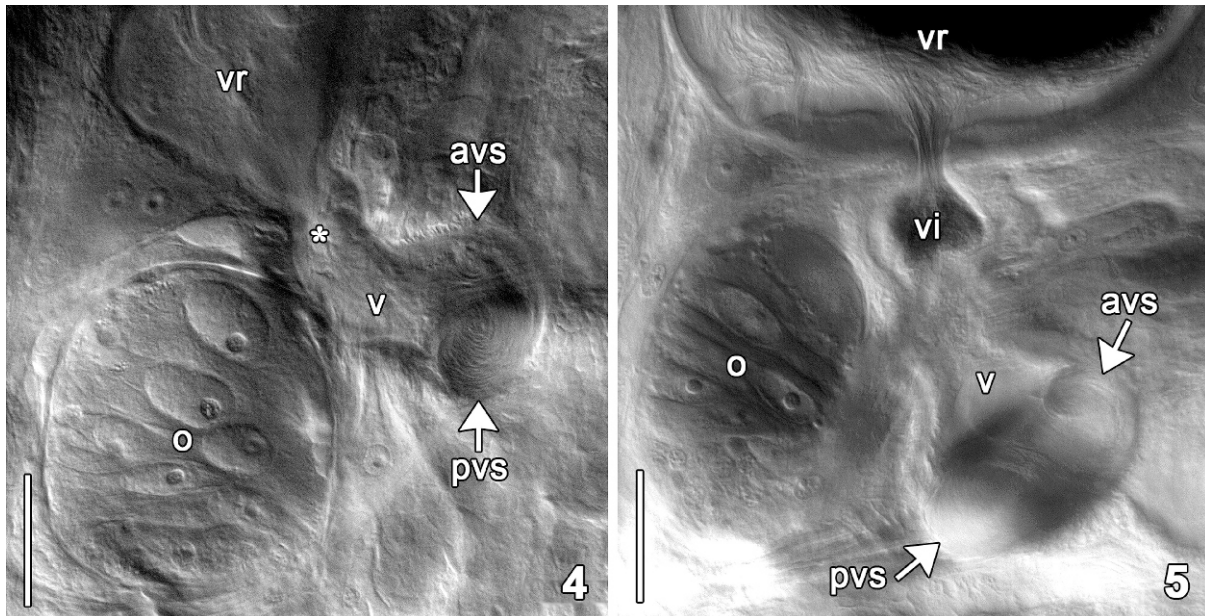
Volonterio (2007) created doubts about the taxonomic validity of *T. cyanoglandula*, when she indicated similarity among the cirri of *T. cyanoglandula*, *T. axenos*, and *Temnocephala bresslaui* Pérez-González, 1949 (synonymized as *T. axenos* by Baer (1931)) (256 μm total length in average - *T. cyanoglandula*, 141 μm total length in average - *T. axenos*, and 280 μm total length in average - *T. bresslaui*). The measurements of the cirrus, prostatic bulb, and body size are similar between *T. cyanoglandula* and *T. bresslaui*, although, Pérez-González (1949) drew and described the female reproductive system with peculiar measurements, which according to Volonterio (2007), did not agree with the measurements of any species already described. *Temnocephala bresslaui* has vesicula intermedia, called 'ootype' by the author, with an average of 243 μm in length, and the vagina has a thick muscular wall which measures an average of 250 μm in length and 150 μm in width, measurements that exclude the possibility of synonymy with *T. axenos*,

whose cirrus is much smaller in length than that of *T. cyanoglandula*. Pérez-González (1949) also has observed seminal receptacles in live specimens and considered these structures as transitory. In *T. cyanoglandula* the vesicula intermedia can be observed in all

specimens. *Temnocephala bresslaui* is probably, a valid species and the re-description of the type specimens is necessary. When the morphometric data of the female organs of *T. cyanoglandula* are compared with those of *T. mertoni*, it is possible to see similarities



Figures 1-3. *Temnocephala cyanoglandula*. (1) Organs of the reproductive system. Anterior portion of the vaginal sphincter (avs), genital atrium (ga), prostatic bulb (pb), cirrus (c), ovary (o), genital pore (gp), posterior portion of the vaginal sphincter (pvs), vesicula intermedia (vi), vagina (v), seminal vesicle (sv), vasa deferentia (vd), and vesicula resorbens (vr). Scale bar = 100 μ m. (2-3) Organs of the female reproductive system. Scale bars = 50 μ m. (2) Anterior portion of the vaginal sphincter (avs), genital atrium (ga), ovary (o), posterior portion of the vaginal sphincter (pvs), vesicula intermedia (*), vagina (v), and vesicula resorbens (vr). (3) Anterior portion of the vaginal sphincter (avs), genital atrium (ga), ovary (o), posterior portion of the vaginal sphincter (pvs), vesicula intermedia (vi), vagina (v), and vesicula resorbens (vr).



Figures 4 and 5. Organs of the female reproductive system of *Temnocephala cyanoglandula* photomicrographed with differential interference contrast (DIC) with Nomarski's prisms. Scale bars = 50 μ m. **(4)** Anterior portion of the vaginal sphincter (avs), ovary (o), posterior portion of the vaginal sphincter (pvs), vesicula intermedia (*), vagina (v), and vesicula resorbens (vr). **(5)** Anterior portion of the vaginal sphincter (avs), ovary (o), posterior portion of the vaginal sphincter (pvs), vesicula intermedia (vi), vagina (v), and vesicula resorbens (vr).

between them. Both species have only one asymmetric vaginal sphincter which is smaller in total diameter in *T. mertoni*, measuring an average of 49 μ m (78 μ m in *T. cyanoglandula*). According to Volonterio (2007), the majority of the specimens of *T. mertoni* have vesicula intermedia, as the author also described for *T. axenos*, the structure is transitory and only 10% have seminal receptacles. The vesicula intermedia has been observed in all specimens of *T. cyanoglandula*, as a relatively large structure (Figs 3 and 5). The cirrus of *T. mertoni* is smaller in length (138 μ m in average) and has a different shape, straight with a slight curve (described as 'sinuous') in the distal portion of the shaft, while in *T. cyanoglandula* the entire cirrus is slightly curved (Fig. 1).

ACKNOWLEDGMENTS

Special thanks are due to Jorge Ernesto de Araújo Mariath and Rinaldo Pires dos Santos, Laboratório de Anatomia Vegetal, Instituto de Biociências, UFRGS, for the permission to use the Leica DMR HC microscope to make the DIC photomicrographs; to CAPES (Coordenação de Aperfeiçoamento do Pessoal de Nível Superior) for the Doctoral Scholarship awarded to SAS (2008-2013); to Luiz Carlos Campos Daudt for the collection and exam of the hosts; to Débora N. Souza and Lucas Casagrande for their help in the laboratory; and, especially, to Philip J. Scholl, for kindly reviewing the English in the several versions of the manuscript.

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Received November 1, 2015.
Accepted December 7, 2015.