



UFRGS
UNIVERSIDADE FEDERAL
DO RIO GRANDE DO SUL



PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

INSTITUTO DE BIOCÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

RICARDO BRUGNERA

**MORFOLOGIA COMPARADA, ONTOGENIA E ALOMETRIA DE ESTRUTURAS
DAS TÍBIAS ANTERIORES E DESCRIÇÃO DE IMATUROS DE PERCEVEJOS
PREDADORES (HEMIPTERA: PENTATOMIDAE: ASOPINAE)**

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Área de concentração: Biologia Comparada

Orientador: Prof. Dr. Augusto Ferrari

Co-orientador: Prof. Dr. Kim Ribeiro Barão

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Aprovada em ____ de _____ de ____.

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A vida é a arte do encontro, embora haja tanto desencontro.

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RESUMO

Estudos morfológicos representam ferramentas básicas para ampliação do conhecimento sobre determinado táxon e abrem caminhos para o desenvolvimento de outros estudos em diversas áreas como taxonomia, filogenia, etologia e ecologia. Neste trabalho, buscamos aprimorar o conhecimento morfológico na família Pentatomidae, investigando as variações presentes nas pernas de percevejos e como elas se desenvolvem ao longo do ciclo de vida. Estudando comparativamente as expansões tibiais e o aparato tibial na subfamília Asopinae, e verificamos que ambas as estruturas possuem uma ampla variação morfológica. As expansões podem estar presentes nas superfícies dorsal e ventral da tíbia, ou apenas na dorsal. A estrutura pode apresentar diferentes tamanhos entre as espécies, podendo ser até duas vezes a largura do eixo central da tíbia como nos gêneros *Cazira* e *Heteroscelis*, ou em outros casos como *Alceorrhynchus*, que se apresenta de forma diminuta. Além disso, as expansões podem ocorrer ao longo de toda a tíbia, ou apenas apicalmente. Em relação ao aparato tibial, verificamos que o número de cerdas é bastante variável entre as espécies analisadas, e que possui relação com o tamanho geral do inseto. Ainda, verificamos que a região circundante à estrutura apresenta morfologia variável. Com base nas variações encontradas, dez caracteres morfológicos foram propostos. Além dos adultos, estudamos a morfologia e alometria da tíbia ao longo da ontogenia em nove espécies de Pentatomidae das subfamílias Asopinae, Pentatominae e Edessinae. Foi constatado que o número de cerdas aumenta em cada estágio de desenvolvimento nas espécies de Asopinae, enquanto nas demais o número se mantém praticamente o mesmo. A alometria do aparato tibial se demonstrou positiva para todas as espécies de Asopinae nos testes realizados, enquanto nas subfamílias Pentatominae e Edessinae a alometria da estrutura foi maioritariamente negativa ou isométrica. Foi demonstrado que assim como em outros insetos, as pernas apresentam um crescimento acelerado em comparação com outras estruturas do corpo, neste caso, da cabeça. Além disso, constatamos que a largura de tíbia apresenta uma queda de tamanho do quinto ínstar para o estágio Adulto, e hipotetizamos que este fenômeno possa ter relação com a perda de estruturas presentes nas pernas das ninfas responsáveis por dar maior suporte ao peso do inseto. Os estágios imaturos de *Tynacantha marginata* Dallas são descritos, estruturas corporais são exploradas e comparadas morfológicamente, e características diagnósticas que permitem a identificação dos ovos e ninfas da espécie são fornecidas.

Palavras-chave: percevejos predadores, pernas, microscopia eletrônica de varredura

ABSTRACT

Morphological studies are basic tools to increase knowledge about a given taxon, opening paths for the development of other studies in several areas such as taxonomy, phylogeny, ethology and ecology. In this work, we aimed to improve the morphological knowledge in the Pentatomidae family, examining the variations present in the legs of stink bugs and how they develop throughout the life-cycle. Comparatively studying the tibial expansions and the foretibial apparatus in the Asopinae subfamily, we found that both structures have a wide morphological variation. The expansions may be present on the dorsal and ventral surfaces of the foretibia, or only on the dorsal surface. The structure can present different sizes between the species, being able to be up to twice the width of the tibial axis as in the genus *Cazira* and *Heteroscelis*, or in other cases like *Alceorrhynchus*, appears in a diminutive form. Besides, the expansions may occur along the entire tibia, or only apically. In relation to the foretibial apparatus, we verified that the number of setae is quite variable among the analyzed species, and that it is related with the general size of the insect. Also, we verified that the region surrounding the structure presents variable morphology. Based on the variations found, ten morphological characters are proposed. In addition to the adults, we studied the morphology and allometry of the foretibia along the ontogeny in nine species of Pentatomidae of the subfamilies Asopinae, Pentatominae and Edessinae. It was observed that the number of setae increases at each stage of development in the Asopinae species, while in the others the number remains practically the same. Allometry of the tibial apparatus was shown to be positive for all Asopinae species in the performed tests, while in the Pentatominae and Edessinae subfamilies the allometry of the structure was mostly negative or isometric. It has demonstrated that, as in other insects, the legs exhibit accelerated growth compared to other structures of the body, in our case, the head. In addition, we verified that tibia width shows a decrease in size of the fifth instar for the adult stage, and hypothesized that this phenomenon is related to the loss of structures present in the legs of the nymphs responsible for giving support to the weight of the insect. The immature stages of *Tynacantha marginata* Dallas are described, body structures are explored and morphologically compared and diagnostic features that allow identification of the eggs and nymphs of the species are provided.

Keywords: predatory stink bugs, legs, scanning electron microscopy

INTRODUÇÃO GERAL ¹

Pentatomidae é a quarta família mais diversa da subordem Heteroptera, representada por mais de 4,700 espécies e 800 gêneros encontrados em todas as regiões zoogeográficas, totalizando nove subfamílias: Aphylinae, Asopinae, Cyrtocorinae, Discocephalinae, Edessinae, Pentatominae, Phyllocephalinae, Podopinae e Styrotarsinae (Grazia *et al.* 2015).

A subfamília Asopinae possui distribuição mundial e apresenta aproximadamente 350 espécies distribuídas em 60 gêneros (Thomas 1992, 1994; Grazia *et al.* 2015). Diferente dos demais pentatomídeos, os asopíneos possuem hábito predador, e muitas vezes são classificados como zoofitófagos por se alimentarem de matéria animal e vegetal. As espécies deste grupo apresentam morfologia atípica em algumas partes do corpo que são utilizadas na predação: o primeiro artículo do lábio robusto, búculas fundidas posteriormente, e a capacidade de estender o lábio totalmente para frente, facilitando a captura das presas (Gross 1975; Gapud 1991). Das espécies de Asopinae que se conhece a biologia, a maioria é generalista, alimentando-se de larvas de Lepidoptera, Diptera, Hymenoptera e Coleoptera, mas algumas outras espécies possuem dietas mais restritas (Schuh & Slater 1995; De Clercq 2000).

Devido à capacidade de predação, várias espécies de Asopinae são utilizadas como controladores biológicos de outros insetos que atacam culturas. Entre os gêneros de maior importância no Brasil destacam-se *Podisus* Herrich-Schaeffer, *Brontocoris* Thomas, *Supputius* Distant e *Tynacantha* Dallas (Pires *et al.* 2015; De Clercq 2000, Sanchez *et al.* 2001, Zanuncio *et al.* 2005, Torres *et al.* 2006, Vacari *et al.* 2013). Por outro lado, há poucos estudos morfológicos para o grupo. Pouco se sabe sobre os mecanismos envolvidos na predação, tanto no estágio adulto quanto nos imaturos (Pires *et al.* 2015). Desta forma, diversas questões relacionadas a diversidade de Asopinae, quanto à sua morfologia e ao hábito predador permanecem em aberto.

A morfologia das pernas de heterópteros é bastante diversa entre os táxons, e normalmente estão relacionadas com hábitos de vida (Lis & Schaefer 2005). A exemplo de predadores, percevejos da família Reduviidae possuem diversas adaptações nas pernas para o hábito predador, desde à utilização de substâncias pegajosas para facilitar a captura das presas (Forero *et al.* 2011), ao uso de pernas raptorais (Weirauch *et al.* 2011). Dentre os pentatomídeos, as pernas de percevejos da subfamília Asopinae chamam a atenção devido à

¹ Formatado conforme as normas do periódico Zootaxa. Veja anexo III.

presença de estruturas peculiares (mas não exclusivas) nas tíbias, as expansões tibiais o aparato tibial.

O aparato tibial é um grupo de cerdas robustas localizadas na superfície ventral da tíbia, aproximadamente a um terço do ápice (Bonatto 1988; Barão *et al.* 2013). A estrutura está presente em quase todas as famílias de Pentatomoidea exceto Urostylididae e Saileriolidae (Grazia *et al.* 2008). Pouco estudada, sua morfologia teve foco em apenas dois trabalhos: Bonatto (1988) que explorou o aparato tibial em uma grande diversidade de famílias de Pentatomoidea, e Barão *et al.* (2013) que explorou a estrutura do forma comparativa, especialmente em relação ao número de cerdas, entre várias espécies de Pentatomidae, Scutelleridae e Tyreocoridae. Os resultados de Barão *et al.* (2013) demonstraram que a estrutura não possui grande variação entre a maioria dos táxons estudados, com exceção da subfamília Asopinae, que apresentou pelo menos três vezes o número de cerdas em relação às demais, levantando a hipótese de uma possível associação da estrutura com o hábito predador desta subfamília.

Em Pentatomidae, as expansões tibiais são estruturas presentes nas tíbias anteriores de grande parte dos asopíneos (Thomas 1992; 1994) e algumas espécies das tribos Alyiini (Ghuri 1988), Catachantini (Ahmad & Kamaluddin 1981) e Myrocheini (Kment 2015) de Pentatominae e Styrotarsinae (Rider, 2000). Entre os heterópteros, estruturas semelhantes são encontradas nas tíbias posteriores de Coreídeos (Fairbairn & Preziosi 1996; Eberhard, 1998), e nas anteriores de Reduvídeos (Gordon & Weirauch, 2016), na qual há hipóteses estabelecidas sobre a sua função. Em Pentatomidae a morfologia da estrutura nunca foi estudada, porém é um importante caracter para identificação de gêneros na subfamília Asopinae (Thomas 1992; Grazia e Schwertner 2008).

Estudos envolvendo o desenvolvimento ontogenético de estruturas nas pernas em heterópteros são escassos. Dos que existem, a maior parte é concentrada em percevejos aquáticos e geralmente sobre estruturas relacionadas à seleção sexual (e.g. Tseng & Rowe 1999; Ohba *et al.* 2006). Insetos hemimetábolos são ótimos modelos para estudos de variação ontogenética e alometria devido aos estágios de desenvolvimento serem bem definidos (Klingenberg & Zimmermann 1992). Variações de determinada região do corpo ao longo da ontogênese podem ajudar a explicar a função e evolução da mesma (Sites *et al.* 1996).

Assim como estudos relacionados a outras estruturas, trabalhos sobre a morfologia externa de ovos e ninfas são bastante escassos. Embora Pentatomidae seja a família de Heteroptera com mais estudos, menos de 1% das espécies neotropicais de Asopinae possui imaturos descritos (Matesco *et al.* 2007; 2009; Brugnera & Grazia 2018). O estudo de

imaturos contribui para diversas áreas, como evolução, taxonomia, filogenia e áreas aplicadas (Bundy & McPherson 2000; Matesco *et al.* 2009; 2014).

O objetivo desta dissertação foi explorar a morfologia das tíbias anteriores de Asopinae, especialmente as expansões tibiais e o aparato tibial a partir de uma quantidade representativa de espécies, assim como verificar as possíveis alterações morfológicas do aparato tibial ao longo da ontogenia e comparar o seu crescimento ontogenético com outras estruturas do corpo. Além disso, descrever os estágios imaturos de *Tynacantha marginata* Dallas, disponibilizando informações que possibilitem a identificação da espécie.

A dissertação está organizada em três capítulos:

O capítulo I explora as variações morfológicas das expansões tibiais e do aparato tibial em 86 e 44 espécies (respectivamente) de Asopinae, utilizando microscopia de luz e eletrônica de varredura. Dez caracteres morfológicos são propostos, a nomenclatura das expansões tibiais é atualizada e uma regionalização do aparato tibial é proposta.

No capítulo II a morfologia e alometria da tíbia anterior são estudadas ao longo da ontogenia, com foco no aparato tibial em nove espécies de Pentatomidae, representadas por três subfamílias: Asopinae, Edessinae e Pentatominae. Variações das estruturas ao longo do crescimento são descritas e hipóteses são levantadas.

E, no capítulo III os ovos e ninfas de *Tynacantha marginata* Dallas são descritos utilizando microscopia de luz e eletrônica de varredura. Estruturas importantes como o sistema odorífero externo é explorado, e ilustrações e fotografias são disponibilizadas para facilitar a identificação da espécie.

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CAPÍTULO I ²

Comparative morphology of selected foretibial traits of Asopinae (Hemiptera: Pentatomidae)

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Abstract

Legs structures are very diverse among Heteroptera groups and usually, these are related to life habits. In the family Pentatomidae, the foretibia is usually cylindrical and little variable among taxa, but the predatory stink bugs of the subfamily Asopinae shows pronounced structures. Using light and scanning electron microscopy the foretibial expansion and the foretibial apparatus of 87 and 42 species (respectively) of Asopinae were studied, representing about 75% of the genera diversity of the subfamily. Both structures show a great variation among the subfamily, and based on that, 10 morphological characters are proposed. The foretibial expansion varies in size and the position in the foretibia. The foretibial apparatus can present two types of setae, and in the majority of species others tibial setae can basally reach the structure. Furthermore, the setae that comprise the foretibial apparatus can vary from 10 to 57 setae among the species of Asopinae. Also, the morphology of cuticle that surrounds the foretibial apparatus can vary. For both structures, the terminologies are reinterpreted.

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Keywords: foretibial apparatus, foretibial expansion, predatory stink bugs, sexual dimorphism

1. Introduction

Heteropteran legs and their structures are diverse, with its morphology usually related with life habits (Eberhard, 1998; Forero *et al.*, 2011). Such variations have been used for the classification of several groups within Heteroptera (Lis and Schaefer, 2005; Grazia *et al.*, 2008; Weirauch, 2008). Compared to other groups of Heteroptera, the legs of the Pentatomoidea show less morphological ornamentations, with the few existing accounts regarding the foretibial apparatus (Barão *et al.*, 2013), the tibial comb (Lis and Schaefer, 2005) and foretibial expansions (Thomas, 1992, 1994).

The foretibial apparatus is a group of two longitudinal rows of stout setae located on the ventral surface of each foretibia, approximately one-third or less from the tibial apex (Bonatto, 1988; Barão *et al.*, 2013), of unknown function. The foretibial apparatus is found in all Pentatomoidea, except Urostylididae and Saileriolidae (Grazia *et al.* 2008). In average, seven setae comprise the foretibial apparatus of Pentatomidae, Scutelleridae, and Thyreocoridae, but species of Asopinae (Pentatomidae) show an average of 16 setae each (Barão *et al.* 2013).

Among the Pentatomoidea, dorsal and/or ventral expansions of the foretibia have been observed only in subfamilies or tribes of Pentatomidae, and are considered by some as diagnostic characters of Asopinae (Grazia & Schwertner, 2008; Thomas, 1992, 1994). Although important for taxonomy, the development of the foretibial expansions has not been explored before and its role in the species natural history is unknown.

Here, we explore the morphological variability of the foretibial apparatus and the foretibial expansions in Asopinae, the only predatory stink-bug subfamily, to test: 1) if Barão *et al.* (2013) observations about foretibial apparatus setal number can be generalized for extended taxon sampling within Asopinae; and 2) whether the foretibial apparatus and foretibial expansions are sexually dimorphic within asopine species. We include the most comprehensive data set of species for comparative morphologic studies in Asopinae to date; propose 10 morphologic characters to summarize the variability of the foretibial apparatus and foretibial expansions, providing illustrations for all characters; and code all studied species to all characters.

2. Material and methods

The described diversity of Asopinae comprises more than 300 species assigned to approximately 63 genera worldwide (Grazia *et al.*, 2015; Thomas, 1992, 1994). We studied the foretibial apparatus in 42 species (33 genera) and the foretibial expansions in 87 species (48 genera), sampling at least one male and one female of each species. Our sampling was based on material available at the Laboratório de Entomologia Sistemática of Universidade Federal do Rio Grande do Sul (UFRGS), borrowed from the collections listed below. A list of the examined Asopinae species is provided in Table 1. Our findings on the foretibial apparatus of Asopinae species were compared to the results of Barão *et al.* (2013) and Bonatto (1988), especially concerning the ultrastructure of the foretibial apparatus.

The specimens used in this study were borrowed from the following institutions: American Museum of Natural History (AMNH – United States of America); Australian Museum (AMS – Australia); Cleveland Museum of Natural History (CLEV – United States of America); Coleção de Entomologia Sistemática do Instituto de Pesquisas da Amazônia (INPA– Brazil); David Rider Collection (DARC - United States of America); Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRG – Brazil); Florida State Collection of Arthropods (FSCA - United States of America); Illinois Natural History Survey (INHS – United States of America); John E. Eger Collection (JEE – United States of America); Milwaukee Public Museum (MCPM – United States of America); Museu de Entomologia Pe. Jesus Santiago Moure (DZUP - Brazil); Museu Nacional do Rio de Janeiro (MNRJ – Brazil); Muséum Zoologique (MZLS – Switzerland); National Museum of Natural History (NMPC – Czech Republic); Staten Island Museum (SIM – United States of America); Naturhistoriska riksmuseet (NHRS – Sweden); University of California, Entomology Research Museum (UCRC – United States of America); Zoological Museum of University of Copenhagen (ZMUC – Denmark); University of Minnesota (UMSP – United States of America); Virginia Museum of Natural History (VMNH – United States of America).

Specimens were studied by optical (OM) and electron scanning (SEM) microscopies. For OM of the foretibial expansion, specimens were cleaned manually with fine brush and isopropyl alcohol 96%; leg articulations were softened with Barber solution and tibiae were stretched to position the foretibial expansions in lateral view, when observing the specimen dorsally (Fig. 1A). Legs were observed in a Nikon AZ100M scope coupled with a DS-Fi2 digital camera and photographed in multiple focal planes, pictures were stacked in a single focused image using the NIS Elements software, the equipment is available at the

Departamento de Zoologia (UFRGS). For SEM of the foretibial apparatus, the forelegs were removed, cleaned manually with a fine brush, and immersed in contact lens solution Renu® for 24 h, then agitated in a sonicator (40 kHz) in water and detergent solution for six minutes, and desiccated at 40° C for 24 h, mounted in stubs and sputter coated with gold. For SEM, legs were observed in the JEOL JSM 6060 at 10 kV in the Centro de Microscopia e Microanálise (CMM) of UFRGS.

We follow Bonatto (1988) and Barão *et al.* (2013) for the terminology of the foretibial apparatus. Additionally, we define and propose a terminology for areas surrounding the foretibial apparatus based on its general appearance (see Results).

Several terms were suggested to the foreleg of Asopinae, especially regarding modifications of the foretibiae: expanded, dilated, foliate, among others (Thomas 1992, 1994). We suggest the more general term “foretibial expansion” to refer to a modified condition of the foretibiae, and to use adjectives to qualify such condition, thus avoiding ambiguous interpretations.

We propose original morphological characters to summarize the observed variability of the foretibial apparatus and expansion. Character statements follow Sereno’s (2007) logical basis, employing the contingent character coding (see Brazeau 2011). Inapplicable data were coded as “-” and not observed data as “?”. Character statements are not polarized and to avoid misinterpretations about polarization, we assigned letters instead of numbers to character states. The following notation for character statements were used in the figures: “X(Y)”, in which X represents the character and Y represents its state. All characters of the foretibial expansion were proposed based in an anterolateral view of the foretibia, which maximizes the observation of diminute foretibial expansions.

The number of setae comprising the foretibial apparatus was counted manually on each image obtained by SEM. Our count of setae included the setae of the foretibial apparatus and the proximal setae (see results), because of the difficulty to distinguish the two setae in some species.

3. Results

1.1 Foretibial expansion

The foretibial expansion is characterized by an enlargement of the tibial tegument, present either on the dorsal or dorsal and ventral surfaces of each foretibia. The dorsal foretibial expansion is situated on the dorsal external margin of each foretibia (Fig. 1B, 2B, C) and is oriented longitudinally along the dorsal surface of the foretibia.

The dorsal foretibial expansion varies among species, from weakly (Fig. 2B-F, L, O-S) to well produced (Fig. 2G-J, M, N) along the foretibial length. Almost half of the analyzed species ($n = 39$; Table 1) shows some developmental degree of the dorsal foretibial expansion.

Ventral foretibial expansions occur only when dorsal expansions are present, and usually when dorsal expansions are well developed (Fig. 2H, I, J). Ventral foretibial expansions are less pronounced than dorsal expansions, usually less than half the foretibial width, and are more commonly restricted to the apical half of foretibial ventral surface (Fig. 2H, I).

For those genera we had access to two or more species, we observed intrageneric variation in foretibial expansions development, and intraspecific variation was not significant. The genera *Alcaeorrhynchus* Bergroth, 1891, *Cazira* Amyot & Serville, 1843, *Coryzorhaphis* Spinola, 1837 (Fig. 2R, S), *Discocera* Laporte, 1833, *Eocanthecona* Bergroth, 1915 (Fig. 2L, M), *Heteroscelis* Latreille, 1829 (Fig. 2J, K), *Glypsus* Dallas, 1851, *Macrorhaphis* Dallas, 1851, *Oplomus* Spinola, 1837 (Fig. 2N-Q), and *Stiretrus* Laporte, 1833, each showed interspecific foretibial expansion variability enabling species identification through comparative size and range of the dorsal expansion.

On the other hand, sexual dimorphism was observed only in *Euthyrhynchus floridanus* (Linnaeus, 1767). The dorsal expansion spans the total length of the foretibia in males (Fig. 2G) whereas in females (Fig. 2F) it is restricted to the apical third, and in the male the dorsal expansion is twice as wide as females dorsal expansion.

1.2 Foretibial apparatus

The asopine foretibial apparatus is characterized by a set of stout setae arranged longitudinally, extending distally on foretibia, forming or not a longitudinal groove, located on the ventral surface of each foretibiae or on the ventral foretibial expansion, approximately one-third from the foretibial apex (fa; Fig. 1). The insertion of each setae of the foretibial apparatus is oblique to the foretibial longitudinal axis.

We observed two types of setae forming the foretibial apparatus. One is curvilinear, in which the setae are rectilinear basally and curved apically, usually forming a longitudinal groove on the foretibial apparatus (Fig. 3A, B, D - I). The other type of seta is characterized by entirely rectilinear setae (Fig. 3C) that usually overlap each other but do not form a longitudinal groove on the foretibial apparatus.

The surface of the foretibia surrounding the foretibial apparatus may be regionalized by its ultrastructure. We propose to divide it into two areas: peripheral (pa; Fig. 3A) and glabrous (ga; Fig. 3A). The peripheral area (pa; Fig. 3A) closely surrounds the foretibial apparatus and is characterized by the presence of longitudinal, semicircular ridges in an elevated (Fig. 3B - D, F, G, I) or flat tegument (Fig. 3H). The tegument of the glabrous area (ga; Fig. 3A) is usually flat and smooth, surrounding the tibial apparatus and the peripheral area, but may present sparse setae, sensilla and pores (Fig. 3J).

Some foretibial setae reach the foretibial apparatus basally, and we refer to them as proximal setae (ps; Fig. 3A). The proximal setae do not comprise the foretibial apparatus, they are thinner than the foretibial apparatus, are usually straight, tapering apically, and never form longitudinal grooves. The proximal setae are similar to the remaining foretibial setae and are distinguished solely based on its distribution reaching the foretibial apparatus.

We found remarkable variation in the amount of seta comprising the foretibial apparatus in Asopinae, ranging from 10 to 57 setae (Table 1), with an average of 23 setae in the subfamily. Out of the 44 species studied, seven showed foretibial apparatuses with less than 15 setae (Table 1). We did not observe significant differences among sexes in the amount of setae composing the foretibial apparatus, with at most five setae of difference among male and female specimens of *Alcaeorrhynchus grandis* (Dallas, 1851) and *Amyotea malabarica* (Fabricius, 1775).

Most analyzed species (39 species) showed proximal setae, as easily observed in *Alcaeorrhynchus grandis* (Fig. 3F), *Machrorhaphis acuta* Dallas, 1851, *Picromerus bidens* (Linnaeus, 1758) (Fig. 3G) and *Stiretrus anchorago* (Fabricius, 1775). Species with a developed ventral foretibial expansion, such as species of *Heteroscelis* and *Cazira*, bear a longitudinal line of setae in the ventral surface of the foretibial expansion, that sometimes overlap the foretibial apparatus setae making it difficult to differentiate and count all setae comprising the foretibial apparatus (Fig. 3I).

1.3 Morphological characters

Variations of foretibial expansion and apparatus were summarized in the character statements listed below. Table 1 contains the coding of each species to each character.

3.3.1 Discrete characters

[1] Foretibia, dorsal external margin, foretibial expansion: (a) present (Fig. 2B-S); (b) absent (Fig. 2A).

- [2] Foretibia, inner view, dorsal external margin, foretibial expansion, maximal width related to width of tibial axis: (a) less than double (Fig. 2B, D-F, K, L, O-S); (b) more than double (Fig. 2C, G-J, M, N).
- [3] Foretibia, inner view, dorsal external margin, foretibial expansion reaching base of tibia: (a) absent (Fig. 2B, D-F, L, O-S); (b) present (Fig. 2C, G-K, M, N).
- [4] Foretibia, inner view, ventral surface, foretibial expansion: (a) present (Fig. 2H-K); (b) absent (Fig. 2D-G, L-S).
- [5] Foretibia, ventral surface, foretibial expansion reaching base of tibia: (a) absent (Fig. 2H, I, K); (b) present (Fig. 2J).
- [6] Foretibia, ventral surface, foretibial apparatus, shape of setae: (a) curvilinear (Fig. 3A, B, D-G); (b) rectilinear (Fig. 3C). The transition area between the proximal setae and the foretibial apparatus can show both types of setae, thus we refer only to the most apically setae of foretibial apparatus.
- [7] Foretibia, ventral surface, proximal setae: (a) present (Fig. 3A-C, F-G); (b) absent (Fig. 3D, E).
- [8] Foretibia, ventral surface, foretibial apparatus, number of rows of setae: (a) one; (b) two (Fig. 3A-J). A foretibial apparatus comprised of a single row of setae was observed only in *Cyrtocoris egeris* Packauskas & Schaefer, 1998 (Cyrtocorinae), *Alitocoris parvus* (Distant, 1880), *Catulona pensa* Rolston, 1992 and *Lincus securiger* Breddin, 1904 (Discocephalinae) (Barão et al. 2013); all species of Asopinae studied showed two rows of setae.
- [9] Foretibia, peripheral area, shape: (a) flat (Fig. 3E, H); (b) elevated (Fig. 3A-D, F - G, I).

3.3.2 Continuous character

- [10] Foretibia, foretibial apparatus, amount of setae (Table 1).

2. Discussion

4.1 Foretibial expansion

The foretibia of some Pentatomidae show expansions of the dorsal and/or ventral surfaces, such as in Strotarsinae (Rider, 2000), and the pentatomine tribes Cathacanthini (Ahmad & Kamaluddin, 1981), Halyini (Ghauri, 1988) and Myrocheini (Kment, 2015). Foretibial expansions are present in some species of Asopinae, and its topological position in these species suggest their homology. Some authors consider the foretibial expansion a taxonomic

informative character at the generic level (e.g. Grazia & Schwertner 2008; Thomas 1992, 1994), especially, its presence or absence is used in identification keys (e.g. Thomas 1992, 1994). Our results also suggest that the development of foretibial expansions are useful to differentiate congeneric species, such as species of *Discocera*, *Eocanthecona*, *Glypsus*, *Heteroscelis*, and *Oplonus*.

Sexual dimorphism of foretibial expansion, either its presence or its development, was not observed in the species studied except for *Euthyrhynchus floridanus*. Stål (1870) highlighted sexual differences in the foretibial apparatus of *E. floridanus* and Thomas (1992) described the presence of sexual dimorphism in populations of *E. floridanus* from North and Central Americas and its absence in South American populations. We could not confirm this disparity among populations because of unavailability of females of *E. floridanus* from South America. The only two males of *E. floridanus* from South America available to us show foretibial expansions considerably larger than females from North and South America.

Although the function of the foretibial expansion remains unknown in Pentatomidae, the usage of similar structures in others groups of Heteroptera has been already observed. A group of Old World Reduviidae, the Salyavatinae, which is specialized in capturing termites, shows expansions in the foretibia that are potentially used in prey capture or feeding (Gordon & Weirauch, 2016). Just as in *E. floridanus*, sexual dimorphism of tibial expanded structures were observed in Coreidae, some studies (e. g. Fairbairn & Preziosi 1996; Eberhard, 1998) show that it can be a result of sexual selection. Generally, morphological structures are not sexually dimorphic in Pentatomidae, but some contrary examples can be observed in wings of *Braunus* Distant, 1899 (Pentatominae) (Barão *et al.* 2016), head of *Coratozygum horridum* (Germar, 1839) (Cyrtocorinae) (Packauskas & Schaefer, 1998) and abdominal ventral surface of some Asopinae genera (Thomas, 1992, 1994).

4.2 Foretibial apparatus

As Bonatto (1988) and Barão *et al.* (2013) highlighted, the foretibial apparatus of asopine species comprises an average of 15 setae (ranging from 13 to 23 setae), whereas the remaining subfamilies show, in average, 6 setae. However, our increased taxon sampling of Asopinae showed species deviating from the forementioned pattern. In at least seven asopine species (six genera) the amount of setae comprising the foretibial apparatus is comparable to other subfamilies of Pentatomidae (Table 1), all these species are relatively small. Indeed, among the studied species, larger species (e.g., *Alcaeorrhynchus grandis*, *Glypsus conspicuus* (Westwood, 1837), *Apoecilus cynicus* (Say, 1831)) show more setae than smaller

species. It suggests that the number of setae and size are isometric in Asopinae. This pattern is not observed in the other taxa studied by Barão *et al.* (2013).

The setae comprising the foretibial apparatus are either curvilinear or rectilinear in Asopinae, without intraspecific variation, and the foretibial apparatus was found to be formed by the two opposing lines of setae. In Barão *et al.* (2013), two species of Pentatominae (*Mormidea quinqueluteum* (Lichtenstein, 1796) and *Mormidea ypsilon* (Fabricius, 1775)) and one of Phyllocephalinae (*Macrina juvenicus* Burmeister, 1835) show rectilinear setae, all the other species show curvilinear setae. The foretibial apparatus of some species of Discocephalinae (*Alitocoris parvus*, *Catulona pensa*, *Lincus securiger*) and Cyrtocorinae (*Cyrtocoris egeris*) is formed of just one line of setae (Barão *et al.* 2013), characteristic not observed in Asopinae.

We observed a series of foretibial setae placed close to the foretibial apparatus, which we named proximal setae. These setae were observed in most asopine species studied, but were not recorded in any other pentatomid subfamily (Bonatto 1988; Barão *et al.* 2013). Bonatto (1988) called “secondary setae” a similar set of setae in the Acanthosomatidae, which can be observed in *Bebaeus punctipes* Dallas, 1851 pictured in Grazia *et al.* (2008). To our knowledge, the high number of setae composing the foretibial apparatus and the several setae scattered on the foretibial surface are traits unique for some asopine species among the Pentatomidae.

Dolling (1981) suggested that the foretibial apparatus could be used for cleaning head appendages, but Bonatto (1988) stated that the diameter of antennae and rostrum is much larger than the groove of the foretibial apparatus, making putative cleaning inefficient, and its function may have been lost throughout evolution. Observing several pentatomid species (R. Brugnera, unpublished data) under different natural and stressing conditions, we could not record any behavior involving the foretibial apparatus, either self-cleaning, feeding or sexual. The function of the foretibial apparatus, if there is, remains unknown.

3. Conclusion

Increased taxon sampling of species of Asopinae showed that former conclusions about the amount of setae of foretibial apparatus in Asopinae do not apply to all its species. Several species showed amount of setae in the foretibial apparatus comparable to the amount of setae in other subfamilies of Pentatomidae. The foretibial expansion is present in several species of Asopinae, and when present is an expansion of the dorsal and/or ventral surfaces of the foretibia. The foretibial expansion proved useful for species and genus level identifications,

and was sexually dimorphic only in one of the studied species. Future studies should focus in allometry of body size and foretibial apparatus and expansions to investigate possible functions of these structures. Also, such structures and its variation can prove useful for phylogenetic studies.

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Table 1. List of examined species with its generalized distribution (Af, Africa; Am, Americas; As, Asia; Cam, Central America; Eu, Europe; Nam, North America; Oc, Oceania; Sam, South America), type of microscopy used to examination (SEM, Scanning electron microscopy; LM, light microscopy) and characters coded to each species. Characters states are not polarized.

Species	Distribution	LM	SEM	Characters										
				Foretibial expansion						Foretibial apparatus				
				1	2	3	4	5	6	7	8	9	10 [\bar{L}/\bar{W} (Mean)]	
<i>Afrius flavirostrum</i> (Signoret, 1861)	Af	x	x	b	-	-	b	-	a	a	b	b	b	24 / 25 (24.5)
<i>Afrius yolofofus</i> (Guérin-Méneville, 1831)	Af	x	x	a	a	a	b	-	?	?	?	?	?	?
<i>Alcaeorrhynchus grandis</i> (Dallas, 1851)	Am	x	x	a	a	a	b	-	a	a	b	b	b	52 / 57 (54.5)
<i>Alcaeorrhynchus phymatophorus</i> (Palisot de Beauvois, 1805)	CAm, NAm	x	x	b	-	-	b	-	?	?	?	?	?	?
<i>Amyotea hamata</i> (Walker, 1868)	Oc	x	x	b	-	-	b	-	?	?	?	?	?	?
<i>Amyotea malabarica</i> (Fabricius, 1775)	As, Oc	x	x	a	a	b	b	-	a	a	b	b	b	15 / 20 (17.5)
<i>Anasida tenebrio</i> Karsch, 1892	Af	x	x	a	a	a	b	-	?	?	?	?	?	?
<i>Andrallus spinidens</i> (Fabricius, 1787)	Af	x	x	a	a	a	b	-	?	?	?	?	?	?
<i>Apateticus lineolatus</i> (Herrich-Schaeffer, 1840)	Af, As, CAm, Nam, Eu, Oc	x	x	b	-	-	b	-	a	a	b	b	b	21 / 18 (19.5)
<i>Apoecilus bracteatus</i> (Fitch, 1856)	Am	x	x	b	-	-	b	-	?	?	?	?	?	?
<i>Apoecilus cynicus</i> (Say, 1831)	Nam	x	x	b	-	-	b	-	?	?	?	?	?	?
<i>Arma custos</i> (Fabricius, 1794)	Nam	x	x	b	-	-	b	-	a	a	b	b	b	36 / 37 (36.5)
<i>Blachia ducalis</i> Walker, 1867	Af, As, Eu	x	x	b	-	-	b	-	a	a	b	b	b	18 / 18 (18)
<i>Brontocoris nigrolimbatus</i> (Spinola, 1852)	As	x	x	a	b	a	a	a	?	?	?	?	?	?
<i>Brontocoris tabidus</i> (Signoret, 1863)	Sam	x	x	b	-	-	b	-	?	?	?	?	?	?
<i>Cazira chiroptera</i> (Herrich-Schaeffer, 1840)	Sam	x	x	b	-	-	b	-	a	a	b	b	b	26 / 28 (27)
<i>Cazira verrucosa</i> (Westwood, 1834)	As	x	x	a	b	b	a	a	?	?	?	?	?	?
<i>Cermatulus nasalis</i> (Westwood, 1837)	As	x	x	a	b	b	a	a	a	a	b	b	b	16 / 16 (16)
<i>Colpothyreus flavolineatus</i> (Blanchard, 1843)	Oc	x	x	b	-	-	b	-	a	a	b	b	b	22 / 23 (22.5)
<i>Comperocoris roehneri</i> (Phillipi, 1862)	SAM	x	x	a	b	b	b	-	?	?	?	?	?	?
<i>Conquistator mucronatus</i> (Uhler, 1897)	SAM	x	x	b	-	-	b	-	?	?	?	?	?	?
<i>Coryzorhaphis carneolus</i> Erichson, 1848	NAM	x	x	b	-	-	b	-	a	a	b	b	b	22 / 22 (22)
<i>Coryzorhaphis cruciata</i> Stal, 1870	SAM	x	x	a	a	a	b	-	b	a	b	b	b	16 / 14 (15)
<i>Damaris splendichilus</i> (Fabricius, 1803)	SAM	x	x	a	a	a	b	-	b	a	b	b	b	18 / 16 (17)
<i>Dinorhynchus dybowskyi</i> Jakovlev, 1876	CAM, SAM	x	x	a	a	b	b	-	?	?	?	?	?	?
<i>Discocera cayennensis</i> Laporte, 1833	Af	x	x	a	a	a	b	-	a	a	b	b	b	40 / ? (40)
<i>Discocera coccinea</i> (Fabricius, 1798)	As	x	x	a	b	b	b	-	?	?	?	?	?	?
<i>Dorycoris pavonivus</i> (Westwood, 1837)	SAM	x	x	a	a	a	b	-	?	?	?	?	?	?
<i>Eocanthoconca concinna</i> (Walker, 1867)	SAM	x	x	a	a	a	b	-	b	a	b	a	a	15 / 15 (15)
<i>Eocanthoconca furcellata</i> (Wolff, 1801)	Af	x	x	b	-	-	b	-	b	b	b	b	b	13 / 11 (12)
	As	x	x	a	b	b	b	-	?	?	?	?	?	?
	As	x	x	a	a	a	b	-	?	?	?	?	?	?

<i>Euthyrhynchus floridanus</i> (Linnaeus, 1767)	Am	x	x	a	a	b	b	-	a	a	b	b	24 / 25 (24.5)
<i>Friarius alluaudi</i> (Schouteden, 1905)	Af	x	x	a	a	b	b	-	?	?	?	?	?
<i>Glypsus bouvieri</i> Schouteden, 1904	Af	x	x	b	-	-	-	-	?	?	?	?	?
<i>Glypsus conspicius</i> (Westwood, 1837)	Af	x	x	a	a	b	b	-	a	a	b	a	? / 35 (35)
<i>Glypsus kuhlgatzii</i> Schouteden, 1904	Af	x	x	b	-	-	-	-	?	?	?	?	?
<i>Glypsus truculentus</i> Walker, 1868	Af	x	x	a	a	b	b	-	?	?	?	?	?
<i>Glypsus vigil</i> (Germar, 1837)	Af	x	x	b	-	-	-	-	?	?	?	?	?
<i>Heteroscelis bimaculata</i> (Walker, 1867)	CAM, SAM	x	x	a	b	b	a	b	?	?	?	?	?
<i>Heteroscelis lepida</i> (Stål, 1862)	Am	x	x	a	b	b	a	b	?	?	?	?	?
<i>Heteroscelis servillei</i> Laporte, 1833	SAM	x	x	a	b	b	a	b	a	a	b	a	10 / 10 (10)
<i>Heteroscelis robustus</i> Thomas, 1992	SAM	x	x	a	a	b	a	b	a	a	b	a	10 / 10 (10)
<i>Hoploxys coerulesus</i> Dallas, 1851	Af	x	x	b	-	-	-	-	?	?	?	?	?
<i>Jalla dumosa</i> (Linnaeus, 1758)	As, Eu	x	x	b	-	-	-	-	a	a	b	b	26 / 24 (25)
<i>Leptolobus murrayi</i> Signoret, 1855	Af	x	x	b	-	-	-	-	?	?	?	?	?
<i>Macrorhaphis leprosa</i> (Germar, 1837)	Af	x	x	a	a	b	b	-	?	?	?	?	?
<i>Macrorhaphis acuta</i> Dallas, 1851	Af	x	x	a	a	b	b	-	a	a	b	b	28 / 28 (28)
<i>Marmessulus brasiliensis</i> Schouteden, 1907	SAM	x	x	b	-	-	-	-	?	?	?	?	?
<i>Marmessulus nigricornis</i> (Stål, 1865)	SAM	x	x	b	-	-	-	-	?	?	?	?	?
<i>Mecosoma mentor</i> (Germar, 1837)	Af	x	x	b	-	-	-	-	?	?	?	?	?
<i>Montroziteriellus falleni</i> (Guérin-Méneville, 1831)	Oc	x	x	b	-	-	-	-	a	a	b	a	24 / 23 (23.5)
<i>Oechalia schellenbergii</i> (Guérin-Méneville, 1831)	As, Oc	x	x	b	-	-	-	-	a	b	b	a	19 / 17 (18)
<i>Oplonus catena</i> (Drury, 1782)	SAM	x	x	a	a	a	b	-	a	a	b	b	22 / 24 (23)
<i>Oplonus cruentus</i> (Burmeister, 1835)	SAM	x	x	a	a	a	b	-	a	a	b	b	30 / 32 (31)
<i>Oplonus salamandra</i> (Burmeister, 1835)	CAM, SAM	x	x	a	a	b	b	-	?	?	?	?	?
<i>Perillus circumcinctus</i> Stål, 1862	SAM	x	x	a	a	b	b	-	?	?	?	?	?
<i>Perillus bioculatus</i> (Fabricius, 1775)	NAM	x	x	b	-	-	-	-	?	?	?	?	?
<i>Perillus exaptus</i> (Say, 1825)	NAM	x	x	b	-	-	-	-	a	a	b	b	16 / 18 (17)
<i>Picromerus bidens</i> (Linnaeus, 1758)	NAM	x	x	b	-	-	-	-	b	b	b	b	22 / 18 (20)
<i>Pinthaeus sanguinipes</i> (Fabricius, 1781)	NAM	x	x	b	-	-	-	-	a	a	b	b	37 / 37 (37)
<i>Platynopiellus septendecimaculatus</i> (Palisot de Beauvois, 1811)	As, Eu, NAM	x	x	a	a	b	b	-	a	a	b	b	42 / 40 (41)
<i>Platynopus melanoleucus</i> (Westwood, 1837)	As, Eu	x	x	a	a	b	b	-	?	?	?	?	?
<i>Podisus aenescens</i> (Stål, 1860)	As	x	x	a	b	b	b	-	?	?	?	?	?
<i>Podisus distinctus</i> (Stål, 1860)	Am	x	x	b	-	-	-	-	?	?	?	?	?
<i>Podisus maculiventris</i> (Say, 1831)	SAM	x	x	b	-	-	-	-	?	?	?	?	?
<i>Podisus nigripinus</i> (Dallas, 1851)	CAM, NAM	x	x	b	-	-	-	-	a	a	b	b	22 / 24 (21)
<i>Podisus rostralis</i> (Stål, 1860)	CAM, SAM	x	x	b	-	-	-	-	a	a	b	b	20 / 20 (20)
	SAM	x	x	b	-	-	-	-	?	?	?	?	?

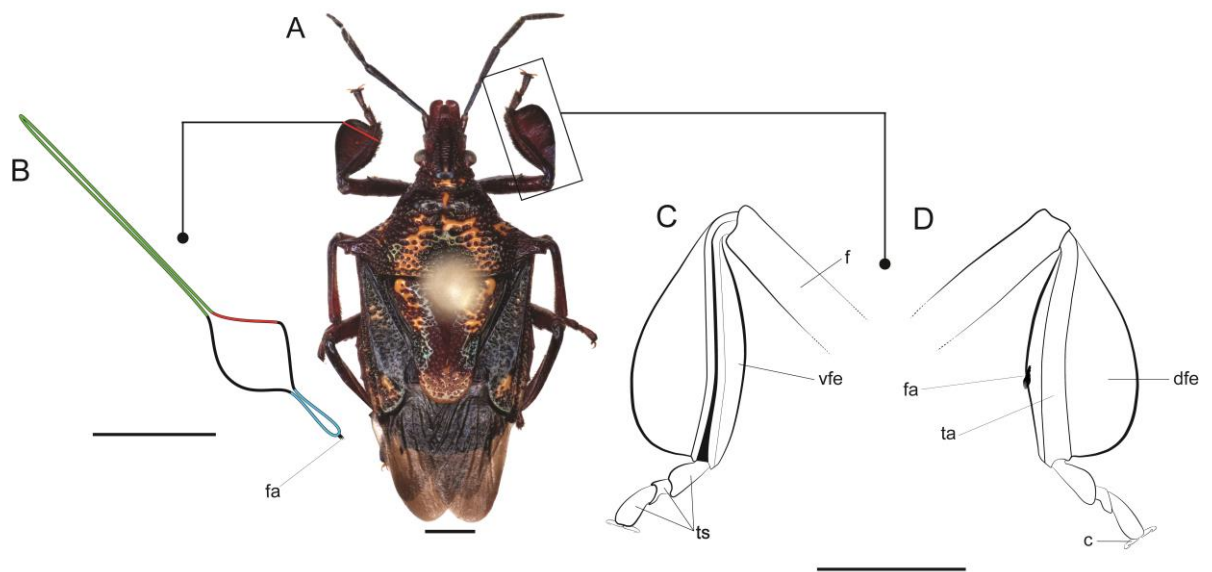


Fig. 1. Foreleg of *Heteroscelis servillei*. (A) Dorsal view of *H. servillei*. (B) Transversal view of the apex of foretibia (green = dorsal external margin expanded; red = dorsal surface; blue = ventral expansion) (the red line on the foreleg indicates the local of the transversal section). (C) Outer view. (D) Inner view. Abbreviations: c, claw; dfe, dorsal foretibial expansion; f, fêmur; fa, foretibial apparatus; vfe, ventral foretibial expansion; ta, tibial axis; ts, tarsus. Scale bars: A, C, D = 1 mm; B = 0,25 mm



Fig. 2. Foretibial expansions of Asopinae. (A – C) Dorsal view. (D – S) Inner view. (A) *Tynacantha marginata*. (B) *Discocera cayennensis*. (C) *Colpothyreus flavolineatus*. (D) *Macrorhaphis acuta*. (E) *Alcaeorrhynchus grandis*. (F) *Euthyrhynchus floridanus* female. (G) *Euthyrhynchus floridanus* male. (H) *Cazira verrucosa*. (I) *Blachia ducalis*. (J) *Heteroscelis servillei*. (K) *Heteroscelis robustus*. (L) *Eocanthecona furcellata*. (M) *Eocanthecona concinna*. (N) *Oplomus mutabilis*. (O) *Oplomus dichrous*. (P) *Oplomus cruentus*. (Q) *Oplomus catena*. (R) *Coryzoraphis leucocephala*. (S) *Coryzoraphis carneolus*. Black arrows indicate the dorsal external margin expanded. Scale bars: 1 mm.

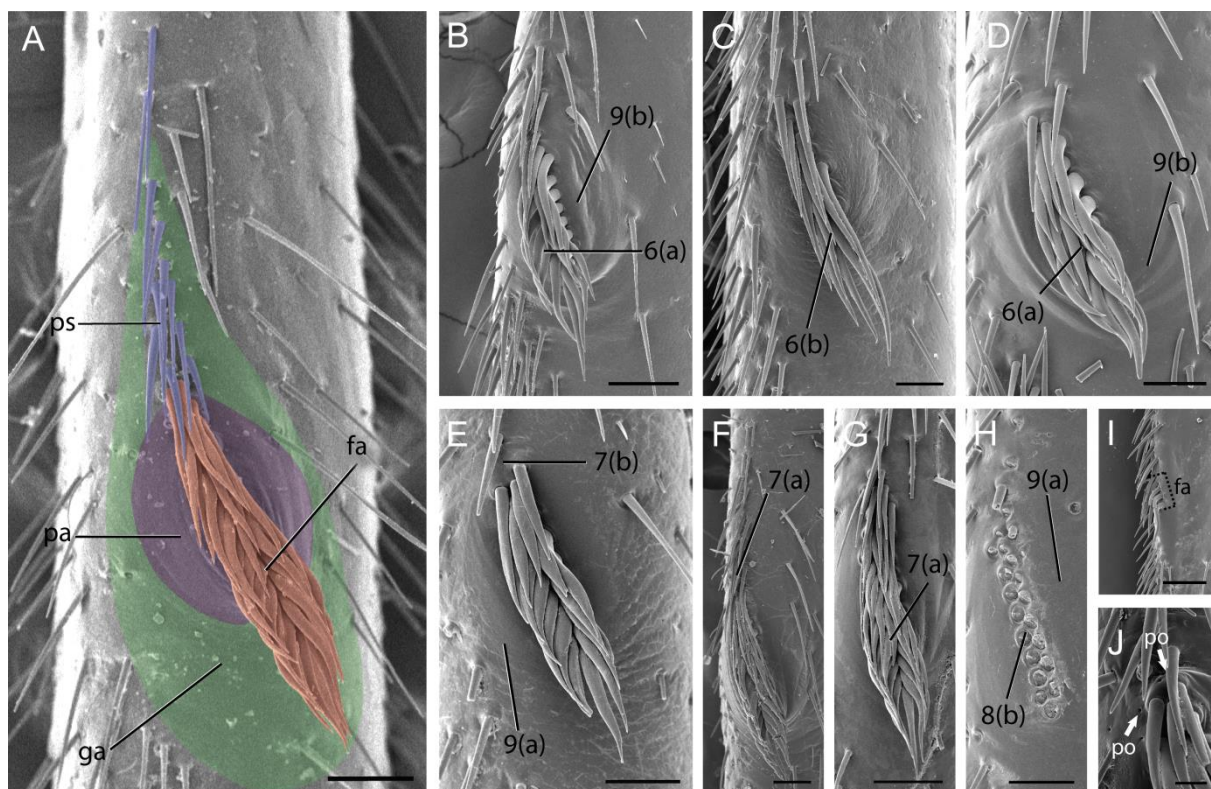


Fig. 3. Foretibial apparatus of Asopinae. (A – J) Ventral view. (A) *Apoecilus cynicus*. (B) *Oplonus catena*. (C) *Discocera coccinea*. (D) *Perillus bioculatus*. (E) *Oechalia schellenbergi*. (F) *Alcaeorrhynchus grandis*. (G) *Picromerus bidens*. (H) *Tylospilus acutissimus*. (I) *Heteroscelis servillei*. (J) *Jalla dumosa*. Abbreviations: fa, foretibial apparatus; ga, glabrous area; pa, peripheral area; po, pores; ps, proximal setae. Scale bars: A, F – I = 100 μm ; B – E = 50 μm ; J = 20 μm .

CAPÍTULO II ³**Ontogenetic trajectories, morphology and allometry of the foretibial apparatus in Pentatomidae (Hemiptera: Heteroptera)****RICARDO BRUGNERA^{1*}, JOSÉ MAURICIO AVENDAÑO FORERO¹, KIM RIBEIRO BARÃO^{2,3}, AUGUSTO FERRARI^{1,4}**

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Abstract

The subfamily Asopinae is the only group of Pentatomidae that have predatory behaviors, feeding mainly on others insects. The foretibial apparatus of the Asopinae species has an increased number of setae comparing to the others groups, however the function of these setae is yet unknown. From measurements, we present the ontogenetic morphology, trajectories and allometry of the foretibial apparatus of nine species of Pentatomidae (four asopines). Besides the foretibial apparatus, others three body structures (two of leg, one of head) were measured in all the nymphal instars and adults, to compare the growth ratio. Our results show that the number of setae of foretibial apparatus has an increase in each instar of Asopinae species, while in the others species the number of setae is practically the same throughout the life-cycle. The allometric coefficients of the foretibial apparatus were positive to all species of Asopinae, and negative or isometric to the phytophagous species. In summary linear regressions between legs and head suggest that the regulation of appendage growth is relatively independent of head growth and possibly other body parts. This study is the first that comprises ontogenetic development and allometry in Pentatomidae.

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Keywords: Asopinae, growth, development, allometric coefficient

1. Introduction

Variation in organismal shape is largely characterized by differences in the relative size of body parts, known as allometry (Huxley 1932; Thompson 1942). Among species, most traits scale proportionately with body size, however, few traits show an exaggerated development during growth (McCullough *et al.* 2015). Variations in shape and size of structures can be influenced by several factors, such as life habits, sexual selection and morphological adaptations (Levin 1992; Eberhard 1998; Weirauch *et al.* 2011; Wang & Dai 2017).

Insects represent an important group for the study of size variation of body parts (see Huxley 1932), especially hemimetabolous insects, such as hemipterans, are excellent models to study allometry because they have well-defined ontogenetic stages (Klingenberg & Zimmermann 1992).

Nymphal stages of many pentatomids are very similar in their overall morphology throughout their development, differing in few structures, such as the dorsal abdominal scent glands, the antennal and tarsal segments, and the development of wing pads (Schaefer *et al.* 1998; Bottega *et al.* 2015; Grazia *et al.* 2015).

Allometric studies in pentatomids are scarce (to our knowledge, only two studies have been published: Brézot *et al.* 1996 and Teuscher *et al.* 2009) and shed limited light on functional morphology and evolution. By the other hand, a vast literature can be found in aquatic heteropterans such as Gerridae and Belostomatidae (e.g. Fairbairn & Preziosi 1994; Tseng & Rowe 1999; Ohba *et al.* 2006). Also, a few studies can be found in Plataspidae (e.g. Stubbins *et al.* 2017) Coreidae (e.g. Eberhard 1998; Rodrigues *et al.* 2005), Lygaeidae (Higgins *et al.* 2009) and Alydidae (e.g. Okada *et al.* 2011), most of them related with sexual traits.

The foretibial apparatus is a group of stout setae present in the foretibia of Pentatomoidea, except in Urostylididae and Saileriolidae (Bonatto 1988; Grazia *et al.* 2008). This structure was explored mainly in four studies: (1) the unpublished thesis of Bonatto (1988), that documented the tibial apparatus in several families of the Pentatomoidea; (2) Grazia *et al.* (2008) coded the foretibial apparatus in their cladistic analysis of the Pentatomoidea; (3) Barão *et al.* (2013) explored, by scanning electron microscopy (SEM), this structure in Pentatomidae, Scutelleridae, and Thyreocoridae; and (4) Brugnera *et al.* (in prep)

studied the foretibial apparatus of the predatory stink bugs (Pentatomidae: Asopinae), proposing five morphological characters.

Although the function of the foretibial apparatus is yet unknown, the number of setae comprising the foretibial apparatus in the Asopinae is higher than in other subfamilies of Pentatomidae (Bonatto 1988; Barão *et al.* 2013, Brugnera *et al.* in prep.). While the average number of setae in Asopinae is 23, the phytophagous groups show an average of only 5 setae (Barão *et al.* 2013; Brugnera *et al.* in prep.). The subfamily Asopinae is the only predatory group of Pentatomidae, feeding mainly of larvae of Lepidoptera and Coleoptera, representing important organisms for biological control (De Clercq, 2000).

In this paper, we explore morphological postembryonic changes of the foretibia, focusing on the foretibial apparatus of nine species of Pentatomidae, representing three subfamilies. Considering that the foretibial apparatus is larger in Asopinae due to the greater number of setae, we used an allometric analysis to verify the growth rate of the structures in relation to other body parts (interocular width and tibial length). Likewise, the ontogenetic trajectory of four body structures is analyzed and the external morphology of the foretibial apparatus in the five nymphal instars and the adult is explored using SEM.

2. Material and Methods

From all the individuals that were used in this study, only the Asopinae species were reared in laboratory. All specimens of Pentatominae were obtained from the Entomological Collection of the Department of Zoology, Federal University of Rio Grande do Sul (UFRGS) and *Edessa rufomarginata* was collected in the field (see Table 1 for the collecting sites).

Adults of Asopinae were collected in different localities of Rio Grande do Sul state, Brazil (Table 1). Specimens were kept in plastic vials and reared at $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$, 70 ± 10 RH, and 12L:12D h photoperiod. Water was provided *ad libitum* to first instar nymphs. Subsequent immature stages and adults were fed, *ad libitum*, with larvae and pupae of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and leaves of *Eugenia uniflora* (Myrtales: Myrtaceae).

All the nymphal stages and adults were fixed in 70% ethanol and stored at 4°C before measurements. The measurements taken are as follows: interocular width (IOW), tibial length (TL), tibial width (TW) and foretibial apparatus length (FAL). To measure the interocular width, the specimens were positioned dorsally, parallel to the focal plane. The right leg of each specimen was removed from the body and positioned, parallel to the focal plane, in internal lateral view, on a glass slide. Measurements were taken in a Nikon AZ100M scope

coupled with a DS-Fi2 using the NIS Elements software. All measurements were taken in millimeters. Individuals with signs of recent moult (e.g., tegument soft and pale) were not measured to avoid potential errors with the size. The number of nymphs and adults measured for each species is available in Table 1.

For allometric analysis, specimens without the four measurements were removed from the data set, thus, the final set comprises 502 individuals (Table 1). Raw data failed the tests of normality and homoscedasticity, observed through Kolmogorov-Smirnov and Levene tests (Zar 2010) respectively, thus measurements were log-transformed. Ontogenetic trajectories were plotted for each structure to compare the growth patterns, from first instar to adult, between the species and describe the observed tendencies. Linear regressions of each variable against TL and IOW, were performed to remove the effect of body size and calculate the allometric equations, in the form $\log y = \log \alpha + \beta \log x$. The slope of the line (β) is the allometric coefficient and indicates if the relationship between the structures is isometric (~ 1), allometric positive (< 1) or allometric negative (> 1). Structure growth rate of every species, were qualitatively compared in order to identify trends and differences in the ontogenetic development of the structures that can be related to life habits or the final morphology. Tests, graphics of linear regression and calculations of allometric equations were performed on STATISTICA 8 software (StatSoft 2008).

For SEM, the right foreleg of two individuals of each instar and adults (one male, one female) of all the analyzed species were removed and immersed in contact lens solution Renu® for 24 h. After, with the help of a sonicator (40 kHz), the forelegs were washed in water and detergent solution for six minutes, and then left to air dry for 24 h. Then, legs were mounted in stubs and sputter coated with gold, and observed in the JEOL JSM 6060 microscope at 10 kV in the Centro de Microscopia e Microanálise (CMM) of UFRGS. Image processing was done in the Adobe Photoshop software. The number of setae comprising the foretibial apparatus was counted manually on each image obtained by SEM (Table 1).

3. Results and Discussion

3.1 Ontogenetic morphology of foretibial apparatus

The ontogenetic development of the foretibial apparatus show more expressive changes in the Asopinae than in the species of phytophagous subfamilies. Except for the first instar, the structure maintains the same morphological pattern of the adults throughout the development in all analyzed species, however, the number of setae changes during the life-cycle in Asopinae (Fig. 1).

The foretibial apparatus morphology of almost all instars of all species resembles the morphology of the foretibial apparatus of adults, as described by Brugnera *et al.* (in prep.) and Barão *et al.* 2013. The foretibial apparatus is organized in two parallel rows of stout setae, generally forming a longitudinal groove in Asopinae. However, in the first instar of Asopinae the setae are not juxtaposed and do not form a parallel row (Fig. 1A, G, M, S, Y). Accordingly, several morphological structures of Pentatomidae show diverse patterns in the first and the remaining instars, such the external scent efferent system, the tricobotria and the shape of the head (see Barão *et al.*, 2015 and Bottega *et al.* 2015).

The number of setae increases throughout ontogeny for all species of Asopinae studied, while in the species of Pentatominae and Edessinae studied the amount of setae shows small variation throughout their life-cycle, with the addition of one or two setae from the first instar through adult. Both phytophagous and predatory species have 3 to 5 setae in the first instar, but in *Podisus* spp., *Supputius cincticeps* and *Tynacantha marginata* there is an increase of about 4 setae per instar, reaching at least 15 setae in the adult (Figs. 1, 2).

As observed in adults of Asopinae (Brugnera *et al.*, in prep.) the morphology of the region surrounding the tibial apparatus can vary among species. The elevated peripheral area is observed since the third instar in *Supputius cincticeps* (Fig. 1I), whereas in *Podisus* species and *Tynacantha marginata* an elevated peripheral area occurs only in the adult (Fig. 1F). In *Edessa rufomarginata* a small elevation is observed in the adult (Fig. 1R). In the others analyzed species the cuticle surrounding the tibial apparatus is flat.

3.2 Ontogenetic trajectories

The growth of the four structures that were object of study in this paper, follow different patterns throughout development. The length of the tibia and the interocular width has less variation between the species. Isolated growth curves, such as *Banasa maculata* (TL) and *Runibia perspicua*, *Edessa rufomarginata* and *Chinavia obstinata* (IOW), due to the fact that these species are particularly small in size (*B. maculata*), or are larger than the others species (Fig. 2).

In relation to the tibial width, except for *Edessa rufomarginata*, all the species that were analyzed have stabilization or a decrease in the width of foretibia from the fifth nymphal instar until adulthood (Fig. 2). Nymphs show small foretibial expansions in the inner and outer dorsal margins of foretibia. In adults, these structures reduce in size or disappear making the shape of tibia cylindrical and thinner. *Edessa rufomarginata* nymphs do not show these expansions, remaining the tibia's shape unaltered during development. We hypothesize that

these foretibial expansions are responsible for making the legs of nymphal stages less flexible. The cuticle of the nymphs is thinner and more flexible than in adults (see Chapman 2013), so that expansion can give more support to the weight. The leg of *Edessa rufomarginata* is harder than the others species since the first instar, so probably the cuticle is thicker and perhaps this feature dispenses the need for expansions.

The measurements of the foretibial apparatuses length of all species show two groups, one formed by the species of Asopinae (*P. pallipes*, *P. nigrispinus*, *S. cincticeps*, *T. marginata*) and another by the phytophagous species. Even though the Asopinae species have a significantly smaller body size than *E. rufomarginata* and *R. perspicua* for example, the final absolute size of the foretibial apparatus is larger in the predatory species (Fig. 2).

The development of insect body size is strongly influenced by the nutritional conditions of the immatures (Emlen & Nijhout 2000). Thus, different trends can be expected in specimens from field. On the other hand, the fact that almost all nymphs used in our study were reared in the same conditions would influence the effect of diet and food quality in the results.

3.3 Allometry

For the ontogenetic allometry of the foretibial apparatus in different species of Pentatomidae, we performed two tests: 1) Comparing the foretibial apparatus with the interocular distance, to verify the growth with other body structure (than any part of the leg) and 2) Comparing the foretibial apparatus with the tibial length, to verify if the structures are dependent on each other in relation to their growth. The same was done for every structure measured.

The allometric coefficients of the foretibial apparatus indicate positive allometry for all Asopinae species, when the regression values were compared with the tibial length (TL) (Fig. 4) and the interocular width (IOW) (Fig. 3). The remaining species other than Asopinae, all the allometric coefficients of the foretibial apparatus (FAL) indicate negative or isometric allometry in relation to the tibial length (TL), and for interocular width (IOW), only *Edessa rufomarginata* and *Runibia perspicua* presented positive allometric coefficients (Table 2, Fig. 3, 4).

Except for the foretibial apparatus of the non-Asopinae species, the tibial width (TW) and the tibial length (TL) have a higher positive allometry in relation with the interocular distance (IOW). Ontogenetic studies with growth in Gerridae (Matsuda 1960) show that the legs appendages become proportionally longer as body size increases. In general, terrestrial walking insects including Pentatomidae, have a positive allometry between legs and body

size. Long legs allow an increased stride length, which enables walking organisms to step over obstacles instead of walking into them (Teuscher *et al.* 2009), besides, insects legs are often adapted to other functions, contingent on their life strategies (Gullan & Cranston 1994), and even the function can change during the life-cycle (Nakajima 2010, 2012), affecting both size and shape.

The growth ratio of leg segments to body length tends to be relatively constant throughout the first four nymphal stages, but significant increases or decreases occur in the final instar (Matsuda 1960, 1961). Our results show a distinction of the first four nymphal instars from the measurements of the leg and head. Fifth instar and adult measurements often overlap, indicating a decrease in growth at this stage and suggesting greater investment in growth in the early stages of life (Fig. 3, 4).

Allometric coefficients of foretibial apparatus length and tibial length suggest that the structures have independent patterns of growth throughout life. In summary linear regressions between legs and head suggest that the regulation of appendage growth is relatively independent of head growth and possibly other body parts, and can be adjusted late in development to reach the final adult morphology (Fairbairn 1990).

4. Conclusion

One of the main adaptations of the Asopinae species to predation is the labium that is strongly incrassate and can be outstretched forward (Grazia *et al.* 2015). The study of Brugnera *et al.* (in prep.) showed that number of setae of the foretibial apparatus is associated with the body size in Asopinae. In Heteroptera, most of the exaggerated traits are involved with sexual selection (e. g. Emberts *et al.* 2017). Against that, the foretibial apparatus is not sexually dimorphic (Brugnera *et al.* in prep.), so that characteristic could be associated with the predatory behavior or with some other structure that is involved in the predatory behavior, like the labium, since our results show that the foretibial apparatus of Asopinae species is more setose than pentatomines since the second instar.

Studies in behavior associated with morphological traits are scarce in Asopinae, the most of them are focused in feeding (e. g. Lemos *et al.* 2005) and reproductive (e. g. Mendonça *et al.* 1997; Zunic *et al.* 2008; Laumann *et al.* 2013) behaviors. More studies in behavior to observe the use of morphological traits involving a great diversity of Asopinae (and others Pentatomoidea) species are needed to verify if the foretibial apparatus can be associated with a behavior or not.

Also, studies involving the structure of the cuticle of legs during the development are needed to test our hypothesis if the expansions present in the tibia of nymphs are responsible to give more support to the weight. Finally, our study is the first that comprises ontogenetic development and allometry in Pentatomidae. More studies involving body size and postembryonic changes should be done to better understand the body growth, thus contributing to several areas such ethology, physiology and evolution.

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Table 1. Informations about the analyzed species.

Specie	Collecting site in Brazil	Geographical coordinates	Number of individuals utilized					Mean of setae number of foretibial apparatus									
			1st	2nd	3rd	4th	5th	Adult	1st	2nd	3rd	4th	5th	Adult			
<i>Podisus nigripinus</i> (Dallas, 1851)	São Domingos do Sul, RS	-28.565639, -51.830667	10	10	10	10	10	10	10	10	10	3	5,5	7,5	9,5	12	16,5
<i>Podisus pallipes</i> (Dallas, 1851)	São Domingos do Sul, RS	-28.565639, -51.830667	9	6	5	10	10	10	10	10	10	3	5,5	7	9,5	13	19
<i>Suputtius cincticeps</i> Stål, 1860	Santo Antônio da Patrulha, RS	-29.750750, -50.480361	10	10	10	10	10	10	10	10	10	3	5,5	7,5	9	12	17
<i>Tynacantha marginata</i> Dallas 1851	Porto Alegre, RS	-30.066806, -51.122306	10	10	10	10	10	10	10	10	10	3	5,5	7,5	9,5	12,5	19,5
<i>Edessa rufomarginata</i> (De Geer, 1773)	Penedo, AL	-10.328528, -36.498833	2	3	3	7	10	10	10	10	10	4	4	4	5	5	5
<i>Banasa maculata</i> Campos e Garbelloto, 2010	Criciúma, SC	-28.707000, -49.409000	10	10	10	10	10	10	10	10	10	3	4	5	5	5	5
<i>Chinavia obstinata</i> (Stål, 1860)	Porto Alegre, RS	-30.100972, -51.256944	10	10	10	10	9	10	10	10	10	4	4,5	5	5	5	5
<i>Mitripus convergens</i> (Herrich-Schäffer, 1842)	Maquiné, RS	-29.665861, -50.208722	10	10	10	10	10	10	10	10	10	3	4	4	4	4	4
<i>Runibia perspicua</i> (Herrich-Schäffer, 1842)	Passo Fundo, RS	-28.230722, -52.405389	10	10	10	10	10	10	10	10	10	5	5	5	5	5	5

Table 2: Regression value and coefficient of determination of the species and structures analyzed. R²= Coefficient of determination.

Tibial length				Interocular width			
Species	Structure	Equation	R ²	Species	Structure	Equation	R ²
<i>Podisus nigrispinus</i>	TW	$y = 0.7105x - 0.6085$	0.8495	<i>Podisus nigrispinus</i>	TW	$y = 1.9793x + 0.2562$	0.9514
	FAL	$y = 1.0879x - 1.3066$	0.9116		FAL	$y = 1.2181x - 0.5529$	0.8267
	IOW	$y = 0.4807x - 0.1292$	0.9514		IOW	$y = 2.2210x - 1.0194$	0.9227
<i>Podisus pallipes</i>	TW	$y = 0.6360x - 0.6666$	0.835	<i>Podisus pallipes</i>	TW	$y = 1.9309x + 0.1253$	0.9066
	FAL	$y = 1.1347x - 1.2881$	0.9077		FAL	$y = 1.2209x - 0.5873$	0.9768
	IOW	$y = 0.5059x - 0.0647$	0.9768		IOW	$y = 2.2155x - 1.1445$	0.8061
<i>Supputius cincticeps</i>	TW	$y = 0.5267x - 0.6932$	0.8126	<i>Supputius cincticeps</i>	TW	$y = 2.0298x + 0.1676$	0.9787
	FAL	$y = 1.0634x - 1.2759$	0.9412		FAL	$y = 1.0956x - 0.6026$	0.8353
	IOW	$y = 0.4821x - 0.0827$	0.9787		IOW	$y = 2.1569x - 1.0978$	0.9197
<i>Tynacantha marginata</i>	TW	$y = 0.6305x - 0.7230$	0.8855	<i>Tynacantha marginata</i>	TW	$y = 1.8377x + 0.2054$	0.9783
	FAL	$y = 1.1595x - 1.3664$	0.9221		FAL	$y = 1.1419x - 0.5946$	0.8414
	IOW	$y = 0.5323x - 0.1109$	0.9783		IOW	$y = 2.1520x - 1.1267$	0.9201
<i>Edessa rufomarginata</i>	TW	$y = 0.7061x - 0.7759$	0.9388	<i>Edessa rufomarginata</i>	TW	$y = 1.8561x + 0.1600$	0.967
	FAL	$y = 0.9068x - 1.5385$	0.899		FAL	$y = 1.3569x - 0.6694$	0.973
	IOW	$y = 0.5210x - 0.0787$	0.967		IOW	$y = 1.6922x - 1.3946$	0.8786
<i>Banasa maculata</i>	TW	$y = 0.4966x - 0.7852$	0.8961	<i>Banasa maculata</i>	TW	$y = 1.8680x - 0.0402$	0.9361
	FAL	$y = 0.9308x - 1.3822$	0.89		FAL	$y = 0.9906x - 0.8008$	0.9565
	IOW	$y = 0.5011x + 0.0157$	0.9361		IOW	$y = 1.7707x - 1.4175$	0.8641
<i>Chinavia obstinata</i>	TW	$y = 0.5568x - 0.6471$	0.9391	<i>Chinavia obstinata</i>	TW	$y = 1.5665x - 0.0065$	0.9865
	FAL	$y = 0.9198x - 1.3922$	0.9397		FAL	$y = 0.8688x - 0.6504$	0.9191
	IOW	$y = 0.6298x + 0.0053$	0.9865		IOW	$y = 1.4500x - 1.3990$	0.9369
<i>Mitripus convergens</i>	TW	$y = 0.4793x - 0.7139$	0.8332	<i>Mitripus convergens</i>	TW	$y = 1.7669x + 0.2417$	0.9491
	FAL	$y = 0.7564x - 1.5088$	0.8766		FAL	$y = 0.8210x - 0.6012$	0.7434
	IOW	$y = 0.5371x - 0.1360$	0.9491		IOW	$y = 1.3773x - 1.3210$	0.8835
<i>Runibia perspicua</i>	TW	$y = 0.5517x - 0.7628$	0.9418	<i>Runibia perspicua</i>	TW	$y = 1.9339x + 0.1440$	0.9688
	FAL	$y = 0.7586x - 1.5252$	0.8822		FAL	$y = 1.0971x - 0.6835$	0.9648
	IOW	$y = 0.5010x - 0.0720$	0.9688		IOW	$y = 1.4994x - 1.4162$	0.8929

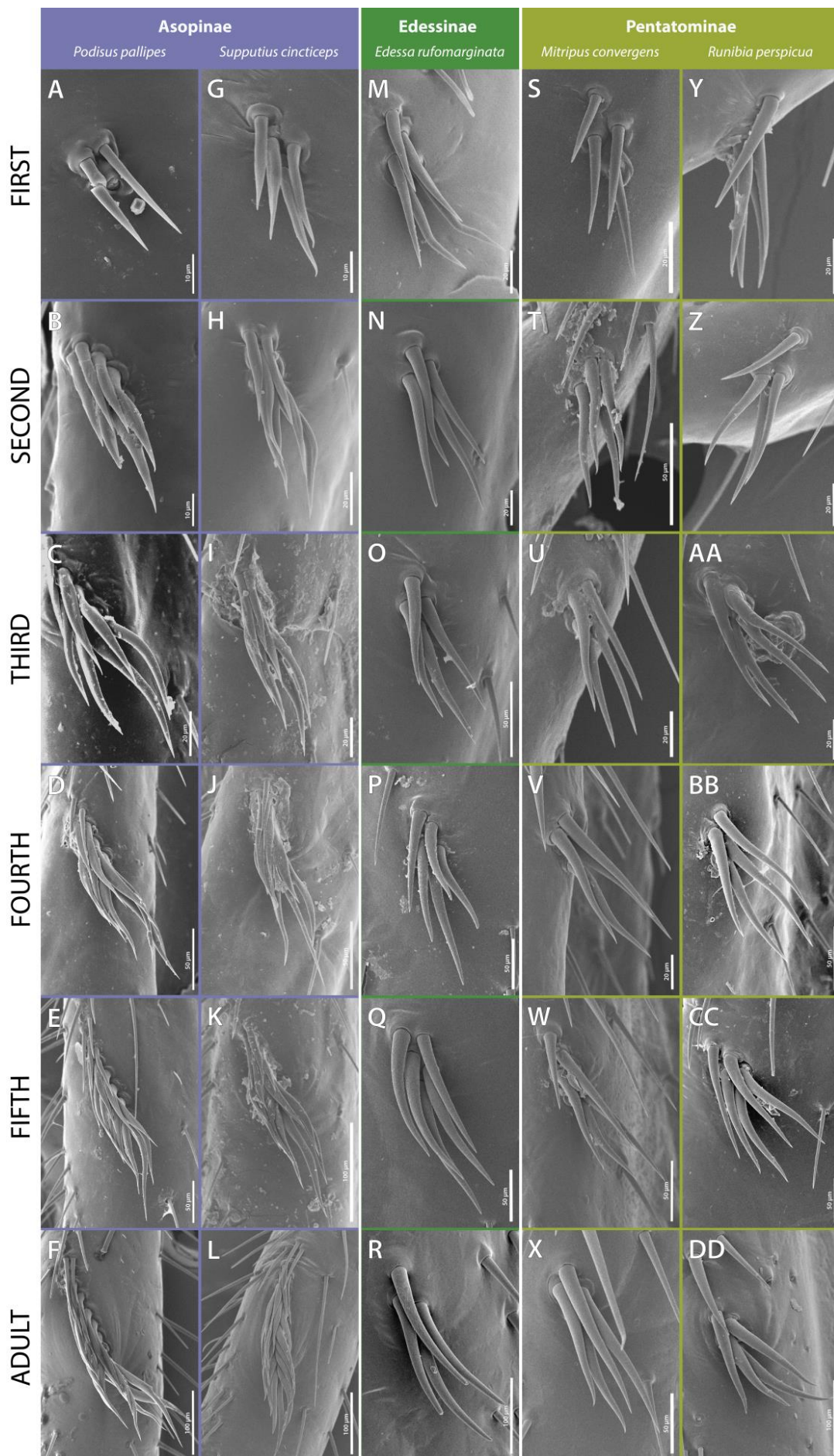


Fig. 1. Scanning electron microscopy of foretibial apparatus in the five nymphal instar and Adult. A-F: *Podisus pallipes*. G-L: *Supputius cincticeps*. M-R: *Edessa rufomarginata*. S-X: *Mitripus convergens*. Y-DD: *Runibia perspicua*.

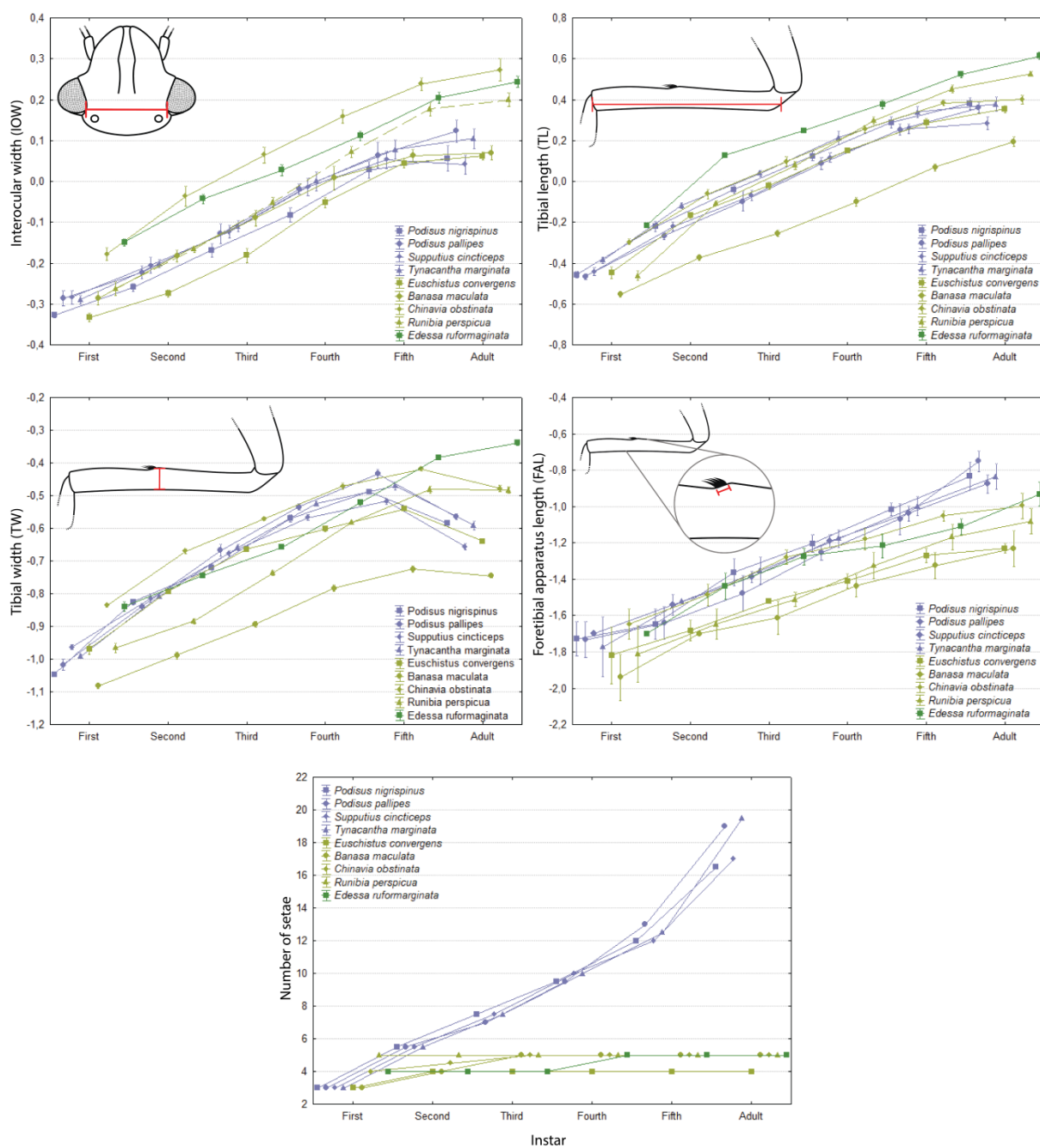


Fig. 2. Ontogenetic trajectories of four structures per instar of Pentatomidae species. The local of measure are indicated in the top left corner of each graph.

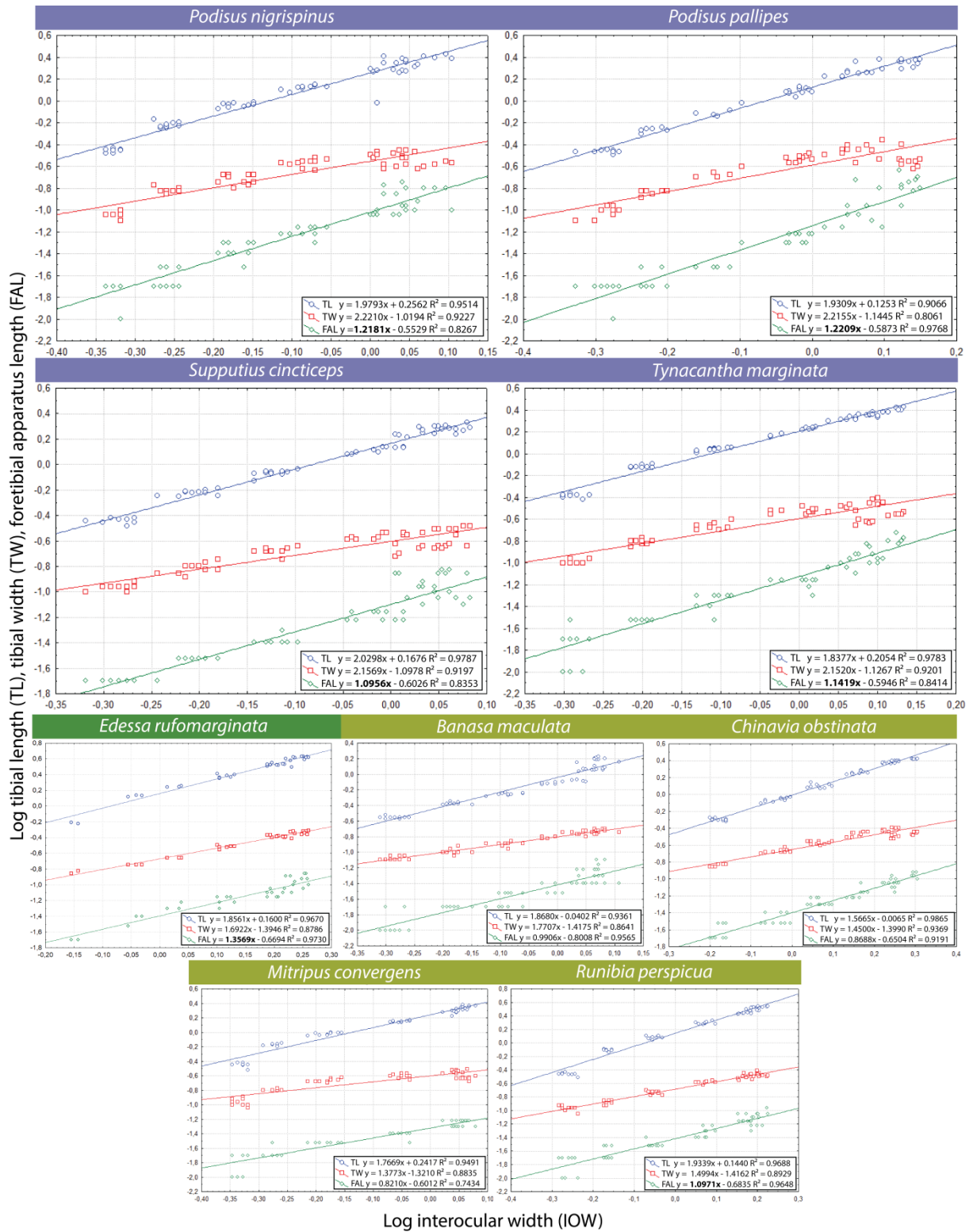


Fig. 3. Ontogenetic allometry plot of tibial length, tibial width and foretibial apparatus length in relation with interocular width of nine species of Pentatomidae. Positive allometric coefficients of foretibial apparatus are represented in bold.

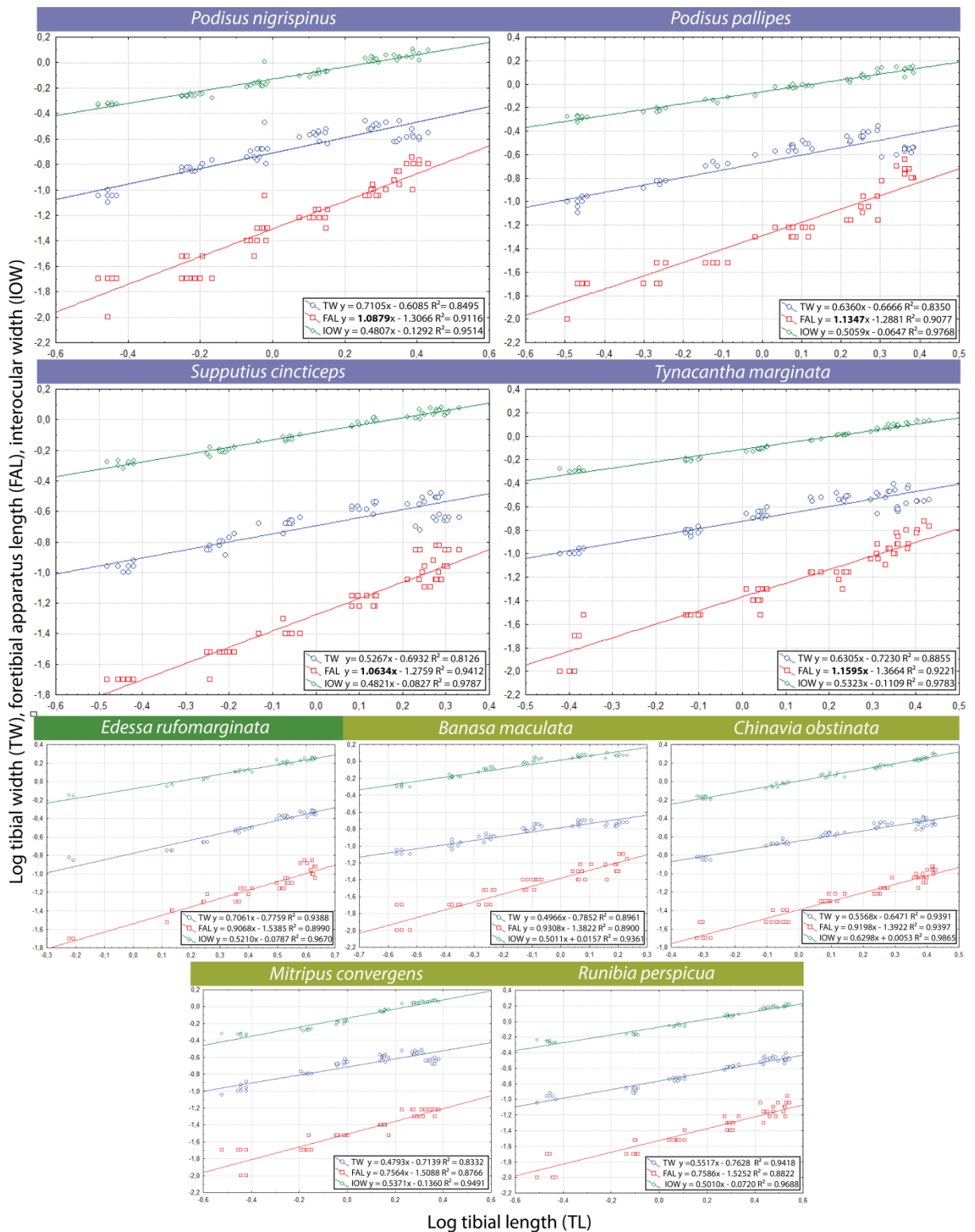


Fig. 4. Ontogenetic allometry plot of tibial width, foretibial apparatus length and interocular width in relation with tibial length of nine species of Pentatomidae. Positive allometric coefficients of foretibial apparatus are represented in bold. Only Asopinae species present positive allometry of foretibial apparatus.

CAPÍTULO III ⁴**External morphology of immature of *Tynacantha marginata* Dallas, 1851 (Hemiptera, Heteroptera, Pentatomidae)**RICARDO BRUGNERA^{1,2} & JOCÉLIA GRAZIA^{1,2}

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Abstract

Immature stages of Neotropical Asopinae have been poorly studied. Here, the external morphology of eggs and nymphs of *Tynacantha marginata* Dallas are described using light and scanning electron microscopy. The egg is barrel-shaped with smooth surface, and long aero-micropylar processes. In the first instar, the color of abdomen varies along the development from yellow to red, the dorsal plates are pale, and the surface of evaporatorium bears spiked projections. From the second, the evaporatorium surface is reticulated. From third instar, the pronotum is orange with black lateral margins. The eggs and nymphs of *T. marginata* are unique among Neotropical Asopinae species of which the morphology is known, allowing the early identification of the species.

Key words: predaceous stink bug, coloration, morphology, ontogeny

Introduction

The neotropical Asopinae genus *Tynacantha* Dallas comprises two species: *T. marginata* Dallas found in South America, and *T. splendens* Distant found in Central America (Thomas 1992). Although *T. marginata* is a common species, frequently captured in field

⁴ Formatado conforme as normas do periódico Zootaxa. Veja anexo III.

samplings (e.g. Campos *et al.* 2009; Bunde *et al.* 2010; Dellapé *et al.* 2017; Melo *et al.* 2017), the nymphs morphology was studied only by De Tella (1951), and Saini & De Coll (1992) provided a picture of a cluster of eggs. Similar to others asopines, *T. marginata* shows a great potential as biological control agent (Sanchez *et al.* 2001), showing an improved longevity compared to others species (Vacari *et al.* 2007; Dalvi *et al.* 2011).

The knowledge of the eggs and nymphs of Pentatomidae contributes to several areas of study, including taxonomy, ecology, phylogeny, and economic aspects (Bundy & McPherson 2000; Matesco *et al.* 2009b). Nevertheless, few species (less than 1%) of new world Asopinae have immature described (Saini 1992; Zanuncio *et al.* 1993; Matesco *et al.* 2009a; Matesco *et al.* 2014) making difficult the early and correct identification of its species.

Here we describe the external morphology of the egg and the five nymphal instars of *T. marginata*, using light and scanning electron microscopy, emphasizing the post-embryonic changes. This study is the first, of a series of papers on immature of Asopinae, which is under development in the “Laboratório de Entomologia Sistemática” (LES), “Universidade Federal do Rio Grande do Sul” (UFRGS), Brazil.

Material and Methods

Adults were collected in Morro Santana, Porto Alegre, Rio Grande do Sul, Brazil (30°04'00.5"S 51°07'20.3"W). In the laboratory, specimens were kept in plastic vials and reared in control chamber at 25 ± 1 °C, 70 ± 10 RH, and 12L:12Dh photoperiod. To the first instar nymphs were offered, only cotton balls moistened with water. The remaining nymphal stages and adults, were feeding with larvae and pupae of *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrionidae) *ad libitum*.

Measurements and morphological data were obtained from ten eggs and ten nymphs of each instar, fixed in 70% ethanol and maintained in freezer. The descriptions of color patterns were carried out *in vivo*. Measurements are presented in millimeters (mean \pm standard deviation [maximum-minimum]), and were obtained according to Barão *et al.* (2015), plus the first segment of labium width (Table 1).

For scanning electron microscopy (SEM) analysis, two eggs and two specimens of each instar (preserved in 70% ethanol) were dehydrated in an increasing concentration of ethanol series (70%, 80%, 90%, and 99.9%) for ten minutes each, and transferred to 100% acetone. Samples were critical point dried (10 cycles), mounted in stubs, sputter-coated with gold and observed by SEM at the “Centro de Microscopia e Microanálise” of UFRGS.

Terminology to general morphology follows Matesco *et al.* 2009b and to dorsal abdominal glands follows Bottega *et al.* (2015) and Vilimová & Kotalová (2012).

Voucher specimens were deposited at the Entomological Collection (UFRS) of the Department of Zoology, Federal University of Rio Grande do Sul.

Results

Egg

Barrel-shaped (Fig. 6); chorium translucent with a black circular stripe at apex of lateral wall and at the top of operculum; pale color after the oviposition, becoming bright orange with the development of the embryo (Figs. 25–26); red eyes and black *ruptor ovis* visible through the operculum before eclosion; nine to 13 white aero-micropylar processes spaced, very long and slender, surrounding the operculum; openings of aero-micropylar processes subapical and outwardly directed (Fig. 7–8); distance between each process almost equal of process length. Surface of chorium smooth under SEM (Fig. 6–7); operculum tenuously delimited. Measurements: egg length (1.2 ± 0.07 [1.3–1.0]); egg width (0.8 ± 0.07 [0.9–0.7]).

First instar

Head, antennae (except intersegmental areas that are pale) thorax and legs dark brown to black; eyes reddish (Fig. 1). Abdomen yellow a day after eclosion, becoming orange and then reddish in late instar; dorsal plates pale; external cuticular structures of dorsal abdominal glands (DAG's) darkened; lateral plates dark grey; last two segments of abdomen dark grey.

Body oval, slightly elongated; surface smooth, without punctures; lateral margin of the body, antennae and legs with setae. Head rounded, clypeus longer than mandibular plates; apex of labium with few sensilla (Fig. 23) and surpassing the meta-coxae. Proportion of antennal segments I < II > III < IV. Lateral margin of the body, antennae, legs and median region of posterior margin of III-VIII ventral segments of abdomen with setae (Fig. 21). Eight pairs of lateral plates in abdomen; 1+1 spiracles in II-VII and 1+1 trichobothria on III-VI abdominal segments; anterior dorsal abdominal glands (DAG1) without evaporatorium; ostioles slit-like (Fig. 14); ostioles of median (DAG2) and posterior (DAG3) attended by cuticular fold; surface of evaporatorium with spiked projections (Fig. 15).

Second instar

Head dark brown to black; labrum and segment I of labium completely black, last three segments pale in the middle; eyes red. Thorax dark brown to black; discal region reddish ventrally. Abdomen red; lateral plates black; last two segments black.

Body oval (Fig. 2); surface smooth; median region of posterior margin of III-VIII ventral segments of abdomen with setae. Clypeus slightly longer than mandibular plates; apex of labium surpassing the hind legs. Pronotal lateral margins expanded and serrated. Abdomen with eight pairs of lateral plates, one pair of dorsal mesolateral plates (located anteriorly), six dorsal median plates and five ventral median plates; dorsal abdominal scent glands in II-IV dorsal median plates; 1+1 spiracles in II-VIII segments and 2+2 trichobothria on III-VII ventral abdominal segments. Characteristics of DAG's are similar to the third instar (see below).

Third instar

Head usually dark, but sometimes with orangish regions (Fig. 30–31). Pronotum orange with lateral margins black (Fig. 3); coxa, trochanter and anterior half of femur orange; ventral margins of head and thorax black with discal area orangish.

Development of wing pads visible (Fig. 11). DAG1 (Fig. 16) surrounded by cuticular wrinkles and evaporatorium (shallow alveoli with low bridges and trabeculae) (Fig. 20). DAG2 (Fig. 17) and DAG3 bearing spout peritreme, surrounded by a reticulate evaporatorium. Color and morphology of the remaining structures similar to the preceding instar.

Fourth instar

First segment of antennae pale. Basal angles of pronotum usually black. Pair of dorsal mesolateral plates smaller than in second and third instars, acquiring drop shape; fusion of sixth dorsal median plate with eighth pair of lateral plates of abdomen.

Body elliptic (Fig. 4). Dorsal sclerotized black areas with tiny and concolour punctures; wing pads reaching posterior margin of metanotum (Fig. 10). Color and morphology of the remaining structures similar to the preceding instar.

Fifth instar

Head frequently with orangish regions (Fig. 30–31); when completely black, usually with an orange spot before the eyes and an orange strip between mandibular plates and clypeus; apex of labium with an increased number of sensilla (Fig. 24) compared to first instar. Posterior margin of pronotum black (Fig. 5); black areas with blue-green iridescent color (Fig. 27) (characteristic also present in adults); ventral plates sometimes concolour with discal area of abdomen.

Body oval; dorsal sclerotized black areas with denser punctuations than in fourth instar; apex of wing pads almost reaching posterior margin of III abdominal segment (Fig. 9); ventral plates covered by comb-like projections (Fig. 22); development of male and female genitalia visible ventrally in the last three segments of abdomen (Figs. 28–29); microsculptures surrounding the ostiole of DAG1, and the suture that interconnects the right and left external scent efferent system (ESES) present (Fig. 18–19). Color and morphology of the remaining structures similar to the preceding instar.

Discussion

Several species of Asopinae are found in natural and modified (i.e. crops) areas, playing an important role as natural enemies (De Clercq 2008). Studies involving morphology of immature are important because allows the early identification, dispensing the need to collect, conserving the populations of these predators.

Nymphs commonly represents an important fraction in studies of taxon sampling (Mendonça, Jr. *et al.* 2009) most of them cannot be identified because the lack of knowledge. Photographs and illustrations are important tools to help the identification (e. g. Oetting & Yonke 1971; Grazia *et al.* 1985; Saini 1992; Zanuncio *et al.* 1993; Costello *et al.* 2002.), although, they should be used with caution, since the color and morphology can vary with the environment and feeding (Schwertner 2002; Matesco *et al.* 2009a).

The egg morphology known so far for some New World Asopinae species show particular characteristics. In *T. marginata* the surface of corium is smooth and the aeromicropylar process are long, a unique pattern among sympatric species already studied. The species of *Podisus* Herrich-Schäffer (Grazia *et al.* 1985; Wolf & Reid 2004; Sá *et al.* 2013; Saini 1994; Lambdin & Lu 1984; Matesco *et al.* 2014), *Brontocoris tabidus* (Signoret) and *Supputius cincticeps* (Stål) (Dimaté *et al.* 2014) show also a long aero-micropylar process but the corium is covered with spines. In *Stiretrus erythrocephalus* (Lepeletier & Serville) the aeromicropylar processes are shorter than in *T. marginata*, dark at base and white at apex, and the spines in corium are present (Saini 1992).

Nymphs of *T. marginata* exhibit a unique morphology. The 1st instar is distinctly different from the others South American species already studied by the presence of dorsal median plates pale. The orange color of pronotum observed from the third instar is similar to the same instar of *Supputius cincticeps* (Zanuncio *et al.* 1993). However, the pronotum of *S. cincticeps* shows ferruginous punctures and it is totally orange while in *T. marginata* the punctures are concolour to the pronotum surface and the lateral margins are black. Also, the iridescent color that is observed in the fifth instar of *T. marginata*, are not present in the others species. Furthermore, the pair of mesolateral plates that are present from second instar in *T. marginata*, are not observed in others species. Oetting & Yonke (1971) illustrated the nymphs of *Striretrus anchorago* (Fabricius) [as *S. fimbriatus* (Germar)] showing dorsal abdominal plates concolour to the surface of abdomen in the second to fourth instars. The iridescence observed on black areas in the fifth instar is so far not mentioned in the literature.

The scent efferent system of the DAGs of Asopinae nymphs were investigated only in the fifth instar of *Alcaeorrhynchus grandis* Dallas (Bianchi *et al.* 2016). In both species the 1st pair is not attended by peritreme, and with incipient evaporatorium. The microsculptures surrounding the ostiole of the anterior external scent efferent system following the suture between the ESES in *T. marginata* are not observed in *A. grandis*.

The sensilla of labium in Asopinae are poorly studied and only in adults. The investigation of sensilla in mouthparts can help to better understand and to answer questions about behavior and feeding that are still unknown (Zacharuk 1991; Parveen *et al.* 2015). The lower number of sensilla observed in the 1st instar of *T. marginata* may be related with the fasting of food in that instar, while in the fifth instar, where the greater number of sensilla is present, the feeding habit is the same of adults.

Females are characterized by a median longitudinal fissure on the VIII abdominal segment, while in males that segment is entire (Greve *et al.* 2003; Matesco *et al.* 2003; Schaefer *et al.* 1998). The differential morphology of sex in nymphs of fifth instar can be useful in biological studies including the sex ratio.

In conclusion, the nymphs of all the instars and eggs of *Tynacantha marginata* present characteristics that make the early identification of the species feasible. Descriptions of other species of Asopinae, including genera of less economic interest are needed, with focus in the differences between species to favor early identification of the species. Description of the ontogenesis of ultrastructural morphology, mainly the dorsal abdominal glands, the ventral abdominal plates, the labium, the trichobothria and the eggs are also needed (Matesco *et al.* 2009a; Barão *et al.* 2015).

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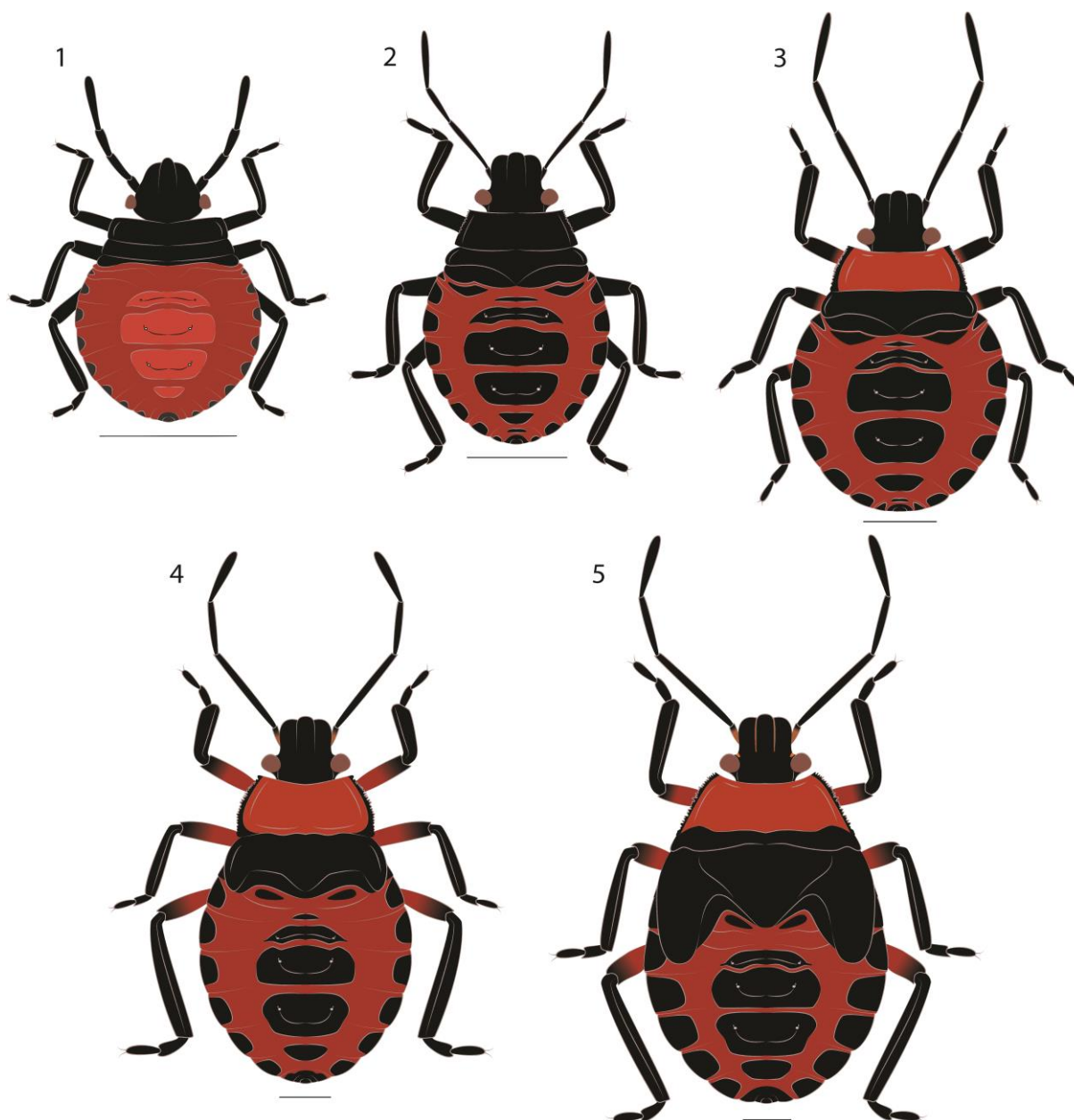
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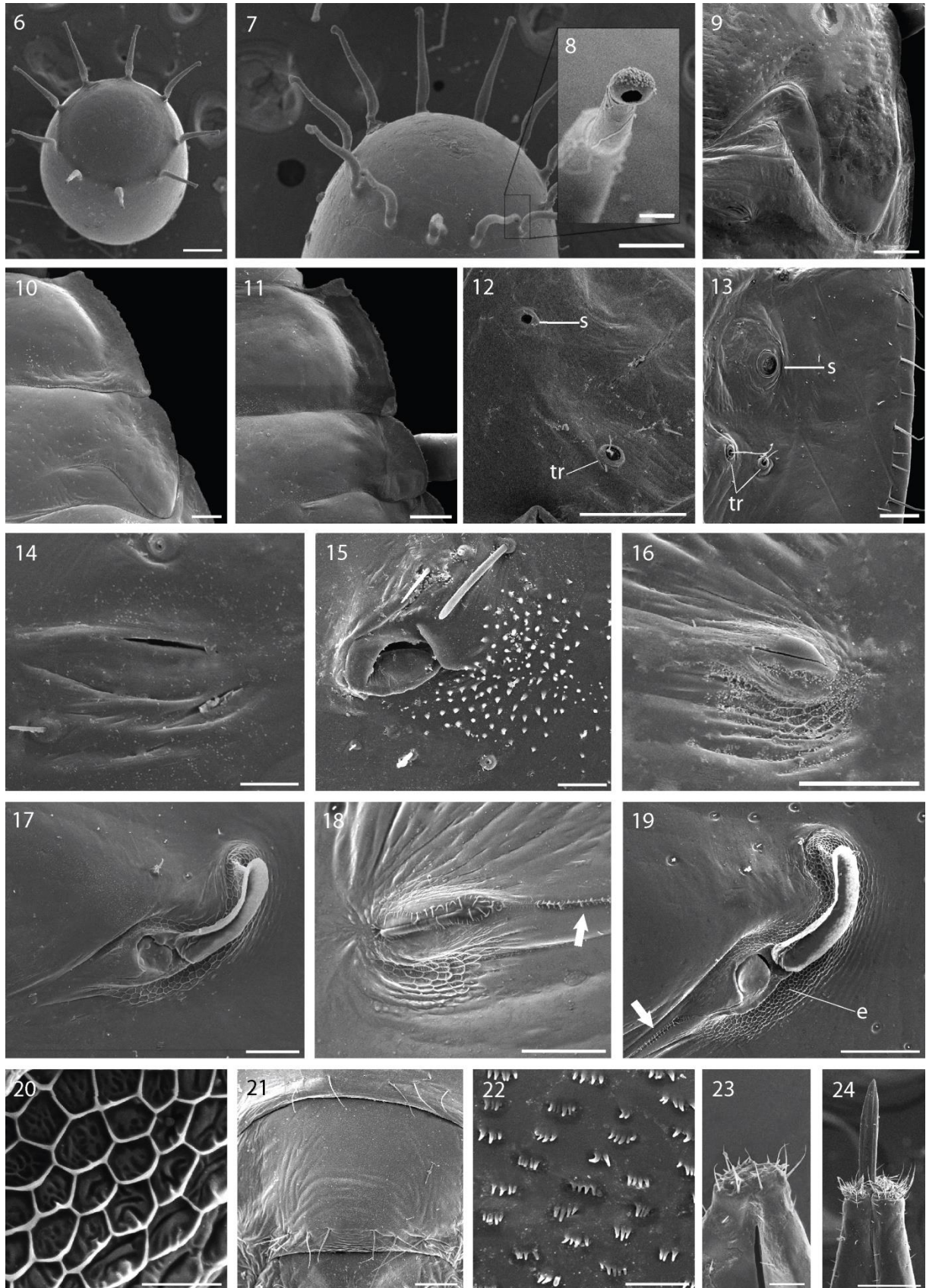
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TABLE 1. Measurements (mean \pm standard deviation [maximum-minimum]), in millimeters, of morphometric characters of nymphs of *Tynacantha marginata*.

Character	First instar	Second instar	Third instar	Fourth instar	Fifth instar
Total length	2.00 \pm 0.03 (2.20–1.79)	2.89 \pm 0.13 (3.56–2.32)	4.31 \pm 0.13 (4.72–3.33)	7.00 \pm 0.11 (7.44–6.56)	7.95 \pm 0.27 (9.13–6.56)
Head length	0.62 \pm 0.01 (0.70–0.52)	0.74 \pm 0.01 (0.79–0.68)	0.96 \pm 0.02 (1.05–0.86)	1.30 \pm 0.03 (1.46–1.12)	1.62 \pm 0.03 (1.74–1.50)
Head width	0.65 \pm 0.01 (0.70–0.60)	0.87 \pm 0.01 (0.92–0.80)	1.18 \pm 0.01 (1.24–1.10)	1.55 \pm 0.01 (1.58–1.48)	1.91 \pm 0.03 (2.05–1.78)
Interocular distance	0.52 \pm 0.01 (0.56–0.48)	0.63 \pm 0.01 (0.66–0.58)	0.80 \pm 0.01 (0.84–0.75)	0.99 \pm 0.01 (1.03–0.96)	1.19 \pm 0.02 (1.28–1.08)
Rostral length	0.88 \pm 0.01 (0.91–0.82)	1.61 \pm 0.02 (1.73–1.52)	2.21 \pm 0.06 (2.40–1.92)	3.22 \pm 0.03 (3.40–3.04)	4.22 \pm 0.08 (4.68–4.01)
Rostral width of I segment	0.14 \pm 0.01 (0.16–0.12)	0.19 \pm 0.01 (0.24–0.16)	0.27 \pm 0.004 (0.30–0.26)	0.40 \pm 0.01 (0.45–0.34)	0.49 \pm 0.01 (0.54–0.42)
Length of I antennal segment	0.11 \pm 0.003 (0.12–0.10)	0.17 \pm 0.004 (0.18–0.14)	0.22 \pm 0.01 (0.27–0.18)	0.28 \pm 0.01 (0.30–0.24)	0.39 \pm 0.01 (0.44–0.34)
Length of II antennal segment	0.26 \pm 0.01 (0.32–0.24)	0.64 \pm 0.01 (0.68–0.60)	1.02 \pm 0.02 (1.10–0.88)	1.56 \pm 0.02 (1.64–0.46)	2.25 \pm 0.04 (2.51–2.06)
Length of III antennal segment	0.22 \pm 0.004 (0.24–0.20)	0.44 \pm 0.004 (0.46–0.43)	0.66 \pm 0.01 (0.72–0.60)	0.95 \pm 0.01 (1.00–0.90)	1.29 \pm 0.03 (1.44–1.20)
Length of IV antennal segment	0.48 \pm 0.004 (0.49–0.46)	0.73 \pm 0.01 (0.78–0.68)	0.91 \pm 0.01 (0.96–0.86)	1.13 \pm 0.01 (1.18–1.08)	1.35 \pm 0.03 (1.50–1.24)
Pronotal length	0.18 \pm 0.01 (0.20–0.14)	0.36 \pm 0.004 (0.37–0.34)	0.60 \pm 0.01 (0.70–0.50)	0.98 \pm 0.02 (1.05–0.90)	1.54 \pm 0.03 (1.64–1.38)
Pronotal width	0.76 \pm 0.02 (0.83–0.60)	1.14 \pm 0.03 (1.22–0.96)	1.76 \pm 0.02 (1.89–1.56)	2.44 \pm 0.04 (2.68–2.32)	3.91 \pm 0.11 (4.56–3.28)
Abdominal width	1.26 \pm 0.03 (1.36–1.08)	1.81 \pm 0.06 (2.08–1.56)	2.78 \pm 0.08 (3.20–2.40)	3.62 \pm 0.05 (3.84–3.37)	4.84 \pm 0.20 (6.00–4.01)



FIGURES 1-5: Dorsal view of nymphs of *Tynacantha marginata*. 1. First instar. 2. Second instar. 3. Third instar. 4. Fourth instar. 5. Fifth instar. Scale bars = 1 mm.



FIGURES 6-24: Immatures of *Tynacantha marginata* under scanning electron microscopy. 6-8. Egg. 6. Antero-lateral view. 7. Lateral view of anterior pole. 8. Magnification of apex of aero-micropylar process. 9-11. Dorsal view of lateral margin of thorax. 9. Fifth instar. 10. Fourth instar. 11. Third instar. 12-13. Spiracles and trichobothria of V ventral abdominal segment. 12. First instar. 13. Fifth instar. 14-20. External scent efferent system. 14. DAG1 of

first instar. 15. DAG2 of first instar. 16. DAG1 of third instar. 17. DAG2 of third instar. 18. DAG1 of fifth instar. 19. DAG2 of fifth instar. 20. Evaporatorium of DAG2 of fifth instar. 21. Dorsal view of VI-VII ventral abdominal segments. 22. Sculpturing of the VI abdominal plate of fifth instar. 23-24. Apex of labium. 23. First instar. 24. Fifth instar. (Arrows = macrosculpture on suture of ESES; e = evaporatorium; s = spiracle; tr = trichobothria). Scale bars = 200, 200, 20, 50, 10, 500, 250, 200, 10, 20, 50, 50, 50, 100, 10, 200, 100, 20, 100 μm , respectively.



FIGURES 25-31. Aspects of *Tynacantha marginata* in colored images. 25-26. Eggs in dorsal view. 27. Habitus of adult and fifth instar nymph. 28-29. Last ventral abdominal segments of fifth instar. 28. Female. 29. Male. 30-31. Dorsal view of head of fifth instar. Scale bars = 2, 0.5, 1, 0.5, 0.5, 0.5, 0.5 mm respectively.

CONCLUSÕES

No capítulo I, apresentamos um estudo morfológico e comparativo sobre as expansões tibiais e o aparato tibial em 86 e 44 espécies (respectivamente) da subfamília Asopinae. Ambas estruturas possuem ampla variação morfológica dentro da subfamília, com base nisso, 10 caracteres morfológicos são propostos, sendo 1 contínuo. Verificamos que conclusões sobre o aparato tibial tomadas em trabalhos anteriores não se aplicam para todas as espécies da subfamília, já que algumas apresentam um número de cerdas comparável a outros grupos. As expansões tibiais estão presentes em grande parte dos asopíneos, e fornecem informações taxonômicas importantes. Sugerimos que as variações observadas nestas estruturas da tibia anterior sejam exploradas em análises filogenéticas.

No capítulo II, investigamos as variações morfológicas e a alometria da tibia anterior em nove espécies de Pentatomidae, com foco no aparato tibial. Nas espécies de Asopinae o aparato tibial possui um crescimento comparativamente mais acelerado que em outros grupos, além disso, o número de cerdas aumenta a cada instar nos percevejos predadores, enquanto nas demais subfamílias estudadas a estrutura possui pouca variação ao longo do ciclo de vida. A largura da tibia diminui consideravelmente de tamanho entre o quinto instar e o estágio adulto, hipotetizamos que esta variação possa ocorrer em função da perda de estruturas presentes nas ninfas. Outros estudos envolvendo desenvolvimento ontogenético, comportamento e alometria são necessários na família Pentatomidae, especialmente da subfamília Asopinae.

No capítulo III, os estágios imaturos de *Tynacantha marginata* Dallas são descritos. Os ovos e todos os instares ninfais apresentam características que tornam possível a distinção das demais espécies de Asopinae que possuem a mesma distribuição geográfica e que se conhece a morfologia. Dentre as principais características diagnósticas se destacam a superfície do cório lisa, e nas ninfas a coloração do pronoto. Mais trabalhos envolvendo imaturos na subfamília são necessários, a fim de contribuir para a morfologia e taxonomia do grupo.

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