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ROSTRO PREDADOR: ALOMETRIA DO LÁBIO DE ASOPINAE
(HEMIPTERA: PENTATOMIDAE)

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Sumário

RESUMO

Asopinae, percevejos predadores inclusos em Pentatomidae, possui algumas características peculiares como a cabeça aparentemente retangular em vista dorsal, lábio marcadamente robusto, alargado e livre, com inserção muito próxima à base do labro, características consideradas diagnósticas para diferenciá-los dos outros pentatomídeos. Apesar do amplo potencial econômico, devido ao interesse sobre esses organismos no controle biológico, a subfamília ainda carece de estudos principalmente de morfologia comparada das estruturas adaptadas para a predação. Comparou-se a morfometria linear e a alometria do lábio entre Asopinae e não-Asopinae (táxons inclusos em Pentatomomorpha e Cimicomorpha).Adicionalmente, realizou-se a reconstrução dos estados de caracteres ancestrais, com base na proporção comprimento/largura labial e um fenograma das relações entre as espécies para esse mesmo carácter. Observou-se que tanto espécies de Asopinae como não-Asopinae possuem alometria negativa em relação à razão comprimento/largura labial, contudo, Asopinae geralmente apresentam uma forte tendência a ter um lábio relativamente mais robusto à medida que o tamanho do corpo aumenta em comparação com a mesma proporção de aumento no grupo não-Asopinae. Essas proporções morfológicas tendem a manter um agrupamento de Asopinae, entretanto não foram encontrados agrupamentos refletidos por ancestralidade comum para as espécies analisadas tanto de Asopinae quanto em não-Asopinae. Os resultados indicam que as pressões seletivas para predadores e fitófagos/onívoros parecem estar sendo conduzidas por diversificação devido aos hábitos alimentares.

PALAVRAS CHAVE: percevejos, morfologia, aparelho bucal, fenograma, filogenia

ABSTRACT

Asopinae, predatory stink bugs included in Pentatomidae, have some peculiar characteristics, such as the apparently rectangular head in dorsal view, markedly robust labium, inserted very close to the base of labrum, all considered diagnostic to differentiate them from other pentatomids. Despite the economic interest on Asopinae due to their potential use in biological control programs, the subfamily still lacks studies on comparative morphology of the structures adapted for predation. Through linear morphometry and allometric studies, a comparison of the labium between Asopinae and other Pentatomomorpha and Cimicomorpha taxons was made; in addition a reconstruction of ancestral character states was performed based on the labial length/width ratio and a phenogram of the relationships between species for the same character. It has been observed that both Asopinae and non-Asopinae species have negative allometry with respect to labial length / width ratio, however, Asopinae generally have a strong tendency to have a relatively more robust labium as body size increases compared to the same proportion of increase in the non-Asopinae group. These morphological proportions tend to maintain a cluster of Asopinae, however no groupings reflected by common ancestry were found for both Asopinae and non-Asopinae analyzed species. The results propose that selective pressures for predators and phytophagous / omnivores seem to be driven by diversification due to feeding habits.

KEYWORDS: stinkbug, morphology, mouthparts, phenogram, phylogeny.

INTRODUÇÃO

Os insetos possuem umas das maiores diversidades morfológicas de aparelhos bucais, adaptados aos mais variados hábitos alimentares (Labandeira 1997; Grimaldi & Engel 2005). Algumas ordens de insetos, tais como Lepidoptera, Diptera e Hemiptera, apresentam adaptações à ingestão de alimentos fluidos, observadas como modificações de diferentes apêndices bucais. Em Hemiptera a presença de um aparelho bucal perfurador-sugador segmentado, associado a sistemas de bombeamento de saliva e alimento, é considerada característica diagnóstica para da ordem (Forero 2008; Grazia & Fernandes 2012). O aparelho bucal dos hemípteros é denominado de rostro, com mandíbulas e maxilas formando estiletes tubulares longos e flexíveis alojadas no lábio, também tubular e segmentado em três ou quatro artículos (Schuh & Slater 1995; Grimaldi & Engel 2005; Grazia & Fernandes 2012).

Dentre os hemípteros, a maior diversidade alimentar é encontrada na subordem Heteroptera, com grupos especializados em alimentar-se de plantas, fungos, artrópodes, pequenos vertebrados e sangue (Schuh & Slater 1995). Essa subordem possui sete infraordens: Enicocephalomorpha, Dipsocoromorpha, Gerromorpha, Nepomorpha, Leptopodomorpha, Cimicomorpha, e Pentatomomorpha. Tal classificação é reconhecida por Schuh (1979) com base principalmente em Cobben (1978) a partir de estudos sobre tendências evolutivas nas estruturas no aparelho bucal e hábitos alimentares (Weirauch & Schuh 2011; Grazia & Fernandes 2012; Panizzi & Grazia 2015). Dentre as infraordens, Enicocephalomorpha é conhecida por seus hábitos predadores ou onívoros. Dipsocoromorpha inclui os menores heterópteros conhecidos e possuem hábitos provavelmente predatórios. Gerromorpha inclui espécies semi-áquaticas exclusivamente predadoras. Nepomorpha reúne as espécies verdadeiramente aquáticas e predadoras, exceto por algumas espécies de Corixidae que se alimentam de algas e material vegetal em decomposição. Leptopodomorpha são predadores. Cimicomorpha inclui espécies predadoras, hematófagas ou fitófagas. Pentatomomorpha corresponde a um dos maiores grupos de Heteroptera e abrange famílias que, além de hábitos fitófagos, podem ser predadores e hematófagos, além de micetófagos, secundariamente derivados. Pentatomomorpha juntamente com Cimicomorpha representam 90% das espécies de heterópteros, provavelmente devido a evolução do hábito alimentar fitófago (Cobben 1978; Spence & Andersen 1994; Grimaldi & Engel 2005; Henry 2009; Weirauch

&Schuh 2011; Grazia & Fernandes 2012;Spangenberg *et al.* 2013; Panizzi & Grazia 2015).

Em Heteroptera o hábito predador é observado em quase todos os grupos considerados basais (Schuh & Slater 1995; Grimaldi & Engel 2005; Li *et al.*2017). A transição da predação para a fitofagia provavelmente ocorreu no antepassado comum a Cimicomorpha e Pentatomomorpha (Grimaldi & Engel 2005; Weirauch & Schuh 2011; Panizzi & Grazia 2015;Li *et al.* 2017), com reversões à predação em algumas famílias dentro das duas infraordens (Li *et al.* 2017). Em Pentatomomorpha, Pentatomidae é uma das famílias mais numerosas e diversas, compartilhando a característica ancestral da infraordem do hábito fitófago, com a exceção da subfamília Asopinae que possui hábito predador.

Asopinae possui 64 gêneros e cerca de 300 espécies descritas, ocorrendo em todas as regiões biogeográficas (Thomas 1992, 1994; De Clercq 2000; Rider 2019). Além de seu hábito predador insetívoro generalista, (isto é, se alimentam de insetos de diferentes ordens) alguns desses percevejos podem apresentar hábito zoofitófago, ou seja, utilizam material vegetal para suprir nutrientes ou aminoácidos não encontrados na presa, ou nos períodos de escassez das mesmas, não causando maiores danos aos tecidos vegetais (Armer *et al.* 1998; Eubanks & Denno 1999; Coll & Guershon 2002; Lemos *et al.* 2009; Torres *et al.* 2010). O hábito zoofitófago é importante para o uso destes insetos no controle biológico, pois permite a manutenção das populações de predadores em períodos onde a população de presas torna-se deficiente (Armer *et al.*1998; Eubanks & Denno 1999; Coll & Guershon 2002; Lemos *et al.* 2009; Torres *et al.* 2010). Sendo assim, Asopinae tem amplo potencial econômico e o interesse sobre as espécies dessa subfamília para controle e manejo biológico de pragas em plantas cultivadas é de crescente interesse no Brasil, assim como em escala mundial (De Clercq *et al.* 1998;Malaguido & Panizzi 1998; Aldrich & Cantelo 1999; Cavalcanti *et al.* 2000;De Clercq 2000, 2008; Vivian *et al.* 2002; Torres *et al.* 2006; Desurmont & Weston 2008; Oliveira *et al.* 2008; Angelini & Boiça-Júnior 2009; Ribeiro *et al.* 2010; Silva-Torres *et al.* 2010; Reddy & Kikuchi 2011; Zibaee *et al.* 2012; Vacari *et al.*2013; Claver & Jaiswal 2013; Bottega *et al.* 2014; Magistrali *et al.* 2014; Pires *et al.* 2015; Zanuncio *et al.* 1994, 2011). Os asopíneos contribuem para o controle populacional de insetos fitófagos, principalmente larvas folívoras, o que possibilita a redução do uso de pesticidas em sistemas agrícolas, os quais são custosos e danosos ao ambiente

(Symondson *et al.*2002; De Clercq 2008;De Bortoli *et al.* 2011; Pires *et al.* 2015). Apesar do potencial econômico das espécies de asopíneos, apenas cerca de 10% foram estudadas quanto aos seus ciclos de vida e hábitos de predação, e uma considerável parcela dos trabalhos publicados referem-se àquelas espécies com maior potencial de manejo de pragas agrícolas, por exemplo, *Podisus maculiventris* (Say, 1831), *P. nigrispinus* (Dallas, 1851), *Perillus bioculatus* (Fabricius, 1775), *Eocanthecona furcellata* (Wolff, 1811) e *Picromerus bidens* (Linnaeus, 1758).

Assim como outros pentatomídeos, os asopíneos possuem corpo ovóide (Figura 1) de tamanho moderado a grande (7 a 25 mm de comprimento) (De Clercq 2008). Entretanto, possivelmente devido ao hábito predador, eles possuem morfologia peculiar da cabeça e pernas, sendo, inclusive, essas características consideradas diagnósticas para diferenciá-los dos outros membros de Pentatomidae. As espécies de Asopinae possuem a cabeça aparentemente retangular em vista dorsal, lábio marcadamente robusto, alargado e livre, com inserção muito próxima à base do labro, capaz de extensão completa à frente, o que possibilita que eles se alimentem de presas ativas; fêmures anteriores dotados de espinho que variam de reduzidos a muito robustos; tíbias anteriores expandidas em algumas espécies; e cerdas do aparato tibial numerosas, variando em número entre os gêneros (Gapud 1991; Thomas 1992, 1994; De Clercq 2008; Barão *et al.* 2013; Brugnera *et al.* 2019).
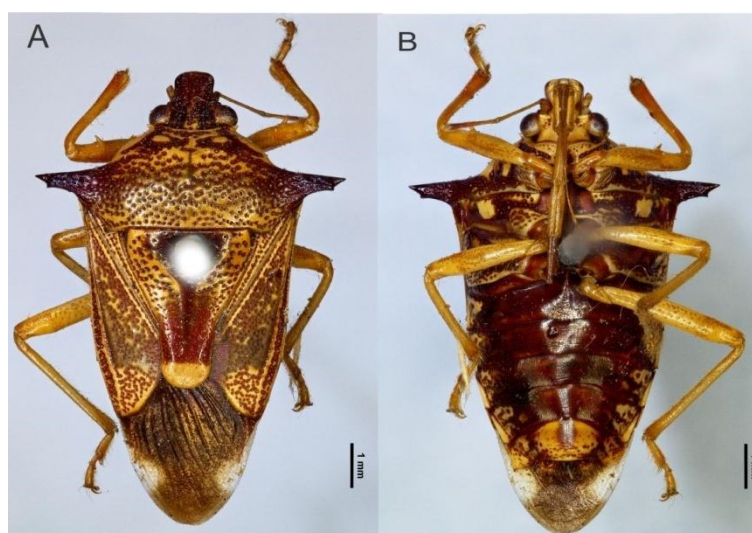

Figura 1. Asopinae: *Montrouzieriellus* sp. (A) vista dorsal; (B) vista ventral. Barra de escala = 1mm (Foto Talita Roell).

A diversidade biológica é dominada pelas diferenças nos tamanhos relativos das estruturas corporais e diferenças anatômicas entre as espécies que compartilham um plano corporal (Raff 1996). A variação no tamanho é um importante determinante para os processos de diversificação evolutiva, uma vez que muitos desses padrões de variação estão sujeitos a seleção natural e/ou sexual. Os insetos são um bom exemplo de diversificação morfológica, eles apresentam variações fenotípicas consideráveis tanto intra- como interespecífica, como por exemplo, os diferentes comprimentos de chifres de machos de besouros *Onthophagus acuminatus* (Emlen 1996) ou as longas probóscides das moscas pertencentes à Nemestrinidae (Karolyi *et al.* 2012), essas adaptações podem esta relacionadas a diversos fatores como locomoção, alimentação (captura ou manuseio de presas), competição na reprodução ou até mesmo defesa (Emlen & Nijhout 2000; Lavine *et al.* 2015). As alterações morfológicas nos organismos vêm sendo frequentemente associadas a estudos de crescimento alométrico para compreender as adaptações evolutivas nessas estruturas (Huxley 1924, 1932; Cock 1966; Gould 1966; Schmidt-Nielsen 1984; Emlen 2008). Brannen *et al.* (2005) através de estudos de alometria comparou a morfologia craniana do primeiro, segundo e terceiro instares de *Agabus disintegratus* mostrando alterações ontogenéticas significativas na arquitetura craniana, evidenciando a capacidade dessas larvas em explorar presas de características diferentes durante cada instar.

A alometria pode ser definida como taxa de crescimento desproporcional entre estruturas corporais de um organismo (Huxley 1924, 1932). Essa taxa de crescimento pode variar entre indivíduos de uma mesma população e mesma faixa etária, conhecido como alometria estática, ao longo do tempo de desenvolvimento, denominada de alometria ontogenética ou ser classificada como alometria evolutiva quando a variação se dá ao longo do ramo de uma filogenia ou entre indivíduos que compartilham um ancestral comum (Klingenberg 1996). As relações alométricas, ou seja, a relação entre o tamanho de um traço morfológico e o tamanho do corpo como um todo é tradicionalmente modelada pela equação alométrica: $y = bx^a$; onde, "x" e "y" são as dimensões de duas estruturas, "a" e "b" são constantes. Huxley (1932) percebeu que se projetado um gráfico de dispersão de duas características de um organismo em crescimento, esse crescimento segue uma linha de tendência curva, contudo, se os dados forem logaritmizados a relação se torna linear. A transformação logarítmica da fórmula alométrica produz uma equação linear simples: $log(y) = log (a) + b \, log (x)$; dessa forma

a constante "*b*" que retorna a inclinação da reta e "*a*" é o intercepto, valor de "*y*" quando "*x*= 0". O tamanho relativo da característica morfológica em relação ao tamanho do corpo é constante (isométrico) quando "*b* = 1", essa condição muda para alometria positiva quando "b > 1" ou caracteriza-se por alometria negativa se "b < 1" (Huxley 1924, 1932; Huxley & Teissier 1936; Cock 1966; Gould 1966; Klingenberg 1996, 2016; Kodric-Brown *et al.*2006; Tobler & Nijhout 2010; Martino & Siena 2012).

O conhecimento atual sobre Asopinae, com relação à morfologia e sistemática, concentra-se em estudos exploratórios de genitália (Leston 1953; Pendergrast 1957; McDonald 1966; Gapud 1991), relatos de diversidade morfológica da área evaporatória (Thomas 1992, 1994) e das pernas (Thomas 1992, 1994; Barão *et al.* 2013; Brugnera *et al.* 2019), e três revisões taxonômicas do grupo, uma fornecida por Schouteden (1907) e outras duas por Thomas (1992, 1994). Além disso, uma avaliação filogenética do grupo com base em dados morfológicos e moleculares está em desenvolvimento (Roell *et al.* in prep.). Entretanto, essa subfamília ainda carece de estudos sobre morfologia comparada visando entender a variação, bem como a evolução morfológica das diferentes estruturas corporais já apontadas como caracteres diagnósticos do grupo. Neste trabalho objetivou-se estudar a morfologia do lábio de Asopinae, comparando com outros táxons de Pentatomomorpha através de morfometria linear e estudos alométricos para inferir as divergências adaptativas devido ao hábito de predação nessa subfamília.

Através de estudos em morfometria linear e alometria foi feita uma comparação do lábio de Asopinae com outros táxons inclusos em Pentatomomorpha. Foi possível observar que tanto espécies de Asopinae como não-Asopinae possuem alometria negativa em relação à razão comprimento/largura labial. Contudo, Asopinae geralmente apresentam uma forte tendência a ter um lábio relativamente mais robusto à medida que o tamanho do corpo aumenta em comparação com a mesma proporção de aumento no grupo não-Asopinae. Além disso, espécies não-Asopinae apresentam maior amplitude de comprimento labial em comparação com Asopinae. Portanto, espécies fitófagas ou onívoras apresentam maior variabilidade alométrica em relação ao tamanho corporal e razão comprimento/largura labial quando comparadas às espécies de Asopinae.

Foi feita uma reconstrução dos estados de caracteres ancestrais, com base na proporção comprimento / largura labial, além disso, um fenograma das relações entre as

espécies foi construído para esses mesmos caracteres. Foi observado que Pentamomorpha mostra uma tendência em direção a faixas de valores mais amplas em relação ao comprimento e largura labial, exceto em Asopinae, que mostram uma tendência ao lábio proporcionalmente mais robusto. O fenograma para a relação comprimento / largura labial mostra uma tendência em agrupar Asopinae, contudo, não foi observada nenhuma correspondência de agrupamentos com as filogenias ou classificações atuais.

Esses resultados indicam que, embora as pressões seletivas possam ter favorecido um lábio menos variável em Asopinae, o mesmo não ocorreu nos demais Pentatomomorpha fitófagos ou onívoros. Além disso, o agrupamento entre as espécies analisadas, Asopinae e não Asopinae parece não ser refletido por ancestralidade comum, mas por diversificação devido aos hábitos alimentares.

REFERÊNCIAS

Aldrich, J.R. & Cantelo, W.W. (1999) Suppression of Colorado potato beetle infestation by pheromone-mediated augmentation of the predatory spined soldier bug, *Podisus maculiventris* (Say) (Heteroptera: Pentatomidae). *Agricultural and Forest Entomology* 1, 209–217. https://doi.org/10.1046/j.1461-9563.1999.00026.x

Andersen, N. M. (1998) Water striders from the Paleogene of Denmark with a review of the fossil record and evolution of semiaquatic bugs (Hemiptera, Gerromorpha). *BiologiskeSkrifter* 50: 1–157.

Angelini, M. R. & Boiça-Júnior, A. L. (2009) Capacidade predatória e atratividade de *Podisus nigrispinus* (Dallas, 1851) (Hemiptera: Pentatomidae) por lagartas de *Dione junojuno* (Cramer, 1779) (Lepidoptera: Nymphalidae) criadas em folhas de genótipos de maracujazeiros. *Revista Ceres*, 56(1), 25–30.

Armer, C.A., Wiedenmann, R.N. & Bush, D.R. (1998) Plant feeding site selection on soybean by the facultatively phytophagous predator *Orius insidiosus*. *Entomologia Experimentalis et Applicata* 86, 109–118. https://doi.org/10.1023/A:1003168909489

Barão, K.R., Ferrari, A. & Grazia, J. (2013) Comparative morphology of selected characters of the Pentatomidae foreleg (Hemiptera, Heteroptera). *Arthropod Structure and Development* 42, 425–435. https://doi.org/10.1016/j.asd.2013.04.004

Bottega, D.B., Souza, B.H.S. de, Silva, A.G. da & Júnior, A.L.B. (2014) Comportamento de *Podisus nigrispinus* (Dallas) (Hemiptera: Pentatomidae) na interação com lagartas de *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) e cultivares de soja com diferentes graus de resistência. *Revista de Agricultura* 89, 53–64.

Brannen, D., Barman, E.H. & Wall, W.P. (2005) An allometric analysis of ontogenetic changes (variation) in the cranial morphology of larvae of Agabus disintegratus (Crotch) (Coleoptera: Dytiscidae). *The Coleopterists Bulletin* 59, 351–360. https://doi.org/10.1649/779.1

Brugnera, R., Barão, K.R., Roell, T. & Ferrari, A. (2019) Comparative morphology of selected foretibial traits of Asopinae (Hemiptera: Heteroptera: Pentatomidae). *Zoologischer Anzeiger* 278, 14–20.

https://doi.org/10.1016/j.jcz.2018.10.011

Cavalcanti, M. das G., Vilela, E.F., Eiras, Á.E., Zanuncio, J.C. & Picanço, M.C. (2000) Interação tritrófica entre *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae), Eucalyptus e lagartas de *Thyrinteina arnobia* (Stoll) (Lepidoptera: Geometridae): I visitação. *Anais da Sociedade Entomológica do Brasil* 29, 697–703.

https://doi.org/10.1590/s0301-80592000000400009

Claver, M.A. & Jaiswal, P. (2013) Distribution and Abundance of Two Predatory Stink Bugs (Pentatomidae: Hemiptera) Associated with Rice Field. *Academic Journal of Entomology* 6, 33–36.

https://doi.org/10.5829/idosi.aje.2013.6.1.72131

Cobben, R.H. (1978) Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies. *Med. Landbouw.,Wageningen* 78(5), 1–407.

Cock, A.G. (1966) Genetical aspects of metrical growth and form in animals. *Q Rev Biol* 41(2), 131–190.

https://doi.org/10.1086/404940

Coll, M. & Guershon, M. (2002) Omnivory in Terrestrial Arthropods: Mixing Plant and Prey Diets. *Annual Review of Entomology* 47, 267–297. https://doi.org/10.1146/annurev.ento.47.091201.145209

De Bortoli, S.A., Otuka, A.K., Vacari, A.M., Martins, M.I.E.G. & Volpe, H.X.L. (2011) Comparative biology and production costs of *Podisus nigrispinus* (Hemiptera: Pentatomidae) when fed different types of prey. *Biological Control* 58, 127–132. https://doi.org/10.1016/j.biocontrol.2011.04.011

De Clercq, P. (2000) Predaceous stinkbugs (Pentatomidae: Asopinae). *In*: Schaefer C.W.& Panizzi, A.R. (Eds),*Heteroptera of economic importance*. CRC Press, Boca Raton, pp 737–789.

De Clercq, P. (2008) Predatory Stink Bugs (Hemiptera: Pentatomidae, Asopinae). *In*: Capinera, J.L. (Ed),*Encyclopedia of insects*. 2nd Ed. Kluwer Academic Publishers, Dordrecht, pp 3042–3045.

De Clercq, P., Merlevede, F., Mestdagh, I., Vandendurpel, K., Mohaghegh, J. & Degheele, D. (1998) Predation on the tomato looper *Chrysodeixis chalcites*

(Esper) (Lep., Noctuidae) by *Podisus maculiventris* (Say) and *Podisus nigrispinus* (Dallas) (Het., Pentatomidae). *Journal of Applied Entomology* 122, 93–98.

Desurmont, G. & Weston, P.A. (2008) Predation by *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae) on Viburnum Leaf Beetle, *Pyrrhalta viburni* (Paykull) (Coleoptera: Chrysomelidae), Under Laboratory and Field Conditions. *Environmental Entomology* 37, 1241–1251.

Emlen, D.J. (1996) Artificial Selection on Horn Length-Body Size Allometry in the Horned Beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). *Evolution* 50, 1219.
https://doi.org/10.2307/2410662

Emlen, D.J. (2008) The Evolution of Animal Weapons. *Annual Review of Ecology, Evolution, and Systematics* 39, 387–413.
https://doi.org/10.1146/annurev.ecolsys.39.110707.173502

Emlen, D.J. & Nijhout, F.H. (2000) The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology* 45, 661–708.
https://doi.org/https://doi.org/10.1146/annurev.ento.45.1.661

Eubanks, M.D. & Denno, R.F. (1999) The ecological consequences of variation in plants and prey for an omnivorous insect. *Ecology* 80, 1253–1266.
https://doi.org/10.1890/0012-9658(1999)080[1253:TECOVI]2.0.CO;2

Forero, D. (2008) The systematics of the Hemiptera. *Revista Colombiana de Entomología* 34, 1–21.

Gapud, V. P. (1991) A generic revision of the subfamily Asopinae, with consideration of its phylogenetic position in the family Pentatomidae and superfamily Pentatomoidea (Hemiptera Heteroptera). *Philippines Entomology.* 8(3), 865–961.

Gould, S. J. (1966) Allometry and size in ontogeny and phylogeny. *Biol. Rev.* 41, 587–640.
https://doi.org/10.1111/j.1469-185X.1966.tb01624.x

Grazia, J. & Fernandes, J. A. M. (2012) Subordem Heteroptera Linnaeus, 1758. *In*: Rafael, J.A., Melo, G.A.R., Carvalho, C.J.K.B., Casari, A.S., Constantino, R. (Eds) *Insetos do Brasil. Diversidade e Taxonomia*. Ed. Holos, Ribeirão Preto, pp 369–405.

Grazia, J., Schuh, R.T. & Wheeler, W.C. (2008) Phylogenetic relationships of family groups in Pentatomoidea based on morphology and DNA sequences (Insecta: Heteroptera). *Cladistics* 24, 932-976. https://doi.org/10.1111/j.1096-0031.2008.00224.x

Grimaldi, D. & Engel M.S. (2005) *Evolution of the Insects*. Cambridge, UK: Cambridge University Press. 13.

Henry, T.J. (2009) Biodiversity of Heteroptera.*In*: Foottit, R., Adler, P. (Eds) *Insect Biodiversity: Science and Society, first edition.* Blackwell Publishing. pp 223-263.

Huxley, J.S. (1924) Constant differential growth-ratios and their significance. *Nature* 114, 895–896.

Huxley, J.S. (1932) *Problems of relative growth*. Johns Hopkins University Press, Baltimore.

Huxley, J.S. & Teissier G. (1936) Terminology of relative growth. *Nature* 137, 780–781.

Karolyi, F., Szucsich, N.U., Colville, J.F. & Krenn, H.W. (2012) Adaptations for nectar-feeding in the mouthparts of long-proboscid flies (Nemestrinidae: Prosoeca). *Biological Journal of the Linnean Society* 107, 414–424. https://doi.org/10.1111/j.1095-8312.2012.01945.x

Klingenberg, C.P. (1996) Multivariate allometry. *In*: Marcus, L.F., Corti, M.,Loy, A., Naylor, G.J.P., Slice, D.E. (Eds) *Advances in morphometrics*. *Vol. 284*. Plenum, New York, pp 23–49.

Klingenberg, C.P. (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. *Dev Genes Evol* 226, 113–137.

Kodric-Brown, A., Sibly, R.M. & Brown J.H. (2006) The allometry of ornaments and weapons. *PNAS*103 (23), 8733–8738. https://doi.org/10.1073/pnas.0602994103

Kment, P. & Vilímova, J. (2010) Thoracic scent efferent system of Pentatomoidea (Hemiptera: Heteroptera): a review of terminology. *Zootaxa* 2706, 1–77. http://dx.doi.org/10.11646/zootaxa.2706.1.1

Labandeira, C. (1997) Insect mouthparts: ascertaining the paleobiology of insect feeding strategies. *Annu. Rev. Ecol. Syst*. 28, 153–193. https://doi.org/10.1146/annurev.ecolsys.28.1.153

Lavine, L., Gotoh, H., Brent, C.S., Dworkin, I. & Emlen, D.J. (2015) Exaggerated Trait Growth in Insects. *Annual Review of Entomology* 60, 453–472. https://doi.org/10.1146/annurev-ento-010814-021045

Lemos, W.P., Zanuncio, J.C., Ramalho, F.S. & Serrão, J.E. (2009) Fat body of the zoophytophagous predator *Brontocoris trabidus* (Het.: Pentatomidae) females: impact of the herbivory. *Micron* 40:635–638.

Leston, D. (1953) On the wing-venation, male genitalia and spermatheca of *Podops inuncta* (F.), with a note on the diagnosis of the subfamily Podopinae Dallas (Hem., Pentatomidae). *Journal of the Society for British Entomology* 4(7), 129–135.

Li, H., Leavengood, J.M., Chapman, E.G., Burkhardt, D., Song, F., Jiang, P., Liu, J., Zhou, X. & Cai, W. (2017) Mitochondrial phylogenomics of Hemiptera reveals adaptive innovations driving the diversification of true bugs. *Proceedings of the Royal Society B: Biological Sciences* 284. https://doi.org/10.1098/rspb.2017.1223

Maddison, W.P. & Maddison, D.R. (2017) Mesquite: a modular system for evolutionary analysis. Version 3.2. Disponível em: http://mesquiteproject.org. (Acesso em 18 de novembro de 2019)

Magistrali, I.C., Costa, E.C., Machado, L.M. & Nadai, J. (2014) Novos registros de Asopinae (Pentatomidae) predadores de lagartas *Nystalea nyseus* (Cramer, 1775) (Lepidoptera: Notodontidae). *Biotemas* 27(2), 209–212.

Mahner, M. (1993) Systema cryptoceratorum phylogeneticum (Insecta, Heteroptera). *Zoologica* 143: 1–302.

Malaguido, A.B. & Panizzi, A.R. (1998) *Alcaeorrhynchus grandis* (Dallas): an eventual predator of *Chlosyne lacinia saundersii* Doubleday & Hewitson on sunflower in Northern Paraná State. *Anais da Sociedade Entomológica do Brasil* 27, 671–674.
https://doi.org/10.1590/s0301-80591998000400024

Martino, S. De & Siena, S. De. (2012) Allometry and growth: A unified view. *Physica A* 391(18), 4302–4307.
http://www.sciencedirect.com/science/article/pii/S0378437112003081

Matesco, V.C., Fürstenau, B.B.R.J., Bernardes, J.L.C., Schwertner, C.F. & Grazia, J. (2009) Morphological features of the eggs of Pentatomidae (Hemiptera: Heteroptera). *Zootaxa*, 1984, 1–30.

http://dx.doi.org/10.11646/zootaxa.1984.1.1

Matesco, V.C., Bianchi, F.M., Fürstenau, B.B.R.J., Da Silva, P.P., Campos, L.A. & Grazia, J. (2014) External egg structure of the Pentatomidae (Hemiptera: Heteroptera) and the search for characters with phylogenetic importance. *Zootaxa* 3768, 351–385.
https://doi.org/10.11646/zootaxa.3768.3.5

McDonald, F. J. D. (1966) The genitalia of North American Pentatomoidea (Hemiptera: Heteroptera). *Quaestiones Entomologicae* 2 (7)–150. 14.

Oliveira, J.E.M., Bortoli, S.A., Miranda, J.E., Torres, J.B. & Zanuncio, J.C. (2008) Predação por *Podisus nigrispinus* (Heteroptera: Pentatomidae) sob efeito da densidade de *Alabama argillacea* (Lepidoptera: Noctuidae) e idades do algodoeiro. *Científica, Jaboticabal* 36, 1 – 9.

Oliveira, J. E. M., Torres, J. B., Carrano-Moreira, A. F. & Ramalho, F. S. (2002) Biologia de *Podisus nigrispinus* predando lagartas de *Alabama argillacea* em campo. *Pesquisa Agropecuaria Brasileira* 37, 7–14.
https://doi.org/10.1590/s0100-204x2002000100002

Panizzi, R.A. & Grazia J. (2015) *True Bugs (Heteroptera) of the Neotropics*. Entomology in Focus 2. Springer.

Pendergrast, J.G. (1957) Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification. *The Transactions of the Royal Entomological Society of London* 109(1), 1–63.
https://doi.org/10.1111/j.1365-2311.1957.tb00133.x

Pires, E.M., Soares, M.A., Nogueira, R.M., Zanuncio, J.C., Moreira, P.S.A. & Oliveira, M.A. (2015) Seven decades of studies with Asopinae predators in Brazil (1930-2014). *Bioscience Journal* 31(5), 1530–1549.

Raff, R. (1996) *The Shape of Life. Genes, Development, and the Evolution of Animal Form*. University of Chicago Press, Chicago.

Reddy, G.V.P. & Kikuchi, R. (2011) Laboratory host range assessment of a predatory pentatomid, *Podisus maculiventris* (Hemiptera: Pentatomidae) for field release on Guam. *Florida Entomologist* 94, 853–858.
https://doi.org/10.1653/024.094.0419

Ribeiro, R.C., Lemos, W.P., Bernardino, A.S., Buecke, J. & Müller, A.A. (2010) Primeira ocorrência de A*lcaeorrhynchus grandis* (Dallas) (Hemiptera:

Pentatomidae) predando lagartas desfolhadoras do dendezeiro no estado do Pará. *Neotropical Entomology* 39, 131–132. https://doi.org/10.1590/s1519-566x2010000100018

Rider, D.A. (2019) Pentatomoidea Home Page. North Dakota: North Dakota State University. Disponível em: http://www.ndsu.nodak.edu/ndsu/rider/Pentatomoidea/ (Acesso em 18 de novembro de 2019)

Schouteden, H. (1907) Family Pentatomidae, subfamily Asopinae (Amyoteinae). *In.* Wystman P.(Eds.) *Genera Insectorum* 52, pp 1-81.

Schmidt-Nielsen, K. (1984) *Scaling: why is animal size so important?*Cambridge University Press, Cambridge

Schuh, R.T. & Slater, J.Á. (1995) *True bugs of the world (Hemiptera: Heteroptera): classification and natural history*. Cornell University Press, Ithaca.

Schuh, R. T. (1979) [Review of] Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies, by R. H. Cobben. *Systematic Zoology* 28: 653-656. 15.

Silva–Torres, C.S.A., Pontes, I.V.A.F., Torres, J.B., & Barros, R. (2010) New records of natural enemies of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Pernambuco, Brazil. *Neotrop. Entomol*. 39, 835-838.

Spangenberg, R., Friedemann, K., Weirauch, C. & Beutel, R. (2013) The head morphology of the potentially basal heteropteran lineages Enicocephalomorpha and Dipsocoromorpha (Insecta: Hemiptera: Heteroptera). *Arthropod Systematics & Phylogeny* 71 (2), 103-136.

Spence, R.J. & Andersen, N.M. (1994) Biology of Water Striders: Interactions Between Systematics and Ecology. *Annu. Rev. Entomol*. 39, 101-28.

Symondson, W.O.C., Sunderland, K.D. & Greenstone, M.H. (2002) Can generalist predators be effective biocontrol angents? *Annual Review of Entomology* 47, 561–594.

Thomas, D.B. (1992) *Taxonomic synopsis of the Asopinae Pentatomidae (Heteroptera) of the Western Hemisphere, Monographs 16*. The Thomas Say Foundation, ESA, Lanham, pp 1–156.

Thomas, D.B. (1994) Taxonomic synopsis of the Old World asopine genera Pentatomidae: Heteroptera). *Insecta Mundi* 8, 145–212.

Tobler, A. & Nijhout, H.F. (2010) Developmental constraints on the evolution of wing-body allometry in Manduca sexta. *Evolution and Development* 12, 592–600.

https://doi.org/10.1111/j.1525-142X.2010.00444.x.

Torres, J.B., Barros, E.M., Coelho, R.R. & Pimentel, R.M.M. (2010) Zoophytophagous pentatomids feeding on plants and implications for biological control. *Arthropod-Plant Interactions* 4, 219–227.

https://doi.org/10.1007/s11829-010-9095-2

Torres, J.B., Zanuncio, J.C. & Moura, M.A. (2006) The predatory stinkbug *Podisus nigrispinus*: Biology, ecology and augmentative releases for lepidopteran larval control in Eucalyptus forests in Brazil. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources* 1. https://doi.org/10.1079/PAVSNNR20061015

Vacari, A.M., De Bortoli, S.A., Goulart, R.M., Volpe, H.X.L., Otuka, A.K. & Veiga, A.C.P. (2013) Comparison of Eggs, Larvae, and Pupae of *Plutella xylostella* (Lepidoptera: Plutellidae) as Prey for *Podisus nigrispinus* (Hemiptera: Pentatomidae). *Annals of the Entomological Society of America* 106, 235–242. https://doi.org/10.1603/an11190

Vivan, L.M., Torres, J.B., De Souza Leão Veiga, A.F. & Zanuncio, J.C. (2002) Comportamento de predação e conversão alimentar de *Podisus nigrispinus* sobre a traça-do-tomateiro. *Pesquisa Agropecuaria Brasileira* 37, 581–587. https://doi.org/10.1590/s0100-204x2002000500002

Weirauch, C. & Schuh, R.T. (2011) Systematics and Evolution of Heteroptera: 25 Years of Progress. *Annu. Rev. Entomol.* 56, 487–510. 16.

Wheeler, W.C., Schuh, R.T. & Bang, R. (1993) Cladistic relationships among higher groups of Heteroptera: congruence between morphological and molecular data sets. *Entomol. Scand.* 24, 121–37.

Xie, Q., Tian, Y., Zheng, L. & Bu, W. (2008) 18S rRNA hyper-elongation and the phylogeny of Euhemiptera (Insecta: Hemiptera). *Molecular Phylogenetics and Evolution* 47, 463–471.

https://doi.org/10.1016/j.ympev.2008.01.024

Zanuncio, J.C., Alves, J.B., Zanuncio, T.V. & Garcia, J.F. (1994) Hemipterous predators of eucalypt defoliator caterpillars. *Forest Ecology and Management*, 65, 65–73.

Zanuncio, J.C., Ferreira, A. M. R. M, Tavares, W. S., Torres, J. B., Serrão, J. E. & Zanuncio, T. V. (2011) Rearing the predator *Brontocoris tabidus* (Heteroptera: Pentatomidae) with *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupa on *Eucalyptus grandis* in the field. *American Journal of Plant Sciences* 2, 449–465. https://doi.org/10.4236/ajps.2011.23052

Zibaee, A., Hoda, H. & Fazeli-Dinan, M. (2012) Role of proteases in extra-oral digestion of a predatory bug, *Andrallus spinidens*. *Journal of Insect Science* 12(51), 1–17. https://doi.org/10.1673/031.012.5101

Capítulo 1

A PREDATOR'S FACE: ALLOMETRY OF THE ASOPINAE LABIUM (HEMIPTERA: PENTATOMIDAE)

A PREDATOR'S FACE: ALLOMETRY OF THE ASOPINAE LABIUM (HEMIPTERA: PENTATOMIDAE)

Abstract

Asopinae includes the predatory stink bugs pertaining to the otherwise phytophagous Pentatomidae. The asopines present peculiar characteristics, considered diagnostic to differentiate them from other pentatomids such as, the apparently rectangular head in dorsal view, and a markedly robust labium, inserted very close to the base of labrum. Despite the wide economic potential, due to the interest of these organisms to biological control programs, the subfamily still lacks studies mainly of comparative morphology of structures adapted to predation. Through linear morphometry and allometric studies, a comparison of the labium between Asopinae and other Pentatomomorpha was made; in addition a reconstruction of ancestral character states was performed based on the labial length / width ratio, along with a phenogram of the relationships between species for the same character. It was observed that both Asopinae and non-Asopinae species have negative allometry with respect to labial length / width ratio, however, Asopinae generally have a strong tendency to have a relatively more robust labium as body size increases compared to the same proportion of increase in the non-Asopinae group. These morphological proportions tend to maintain a cluster of Asopinae, however no groupings reflected by common ancestry were found in the phenogram for both Asopinae and non-Asopinae. Selective pressures for both predators and phytophagous / omnivores seem to be driven by diversification due to feeding habits.

KEYWORDS: stinkbug, morphology, mouthparts, phenogram, phylogeny.

Introduction

The hemipterans have adaptations to feed on fluids, observed externally as modifications of the different mouthpart appendages into a segmented piercing-sucking apparatus, associated with saliva and food pumping systems. This specialized feeding apparatus, known as rostrum, is a synapomorphy of the order (Weirauch & Schuh 2011). The rostrum is formed by a three- or four-segmented tubular labium that houses mandibles and maxillae modified into long and flexible tubular stylets (Schuh & Slater 1995; Grimaldi & Engel 2005; Grazia & Fernandes 2012). The suborder Heteroptera presents the highest feeding strategies diversity among the Hemiptera, with groups specialized in feeding on plants, fungi, arthropods, small vertebrates, and blood (Schuh & Slater 1995). Heteroptera comprises seven infraorders: Enicocephalomorpha, Dipsocoromorpha, Gerromorpha, Nepomorpha, Leptopodomorpha, Cimicomorpha, and Pentatomomorpha. This classification was proposed by Schuh (1979) based mainly in Cobben (1978) from studies on evolutionary trends regarding the morphology of the mouthparts, and life and feeding habits (Weirauch & Schuh 2011; Grazia & Fernandes 2012; Panizzi & Grazia 2015).

Almost all the groups that are considered basal in Heteroptera present predatory habits (Schuh & Slater 1995; Grimaldi & Engel 2005; Li *et al*. 2017). The transition from predation to phytophagy probably occurred in the common ancestor of Cimicomorpha and Pentatomomorpha (Grimaldi & Engel 2005; Weirauch & Schuh 2011; Panizzi & Grazia 2015; Li *et al*. 2017), with reversions to predation in some families within the two infraorders (Li *et al*. 2017). Pentatomidae, one of the most diverse families in Pentatomomorpha, has most of its subgroups sharing the ancestral infraorder characteristic of phytophagy, with the exception of the subfamily Asopinae whose species have predatory habits.

The Asopinae comprise 64 genera and about 300 species, occurring in all biogeographical regions (Thomas 1992, 1994; De Clercq 2000; Rider 2019). In addition to their general insectivorous habits, feeding on insects of different orders, some of these stink bugs may present zoophytophagy, using plant fluids as sources of nutrients or amino acids not found in their preys, or to stay healthy when preys are scarce, causing no great damage to plant tissues (Armer *et al*. 1998; Eubanks & Denno 1999;

Coll & Guershon 2002; Lemos *et al.* 2009; Torres *et al.* 2010). Species in Asopinae are of great interest and have broad economic potential because of their role in biological control programs and management of pests in cultivated plants on a global scale (Malaguido & Panizzi 1998; De Clercq 2000, 2008; Pires *et al.* 2015).

As with other pentatomids, the Asopinae have moderate to large size ovoid body ranging in length from 7 to 25 mm (De Clercq 2008). However, possibly due to the predatory habits, they have a peculiar morphology of the head and legs, and these characteristics are diagnostic to differentiate them from other Pentatomidae. The most remarkable characteristic of asopines is the markedly robust and not particularly long, labium, inserted very close to the base of labrum, and capable of full extension forward allowing them to feed on active preys (Gapud 1991; Thomas 1992, 1994; De Clercq 2000, 2008).

Biological diversity is dominated by differences in relative body structure sizes and anatomical differences between species sharing a body plane (Raff 1996). Variation in size is an important determinant of evolutionary diversification processes since many morphological features are subject to natural or sexual selection. Insects are a good example of morphological diversification, they have considerable phenotypic variations both intraspecific and interspecific, such as the different horn lengths of male beetles *Onthophagus acuminatus* (Emlen 1996) or the long proboscis of flies belonging to Nemestrinidae (Karolyi *et al.* 2012), these adaptations may be related to various factors such as locomotion, feeding (prey capture or handling), reproduction competition or even defense (Emlen & Nijhout 2000; Lavine *et al.* 2015). These features have often been subject of allometric growth studies to help understand their evolutionary adaptations (Cock 1966; Gould 1966; Huxley 1924, 1932; Schmidt-Nielsen 1984; Emlen 2008). Moreira *et al.* (2017) showed that the prothoracic legs in heliconian butterflies decreased proportionally in size over evolutionary time, with the evolution of this structure based on losses and fusions of podites, in association with negative size allometry. Brannen *et al.* (2005) through allometric studies compared the cranial morphology of the first, second and third instars of *Agabus disintegratus* showing significant ontogenetic changes in cranial architecture, evidencing the ability of these larvae to explore prey with different characteristics.

Allometry can be defined as disproportionate growth rate between body structures of an organism (Huxley, 1924, 1932). This growth rate may vary between individuals in the same population and age group, known as static allometry, along the development time, called ontogenetic allometry or be classified as evolutionary allometry when variation occurs along the branch of a phylogeny or between individuals who share a common ancestor (Klingenberg, 1996). An allometric relationships, that is, the relationship between a body structure and the relative size of an organism, is traditionally modeled by the equation: $y = bx^a$; where "x" and "y" are the dimensions of two structures, "a" and "b" are constants. The logarithmic transformation of the allometric formula produces a simple linear equation: $log(y) = log\ (a) + b\ log\ (x)$; thus the constant "b" that returns the slope of the line and "a" is the intercept, value of "y" when "x = 0" (Huxley 1932). The relative size of the morphological feature relative to body size is constant (isometric) when "b = 1", this condition changes to positive allometry when "b> 1" or is characterized by negative allometry if "b <1" (Cock, 1966; Gould, 1966; Huxley, 1924, 1932; Huxley & Teissier, 1936; Klingenberg, 1996, 2016; Kodric-Brown *et al.,* 2006 ; Tobler & Nijhout, 2010; Martino & Siena, 2012).

The current knowledge about Asopinae, with respect to morphology and systematics, focus on exploratory studies of their genitalia (Gapud 1991; Leston 1953; McDonald 1966; Pendergrast 1957), reports on the morphological diversity of evaporatorium (Thomas 1992, 1994) and legs (Thomas 1992, 1994; Barão *et al.* 2013; Brugnera *et al.* 2019), and three taxonomic revisions (Schouteden 1907; Thomas 1992, 1994). However, this subfamily still lacks studies on comparative morphology in order to understand the variation, as well as the morphological evolution of the different body structures already identified as diagnostic characters to the group. Thus, this work aimed to perform a linear morphometry and allometric evaluation of the labium in Asopinae to help understand the adaptive divergences due to predation habits in this subfamily. We hypothesised that: (i) feeding habits influence the morphology in relation to labial length and width in Pentatomomorpha; (ii) the proportion the labial length/width ratio of predatory species is greater than the same proportion in phytophagous or omnivorous species; (ii) the labial length/width ratio will show positive allometric growth in Asopinae.

Material and methods

We used specimens from dry collections belonging to the following institutions. Acronyms according to Evenhuis (2019): American Museum of Natural History (AMNH – USA); Australian Museum (MAS – Australia); Cleveland Museum of Natural History (CLEV – USA); Coleção de Entomologia Sistemática do Instituto de Pesquisas da Amazônia (INPA – Brazil); Departamento de Zoologia da Universidade Federal do Rio Grande do Sul (UFRG – Brazil); Illinois Natural History Survey (INHS – USA); John E. EgerCollection (JEE – USA); Milwaukee Public Museum (MPM – USA); Museu Nacional do Rio de Janeiro (MNRJ – Brazil); Museé Zoologique (MZLS – Switzerland); National Museum of Natural History (NMPC – Czech Republic); Staten Island Museum (SIM – USA); Swedish Museum of Natural History (SMNH – Sweden); University of California, Entomology Research Museum (UCRC – USA); University of Copenhagen (ZMUC – Denmark); University of Minnesota (UMSP – USA); Virginia Museum of Natural History (VMNH – USA); Museu de Zoologia da Universidade de São Paulo (MZUSP– Brazil); and Coleção de Hemiptera do Museu de Zoologia da Universidade Estadual de Campinas (ZUEC– Brazil). Dichotomous keys were used for specimen identification at the lowest possible taxonomic level when necessary (Carvalho & Ferreira 1995; Froeschner 1981; Panizzi & Grazia 2015; Schuh & Slater 1995).

A total of 28 species from the following families of Pentatomomorpha were measured: Coreidae, Rhyparochromidae, Alydidae, Dinidoridae, Pyrrhocoridae, Geocoridae, Rhopalidae, Acanthosomatidae, Berytidae, Largidae, Lygaidae, Scutelleridae, Cydnidae, Tessaratomidae and Pentatomidae. We also measured one Miridae species (Cimicomorpha) (Table 1). For each of these families a single species was chosen as representative, except for Pentatomidae for whom we selected one species of Pentatominae and 13 species of Asopinae (Table 1).

Morphological measurements of the length and width of the labial segments as well as the mesofemur length (n=10/sex/species) were obtained. The length of each labial segment was measured in ventral view or in lateral view when necessary, and the maximum anterior and posterior width measures were taken in ventral view (Figure 1). Mesofemur has little inter-specific morphological variation, so this appendix was obtained as a standard size measurement for comparison where its largest length was

measured in dorsal view (Figure 1). All measurements were made on adult specimens using a stereomicroscope with a graduated reticulum. For the measurements of the labial segments we calculated the mean of the total length for each segment, for the width of each labial segment we calculated the means for each anterior and posterior widths. For the measurements of the mesofemur we calculated the mean of its dorsal length.

Linear measurements were transformed by the formula (ln N)+4 prior to the regression analyses in order to avoid negative numbers in the results. We then applied a linear regression where the labial length/ width ratio is the dependent variable and the median femur length is the independent covariable. Due to the differences in size often found in males and females in Pentatomidae, both sexes were individually analyzed for labial length / width ratio, and then compared to each other (n=10/sex/species), then both sexes were combined for further analysis. In order to verify differences between Asopinae and other taxa, the means of each Asopinae species were used, then these measurements were combined into a single group, similarly the means of the non-Asopinae species were grouped, and this nomenclature (Asopinae and non-Asopinae) was adopted throughout the work. The mean values of both groups were compared using the T-test. Slopes and intercepts were compared with covariance analysis (one-way ANCOVA). All comparisons were made using GraphPad Prism® software (GraphPad Software 5, San Diego, CA, USA).

In order to infer the evolutionary changes of the labium throughout the phylogenetic history in Pentatomomorpha, we performed an ancestral character states reconstruction on a molecular phylogeny. A total of 20 Pentatomomorpha taxa were sampled, plus one species of Cimicomorpha used to root the tree (Table 2). The sequences used in the molecular analysis were retrieved from Genbank. To generate our dataset we followed Weirauch et al (2018), who suggested the three ribosomal markers consistently used within Heteroptera, i.e., 16S rDNA, 18S rDNA and 28S rDNA, have been show to resolve phylogenies at high taxonomic level within the suborder. We additionally selected the mitochondrial marker cytochrome oxidase subunit 1 (COI) (Li *et al.* 2012). The families are represented by a single selected species, except for the Pentatomidae due to our focus in Asopinae. Due to the lack of molecular data for the species selected for morphology studies, we assumed the family monophyly and species with pre-existing GenBank data were selected to represent previously used families. The

species were selected intending to avoid including missing data in the taxon-character matrix (Table 2).

Alignments of the sequences were performed using Mafft 7 (Katoh et al. 2017), online version (http://mafft.cbrc.jp/alignment/server/index.html), applying the strategy "Auto". The appropriate substitution model was chosen for each molecular marker using jModelTest v2.1.6 (Darriba et al., 2012; Guindon and Gascuel, 2003) via the Bayesian information criterion (BIC) as suggested by Luo et al. (2010), also implemented in jModeltest 2.1.5. Alignments were concatenated in a single matrix using SequenceMatrix 1.8 (Vaidya *et al.*, 2011), recoding external gaps as question marks, and the final matrix was exported in both Phylip for RAxML analyses on CIPRES (see below).

Maximum-Likelihood analysis was performed on the matrix using RAxML-HPC2 at CIPRES Science Gateway (Miller et al., 2012) (www.phylo.org/portal2/). The GTR-CAT model optimizes site-specific evolutionary rates. Nodal support was assessed with automatic Stop Bootstrapping Automatically with Majority Rule Criterion (autoMRE).

For the ancestral character states reconstruction, the character labial length / width ratio was estimated by Maximum-likelihood as a continuous character assuming a Brownian motion pattern of evolution along the tree. For the cluster analysis, a phenogram of relationships between species was constructed for the characters labial length / width ratio and femur length based on Mahalanobis distances by the unweighted pair group mean (UPGMA) method. Reconstructions were performed usingR® software (R Foundation for Statistical Computing, Vienna, Austria) using "ape" (Paradis & Schliep 2018), "phylogram" (Wilkinson &Davy 2018), "phytools" (Revell 2012) and"vegan: Community Ecology" (Oksanen *et al.* 2013) packages.

Results and discussion

Sexual size dimorphism is reported in many arthropods, and such dimorphism may be related to the differential phenotypic plasticity between the sexes and the adaptations of both sexes in their different roles in sexual selection (Fairbairn, 1997;

Stillwell *et al*. 2010). The comparison of male and female allometric coefficient analyses did not show significant differences between slopes of males and females of the same species (*P*>0.05), except for *Oplomus catena* (Table S1).

Asopinae is known for some remarkable morphological characteristics that differ from other stinkbugs, such as the labium markedly robust and not particularly long, never exceeding the abdominal base (De Clercq 2000, 2008; Thomas 1992, 1994). An allometry test for linear measurements showed isometry between the total labial length and mesofemur length both for Asopinae (F_1= 0.8971, P = 0.354) and non-Asopinae (F_1= 0.0005, *P*= 0.981) (Table 3). In the same way we found isometry between the labial width and mesofemur length for both groups (F_1= 1.0344, P = 0.320 for Asopinae and F_1 = 1.8377, P = 0.186 for non-Asopinae) (Table 3). A comparison between the slopes of the two groups showed no significant difference for both variables (F_1< 0.001, P = 0.9973 for length and F_1 = 1.36698, *P*= 0.2543 for width). A T-test for the two variables (total length and average labial width) revealed the means of both characteristics are significantly different between Asopinae and non-Asopinae (*P*<0.05) (Table 4). We observed that the larger species in Asopinae have relatively wider labium compared to a more variable non-Asopinae, and the later have greater amplitude of labial length compared to Asopinae (Figure 2). Therefore, phytophagous or omnivorous species present greater variability in relation to body size and labial length and labial width when compared to Asopinae species (Figure 2).

The relation between the labial length / width ratio and femur resulted negative allometry in all analyzed species (*P*<0.005), except for *Geocoris* sp., *Jalysus* sp., and *Pycnoderes* sp. due to the presence of outliers (Table 5). These three species also resulted negative allometric after the removal of outliers (Table 5). When the species are grouped into Asopinae and non-Asopinae and compared to each other in relation to labial length / width ratio, the differences between the slopes are not significant (F_1 = 1.03949, P = 0,3177), but the difference between intercepts are significant (*P*<0.05). The T-test showed significant differences between the means of the two groups (*P*< 0.05) (Table 4). A negative inclination of the slope was observed in the Asopinae, showing a low ratio of labial length / width (Figure 2). We observed that Asopinae usually present a strong tendency to have a relatively more robust labium as body size increases compared to the same proportion of increase in the non-Asopinae group (Figure 2). Phytophagous species included in Pentatomomorpha and Cimicomorpha

(like other Hemiptera) feed on plant material using their mouthparts to puncture tissue and suck leaf cell or seed contents or vascular fluids, these specimens generally find little or no plant resistance beyond the rigidity of the cell wall (Schaefer & Panizzi 2000). Asopinae, different from other taxons in Pentatomorpha, has broad and free labium, inserted very close to the base of the labrum, and capable of full extension forward (De Clercq 2000, 2008; Thomas 1992, 1994). The predatory behavior in Asopinae consists to approach the prey with the rostrum extended, then attack by inserting the stylets in the prey, however different prey types can make abrupt movements in defense against the predator's attack (Lemos *et al*. 2005; Silva *et al*. 2012). The increase in the caliber of the mouthparts may reflect adaptations to this predation behavior, giving greater support to resist the impact caused by the capture of live prey.

The maximum likelihood phylogenetic hypothesis that we infer recovered relationships between families representatives similar to those found in the literature (Li *et al*. 2017; Weirauch *et al*. 2018) (Figure S1). The ancestral character states reconstruction recovered a phylogeneticaly structured evolution of the labium, considered here as the character of labial length / width ratio. The lowest trait values were observed in the outgroup (Miridae), intermediate values were found in the Pentatomoidea clade, whereas the Coreidoea, Pyrrhocoroidea, and Lygaeoidea clade tend to high values (Figure 3). The Pentamomorpha show a trend towards wider value ranges in relation to both the length and width of the labium, except for the Asopinae which show a trend to proportionally wider (low values) labium (Figure 3). Low values in the labial trait were also observed in Dinidoridae and Tessaratomidae, showing a particular variation in the labial length / width ratio in these families due to the relatively short rostrum of their species.

The phenogram for the characters labial length / width ratio and femur shows a trend towards grouping the Asopinae (Figure 4, cluster A). However, two non-Asopinae species clustered in this group: *Dinidor mactabilis* (Dinidoridae) and *Piezosternum subulatum* (Tessaratomidae). These species have a very short labium compared to other species of similar sizes (Table 1). Therefore, it is likely that the grouping of these species with Asopinae is due to the proportions resulted from short rather than wide labium. Two species of Asopinae clustered outside the largest Asopinae grouping: *Discocera coccinea* and *Euthyrhynchus floridanus* (Figure 4, cluster B). It is possible

this morphological distance is because these species have relatively narrow labium compared to other asopine species of similar sizes. The remainder species clustered without any correspondence with current phylogenies or classification.

These results indicate that while selective pressures may have acted favoring a less variable labium in Asopinae when we analyze the labial length/width ratio, the same have not occurred in the remainder phytophagous or omnivorous pentatomomorphans. The clustering between the species analyzed, both Asopinae and non-Asopinae, seems to be reflected by diversification due to feeding habits. Species included in Pentatomomorpha are generally recognized by phytophagous habits, apart from some reports of predation tendency in Berytidae and Geocoridae, and the Asopinae (Pentatomidae) with obligatory predatory habits (Panizzi & Grazia 2015). The phytophagous species have great morphological variation in relation to the proportions of the labium, a characteristic probably related to particularities in their main food that can be different plant groups and parts. As examples we can mention species included in Acanthosomatidae that feed on young tissues and reproductive parts of the host plant. (Schaefer & Ahmad 1987). Dinidoridae can feed on both reproductive and vegetative parts (Schaefer & Panizzi 2000; Panizzi & Grazia 2015). Pyrrhocoridae usually feed on mature seed or seed secretions (Schaefer & Panizzi 2000; Panizzi & Grazia 2015). Members of the Scutelleridae family can feed on stems, leaves, flowers and fruits of the host plant (Panizzi & Grazia 2015). Although they feed on different plant parts, sucking insects find little or no resistance to the plant, as these organisms often have external defense against predators (such as spine or trichomes), so these insects can easily access plant nutrients without major impacts to their mouthparts (Schaefer & Panizzi 2000). The Asopinae, in addition to the predatory habit, have different morphological features and behaviors when compared to other pentatomomorphans. The behavior of approaching the prey with the rostrum extended forward, and after prey fixation, the resistance against abrupt movements of the prey with the rostrum still extended (Lemos *et al*. 2005; Silva *et al*. 2012), may reflect a greater energy investment in the mouthparts, as a result of greater robustness. Therefore, the increase in caliber of the Asopinae mouthparts could be related to the specialization in feeding habits, whereas these stinkbugs have the generalist isetivore predatory habit, feeding on insects of several orders, exclusive behavior for this subfamily included in Pentatomidae.

Supporting Information

Appendix 1. Table S1. Comparison of male and female allometric coefficient for the labial length / width ratio when regressed on the femur for different species of Pentatomomorpha and selected outgroup (*Pycnoderes* sp., Cimicomorpha). Values in red indicate statistical significance (one-way ANCOVA, alpha= 0.05).

Appendix 2. Figure S1. Maximum likelihood tree from the RAxML analysis of molecular dataset.

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References

Normas editoriais: *Zootaxa*

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Armer, C.A., Wiedenmann, R.N. & Bush, D.R. (1998) Plant feeding site selection on soybean by the facultatively phytophagous predator *Orius insidiosus*. *Entomologia Experimentalis et Applicata* 86, 109–118. https://doi.org/10.1023/A:1003168909489

Barão, K.R., Ferrari, A. & Grazia, J. (2013) Comparative morphology of selected characters of the Pentatomidae foreleg (Hemiptera, Heteroptera). *Arthropod Structure and Development* 42, 425–435. https://doi.org/10.1016/j.asd.2013.04.004

Brannen, D., Barman, E.H. & Wall, W.P. (2005) An allometric analysis of ontogenetic changes (variation) in the cranial morphology of larvae of Agabus disintegratus (Crotch) (Coleoptera: Dytiscidae). *The Coleopterists Bulletin* 59, 351–360.

https://doi.org/10.1649/779.1

Brugnera, R., Barão, K.R., Roell, T. & Ferrari, A. (2019) Comparative morphology of selected foretibial traits of Asopinae (Hemiptera: Heteroptera: Pentatomidae). *Zoologischer Anzeiger* 278, 14–20.
https://doi.org/10.1016/j.jcz.2018.10.011

Carvalho, J.C.M, & Ferreira, P.S.F. (1995). Mirídeos Neotropicais, CCCXC: chave para os gêneros Neotropicais de Bryocorinae Baerensprung, 1860 (Heteroptera). *Revista Ceres* 42(243), 469-496.

Cobben, R.H. (1978) Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies. *Med. Landbouw.,Wageningen* 78(5), 1–407.

Cock, A.G. (1966) Genetical aspects of metrical growth and form in animals. *Q Rev Biol* 41(2), 131–190.

Coll, M. & Guershon, M. (2002) Omnivory in Terrestrial Arthropods: Mixing Plant and Prey Diets. *Annual Review of Entomology* 47, 267–297.
https://doi.org/10.1146/annurev.ento.47.091201.145209

Darriba, D., Taboada, G.L., Doallo, R., & Posada, D. (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nature methods*, *9*(8), 772.

De Clercq, P. (2000) Predaceous stinkbugs (Pentatomidae: Asopinae). *In*: Schaefer C.W. & Panizzi, A.R. (Eds), *Heteroptera of economic importance*. CRC Press, Boca Raton, pp 737–789.

De Clercq, P. (2008) Predatory Stink Bugs (Hemiptera: Pentatomidae, Asopinae). *In*: Capinera, J.L. (Ed), *Encyclopedia of insects*. 2nd Ed. Kluwer Academic Publishers, Dordrecht, pp 3042–3045.

Emlen, D.J. (1996) Artificial Selection on Horn Length-Body Size Allometry in the Horned Beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). *Evolution* 50, 1219.
https://doi.org/10.2307/2410662

Emlen, D.J. (2008) The Evolution of Animal Weapons. *Annual Review of Ecology, Evolution, and Systematics* 39, 387–413.
https://doi.org/10.1146/annurev.ecolsys.39.110707.173502

Emlen, D.J. & Nijhout, F.H. (2000) The development and evolution of exaggerated morphologies in insects. *Annual Review of Entomology* 45, 661–708.
https://doi.org/https://doi.org/10.1146/annurev.ento.45.1.661

Eubanks, M.D. & Denno, R.F. (1999) The ecological consequences of variation in plants and prey for an omnivorous insect. *Ecology* 80, 1253–1266. https://doi.org/10.1890/0012-9658(1999)080[1253:TECOVI]2.0.CO;2

Evenhuis, N.L. (2019) *Abbreviations for insect and spider collections of the world*. Available at: http://hbs.bishopmuseum.org/codens/codens-inst.html (accessed 18November 2019)

Fairbairn, D.J. (1997) Allometry For Sexual Size Dimorphism: Pattern and Processin the Coevolution of Body Sizein Males and Females. *Annu. Rev. Ecol. Syst. 28, 659–87.*

Froeschner, R.C. (1981) Heteroptera or true bugs of Ecuador. *Smithsonian contributions to zoology*, no. 322

Gapud, V. P. (1991) A generic revision of the subfamily Asopinae, with consideration of its phylogenetic position in the family Pentatomidae and superfamily Pentatomoidea (Hemiptera Heteroptera). *Philippines Entomology.* 8(3), 865–961.

Gould, S. J. (1966) Allometry and size in ontogeny and phylogeny. *Biol. Rev.* 41, 587–640. https://doi.org/10.1111/j.1469-185X.1966.tb01624.x

Grazia, J. & Fernandes, J. A. M. (2012) Subordem Heteroptera Linnaeus, 1758. *In*: Rafael, J.A., Melo, G.A.R., Carvalho, C.J.K.B., Casari, A.S., Constantino, R. (Eds) *Insetos do Brasil. Diversidade e Taxonomia*. Ed. Holos, Ribeirão Preto, pp 369–405.

Grimaldi, D. & Engel M.S. (2005) *Evolution of the Insects*. Cambridge, UK: Cambridge University Press. 13.

Guindon, S., & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic biology*, *52*(5), 696-704.

Huxley, J.S. (1924) Constant differential growth-ratios and their significance. *Nature* 114, 895–896.

Huxley, J.S. (1932) *Problems of relative growth*. Johns Hopkins University Press, Baltimore.

Huxley, J.S. & Teissier G. (1936) Terminology of relative growth. *Nature* 137, 780–781.

Karolyi, F., Szucsich, N.U., Colville, J.F. & Krenn, H.W. (2012) Adaptations for nectar-feeding in the mouthparts of long-proboscid flies (Nemestrinidae: Prosoeca).

Biological Journal of the Linnean Society 107, 414–424. https://doi.org/10.1111/j.1095-8312.2012.01945.x

Katoh, K., Rozewicki, J., & Yamada, K.D. (2017) MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings in bioinformatics. bbx108.

https://doi.org/10.1093/bib/bbx108.

Klingenberg, C.P. (1996) Multivariate allometry. *In*: Marcus, L.F., Corti, M., Loy, A., Naylor, G.J.P., Slice, D.E. (Eds) *Advances in morphometrics*. *Vol. 284*. Plenum, New York, pp 23–49.

Klingenberg, C.P. (2016) Size, shape, and form: concepts of allometry in geometric morphometrics. *Dev Genes Evol* 226, 113–137.

Kodric-Brown, A., Sibly, R.M. & Brown J.H. (2006) The allometry of ornaments and weapons. *PNAS* 103 (23), 8733–8738.

https://doi.org/10.1073/pnas.0602994103

Lavine, L., Gotoh, H., Brent, C.S., Dworkin, I. & Emlen, D.J. (2015) Exaggerated Trait Growth in Insects. *Annual Review of Entomology* 60, 453–472. https://doi.org/10.1146/annurev-ento-010814-021045

Lemos, W.P., Zanuncio, J.C., Ramalho, F.S. & Serrão, J.E. (2009) Fat body of the zoophytophagous predator *Brontocoris trabidus* (Het.: Pentatomidae) females: impact of the herbivory. *Micron* 40:635–638.

Lemos, W.P, Zanuncio, J.C. & Serrão, J.E. (2005) Attack Behavior of *Podisus rostralis* (Heteroptera: Pentatomidade) Adults on Caterpillars of *Bombyx mori* (Lepidoptera: Bombycidae). *Brazilian Archives of Biology and Technology*. 48(6), 975-981.

Leston, D. (1953) On the wing-venation, male genitalia and spermatheca of *Podops inuncta* (F.), with a note on the diagnosis of the subfamily Podopinae Dallas (Hem., Pentatomidae). *Journal of the Society for British Entomology* 4(7), 129–135.

Li, H., Leavengood, J.M., Chapman, E.G., Burkhardt, D., Song, F., Jiang, P., Liu, J., Zhou, X. & Cai, W. (2017) Mitochondrial phylogenomics of Hemiptera reveals adaptive innovations driving the diversification of true bugs. *Proceedings of the Royal Society B: Biological Sciences* 284. https://doi.org/10.1098/rspb.2017.1223

Li, M., Tian, Y., Zhao, Y. & Bu, W. (2012) Higher level phylogeny and the first divergence time estimation of heteroptera (insecta: Hemiptera) based on multiple genes. *PLoS ONE* 7.

https://doi.org/10.1371/journal.pone.0032152

Luo, A., Qiao, H., Zhang, Y., Shi, W., Ho, S. Y., Xu, W., Zhang, A. & Zhu, C. (2010) Performace of criteria for selecting evolutionary models in Phylogenetics: a comprehensive study based on simulated datasets. *BMC Evolutionary Biology*, 10,1.

Malaguido, A.B. & Panizzi, A.R. (1998) *Alcaeorrhynchus grandis* (Dallas): an eventual predator of *Chlosyne lacinia saundersii* Doubleday & Hewitson on sunflower in Northern Paraná State. *Anais da Sociedade Entomológica do Brasil* 27, 671–674.

https://doi.org/10.1590/s0301-80591998000400024

Martino, S. De & Siena, S. De. (2012) Allometry and growth: A unified view. *Physica A* 391(18), 4302–4307.

http://www.sciencedirect.com/science/article/pii/S0378437112003081

McDonald, F. J. D. (1966) The genitalia of North American Pentatomoidea (Hemiptera: Heteroptera). *Quaestiones Entomologicae* 2 (7)–150. 14.

Miller, M. A., Pfeiffer, W. & Schwartz, T. (2012) The CIPRES science gateway: enabling high-impact science for phylogenetics researchers with limited resources. In *Proceedings of the 1st Conference of the Extreme Science and Engineering Discovery Environment: Bridging from the eXtreme to the campus and beyond* (p. 39). ACM.

Moreira, G.R.P., Silva, D.S. & Gonçalves, G.L. (2017) Comparative morphology of the prothoracic leg in heliconian butterflies: Tracing size allometry, podite fusions and losses in ontogeny and phylogeny. *Arthropod Structure and Development* 46, 462–471.

https://doi.org/10.1016/j.asd.2017.03.008

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, R., McGlinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens,H., Szoecs, E., & Wagner, H. (2019). Vegan: Community Ecology Package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan

Panizzi, R.A. & Grazia J. (2015) *True Bugs (Heteroptera) of the Neotropics*. Entomology in Focus 2. Springer.

Paradis, E. & Schliep, K. (2018) ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526-528. *Bioinformatics* 35, 526–528. https://doi.org/10.1093/bioinformatics/bty633/5055127

Pendergrast, J.G. (1957) Studies on the reproductive organs of the Heteroptera with a consideration of their bearing on classification. *The Transactions of the Royal Entomological Society of London* 109(1), 1–63. https://doi.org/10.1111/j.1365-2311.1957.tb00133.x

Pires, E.M., Soares, M.A., Nogueira, R.M., Zanuncio, J.C., Moreira, P.S.A. & Oliveira, M.A. (2015) Seven decades of studies with Asopinae predators in Brazil (1930-2014). *Bioscience Journal* 31(5), 1530–1549.

Raff, R. (1996) *The Shape of Life. Genes, Development, and the Evolution ofAnimal Form*. University of Chicago Press, Chicago.

Revell, L.J. (2012) phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* 3, 217–223. https://doi.org/10.1111/j.2041-210X.2011.00169.x

Rider, D.A. (2019) Pentatomoidea Home Page. North Dakota: North Dakota State University. Available from: http://www.ndsu.nodak.edu/ndsu/rider/Pentatomoidea/ (accessed 18November 2019)

Schaefer, C.W., Ahmad, I. (1987) The food plants of four pentatomoid families (Hemiptera:Acanthosomatidae, Tessaratomidae, Urostylidae, and Dinidoridae). *Phytophaga* 1, 21–34.

Schaefer C.W., Panizzi, A.R. (2000) *Heteroptera of economic importance*. CRC Press, Boca Raton.

Schouteden, H. (1907) Family Pentatomidae, subfamily Asopinae (Amyoteinae). *In.* Wystman P.(Eds.) *Genera Insectorum* 52, pp 1-81.

Schmidt-Nielsen, K. (1984) *Scaling: why is animal size so important?* Cambridge University Press, Cambridge

Schuh, R.T. & Slater, J.Á. (1995) *True bugs of the world (Hemiptera: Heteroptera): classification and natural history*. Cornell University Press, Ithaca.

Schuh, R. T. (1979) [Review of] Evolutionary trends in Heteroptera. Part II. Mouthpart-structures and feeding strategies, by R. H. Cobben. *Systematic Zoology* 28: 653-656. 15.

Silva, R.B., Corrêa, A.S., Lucia, T.M.C.D., Pereira, A.I.A., Cruz, I. & Zanuncio, J.C. (2012) Does the aggressiveness of the prey modify the attack behavior of the predator *Supputius cincticeps* (Stål) (Hemiptera, Pentatomidae)?*Revista Brasileira de Entomologia* 56(2), 244–248.

Stillwell, R.C., Blanckenhorn, W.U., Teder, T., Davidowitz, G. & Fox, C.W. (2010) Sex Differences in Phenotypic Plasticity Affect Variationin Sexual Size Dimorphismin Insects: From Physiologyto Evolution. *Annu. Rev. Entomol.* 55, 227–45.

Thomas, D.B. (1992) *Taxonomic synopsis of the Asopinae Pentatomidae (Heteroptera) of the Western Hemisphere, Monographs 16*. The Thomas Say Foundation, ESA, Lanham, pp 1–156.

Thomas, D.B. (1994) Taxonomic synopsis of the Old World asopine genera Pentatomidae: Heteroptera). *Insecta Mundi* 8, 145–212.

Tobler, A. & Nijhout, H.F. (2010) Developmental constraints on the evolution of wing-body allometry in Manduca sexta. *Evolution and Development* 12, 592–600. https://doi.org/10.1111/j.1525-142X.2010.00444.x.

Torres, J.B., Barros, E.M., Coelho, R.R. & Pimentel, R.M.M. (2010) Zoophytophagous pentatomids feeding on plants and implications for biological control. *Arthropod-Plant Interactions* 4, 219–227. https://doi.org/10.1007/s11829-010-9095-2

Vaidya, G., Lohman, D.J., & Meier, R. (2011) Sequence Matrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics*, *27*(2), 171-180.

Weirauch, C. & Schuh, R.T. (2011) Systematics and Evolution of Heteroptera: 25 Years of Progress. *Annu. Rev. Entomol.* 56, 487–510. 16.

Weirauch, C., Schuh, R.T., Cassis, G., & Wheeler, W.C. (2018). Revisiting habitat and lifestyle transitions in Heteroptera (Insecta: Hemiptera): insights from a combined morphological and molecular phylogeny. *Cladistics*, *35*(1), 67-105.

Wilkinson, S.P. & Davy, S.K. (2018) phylogram: an R package for phylogenetic analysis with nested lists. *Journal of Open Source Software* 3, 790. https://doi.org/10.21105/joss.00790

Table 1. Mean of measures for mesofemur, total labial length, and labial width mean of species belonging to Asopinae and non-Asopinae included in Pentatomomorpha and Cimicomorpha (*Pycnoderes* sp.) with their feeding habits. All measurements are in millimeters.

Asopinae	Genera/Species	Habit	Mesofemur	Total labial length	Labial width
	Alcaeorrhynchus grandis (Dallas, 1851)	predator	4.751	7.027	0.879
	Andrallus spinidens (Fabricius, 1787)	predator	3.461	5.153	0.485
	Apoecilus cynicus (Say, 1831)	predator	4.527	5.681	0.693
	Arma custos (Fabricius, 1794)	predator	3.003	4.856	0.354
	Brontocoris tabidus (Signoret, 1863)	predator	3.403	5.091	0.520
	Discocera coccinea (Fabricius, 1798)	predator	3.389	4.500	0.273
	Euthyrhynchus floridanus (Linnaeus, 1767)	predator	3.848	6.847	0.401
	Oplomus catena (Drury, 1782)	predator	3.471	4.475	0.419
	Perillus bioculatus (Fabricius, 1775)	predator	2.693	3.269	0.330
	Podisus nigrispinus (Dallas, 1851)	predator	2.576	4.291	0.358
	Stiretrus anchorago (Fabricius, 1775)	predator	2.459	3.386	0.302
	Supputius cincticeps (Stål, 1858)	predator	2.329	4.228	0.312
	Tynacantha marginata Dallas, 1851	predator	2.885	4.983	0.324

Non-Asopinae	Genera/Species	Habit	Mesofemur	Total labial length	Labial width
Coreidae	*Anasa varicornis* (Westwood, 1842)	phytophagous	4.102	4.753	0.185
Rhyparochromidae	*Cryphula sp.*	phytophagous	1.000	1.787	0.075
Alydidae	*Cydamus sp.*	phytophagous	2.158	3.516	0.158
Dinidoridae	*Dinidor mactabilis* (Perty, 1833)	phytophagous	3.624	3.269	0.265
Pyrrochoridae	*Dysdercus sp.*	phytophagous	3.152	5.774	0.236
Geocoridae	*Geocoris sp.*	omnivorous	0.915	1.669	0.073
Rhopalidae	*Harmostes sp.*	phytophagous	1.386	2.170	0.095
Acanthosomatidae	*Hellica nítida* Haglund, 1868	phytophagous	1.280	1.819	0.143
Berytidae	*Jalysus sp.*	omnivorous	3.330	2.524	0.076
Largidae	*Largus sp.*	phytophagous	3.985	4.035	0.209
Pentatomidae	*Nezara viridula* (Linnaeus 1758)	phytophagous	3.735	5.718	0.310
Lygaidae	*Nysius sp.*	phytophagous	1.308	2.439	0.071
Scutelleridae	*Pachycoris torridus* (Scopoli, 1772)	phytophagous	3.732	6.919	0.395
Cydnidae	*Pangaeus* sp.	phytophagous	2.200	3.394	0.170

Table 1. continuation.

Tessaratomidae	*Piezosternum subulatum* (Thunberg, 1783)	phytophagous	4.318	3.558	0.314
Miridae	*Pycnoderes sp.*	phytophagous	0.736	0.597	0.073

Table 2. Classification and GenBank accession numbers of the taxa included in the phylogenetic analyses. – , not applicable.

Infraorder	Superfamily	Family (Subfamily)	Species	COI	18S rDNA	28S rDNA	16S rDNA
Cimicomorpha		Miridae	*Orthotylus flavosparus*	JQ004042	JQ004030	JQ004034	JQ004038
Pentatomomorpha	Pentatomoidea	Acanthosomatide	*Stauralia compuncta*	AY253001	AY252269	AY252513	AY252741
		Cydnidae	*Microporus nigrita*	MF162975.1	KJ535880.1	KJ535880.1	JQ029137.1
		Dinidoridae	*Cyclopelta obscura*	MH707339.1	KJ522641.1	KJ522642.1	JQ029146.1
		Pentatomidae (Asopinae)	*Oechalia schellenbergii*	EF641159.1	EF641206.1	EF641185.1	EF641133.1
		Pentatomidae (Asopinae)	*Picromerus lewisi*	KC155928.1	KJ535896.1	KJ535891.1	KC155950.1
		Pentatomidae (Asopinae)	*Podisus maculiventris*	AY252953.1	AY252208.1	AY252457.1	–
		Pentatomidae (Pentatominae)	*Hypogomphus* sp.	AY253007	AY252278	AY252522	AY252750
		Pentatomidae (Pentatominae)	*Brochymena* sp.	AY252918	AY252137	EF641187	AY252655
		Scutelleridae	*Coleotichus costatus*	AY253005	AY252274	AY252518	AY252746
		Tessaratomidae	*Tessaratoma papillosa*	AY252948.1	AY627312.1	–	AY986806.1
	Coreoidea	Alydidae	*Leptocorisa acuta*	AY252956	AY627322	AY252462	AY252691
		Coreidae	*Maevius indecorus*	AY252957	AY252214	AY252463	AY252692
		Rhopalidae	*Leptocoris augur*	KU234090.1	KP142861.1	KX523434.1	KX523378.1
	Lygaeoidea	Berytidae	*Neoneides muticus*	AY253130	AY252412	AY252631	AY252892
		Geocoridae	*Germalus* sp.	AY252930.1	AY252423.1	AY252642.1	AY252669.1
		Lygaeidae	*Kleidocerys* sp.	AY253128	AY252410	AY252629	AY252890
		Rhyparochromidae	*Udeocoris nigroaeneus*	AY252994	AY252262	AY252505	AY252735
	Pyrrhocoroidea	Largidae	*Physopelta gutta*	EU427343.1	KP142865.1	KJ461255.1	AY139970.1
		Pyrrhocoridae	*Pyrrhocoris apterus*	KX821862.1	KX821833.1	KX821848.1	KX821818.1

Table 3. Allometric coefficients of labial length (mm) and labial width (mm) in relation to mesofemur length among Asopinae and non-Asopinae, and comparison of the slope with an isometric line (Isometry comp.). Regression analysis, one-way ANCOVA, α = 0.05.

	Body parts	Regression parameter		Isometry comp.	
		Slope ± CI	Intercept ± CI	F	P
Asopinae	labial length	1.246 ± 0.2592	0.8439 ± 0.8737	0.8971	0.354
	labial width	1.289 ± 0.2842	-3.545 ± 1.470	1.0344	0.320

Table 3. continuation.

Non-Asopinae	labial length	1.005 ± 0.2292	0.7972 ± 0.654	0.0005	0.981
	labial width	0.7893 ± 0.1554	-1.665 ± 0.7490	1.8377	0.186

Table 4. T-test for the variables (total length, average labial width and labial length / width ratio) among Asopinae and non-Asopinae. Measurements are in millimeters and then transformed by the formula (ln N)+4. SEM = standard error of the mean.

Body parts	Asopinae	Non-Asopinae	
	Mean ± SEM		**P**
labial length	5.574 ± 0.06381	5.068 ± 0.1521	0,0063
labial width	3.117 ± 0.09827	2.110 ± 0.1508	<0.0001
labial length / width ratio	6.464 ± 0.06836	6.968 ± 0.1001	0,0003

Table 5. Allometric coefficients of labial length / labial width ratio for Asopinae and non-Asopinae, and comparison of the slope with an isometric line (Isometry comp.). Regression analysis,one-way ANCOVA, alpha= 0.05.Values in parentheses represent analysis excluding outliers.

Asopinae	Regression parameter	Isometry comp.	
	Slope ± CI	**F**	***P***
Alcaeorrhynchus grandis	-0.1422 ± 0.07468	233.918	<0.0001
Andrallus spinidens	-0.1940 ± 0.1402	72.5045	<0.0001
Apoecilus cynicus	-0.7503 ± 0.1841	90.3779	<0.0001
Arma custos	-0.4662 ± 0.1327	122.0390	<0.0001
Brontocoris tabidus	-0.1955 ± 0.3196	13.9899	0.0006385
Discocera coccinea	0.04461 ± 0.1911	24.9885	<0.0001
Euthyrhynchus floridanus	-0.4195 ± 0.1382	105.4770	<0.0001
Oplomus catena	-0.3774 ± 0.07547	333.0350	<0.0001
Perillus bioculatus	-0.1114 ± 0.1484	56.0560	<0.0001
Podisus nigrispinus	-0.1833 ± 0.1360	75.7487	<0.0001
Stiretrus anchorago	-0.07618 ± 0.09787	120.9120	<0.0001
Supputius cincticeps	-0.3772 ± 0.06200	493.4530	<0.0001
Tynacantha marginata	-0.01627 ± 0.08645	138.1920	<0.0001
Non-Asopinae	**Regression parameter**	**Isometry comp.**	
	Slope ± CI	**F**	***P***
Anasa varicornis	-0.1416 ± 0.1619	49.7514	<0.0001
Cryphula sp.	0.4414 ± 0.1724	10.5032	0.0025868
Cydamus sp.	0.01039 ± 0.2951	11.2455	0.001889
Dinidor mactabilis	0.1193 ± 0.1596	30.4468	<0.0001
Dysdercus sp.	-0.1835 ± 0.1407	70.7866	<0.0001
Geocoris sp.	-0.1905 ± 0.8587 (0.3112 ± 0.5549)	1.92238 (5.583)	0.1741 (0.02399)

Table 5. continuation.

Harmostes sp.	0.2023 ± 0.2441	10.6775	0.002388	
Hellica nitida	-0.1135 ± 0.2274	23.9836	<0.0001	
Jalysus sp.	0.3396 ± 0.4594 (0.3068 ± 0.2193)	2.06646 (9.99281)	0.1592 (0.003297)	
Largus sp.	0.2390 ± 0.1066	50.9801	<0.0001	
Nezara viridula	-0.1326 ± 0.1052	115.9220	<0.0001	
Nysius sp.	0.2901 ± 0.3054	5.4016	0.02588	
Pachycoris torridus	0.2331 ± 0.2462	9.7042	0.003599	
Pangaeus sp.	0.2192 ± 0.2468	10.0073	0.003163	-
Piezosternum subulatum	0.1411 ± 0.1932	19.7562	<0.0001	-
Pycnoderes sp.	0.3484 ± 0.3784 (-0.2890 ± 0.2406)	2.96476 (28.6974)	0.09368 (<0.0001)	-
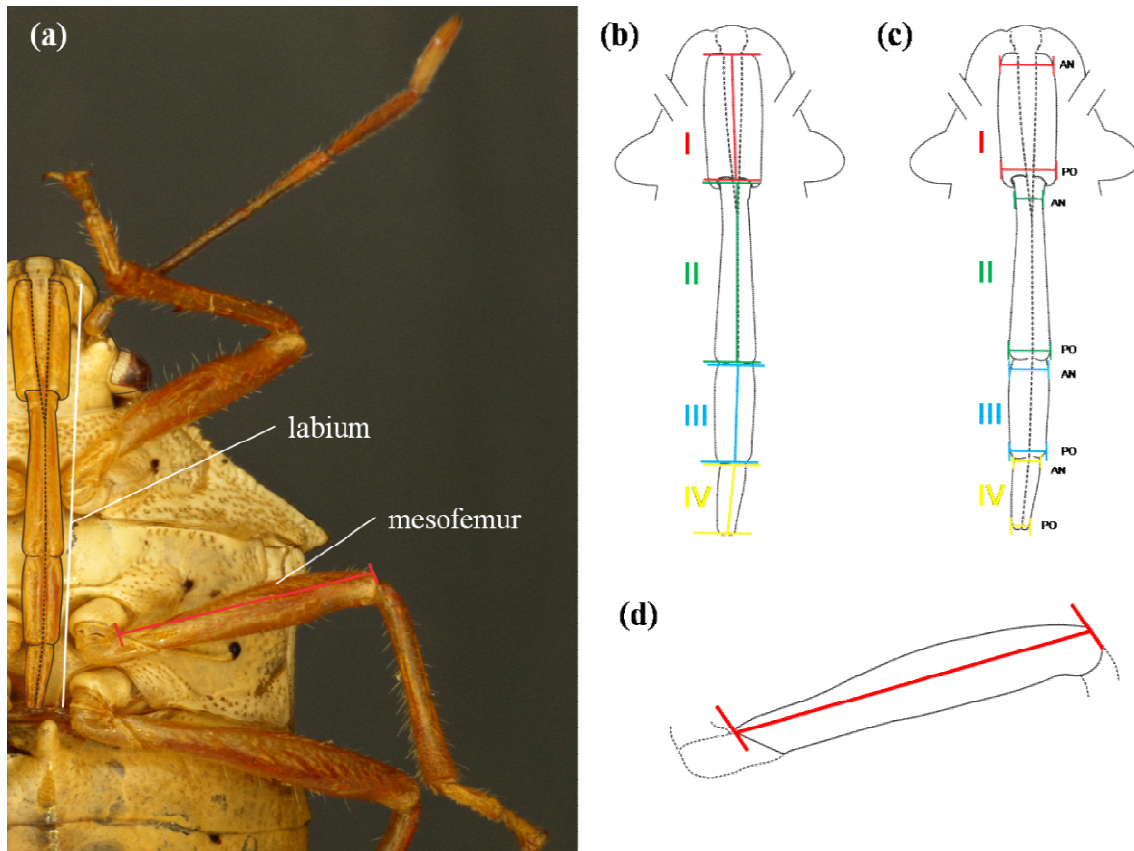
Figure 1. Measurements obtained from labium and mesofemur in Asopinae and non-Asopinae (a) *Supputius cincticeps* ventral view (b) length of each labial segment (c) maximum anterior (AN) and posterior (PO) width obtained by labial segments (d) largest length of mesofemur in dorsal view. I, II, II, IV = first, second, third and fourth labial segments respectively.
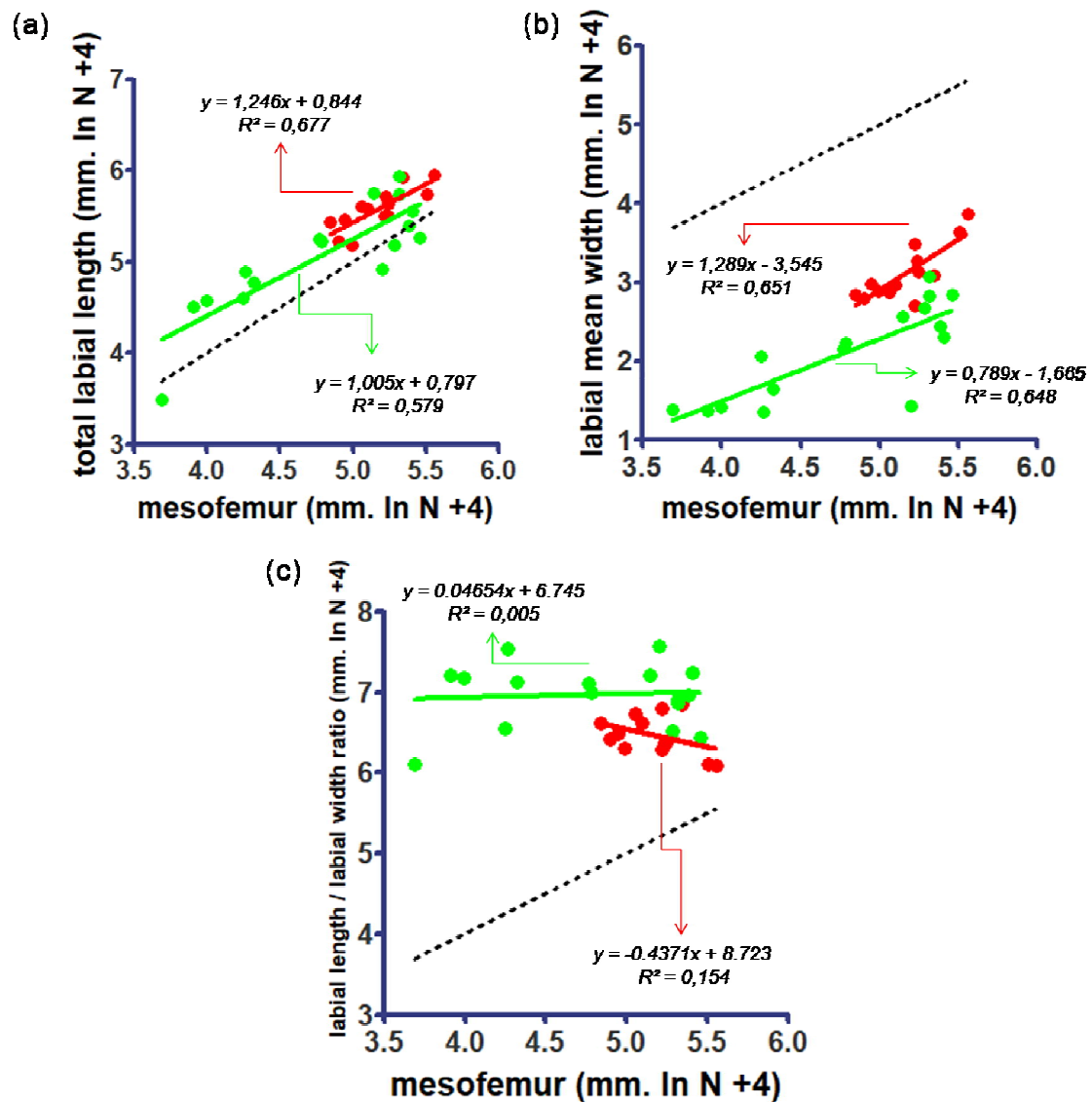
Figure 2. Variation of (a) labial length, (b) labial mean width and (c) labial length / width ratio in relation to mesofemur length among Asopinae (red) and non-Asopinae (green). Isometry is indicated by dashed black line.
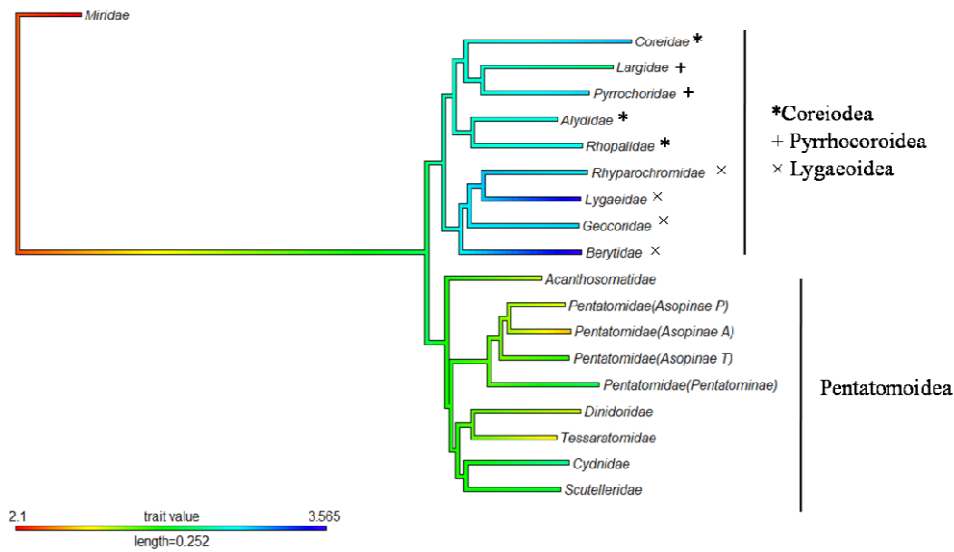
Figure 3. Ancestral character states reconstruction for the character labial length / width ratio on a maximum likelihood tree from the RAxML analysis of molecular dataset.Species used and morphological values based on the Table 3. (Asopinae P) *Podisus nigrispinus,* (Asopinae T) *Tynacantha marginata*, (Asopinae A) *Andrallus spinidens.*
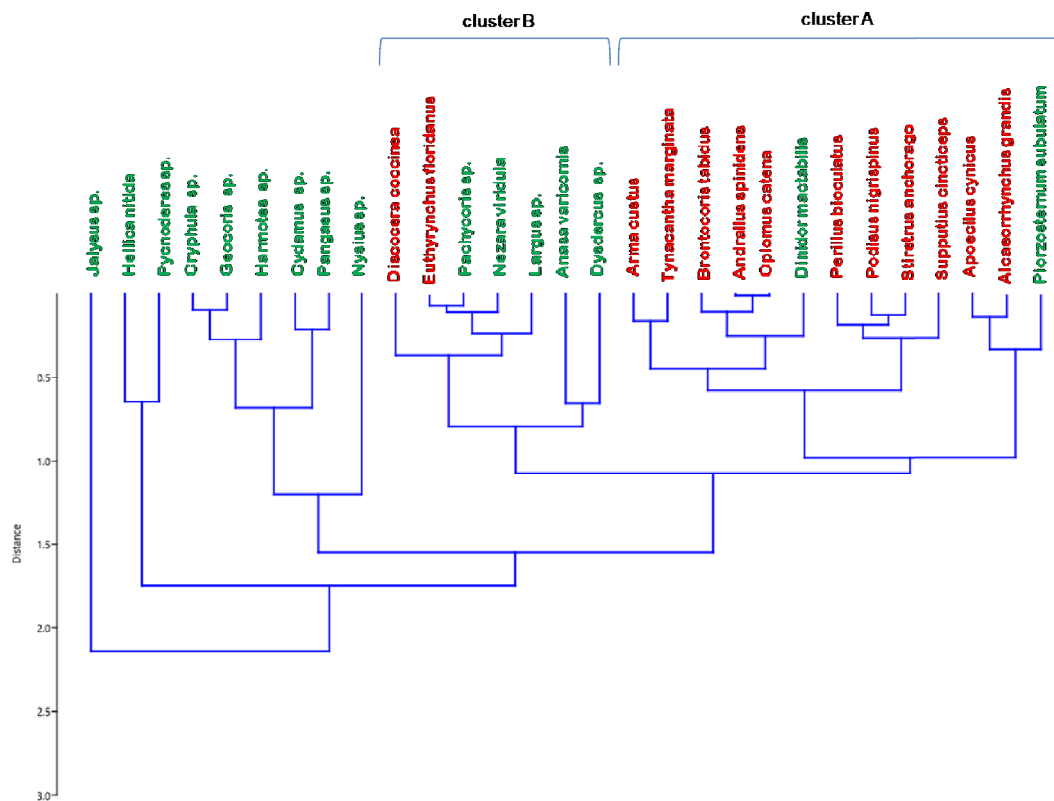
Figure 4. Phenogram showing the linear morphometric relationships among Asopinae (red) and non-Asopinae (green), based on Mahalanobis distances. Clustering method UPGMA.

Supporting Information

Appendix 1.

Table S1. Comparison of male and female allometric coefficient for the labial length / width ratio when regressed on the mesofemur length in different species of Pentatomomorpha and selected outgroup (*Pycnoderes* sp., Cimicomorpha). Values in red indicate statistical significance (one-way ANCOVA, α = 0.05).

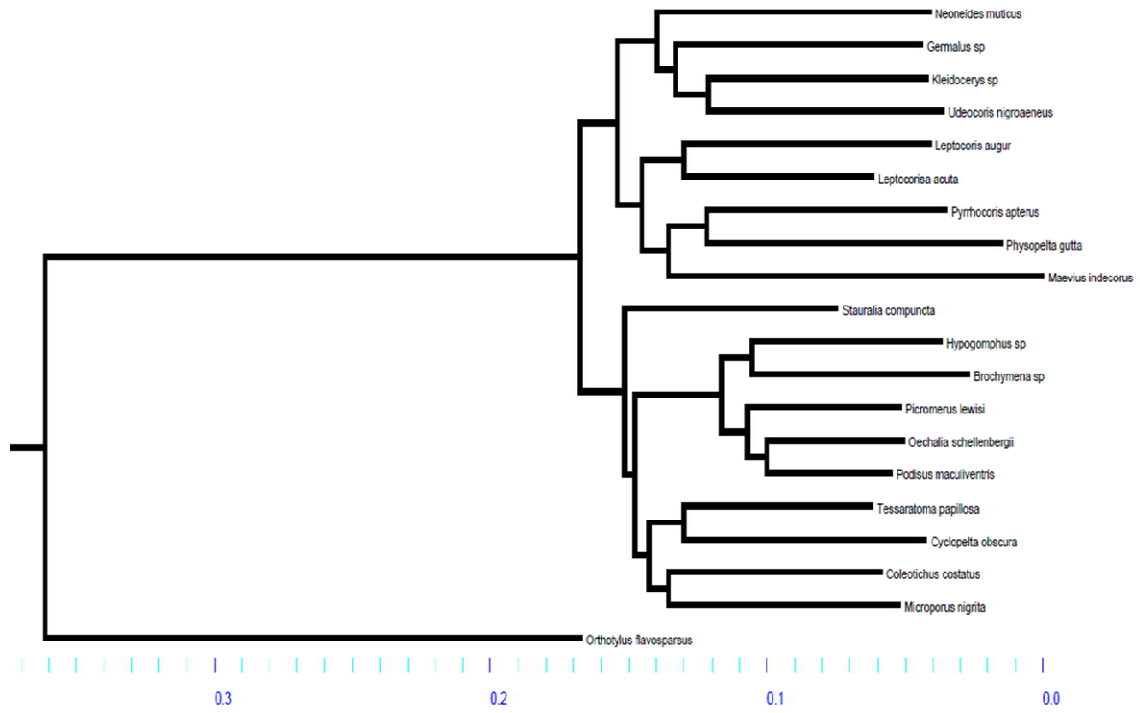|	*non-Asopinae*			*Asopinae*		
Family	**Genera/Species**	**F**	***p***	**Species**	**F**	***p***
Coreidae	*Anasa varicornis*	2.323	0.147	*Alcaeorrhynchus grandis*	2.479	0.135
Rhyparochromidae	*Cryphula* sp.	0.959	0.342	*Andrallus spinidens*	1.820	0.196
Alydidae	*Cydamus* sp.	0.254	0.620	*Apoecilus cynicus*	0.044	0.837
Dinidoridae	*Dinidor mactabilis*	1.698	0.210	*Arma custos*	0.781	0.390
Pyrrochoridae	*Dysdercus* sp.	3.599	0.076	*Brontocoris tabidus*	0.043	0.840
Geocoridae	*Geocoris* sp.	0.228	0.639	*Discocera coccinea*	1.037	0.324
Rhopalidae	*Harmostes* sp.	2.827	0.112	*Euthyrhynchus floridanus*	0.185	0.673
Acanthosomatidae	*Hellica nitida*	0.129	0.724	*Oplomus catena*	4.812	0.043
Berytidae	*Jalysus* sp.	0.148	0.706	*Perillus bioculatus*	1.148	0.299
Largidae	*Largus* sp.	3.908	0.066	*Podisus nigrispinus*	0.067	0.798
Pentatomidae (Pentatominae)	*Nezara viridula*	0.009	0.922	*Stiretrus anchorago*	2.392	0.141
Lygaidae	*Nysius* sp.	2.949	0.105	*Supputius cincticeps*	0.374	0.549
Scutelleridae	*Pachycoris torridus*	1.310	0.269	*Tynacantha marginata*	0.101	0.753
Cydnidae	*Pangaeus* sp.	2.119	1.65	-	-	-
Tessaratomidae	*Piezosternum subulatum*	0.092	0.765	-	-	-
Miridae	*Pycnoderes* sp.	3.775	0.070	-	-	-

Appendix 2.

Figure S1. Maximum likelihood tree from the RAxML analysis of molecular dataset.

CONCLUSÕES GERAIS

Asopinae geralmente apresentam uma forte tendência a ter um lábio relativamente mais robusto à medida que o tamanho do corpo aumenta em comparação com a mesma proporção de aumento em não-Asopinae. A maior variabilidade morfológica encontra-se em espécies não-Asopinae, consideradas fitófagas ou onívoras em alguns casos excepcionais. Essas espécies possuem maior amplitude quanto ao comprimento e largura do aparelho bucal, característica provavelmente relacionada a particularidades em seus principais alimentos, que podem ser de diferentes grupos e partes de plantas. A maior robustez do lábio em Asopinae pode refletir um maior investimento em energia no aparelho bucal, o que estaria relacionado à especialização em alimentação devido a seu hábito predatório insetívoro generalista, característica exclusiva dessa subfamília dentro de Pentatomidae.