Universidade Estadual de Londrina

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METAZOÁRIOS SIMBIONTES DE AEGLA LEACH, 1820 (CRUSTACEA, ANOMURA)

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Biológicas, da Universidade Estadual de Londrina, como requisito parcial à obtenção do título de Mestre em Ciências Biológicas (Biodiversidade e Conservação de Habitats Fragmentados).

Orientadora: Profª. Drª. Profª. Drª. Aline Aguiar
Co-orientador: Prof. Dr. Gustavo Monteiro Teixeira

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FORMATAÇÃO DA DISSERTAÇÃO

A dissertação está estruturada em dois capítulos. O primeiro capítulo, intitulado "A new species of Temnocephala (Platyhelminthes: Temnocephalidae), an ectocommensal of Aegla lata (Crustacea; Anomura)", será submetido à revista Zootaxa (Qualis da CAPESBiodiversidade B1) e trata-se de uma descrição de uma espécie nova de Temnocephala associada a uma espécie de eglídeo recentemente enquadrada no grau de ameaça 'criticamente ameaçada'.

O segundo capítulo, intitulado "Stratiodrilus circensis (Polychaeta, Histriobdellidae), a widely distributed ectocommensal of freshwater decapods: morphological variations from seven populations", também será submetido para publicação na revista Zootaxa. Esse capítulo apresenta um estudo das principais características morfológicas de Stratiodrilus circensis, bem como reporta uma grande variação morfológica entre diferentes populações de Stratiodrilus associada a seis espécies de hospedeiros eglídeos de riachos de diferentes bacias hidrográficas do Estado do Paraná.

A introdução geral e os capítulos seguem as normas da revista Zootaxa.

ROSA, Jheimison Junior da Silva. **Metazoários Simiontes de *Aegla* Leach, 1820 (CrustaceaAnomura)**. 2018. 3 f. Dissertação (Mestrado em Ciências Biológicas) - Universidade Estadual de Londrina, Londrina, 2018.

RESUMO GERAL

Os caranguejos anomuros do gênero *Aegla* Leach, 1820 são um exemplo de fauna neotropical endêmica que é ameaçada de extinção, sendo provavelmente o grupo de decápodos dulcícolas mais ameaçados da América do Sul. Esses crustáceos apresentam área de distribuição desde o sudeste do Brasil até a região central do Chile. Embora várias espécies de eglídeos tenham sido descritas nos últimos anos, os simbiontes metazoários associados com espécies de *Aegla* são ainda pouco conhecidos. Portanto, estudos de inventários são de extrema importância para o conhecimento dessa fauna, principalmente em relação àqueles hospedeiros que se enquadram em algum grau de ameaça. Neste sentido, o objetivo desse estudo foi inventariar os platelmintos do gênero *Temnocephala* Blanchard, 1849, e os poliquetas do gênero *Stratiodrilus* Haswell, 1900, associados a populações de eglídeos. Este estudo amostrou exemplares de sete populações de *Aegla* (rio Couro, rio Preto, riacho do Bule, ribeirão Jacutinga, rio Pinhão e rio Ema) presentes em quatro bacias hidrográficas distintas (bacias dos rios Pirapó, Tibagi, Ivaí e Iguaçu), localizadas na região sul do Brasil. Há poucos registros de espécies de *Aegla* para o norte e noroeste do estado do Paraná, principalmente devido à falta de amostragem. Os simbiontes coletados foram identificados até o nível de espécie, baseando-se em trabalhos de revisão e de descrição. Os métodos de fixação e preparação de lâminas para análise de estruturas morfológicas foram implementados de acordo com o gênero de cada simbionte. No primeiro capítulo, uma nova espécie de temnocefalídeo foi descrita em associação com *Aegla lata* Bond-Buckup & Buckup, 1994, em Londrina, Região Sul do Brasil. Essa espécie diferencia-se das demais principalmente pela forma das placas sinciciais, forma do cirro e plano de fratura e ornamentação dos ovos. A nova espécie é a vigésima descrita do Brasil e a sétima espécie reportada em associação com hospedeiros eglídeos. No segundo capítulo, foi feito um estudo da variação morfológica em *Stratiodrilus circensis* Steiner & Amaral, 1999*,* incluindo os hospedeiros reportados e área de ocorrência das populações. *Stratiodrilus circensis* é uma espécie amplamente distribuída na região Neotropical, associada a muitas espécies de decápodos dulcícolas. Neste capítulo, nós discutimos as variações morfológicas das populações amostradas e os caracteres de valor taxonômico de *S. circensis*. Além disso, a partir de análises morfológicas nós sugerimos que as populações de *S. circensis* aqui estudadas constituem um grupo de espécies crípticas. Em suma, esse estudo contribui para o aumento do conhecimento da fauna de simbiontes que estabelecem associação com espécies de *Aegla*.

Palavras-chave: Ectocomensal. Decápodos dulcícolas. *Stratiodrilus*. *Temnocephala*.

ROSA, Jheimison Junior da Silva. **Metazoan Symbionts of *Aegla* Leachi, 1820 (Crustacea, Anomura)**. 2018. 93 pp. Master Dissertation (Master in Biological Sciences) - Universidade Estadual de Londrina, Londrina, 2018.

GENERAL ABSTRACT

Anomuran crabs of the genus *Aegla* Leach, 1820 are an endemic Neotropical fauna, being probably the most threatened freshwater decapod crustaceans in South America. These crustaceans are found from the Southeastern Brazil to Central Chile. Although many aeglid species were described in the last few years, their metazoan symbionts are still little known. Therefore, inventory studies are extremely important for the knowledge of this symbionts because many hosts species are in any degree of threat. We collected specimens of aeglid of the seven population from the four river basins to investigate the flatworms of the genus *Temnocephala* Blanchard, 1849, and the polychaetes of the genus *Stratiodrilus* Haswell, 1900. This study sampled seven populations of *Aegla* in the Pirapó, Tibagi, Ivaí, and Iguaçu river basins. The symbionts collected were identified down to the species level with review and description papers. For the morphological study, some specimens were fixed in ethanol 70% and mounted in lactic acid semi-permanet slides for internal morphometry. In the first chapter, a new temnocephalan species was described associated with *Aegla lata* Bond-Buckup & Buckup, 1994, collected in Bule Stream, Londrina, Southern Brazil. This new species differed from the other termnocephalan species due to the shape and size of syncytial plates, the shape of the cirrus, and the plane of eggs fracture and ornamentation. The new species is the twentieth described in Brazil and the seventh associated with aeglid hosts. In the second chapter, we studied the morphological variation in *Stratiodrilus circensis* Steiner & Amaral, 1999, reporting the hosts' distribution and area of occurrence of the populations for each river basin. *Stratiodrilus circensis* is a widely distributed species in the Neotropical region, and it is associated with several species of freshwater decapods. Here, we discussed the morphological variations of the sampled populations and characters of the taxonomic value of *S. circensis*. Also, based on morphological analyses we suggest that the populations of *S. circensis* studied herein compound a group of cryptic species. In summary, this study contributes to the increasing the knowledge of the symbiotic fauna that occurs in association with species of *Aegla*.

Key words: Aeglids. Freshwater decapods. *Stratiodrilus*. *Temnocephala*.

SUMÁRIO

INTRODUÇÃO GERAL

Os caranguejos anomuros da família Aeglidae (Crustacea, Anomura) são exemplo de uma fauna dulcícola endêmica da América do Sul que é muito vulnerável a mudanças ambientais nas nascentes dos cursos d'água (Bond-Buckup *et al.* 2008). A família é representada por apenas um gênero atual, *Aegla* Leach, 1820, com 85 espécies descritas (Moraes *et al.* 2017). No entanto, é provável que esse número aumente nos próximos anos, já que somente na última década foram descritas 19 espécies (Bueno *et al.* 2017; Santos *et al.* 2017).

O gênero *Aegla* apresenta, como limite norte de distribuição, o município de Claraval, estado de Minas Gerais, Brasil, e como limite sul a Ilha de Duque de York, no Chile (Bueno *et al.* 2007; Oyanedel *et al.* 2011). Há registros de ocorrência ao longo do Brasil, Bolívia, Paraguai, Uruguai, Argentina e Chile (Bond-Buckup & Buckup 1994). Esses anomuros têm hábitos bentônicos e podem ser encontrados em arroios, riachos, rios, lagoas e cavernas da região subtropical da América do Sul, sendo considerados elos importantes na cadeia trófica dos ecossistemas aquáticos continentais (Bond-Buckup 2003; Bond-Buckup & Buckup 1994).

A maioria das espécies de eglídeos é encontrada em pequenos riachos de uma única bacia hidrográfica ou em áreas muito restritas de riachos de cabeceira em locais com poucos registros de ocorrência, o que aumenta o nível de ameaça e a suscetibilidade desse grupo a extinções locais (Bücker *et al.* 2008; Magris *et al.* 2010). Por serem muito sensíveis à alterações no ambiente os eglídeos são considerados indicadores de qualidade de habitat (Bond-Buckup & Buckup 1994; Pérez-Losada *et al.* 2009). Além disso, os eglídeos podem estar sujeitos aos efeitos de diversos contaminantes presentes nos ambientes, já que possuem uma dieta caracteristicamente omnívora, consumindo plantas em decomposição, macrófitas, pequenos invertebrados aquáticos e até mesmo sedimento do fundo do riacho (Bueno & Bond-Buckup 2004; Castro-Souza & Bond-Buckup 2004).

A poluição de riachos a partir de efluentes agrícolas e industriais, a alteração e fragmentação de habitat e a degradação da vegetação ripária de riachos estão entre as principais ameaças à biodiversidade de eglídeos, sendo potencialmente preocupante no caso de espécies com área de distribuição restrita (Santos *et al.* 2017). Assim, os esforços para a conservação desses ambientes são de extrema urgência, já que os eglídeos são considerados os crustáceos decápodos dulcícolas mais ameaçados da América do Sul (Bueno *et al.* 2016). Além disso, cerca de 70% das espécies de eglídeos estão alocadas em algum grau de ameaça, sendo 20% das espécies consideradas criticamente ameaçadas (Santos *et al.* 2017).

Muitos estudos têm sido feitos com espécies de *Aegla*, como aqueles em relação à origem e dispersão (Pérez-Losada *et al.* 2004), biologia populacional (Bueno *et al.* 2007; Dalosto *et al.* 2014), biologia reprodutiva e desenvolvimento (Bueno & Shimizu 2008; Rocha *et al.* 2010), morfometria geométrica (Marchiori *et al.* 2014), comportamento (Martin & Felgenhauer 1986) e conservação (Pérez-Losada *et al.* 2009). No entanto, nos últimos anos muitas espécies têm sido descritas e algumas têm sido consideradas complexos de espécies, levando a um crescimento do interesse acerca da diversidade e do grau de ameaça desses crustáceos anomuros (Bueno *et al.* 2017; Crivellaro *et al.* 2017; Moraes *et al.* 2016; Santos *et al.* 2010; 2013; 2015; 2017).

Se por um lado o conhecimento da diversidade da fauna de eglídeos ainda é insuficiente, por outro a fauna de simbiontes associada a esses crustáceos é ainda menos estudada. Overstreet (1983) definiu um simbionte como aquele organismo que 'vive junto' de outro organismo; e um organismo comensal aquele que se beneficia de alguma forma da associação de um determinado hospedeiro, mas sem depender deste metabolicamente (Overstreet 1983). Assim, um ectocomensal caracteriza-se como um simbionte externo que infesta o hospedeiro e o utiliza como substrato (Overstreet 1983). Embora as complexas relações entre simbiontes e hospedeiros crustáceos sejam pouco conhecidas, diversos grupos como rotíferos, protozoários, anelídeos, ostrácodos, copépodos, nemátodos e platelmintos já foram reportados na literatura por estabelecer associação com algumas espécies de decápodos e isópodos (Steiner & Amaral 1999). Da mesma forma, os eglídeos já foram reportados como hospedeiros de espécies de turbelários (Martínez-Aquino *et al.* 2014a), poliquetas (Steiner & Amaral 1999) e digenéticos (Rauque *et al.* 2013).

Assim, dentre a fauna associada aos eglídeos existem diferentes estratégias de vida conforme o grupo de simbionte. Os parasitas digenéticos podem estar dentro do tecido do hospedeiro, como por exemplo, na base dos filamentos branquiais (Rauque *et al.* 2013); já os simbiontes metazoários que não infectam o tecido do hospedeiro podem ocorrer na superfície do corpo (cabeça, tórax, abdome e apêndices), dentro da câmara branquial, nos epipoditos, na superfície interna da carapaça (Steiner & Amaral 1999), ou ainda sobre as massas de ovos dos hospedeiros (Glasby & Timm 2008).

Infestações mistas de platelmintos do gênero *Temnocephala* Blanchard, 1849 e anelídeos do gênero *Stratiodrilus* Haswell, 1900 já foram reportadas na literatura, sendo estes os grupos que mais foram relatados na literatura em estabelecer associações com eglídeos (Dioni 1972). Esses dois gêneros são comumente relatados na literatura como 'ectocomensais' (Amato *et al.* 2003; Steiner & Amaral 1999). Por conseguinte, tanto

Temnocephala quanto *Stratiodrilus* utilizam os seus hospedeiros como um substrato para completar o ciclo de vida, na medida em que depositam seus ovos no hospedeiro (Vila & Véliz 2014; Martínez-Aquino *et al.* 2014b), e se alimentam de outros organismos que estão na carapaça (Dioni 1972). Isso evidencia a dependência do sucesso do ciclo de vida de *Temnocephala* e *Stratiodrilus* em relação a seus hospedeiros (Villela 2002; Vila & Véliz 2014).

Temnocephala

A ordem Temnocephalida abriga turbelários ectocomensais estritamente associados a hospedeiros dulcícolas (Martínez-Aquino *et al.* 2016). Dentro de Temnocephalida, a família Temnocephalidae é a mais diversa, com alta diversidade de espécies hospedeiras na região Neotropical (Martínez-Aquino *et al.* 2014a). Os representantes de *Temnocephala* são ectocomensais de uma ampla gama de hospedeiros, incluindo crustáceos, moluscos, insetos aquáticos e quelônios (Damborenea & Cannon 2001a). Esses organismos são endêmicos da região Neotropical, possuem certa afinidade por algumas famílias de hospedeiros e sua distribuição segue o padrão de distribuição dos organismos aos quais costumam associar-se (Martínez-Aquino *et al.* 2014a; 2014b; Martínez-Aquino *et al.* 2016).

Os representantes do gênero *Temnocephala* podem ser reconhecidos pelas seguintes características morfológicas gerais: ocelos com pigmento vermelho; epiderme composta de placas sinciciais; poros excretores sempre dentro das placas sinciciais excretoras dorsolaterais; dois pares de testículos laterais e posteriores ao intestino e ausência de saco ejaculatório (Damborenea & Cannon 2001a).

Martínez-Aquino *et al.* (2014a) realizaram um extenso inventário das espécies de *Temnocephala* da região Neotropical encontrados em gastrópodes (Caenogastropoda), insetos (Hemiptera, Megaloptera, Plecoptera e Trichoptera), malacóstracos (Decapoda) e répteis (Testudines), e compilaram ao todo 31 espécies. No entanto, desde o trabalho de Martínez-Aquino *et al.* (2014a) quatro espécies novas foram descritas, sumarizando 35 espécies, sete dessas associadas à eglídeos (Ponce de Leon *et al.* 2015; Seixas *et al.* 2015a; 2015b; Ponce de Leon & Volonterio 2018). Os crustáceos são os hospedeiros com a maior diversidade de espécies de *Temnocephala* associada (21 espécies), seguidos pelos moluscos (6 espécies) e insetos (5 espécies) (Martínez-Aquino *et al.* 2014a; Ponce de Leon *et al.* 2015; Seixas *et al.* 2015a; 2015b; Ponce de Leon & Volonterio 2018). No entanto, os temnocefalídeos têm sido relatados na literatura como organismos que abrigam uma extensa fauna ainda não catalogada e necessitada de investigação para o conhecimento da sua biodiversidade (Martínez-Aquino *et al.* 2014a).

Stratiodrilus

A família Histriobdellidae é representada por pequenos ectocomensais associados a hospedeiros decápodos e isópodos (Steiner & Amaral 1999). Essa família é composta por três gêneros: *Histriobdella* van Beneden, 1858, encontrado sobre ovos de lagostas da América do Norte e Europa; *Steineridrilus* Zhang, 2014 (=*Dayus* Steiner & Amaral, 1999), encontrado nos pleópodos de isópodos marinhos da África do Sul; e *Stratiodrilus*, encontrado nas câmaras branquiais, na face interna do abdomen, sobre os pleópodos e massas de ovos de crustáceos de água doce da América do Sul, Madagascar e Austrália (Steiner & Amaral 1999). *Histriobdella* e *Steineridrilus* são gêneros monospecíficos, enquanto que *Stratiodrilus* é o gênero mais especioso, estabelecendo associação com uma grande variedade de hospedeiros e localidades no Hemisfério Sul (Steiner & Amaral 1999).

A distribuição das espécies de *Stratiodrilus* é caracteristicamente gonduânica (Glasby & Timm 2008), e todas as espécies estão associadas a hospedeiros decápodos dulcícolas das famílias Aeglidae, Parastacidae e Trichodactylidae (Rosa *et al.* 2018). Além disso, a diversidade de espécies de *Stratiodrilus* pode estar associada à distribuição de seus hospedeiros (Amaral & Morgado 1997), já que cada associação entre hospedeiro e ectocomensal é exclusiva em cada região biogeográfica em que *Stratiodrilus* ocorre (Rosa *et al.* 2018).

O gênero *Stratiodrilus* possui características morfológicas distintas daquelas encontradas nos demais poliquetas errantes, as quais permitiram a adaptação para uma estratégia de vida simbiótica (Steiner & Amaral 1999). Vila & Bahamonde (1985) reportaram a presença de um corpo de tamanho reduzido, glândulas adesivas na região posterior do corpo e ausência de parapódios e cerdas como características que indicam um alto grau de especialização evolutiva de *Stratiodrilus*.

Os estudos com *Stratiodrilus* são reduzidos e apenas 11 espécies foram descritas desde a descrição do gênero por Haswell (1900). A revisão mais recente de *Stratiodrilus* foi elaborada por Steiner & Amaral (1999), e as duas últimas espécies descritas para o gênero são *Stratiodrilus vilae* Amato, 2001 e *Stratiodrilus brevicirrus* Amato, Daudt & Amato, 2004.

Apesar de *Temnocephala* e *Stratiodrilus* serem os dois gêneros mais citados em associação com eglídeos (Dioni 1972), estudos acerca da biologia, morfologia e conservação desses simbiontes ainda são insuficientes. Por outro lado, embora a fauna de hospedeiros eglídeos seja relativamente bem estudada, muitas espécies estão sujeitas a diversas fontes de ameaça, como perda e diminuição de habitat, contaminação de riachos e desmatamento da vegetação ripária (Santos *et al.* 2017). Além disso, há espécies de eglídeos consideradas criticamente ameaçadas e distribuídas numa estreita área de ocorrência, como referido para *Aegla lata* Bond-Buckup & Buckup, 1994 em Santos *et al.* (2017). Nesse contexto, se considerarmos que algumas espécies de *Aegla* podem ser extintas e que os simbiontes geralmente enfrentam as mesmas ameaças que seus hospedeiros (Colwell *et al*. 2012), há uma urgência em se implementar políticas conservacionistas para a proteção da biodiversidade de eglídeos e simbiontes metazoários associados.

Assim, o presente estudo tem como objetivos descrever uma espécie nova de *Temnocephala* e estudar a morfologia de sete populações de *Stratiodrilus* associados a diferentes espécies de *Aegla* do Estado do Paraná.

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CAPÍTULO 1

A new species of *Temnocephala* (Platyhelminthes: Temnocephalidae), an ectocommensal of *Aegla lata* (Crustacea: Anomura)

Artigo a ser submetido ao periódico *Zootaxa*

A new species of *Temnocephala* (Platyhelminthes: Temnocephalidae), an ectocommensal of *Aegla lata* (Crustacea: Anomura)

Abstract

Symbiotic turbellarians of the genus *Temnocephala* Blanchard, 1849 are endemic to the Neotropics, being associated with many hosts, including anomuran crustaceans of the genus *Aegla* Leach, 1820. Although several species have been described in Brazil in the last few years, only *Temnocephala axenos* Monticelli, 1899 was already reported in the State of Paraná. This study aimed to describe a new species of *Temnocephala* from State of Paraná, Southern Brazil. The aeglid hosts were collected in a small headwater stream, in Londrina, Southern Brazil, and the helminths were recovered from the host in the laboratory. The specimens were stained and cleared and then mounted with Hoyer's medium. Some specimens were analyzed with scanning electron microscopy to observe the epidermal mosaic. Eighty-eight percent of 86 specimens of *Aegla lata* Bond-Buckup & Buckup, 1994 collected had temnocephalans associated. In average, each specimen of *A. lata* had 18.55±2.78 specimens of *Temnocephala* sp. nov, associated, while the mean abundance of the population was 16.30±2.54. *Temnocephala* sp. nov. resembles *Temnocephala mertoni* Volonterio, 2007, *T. axenos*, and *Temnocephala talicei* Dioni, 1967a regarding general body shape. The new species can be distinguished from its congeners by the combination of the following characters: syncytial plates elongated; excretory pore in a subterminal level, displaced toward the inner side of the plate; four paranephrocytes. Moreover, this new species has two vaginal sphincters, eggs with tuberous ornamentation, testes without lobes, cirrus slightly curved with non-sinuous distal portion, the distal portion of the introvert with a pronounced swelling, a proximal wall of introvert thick-walled, introvert base with small teeth-like protuberances, and shaft base with a thicker rim. Although *Temnocephala* sp. nov. shares some characteristics with other species the shape of syncytial plates, the shape of cirrus with a thickening in the proximal base of the introvert, and the ornamentation of eggs are probably found only in the new species. Therefore, *Temnocephala* sp. nov. is the seventh reported associated with aeglid hosts, and the twentieth reported for Brazil.

Key words: Temnocephalidae, symbiotic turbellarians, Aeglidae.

Introduction

Within the Temnocephalida, Temnocephalidae Monticelli, 1899 is the most diverse group of symbiotic turbellarians (Damborenea & Brusa 2009). *Temnocephala* Blanchard, 1849—the type genus of the family—is endemic from the Neotropical region and it is found in association with crustaceans, mollusks, chelonians and insect hosts (Damborenea & Cannon 2001). Among these, decapod crustaceans are the hosts with the highest species richness associated with temnocephalans, as well as the host group that most influenced the distribution patterns of these helminths in the Neotropical region (Martínez-Aquino *et al.* 2016).

The latest study on freshwater symbiotic temnocephalans from the Neotropics

(Martínez-Aquino *et al*. 2014a) reported 31 species belonging to *Temnocephala*. However, since then four more species were described: *Temnocephala dionii* Ponce de Leon, Beron & Volonterio, 2015, from Southern Argentina, commensal of the freshwater anomuran *Aegla neuquensis* Schmitt; *Temnocephala euryhalina* Seixas, Amato & Amato, 2015, from Northern Brazil, commensal of the mollusk *Neritina zebra* (Bruguière); *Temnocephala stoneflyi* Seixas, Amato & Amato, 2015, from Southeastern Brazil, commensal of the immature insect *Kempnyia reticulata* (Klapálek); and *Temnocephala gargantua* Ponce de Leon & Volonterio, 2018, from Uruguay, commensal of *Aegla platensis* Schmitt and *Aegla uruguayana* Schmitt. From these 35 species of *Temnocephala*, 21 are associated with crustaceans and seven of them are commensals of anomuran aeglids.

Nineteen species of *Temnocephala* occur in Brazil, but only two of them are associated with aeglid hosts: *Temnocephala cyanoglandula* Amato, Amato & Daudt, 2003, from State of Rio Grande do Sul, associated with *Aegla serrana* Buckup & Rossi; and *Temnocephala axenos* Monticelli, 1899, found in Brazil, Uruguay and Argentina associated with several species of *Aegla* Leach and *Parastacus* Huxley (Martínez-Aquino *et al*. 2014a). In the State of Paraná, there is only one occurrence record for a temnocephalan species, *T. axenos* associated with *Aegla castro* Schmitt (Martínez-Aquino *et al*. 2014a).

Species of *Temnocephala* were reported to have host specificity since each species are associated with a single species of *Temnocephala* or with closely related hosts (Martínez-Aquino *et al.* 2014b). Also, temnocephalan species can be diagnosed by the shape of dorsolateral ‘excretory’ syncytial plates (DLSPs), the position of excretory pore inside this plate, and shape of cirrus (Seixas *et al*. 2015c). These characters follow a pattern according to the host group, as reported for the shape of DLSPs (Seixas *et al*. 2015c) and the shape of cirrus (Garcés *et al*. 2013).

The present study describes a new species of *Temnocephala* from the State of Paraná, as well as report the first occurrence record of a temnocephalan species in *Aegla lata* Bond-Buckup & Buckup, 1994, a critically endangered freshwater anomuran crustacean (Santos *et al*. 2017).

Material and methods

Eighty-six specimens of *Aegla lata* were collected between May 2014 and June 2015 in Bule Stream, a small headwater stream inside Mata dos Godoy State Park, Londrina, Upper Paraná River Basin, in Londrina, State of Paraná, Brazil (23º26’60”S, 51º15’06”W). Crabs were collected (Collection licenses: SISBIO 36819-1, SISBIO 33578-1 and IAP 32.14) with the aid of hand nets of 90 cm diameter and 1 mm mesh size. All hosts were immediately individualised in bottles containing stream water and transported alive to the laboratory.

A total of 1,206 temnocephalan specimens were collected under a stereomicroscope. Some specimens were fixed in 70% ethanol under slight cover slip pressure for the analysis of internal morphological structures. Some specimens were stained with Delafield's hematoxylin (Amato & Amato 2005) and others with chloride-carmine (Amato *et al.* 1991). They were cleared with eugenol oil or lactic acid, and then mounted in Canada balsam (Amato & Amato 2005) or preserved in 70% ethanol. Eggs were removed from the surface of the hosts and preserved in 70% ethanol (Volonterio 2007). Some eggs were mounted in Hoyer's to examine the opercular plates. Whole-mounted specimens, dissected cirri and eggs were photomicrographed under Opton microscope coupled to the imaging system BELview, software version 6.2.3.0.

The terminology used to describe the reproductive structures follows Cannon (1993) and Damborenea & Brusa (2008). The morphology of some helminths was evaluated by scanning electron microscopy (SEM). First, they were fixed in absolute hot alcohol, dehydrated in graded alcohol series and dried to a critical point with liquid CO_2 in CPD 020 (Balzer Union). Then they were mounted on an aluminium stub using conductive double-sided tape, coated with gold-palladium and examined with the use of a Quanta 200 scanning electron microscope (FEI Company; from Center of Electron Microscopy of Botucatu, State of São Paulo, Brazil) (following Allison *et al.* 1972). For the morphometry study of dorsolateral 'excretory' syncytial epidermal plates (DLSPs) we used the methodology proposed by Seixas *et al.* (2015c), which study considered the position of the excretory pore in relation to the boundaries of the DLSPs. The distances from excretory pore to the anterior and posterior boundaries of DLSPs, and the distances from excretory pore to the internal and external boundaries of DLSPs were taken (Seixas *et al.* 2015c). The sum of this measurements represents the total length and the total width of the plates, respectively.

The description was based on 27 specimens. Description of DLSPs was based on eight specimens. All measurements are presented in micrometres (µm); minimum and maximum numbers are followed (between parentheses) by the mean, the standard deviation values, and the number of specimens measured for a given character. Measurements of cirrus dimension followed Amato *et al.* (2003). Intestinal sac length was made from the average length of the two lobes of the intestinal sac.

Ecological descriptors such as prevalence (P%), mean abundance (MA), and mean intensity (MI) were calculated according to Bush *et al.* (1997), using standard error values.

The hosts were deposited in Crustacean Collection of the Museum of Zoology of the Londrina State University (MZUEL), State of Paraná, Brazil. The helminths were deposited in Helminthological Collection of the Oswaldo Cruz Institute, FIOCRUZ (CHIOC), State of Rio de Janeiro, Brazil; and in Helminthological Collection of the São Paulo State University, Institute of Biosciences, UNESP (CHIBB), State of São Paulo, Brazil.

Results

From the specimens of *Aegla lata* collected from Bule Stream (Fig. 1) we recovered 1206 temnocephalans (Fig. 2), of which five whole-mounted in Hoyer's; 14 whole-mounted in permanent slides with Canada balsam; 13 temporary slides; eight specimens mounted on stubs for SEM. Description of eggs were based on 11 permanent slides mounted with Hoyer's (Figs. 2c–g).

Temnocephala sp. nov.
(Figs. 2–7)

Type specimens: Holotype: stained with hematoxylin, preserved in 70% ethanol. Deposited at catalogue number:

Paratypes: 26 specimens, stained with chloride carmine or hematoxylin, preserved in 70% ethanol. Deposited at catalog number:

The holotype and paratypes will be deposited in Helminthological Collection of the Oswaldo Cruz Institute, FIOCRUZ (CHIOC), State of Rio de Janeiro, Brazil; and in Helminthological Collection of the São Paulo State University, Institute of Biosciences, UNESP (CHIBB), State of São Paulo, Brazil.

Site of infestation: Adult and young temnocephalans were found on the following structures of the crustacean: dorsal carapace, pereopods, the base of the mouth appendages, ocular orbit, surrounding eye peduncle. Eggs were deposited on the general body surface of hosts, mainly on the ventral side of pereopods, never in the internal part of the abdomen or thoracic sternites (Fig. 2b).

Type host: *Aegla lata* Bond-Buckup & Buckup, 1994 (Fig. 2a). The specimens will be deposited in Crustacean Collection of the Museum of Zoology of the Londrina State University (MZUEL), State of Paraná, Brazil.

Type-locality: Bule Stream (23º26'60"S, 51º15'06"W), a small stream inside Mata dos Godoy State Park, Upper Paraná River Basin, in Londrina, State of Paraná, Brazil.

Ecological descriptors: P=88%; MA=16.30±2.54; MI=18.55±2.78; min=0; max=133.

Diagnosis

Temnocephala sp. nov. differs from congeners by carrying the following set of characteristics: (1) dorsolateral ‘excretory’ syncytial epidermal plates elongated with anterior portion wider and posterior portion narrower; (2) excretory pore in a subterminal level, displaced toward the inner side of the plate; (3) four paranephrocytes; (4) two vaginal sphincters; (5) eggs with tuberous ornamentation; (6) testes without lobes; (7) cirrus slightly curved with non-sinuous distal portion; (8) distal portion of introvert with a pronounced swelling (8) proximal wall of introvert thick-walled; (9) introvert base with small teeth-like protuberances; and (10) shaft base with a thicker rim. See Table 1 and Remarks for comparisons.

Description

Body shape elliptical, wider at gonopore level, narrower at adhesive disk level (Fig. 3a); five medium-sized tentacles (Figs. 3a and 3b); whitish on live and preserved specimens. Body length without tentacles, 1360.69–3346.55 (1996.85, 435.52, n = 22); body width 887.92–1696.04 (1221.19, 224.83, n = 19); adhesive disk ventral, subterminal, pedunculate, partially covered by body (Figs. 3a and 3c), wider than longer, length 215.05–587.02 (377.09, 98.18, n = 20) and width 301.10–687.84 (458.25, 114.06, n = 20); peduncle perpendicular to the longitudinal axis of body surface (Figs. 4a and 4b). Ratio between body length and adhesive disk length 1:0.16–0.34 (1:0.21, n = 8); ratio between body width and adhesive disk width 1:0.35–0.50 (1:0.41, n = 5), ratio between body length and distance from gonopore to base of tentacles 1:0.41–0.57 (1:0.48, n = 10). Eye spots elliptical, with red pigment in live specimens. Epidermal mosaic with two, dorsolateral, post tentacular, elongated, ‘excretory’ syncytial epidermal plates (DLSPs) (Figs. 4c and d); anterior boundary of the plate convex, posterior boundary narrow; proximal boundary slightly convex or straight; distal boundary of each plate not visible in dorsal view (Figs. 4c and d); dorsolateral ‘excretory’ syncytial epidermal plates wider at anterior region, extending from base of first and fifth tentacles to the first and second thirds of body (Fig. 4c); left plate 222,00–374.91 (329.25, 72.03, n = 4) long, and 55.56–122.43 (77.79, 31.30, n = 4) wide; right plate 343.33–458.65 (400.38, 57.34, n = 4) long, 44.58–142.99 (98.53, 42.55, n = 4) wide. Excretory pore inside of the DLSPs in a subterminal level displaced toward the inner side of the plate (Figs. 4c and d; Table 2). Secretion bodies observed in the tegument of the adhesive disk of some specimens, with irregular shape (Fig. 3g).

Alimentary system. Mouth surrounded by a robust muscular sphincter (Fig. 3); mouth at first third of body; pharynx with anterior portion wider than posterior (Fig. 3); rounded in some specimens; pharynx wider than long, 258.17–630.83 (407.77, 120.99, n = 22) long, 311.03–657.09 (446.07, 109.66, n = 22) wide; pharynx longer than wide in 28% of specimens; intestinal sac on the middle part of the body, large, 448.05–1068.01 (615.23, 156.10, n = 13) maximum length, 831.04–1458.59 (1028.69, 190.47, n = 13) wide (Fig. 3a), with a markedly central constriction, (Fig. 3a); with septations best observed in young specimens, ill-defined in adults.

Excretory system. Excretory ampullae small, elliptical, at the level of the mouth, with excretory pore directed anteriorly in the ampullae (Figs. 5a and 5b).

Glands. Rhabditogenic producing glands numerous, spherical, present in lateral fields of body (Figs. 5c–e), extending from anterior portion of pharynx to posterior portion of posterior testes; rhabditogenic tracts difficult to observe, uniting just before entering tentacles (Figs. 5c and 5f); tentacular glands in anterolateral portion of pharynx (Fig. 5f); cyanophilous glands absent; Haswell's cells two, inconspicuous, of relatively different diameters in some specimens, 68.17–125.30 (89.75, 26.71, n = 4) right cell diameter, 67.44–108.56 (88.39, 19.44, n = 4) left cell diameter (Fig. 5a). Esophageal glands elongated, in the space between anterolateral portion of intestinal sac and posterolateral portion of pharynx, not forming a collar, stained reddish-purple (Fig. 3f). Ootype glands not observed. Disk glands posterior to genital complex, scattered between and posterior to posterior testes (Figs. 3c and 6a). Large glandular disc cells (paranephrocytes?) four, lobed, anterior pair more central than posterior, between anterior testes; posterior pair posterior to posterior testes (Figs. 5d and 6a).

Reproductive system. **Female.** Gonopore in the region of intestinal sac invagination, anterior to the level of anterior testes (Fig. 6a); reproductive complex difficult to observe (Fig. 6); ovary ovoid, posterior to the intestinal sac, 140.62–153.16 (147.58, 6.43, n = 4) long, 109.63–127.18 (119.5, 8.14, n = 4) wide (Figs. 6a–c); oviduct short, 17.00 (measured in the holotype) opening into posterior portion of vagina just behind seminal receptacles (Fig. 6c); at least one, small, spherical seminal receptacle was observed (Fig. 6d); vitellaria dorsal, branched, well superposed to the intestinal sac in adult specimens, terminal portions of the branches turning toward its ventral side, with a marked constriction in the central region, following the shape of intestinal sac (Figs. 3a–c); young specimens with the branches not reaching the distal limit of intestinal sac. Vagina with two sphincters, vaginal tube parallel to the longitudinal axis of body, with posterior portion curved towards to resorbens vesicle, resembling the shape of letter 'J', 162.54 long (Fig. 6d); length of vagina between anterior and posterior sphincters 59.94; maximum wall thickness between anterior and posterior sphincters 22.42. Genital atrium is difficult to observe. Sphincters two, circular, well developed; anterior sphincter symmetrical, 46.86 total width, left 27.85 diameter, right 29.52 diameter; posterior sphincter asymmetrical, 72.64 total width, left 41.48 diameter, right 20.08 diameter (Fig. 6d). Resorbens vesicle semispherical, 97.50–205.37 (151.44, 76.28, n = 2) long, 120.83–213.19 (167.01, 65.31, n = 2) wide (Figs. 6b and 6d); proximal side flattened, thick-walled, placed in space left by posterior constriction of intestinal sac. Conduct connecting vagina and resorbens vesicle 29.76 long, 8.45 wide (measured in holotype) (Fig. 6d). Eggs deposited on general body surface of hosts, mainly on ventral side of pereopods (Fig. 2), never in internal part of abdomen or thoracic sternites; sessile eggs (Figs. 4–6), 496.85–651.90 (577.21, 49.34, n = 11) long, 259.71–400.15 (340.02, 51.24, n = 11) wide; elliptical; ornamentation inconspicuous with a small subapical tuberous projection, non-filamentous (Figs. 2c–g); opercular plates medium-sized, slightly oblique to the longitudinal axis of the eggs (Fig. 2g); plane of fracture slightly oblique (Figs. 2e and 2g).

Male. Testes four, ovoid, medium-sized, with the slightly oblique position; testes surface smooth, without lobes; anterior pair smaller than posterior (Figs. 2a, 2c, and 5d). Anterior pair of testes posterolateral to intestinal sac, slightly oblique to the longitudinal axis of body, more external than posterior pair; left anterior testis 97.09–185.12 (152.72, 23.55, n = 15) long, 62.90–150.04 (99.14, 25.56, n = 15) wide; right anterior testis 78.88–261.96 (159.90, 47.41, n = 18) long, 64.10–207.00 (120.93, 39.18, n = 18) wide (Fig. 5d). Posterior pair of testes always posterior to intestinal sac, slightly oblique to the longitudinal axis of body, more central than anterior pair; left posterior testis 77.48–313.22 (177.69, 63.22, n = 19) long, 148.89–367.35 (242.26, 75.06, n = 19) wide; right posterior testis 75.39–296.14 (174.35, 55.60, n = 21) long, 99.71–397.55 (236.47, 79.17, n = 21) wide (Figs. 3c and 5d). Deferent vessels originate on middle and inner region of both posterior testes (Figs. 6a and 6b), right long, left short, uniting to form a pear-shaped, thick-walled seminal vesicle, 72.43–211.55 (137.37, 42.98, n = 16) long, 32.24–177.56 (69.95, 34.07, n = 16) wide, 14.82 maximum wall thickness (measured in holotype) (Fig. 6b). Prostatic bulb ovoid, 50.26–221.17 (118.92, 44.98, n = 20) long, 46.83–108.59 (74.76, 17.33, n = 20) wide (Fig. 6b). Prostatic vesicle and prostatic glands not observed. Cirrus straight in frontal view (Fig. 7a), slightly curved in lateral view, more curved near base of introvert, with a non-sinuous distal portion (Fig. 7b), 129.89–215.84 (169.70, 23.19, n = 25) long; shaft slightly curved, more curved at distal portion (Fig. 7b), proximal shaft base with a conspicuous thicker rim, directed outwards (Figs. 7a, 7b, and 7d), 113.71–171.43 (148.40, 15.14, n = 16) long, shaft base 41.67–80.39 (53.32, 9.16, n = 17) wide; introvert with fine spines with distal portion of introvert wider than proximal, with a pronounced swelling (Figs. 7a, 7b, 7c, 7e, and 7f), 21.24–49.50 (34.67, 9.65, n = 16) long, 7.26–18.68 (11.34, 3.28, n = 17) introvert base width, 10.44–27.55 (15.82, 4.85, n = 17) introvert maximum width; introvert never observed more extroverted than is shown in Figure 7c. In lateral, there are about 11 spines per row in the introvert (Figs. 7e and 7f). Proximal limit of the introvert with very small teeth-like protuberances (Fig. 7f); region between the proximal limit of introvert and base of proximal spines slightly thick-walled outwards (Figs. 7e and 7f). Ratio between length of introvert and cirrus length 1:0.11–31 (1:0.19, n = 18); ratio between shaft base and cirrus length 1:0.16–0.41 (0.29).

Distribution. Known only for its type locality (Fig. 1).

Remarks

The species described here was compared in more detail with species of *Temnocephala* that occur in Brazil, or those that shared intimately related hosts, or those that share some morphological similarity with the new species, as shown in Tables 1–3.

Temnocephala sp. nov. is more similar to *Temnocephala mertoni* Volonterio, 2009, *Temnocephala talicei* Dioni, 1967a, and *T. axenos* regarding to general body shape. *Temnocephala* sp. nov. differs from *T. mertoni* and *T. talicei* mainly by the presence of four paranephrocytes (*vs.* two paranephrocytes), cirrus slightly curved (*vs.* cirrus straight), two vaginal sphincters (*vs.* one vaginal sphincter), testes without lobes (*vs.* testes lobed), and a large adhesive disk (*vs.* small adhesive disk). In contrast, *Temnocephala* sp. nov. differs from *T. axenos* mainly by having an elliptical body shape (*vs.* rounded body shape), testes without lobes (*vs.* testes slightly lobed), intestinal sac with septations ill-defined (*vs.* conspicuous septations), and by the shape of cirrus and DLSPs.

Compared to *Temnocephala mertoni* and *T. talicei*, *Temnocephala* sp. nov. is larger in several morphological structures, such as adhesive disk length and width, pharynx length and width, vagina length and width, cirrus length, introvert length, and egg length and width (Table 3). *Temnocephala* sp. nov. is larger than *T. axenos* with respect to pharynx length, vagina length and width, prostatic bulb length and width, cirrus length, introvert length, introvert maximum width, and egg length and width (Table 3).

The ratio between body length and adhesive disk length (1:0.21) and the ratio between body width and adhesive disk width (1:0.41) of *Temnocephala* sp. nov. is larger than those found in *T. mertoni* (*vs.* 1:0.14 and 1:0.29, respectively). However, the ratio between body length and adhesive disk length (1:0.28) of *T. axenos* were higher than the new species, while the ratio between body width and adhesive disk width (1:0.36) were lower than

Temnocephala sp. nov. The ratio between body length and adhesive disk length of *T. talicei* were the same found for the new species.

Although the total body length has no taxonomic value (Seixas *et al*. 2010b) we found that the average of body length of *Temnocephala* sp. nov. was higher than *Temnocephala kingsleyae* Damborenea, 1994, *Temnocephala lutzi* Monticelli, 1913 (specimens studied by Amato *et al*. 2005), *T. mertoni, T. axenos*, and *T. talicei*; and the average of body width was higher than *T. lutzi* (specimens studied by Amato *et al*. 2005), *T. mertoni*, and *T. talicei* (Table 3).

The DLSPs of the new species is more similar in shape with *Temnocephala pignalberiae* Dioni, 1967b, *T. mertoni*, and *T. talicei*, by being elongated with wider portion anteriorly, and by having the excretory pore in a subterminal level displaced toward to the inner side of the plate (evidenced by a low value of D measure, Table 2). However, the DSLPs of the *T. pignalberiae* differ from the new species by having the distal boundary of the plate visible in dorsal view (*vs.* distal boundary of the plates not visible in dorsal view), and by having approximately twice the length of the DLSPs of the *Temnocephala* sp. nov. (Table 2). The DLSPs of *T. talicei* differs from the new species by the presence of a concave proximal boundary of the plate (*vs.* proximal boundary slightly convex or straight). Although the shape of DLSPs of *T. mertoni* and *T. talicei* is similar to the new species the authors did not mentioned if they used scanning electron microscopy to draw the DLSPs, as well as they did not present the measures of the length of DLSPs. In addition, *Temnocephala* sp. nov. differs from most of species studied by Seixas *et al*. (2015c) by having the excretory pore in a more proximal position (evidenced by a low value of D measure, Table 2).

Temnocephala sp. nov. is more similar in shape of the cirrus with *T. cyanoglandula*, *T. lutzi*, *T. mertoni*, and *T. axenos*. However, the cirrus of the new species differs from *T. cyanoglandula* and *T. lutzi* by having both the sides of the shaft curved (*vs.* one of the sides of the shaft nearly straight and the other curved); differs from *T. cyanoglandula*, *T. lutzi*, and *T. axenos* by having the region between proximal limit of introvert and base of proximal spines thick-walled outwards (*vs.* without a conspicuous thickening in the proximal wall of introvert); and differs from *T. cyanoglandula* by the presence of a thicker rim at shaft base (*vs.* absence of a conspicuous thickening at shaft base). The cirrus of *T. mertoni* is similar to the cirrus of the new species by the presence of a conspicuous thickening at shaft base and by having the region between the proximal limit of introvert and base of proximal spines thick-walled outwards. However, the cirrus of *T. mertoni* is straight and has a sinuous distal portion of the shaft, which is completely different to the new species (*vs.* distal portion of the shaft non-sinuous, slightly curved, more curved at distal portion). Also, the cirrus of *T. pignalberiae*, *Temnocephala iheringi* Haswell, 1893, and *T. kingsleyae* are different from the cirrus of the new species by being straight (*vs*. slightly curved). The new species has the swelling in the distal region of introvert more pronounced than in *T. lutzi*, *T. mertoni* and *T. axenos*, as evidenced by the higher average of introvert maximum width (Table 1).

The number of spines per row in the introvert of the new species (11 spines) is larger than *T. pignalberiae* (without spines), *T. iheringi* (seven spines), *Temnocephala rochensis* Seixas, Amato & Amato, 2010a (six spines), *T. mertoni* (10 spines), *T. axenos* (10 spines), and *T. talicei* (10 spines); and is lower than *T. kingsleyae* (12 spines) and *T. lutzi* (12–15 spines). Yet, regarding to size, the cirrus of the new species is longer than the cirrus of *T. pignalberiae*, *T. kingsleyae*, *T. lutzi*, *T. mertoni*, *T. axenos*, and *T. talicei*, and shorter than the cirrus of *T. cyanoglandula*, *T. iheringi*, and *T. rochensis* (Table 3).

The set of cirrus characteristics of the *Temnocephala* sp. nov. is probably found only in this species due to: both the sides of the cirrus are slightly curved, shaft base with a thicker rim, shaft slightly curved with a non-sinuous distal portion, region between proximal limit of introvert and base of proximal spines thick-walled outwards, and distal portion of introvert with a pronounced swelling.

The eggs of *Temnocephala* sp. nov. differs from those found for *T. axenos* by having a plane of fracture slightly oblique (*vs*. longitudinal) and by the presence of medium-sized opercular plates (*vs*. large opercular plates). Although the ornamentation of eggs in *Temnocephala* sp. nov. is not so conspicuous, this tuberous ornamentation is probably found only in this species, since in *T. cyanoglandula*, *T. pignalberiae, T. rochensis, T. lutzi*, *T. mertoni, T. axenos* and *T. talicei* there are delicate filaments as ornamentation.

Some structures were difficult to observe in most of the studied specimens, such as, seminal receptacles, vagina and genital atrium.

Discussion

Monticelli (1899) described the first temnocephalan species in Brazil, *T. axenos* commensal with *Aegla laevis* (Latreille), from Blumenau, State of Santa Catarina. Also, *T. axenos* was the first temnocephalan species reported to the State of Paraná, commensal with *A. castro* in Curitiba (Pérez-González 1949). Nevertheless, it is probable that the host species studied by Monticelli (1899) was identified incorrectly since *A. laevis* does not occur in the Eastern Andes (Bond-Buckup 2003). Moreover, in the study of Boos *et al*. (2012) it was reported the occurrence of *Aegla jarai* Bond-Buckup & Buckup, in Blumenau, State of Santa Catarina, which could suggest that the type-host for *T. axenos* is *A. jarai*. Another record of a temnocephalan species in State of Paraná was *Temnocephala* sp. in association with *Aegla castro* from Varanal Stream, Tibagi River Basin (Guerrero-Ocampo & Kishino 2008).

Temnocephala sp. nov. is the first species commensal with *A. lata*, as well as the first temnocephalan species described in the State of Paraná. *Aegla lata* was firstly reported at Ponta Grossa, State of Paraná (Bond-Buckup & Buckup 1994), and since then, it has been mentioned as “not found in their restricted areas of occurrence anymore” by Pérez-Losada *et al*. (2004: 768), and later assigned as “extinct in the wild” by Pérez-Losada *et al*. (2009: 697). Moreover, even after this species has been found again in the Mata dos Godoy State Park, in Londrina, State of Paraná (Galves *et al*. 2007), it was considered as “critically endangered” by Santos *et al*. (2017: 3). However, given that *A. lata* has a relatively wide distribution area (from Ponta Grossa to Londrina) and the species occurs at least in one protected area (Mata dos Godoy State Park), the threat status of this species needs to be reviewed. As *A. lata* is found only in a restricted area of occurrence, it is possible that *Temnocephala* sp. nov. is a microendemic species, that is, those species recorded for only one locality (Martínez-Aquino *et al*. 2014a). However, the lack of occurrence records of temnocephalan species in the State of Paraná may be explained more by the scarce sampling of aeglid hosts (and symbiotic fauna) than by the absence of temnocephalans in the region.

To date, *Hydromedusa tectifera* Cope (a chelonian) and *Aegla neuquensis* are the hosts with the highest temnocephalan species richness, with four *taxa* each (Martínez-Aquino *et al*. 2014a; Ponce de León *et al*. 2015). On the other hand, *Temnocephala chilensis* (Moquin-Tandon, 1846), and *T. talicei* are associated with a great variety of aeglids, showing a high degree of specificity for aeglid hosts (Martínez-Aquino *et al*. 2014a; Volonterio 2009).

Host specificity has been reported for temnocephalans so that each species stablish association with a single host species or with phylogenetically related hosts that have a shared evolutionary history (Martínez-Aquino *et al*. 2014b). In the same way, the shape and dimension of dorsolateral ‘excretory’ syncytial epidermal plates (DLSPs), as well as the position of an excretory pore within the DLSPs of each temnocephalan species was reported in the literature as characters that follow a specific pattern for each host group (Seixas *et al*. 2015c). Our results showed that the morphological pattern of DLSPs of *Temnocephala* sp. nov. are similar to those found in *T. pignalberiae*, *T. mertoni* and *T. talicei*, all of them commensal with crustacean hosts.

Despite the similarities within host group, the DLSPs are very specific structures that vary between species (Damborenea & Cannon 2001). Several authors reported that the DLSPs are important characters of taxonomic value for species identification of *Temnocephala* (Damborenea & Cannon 2001; Amato *et al*. 2006; Volonterio 2007; Volonterio 2009). Even so, many studies did not describe in detail the DLSPs and did not provide SEM images for future comparisons. For instance, Volonterio (2007) and Volonterio (2009) represent the DLSPs of the specimens they studied, and they referred that the DLSPs of *T. talicei* were identical to those found in *T. mertoni*. However, the authors did not inform if they used SEM images to make their drawings. Therefore, because the DLSPs have unique characteristics between different species (Seixas *et al*. 2015c), and by having little variation within the same species, the detailed study and morphometry of this plates are of great importance for the identification of species, especially those with similar cirrus morphology or those that share the same hosts group (Seixas *et al*. 2015c).

Although the genital system is homogeneous among the Neotropical temnocephalans (Damborenea & Cannon 2001), the morphology of the cirrus remains the most valuable taxonomic character to discriminate temnocephalan species (Damborenea & Cannon 2001; Volonterio 2007), since it is a non-deformable structure with constant general morphology (Seixas *et al*. 2015c). Another structure that has been reported to be common in all Neotropical temnocephalans is the red pigmentation in the eyes (Damborenea & Cannon 2001). However, in the redescription of *T. iheringi* made by Seixas *et al*. 2010b the authors stated they do not found red pigmentation in the specimens they studied and referred that *T. iheringi* is the only species that have no red pigmentation.

Some structures are difficult to observe and are often not mentioned in descriptions (Damborenea & Cannon 2001), for instance, nervous system, genital atrium, secretion bodies, esophageal glands, oviduct and conduct connecting vagina and resorbens vesicle. Furthermore, even the whole genital complex was reported as difficult to observe by Amato *et al*. (2003) for *T. cyanoglandula*. Here, genital atrium, vagina and rhabditogenic tracts were the features less observed in the specimens studied. The seminal receptacles were also mentioned as structures difficult to observe, as in the specimens of *T. axenos* studied by Volonterio (2007), and in *T. lutzi* and *T. kingsleyae* studied by Damborenea (1994). Similarly, seminal receptacles were rarely observed in specimens of *Temnocephala* sp. nov. In addition, we observed that the red pigments of the eyespots dissolve when specimens are fixed in ethanol.

Although new methodologies have been developed and new characters of taxonomic importance have been chosen for the *Temnocephala* study (Seixas *et al*. 2015c), there are still many species to be described in the Neotropical region (Martínez-Aquino *et al*. 2014a). Thus, the wide distribution range of this genus, extending from Mexico to Chile and Argentina, plus the increasing of number of described species in recent years (Martínez-Aquino *et al*. 2014a) may suggest that the diversity of these symbiotic organisms is still very much underestimated. In addition, although the host diversity associated with *Temnocephala* species in the Neotropics is high, there remain a large number of potential hosts that have not been studied regarding the fauna of associated metazoan symbionts (Martínez-Aquino *et al*. 2014a). Even though nineteen species of Neotropical temnocephalans has been described in Brazil, it is likely that these numbers are far from demonstrating both the species richness and the ectocommensal-host association diversity within this group. Moreover, it is interesting to note that some species described in Brazil have very distinct type hosts, occurrence areas, and life habits, such as *T. euryhalina*, which is associated with euryhaline gastropods present in large rivers, and *T. stoneflyi*, which is associated with small insect larvae from small streams.

In summary, we described a new species of *Temnocephala* in association with a host with restricted distribution, *A. lata*. Also, the type locality of the new species is located in an area of State of Paraná where there are scarcely occurrence records for aeglids. Thus, we reinforce the importance to continue inventory work with temnocephalans, especially those species whose hosts are in some degree of threat and have restricted area of occurrence, such as *A. lata*. Moreover, since most of temnocephalans diversity remains yet to be described (Martínez-Aquino *et al*. 2014a), we encourage the study of these animals using distinct methods of analysis, such as histology, SEM, detailed morphometry of DLSPs, to better characterize the morphology of these freshwater symbiotic Platyhelminthes.

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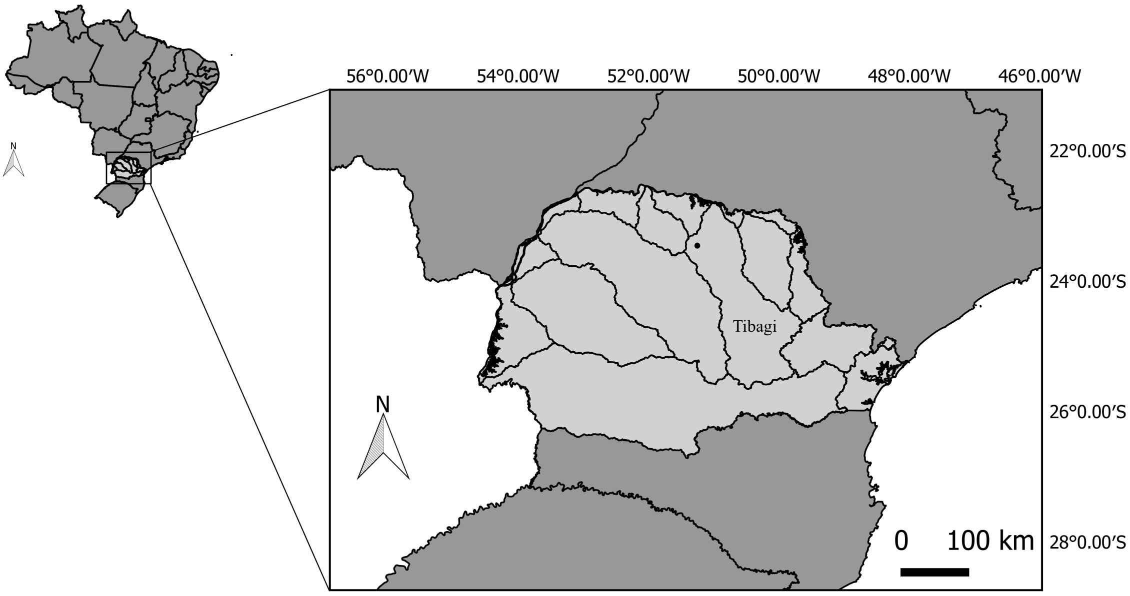

FIGURE 1. Type-locality of *Temnocephala* sp. nov. Sampling point of the aeglid's population from Bule Stream (black circle), Tibagi River Basin (Tibagi).

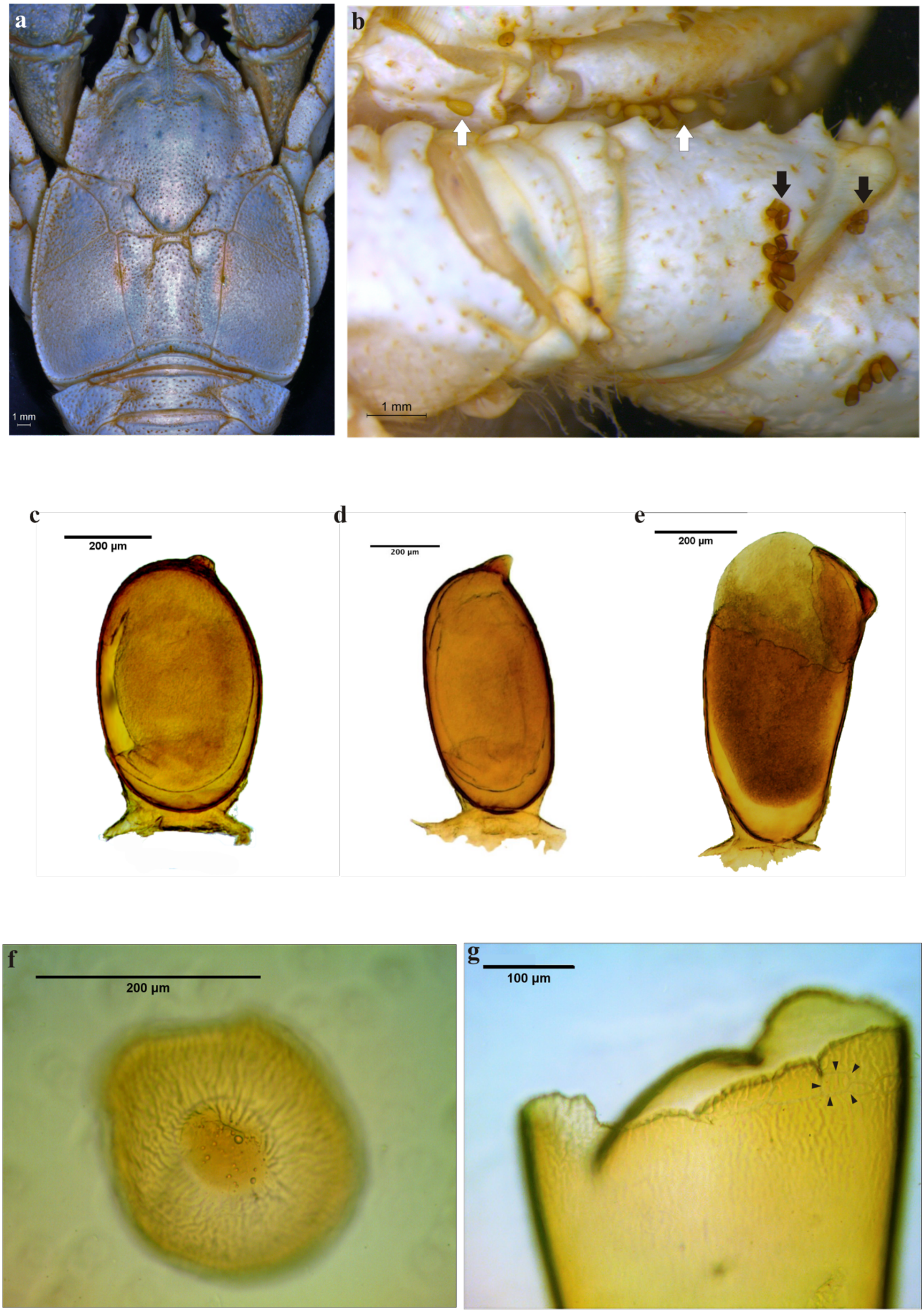

FIGURE 2 (a–g). Host, site of infestation and eggs of *Temnocephala* sp. nov. (a) *Aegla lata* in dorsal view; (b) ventral view of the left chelipod showing hatched (black arrows) and unhatched (white arrows) eggs; (c and d) unhatched eggs, showing the tuberous ornamentation; (e) egg during hatching, showing the plane of fracture slightly oblique; (f) top of the egg in dorsal view; (g) hatched egg showing the medium-sized opercular plates (black head arrows).

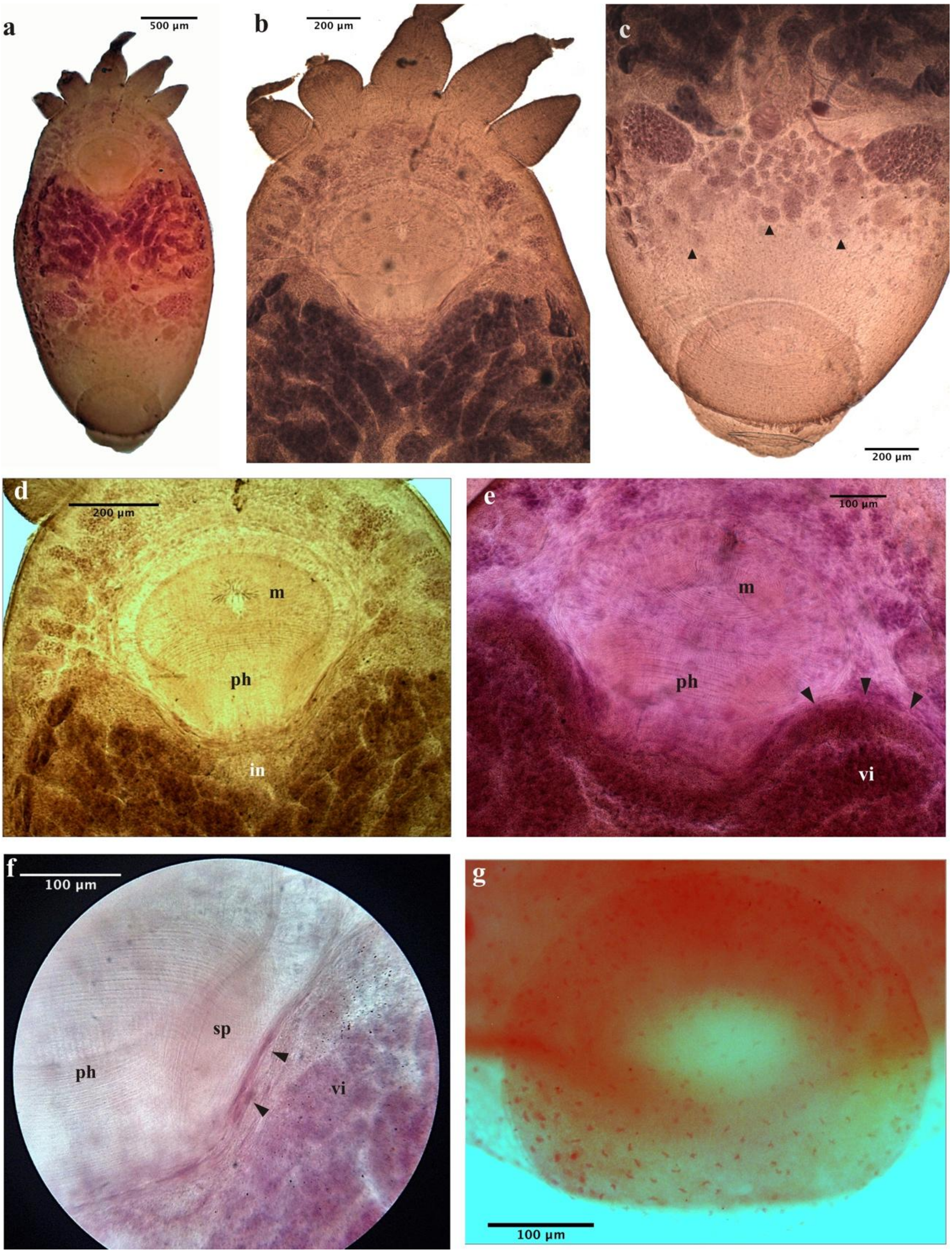

FIGURE 3 (a–g). General body shape of *Temnocephala* sp. nov., ventral view. (a–d, f, g) Specimens stained with Delafield's hematoxylin; (e) specimen stained with chloride carmine. (a) whole ventral view; (b) anterior portion of body; (c) posterior portion of body, showing tentacular glands scattered posterior to posterior testes (black head arrows); (d–e) pharynx at anterior portion of body, with anterior portion wider than posterior, mouth surrounded by a strong muscular sphincter; (e) anterior limit of intestinal sac (black head arrows); (f) posterior portion of pharynx showing esophageal glands (black head arrows); (g) secretion bodies of irregular shape in adhesive disk. (in) Intestinal sac; (ph) pharynx; (m) mouth; (vi) vitellaria.

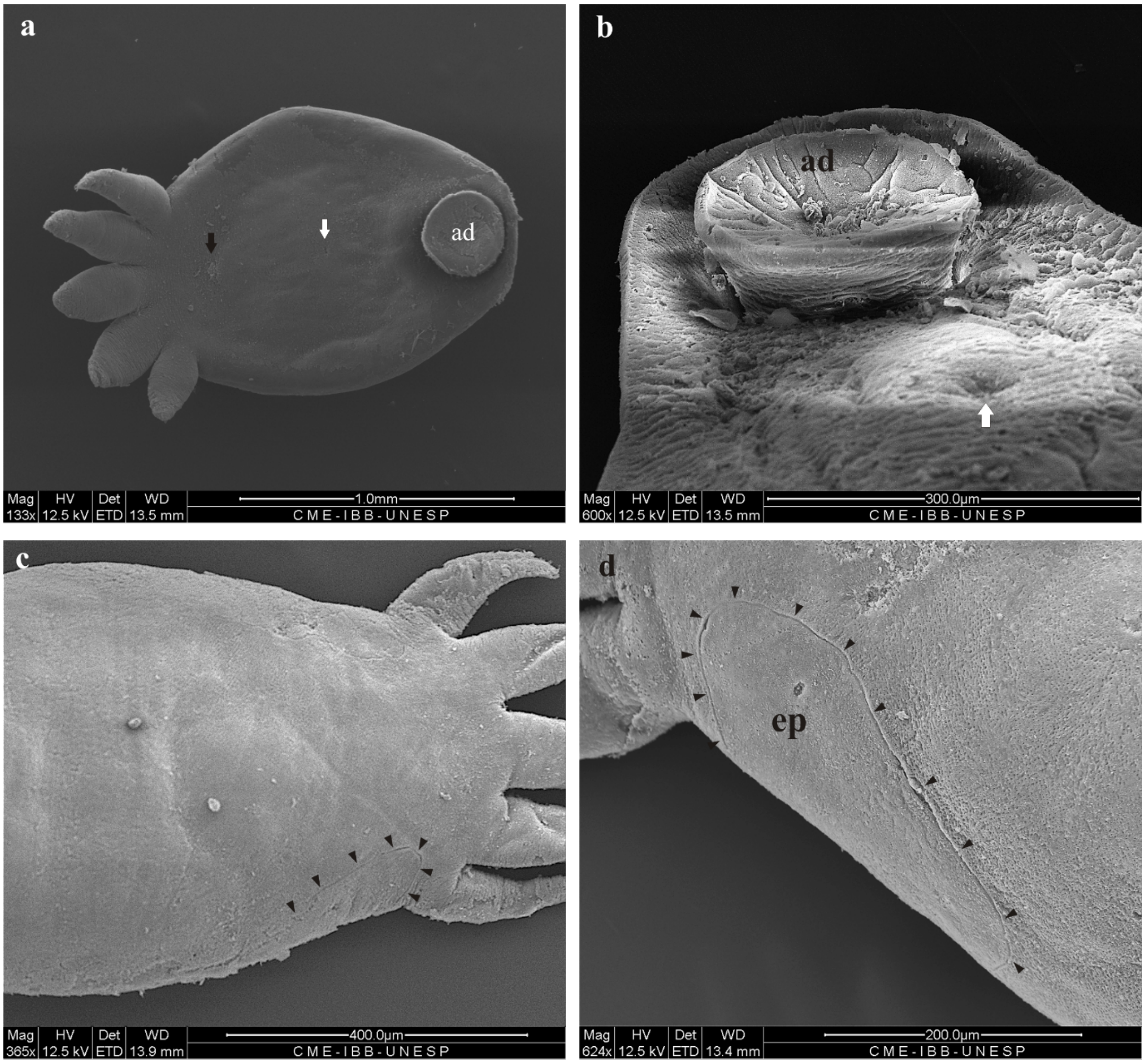

FIGURE 4 (a–e). Scanning electron microscopy of *Temnocephala* sp. nov. (a–b) Ventral side of body showing the adhesive disk perpendicular to the longitudinal axis of body, showing gonopore (white arrow) and mouth (black arrow); (c) dorsal side of body, showing the anterior and internal boundaries of the right DLSP (black head arrows); (d) boundaries of the left DLSP (black head arrows), showing the excretory pore toward the inner side of the DLSP; (e) boundaries of the right DLSP (black head arrows), showing the excretory pore. (ad) Adhesive disk; (ep) excretory pore.

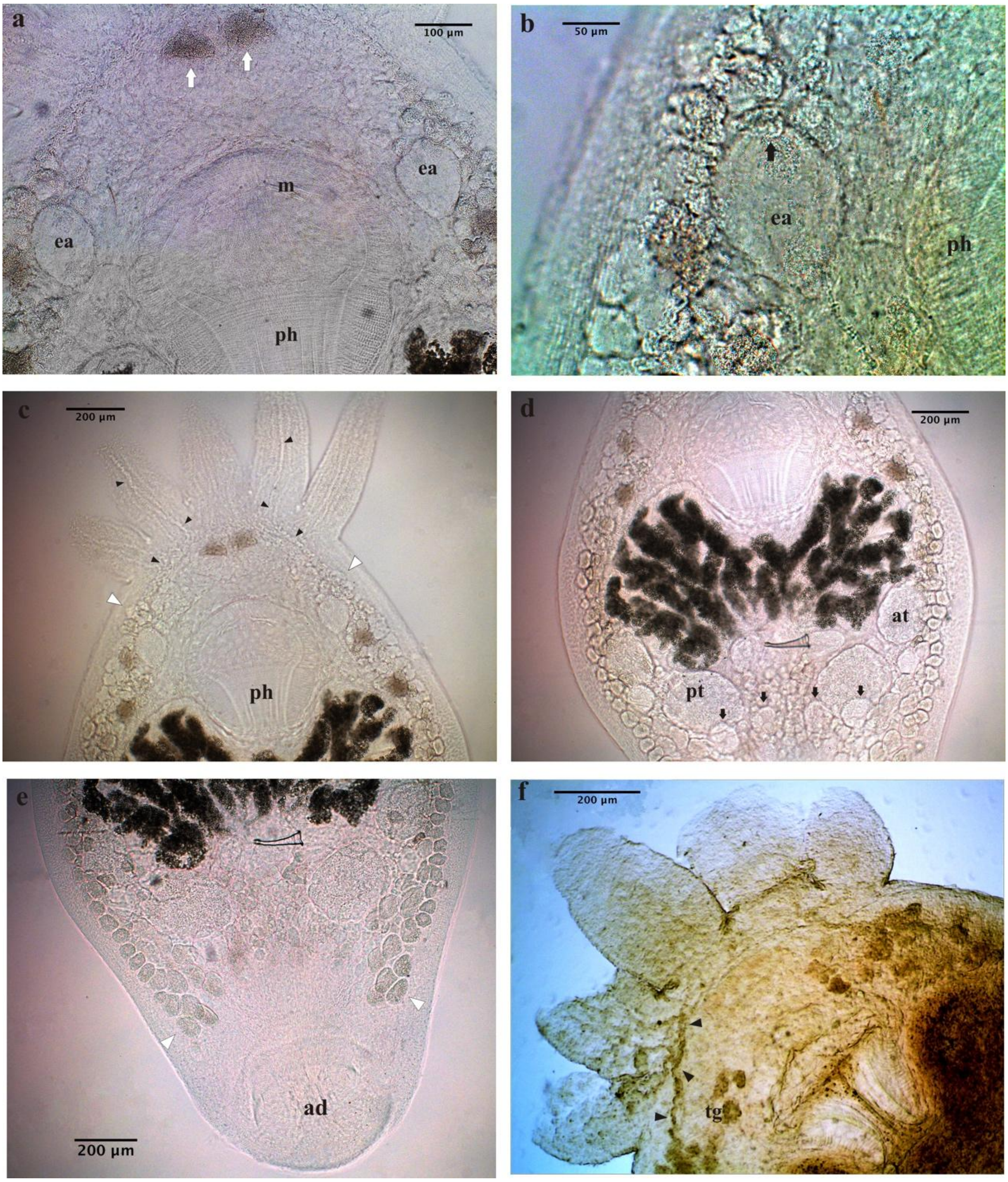

FIGURE 5 (a–f). Glands of *Temnocephala* sp. nov. (a–e) specimens stained with Delafield's hematoxylin and cleared with lactic acid; (f) specimens stained with Delafield's hematoxylin and cleared with eugenol oil. (4a) Two elliptical, excretory ampullae at level of mouth, with detail for the two Haswell's cells (white arrows); (b) right excretory ampullae showing the excretory pore directed anteriorly (black arrow); (c) anterior portion of body showing the rhabdite tracts (black head arrows) and the anterior limit of rhabdites (white head arrow); (d) middle portion of body showing the rhabdites along the lateral fields, with detail for the four paranephrocytes (black arrows); (e) posterior extension of rhabdites (white head arrows); (f) anterior region showing tentacular glands (tg) and rhabdite tracts (black head arrows). (ad) Adhesive disk; (at) anterior testes; (ea) excretory ampullae; (m) mouth; (ph) pharynx (ph); (pt) posterior testes; (tg) tentacular glands.

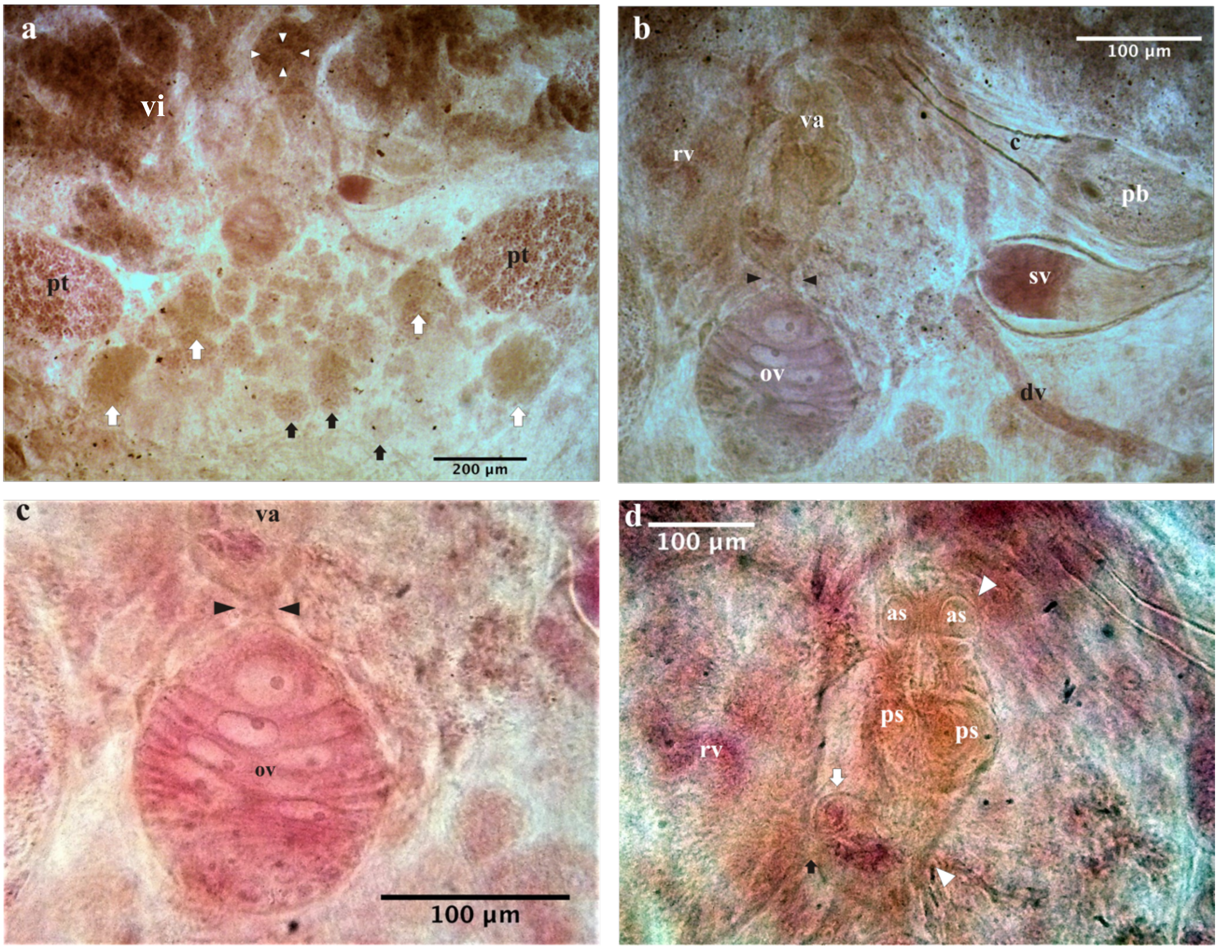

FIGURE 6 (a–d). Reproductive complex of *Temnocephala* sp. nov., in ventral view, stained with Delafield's hematoxylin and cleared with eugenol oil. (a) Reproductive complex showing the posterior portion of vitellaria, gonopore (white head arrows), posterior testes without lobes, and four paranephrocytes (white arrows), stained in light brown; (b) female and male reproductive complex in detail; (c) ovary opening into posterior portion of vagina just behind seminal receptacles by a short oviduct (black head arrows; (d) vagina curved towards to resorbens vesicle, showing the anterior and posterior limits (white head arrows), anterior symmetrical sphincter, posterior asymmetrical sphincter, conduct connecting vagina and resorbens vesicle (black arrow), and at least one seminal receptacle (white arrow). (as) anterior sphincter; (c) cirrus; (dv) deferent vessels; (ov) ovary; (pb) prostatic bulb; (ps) posterior sphincter; (pt) posterior testes (rv) resorbens vesicle; (sv) seminal vesicle; (va) vagina; (vi) vitellaria.

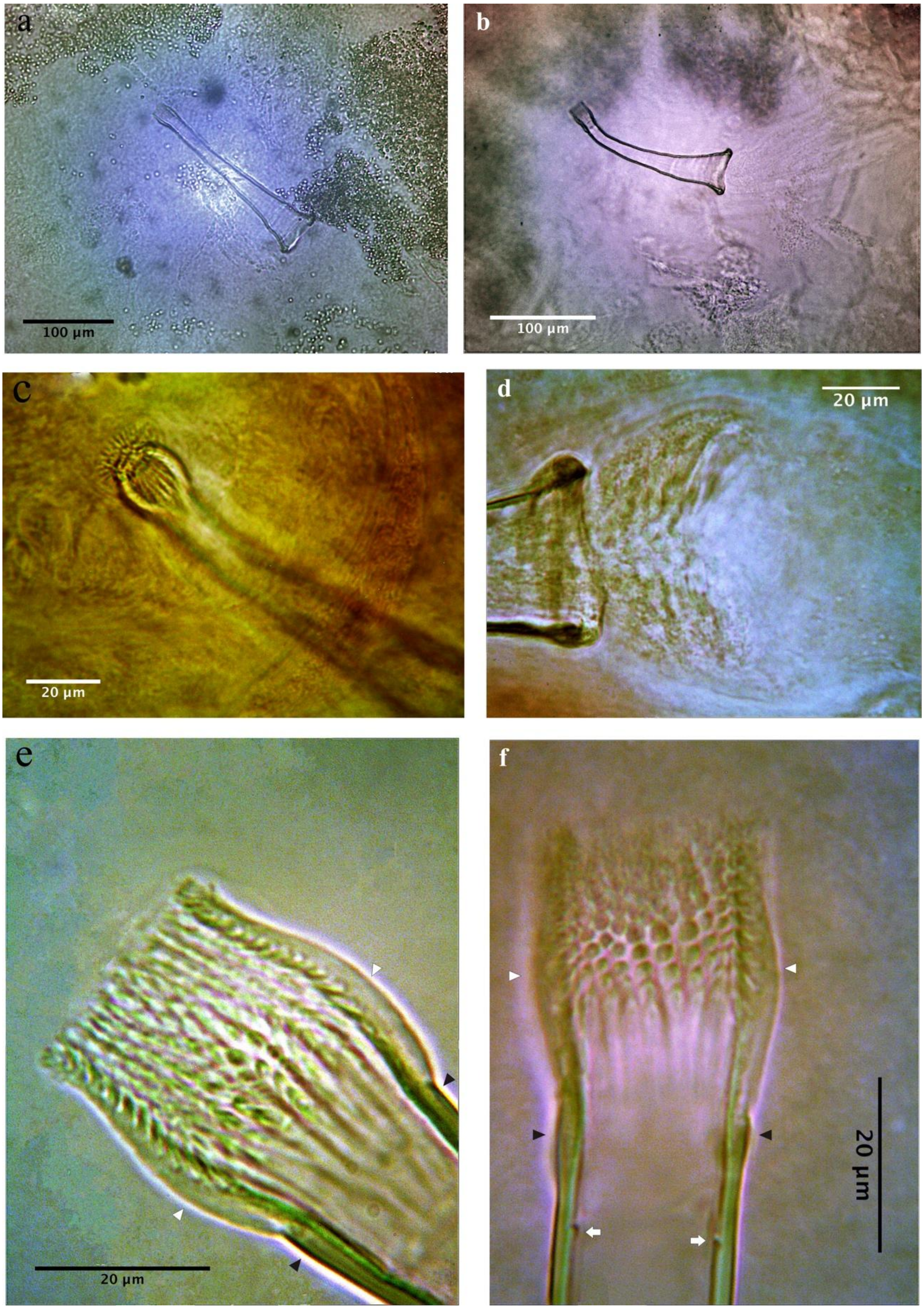

FIGURE 7 (a–f). Morphology of the cirrus of *Temnocephala* sp. nov. (a–f) Preparations with Delafield's hematoxylin and lactic acid. (a) Cirrus straight in frontal view; (b) cirrus slightly curved in lateral view; (c) introvert of the cirrus with fine spines, extroverted; (d) shaft base with a conspicuous thicker rim, directed outwards; (e) lateral view of a dissected cirrus, showing the rows of spines in the introvert and the swelling at distal portion of introvert (white head arrows); (f) lateral view of a dissected cirrus, with detail for the swelling at distal portion of introvert (white head arrows), small teeth-like protuberances at proximal limit of introvert (white arrows), and the thickening at region between the base of spines and proximal limit of introvert (black head arrows).

TABLE 1. Species of *Temnocephala* and the characteristics shared with *Temnocephala* sp. nov. Hyphens mean the characteristics not informed in the literature. AP–State of Amapá, MS–State of Mato Grosso do Sul, MT–State of Mato Grosso, PA–State of Pará, PR–State of Paraná, RS–State of Rio Grande do Sul, SC–State of Santa Catarina, SP–State of São Paulo.

Species	***Temnocephala axenos***	***Temnocephala cyanoglandula***	***Temnocephala iheringi***	***Temnocephala kingsleyae***	***Temnocephala lutzi***	***Temnocephala mertoni***	***Temnocephala pignalberiae***	***Temnocephala rochensis***	***Temnocephala talicei***	***Temnocephala* sp. nov.**
General shape of DLSPs	small, elliptical	elliptical	elliptical	-	elliptical	elongated	elongated	large, rounded	elongated	elongated
Position of excretory pore within the DLSP	subterminal level, inner side of the plate	equatorial level, inner side of the plate	central	-	central	subterminal level, inner side of the plate	subterminal level, inner side of the plate	equatorial level, outer side of the plate	subterminal level, inner side of the plate	subterminal level, inner side of the plate
Number of paranephrocytes	4	-	4	-	2	2	2	4	2	4
Shape of cirrus	slightly curved	slightly curved	short, straight	slightly curved	short, slightly bent anteriorly	straight, with sinuous distal portion	straight, unarmed, without spines	long, slightly curved	straight	slightly curved
Thickening in the proximal wall of introvert	absent	-	base of swelling	-	-	present	-	-	absent	present
Number of vaginal sphincters	2, proximal asymmetrical, distal symmetrical	-	1	1	not observable	1	1	1	1, markedly asymmetrical	2, proximal asymmetrical, distal symmetrical

Table 1 continued.

Ornamentation of eggs	subapical, short filament	-	-	-	subterminal filament	subterminal, medium-sized filament	delicate, long subterminal filament	long, apical, filament	subapical filament	subapical, tuberous ornamentation
Plane of fracture	horizontal	apparently horizontal	-	-	-	oblique	-	-	oblique	slightly oblique
Distribution	Brazil (PR, SP, SC, RS), Argentina, Uruguay	Brazil (RS)	Brazil (RS, MS), Argentina, Uruguay	Brazil (AP)	Brazil (PA, SP, RS), Uruguay, Peru	Uruguay	Brazil (SP, MT), Argentina, Uruguay	Brazil (RS), Uruguay	Argentina, Uruguay, Paraguay	Brazil (PR)
Host group	crustacean	crustacean	mollusk	crustacean	crustacean	crustacean	crustacean	mollusk	crustacean	crustacean
Reference	Volonterio (2007)	Amato *et al.* (2003)	Seixas *et al.* (2010b)	Damborenea (1994)	Amato *et al*. (2005)	Volonterio (2007)	Amato *et al.* (2010)	Seixas *et al.* (2010a)	Volonterio (2009)	Present study

TABLE 2. Comparison of measurements of the dorsolateral 'excretory' syncytial epidermal plates between *Temnocephala* sp. nov. and the species studied by Seixas et al. (2015c).

Species	**A**	**B**	**C**	**D**	**E**	**A+B**	**C+D**	**X Ratio**	**Y Ratio**
Temnocephala caddisflyi	79.00	282.67	130.67	45.00	7.67	361.67	175.67	1:5	1:7.2
Temnocephala curvicirri	73.58	222.20	165.54	26.68	-	295.78	192.22	1:7.3	1:7.4
Temnocephala cyanoglandula	136.55	147.89	150.84	60.92	17.65	284.44	211.76	1:9.2	1:10.3
Temnocephala haswelli	95.09	132.66	65.03	139.88	13.58	227.75	204.91	1:9.5	1:6.5
Temnocephala iheringi	87.95	118.47	53.21	78.71	23.49	206.42	131.92	1:11.8	1:10.3
Temnocephala lutzi	73.47	66.53	41.08	46.27	27.20	140.00	87.35	1:6.6	1:7
Temnocephala minutocirrus	98.80	86.55	57.83	45.58	8.43	185.35	103.41	1:5.6	1:7
Temnocephala pignalberiae *	165.00	356.25	127.50	76.88	23.13	521.25	204.38	1:5	1:9.1
Temnocephala pignalberiae **	191.72	473.96	209.46	84.62	142.01	665.68	294.08	1:3.7	1:5.7
Temnocephala rochensis	182.34	188.22	80.06	150.65	78.75	370.56	230.71	1:7.5	1:6.7
Temnocephala trapeziformis	70.90	29.48	145.52	66.79	-	100.38	212.31	1:17.3	1:6.7
Temnocephala sp. nov.	113.745	251.07	55.04	34.69	-	364.81	88.16	1:5.5	1:13.8

Abbreviations. A, distance of the anterior portion of the DLSP from the excretory pore; B, distance of the posterior portion of the DLSP from the excretory pore; C, width of the external limit of the DLSP from the excretory; D, width of the internal limit of the DLSP from the excretory pore; E, length of the portion exceeding the tentacles' limit with the body; (A+B), total length of the DLSP; (C+D), total width of the DLSP; X Ratio, total body length/total length of the DLSP; Y Ratio, total body width/total width of the DLSP.

* Population from Poconé, State of Mato Grosso.

** Population from Bebedouro, State of São Paulo.

TABLE 3. Average measurements of morphological structures of species of *Temnocephala* compared in this study. Hyphens mean the characteristics not informed in the literature. When the average value is not informed in the literature, we present the minimum and maximum numbers.

Measurement	*Temnocephala axenos*[1]	*Temnocephala cyanoglandula*[2]	*Temnocephala iheringi*[3]	*Temnocephala kingsleyae*[4]	*Temnocephala lutzi*[5]	*Temnocephala mertoni*[6]	*Temnocephala pignalberiae*[7]	*Temnocephala rochensis*[8]	*Temnocephala talicei*[9]	*Temnocephala* sp. nov.
Body length	1754.4	2610	2437	1840	931	1260.6	2490	2800	1547	1996.85
Body width	1439.6	2180	1358	1330	605	639.1	1680	1540	1039.3	1221.19
Adhesive disk length	479.3	606	581	490	244	170.4	597	689	303.2	377.09
Adhesive disk width	509.6	606	701	280	244	185.9	603	799	323.3	458.25
Pharynx length	315.1	257	445	250–360	205	207.4	499	-	268.5	407.77
Pharynx width	447.9	366	409	330–410	233	238.4	441	-	313.3	446.07
Ovary length	-	-	109	60–120	57	-	107	-	-	147.58
Ovary width	-	-	169	40–120	55	-	110	-	-	119.5
Vagina length	70.4	-	163	-	-	74.6	107	128	119.1	162.54 *
Vagina width	49.3	-	84	48–94	-	48.7	83	75	50.8	72.64 *
Prostatic bulb length	107.9	182	182	20–290	55	108.3	160	240	-	118.92
Prostatic bulb width	67.9	88	169	80–140	44	82.4	92	145	-	74.76
Cirrus length	141.4	256	193	160	96	138	105	243	125.5	169.70

Table 3 continued.

Shaft length	-	224	140	-	68	-	85	204	-	148.40
Shaft base	-	64	122	58	37	46	34	84	60.4	53.32
Introvert length	23.9	31	53	23	26	27.2	18	39	26.3	34.67
Introvert base	-	-	22	17	-	-	13	20	-	11.34
Introvert maximum width	11.9	-	35	21	10	14.1	15	25.5	18.2	15.82
Egg length	281	611 ** 782 ***	-	-	343	504.5	-	477	441.7	577.21
Egg width	255.6	-	-	-	201	237.9	-	247	219.5	340.02

[1] Volonterio (2007).
[2] Amato *et al*. (2003).
[3] Seixas *et al*. (2010b).
[4] Damborenea (1994).
[5] Amato *et al*. (2005).
[6] Volonterio (2007).
[7] Amato *et al*. (2010).
[8] Seixas *et al*. (2010a).
[9] Volonterio (2009)
* Measurements from holotype.
** Measurement from sessile eggs.
*** Measurements from pedunculate eggs.

CAPÍTULO 2

Stratiodrilus circensis (Polychaeta, Histriobdellidae), a widely distributed ectocommensal of freshwater decapods: morphological variations from seven populations

Artigo a ser submetido ao periódico *Zootaxa*

Stratiodrilus circensis **(Polychaeta, Histriobdellidae), a widely distributed ectocommensal of freshwater decapods: morphological variations from seven populations**

Abstract

Stratiodrilus circensis is widely distributed in South America when compared to other species of the genus. Also, this species was reported to occur in association with several decapod hosts from Brazil, Argentina, and Chile. As this species has a wide distribution and a variety of associated hosts, plus the lack of morphological studies, the present study aims to evaluate the characters used to diagnose *S. circensis* as well as to assess if morphological variations may indicate the existence of cryptic species. Over the period of August 2014 to June 2017 seven populations of aeglid hosts, belonging to four river basins from Southern Brazil were collected: populations from the streams Couro, Jacutinga, Bule, and tributary of Preto River (Tibagi River Basin); from Ema stream (Pirapó River Basin); from Pinhão river (Iguaçu River Basin); and from Lageadão River (Ivaí River Basin). The specimens of *S. circensis* were collected using protocols to prevent the body contraction. They were mounted in permanent and temporary slides and photomicrographed with an image system to take the measurements of morphological structures. We analyzed the morphometric data with a Principal Component Analysis. Then, we selected the morphometric variables that explained the most of variation, and we compared the data of five populations for each of these variables. Based on information available in the literature, the specimens collected was indicated to be *S. circensis*. However, our findings showed morphological variations concerning shape and size in the extension of the jaw apparatus in relation to the first trunk segment, length of antennae, lengths of lateral and posterior cirri, length of adjacent conical ventral lobes, and length of tubercles. Among all morphometric variables, the Principal Component Analysis showed that most of the variation was explained by the lengths of antennae and cirri. Also, for five populations we found statistic differences regarding the lengths of all cirri and antennae. Our results reported new morphological variations for *S. circensis*, as well as showed that some structures seem to be retractile which, in turn, should be considered cautiously. Also, morphological analyzes suggested the existence of at least three morphologically distinct populations of *S. circensis.* These populations are geographically isolated and are associated with three host species. Besides, based on the wide distribution of *S. circensis*, morphological variation, and the variety of hosts associated we suggest that the populations studied herein constitute a set of cryptic species.

Key words: *Aegla*, freshwater polychaetes, Histriobdellids, Southern Brazil.

Introduction

Histriobdellidae Vaillant, 1890, is a highly specialized family of small ectocommensal polychaetes that make interactions with marine and freshwater isopod and decapod crustaceans (Steiner & Amaral 1999). They differ from other polychaetes mainly by lacking eyes, chaetae, true parapodia, and aciculae (Steiner & Amaral 1999).

Moreover, these histriobdellids have some features that evolved from the Errantia pattern (Steiner & Amaral 1999) and contribute to their typical symbiotic way of life, such as, a small vermiform body shape; head conspicuous, clearly separated from rest of body; coelom reduced; sexes separated with sexual dimorphism when mature; adhesive glands at the posterior end of body; and male copulatory organ (Vila & Bahamonde 1985). Within Histriobdellidae, *Stratiodrilus* Haswell, 1900, is the most speciose genus (11 known species) (Rosa *et al*. 2018). *Stratiodrilus* is a little studied genus. The first species was described in the late 19th century—*Stratiodrilus tasmanicus* Haswell, 1900—and the last was described more than a decade ago—*Stratiodrilus brevicirrus* Amato, Daudt & Amato, 2004.

This genus successfully invaded continental waters before the breakup of Gondwanna continent (Glasby & Timm 2008), becoming well distributed in South hemisphere, being found in South America, Madagascar, and Australia (Haswell 1900). Some species of *Stratiodrilus* have a restricted area of occurrence. *Stratiodrilus arreliai* Amaral & Morgado, 1997, for example, is found only in association with *Aegla perobae* Hebling & Rodrigues which, in turn, is exclusive to the Peroba Grot, Paraná River Basin. However, *Stratiodrilus circensis* Steiner & Amaral, 1999 was indicated to occur on several hosts and geographical localities in Brazil, Argentina, and Chile (Daudt & Amato 2007). Furthermore, the wide distribution plus the variety of hosts reported from different river basins suggest that *S. circensis* is not a single species (Rosa *et al*. 2018).

The species of *Stratiodrilus* were historically differentiated by qualitative and quantitative features, such as, the number and pattern of segmentation of lateral cirri; number of tubercles located in the posterior locomotor appendages; and by the extension of the jaw apparatus in relation to the first trunk segment (see Vila & Bahamonde 1985; Amaral & Morgado 1997; Steiner & Amaral 1999; Amato 2001; Amato *et al*. 2004). In addition, the adjacent lobes associated to posterior cirri were reported to be taxonomically important by Steiner & Amaral (1999). However, most of descriptions did not present detailed morphometric studies in order to observe possible morphological variations in their populations. Therefore, there is a need for additional studies that provide drawings with high quality to observe small structures, such as adjacent lobes and tubercles.

Stratiodrilus circensis may be identified by the presence of the posterior cirri associated with an adjacent conical, ventral lobe; two pairs of tubercles; and the absence of anal conical, ventral lobes (Steiner & Amaral 1999). Few studies reported morphological variation in species of *Stratiodrilus*, with mention to the study of Daudt & Amato (2007), which found differences in size of antennae, cirri, body, adjacent lobes, and tubercles in the specimens they studied.

However, the characters used to diagnose species of *Stratiodrilus* do not allow precise and safe identification and the measurements presented in descriptions are usually inaccurate and are referred to as proportions of other parts of the body. Thus, taking into account the possibility of *S. circensis* not being a single species, our objectives were (1) to assess the characters used to diagnose *S. circensis* from seven populations proposing new morphometric analyses; as well as (2) to assess whether morphological variations may indicate the existence of cryptic species.

Material and methods

Seven populations of aeglid hosts from four different river basins from the State of Paraná were sampled: Tibagi River Basin, Iguaçu River Basin, Ivaí River Basin, and Pirapó River Basin (Collection licenses: 36819-1 and 33578-1) (Fig. 1). In the Tibagi River Basin, specimens of *Aegla castro* Schmitt were collected (three expeditions) from July 2016 to May 2017 from Couro Stream (CO) (23º57’15”S, 51º07’00”W); and in May 2017 from a stream tributary of Preto River (PT) (23º53’55”S 51º10’6”W), at Mauá da Serra. *Aegla lata* Bond-Buckup & Buckup was collected (11 expeditions) from August 2014 to June 2015 from Bule Stream (BU) (23º26’60”S, 51º15’06”W), at Londrina. *Aegla* sp. 1 was collected in June 2017 from Jacutinga Stream (JA) (23º13’31”S, 51º12’39”W), at Londrina. In the Iguaçu River Basin, specimens of *Aegla parana* Schmitt were collected in June 2017 from Pinhão River (PI) (25º33’14”S, 51º26’23”W), at Guarapuava. In the Ivaí River Basin, specimens of *Aegla* sp. 2 were collected (four expeditions) from July 2015 to September 2015 from Lageadão River (LA) (24º01’33”S, 51º36’47”W), at Borrazópolis. In the Pirapó River Basin, specimens of *Aegla* sp. 3 were collected in November 2016 from Ema Stream (EM) (23º19’45”S, 51º26’03”W), at Rolândia.

Aeglid hosts were collected with hand nets, separated in bottles with stream water, and transported alive to the laboratory. Specimens of *Stratiodrilus* were collected after the immersion of hosts in stream water with menthol, to prevent body contraction (Amato *et al.* 2004). Then, they were searched on the carapace, in branchial chambers, and in the ventral face of the abdomen of hosts, and they were fixed with 70% ethanol. We also mounted some slides *in vivo* with stream water and coverslip. Some specimens were cleared in eugenol oil or lactic acid in temporary slides. Whole-mounted specimens were photomicrographed under Opton microscope coupled to an imaging system. The photomicrographic images were taken and measured with BELview software version 6.2.3.0.

The morphology of female and male polychaets of the seven host’s populations were described together in a general description. The sexual characteristics are presented separately. The following measurements were taken: body length, head length, jaw apparatus length, fulcrum length, length of median antenna (T1), length of first pair of antennae (T2), length of second pair of antennae (T3), length of first lateral pair of cirri (C1), length of second lateral pair of cirri (C2), length of third lateral pair of cirri (C3), length of posterior pair of cirri (C4), length of posterior locomotor appendages (PLA), penis length and width, and clasper length and width. All measurements are in micrometers (µm); minimum and maximum numbers are followed (between parentheses) by the mean and the standard deviation values. The terminology used to describe the cirri, antennae, adjacent lobes, and locomotor appendages follows Amato (2001).

Statistical analysis

Ecological descriptors such as prevalence (P%), mean abundance (MA), and mean intensity (MI) were calculated according to Bush *et al*. (1997).

A principal component analysis (PCA) was performed for all measurements taken from seven populations of *Stratiodrilus circensis*, to infer which morphological characters could better explain the most of variations among populations. PCA evidenced that the measurements antennae length (T1–T3) and cirri length (C1–C4) were responsible for most of data variation. Then, we selected these measurements and we compared each one of them for five populations (CO, PT, BU, JA, and LA). We used Analysis of variance (ANOVA) or Kruskal-Wallis test depending on assumptions of normality and homogeneity of variances. In comparisons we disregarded the EM and PI populations due to their low sample size.

The measurements T1 length, C1 length, and T2 length were compared for the five populations to test the hypothesis that there are significant differences among these populations. For these measurements we used an Analysis of variance (ANOVA) followed by Tukey test. In the same way, the measurements C2 length, C3 length, C4 length, and T3 length were compared for the five populations to investigate if there are significant differences among these populations. For these measurements we used Kruskal-Wallis test followed by Dunn's test. Tukey test and Dunn's test were carried out to find which populations differ from the others with respect each measurement.

PCA was performed with the software Canoco version 4.5 and the comparisons among the populations were carried out with the software SigmaStat 3.2.

Results

The total number of specimens of aeglid hosts and ectocommensals collected in each locality are presented in Table 1. Specimens of *Stratiodilus circensis* from all populations were found in the branchial chambers and ventral face of the abdomen of hosts. All host specimens of the populations from JA and PT were associated with at least one specimen of *Stratiodrilus circensis* (prevalence = 100%) (Table 1).

Based on information available in the literature, *Stratiodrilus circensis* may be identified by carring the median antenna (T1) and the first lateral pair of antennae (T2) both unsegmented; second lateral pair (T3) simple, bisegmented; cirrus C1 to C4 simple, unsegmented, longer than first pair of antennae; jaw apparatus not reaching first trunk segment; adjacent conical, ventral lobes (CvLb) half the length of cirrus C4; anal, conical, ventral lobes absent; and one pair of tubercles at each posterior locomotor appendage (PLA) (Table 2). However, in live specimens herein reported a certain mobility of the jaw apparatus was observed, and thus, the extension of this structure in relation to first trunk segment varied between and within the populations (Table 2).

The general shape and number of structures of the specimens studied here allowed us to diagnose them as *S. circensis* (Table 2). However, we found some morphological variations among the examined populations that never had been reported for *S. circensis*.

Measurements of 75 whole-mounted specimens from the seven populations are presented in Table 3.

Stratiodrilus circensis Steiner & Amaral, 1999

(Figs. 2–6)

Description

Body divided into head, trunk and posterior region (Fig. 2). Head conspicuous, divided from rest of body, with seven appendages and jaw apparatus. Trunk elongated, with the anterior and posterior region of the trunk narrower than its median region; with cirri laterally. Posterior region of the body divided into appendages directed laterally (Fig. 2). Total body length averages among seven populations ranged from 957.0±130.6 (EM) to 1649.7±138.6 (PI). Smallest and largest specimens found reached 779.7 (EM) and 1747.4 (PI) in length, respectively (Table 3).

Head: with seven appendages, of which five antennae (T1, T2, T3) and two ventral anterior locomotor appendages (AL) (Fig. 3). The head length averages varied from 169.2±8.4 (BU) to 234.6±13.9 (PI), being the smallest and largest ranges 157.0 (BU) and 244.4 (PI), respectively (Fig. 3). The ratio body length and head length was, in average, lower in PI (1:0.14) and higher in EM (1:0.19) (Table 3).

Jaw apparatus: heavily chitinous, with four maxillae, two mandibles and one fulcrum. Mandibles thicker than fulcrum. Pair of mandibles observed slightly apart, slightly curved in its median region, or parallel to each other (Figs. 3a, 3f, 3c, respectively). Jaw apparatus length averages varied from 142.7±7.4 (JA) to 180.2±3.0 (PI), and the smallest and largest ranges were 122.5 (LA) and 182.3 (PI), respectively (Table 3). The ratio head length and jaw apparatus length was, in average, lower in PI (1:0.77) and higher in LA and BU (1:0.87). Extension of jaw apparatus in relation to first trunk segment varying among populations (Fig. 3).

Fulcrum: straight, thinner than mandibles (Fig. 3). Extension of fulcrum about jaw apparatus varied among populations. Posterior end of fulcrum not reach or exceeds the level of the posterior end of the mandibles (Figs. 3d and 3b, respectively). The fulcrum length averages among the populations varied from 79.5±26.1 (EM) to 113.6±2.8 (PI). The rami are a complex of small pieces that support the four maxillae at the distal end of jaw apparatus. Rami with four teeth hooked-shaped, fulcrum never observed more extroverted than is shown in figure 3d.

Antennae (T1–T3): five antennae, very long (Fig. 3). Median antenna (T1) slightly shorter than a first lateral pair (T2), both unsegmented. T2 with a dilated base (Figs. 3f and 3g). The lowest and highest averages of T1 length were 35.6±4.1 (EM) and 48.9±3.1 (LA), respectively, while for T2 length the lowest and highest averages were 39.1±6.3 (EM) and 52.4±0.3 (PI), respectively (Table 3). T3 bisegmented, collapsible, without dilated tips, with the distal segment always shorter than the proximal one. T3 length averages ranged from 85.9±4.9 (EM) to 129.9±4.5 (PI) (Table 3).

Anterior locomotor appendages (AL): two, ventrolaterally on the head, retractile, quite mobile, hardly observed entirely protruded in fixed specimens (Fig. 3). Distal end with a fan-shaped structure composed of several small adhesive glands in their distal border (Fig. 3h).

Lateral cirri (C1–C3): three pairs of lateral, unsegmented, long cirri (C1, C2, and C3), longer than median (T1) and first lateral pair of antennae (T2) (Fig. 4). Lateral cirri with thickening at base (Fig. 4e). The lowest averages of C1, C2 and C3 length were 49.9±8.2 (CO), 52.6±10.2 (CO), and 48.1±9.9 (EM), respectively, while the highest values for C1, C2, and C3 were 86.3±4.5 (PI), 98.5±3.3 (PI), and 111.9±17.3 (PI), respectively (Table 3).

Posterior cirri (C4): one pair of posterior, unsegmented, long cirri (C4), with an adjacent conical, ventral lobe (CvLb), whose structure is of a different constitution from the cirri (Fig. 3g). An expansion at the base of C4 was observed in some specimens, as shown in Figure 3g.

Adjacent conical, ventral lobes (CvLb): the adjacent conical, ventral lobes were the structures that most varied among and within the populations in this study. CvLb adjacent to cirrus C4. CvLb with different lengths from short rounded to long (Fig. 5).

Posterior locomotor appendages (PLA): bearing one pair of tubercles in each one (Fig. 5). Distal ends of the PLA flattened dorso-ventrally, rounded, with small tooth-shaped projections in their contour with adhesive secretions (Fig. 5). The average of PLA length varied from 244.8±23.3 (EM) to 400.9±31.7 (PI), being the lowest and highest ranges 213.9 (CO) and 423.3 (PI), respectively (Table 3).

Tubercles: all the populations presented two pairs (one pair in each PLA) of easily visible tubercles (Fig. 5). The tubercles of specimens from PI were longer than those found in other populations (Fig. 5g).

Female sexual characters: birth pores immediately behind C3 where eggs are released (Figs. 4a, 4c, and 4e). The number of eggs per female varied among populations, with at least one female per population carrying at least one egg (Table 4). One specimen from PI had six eggs in its body cavity (Figs. 2e and 2f).

Male sexual characters: penis heavily chitinous, with the tip of the bezel and a central duct (Figs. 5 and 6). The most prolonged penis was found in a specimen from PI (132.1, Table 4), while the shortest was found in a specimen from JA (56.6) (Table 4). Penis width ranged from 12.6 (LA) to 32.2 (BU), and the ratio penis length and penis width varied, in average, from 1:0.17 (PT) to 1:0.33 (BU) (Table 4). Penis with a tissue cover when it is not entirely protruded (Figs. 6b and 6d). Claspers cylindrical, wider at base, difficult to observe entirely protruded from inside their lateral pouches (Figs. 6a, 6e and 6f); with a large unicellular gland (Fig. 6f); distal ends with four lobe-like projections (Fig. 6f). Only three populations presented specimens with claspers entirely protruded, as presented in Table 4.

Remarks

We found morphological variations that need to be taxonomically considered, since some characters may be imprecise to diagnose species of *Stratiodrilus*. For example, the present study reported different lengths of adjacent conical ventral lobes in the seven populations, as well as Daudt & Amato (2007) reported for the population they studied. Likewise, an extension of jaw apparatus about first trunk segment has been used in the descriptions as an important character, but in our study, it was observed with variations.

Jaw apparatus was meticulously described by Haswell (1900) as a complex set of both strong and delicate chitinous pieces, that lies ventral to the digestive tube. The jaw apparatus of the populations of this study varied concerning both the level of fulcrum protraction and the extension of the posterior end of the mandibles relatively to the first trunk segment. We observed specimens of BU and PI with jaw apparatus reaching first trunk segment (e. g. Fig. 3e), whereas the other five populations with the extension of jaw apparatus varying within populations, with some specimens reaching and not reaching first trunk segment (Figs. 2c and 2f). This led us to propose that these variations observed in our specimens are the result of the motility between fulcrum and jaw apparatus, by the presence of muscles that involve them. Indeed, Haswell (1900) reported the presence of striated and non-striated bundles of several muscles that allow independent movements between the fulcrum and jaw apparatus. Although Haswell (1900) did not adequately elucidate the mode of action of these muscles, he postulated that these striated bundles allow rapid and robust movements of the fulcrum over the mandibles and that the set of the striated and non-striated bundles would be involved in sharp biting movements of the jaw apparatus. Thus, we suggest that the length of the jaw apparatus (if it reaches or does not reach the first trunk segment) may be associated to the contraction of the complex musculature that Haswell (1900) referred.

Steiner & Amaral (1999) reported that the antennae T1 and T2 are of equal length. However, in our study T2 was always longer than T1. We also observed sensory cilia at the end of T2 agreeing with Haswell (1900) that reported these structures as “(...) very fine non-motile sensory cilia, arranged in a circlet or spiral” (Haswell 1900; 301). Although these sensory cilia occur in all antennae and cirri (Haswell 1900) in our study, they were complicated to observe.

In this study, we observed specimens that had the anterior locomotor appendages entirely protruded or slightly retracted, even in the same population (Fig. 3). Anterior locomotor appendages were reported to be retractile for the genus *Stratiodrilus* by Haswell (1900) and as “(...) being capable of becoming completely withdrawn in the interior of the head” (Haswell 1900; 301). Steiner & Amaral (1999) pointed out that AL were retractile, but they did not draw or found any specimen of *S. circensis* with these structures entirely protruded. Moreover, at the end of AL, we observed a fan-shaped structure that Haswell (1900) called as a “(...) slightly expansion, apparently of the nature of a sucker” (Haswell 1900; 301). These small structures were not observed easily in all specimens, probably to due to their retractile and motile nature. However, observing different angles this structure seems more to be a flattened flap in the form of a half circle with numerous papilliform structures in its distal contour than a typical sucker (Fig. 3h). It was also possible to observe in live specimens several small ducts that originate from the base to the distal tip of AL, as reported by Haswell (1900).

Amato *et al*. (2004), when describing *Stratiodrilus brevicirrus*, reported the existence of an expansion at the base of the cirrus C4. They considered this character as diagnostic for *S. brevicirrus*. However, we also found a very similar structure to those described by Amato *et al*. (2004) in some of our specimens (Fig. 5g), that has never been informed for other species of *S. circensis* (Table 2). Furthermore, it is important to highlight that since we have found this structures in lateral cirri (C1, C2 and C3) and antennae (T2) as well. This expansion was very subtle to observe so that it was visible only as an inconspicuous line crossing the cirrus transversally near to its base. Thus, it is possible that these types of expansions had been neglected in most of the descriptions, and it is also possible that they are common structures in other cirri and antennae of other species and not only in cirri C4 of *S. brevicirrus*.

The shape and size of lobes associated with cirrus C4 have been reported in the literature as taxonomically essential features for differentiation of *Stratiodrilus* spp. (Steiner & Amaral 1999; Table 2). These lobes have previously been considered by Amaral & Morgado (1997) and Steiner & Amaral (1999) as a double cirrus C4 (Table 2). However, Amato (2001) reported that the nature of the CvLb are completely different from the other cirri, and stated that the cirrus C4 could not be double, but a cirrus C4 associated with an adjacent conical, ventral lobe. The specimens of this study showed CvLb associated with cirri C4, as reported in the original description of *S. circensis*. However, we observed a considerable variation in the length of these lobes among and within populations. In the specimens from CO we observed a gradient of lengths of adjacent lobes to C4, from short, rounded lobes to long CvLb (Figs. 5a and 5b). The adjacent lobes to C4 of specimens from PT were either short, rounded, with a wider base and a small projection on their distal end or long, conical, with a wider base (Figs. 5c and 5d). The CvLb observed in the specimens from BU were long, conical, half the length of C4, with wider base (Fig. 5e). The CvLb of specimens from JA were long CvLb, half the length of C4, with a wider base (Fig. 5f). In the specimens from PI the CvLb were similar to those found in specimens from JA, being long, half the length of C4, with a wider base (Fig. 3i). The adjacent lobes to C4 of the specimens from LA were similar to those found in specimens from CO, being short, rounded lobes (Fig. 5h), with a wider base and a small projection on their distal end in some specimens (Fig. 5i). The specimens from EM presented a long CvLb, half the length of C4, with a wider base (Fig. 5j).

Morphological variation in the length of CvLb was also observed in two populations of *S. circensis* studied by Daudt & Amato (2007). Also, we found some specimens that presented the CvLb with a wide base followed by a delicate projection, appearing to be a structure protruding out of the base of CvLb (Fig. 5i), while in other specimens we did not found this type of wide base. In fact, if the CvLb presents a different constitution about the cirri which we could observe with different lengths, we may suggest that the lobes associated with C4 may be retractile structures. The CvLb of all populations seemed to be retractile, since we could observe, for example, specimens from JA, LA, and PT with different lengths of CvLb even in the same specimen (e.g. Figs. 5c and 5d).

In some specimens, we were able to distinguish the presence of a wide base of CvLb plus a thickening at the base of the cirrus C4. Because these two types of structures are in the same viewing plane, the distinction becomes very subtle (Fig. 5g). Therefore, the use of CvLb length about C4 should be used with caution for diagnosing species of *Stratiodrilus*, since these structures may show different lengths. A correction of CvLb's morphology has already been made for *Stratiodrilus arreliai* by Steiner & Amaral (1999). In the original description of *S. arreliai* the authors described the cirrus C4 as single and non-segmented, with basal support (Amaral & Morgado 1997; see Fig. 1). Afterwards, Steiner & Amaral (1999) redescribed this species and reported the occurrence of small papilliform lobes associated with a simple and non-segmented cirrus C4 (Steiner & Amaral 1999; see Fig. 1).

Just as CvLb were used as taxonomically important characters, the AcvLb (anal conical, ventral lobes) was also used for this purpose. Although AcvLb has been previously considered as cirrus C5 by Amaral & Morgado (1997) and Steiner & Amaral (1999), they are of a different structure from cirri, as are the CvLb (Amato 2001). To date, only *Stratiodrilus pugnaxi* Vila & Bahamonde, 1985 and *Stratiodrilus vilae* Amato, 2001 are recognized for having anal conical, ventral lobes. However, in the study of Daudt & Amato (2007) the authors represented in their drawings and figures a structure which appears to be very similar to the AcvLb (Daudt & Amato 2007; see Figs. 4, 6, 9–12) that Amato (2001) described for *S. vilae*. Although there is no mention or discussion of these structures in the text, the presence of AcvLb (called cirrus C5 by Steiner & Amaral, 1999) is not mentioned in the original description of *S. circensis*.

Steiner & Amaral (1999) described tubercles as small rounded structures, always simple. Species of *Stratiodrilus* may have none, one, or two tubercles on the distal end of PLA. *Stratiodrilus circensis*, *S. vilae*, and *S. brevicirrus* have two pairs of these structures. In fact, all the populations studied by us had two pairs of tubercles, most of which easily visible, small, rounded tubercles. However, the population from PI presented the tubercles longer than other populations, similarly those found in the populations studied by Daudt & Amato (2007).

In our study, we found a female specimen carrying six eggs inner their body cavity. This finding goes against what Haswell (1900) proposed for *Stratiodrilus*, whose number of eggs per female is commonly one, rarely two. Also, Haswell (1900) postulated that there is usually one egg of predominant size over the other that is being formed. In contrast, in the female of the population from PI the six eggs had relatively similar size.

Claspers are retractile, large, complex structures that lie laterally to each side of the body. They are not commonly mentioned in previous descriptions, and they were considered absent in South American species of *Statriodrilus* by Roubaud (1962). Indeed, Steiner & Amaral reported that of all South American specimens of *Stratiodrilus* examined by them just one had structures similarly with claspers. Daudt & Amato (2007) referred just two specimens with claspers. We observed eight specimens of *S. circensis* with claspers entirely protruded (Table 4; Fig. 6e), and some specimens with these organs partially protruded (Fig. 6a). The large unicellular glands located at the interior of each clasper reported by Haswell (1900) were also observed (Fig. 6f).

Morphometric analyses

The PCA performed with the morphometric data of the seven populations (measurements of structures presented in Table 3) extracted two axes that explained 73.9% of the total data variance (Fig. 7); axis 1 accounted for 51.1% and axis 2 for 22.8%. In the first component (PC1) were grouped the variables T1 length, T2 length, T3 length, C1 length, C2 length, C3 length, and C4 length, while in the second component (PC2) were grouped the variables Body length, Head length, Jaw apparatus length, Fulcrum length, and PLA length (Fig. 7; Table 5). The morphological structures T1, T3, C1, C2, C3, and C4 presented loading values close each other (Table 5), suggesting proximity among these variables. About the first component (PC1), T3 length had the highest loading value, while for the second component (PC2), Head length had the highest loading value (Fig. 7; Table 5).

The morphometric variables that explained the most of the variation (T1 length, T2 length, T3 length, C1 length, C2 length, C3 length, and C4 length) were taken and each one of them was compared among the five populations (CO, JA, LA, BU, and PT). We found statistical differences among the five populations for all morphometric variables compared (Table 3). In pairwise comparisons, T1 length, T3 length, C1 length, and C2 length from LA differed from other four populations, being the longest antennae and cirri among the four populations compared. The C3 length and C4 length from LA differed from CO, PT, and BU and CO, BU, and JA, respectively. On the other hand, C1 length and C2 length from CO were the smallest cirri among the populations compared.

Discussion

Eleven species of *Stratiodrilus* are known worldwide (Amato *et al*. 2004; Rosa *et al*. 2018). This genus is found in several localities along South America, Madagascar and Australia (Steiner & Amaral 1999). *Stratiodrilus circensis* is endemic to the Neotropical region and has the widest distribution reported for the genus, being found from Bule Stream, the State of Paraná, Brazil to Chiloé Island, Chile (Steiner & Amaral 1999; Rosa *et al*. 2018). In addition, *S. circensis* was reported to occur in association with several species of aeglid hosts from different river basins (Steiner & Amaral 1999).

Ecological descriptors are rarely reported for species of *Stratiodrilus*. For instance, Daudt & Amato (2007) reported a prevalence of 70% and mean intensity of 13.3±10.4 for *S. circensis*. In our study, the populations presented values of ecological descriptors both higher and lower than those found for the population studied by Daudt & Amato (2007). The population from a tributary of Preto River (PT) and Jacutinga Stream (JA) had the highest prevalences, followed by the populations from Couro Stream (CO) and Lageadão River (LA). Specimens from PT had the highest mean abundance and mean intensity, followed by the population from CO. The host specimens from Bule Stream (BU) and Ema Stream (EM) had lower prevalences, while the populations from Pinhão River (PI), JA, and BU had lower mean intensities.

In this study we collected specimens of *Stratiodrilus* from seven localities, belonging to four river basins from the State of Paraná, of which we recognized six species of aeglid hosts. Based on information available in the literature, the ectocommensals from the seven populations were identified as *S. circensis*. The species that are similar to *Stratiodrilus circensis* are *Stratiodrilus arreliai*, *Stratiodrilus vilae*, and *Stratiodrilus brevicirrus* concerning the general body shape, shape of cirri and antennae, and by the presence of tubercles at the distal region of PLA. However, *S. circensis* differs from *S. arreliai* by the presence of two pairs of tubercles in each PLA (*vs.* one pair); differs from *S. vilae* by the absence of AcvLb (*vs.* presence of AcvLb); and differs from *S. brevicirrus* by the presence of CvLb associated with C4 (*vs.* CvLb absent).

Although the specimens collected in this study were indicated to be *S. circensis* we found morphological variations among the populations, which were mainly in the shape and size of conical, ventral lobes adjacent do C4 (CvLb), in the extension of jaw apparatus relatively to first trunk segment, and in the length of antennae and cirri. However, we considered that the extension of jaw apparatus and the size of CvLb are characteristics that should be analyzed with caution, since we observed variations even among specimens of the same population, as previously pointed out.

In this study, we found the highest range for body length and head length for a species of *Stratiodrilus*, as well as the longest specimen ever reported in the literature. Although the ranges of body length and jaw apparatus length are usually referred in the descriptions, most of the measurements of other structures, such as antennae and cirri, are not presented in detail. For example, in the study of Steiner & Amaral (1999) the size of cirri and antennae are presented as proportions of other body structures. Therefore, we presented several measurements that are not commonly carried out in descriptions, and we encourage the standardization of fixation protocols as well as the adoption of additional morphological structures of taxonomic value to the study of *Stratiodrilus*' morphology.

Regarding head size, the specimens from EM and PI had the largest and smallest head lengths about body length, respectively. Concerning jaw apparatus size, the specimens from LA and BU had the most extensive jaw apparatus lengths when compared to their head lengths. In contrast, the specimens from PI had the smallest jaw apparatus lengths when compared to their head lengths. In fact, the largest specimens found in this study (PI) had the smallest head length relatively to body length, and the smallest jaw apparatus length relatively to head length. We also report a certain degree of distance between the mandibles in some specimens, which may be due to the mechanism of movement of the fulcrum over the pair of mandibles. The ratio between the penis length and penis width showed that specimens from BU had the shortest and widest penis, while specimens from PT had the longest and narrowest penis.

The extension of jaw apparatus relatively to the first trunk segment was used as an important taxonomic character in the literature (see Steiner & Amaral 1999; Amato *et al.* 2004). However, Haswell (1900) demonstrate that there is a complex musculature surrounding the jaw apparatus, suggesting that these muscles may displace the complex during biting movements. Thus, the end of jaw apparatus can reach the first trunk segment as the muscle contraction. We observed this type of variation in some of our specimens—even in the same population—although this variation is not reported for *S. circensis*. Therefore, we suggest the fact that the mandibles reach or do not reach the first trunk segment may be an imprecise measure and should be considered with caution to differentiate species of

Stratiodrilus. So, we propose a ratio that compares the jaw apparatus length with head length, to measure the portion of the head length that the jaw apparatus occupies.

Morphological studies with *Stratiodrilus* are scarce, and most of the descriptions are little detailed and present figures with low quality. Also, studies on the morphology of *Stratiodrilus* are difficult because authors used different terminologies to describe structures. For example, Steiner & Amaral (1999) considered that the cirri C4 may be “double”, while Amato (2001) considered it may be associated with adjacent conical, ventral lobes; Steiner & Amaral (1999) considered that the cirri C5 may be “double”, while Amato (2001) considered that cirri C5 are not true cirri, but anal conical, ventral lobes (AcvLb). Intraspecific morphological variations are rarely reported, except for the study of Daudt & Amato (2007).

Considering the lack of detailed measurements in the literature plus the controversies on the terminology of taxonomic importance structures, this study presented morphometric data and pointed out the morphological structures that most varied among the seven populations studied by us. Thus, using a principal component analysis (PCA), we found that the first and second components explained most of the variation. The first component accounted more than half of the data variation, grouping all the variables of cirri lengths (C1, C2, C3, and C4) and all the variables of antennae lengths (T1, T2, and T3). The second component grouped the measurements of jaw apparatus length and general body size (body, head, jaw apparatus, fulcrum, and PLA lengths). Interestingly, the body and head lengths were grouped in the second component, suggesting that the variation of cirri and antennae lengths were influenced more by morphological differences between the populations than by variations of the body size of the individuals.

Then, with an analysis of variance (ANOVA) comparing cirri and antennae lengths for five populations, we found that all these measurements varied statistically among these populations. The differences in the size of cirri and antennae were also noted qualitatively since we observed that specimens of the population from CO had cirri clearly smaller than those found in specimens from LA. Also, pairwise comparisons revealed that specimens from LA and CO had the longest and the shortest antennae and cirri, respectively. It is important to note that specimens of *Stratiodrilus* from LA and CO are associated with different species of aeglid hosts (*Aegla* sp. and *Aegla castro*, respectively), which populations are isolated from each other, belonging to streams of different river basins (Ivaí and Tibagi river basins, respectively).

Although the specimens from PI presented characteristics that allowed us to diagnosed them as *S. circensis*, they had the general body morphology conspicuous when compared to other six populations. Moreover, the specimens from PI of our study were the longest ever reported in the literature, as well as the only associated with *Aegla parana* to date (Rosa *et al*. 2018).

Furthermore, considering the possibility of some morphological structures being retractile—such as CvLb—and the variations in the extension of jaw apparatus, there is a need to evaluate the taxonomic importance characters used until today for diagnosing species of *Stratiodrilus*. In addition, our findings showed that even though the seven populations could be identified as *S. circensis*, there was a morphological variation regarding the size of antennae and cirri among populations. Thus, though the size of cirri and antennae have not usually been considered as important taxonomic characters, we propose that these structures become better explored in the subsequent descriptions since their size varied significantly in our study. Detailed measurements of antennae and cirri can provide relevant information about the morphology of *Stratiodrilus* as well as could help in the diagnosis of species of the genus. On the other hand, we suggest that shape and size of CvLb should be considered with caution, since they may be retractile structures.

Species of *Stratiodrilus* were reported in the literature by having a certain specificity of host and by being identified according to their geographical distribution (Amaral & Morgado 1997). In fact, distribution of hosts is crucial to understand the distribution patterns of species of *Stratiodrilus*, since these ectocommensals complete their entire life cycle in the host (Vila & Véliz 2014).

Although aeglids have a wide distribution range in South America, most of the species are restricted to small headwater streams of only one river basin or few adjacent drainages (Morrone 1996; Bueno *et al.* 2016). Thus, if the hosts are geographically isolated in different drainages, the populations of *Stratiodrilus* that are associated with these hosts will also be. Therefore, taking into account that life cycle of *Stratiodrilus* is completely dependent on the life cycle of their hosts (Vila & Véliz 2014), we may assume that biology features of aeglid hosts, such as behaviour, physiology, growth and reproduction may directly affect the *Stratiodrilus*' biology. Also, if the hosts collected in this study belong to different species, which populations are geographically isolated, it may be that each host species provide different microhabitats and selective pressures for the speciation of *Stratiodrilus*.

Despite the populations from EM and PI have not been used in the comparisons, the morphological variations found in this study are enough to suggest the existence of three morphologically distinct populations: the population associated with *A. castro* from CO, Tibagi River Basin; the population associated with *Aegla* sp. 2 from LA, Ivaí River Basin; and the population associated with *A. parana* from PI, Iguaçu River Basin.

Thus, based on morphological differences discussed previously, plus the geographic isolation of these populations (from different drainage systems), and the distinct host species associated, we can infer these three morphologically distinct populations compound a set of cryptic species. Therefore, we believe that molecular studies and scanning electron microscopy images will complement and elucidate taxonomic issues on these organisms.

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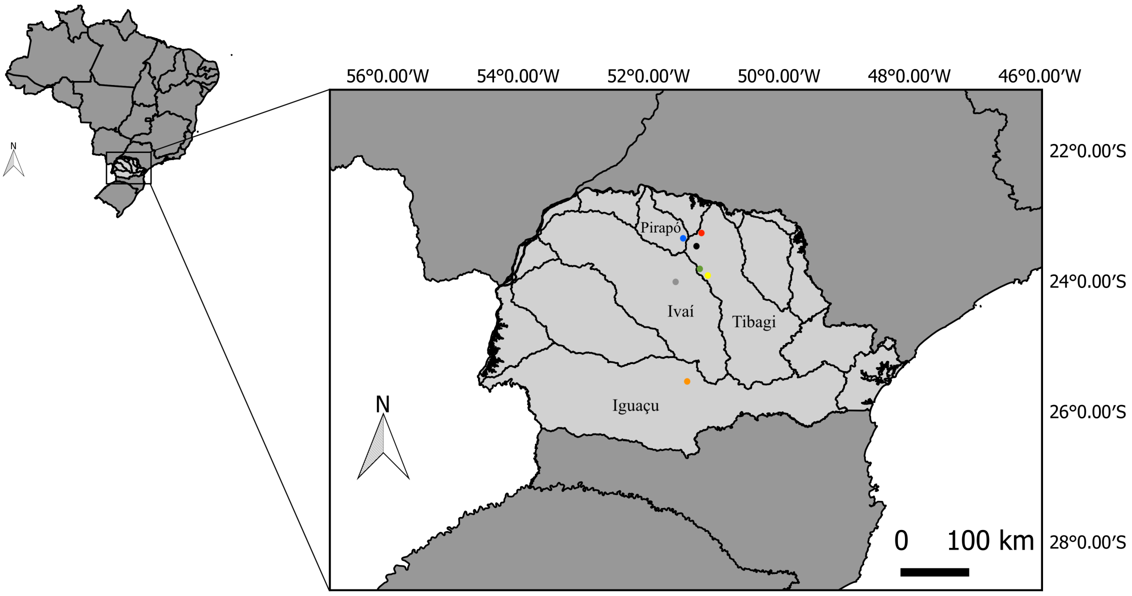

FIGURE 1. Sampling area of the seven populations of aeglid hosts from four river basins of State of Paraná. Jacutinga Stream (red circle); Ema Stream (blue circle); Bule Stream (black circle); Couro Stream (green circle); tributary of Preto River (yellow circle); Lageadão River (gray circle); and Pinhão River (orange circle). Name of river basins are indicated in the figure; (Pirapó) Pirapó River Basin; (Tibagi) Tibagi River Basin; (Ivaí) Ivaí River Basin; and (Iguaçu) Iguaçu River Basin.

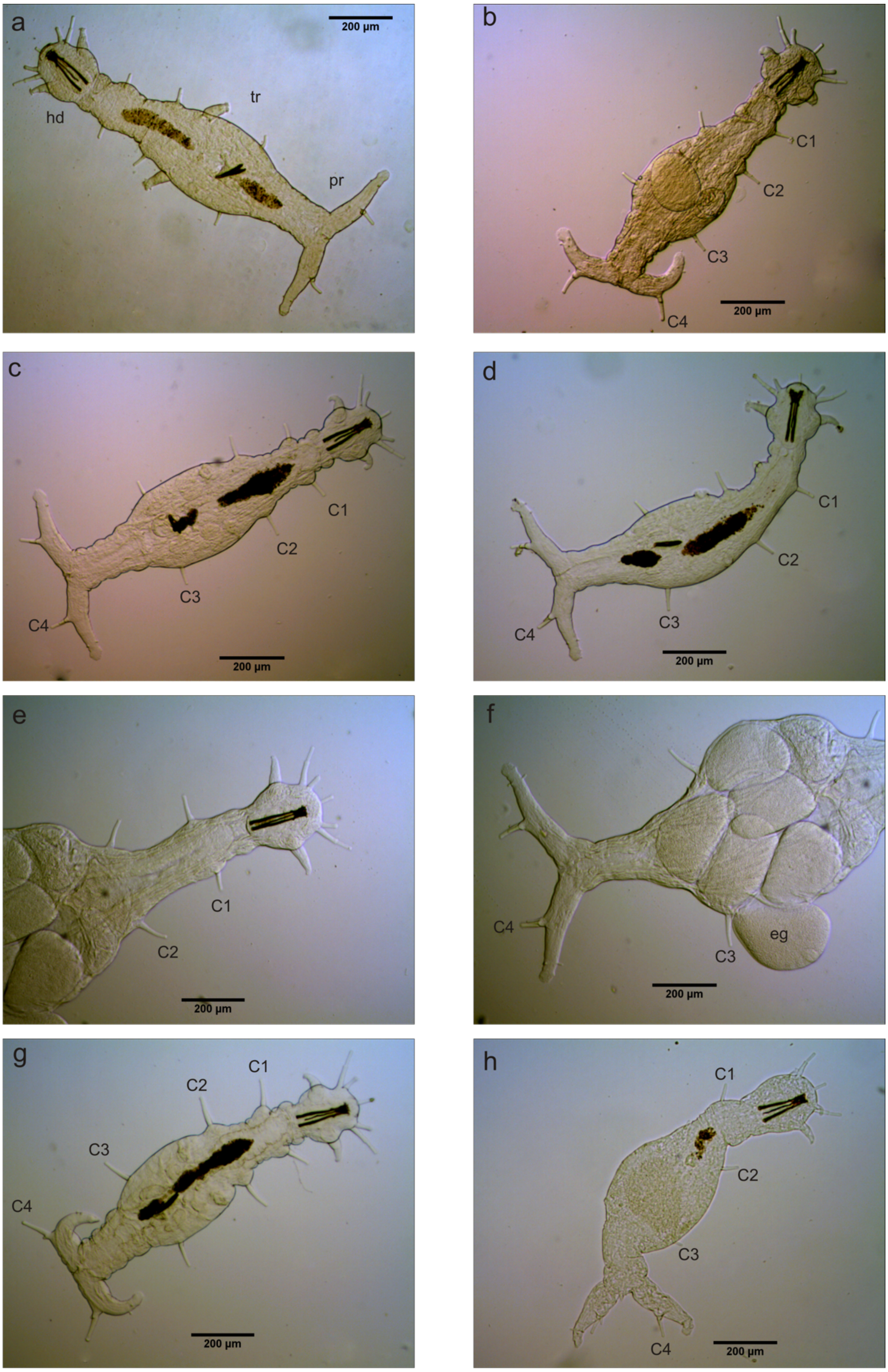

FIGURE 2a–h. General body shape of male and female specimens of *Stratiodrilus circensis*. (a) Male specimen associated with *Aegla castro* from Couro Stream (CO); (b) female specimen associated with *Aegla castro* from a tributary of Preto River (PT); (c) male specimen associated with *Aegla lata* from Bule Stream (BU); (d) male specimen associated with *Aegla* sp. 1 from Jacutinga Stream (JA); (e–f) female specimen associated with *Aegla parana* from Pinhão River (PI); (g) male specimen associated with *Aegla* sp. 2 from Lageadão River (LA); (h) female specimen associated with *Aegla* sp. 3 from Ema Stream (EM). (C1, C2, C3) Lateral pair of cirri; (C4) posterior pair of cirri; (eg) egg; (hd) head; (pr) posterior region of body; (tr) trunk.

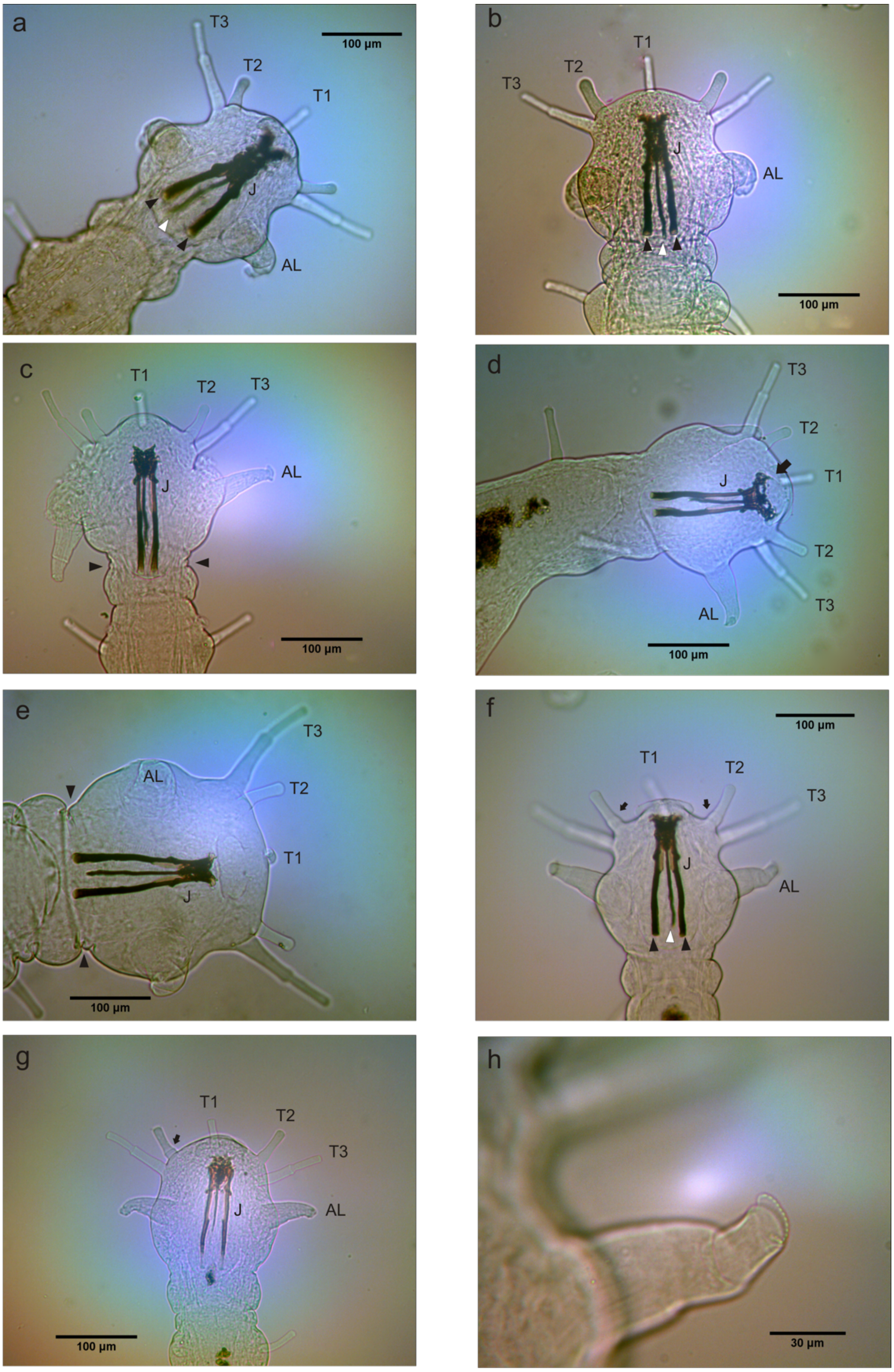

FIGURE 3a–h. Anterior region of body of specimens of *Stratiodrilus circensis*. (a–b) Specimens from CO and PT showing the posterior end of fulcrum (white head arrows) posteriorly to pair of mandibles (black head arrows); (c–d) specimens from JA showing the posterior end of jaw apparatus excceding the first trunk segment (black head arrows), and fulcrum entroverted (black arrow); (e) specimen from PI showing the posterior end of jaw apparatus reaching the first trunk segment (black head arrows); (f) specimen from LA showing the posterior end of fulcrum (white head arrows) anteriorly to pair of mandibles (black head arrows), and an expansion at base of T2 (black arrow); (g) specimen from EMA with detail for the expansion at base of T2 (black arrow); (h) left anterior locomotor appendage showing the distal end with a fan-shaped structure. (AL) Anterior locomotor appendages; (J) jaw apparatus; (T1) median antenna; (T2) first pair of antennae; (T3) second pair of antennae.

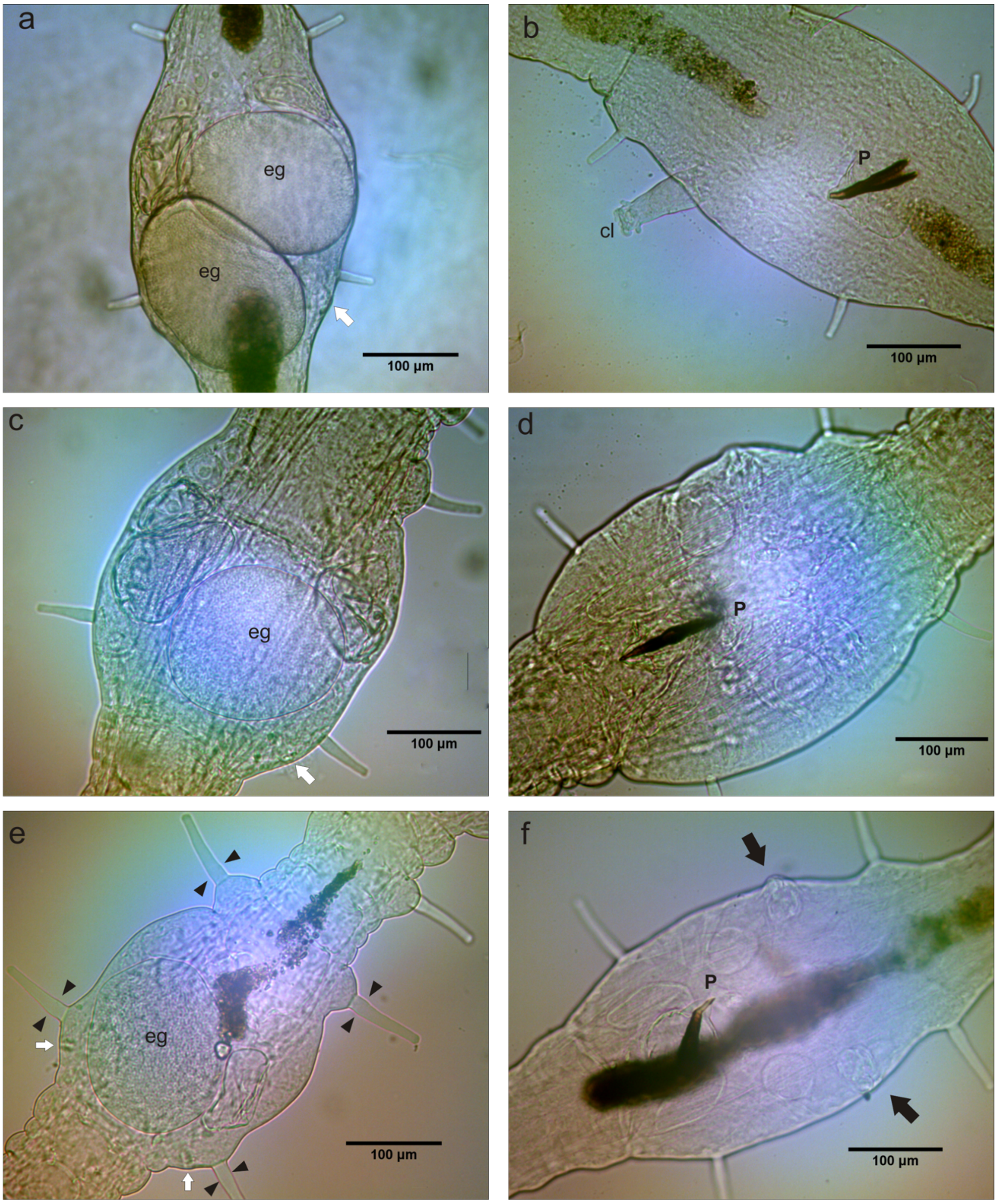

FIGURE 4a–f. Middle region of body of female and male specimens of *Stratiodrilus circensis*. (a) Female specimen from CO showing the eggs and birth pore (white arrow); (b) male specimen from CO showing the penis and clasper in lateral view; (c) female specimen from PT showing the egg and birth pore (white arrow); (d) male specimen from PT with detail of penis; (e) female specimen from LA with detail for expansions at base of cirri (black head arrows); (f) male specimen from LA showing the lateral pouches of claspers (black arrows). (eg) Eggs; (P) penis.

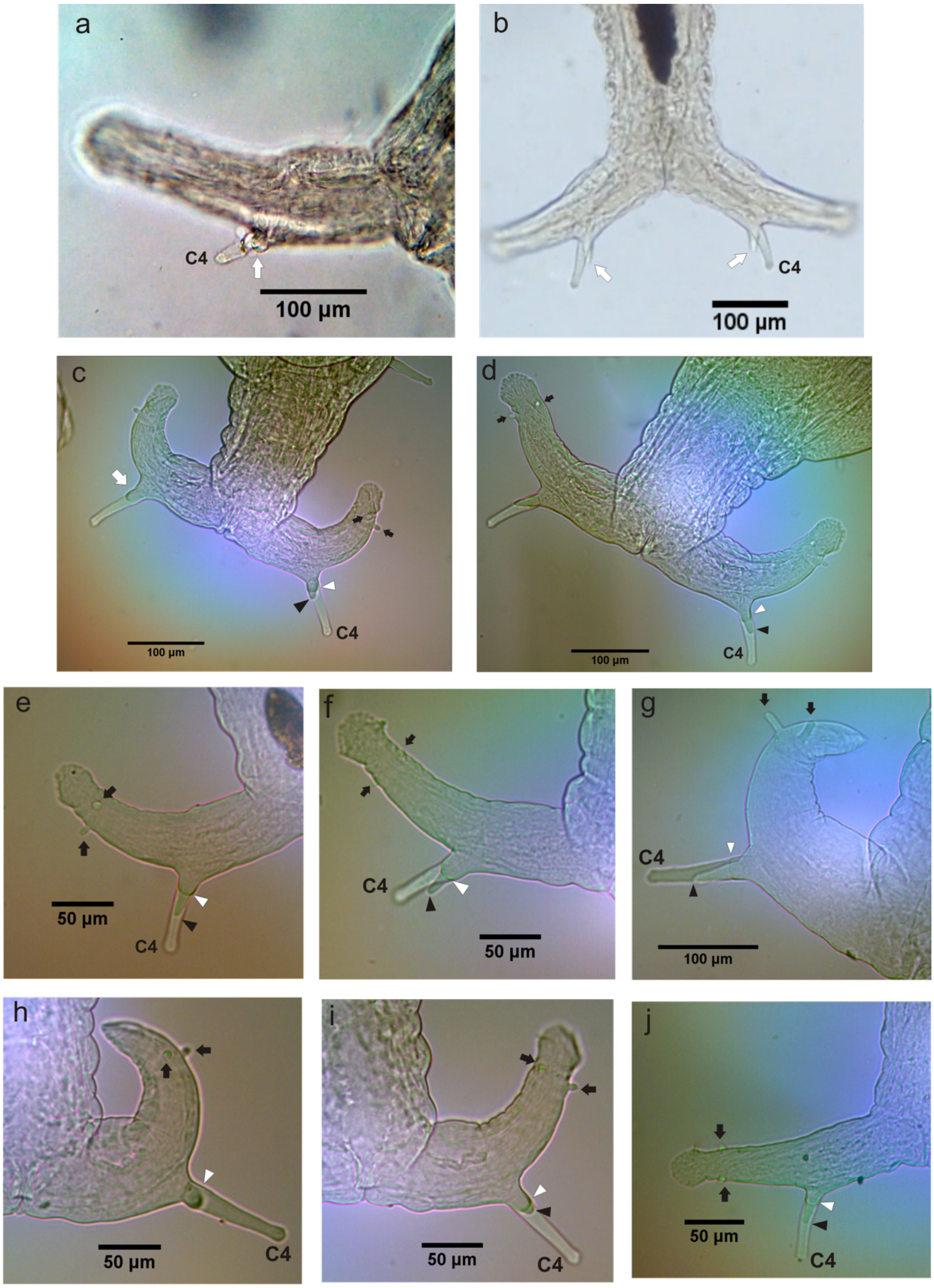

FIGURE 5a–j. Posterior region of body of specimens of *Stratiodrilus circensis* with detail for adjacent lobes, cirri C4, and one pair of tubercles (black arrows) in each posterior locomotor appendage (PLA). (a) Specimen from CO with a short, rounded lobe adjacent to C4 (white arrow); (b) specimen from CO with long adjacent lobes; (c) specimen from PT with a short rounded lobe (white arrow), a short, rounded lobe with a wider base (white head arrow), and a small projection on its distal end (black head arrow); (d) specimen from PT with long, conical lobes (black head arrow) with a wider base (white head arrow); (e) specimens from BU with long, conical lobes (black head arrow) with wider base (white head arrow); (f) specimens from JA with long, conical lobes (black head arrow) with a wider base (white head arrow); (g) specimen from PI with long tubercles (black arrows), long conical lobes (black head arrow), and an expansion at base of C4 (white head arrow); (h) specimen from LA with a short, rounded lobe (white head arrow); (i) specimen from LA with a short, rounded lobe with a wider base (white head arrow) and a small projection on its distal end (black head arrow); (j) specimen from EM with long, conical lobes (black head arrow) with a wider base (white head arrow). (C4) Posterior pair of cirri.

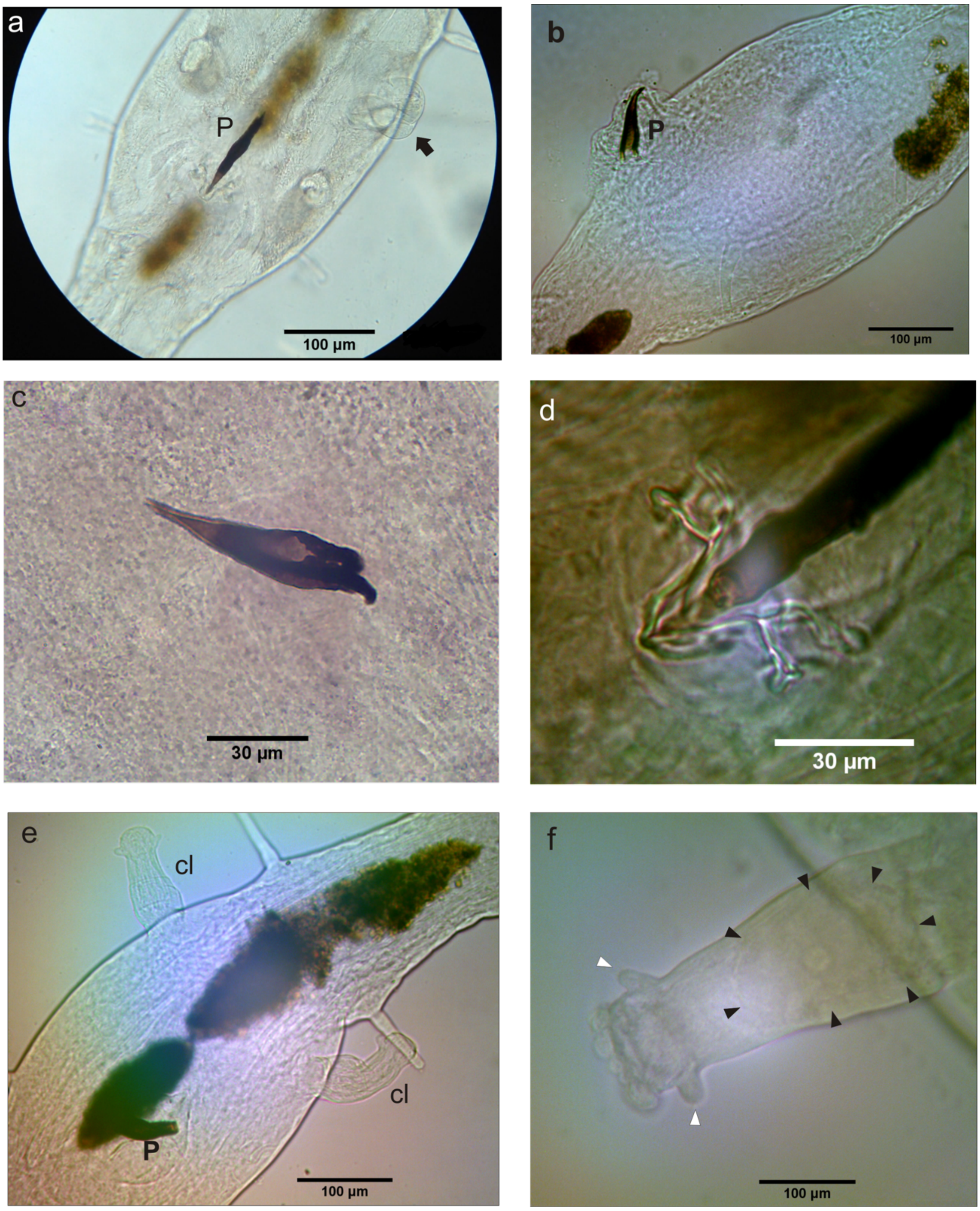

FIGURE 6a–f. Middle region of body with detail for male sexual structures of specimens of *Stratiodrilus circensis*. (a) Specimen from CO showing the penis in frontal view and a clasper partially protruded (black arrow); (b) specimen from JA with penis in lateral view; (c) penis of a specimen from BU with detail for the tip in bezel and a central duct; (d) penis os a specimen of LA with a tissue cover at distal end; (e) specimen from LA with claspers entirely protruded; (f) right clasper entirely protruded of a specimen from CO, with detail for a large unicellular gland (black head arrows). (cl) Claspers; (P) penis.

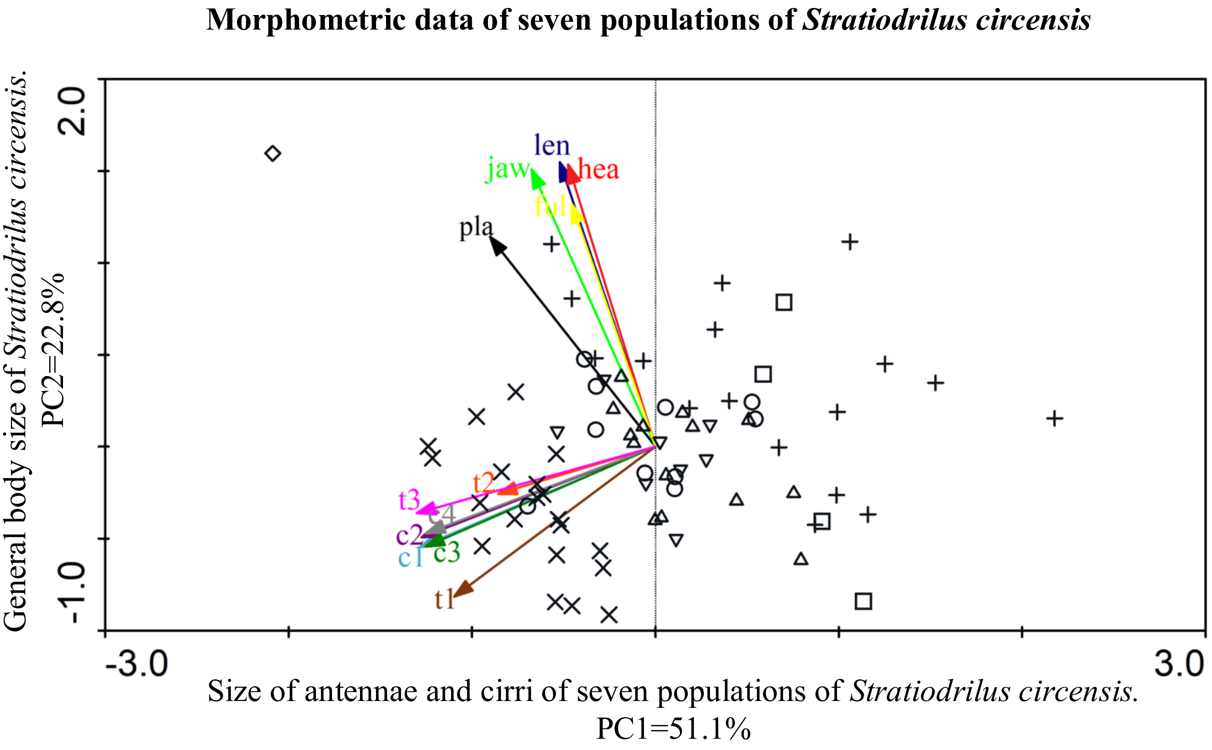

FIGURE 7. Biplot obtained from a Principal Analysis (PCA) of morphometric data of populations from Couro Stream (cross), tributary of Preto River (circle), Bule Stream (triangle up), Jacutinga Stream (triangle down), Pinhão River (diamond), Lageadão River (X), and Ema Stream (square). The symbols represent the following morphological structures: Body length (len), Head length (hea), Jaw apparatus length (jaw), Fulcrum length (ful), median antenna length (t1), length of first pair of antennae (t2), length of second pair of antennae (t3), length of first pair of lateral cirri (c1), length of second pair of lateral cirri (c2), length of third pair of lateral cirri (c3), length of posterior cirri (c4), and posterior locomotor appendages length (pla).

TABLE 1. Ecological descriptors of specimens of *Stratiodrilus circensis* from seven populations of aeglids from State of Paraná (CO–Couro Stream; PT–tributary of Preto River; BU–Bule Stream; JA–Jacutinga Stream; PI–Pinhão River; LA–Lageadão River; and EM–Ema Stream). Total number of hosts collected in each locality (NH). Total number of specimens of *S. circensis* collected in each population of aeglid hosts (NS). Standard error values were used.

Host	**River basin**	**NH**	**Prevalence (%)**	**NS**	**Mean abundance (min–max)**	**Mean intensity (min–max)**
Aegla castro Schmitt (CO)	Tibagi	90	97.8	3355	37.3±3.1 (0–130)	38.1±3.1 (1–130)
Aegla castro Schmitt (PT)	Tibagi	4	100.0	172	43.0±12.6 (20–79)	43.0±12.6 (20–79)
Aegla lata Bond-Buckup & Buckup (BU)	Tibagi	74	62.2	252	3.4±0.6 (0–22)	5.5±0.8 (1–22)
Aegla sp. 1 (JA)	Tibagi	7	100.0	37	5.3±1.8 (1–14)	5.3±1.8 (1–14)
Aegla parana Schmitt (PI)	Iguaçu	10	80.0	44	4.4±1.5 (0–13)	5.5±1.6 (1–13)
Aegla sp. 2 (LA)	Ivaí	24	95.8	497	20.7±5.4 (0–117)	21.6±5.5 (1–117)
Aegla sp. 3 (EM)	Pirapó	22	68.2	237	10.8±3.9 (0–78)	15.8±5.3 (1–78)

TABLE 2. Morphological characterization of species of *Stratiodrilus*, adapted from Steiner & Amaral (1999), with an adaptation with the terminology of Amato (2001). A–absent; AcvLb–anal conical ventral lobes; Bs–bisegmented; CvLb–conical ventral lobes; Do–double; Fo–forked; Si–simple; and Us–unsegmented.

Species	Reference	Antennae			Cirri				Tubercles	CvLb adjacent to C4	AcvLb
		T1	T2	T3	C1 to C3	C4	Expansion at the base of C4	C5			
Stratiodrilus. tasmanicus	Haswell (1900)	Us	Bs	Bs	Si, Bs	Si, Us	Not informed	A	A	1 pair	Not informed
Stratiodrilus novaehollandiae	Haswell (1913)	Us	Bs	Bs	Si, Bs	Si, Bs	Not informed	A	A	1 pair	Not informed
Stratiodrilus platensis	Cordero (1927)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	A	A	Not informed
Stratiodrilus haswelli	Harrison (1928)	Us	Bs	Bs	Si, Us, Fo	Si, Bs	Not informed	A	1 pair	A	Not informed
Stratiodrilus aeglaphilus	Vila & Bahamonde (1985)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	1 pair	A	Not informed
Stratiodrilus pugnaxi	Vila & Bahamonde (1985)	Us	Us	Bs	Si, Us	[1] Do, Us	Not informed	[2] Do, Us	1 pair	Not informed	Not informed
Stratiodrilus arreliai	Amaral & Morgado (1997)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	1 pair	1 pair	Not informed
Stratiodrilus robustus	Steiner & Amaral (1999)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	A	1 pair	Not informed
Stratiodrilus circensis	Steiner & Amaral (1999)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	2 pairs	1 pair	Not informed
* *Stratiodrilus circensis*	Daudt & Amato (2007)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	2 pairs	1 pair	[3] A
Stratiodrilus circensis	Present study	Us	Us	Bs	Si, Us	Si Us	Present	A	2 pairs	1 pair	A
Stratiodrilus vilae	Amato (2001)	Us	Us	Bs	Si, Us	Si, Us	Not informed	A	2 pairs	1 pair	1 pair
Stratiodrilus brevicirrus	Amato *et al.* (2004)	Us	Us	Bs	Si, Us	Si, Us	Present	A	2 pairs	A	A

* Populations of *Stratiodrilus circensis* from Divisa river and Carvalho river, Rio Grande do Sul State.

** *Stratiodrilus circensis* from the seven populations.

[1] Amaral & Morgado (1997) and Steiner & Amaral (1999) reffered the cirrus C4 of *Stratiodrilus pugnaxi* as double. However, Amato (2001) stated that, in some species, the cirrus C4 may be associated with and adjacent conical, ventral lobe (CvLb), which is completely different in constitution when compared with cirri.

[2] Amato (2001) stated that the pair of cirri C5 referred by Amaral & Morgado (1997) and Steiner & Amaral (1999) for *S. pugnaxi* is, in fact, a pair of anal, conical, ventral lobes (AcvLb)

[3] Daudt & Amato, 2007 not informed in the text the AcvLb, but represented in their drawnings some structures that ressembles the AcvLb.

TABLE 3. Measurements (in μm) of general structures of specimens of *Stratiodrilus circensis* from seven populations of aeglids from State of Paraná (CO–Couro Stream; PT–tributary of Preto River; BU–Bule Stream; JA–Jacutinga Stream; PI–Pinhão River; LA–Lageadão River; and EM–Ema Stream). Specimens of *S. circensis* from five localities (CO, PT, BU, JA, and LA) had some morphological structures compared by ANOVA (T1, C1, and T2) and Kruskal-Wallis test (C2, C3, C4, and T3). All measurements are in micrometers. Minimum and maximum numbers are followed (between parenthesis) by averages ± standard deviation values. Hyphens mean the morphological structures not compared among populations.

	Tibagi River Basin				**Iguaçu River Basin**	**Ivaí River Basin**	**Pirapó River Basin**	
Structure	**CO** ***Aegla castro*** **n=18**	**PT** ***Aegla castro*** **n=8**	**BU** ***Aegla lata*** **n=14**	**JA** ***Aegla* sp. 1** **n=8**	**PI** ***Aegla parana*** **n=2**	**LA** ***Aegla* sp. 2** **n=21**	**EM** ***Aegla* sp. 3** **n=4**	**Comparisons**
Body length	866.6–1184.4 (1054.7±93.0)	887.0–1100.7 (998.9±71.8)	797.5–1166.8 (1018.7±101.6)	892.6–1162.7 (1037.1±81.2)	1551.7–1747.7 (1649.7±138.6)	843.8–1144.0 (1004.6±94.5)	779.7–1075.0 (957.0±130.6)	–
Head length	158.6–197.4 (175.8±13.5)	163.4–189.6 (178.2±10.2)	157.0–183.0 (169.2±8.4)	164.8–185.6 (175.8±7.3)	224.8–244.4 (234.6±13.9)	157.6–185.0 (170.8±7.3)	164.4–192.8 (178.3±13.8)	–
Ratio body and head length	1:0.15–0.19 (1:0.17)	1:0.17–0.20 (1:0.18)	1:0.14–0.20 (1:0.17)	1:0.15–0.19 (1:0.17)	1:0.13–0.16 (1:0.14)	1:0.15–0.20 (1:0.17)	1:0.16–0.21 (1:0.19)	–
Jaw apparatus length	130.7–185.6 (151.2±14.1)	135.5–157.9 (148.3±6.9)	137.1–158.5 (146.4±7.3)	130.3–151.1 (142.7±7.4)	178.2–182.3 (180.2±3.0)	122.5–165.6 (148.3±9.0)	129.5–150.9 (144.0±10.0)	–
Ratio head and jaw apparatus length	1:0.78–0.96 (1:0.86)	1:0.71–0.92 (1:0.83)	1:0.78–0.97 (1:0.87)	1:0.74–0.87 (1:0.81)	1:0.73–0.81 (1:0.77)	1:0.74–0.94 (1:0.87)	1:0.78–0.86 (1:0.81)	–
Fulcrum length	70.4–113.5 (88.7±10.3)	84.6–98.0 (91.0±4.9)	76.4–96.8 (85.4±7.5)	75.6–95.3 (86.4±6.0)	111.6–115.6 (113.6±2.8)	61.8–110.3 (87.0±13.6)	52.0–103.6 (79.5±26.1)	–

Table 3 continued.

Length of median antenna (T1)	22.2–42.4 (36.4±5.4)	32.4–47.2 (42.1±4.8)	34.9–45.9 (40.4±3.5)	33.8–45.8 (42.1±3.6)	46.6*	43.6–54.7 (48.9±3.1)	30.1–39.4 (35.6±4.1)	p≤0.001
Length of first pair of antennae (T2)	37.5–50.8 (44.2±4.1)	35.2–54.8 (48.3±6.1)	39.7–56.4 (47.0±4.9)	38.4–49.6 (44.2±3.2)	52.2–52.7 (52.4±0.3)	42.1–60.8 (49.5±4.2)	30.7–45.2 (39.1±6.3)	p≤0.004
Length of second pair of antennae (T3)	66.3–112.3 (92.6±11.5)	86.3–103.5 (97.7±5.2)	88.5–109.2 (98.8±6.2)	93.0–105.5 (98.4±4.0)	126.8–133.1 (129.9±4.5)	103.9–120.7 (113.4±5.1)	80.0–91.9 (85.9±4.9)	p≤0.001
Length of first lateral pair of cirri (C1)	30.2–63.4 (49.9±8.2)	55.2–63.9 (59.6±2.6)	50.0–71.9 (61.2±6.3)	60.1–71.9 (65.8±4.3)	83.1–89.4 (86.3±4.5)	66.5–89.4 (78.3±5.7)	48.8–57.1 (52.4±3.4)	p≤0.001
Length of second lateral pair of cirri (C2)	32.8–71.1 (52.6±10.2)	54.5–69.8 (62.7±4.5)	57.5–74.8 (64.9±4.6)	49.9–77.3 (64.7±8.8)	96.2–100.9 (98.5±3.3)	68.3–94.9 (83.7±6.5)	51.9–64.9 (59.8±5.7)	p≤0.001
Length of third lateral pair of cirri (C3)	36.5–72.3 (52.6±10.3)	51.1–64.5 (59.5±4.8)	51.8–64.6 (59.9±3.9)	60.3–76.8 (67.8±5.0)	99.7–124.1 (111.9±17.3)	71.8–101.8 (82.0±8.1)	35.7–58.8 (48.1±9.9)	p≤0.001
Length of posterior pair of cirri (C4)	40.3–82.2 (58.2±13.5)	80.7–90.3 (83.9±3.7)	54.8–79.8 (66.5±6.4)	63.0–81.0 (73.6±5.2)	101.6–112.6 (107.1±7.7)	81.7–105.7 (92.1±6.7)	59.8–66.4 (63.3±2.9)	p≤0.001
Length of anterior locomotor appendage (PLA)	213.9–301.0 (265.9±23.0)	258.0–306.4 (278.9±18.4)	239.4–291.5 (266.4±17.0)	253.6–297.1 (273.2±15.9)	378.5–423.3 (400.9±31.7)	243.5–303.1 (272.2±18.4)	223.1–276.8 (244.8±23.3)	–

*Measure obained from a single specimen.

TABLE 4. Number of eggs and measurements of male sexual structures of specimens of *Stratiodrilus circensis* from seven populations of aeglids from State of Paraná (CO–Couro Stream; PT–tributary of Preto River; BU–Bule Stream; JA–Jacutinga Stream; PI–Pinhão River; LA–Lageadão River; and EM–Ema Stream). Measurements of penis and claspers are in micrometers. Minimum and maximum numbers are followed (between parenthesis) by averages ± standard deviation values. Hyphens mean measurements not provided.

	Tibagi River Basin				**Iguaçu River Basin**	**Ivaí River Basin**	**Pirapó River Basin**
Structure	**CO** ***Aegla castro***	**PT** ***Aegla castro***	**BU** ***Aegla lata***	**JA** ***Aegla* sp. 1**	**PI** ***Aegla parana***	**LA** ***Aegla* sp. 2**	**EM** ***Aegla* sp. 3**
Number of eggs	0–3 (n=11)	1 (n=4)	0–5 (n=10)	0–1 (n=2)	6 (n=1)	1 (n=9)	0–1 (n=3)
Penis length	95.6–122.8 (109.3±10.3) (n=7)	90.5–121.3 (112.2±12.5) (n=5)	68.9–74.8 (72.5±3.0) (n=5)	56.6–110.7 (93.3±11.0) (n=6)	132.1 (n=1)	71.9–107.2 (85.3±11.1) (n=12)	76.4 (n=1)
Penis width	15.9–28.5 (23.6±4.0) (n=7)	15.5–23.6 (18.8±3.0) (n=5)	16.4–32.2 (24.0±6.2) (n=5)	17.2–25.6 (19.7±3.0) (n=6)	26.4 (n=1)	12.6–31.5 (17.5±5.4) (n=12)	15.1 (n=1)
Ratio penis length and penis width	1:0.15–0.26 (0.22) (n=7)	1:0.14–0.20 (0.17) (n=5)	1:0.22–0.43 (0.33) (n=5)	1:0.17–0.31 (0.21) (n=6)	1:0.20 (n=1)	1:0.15–0.44 (0.21) (n=12)	1:0.20 (n=1)
Clasper length	90.4–117.9 (105.2±11.4) (n=4)	84.7–89.1 (86.9±3.1) (n=2)	-	116.8–137.6 (127.2±14.7) (n=2)	-	-	-
Clasper width	41.0–50.7 (44.2±4.5) (n=4)	48.3–55.2 (51.7±4.9) (n=2)	-	45.2–51.2 (48.2±4.3) (n=2)	-	-	-

TABLE 5. Loading values obtained from principal component analysis (PCA) of morphological structures from seven populations of *Stratiodrilus circensis* from State of Paraná.

Morphological structure	PC1	PC2
Body length	0.432	0.720
Head length	0.374	0.737
Jaw apparatus length	0.505	0.703
Fulcrum length	0.361	0.621
Length of median antenna (T1)	0.777	-0.435
Length of first pair of antennae (T2)	0.583	-0.141
Length of second pair of antennae (T3)	0.915	-0.196
Length of first lateral pair of cirri (C1)	0.905	-0.297
Length of second lateral pair of cirri (C2)	0.903	-0.263
Length of third lateral pair of cirri (C3)	0.896	-0.209
Length of posterior pair of cirri (C4)	0.877	-0.254
Length of anterior locomotor appendage (PLA)	0.658	0.533
Proportion variance	0.511	0.228
Cumulative variance	0.511	0.739

CONCLUSÃO

A fauna de simbiontes metazoários associada a espécies de *Aegla* ainda é insuficientemente estudada. Dentre esses organismos, os temnocefalídeos são provavelmente o grupo mais estudado, com maior número de espécies descritas, com maior diversidade de hospedeiros não-eglídeos e com maior distribuição já reportada na região Neotropical. Por outro lado, *Stratiodrilus* é encontrado em várias localidades da América do Sul, Madagascar e Austrália, é reportado por estabelecer associação apenas com crustáceos dulcícolas, e tem um número reduzido de espécies conhecidas.

Há uma necessidade em se melhorar as técnicas de análise morfológica que promovam ilustrações e fotos de qualidade, para ajudar na identificação de espécies. Além disso, a implementação de medidas detalhadas e a padronização dos protocolos de fixação contribuem para a compararação de caracteres morfológicos. No entanto, estudos moleculares podem fornecer grande contribuição para a identificação segura e espécies ea delimitação de grupos de espécies crípticas.

Nosso estudo contribui para o aumento do conhecimento das espécies de *Temnocephala* associadas a eglídeos e reporta a primeira espécie descrita no estado do Paraná. Além disso, como *A. lata* foi recentemente enquadrada como uma espécie criticamente ameaçada (Santos *et al.* 2017) e encontrada apenas em pequenos riachos de um remanescente de Mata Atlântica, o inventário dos simbiontes associados a essa espécie é de extrema importância para o conhecimento da diversidade biológica. No entanto, o fato de não terem sido reportadas espécies de *Temnocephala* para a região norte e noroeste do Paraná não quer dizer necessariamente que esses organismos não ocorram nessa região, mas pode ser um reflexo da falta de amostragem de seus potenciais hospedeiros.

Portanto, *Temnocephala* sp. nov. é a vigésima espécie descrita no Brasil e a sexta espécie associada a hospedeiros eglídeos. A nova espécie pode ser identificada principalmente pela forma e tamanho das placas sinciciais dorsolaterais, pelo formato do cirro, e pela ornamentação conspícua dos ovos.

Além disso, nós apresentamos um estudo da variação morfológica de sete populações de *S. circensis* associados a seis espécies de hospedeiros eglídeos provenientes de quatro bacias hidrográficas. Esse estudo é o primeiro a apresentar medidas detalhadas do comprimento das antenas e dos cirros para uma espécie de *Stratiodrilus*, assim como o primeiro a reportar diferenças estatísticas com respeito a dimensão dessas estruturas entre diferentes populações de *S. circensis*. Nossos resultados também mostraram que alguns caracteres de valor taxonômico para o diagnóstico de *S. circensis*—como forma e comprimento dos lobos adjacentes cônicos ventrais e a extensão da mandíbula em relação ao primeiro segmento do corpo—devem ser considerados com cautela, já que dentro de uma mesma população foi possível observar diferentes formatos e tamanhos dessas estruturas. Além disso, as análises morfométricas evidenciaram que o comprimento dos cirros e das antenas são caracteres importantes para o estudo da morfologia de *Stratiodrilus*, e que as diferenças estatísticas encontradas são suficientes para discriminar pelo menos três populações morfologicamente distintas. As diferentes espécies de hospedeiros eglídeos reportados aliado ao isolamento geográfico dessas populações sugerem que as variações morfológicas encontradas são suficientes para considerar que as populações de ectocomensais amostradas constituem um grupo de espécies crípticas.

Assim, a adoção de diferentes métodos para a visualização de estruturas morfológicas, a padronização de protocolos de fixação e a implementação de estudos morfométricos detalhados contribuem para o conhecimento da diversidade de simbiontes associados à eglídeos, assim como para a validação de caracteres morfológicos utilizados nas descrições. Além disso, estudos de inventário de *Temnocephala* e *Stratiodrilus* quanto de seus potenciais hospedeiros são importantes para aumentar o conhecimento dessa fauna de simbiontes pouco amostrada.