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ARTICLE

The epidermal 'excretory' syncytial plates in species of *Temnocephala* (Platyhelminthes, Temnocephalida): Proposal of a new methodology

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ABSTRACT: (The epidermal 'excretory' syncytial plates in species of *Temnocephala* (Platyhelminthes, Temnocephalida): Proposal of a new methodology). The epidermis of temnocephalids is formed by a mosaic of syncytial plates as revealed by electron microscopy and/or 5% silver nitrate impregnation. The variable form and size have been observed but the value of the epidermal 'excretory' syncytial plates (EPs) in the identification of *Temnocephala* species was never properly discussed. Ten species of *Temnocephala* were studied using images taken with a scanning electron microscope (SEM), describing a morphometrical method to allow the comparison, and thus the evaluation the EPs as a specific character in the identification of species of *Temnocephala*. The measurements (length of the anterior and posterior portions of the plate relative to the excretory pore, width of the internal and external limits, length of the portion exceeding the tentacles, total width, and total length) were obtained using AxioVision® Zeiss LWE 4.7.2 (AVZ) software. The EPs of the analyzed species were different revealing unique characteristics thus allowing their use as specific characters within *Temnocephala*. There was a pattern for each host group (Mollusca, Crustacea, Insecta, and Chelonia); the exceptions were the crustacean species and one insect species. The intraspecific variation study in *Temnocephala trapeziformis* demonstrated that this character varied minimally within the species. The SEM images and the AVZ software measurements provided good results for the characterization of the species of *Temnocephala*. The cirrus remained the most important specific character within *Temnocephala*. The traditional morphometric method was sufficient to demonstrate several specific EP characteristics. The EPs should always be clearly described and illustrated following the now proposed methodology for *Temnocephala* species identification.
Key words: Neotropical Region, new specific diagnostic character, symbiosis; syncytia, traditional morphometry.

RESUMO: (As placas epidérmicas sinciciais 'excretoras' em espécies de *Temnocephala* (Platyhelminthes, Temnocephalida): Proposta de uma nova metodologia). A epiderme dos representantes da ordem Temnocephalida é formada por um mosaico de placas epidérmicas sinciciais evidenciado através da microscopia eletrônica e da impregnação com nitrato de prata 5%. A variação de forma e tamanho do par de sincícios pós-tentaculares, ou 'placas excretoras' (PEs), entre as espécies já foram observados, mas o valor das PEs na identificação de espécies de *Temnocephala* nunca foi adequadamente discutido. Dez espécies de *Temnocephala* foram analisadas através de imagens obtidas em microscopia eletrônica de varredura (MEV), descrevendo um método morfométrico que permite a comparação, e avaliação, das PEs como carácter específico. As medidas (comprimento da porção anterior e posterior, largura do limite interno e externo, comprimento da porção da placa acima do limite dos tentáculos, comprimento e largura totais) foram obtidas no programa AxioVision Zeiss LE 4.7.2 (AVZ). As PEs das espécies estudadas diferiram entre si e possuem características únicas que fazem delas bons caracteres diagnósticos específicos. Possuem um padrão por grupo hospedeiro; as exceções são as espécies epibiontes em crustáceos e uma espécie epibionte em insetos. O estudo da variação intraespecífica de *Temnocephala trapeziformis* mostrou que este caráter tem pouca variação dentro de uma mesma espécie. A visualização das PEs em MEV e as medidas obtidas através do programa AVZ produziram bons resultados na caracterização das espécies. O estudo morfométrico tradicional foi suficiente para evidenciar as características específicas das PEs. Estas devem ser claramente descritas e ilustradas, seguindo a metodologia agora proposta, para permitir a utilização deste caráter diagnóstico na identificação das espécies de *Temnocephala*.
Palavras-chave: morfometria tradicional, novo carácter diagnóstico específico, Região Neotropical, simbiose, sincícios.

INTRODUCTION

Williams (1975, 1978, 1979, 1980, 1982, 1985, 1986, and 1992) recognized that the body covering of temnocephalans showed a high degree of regional differentiation and functional specialization, suggesting that the epidermis in Temnocephalida could be more complex than a simple epithelium. In fact, Temnocephalida possess an epidermis made of several syncytia (Williams 1980, Joffe *et al*. 1996, Damborenea & Cannon 2001). The presence of this multi-syncytial epidermis was established as the basal characteristic linking the organisms of this order (Cannon & Joffe 2001).

Joffe *et al.* (1995) and Sewell & Cannon (1998) developed detailed studies about *Didymorchis* syncytial epidermis using specimens impregnated with 5% silver

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nitrate. Other studies, mainly until the year 2000, were done with a scanning electron microscope (SEM) or a transmission electron microscope (TEM) (Damborenea & Cannon 2001, Seixas *et al.* 2014).

Damborenea & Cannon (2001) indicated the number of syncytial plates as the character which, together with other characters, separated the Neotropical from the Australasian specimens in the genus *Temnocephala* Blanchard, 1849. In this study, the Australasian species were grouped in the new genus *Temnosewellia* Damborenea & Cannon, 2001. The authors named the four syncytial plates found in every *Temnocephala* species (Fig. 1A), including the paired post-tentacular syncytial plates (PTS) (Fig. 1A - b). In line with the proposed osmoregulatory/excretory function of the PTS, Damborenea & Cannon (2001) used the term epidermal 'excretory' syncytial plates. We follow this terminology and hereafter abbreviate the epidermal 'excretory' syncytial plates as EPs. Damborenea & Cannon (2001) showed the pattern of these syncytia in seven Neotropical species: *Temnocephala chilensis* (Moquin-Tandon, 1846), *Temnocephala talicei* Dioni, 1967, *Temnocephala decarloi* Moretto, 1978, *Temnocephala pignalberiae* Dioni, 1967, *Temnocephala microdactyla* Monticelli, 1903, *Temnocephala iheringi* Haswell, 1893, and *Temnocephala digitata* Monticelli, 1902. The authors stated that the EPs always enclosed the excretory pore, and, observed that their shape and form were variable between species, without emphasizing the specific value of the EPs.

Sewell *et al.* (2006) recognized the epidermal syncytial plates as a valuable character to discriminate taxa at the higher level, but considered them as less useful at generic and specific levels. Nonetheless, they included the EPs in their revision of the Australian temnocephalans as a potential character of specific diagnostic value.

The only work about the value and relevance of specific characters of temnocephalans (Damborenea 1991) used in the identification of the species of *Temnocephala* was done prior to the discovery and study of the EPs. The significant plasticity, due to the high capacity of muscular contraction and the absence of a rigid body cover, creates differences in relation to morphometry depending upon the technique employed. The cirrus is the only rigid structure and therefore of constant general morphology (except for small intraspecific variations) for each species. Thus, the morphology of the cirrus constitutes one of the few characters used once the juveniles leave the egg, and is the most valuable taxonomic character for species identification.

The need for new specific characters to separate species within *Temnocephala* has been pointed out by Volonterio (2007), above all, to help to differentiate species with similar cirrus morphometry. This is also true for species found on the same individual host or in the same host group. The author also suggested the description of the shape and extension of the EPs, among other characters as well as all organs of the feminine reproductive system.

When using techniques to demonstrate the EPs (Romeis 1968, Cannon & Sewell 1995, Joffe *et al.* 1995), many authors did not document the observed results,

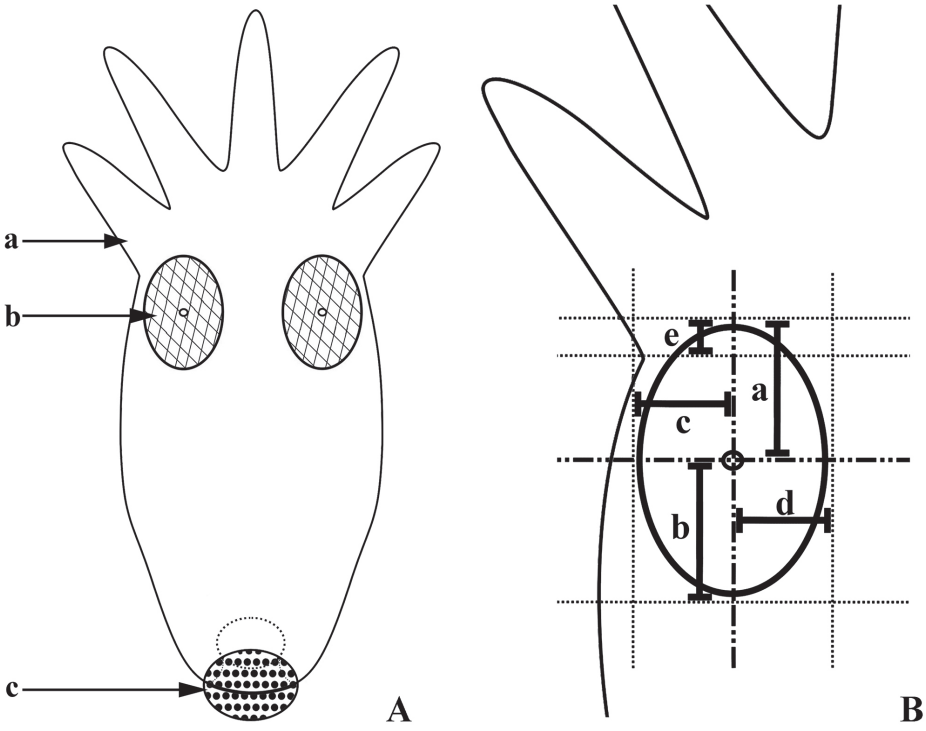

Figure 1. A. Diagram of the syncytial plates found in *Temnocephala*, showing the body syncytia (a), two post tentacular 'excretory' syncytia (b), and adhesive disc syncytia (c). **B.** Measurements of the post tentacular 'excretory' syncytia (EP) obtained in AxioVision Zeiss LE 4.7.2 (AVZ) software. Length of the anterior (a), and posterior portions (b) of the EP relative to the excretory pore; width of the external (c), and internal (d) limits of the EP relative to the excretory pore; length of the portion exceeding the tentacles' limit (e) with the body.

and limited themselves to show a simple diagram or a short description in the text. This fact makes it difficult to use this character, as few works have described the EPs using a method which could allow future comparisons; the specific value of the EPs has been little exploited.

The present work has the main purpose of comparing the EPs of some Neotropical species, describing a morphometric method to allow the comparison, and thus, evaluating the EPs as a specific diagnostic character in the identification of species of *Temnocephala*.

MATERIAL AND METHODS

Ten species of *Temnocephala* had their EPs studied through SEM images included in original species descriptions or re-descriptions done in the 'Laboratório de Helmintologia, UFRGS'. Due to the small number of images in each manuscript, SEM images from 10 specimens of *Temnocephala trapeziformis* Amato, Amato & Seixas, 2006 were obtained for the intraspecific variation study. All the studied species, their host, and the references, where the localities could be consulted, are summarized in Table 1.

For the observation of the EPs with SEM, helminths were fixed with hot (90 °C) 10% phosphate buffered formalin; dehydrated with an ethanol/acetone series and critical point dried (BAL-TEC CPD030). The specimens were gold sputtered (BAL-TEC SCD050), observed and photomicrographed with a JEOL (JSM-6060) SEM at the 'Centro de Microscopia Eletrônica da UFRGS (CME)'.

The images of the EPs were vectorially treated in CorelDraw X5® and the drawings edited in Adobe Photoshop® CC, where the limit between the EPs and guidelines for the measurements in relation to the excretory pore and the limit between the tentacles and the body were drawn (Fig. 1B). Based on these images, the EPs were measured using the AxioVision Zeiss LE 4.7.2 software. The measurements taken were: 1. the total length of the EP corresponds to the sum of the length of the anterior (Fig. 1B - a) and the posterior portions (Fig. 1B - b) of the EP relative to the excretory pore; 2. the total width of the EP corresponds to the sum of the width of the external (Fig. 1B - c), and the internal limits (Fig. 1B - d) of the EP relative to the excretory pore; and 3. the length of the portion exceeding the tentacles' limit with the body (Fig. 1B - e). The relationship between total body length and width (without tentacles) and the length and width of the EPs was also analyzed.

For the comparison between the EPs, 10 species of *Temnocephala* were grouped as follows: 1. Host group: a) mollusks (*T. iheringi*, *Temnocephala rochensis* Ponce de León, 1979*,* and *Temnocephala haswelli* Ponce de León, 1989); b) insects (*Temnocephala curvicirri* Amato & Amato, 2005, *Temnocephala minutocirrus* Amato, Amato & Seixas, 2007*,* and *Temnocephala caddisflyi* Amato, Amato & Seixas, 2011), and c) crustaceans (*Temnocephala cyanoglandula* Amato, Amato & Daudt, 2003*, Temnocephala lutzi* Monticelli, 1913, *T. trapeziformis, T. pignalberiae,* and *Temnocephala longivaginata* Seixas, Amato & Amato, 2011); 2. Same individual host (concurrent infestations): a) ectosymbionts on *Pomacea canaliculata* (Lamarck, 1822) (*T. iheringi*, *T. rochensis,* and *T. haswelli*), and, b) ectosymbionts on *Trichodactylus fluviatilis* Latreille, 1828 (*T. lutzi* and *T. trapeziformis*); 3. Same host species, at different localities: a) *T. pignalberiae* ectosymbiont on *Dilocarcinus pagei* Stimpson, 1861 of Poconé, MT and b) *T. pignalberiae* ectosymbiont on *D. pagei* of Bebedouro, SP; 4. Intraspecific variation: *T. trapeziformis*.

RESULTS

Description of the epidermal 'excretory' syncytial plates

The measurements of the EPs in relation to the excretory pore and the plate limits in relation to the tentacle limits showed differences among the 10 species studied (Table 2).

The EPs of *T. iheringi* (Fig. 2A), *T. lutzi* (Fig. 2B), and *T. minutocirrus* (Fig. 2C) are longer than wide, similar to an ellipsis. In *T. iheringi* the posterior portion is narrower and longer in length than the anterior portion; the excretory pore, almost central, is closer to the external limit of the EP. Eleven percent of the total length is beyond the limit of the tentacles with the body (Fig. 2A). In *T. lutzi* and *T. minutocirrus*, the anterior portion of the EP is slightly longer than the anterior portion (Table 2), and the excretory pore is central. *Temnocephala lutzi* has 19% of the EPs total length beyond the limit between

Table 1. Species of *Temnocephala* that have their 'excretory' plates (EPs) analyzed in the present work, their hosts, and the references, where the localities could be consulted.

Species	Hosts	Reference
Temnocephala iheringi Haswell, 1893	*Pomacea canaliculata* (Lamarck, 1822)	Seixas *et al.* 2010a
Temnocephala lutzi Monticelli, 1913	*Trichodactylus panoplus* (von Martens, 1869)	Amato *et al.* 2005
Temnocephala pignalberiae Dioni, 1967	*Dilocarcinus pagei* Stimpson, 1861	Amato *et al.* 2010
Temnocephala rochensis Ponce de León, 1979	*Pomacea canaliculata* (Lamarck, 1822)	Seixas *et al.* 2010b
Temnocephala haswelli Ponce de León, 1989	*Pomacea canaliculata* (Lamarck, 1822)	Seixas *et al.* 2010c
Temnocephala cyanoglandula Amato, Amato & Daudt, 2003	*Aegla serrana* Buckup & Rossi, 1977	Amato *et al.* 2003
Temnocephala curvicirri Amato & Amato, 2005	*Belostoma* spp.	Amato & Amato 2005
Temnocephala trapeziformis Amato, Amato & Seixas, 2006	*Trichodactylus fluviatilis* Latreille, 1828	Amato *et al.* 2006
Temnocephala minutocirrus Amato, Seixas & Amato, 2007	*Cryphocricos granulosus* De Carlo, 1967	Amato *et al.* 2007
Temnocephala caddisflyi Amato, Amato & Seixas, 2011	*Barypenthus* sp.	Amato *et al.* 2011

tentacles and body (Fig. 2B), and in *T. minutocirrus*, the epidermis which covers the delimited area of the EPs has a corrugated aspect, different than the epidermis in other body syncytia. Four-and-a-half percent of its length lies above the limit of tentacles with the body (Fig. 2C).

The EPs in *T. pignalberiae,* collected in Bebedouro, SP (Fig. 2D) and in Poconé, MT (Fig. 2E), are the longest EPs of all species of *Temnocephala* studied in the present work. The length is much longer than the width and the distance of the excretory pore to the posterior portion is three times larger than to the anterior portion. The excretory pore is in the anterior portion and closer to the internal limit of the plates. Specimens from Bebedouro had the largest EPs (Table 2), and 21% of the EPs total length lies above the limit between body and tentacles (Fig. 2D). The EPs of specimens from Poconé are similar to those specimens from Bebedouro, although smaller in size (total length and width). Only 4% of the EPs total length lies above the limit between the body and the tentacles (Fig. 2E).

Temnocephala rochensis (Fig. 2F), *T. haswelli* (Fig. 2G), and *T. cyanoglandula* (Fig. 2H) have rounded EPs. In *T. rochensis,* the EPs are a bit longer than wide; the anterior and posterior portions are of similar size; the

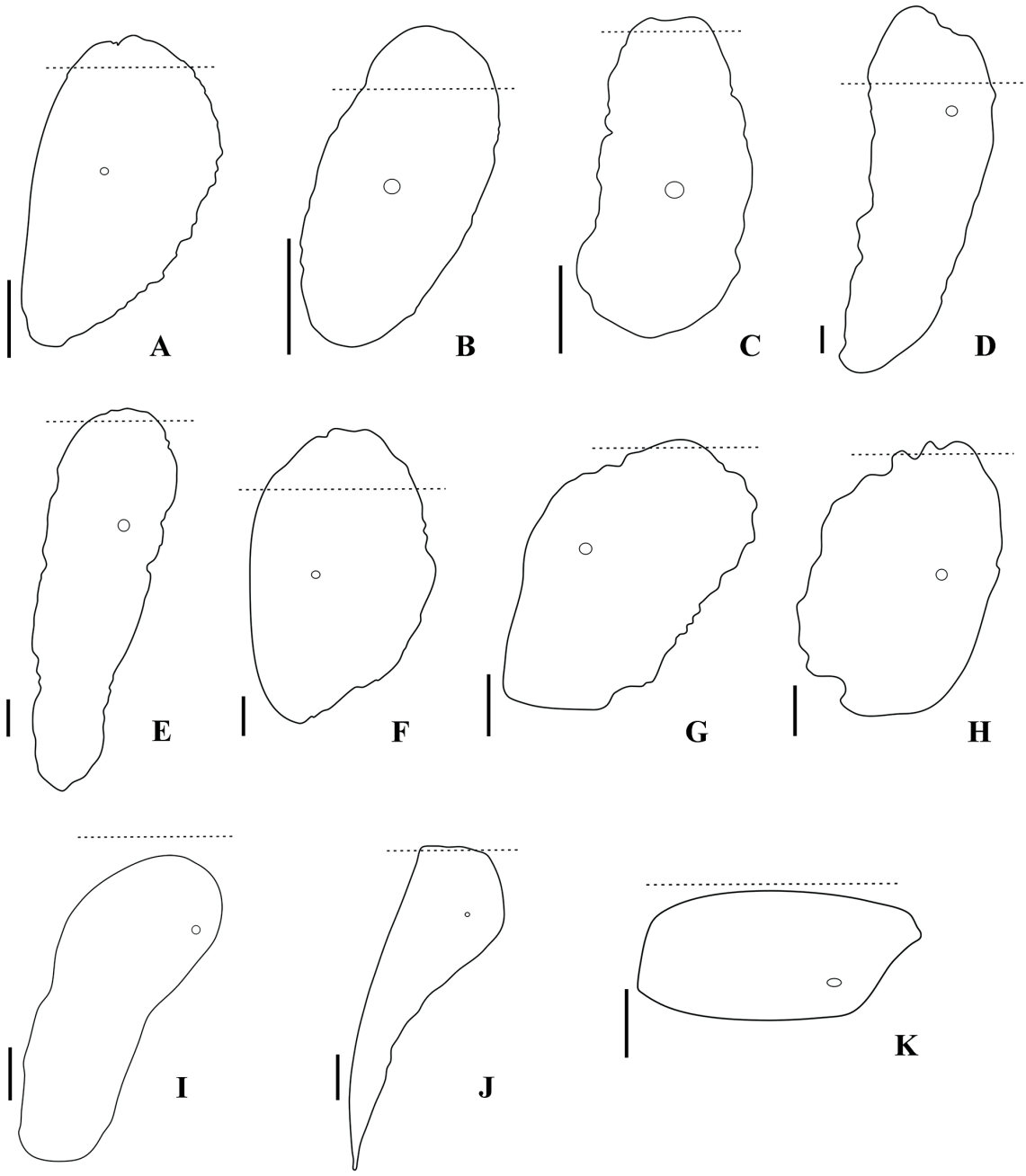

Figure 2. Diagram of scanning electron microscope images of the epidermal 'excretory' syncytial plates of 10 species of *Temnocephala* studied in the present work, showing the portion exceeding the tentacles' limit with the body (dotted lines). Bars = 50 µm. **A.** *Temnocephala iheringi.* **B.** *Temnocephala lutzi*. **C.** *Temnocephala minutocirrus.* **D.** *Temnocephala pignalberiae* (collected in Bebedouro, SP). **E.** *Temnocephala pignalberiae* (collected in Poconé, MT). **F.** *Temnocephala rochensis.* **G.** *Temnocephala haswelli.* **H.** *Temnocephala cyanoglandula.* **I.** *Temnocephala curvicirri.* **J.** *Temnocephala caddisflyi.* **K.** *Temnocephala trapeziformis*.

Table 2. Measurements (μm) of the 'excretory' plates (EPs) of 10 species of *Temnocephala* analyzed with the AxioVision Zeiss LE 4.7.2. software.

Species	A	B	C	D	E	(A+B)	(C+D)	X Ratio	Y Ratio
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 (Bebedouro, SP)	191.72	473.96	209.46	84.62	142.01	665.68	294.08	1:3.7	1:5.7
Temnocephala pignalberiae (Poconé, MT)	165.00	356.25	127.50	76.88	23.13	521.25	204.38	1:5	1:9.1
Temnocephala rochensis	182.34	188.22	80.06	150.65	78.75	370.56	230.71	1:7.5	1:6.7
Temnocephala haswelli	95.09	132.66	65.03	139.88	13.58	227.75	204.91	1:9.5	1:6.5
Temnocephala cyanoglandula	136.55	147.89	150.84	60.92	17.65	284.44	211.76	1:9.2	1:10.3
Temnocephala curvicirri	73.58	222.20	165.54	26.68	-	295.78	192.22	1:7.3	1:7.4
Temnocephala caddisflyi	79.00	282.67	130.67	45.00	7.67	361.67	175.67	1:5	1:7.2
Temnocephala trapeziformis	70.90	29.48	145.52	66.79	-	100.38	212.31	1:17.3	1:6.7

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

excretory pore is slightly displaced to the external limit of the plates. Twenty-one percent of the total length of the plate is beyond the limit of the tentacles with the body (Fig. 2F). The EPs of *T. haswelli* also have similar total length and width, but the width of the anterior portion is larger. The excretory pore is located at the anterior portion, near the external limit of the plates. Six percent of the total length of the plates lies above the tentacles' limit with the body (Fig. 2G). In *T. cyanoglandula*, the EPs have irregular borders and their length and width are similar. The excretory pore is almost central, slightly displaced to the internal limit of the plates. Six percent of the total length of the plates is beyond the tentacles' limit with the body (Fig. 2H).

Temnocephala curvicirri (Fig. 2I) and *T. caddisflyi* (Fig. 2J) have EPs with the format of a horse saddle, much longer than wide, and with the widest portion at the anterior end (Table 2). The EPs of both species are similar and the excretory pore is in the anterior portion of the plate, nearer to its internal limit. In *T. curvicirri,* the entire EPs are located below the limit of the tentacles (Fig. 2I) and in *T. caddisflyi*, the EPs have their length twice the size of their width, and 2% of the total length of the plate lies above the tentacles' limit (Fig. 2J).

Temnocephala trapeziformis (Fig. 2K) has the most peculiar EPs among the 10 species studied. Similar to a trapezium, it is the only EP which is wider than long. It is the only species where the excretory pore is located in the posterior portion of the EP, near the internal limit. The entire plate is located below the tentacles' limit with the body (see the study of the intraspecific variation) (Fig. 2K).

Description of the epidermal 'excretory' syncytial plates of species on the same host group

When the EPs between host groups are compared the group pattern is observed, although there are a few exceptions. Usually, the format and measurements relative to the excretory pore are similar and the species vary in the relationship of total body length/length of the EPs and total body area/area of the 'excretory' plate. In the ectosymbiont species living on mollusks (*T. iheringi*, *T. rochensis,* and *T. haswelli* ectosymbiont on *P. canaliculata*), the round format predominates, but in some details it is possible to differentiate the plates of these species. *Temnocephala rochensis* has the EPs nearly round and larger (area and total body length / length of the EPs ratio) (Table 2), than those of the other two species (Fig. 2F). The difference is still larger among species which live on the same host and habitat, *T. iheringi* (Fig. 2A) and *T. haswelli* (Fig. 2G).

The species ectosymbiont on insects have the EPs large and with a horse-saddle format, with the exception of *T. minutocirrus* which has elliptic EPs, with a corrugated surface. The EPs of *T. caddisflyi* and *T. curvicirri* follow the pattern of the group and are similar, although the plates of the former species are longer. This difference is evidenced when the relationship between total body length/length of the EPs, as *T. curvicirri* has greater total body length.

The format of the EPs among the ectosymbionts of crustaceans showed great variation, thus not allowing the establishment of a pattern for the group.

Epidermal 'excretory' syncytial plates of species on the same host (concurrent infestations)

The species *T. iheringi* and *T. haswelli*, ectosymbionts of *P. canaliculata* (co-occurring in the locality of Arrozeira, RS), can be differentiated based on this character. The EPs of *T. iheringi* (Fig. 2A) have an elliptic format and are the smallest among the three ectosymbiont species living on mollusks. The EPs of *T. haswelli* (Fig. 2G) differ from the EPs of *T. iheringi* by having the posterior portion wider and more rounded.

The species co-occurring on *Tr. fluviatilis* have differences still more pronounced. The EPs of *T. lutzi* are small, elliptic and longer than wide. *Temnocephala trapeziformis* is the only species where the EPs have the width much greater than the length.

Table 3. Measurements (µm) of the 'excretory' plates (EPs) of 10 specimens of *Temnocephala trapeziformis*.

Specimens	A	B	C	D	(A+B)	(C+D)
1	70.90	29.48	145.52	66.79	100.38	212.31
2	87.90	25.93	96.91	98.77	113.83	195.68
3	52.20	14.93	70.15	60.45	67.13	130.60
4	60.45	15.67	50.77	68.66	76.12	119.43
5	52.94	29.41	58.50	37.91	82.35	96.41
6	53.28	26.57	71.50	38.36	79.85	109.86
7	50.78	15.60	63.27	42.41	66.38	105.68
8	33.25	8.34	35.97	26.69	41.59	62.66
9	48.21	18.85	68.65	55.16	67.06	123.81
10	50.40	15.61	44.67	52.37	66.01	97.04
AVERAGE ± SD	**56.03 ± 15**	**20.04 ± 7**	**70.59 ± 31**	**54.76 ± 21**	**76.07 ± 20**	**125.35 ± 46**

Abbreviations: A, distance of the anterior portion of the EP from the excretory pore; B, distance of the posterior portion of the EP from the excretory pore; C, width of the external limit of the EP from the excretory pore; D, width of the internal limit of the EP from the excretory pore; (A+B), total length of the EP; (C+D), total width of the EP.

Epidermal 'excretory' syncytial plates of species of hosts collected from different localities

The EPs of specimens of *T. pignalberiae* collected from distant localities (approximately 1,300 km between Bebedouro, SP and Poconé, MT) are similar. The format and proportions of the total body length and width in relation to the excretory pore are almost identical (Figs 2D and 2E). The specimens collected in Bebedouro, SP, have the largest measurements. The relation between total body width/total width of the EP observed in specimens collected in Bebedouro (1:6) and Poconé (1:9) reflect the differences in body size (Table 2). The EPs of specimens from Bebedouro have 21% of their total length beyond the limit between the body and the tentacles, while the specimens from Poconé have only 4%.

Intraspecific variation of the epidermal 'excretory' syncytial plates in Temnocephala trapeziformis

The study of the intraspecific variation of the EPs in *T. trapeziformis* showed that this character has little variation within the same species (Table 3). The total width of the EPs (average 125 µm) is larger than the total length (average 76 µm) in 100% of the samples. The length of the anterior portion is always larger, *i.e.*, the excretory pore is always placed in the posterior portion of the plate (Fig. 2K). The width of the external and internal limits in relative to the excretory pore is quite variable, but in 70% of the samples the excretory pore is closer to the internal limit of the plate. In the whole sample, the entire plate is located below the limit between the tentacles and the body.

DISCUSSION

The methodology to describe the EPs based on measurements obtained with the software AxioVision Zeiss LE 4.7.2 from images made with SEM were effective in the characterization of the species of *Temnocephala*. Damborenea & Cannon (2001) and Volonterio (2007) recognized the necessity of new specific characters. The study of EPs does not invalidate (or change) the assertions made by Damborenea (1991) during the revision of the specific characters for the species of *Temnocephala,* while the cirrus continues as the most important character in the identification of these species. There are few records of concurrent infestations: *T. lutzi* and *T. trapeziformis* occur jointly in *Tr. fluviatilis* (Amato *et al.* 2005, 2006) and *T. iheringi* and *T. haswelli* in *P. canaliculata* (Seixas *et al.* 2010a, 2010c). On these occasions, the differences in the EPs were of fundamental importance in the separation of infrapopulations. Images with SEM are of utmost importance to the visualization of the EPs for this type of study. The 5% silver nitrate method of impregnation (Romeis 1968, Joffe *et al.* 1995), usually employed by earlier studies (Joffe & Cannon 1998, Amato *et al.* 2005), does not offer a complete image of this character. When a specimen is pressed between slide and coverslip the body is slightly deformed by the compression and the limits of the plate are not completely visible, making the application of the proposed method impossible.

In the species which are ectosymbionts on crustaceans the pattern, format, and size of the EPs vary. In this group, there are the smallest plates (*T. lutzi*), the longest plates (*T. pignalberiae*), and the only plates where the width is larger than the length (*T. trapeziformis*). The large variation was somehow expected as the temnocephalans had crustaceans as their first host group, possibly a parastacid crayfish (Schaefer 1971, Cannon 1991), so this long coexistence may have led to a greater variation of formats and sizes of the EPs within this group. An example is the great variety of Australasian temnocephalids, which are ectosymbionts on crustaceans, and exceed the total number of species described in the Neotropical Region (Cannon 1993, Sewell *et al.* 2006). Even in the Neotropical Region the number of species ectosymbiont on crustaceans is larger than in the other host groups known to date. Among the ectosymbionts on insects, *T. minutocirrus* is the only species which does not show the EPs according to the host group pattern. This species also differs in relation to the cirrus and other organs of the reproductive system. In the description of *T. caddisflyi*, Amato *et al.* (2011) detailed the reproductive system of the species, calling it 'complex', documenting how the

peculiarities of the reproductive system are shared with those of *T. curvicirri* (also ectosymbiont on insects). However, *T. minutocirrus* has the smallest cirrus so far described for species of *Temnocephala* and other organs much simpler than those described for other species mentioned above. Thus, the difference in the pattern of the EPs in *T. minutocirrus* is, in a way expected, as all the other specific characters (mainly the cirrus) also differ from the host group patterns.

The variation found in *T. pignalberiae* (from different localities) was low and basically related to the area and total body length / EPs ratio (expected by the normal variation on body size) and the portion exceeding the tentacles; however, the shape / form and the position of the excretory pore given by the measurements of the EPs were similar. The intraspecific variation study with *T. trapeziformis* also shows good results; in 100% of the samples, the EPs were wider than longer and the entire plate was located below the limit between the tentacles and the body. The variation in absolute measurements can be explained by the normal variation in body size among specimens.

Even with the general syncytial epidermic mosaic having a pattern (Joffe & Cannon 1998, Damborenea & Cannon 2001), the EPs vary in format and size. The format and size in the present study were analyzed following the traditional morphometry proposition, which is the study of the variation of the distance measurements, either between anatomic points or extremes of characters. This method allows the EPs to be used as specific diagnostic characters in the species descriptions of *Temnocephala*.

The EPs should be clearly described and illustrated in articles to be published, as well as the images of the cirrus showing fine details of the introvert obtained with differential interference contrast (DIC) microscope with Nomarski prisms (Seixas *et al.* 2010a). Both are necessary to species identification and comparison with SEM images of the EPs published by other authors. The measurements, as proposed above should always be included in future descriptions of *Temnocephala* species. It is impossible to apply the present methodology to papers which only have the diagrammatic representation of the plates, where some of the limits of the plates are lacking as well as the indication of the methods used to produce such images.

It should be possible to distinguish species in comparative studies when the EPs are described correctly. When the distinction of the format is not so evident, the position of the excretory pore given by the measures of the length of the anterior and posterior portions and the width of the internal and external limits of the EPs is a specific character for the differentiation and identification of the species. This character is of considerable aid in situations where other characters are difficult to observe, in concurrent infestations where it is impossible to see the cirrus of the specimens in the entire sample, and even allows the identification of the host group in samples with unknown origin.

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