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

ORIGINAL ARTICLE /ARTÍCULO ORIGINAL

A NEW SPECIES OF *TEMNOCEPHALA* BLANCHARD (PLATYHELMINTHES, TEMNOCEPHALIDA) ECTOSYMBIONT OF *KEMPNYIA RETICULATA* (KLAPÁLEK) (INSECTA, PLECOPTERA) IMMATURES FROM BRAZIL

UMA NOVA ESPÉCIE DE *TEMNOCEPHALA* (PLATYHELMINTHES, TEMNOCEPHALIDA) ECTOSIMBIONTE SOBRE IMATUROS DE *KEMPNYIA RETICULATA* (KLAPÁLEK) (INSECTA, PLECOPTERA) DO BRASIL

UNA NUEVA ESPECIE DE *TEMNOCEPHALA* (PLATYHELMINTHES, TEMNOCEPHALIDA) ECTOSIMBIONTE SOBRE INMATUDOS DE *KEMPNYIA RETICULATA* (KLAPÁLEK) (INSECTA, PLECOPTERA) DE BRASIL

Samantha A. Seixas[1]; José F. R. Amato[2] & Suzana B. Amato[3]

[1]Setor de Evolução e Ecologia, Programa de Pós-Graduação em Ambiente e Desenvolvimento, Centro Universitário UNIVATES, Rua Avelino Tallini 171, Prédio 8, salas:102/104, Bairro Universitário, CEP 95900-000, Lajeado, RS, Brasil. *PNPD/CAPES* Post-Doctoral Fellow. E-mail: seixas.sa@gmail.com
[2]Rua Cabral, 875/301, Bairro Rio Branco, CEP 90420-121, Porto Alegre, RS, Brasil. E-mail: josefelipeamato@gmail.com
[3]Laboratório de Helmintologia, Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Rio Grande do Sul, Av. Bento Gonçalves 9500, Prédio 43435, sala 208, Bairro Agronomia, CEP 91501-970, Porto Alegre, RS, Brasil. E-mail: sbamato@ufrgs.br

Neotropical Helminthology, 2015, 9(2), jul-dec: 277-284.

ABSTRACT

Temnocephalans were found on immature *Kempnyia reticulata* (Klapálek) collected in a first order section of Córrego Bonito (19°58'28,4"S, 40°31'54,4"W), in the Estação Biológica de Santa Lúcia (EBSL), Santa Teresa, State of Espírito Santo, Brazil. Of 29 immature specimens examined, 5 (17.24%) were positive for specimens of *Temnocephala* Blanchard, 1849. Juveniles and adult parasites were found on the head, mesonotum and, median legs of the hosts. Eggs were found in larger numbers in the intrathoracic gills. The most distinctive characters of the *Temnocephala stoneflyi n. sp.* were in the cirrus and the dorsolateral 'excretory' syncytial epidermal plates (EPs). The comparison of the general anatomy and, in particular, the morphology of the cirrus and EPs with those of *Temnocephala curvicirri* Amato & Amato, 2005 described from aquatic heteropterans and *Temnocephala caddisflyi* Amato, Amato & Seixas, 2011 described from trichopterans, showed that although these characters are similar, they are not equal, differing primarily in size and morphology of the cirrus, that has an intermediary size between the three species and smaller EPs, which also have a different format. The egg deposition sites are different and this is the third species with the reproductive system characterized as being 'complex'.

Keywords: aquatic insects - Neotropic Region - plecopterans - taxonomy - Temnocephalidae.

RESUMO

Temnocefalídeos foram encontrados sobre imaturos de *Kempnyia reticulata* (Klapálek, 1916) coletados no Córrego Bonito, em uma seção de primeira ordem (19°58'28,4"S, 40°31'54,4"W), na Estação Biológica de Santa Lúcia (EBSL), Santa Teresa, Espírito Santo, Brasil. Vinte e nove imaturos foram examinados, cinco (17.24%) estavam positivos para espécimes de *Temnocephala* Blanchard, 1849. Juvenis e adultos foram encontrados sobre a cabeça, o mesonoto e as pernas médias de seus hospedeiros. Ovos foram encontrados em grande número sobre as brânquias intratorácicas. Os caracteres mais distintivos de *Temnocephala stoneflyi n. sp* foram o cirro e o par de sincícios pós-tentaculares, ou placas excretoras (PEs). A comparação da anatomia geral e, em particular, da morfologia do cirro e das PEs com *Temnocephala curvicirri* Amato & Amato, 2005 epibionte sobre heterópteros aquáticos e *Temnocephala caddisflyi* Amato, Amato & Seixas, 2011 epibionte sobre tricópteros, mostrou que, embora sejam de mesmo tipo e natureza, estes caracteres não são iguais, diferindo principalmente em tamanho e morfologia do cirro, que possui um tamanho intermediário entre as três espécies, e PEs menores e de formato diferente. Os locais de postura dos ovos diferem e esta é a terceira espécie com o sistema reprodutor caracterizado como 'complexo'.

Palavras-chave: insetos aquáticos - plecópteros - Região Neotropical - taxonomia - Temnocephalidae.

INTRODUCTION

Temnocephala decarloi Moretto, 1978 was the first species described as epibiont on insects. It was found on *Belostoma cummingsi* De Carlo, 1935 collected in Otamendi, Delta del Paraná, Província de Buenos Aires, Argentina (Moretto, 1978). The species was recorded again in Argentina by Damborenea & Cannon (2001). Vianna & De Melo (2002) recorded temnocephalans on belostomatids and naucorids in the State of Minas Gerais, Brazil. Three species have been described as epibionts on insects in Brazil. Amato & Amato (2005) described *Temnocephala curvicirri* Amato & Amato, 2005 on two species of *Belostoma* Latreille, 1807; and Amato *et al. (*2007) described *Temnocephala minutocirrus* Amato, Seixas & Amato, 2007 on *Cryphocricos granulosus* De Carlo, 1967, a naucorid hemipteran. Both records were made in the State of Rio Grande do Sul, Brazil. *Temnocephala caddisflyi* Amato, Amato & Seixas, 2011 was described as epibiont on 'caddisfly' larvae (Trichoptera) in the State of Minas Gerais, Brazil. Amato *et al.* (2011) made an extensive study of the peculiar reproductive system of the species *T. curvicirri* and *T. caddisflyi*, which, most probably, is shared by *T. decarloi*.

Species of *Kempnyia* Klapálek, 1914 are endemic to the southern half and central regions of Brazil. The immatures are usually found in the litter retained by currents, although they can also be found in puddles with low current and accumulated litter, which are formed during periods of drought (Bispo & Froehlich, 2004). Avelino-Capistrano *et al.* (2013) recorded the presence of temnocephalans epibiont on immatures of *Kempnyia reticulata* (Klapálek, 1916) collected in the State of Espírito Santo, without describing the species.

The present study describes a new species of *Temnocephala* epibiont on *K. reticulata* found in the 'Reserva Biológica de Santa Lúcia', Santa Teresa, State of Espírito Santo, Brazil.

MATERIAL AND METHODS

Twenty-nine immature *K. reticulata* were collected from July 2008 to February 2009 in a first order section of Córrego Bonito (19°58'28,4"S, 40°31'54,4"W), in the 'Estação Biológica de Santa Lúcia (EBSL)', Santa Teresa, State of Espírito Santo, Brazil. The insects were collected by Avelino-Capistrano, examined for temnocephalans and deposited in the Coleção do Departamento de Entomologia, Museu Nacional, Universidade Federal do Rio de Janeiro (MNRJ). Hosts with temnocephalans were sent to the Laboratório de Helmintologia, Universidade Federal do Rio Grande do Sul. The temnocephalans from plecopterans were studied through a series of techniques fully described by Amato *et al.* (2007) and Seixas *et al.* (2010), focusing especially on the: 1. morphology of the cirrus structure; 2. shape of individual eggs as well as the areas of egg deposition on the host; and, 3. paired, dorsolateral, post-tentacular 'excretory' syncytial plates (EPs). Photomicrographs were taken with a Zeiss Axiolab microscope, a Leica DMR Hc Microscope, and Nomarski interference contrast prisms. The photographic images and line drawings were scanned and prepared using Adobe's *Photoshop*® CC and CorelDraw X5®, respectively. The image of the cirrus with total focus was obtained with Helicon Focus® 5.3.7.2 software. Measurements are in micrometers (μm) unless otherwise indicated 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 2). Measurements of cirrus were taken from extracted cirri mounted in Faure´s mounting medium (F), and follow Amato *et al.* (2011). Measurements of the introvert and shaft curvature angles were made with Zeiss Axiovision® 4.8 software. Specimens, as well as slides containing individual cirri mounted in Faure´s mounting medium, 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 Helmintologia', Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS. The remainder host specimens were deposited in the 'Coleção Helmintológica do Laboratório de Helmintologia', UFRGS, Porto Alegre, RS.

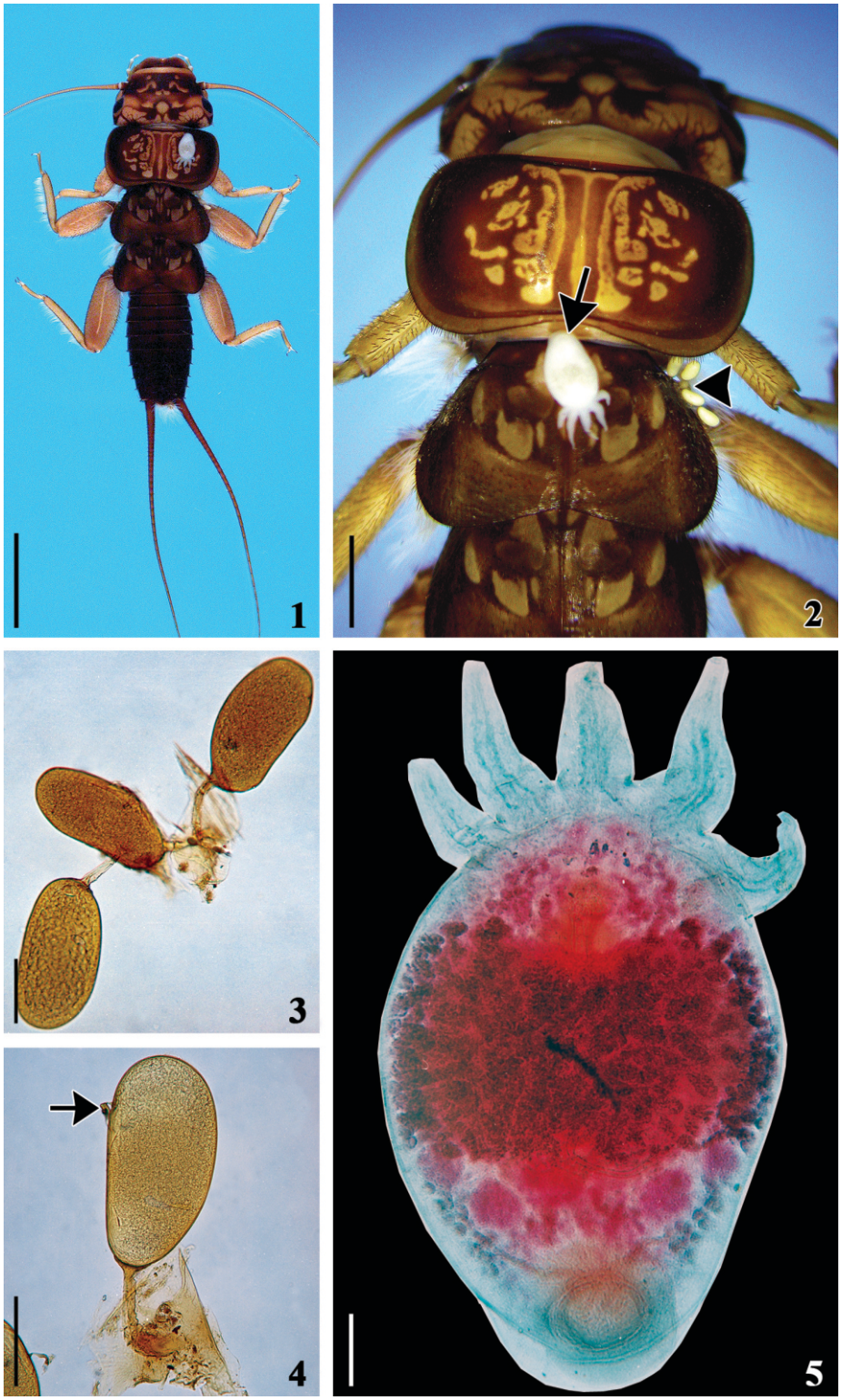
RESULTS

Temnocephala stoneflyi n. sp.
(Figs 3 - 11)
Description. Based on 4 specimens collected: 2 whole mounted adults; 1 specimen mounted on a stub for SEM; 2 dissected cirri mounted in (F).

External characteristics. Red pigmentation of the eyes absent. Two small rounded EPs, slightly longer than wide (Fig. 6), right plate 166.34 (n = 1) long, 122 (n = 1) wide; Excretory pore in the anterior portion of the plate (Fig. 6 - ep).

Female reproductive system not observed.

Male reproductive system. Cirrus classified as 'complex', composed of long shaft and long introvert, both curved (lateral view), directed ventrally, 697.5 (*n* = 1) long, shaft curved (approximately 169º) 340 (*n* = 1) long, shaft maximum width at base 30 (*n* = 1) (Fig. 18); introvert curved (approximately 133º) at mid-region, introvert total length (ventral) 157.5 (*n* = 2), introvert total length (dorsal) 200-357.5 (279, *n* = 2, 111), 130 (*n* = 2), ventral portion without spines, 155-160 (157.5, *n* = 2, 3) long, introvert´s swelling portion length (ventral side) 27.5 (*n* = 2), introvert´s swelling portion length (dorsal side) 40-47.5 (44, *n* = 2, 5). Ratio



Figures 1-2. Immature of *Kempnyia reticulata*. **(1)** Specimen infested with temnocephalids, dorsal view. Bar = 5 mm. **(2)** Detail of the temnocephalid (arrow) and of the eggs (arrow head) fixed to the host. Bar = 2 mm. **Figures 3-5.** *Temnocephala stoneflyi n. sp.* **(3)** Pedunculated eggs. Bar = 250 µm. **(4)** Detail of a pedunculated egg and filament (arrow). Bar = 250 µm. **(5)** Adult specimen mounted *in toto*, stained with acetic carmine/fast green. Bar = 250 µm.

between total length of cirrus/ maximum width of shaft´s base 23: 1; ratio between total length of cirrus/ total length of introvert 2.5: 1.

Taxonomic summary.

Type host: Immature *Kempnyia reticulata* (Klapálek, 1916) (Insecta, Plecoptera, Perlidae).

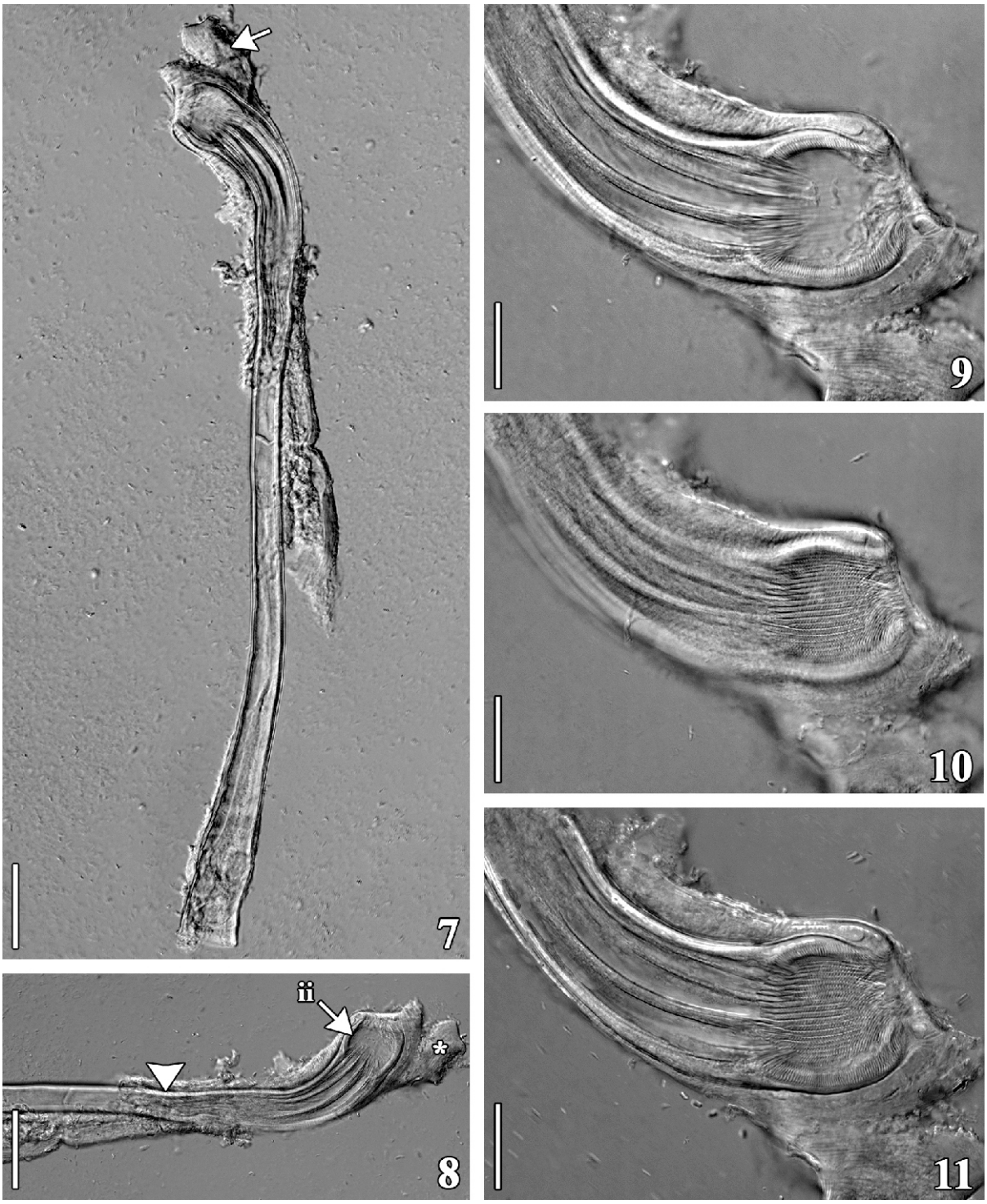
Type locality: Córrego da Divisa (19°58'28,4"S, 40°31'54,4"W), 'Reserva Biológica de Santa Lúcia', Santa Teresa, State of Espírito Santo, Brazil.

Site of infestation: Juveniles and adults of *T. stoneflyi n. sp.* were living on the head, mesonotum and, median legs of the immatures.

Prevalence: 17.2%.

Helminth specimens deposited: 'Coleção Helmintológica do Instituto Oswaldo Cruz': CHIOC 38203 (SBA 3113-1-1holotype); CHIOC 38204 (SBA3112-1-1- cirrus).

Figure 6. Scanning Electron Microscopy (SEM) of the right post-tentacular 'excretory' syncytial plate of *Temnocephala stoneflyi n. sp.* showing the excretory pore (ep). Bar = 20 μm.



Figures 7-11. Cirrus of *Temnocephala stoneflyi n. sp.* seen with DIC. **(7)** Cirrus 'complex' showing the retractor muscle which projects toward the front of the cirrus (arrow). Bar = 50 µm. **(8)** Limit of introvert-shaft (arrow head), portion of the retractor muscle which projects toward the front of the cirrus (asterisk) and the introvert inflation. Bar = 50 µm. **(9-10)** Sequence of different focus views of the distal portion of the cirrus, showing the introvert inflation and the small spines; long spines which are in the internal wall of the structure. Bar = 20 µm. **(11)** Image created by the Helicon Focus® software 5.3.7.2 made from the sequence of picture stacking of the distal portion of the cirrus. Bar = 20 µm.

DISCUSSION

As observed in *T. caddisflyi* and *T. curvicirri*, the species epibiont on immatures of *K. reticulata* has a cirrus classified as 'complex', and, most probably, it must also have a 'complex' vagina. The feminine reproductive system of *T. stoneflyi n. sp.* could not be clearly seen, although the 'complex' vaginas, as described for the epibionts found on belostomatids and trichopterans are quite similar (Amato & Amato, 2005; Amato *et al.,* 2011). When the 'complex' cirrus of the two species of epibionts found on insects are compared with that of *T. stoneflyi n. sp.*, we observed that the new species has the total size of the cirrus of an intermediary size among the three (1003 µm in average - *T. curvicirri*; 560 µm in average - *T. caddisflyi,* and 697 µm in average - *T. stoneflyi* n. sp.) and the largest total length of the introvert (155 µm in average - *T. curvicirri*; 140 µm in average - *T. caddisflyi* and 157 µm in average - *T. stoneflyi* n. sp.).

Amato *et al.* (2011) mentioned this species (as unpublished data) when they compared the shape of the inflation of the introvert among the species with cirrus classified as 'complex'. *Temnocephala caddisflyi* and *T. curvicirri* have approximately the same number of spine rows and the number of spines per row, as well as a similar shape (low cylinder in *T. caddisflyi* and tall cylinder in *T. curvicirri*). The inflation of the introvert of *T. stoneflyi* n. sp. has size and number of spine rows similar to those two species mentioned above, although the shape is globular (Figs 9-11). When curvature angles of the shaft are compared (158º – *T. caddisflyi*; 129º – *T. curvicirri*, and 169º – *T. stoneflyi* n. sp.), it is possible to see that the epibionts found on plecopterans have the shaft with the smallest curvature (almost straight), while *T. curvicirri* shows the largest curvature among the three species. The inverse occurs when the curvature angle of the introvert is analyzed (135º – *T. caddisflyi*; 145º – *T. curvicirri*, and 133º – *T. stoneflyi n. sp.*). The epibionts of the *K. reticulata* immatures have the largest introvert curvature among the three species. The shaft is smaller in the specimens of *T. stoneflyi n. sp.* and the relationship between the lengths of the introvert/length of shaft (2.5:1) is smaller than that found in *T. caddisflyi* (2.8:1) and in *T. curvicirri* (4.5:1). *Temnocephala curvicirri* and *T. caddisflyi* have EPs with a similar shape, but different size, which go beyond the equatorial line of the body in *T. caddisflyi* (Amato & Amato 2005; Amato *et al.* 2011). Comparing the EPs of the two species with those of *T. stoneflyi n. sp.*, the new species has the excretory plates of different shape and size (Fig. 6), they are rounded while the excretory pore is in the anterior half of the plate. The excretory plates are smaller in length (166 µm) than those of *T. caddisflyi* (368 µm in average) and *T. curvicirri* (492 µm in average).

Just a few specimens of *T. stoneflyi n. sp.* were sent to the 'Laboratório de Helmintologia, UFRGS' and the majority were not correctly fixed. Given the small number of samples, the female reproductive system was not described.

ACKNOWLEDGMENTS

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 Fernanda Avelino-Capistrano at the 'Departamento de Entomologia, Museu Nacional da Universidade Federal do Rio de Janeiro' for the collection of the insect hosts; to Adriano Cavalleri for the help with the Helicon Focus® 5.3.7.2 software; to the staff of the 'Centro de Microscopia Eletrônica', UFRGS for the SEM operation; to CAPES (Coordenação de Aperfeiçoamento do Pessoal de Nível

Superior) for the Doctoral Scholarship awarded to SAS (2008-2013); 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.

BIBLIOGRAPHIC REFERENCES

Amato, JFR & Amato, SB. 2005. *New species of Temnocephala Blanchard (Platyhelminthes, Temnocephalida) ectosymbiont on giant water bugs, Belostoma* spp. *(Hemiptera, Belostomatidae) from southern Brazil. Revista Brasileira de Zoologia, vol.* 22, pp.107-118.

Amato, JFR, Seixas, SA & Amato, SB. 2007. *A new species of* Temnocephala *Blanchard (Platyhelminthes, Temnocephalida) ectosymbiont on creeping water bugs,* Cryphocricos granulosus *De Carlo (Hemiptera, Naucoridae) from southern Brazil.* Revista Brasileira de Zoologia, vol. 24, pp. 1043–1051.

Amato, JFR, Amato, SB, Seixas, SA, Vidigal, THDA & Andrade, CP. 2011. *Trichoptera — the newest insect order host of temnocephalans (Platyhelminthes, Temnocephalida) and the description of a new species of Temnocephala from Brazil. Zootaxa, vol.* 2975, pp. 47–58.

Avelino-Capistrano, F, Barbosa, LS & Cunha, AM. 2013. *First occurrence of* Temnocephala *(Platyhelminthes: Temnocephalida) in immatures of* Kempnyia reticulata *(Enderlein) (Plecoptera: Perlidae).* EntomoBrasilis, vol. 6, pp. 91-93.

Bispo, PC & Froehlich, CG. 2004. *Perlidae (Plecoptera) from Intervales State Park, São Paulo State, Southeastern Brazil. Aquatic Insects*, vol. 26, pp. 97–114.

Damborenea, MC & Cannon, LRG. 2001. *On neotropical Temnocephala (Platyhelminthes).* Journal of Natural History, vol. 35, pp. 1103-1118.

Moretto, HJA. 1978. *Presencia de* Temnocephala *(Temnocephalida, Platyhelminthes) en hemipteros acuáticos.* Ciencia e investigación, vol. 34, pp. 95-99.

Seixas, SA, Amato, JFR & Amato, SB. 2010. *Redescription of* Temnocephala *iheringi (Platyhelminthes: Temnocephalida) based on specimens from Pomacea canaliculata (Mollusca: Ampullariidae) of the State of Rio Grande do Sul: the possible type host and type locality.* Zoologia, vol. 27, pp. 245-257.

Vianna, GJC & De Melo, AL. 2002. *Aquatic heteroptera as host of* Temnocephala *Blanchard (Platyhelminthes: Temnocephalidae) in Minas Gerais, Brazil.* Lundiana, vol. 3, pp. 151-153.

Received June 23, 2015.
Accepted September 25, 2015.