FILIPE MICHELS BIANCHI

Filogenia e sistemática de *Euschistus* Dallas (Hemiptera: Pentatomidae) e gêneros relacionados: hipóteses baseadas em moléculas

Tese apresentada ao Programa de Pós-Graduação em Biologia Animal, Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como requisito parcial à obtenção do título de Doutor em Biologia Animal.

Área de concentração: Biologia Comparada

Orientador: Prof. Dr. Luiz Alexandre Campos

Coorientador: Profa. Dra. Maríndia Deprá

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Dr. José Ricardo I. Ribeiro

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Dra. Juliana Cordeiro

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Dr. Marcio Borges-Martins

À minha família, a qual nunca poupou motivação e incentivo em cada passo desta jornada.

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“Só sei que nada sei por completo;
Só sei que nada sei que só eu saiba;
Só sei que nada sei que eu não possa vir a saber;
Só sei que nada sei que outra pessoa não saiba;
Só sei que nada sei que eu e outra pessoa não saibamos juntos”

Mario Sergio Cortella sobre Sócratres

Sumário

RESUMO

Os heteropteros são o maior grupo de insetos hemimetábolos. Pentatomidae é composta por cerca de 4.700 espécies com hábito predominantemente fitosuccívoro. Estudos filogenéticos nesta família são escassos, sendo os agrupamentos taxonômicos baseados principalmente em similaridades morfológicas. *Euschistus* Dallas é um dos mais especiosos gêneros de Pentatomidae e tem distribuição no novo mundo. Algumas espécies do gênero podem causar danos a plantas cultivadas, sendo consideradas espécies pragas. Nesta tese, são descritas três espécies dentro de Carpocorini: *Euschistus.* (*Euschistus*) *baranowskii* sp. nov., *Euschistus* (*Mitripus*) *saramagoi* sp. nov., e *Ladeaschistus borgesi* sp. nov. Também são abordadas as relações entre quatro os subgêneros de *Euschistus* (*Euschistus*, *Euschistomorphus*, *Lycipta, Mitripus*) e gêneros relacionados em uma análise filogenética de evidência total utilizando quatro marcadores meleculares e 85 caracteres morfológicos. *Euschistus* é recuperado como não monofilético, sendo os subgêneros *Euschistomorphus* e *Mitripus* indicados como taxa fora de *Euschistus*. O relacionamento entre *Ladeaschistus*, *Sibaria* e *Euschistus* (*Mitripus*) é corroborado. Com base nos resultados, *Mitripus* new rank é elevado a gênero, e *Adustonotus* gen. n. é proposto. Uma análise filogenética utilizando seis marcadores moleculares é realizada para testar a hipótese de monofilia do grupo-*Euschistus* proposto inicialmente por L. Rolston. Este agrupamento de gêneros não foi recuperado na nossa análise, no entanto, relações genéricas destes carpocorineos, que até então eram baseadas em similaridades taxonômicas, agora possuem uma hipótese filogenética testada. Analisamos a evolução de caractéres de genitália interna de fêmea. As estruturas da espermateca apresentam grande variação dentro da nossa amostra. A reconstrução de estado ancestral detectou muitas mudanças de estado para maior parte dos caracteres.

1 Introdução Geral

Os percevejos verdadeiros (Heteroptera), são considerados o maior grupo de insetos hemi-metábolos, compreendendo mais de 40.000 espécies nominais, e tem sido foco de estudos básicos e aplicados (Panizzi & Grazia, 2015). O grupo compreende vasta diversidade morfológica, presentes em ambientes aquático e terrestre; hábtos de vida sedentário e ativos. Os hábtos alimentares estão relacionados com fitofagia, micofagia, hematofagia, e tais comportamentos tem impacto na saúde humana, como também causam danos a plantas cultivadas. A subordem Heteroptera é dividida em sete infraordens: Enicocephalomorpha, Dipsocoromorpha, Gerromorpha, Nepomorpha, Leptopodomorpha, Cimicomorpha e Pentatomomorpha (Weirauch & Schuh, 2011).

A primeira hipótese filogenética de Heteroptera, adotando método cladístico explícito e claro foi desenvolvida por Schuh (1979) ao reinterpretar caracteres apresentados na proposta de Cobben (1968). Desde então, poucas novas hipóteses foram propostas, sendo elas baseadas em conjuntos de dados de diferentes naturezas. Por exemplo, Wheeler *et al*. (1993) utilizaram dados moleculares (18S rDNA) e morfológicos; Mahner (1993) apenas dados morfológicos; Xie *et al.* (2008) apenas dados moleculares (18S rDNA). Cada uma dessas análises traz hipóteses distintas das relações entre as infraordens (para históricos mais completos, veja Weirauch & Schuh, 2011; Ferrari *et al.*, 2015).

A proposição de um táxon com composição similar a atual Pentatomoidea reporta a Leach, 1815 (Leston, 1953). A superfamília é composta por heterópteros que formam o maior grupo de espécies dentro de Pentatomomorpha (Henry, 1997), compreendendo mais de 7.000 nomes (Grazia *et al.*, 2008). Estudos cladísticos suportam a monofilia do grupo, tanto os trabalhos que utilizaram dados morfológicos (Gapud, 1991; Henry, 1997), quanto moleculares (Li *et* *al.*, 2005; Wu *et al.*, 2016). Grazia *et al.* (2008) utilizaram dados morfológicos e moleculares, para análises de máxima parcimônia e máxima verossimilhança. Nesse estudo, quinze famílias são reconhecidas dentro do clado Pentatomoidea, corroborando a monofilia dessa superfamília. As sinapomorfias que suportam Pentatomoidea incluem: escutelo ultrapassando a metade do comprimento do abdome, tricobótrios abdominais pareados e localizados lateralmente à linha dos espiráculos, abertura da cápsula genital dos machos direcionada posteriormente, ovos em forma de barril, ovóides ou esféricos (Grazia *et al.*, 2008).

Popularmente conhecidos como percevejos-de-planta ou percevejos-do-mato, os pentatomóideos são facilmente reconhecidos pelo corpo de tamanho médio, usualmente variando de 2 a 20 mm, de forma oval a elíptica, antenas geralmente com cinco segmentos e escutelo amplo. Apresentam coloração negra, castanha e tons escuros, compondo padrões crípticos, podendo ser também de cores vivas como verde, vermelho, alaranjado e possuir detalhes brilhantes e traços aposemáticos (Grazia *et al*,. 1999). O hábito fitosuccívoro é aparentemente uma condição ancestral, compartilhada entre os pentatomomorfos (Grazia *et al.* 1999; Grazia *et al.*, 2015b). Usualmente, a nutrição é obtida diretamente do floema em diversas partes da planta, e podem se alimentar em folhas, flores, sementes, frutos e raízes. Dois grupos constituem exceções ao hábito fitófago; Asopinae (Pentatomidae), cujas espécies são predadoras de outros artrópodes (Grazia *et al.*, 2015b), e Canopidae, cujos representantes são micetófagos (McHugh, 1994). Os pentatomóideos são exclusivamente terrestres e habitam todas as regiões biogeográficas do globo. Na região Neotropical, são registradas dez famílias: Acanthosomatidae, Canopidae, Cydnidae, Dinidoridae, Megarididae, Pentatomidae, Phloeidae, Scutelleridae, Tessaratomidae e Thyreocoridae (Grazia & Schwertner, 2011).

Pentatomidae é a maior família entre os pentatomóideos e a quarta maior em Heteroptera (Grazia *et al.*, 1999). O grupo inclui mais de 4.700 espécies distribuídas em quase 900 gêneros e 10 subfamílias (Rider, 2016). Destas, Asopinae, Pentatominae, Cyrtocorinae, Discocephalinae, Edessinae e Stirotarsinae ocorrem na região Neotropical; as quatro últimas com distribuição exclusivamente no neotrópico (Grazia & Schwertner, 2011 Grazia *et al.*, 2015). Os pentatomídeos são considerados polífagos, e apresentam importância econômica por terem como suas plantas hospedeiras espécies cultivadas em todo o mundo. Algumas espécies são consideradas pragas agrícolas (Panizzi *et al.*, 2000). A maior parte dos estudos que envolvem representantes da família é restrita a tais espécies (e. g. Moraes *et al.*, 2005; Cullen & Zalom, 2005; Borges *et al.*, 2015). Desta maneira, para estas espécies há um maior conhecimento de morfologia, bioquímica, ciclos biológicos, nutrição e etologia.

Teste da monofilia dos pentatomídeos vem sendo publicados, e.g. Gapud (1991) que usa caracteres morfológicos, Wu *et al.* (2016) utilizaram caracteres molecular, Grazia *et al.* (2008) que utilizam ambas fontes de informação para construção filogenética. A ampliação do número de caracteres no conjunto dados morfológicos e marcadores moleculares eventualmente utilizados estão sendo explorados a fim de elucidar as relações do grupo taxonômico. No entanto, a monofilia de Pentatomidae ainda está longe de ser um consenso na literatura. Também, as propostas de agrupamentos taxonômicos dentro de Pentatomidae carecem de testes de hipóteses. As filogenias para níveis taxonômicos mais baixos são escassos para a família (Grazia *et al.*, 2008).

Carpocorini teve sua proposição em 1866 por Mulsant & Rey, todavia, ainda não há uma diagnose formal da tribo. Ao longo dos anos diferentes autores propuseram distintas composições de Carpocorini (i.e. Stål, 1876; Distant, 1902; Ahmad *et al.*, 1974; Gross, 1975). Atualmente os carpocoríneos são a maior tribo dentro da subfamília nominal, e compreendem mais de 100 gêneros e aproximadamente 450 especies (*sensu* Rider 2016). Contudo, quase metade destes gêneros é monotípico. Por outro lado, quatro gêneros, *Euschistus* Dallas 1851, *Mormidea*

Amyot & Serville, 1843, *Aeliomorpha* Stål, 1858 e *Dichelops* Spinola 1837 compreendem mais de um terço das espécies da tribo.

Euschistus *Dallas, 1851*

Euschistus Dallas, 1851 é um dos maiores gêneros da Pentatomidae, com 76 espécies, das quais nove tem distribuição originalmente neártica e o restante neotropical (Weiler *et al.*, *in press*). São popularmente conhecidos como percevejos-castanhos, pois dorsalmente são castanhos ou negros, em algumas espécies com tons avermelhados, ventralmente são mais claros e podem apresentar coloração vermelha ou verde. O tamanho varia de 7 a 14 mm de comprimento. Um conjunto de caracteristicas morfológicas pode ser usado como uma diagnose primária para *Euschistus*, tais como: metasterno não pronunciado, peritrema atingindo menos de 2/5 a distância entre o ostíolo e a margem lateral do segmento toráxico, fêmures não armados, largura da base do escutelo maior que o dobro da largura do escutelo na intersecção com o ápice da veia frenal, olhos contíguos com o pronoto, superfície da tíbia sulcada, placas mandibulares ou clípeo, ou ambos arredondados apicalmente e usualmente igual em comprimento, búcula evanescente ou truncada na base da cabeça, e antena com cinco segmentos (Rolston, 1974).

Euschistus é subdividido em quatro subgêneros: o nominal, com 53 espécies; *Lycipta* Stål 1862, 12 espécies; *Euschistomorphus* Jensen-Haarup 1922, monotípico; *Mitripus* Rolston 1978, 10 espécies. Os subgêneros foram propostos com base em caracteres diagnósticos principalmente estruturação geral do corpo e genitália externa de macho.

Euschistus (*Lycipta*) foi descrito com o *rank* de gênero por Stål (1862a) para incluir três espécies do gênero *Euschistus*, *Euschistus illotus* Stål, 1860; *Euschistus triangulator* (Herrich-Schäffer, 1842) e *Euschistus scabricornis* (Herrich-Schäffer, 1844). Estas espécies foram agrupadas como gênero por apresentar um incisão entre o clípeo e as placas mandibulares, detículo na margem anterolateral do pronoto e membrana do hemelitro com veias não paralelas. Ainda no mesmo ano, Stål (1862b) trata *Lycipta* como subgênero de *Euschistus*, remove *E. scabricosnis* tratando a espécie como *insertis generis*, e descreve outras quatro espécies no subgênero. Posteriormente, diversos atos taxonômicos foram tomados para as espécies que compõem os subgêneros, tais como homonímias [e.g. *Euschistus luridus* renomeado como *Euschistus* (*Lycipta*) *aceratus* (Berg, 1894)], transferências [e.g. *Euschistus monrosi* Pirán, 1963 foi transferido de *Berecynthus* Stål, 1862 por Grazia & Hildebrand (1982)] e descrições (veja a seguir). Atualmente as espécies que compõe o subgênero *Lycipta* são: *E.* (*Lycipta*) *aceratos*, *E.* (*L.*) *circumfusus* Berg, 1883, *E.* (*L.*) *cornutus* (Dallas, 1851), *E.* (*L.*) *cribarius* Stål, 1872, *E.* (*L.*) *illotus* Stål, 1860, *E.* (*L.*) *imitator* Berg, 1978, *E.* (*L.*) *longicornis* Grazia & Hildebrand, 1982, *E.* (*L.*) *machadus* Rolston, 1982, *E.* (*L.*) *picticornis* Stål, 1872, *E.* (*L.*) *sharpi* Bergroth, 1891, *E.* (*L.*) *triangulator* (Herrich- Schäffer, 1842) e *E.* (*L.*) *riograndensis* Weiler & Grazia, 2011.

Euschistus longiceps Berg, 1891 foi descrito com base em uma única fêmea da Patagônia, Argentina, e depositada no Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires. Nesta descrição, Berg (1891) ressaltou a forma peculiar da cabeça, no entanto a ausência das antenas o impedediu de descrever um novo gênero. Jensen-Haarup (1922) na posse de uma pequena série de espécies da Provícia de Mendonza, Argentina, pode descrever a morfologia das antenas e foi categórico ao afirmar “... this material enables me to declare with certainty that the building of the antennae in no way justifies a generic separation from the other *Euschistus* species” (“... este material me permite declarar com certeza que a construção das antenas de maneira alguma justifica uma separação genérica das outras espécies de *Euschistus*”) (Jensen-Haarup, 1922 p. 10). Baseado no excepcional prolongamento das placas mandibulares, que se extendem além do clípeo, e a sobreposição apical, Jensen-Haarup (1922) propôs o subgênero *Euschistomorphus* para incluir *E. longiceps* e descreveu uma nova espécies *E. (Euschistomorphus) albidus*. Esta espécie foi transferida por Grazia (1988) para *Prionotocoris* Kormilev, 1955, remanescendo o subgênero *Euschistomorphus* como monotípico.

Rolston (1978) descreve o subgênero *Mitripus* com base na forma e estruturações presentes na genitália interna e externa dos machos e pelo alcance e orientação das jugas. Para compor este grupo, foram transferidas do subgênero nominal, as espécies: *Euschistus (Mitripus) acutus* Dallas, 1851, *E. (M.) anticus* Stål, 1860, *E. (M.) convergens* (Herrich-Schäffer, 1842), *E. (M.) latus* (Dallas, 1852), *E. (M.) legionarius* Breddin, 1914, *E. (M.) tauricornis* Stål, 1872, e a descrita no mesmo trabalho *E. (M.) grandis*. Posteriormente foram incluídas *E. (M.) hansi* Grazia, 1987, *E. (M.) paranticus* Grazia, 1987 e *E. (M.) irroratus* Bunde *et al.*, 2006. Ao descrever *E. (M.) paranticus* e *E. (M.) hansi*, Grazia (1987) chama a atenção para semelhança da morfologia geral e da genitália dos machos, em especial dos parâmeros e pigóforo, que junto com *E. (M.) anticus* possuem um padrão único no subgênero de escavação na margem ventral do pigóforo em forma de “U”, sendo mais escavado em *E. (M.) anticus* e menos em *E. (M.) paranticus*. Na descrição de *E. (M.) irroratus* Bunde, Grazia & Mendonça, 2006, mais recente para o subgênero, Bunde *et al.*, (2006) relacionam a morfologia desta com *E. (M.) paranticus* e *E. (M.) hansi*. Estas quatro espécies formam um complexo, ao menos morfológico, estreitamente relacionado dentro de *Euschistus*.

As hipóteses de relações dentro do gênero ainda são infatisfatórias, uma vez que baseadas principalmente em agrupamentos taxômicos sem teste cladístico. *Euschistus* (*Lycipta*) é a exceção, recentemente testado por Weiler *et al.* (2016) que redefiniu os limites de *Euschistus* (*Lycipta*) baseando em caracteres de pigóforo e dutos ectodérmicos da genitália feminina; e para tal transfere *Euschistus monrosi* para o gênero nominal. Weiler *et al.*(2016) também sugerem o subgênero *Mitripus* relacionado com *Ladeaschistus* Rolston 1973 e não fazendo parte do clado *Euschistus*.

Gêneros historicamente relacionados a Euschistus

Ao longo da história taxonômica das espécies e gêneros de Carpocorini alguns agrupamentos de taxa foram sugeridos (e. g. grupo *Mecocephala*). Estes grupos possuem afinidades morfológicas que até então não foram objetos de estudo filogenético.

Por se tratar de um gênero especioso e com ampla diversidade morfológica, alguns gêneros da tribo têm sido relacionados à *Euschistus*. Os gêneros *Agroecus* Dallas, 1851, *Berecynthus*, *Dichelops*, *Euschistus*, *Galedanta* Amyot & Serville, 1843, *Hymenarcys* Amyot & Serville, 1843, *Menecles* Stål, 1867, *Padaeus* Stål, 1862, *Proxys* Spinola, 1837, *Sibaria* Stål, 1872, e *Tibraca* Stål, 1860 foram agrupados por Rolston (1974) por semelhanças de cor, tamanho e aspecto geral do corpo. Uma vez que este grupo de espécie está frequentemente relacionado com plantas cultivadas, Rolston (1974) elaborou uma chave de identificação. Posteriormente, outros gêneros foram sugeridos como pertencentes ao grupo (e.g. *Coenus* Dallas, 1851, *Oenopiella* Bergroth, 1891). Além disto, o reconhecimento de supostas linhagens dentro de *Euschistus* e estes gêneros relacionados, elevaram outros taxa, incorporando-os no grupo *Euschistus* (e.g. *Mcphersonarcys* Thomas, 2012, and *Spinalanx* Rolston & Rider, 1988). Um exemplo pertinente é *Ladeaschistus* Rolston, 1973, pois discussões acerca desde gênero estão presentes no segundo capítulo desta tese.

Ladeaschistus foi originalmente proposto para incluir três espécies de *Euschistus*, (i. e. *Euschistus armipes* Stål, 1872, *Euschistus bilobus* Stål, 1872 e *Euschistus trilobus* Stål, 1872, e uma nova espécies *Ladeaschistus boliviensis* Rolston, 1973. Todas estas espécies estão distribuídas na América do Sul, e compartilham as seguintes estruturas: robusto tubérculo na base do fêmur, margem superior do pigóforo tectiforme e parâmetros inversamente curvados quando comparados com outros *Euschistus*. Para dar maior suporte a sua decisão taxonômica, Rolston comparou a morfologia genital das espécies deste novo gênero proposto com a espécie tipo *E. (E.) tristigmus* (Say, 1831) e *E. (M.) hansi* (citado como *Euschistus anticus*), e concluiu "the genital morphology of both sexes demonstrates that *Ladeaschistus* species form a homogeneous group whose phylogenetic relationship is much closer to a group of South American *Euschistus* of which *E. anticus* is representative than it is to the type species *E. tristigmus.*" ("a morfologia genital de ambos os sexos demonstra que as espécies de *Ladeaschistus* formam um grupo homogêneo os quais são mais filogeneticamente próximos de um grupo de *Euschistus* da América do Sul, que *Euschistus anticus* é a uma espécie representativa do que é com a espécie tipo *Euschistus* (*Euschistus*) *tristigmus*.) (Rolston, 1973 p. 104).

As hipóteses filogenéticas estão se tornando cada vez mais fundamentais para o avanço de diferentes áreas relacionadas à biologia, tais como ecologia e biologia evolutiva. Uma infinidade de características dos taxa, desde traços morfológicos a comportamento ecológicos, traz consigo a história filogenética de uma espécie (Rabosky, 2015). As relações do grupo *Euschistus*, até então, não foram submetidas a um teste filogenético, sendo este agrupamento e suas relações baseados em comparações morfológicas. As filogenias dentro de Pentatomidea são poucas, e hipóteses baseadas em testes filogenéticos são pertinentes para o desenvolvimento do conhecimento (Grazia *et al.*, 2008).

2 Objetivos

Objetivo Geral

Testar, a partir de métodos filogenéticos, a corrente classificação de *Euschistus* e suas relações filogenéticas com os gêneros relacionados.

Objetivos específicos

- Descrever duas espécies de *Euchistus* e um de *Ladeaschistus*
- Propor hipótese filogenética para as relações dos subgêneros de *Euschistus*.
- Explorar a hipótese de relacionamento proposta por L. Rolston entre *Euschistus* (*Mitripus*), *Sibaria* e *Ladeaschistus*, e testá-la filogeneticamente com dados moleculares e morfológicos.
- Gerar uma hipótese filogenética para o grupo *Euschistus* proposto inicalmente por L. Rolston.
- Fornecer uma visão evolutiva das estruturas da espermateca de *Euschistus* e gêneros relacionados.

3 Estrutura da Tese

Esta tese está estruturada em capítulos que correspondem a artigos desenvolvidos independentemente. Cada um deles, no entanto, usa métodos ou técnicas que desenvolveram base prática e teórica para melhor execução do capítulo seguinte. Os capítulos abordam respectivamente: taxonomia, sistemática filogenética usando dados moleculares e morfológicos combinados, e sistemática filogenética com viés evolutivo voltado para a morfologia.

Capítulo I

New species of *Euschistus* (*Euschistus*) from Jamaica, *Euschistus* (*Mitripus*) and *Ladeaschistus* from southern of South America (Hemiptera, Heteroptera, Pentatomidae, Carpocorini) [Novas espécies de *Euschistus* (*Euschistus*) da Jamaica, *Euschistus* (*Mitripus*) e *Ladeaschistus* do sul da América do sul (Hemiptera, Heteroptera, Pentatomidae, Carpocorini)]

Neste capítulo são descritos três novas espécies do grupo; *Euschistus.* (*Euschistus*) *baranowskii* sp. nov., uma espécie da Jamaica; *Euschistus* (*Mitripus*) *saramagoi* sp. nov., com distribuição para o sul do Brasil; *Ladeaschistus borgesi* sp. nov. com distribuição para o sul da América do Sul.

Capítulo II

Phylogenetic insights from morphology and molecules within Pentatomidae: the split of a speciose genus in Carpocorini (Hemiptera: Heteroptera) [Perspectiva filogenética a partir de morfologia e moléculas dentro de Pentatomidae: a divisão de um gênero especioso em Carpocorini (Hemiptera: Heteroptera)]

Neste capítulo construimos uma análise filogenética utilizando 85 caracteres morfológicos combinados com 2330 bp de 4 marcadores moleculares para testar as relações entre os subgêneros de *Euschistus* e gêneros relacionados. Nossa hipótese filogenética não reconhece *Euschistus* como monofilético. Os subgêneros *Euschistomorphus* e *Mitripus* são indicados como linhagems fora de *Euschistus*. Com base nos resultados o subgênero *Mitripus* é elevado a gênero, e *Adustonotus* gen. n. é proposto.

Capítulo III

Molecules enlightening over neglected structures: Exploring the female genital evolution of *Euschistus* (Heteroptera: Pentatomidae) [Moléculas esclarecendo estruturas negligenciadas: explorando a evolução da de genitália de fêmea de *Euschistus* (Heteroptera: Pentatomidae)]

Neste último capítulo utilizamos seis marcadores moleculares para testar a hipótese de relacionamento do grupo *Euschistus*, proposto por L. Rolston. Também buscamos trazer elucidações sobre a evolução de estruturas selecionadas da espermateca. O grupo *Euschistus* não é suportato como monofilético. Além disto, as relações genéricas destes carpocoríneos, que até então eram baseadas em similaridades taxonômicas, agora possuem uma hipótese testada, e. g. *Mormidea* e *Oebalus*. As estruturas da espermateca apresentam grande variação dentro da nossa amostra. Nossa reconstrução de estado ancestral detectou muitas mudanças de estado para maior parte dos caracteres.

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5 CAPÍTULOS (ARTIGOS)

Se podes olhar, vê. Se podes ver, repara.

José Saramago

Sempre imaginei que o paraíso será uma espécie de biblioteca.

Jorge Luis Borges

Capítulo I

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New species of *Euschistus (Euschistus)* from Jamaica, *Euschistus (Mitripus)* and *Ladeaschistus* from southern of South America (Hemiptera, Heteroptera, Pentatomidae, Carpocorini)

ALANA CIOATO[1], FILIPE MICHELS BIANCHI[1], JOSEPH EGER[2] & JOCÉLIA GRAZIA[1]
[1]*Universidade Federal do Rio Grande do Sul, Dep. Zoologia, Av. Bento Gonçalves 9500, 91501-970, Prédio 43435, Porto Alegre RS, Brasil.*
[2] *Dow AgroSciences LLC, 2606 S. Dundee St., Tampa, FL 32629, USA.*

alana.cioato@ufrgs.br, bianchi.fm@hotmail.com, jeeger@dow.com, jocelia@ufrgs.br

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Abstract:

New species of *Euschistus (Euschistus)* from Jamaica and *Euschistus (Mitripus)* and *Ladeaschistus* from southern of South America (Hemiptera, Heteroptera, Pentatomidae, Carpocorini). Three new species of the Carpocorini are described: *Euschistus (Euschistus) baranowskii* sp. nov. from Jamaica, *Euschistus (Mitripus) saramagoi* sp. nov. from Brazil, and *Ladeaschistus borgesi* sp. nov. from Argentina, Bolivia and Brazil. Comparative illustrations of external and internal genitalia of the species are provided.

Key words: Brown stinkbug, Carpocorini, new species, *Euschistus (Euschistus) baranowskii*, *Euschistus (Mitripus) saramagoi*, *Ladeaschistus borgesi*

Introduction

The numbers of species-level taxa that remain undescribed is being debated by researchers (Novotny et al. 2002). Probably there are many more than many researches expect that need to be recognized and described. Identifying and classifying the global biodiversity is an old task to the science. However biodiversity is decreasing faster than ever before (Amato & DeSalle 2012). Concern over the loss of knowledge about these species has resulted in the use of different techniques to generate taxonomic hypothesis. Nevertheless the present or predicted levels of manpower may not handle easily different techniques simultaneously (Will & Rubinoff 2004), given the limitations for obtaining the necessary kind of data to base a holistic and robust species description. Around 30% of tropical arthropods sampled are represented by only one specimen (Coddington et al. 2009). Among invertebrates one out of six newly described species is only known from one specimen (Lim et al. 2012).

Euschistus Dallas, 1851 the largest genus within Carpocorini, containing seventy-six species; contained in four subgenera: *Euschistus*, *Euschistomorphus* Jensen-Haarup, 1922, *Lycipta* Stål, 1862 and *Mitripus* Rolston, 1978. The genus is broadly spread in Nearctic and Neotropical regions and members of the genus are generally known as brown stink bugs. This genus is diagnosed primarily by the following characters: metasternum not produced, peritreme reaching less than 2/5 of the distance from ostiole to lateral margin of segment, superior surface of femora unarmed apically and inferior surface also unarmed, scutellar width at end of frena ½ or less of basal scutellar width, eyes contiguous with pronotum, superior face of tibiae sulcate, juga and tylus or both rounded apically and usually subequal in length, antennae five-segmented. The external morphology within the genus is variable, the body length ranging from 7 to 14 mm and humeral angles being undeveloped to strongly projected as spine or obtuse projection. These characters along with pygophore morphology have been used to define subgenera, and to sort out groups of species within the genus (e.g. *E. sulcacitus* group, *E. crenator* group, see Rolston 1974). The nominal subgenus can be considered a taxonomic dumping ground, since the species belonging to it have been included because they do not share the diagnostic characters of the remaining subgenera.

The subgenus *Mitripus* was proposed by Rolston (1978) based mainly on male genital structures; one pair of small tubercles located on segment X about midway between base and apex of segment X; thecal processes originating within theca; and the lateral walls of the genital cup each bearing a large carina or carinae. *Mitripus* includes the 10 following species: *E. (M.) acutus* Dallas, 1851; *E. (M.) anticus* Stål, 1860; *E. (M.) convergens* (Herrich-Schaeffer, 1842); *E. (M.) grandis* Rolston, 1978; *E. (M.) hansi* Grazia, 1987; *E. (M.) irroratus* Bunde et al. 2006; *E. (M.) latus* (Dallas, 1852); *E. (M.) legionarius* Breddin, 1914; *E. (M.) paranticus* Grazia, 1987; and *E. (M.) tauricornis* Stål, 1872.

Ladeaschistus Rolston, 1973 was proposed to include three species of *Euschistus* in which the femora are armed on the inferior face with conspicuous tubercles, superior ridge of the pygophore is tectiform, and parameres are inversely bent. Included species are *Ladeaschistus armipes* (Stål, 1872), *L. bilobus* (Stål, 1872) and *L. trilobus* (Stål, 1872) and *L. boliviensis* Rolston, 1973.

Both *Ladeaschistus*, and *Mitripus* are exclusively South American in distribution. Rolston (1973), based on the genitalia of both sexes, indicated that *Ladeaschistus* was a homogeneous group. He later indicated that *Ladeaschistus* was related to the South American subgenus *Euschistus* (*Mitripus*) Rolston (1978).

Based on newly found specimens, new species of *Euschistus* (*Euschistus*), *Euschistus* (*Mitripus*) and *Ladeaschistus* are described and the genitalia are illustrated.

Material and methods

The material studied belongs to the following collections (acronyms according to Evenhuis (2014): Coleção do Departamento de Zoologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (UFRG); Florida State Collection of Arthropods, Division of Plant Industry, Gainesville, Florida, USA (JEEC); Instituto Fundación Miguel Lillo, Tucumán, Argentina (IFML); Museo Argentino de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina (MACN); Museu Nacional do Rio de Janeiro, Rio de Janeiro, Brazil (MNRJ); Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil (MZSP).

The genitalia terminology follows Weiler et al. (2011). Description, measurements of 15 morphological parameters and illustrations were performed using a camera lucida coupled to a stereomicroscope. Illustrations were drawn with black pigment ink pen, digitally scanned, and prepared with Adobe Photoshop® and vectorized with Adobe Illustrator®. The measurements are in millimeters (mean, minimum and maximum). Genitalia were prepared using potassium hydroxide (10% or supersaturated heated solution) and stained with Congo red. Pictures were taken using a stereomicroscope with Nikon AZ 100M coupled, with the software NIS - Elements Advanced Research.

Results

Euschistus (Euschistus) baranowskii **sp. nov. Eger & Bianchi**

Etymology: This species is named for R. M. Baranowski who collected extensively in the Caribbean and greatly advanced our knowledge of Heteroptera from that region. The specimen on which the species was based was donated to the Florida State Collection of Arthropods by Dr. Baranowski.

Distribution: Jamaica: Saint Catherine Parish.

Type locality: Linstead, Saint Catherine Parish, Jamaica.

Type material: Holotype: ♀ JAMAICA, *Saint Catherine Parish*: Linstead, 6.IV.1971, R. M. Baranowski col. Black light trap (FCSA)

Description: (Fig. 1) Dorsal surface light brownish-yellow; punctures dark brown, equally distributed but smaller and shallower in fascia on median third of pronotum . Humeri strongly produced laterally. Ventral surface light brownish-yellow, punctures on head and thorax sparse, shallow and concolorous, abdomen unpunctured. Antennae uniformly light brownish-yellow.

Head: Longer than wide, surface light brownish-yellow, dorsal punctures darker and deeper than those on venter. Mandibular plates subequal to clypeus, both obtuse at apex. Antennae uniform light brownish-yellow, segment I with small dots, darker and wider than the segments II-V. Bucculae evanescent at base of head. Rostrum reaching the metacoxae.

Thorax: Pronotum with anterolateral margins denticulate, denticles small, closely spaced but not overlapping; humeri strongly produced laterally, falcate, posterior margin of humeral angle bearing a black punctate spot. Scutellum small, fovea present in basal angles, lateral margins more densely punctured than disc; small ivory callus present at apex. Membrane of hemelytra clear. Ventrally with dark spot at supracoxal cleft of each pleurite, plus at middle of each metaepimeron. Evaporatorium unpunctured, extending more than halfway from ostiole to lateral margin; peritreme spout-shaped. Metasternum carinate. Legs concolorous with thoracic ventral surface femora and tibiae with small dark dots.

Abdomen: Conexivum uniformly light brownish-yellow, ventrally bearing a dark spot at anterolateral margin. Spiracles concolorous with abdomen.

Female Genitalia: (Fig. 4-5) Gonocoxites 8 with sutural margin straight, sutural angle acute and elevated, projected over gonocoxites 9, posterior margin indented where anterolateral angles of gonocoxites 9 fit. Gonocoxites 9 surface slightly convex, anterior and posterior margins continuous and convex. Laterotergites 8 strongly acute at apex, clearly surpassing the posterior margin of laterotergites 9; laterotergites 9 clearly surpassing dorsal band that unites laterotergites 8, lateral margins non-emarginated, inner margins straight.

Measurements: (n=1) Body length 13.30; abdominal width 7.30; head length 2.55; length before eyes 1.38; head width 2.39; interocular distance 1.41; length of antennal segments: I 0.90; II 1.00; III 1.89; IV 1.92; V 1.87; pronotum length 3.19; pronotum width 9.43; scutellum length 5.15; scutellum width 7.70.

Comments: This is a relatively large species within the genus *Euschistus* and as such resembles the larger species such as *E. servus* (Say), *E. heros* (L.), and *E. rugifer* Stål. However, the humeri in these species are not as strongly produced and none of these species have falcate humeri. The falcate humeri will also separate this species from all other species occurring in the Caribbean.

Euschistus (Mitripus) saramagoi **sp. nov. Bianchi, Cioato & Grazia**

Etymology: Named in honor of José Saramago, for his contribution to literature. This late Portuguese writer, who had received the Nobel Prize in Literature 1998, was responsible for the spread of prose in Portuguese around the word.

Distribution: Brazil, Rio Grande do Sul, São Francisco de Paula; Santa Catarina, Mondaí.

Type locality: Mondaí, Santa Catarina, Brazil.

Type material. Holotype ♂. BRAZIL, *Santa Catarina* Mondaí. 14.II.2013, Botega, C. (UFRG).

Paratypes. BRAZIL, Rio Grande do Sul: São Francisco de Paula (FLONA). 2♂ (one without pygophore), 18.XII.2010, Bianchi, F. M. col. [-29.4281, -50.3903] (UFRG). 1♀ (without abdomen), 31.I.2007, Fürstenau, B. B. & Weiler, L. M. col (UFRG). 1♀, 18.XII.2010, Bianchi, F. M. col. [-29.4281, -50.3903] (UFRG), 4♀, 2012, Bianchi, F. M. col. [-294477, -50.5838] (UFRG).

Description: (Fig. 2) Dorsal surface light brown, head and anterior third of pronotum dark brown; punctures dark brown, less numerous on hemelytra. Humeri obtuse. Ventral surface pale yellow, sparsely and shallowly punctured. Antennae pale yellow with black stripes.

Head: Longer than wide; dorsal surface dark brown. Mandibular plates surpassing clypeus, apices convergent, obtuse. Antennae pale yellow, black stripes at apex of segments I and III segments and most of IV and V. Bucculae evanescent at base of head. Rostrum reaching metacoxae.

Thorax: Pronotum with anterior third darker than disc; anterolateral margins dark, denticulate at least along anterior third; humeri developed, obtuse, directed anterolaterally; posterior margin of humeri often reddish; cicatrices of pronotum bearing callus at basal angles, concolorous. Scutellum with small fovea present at basal angles; 1+1 areas more densely punctured at the disc. Membrane of hemelytra clear. Ventrally with dark spot at supracoxal cleft of each pleurite, plus at middle metaepimeron. Metasternum carinate. Legs straw yellow, with coarse dark dots on femora and tibiae.

Abdomen: Conexivum dark brown, bearing a pale yellow spot between anterior and posterior margins. Spiracles black.

Male Genitalia: (Figs. 6-11) Ventral rim of pygophore concave, in "U"-shaped excavation; lateral margins subrectilinear to parallel. Dorsal rim with median third concave, lateral margins subrectilinear. Posterolateral angles of pygophore with clear carina and with bristle tufts. Carina along dorsal rim obsolete; genital cup with superior process of dorsal rim of pygophore (anterior carina and posterior carina of Rolston 1978) in a deeper carina, subtriangular, and partly hidden by the paramere. (Fig. 12) Parameres falciform. Phallus. (Figs. 13-15) Processes of phalloteca sinuous, thickened at base, placed laterally and surpassing the middle of the processus vesicae. Conjunctiva with lateral lobes globose and a large median lobe. Ductus seminis distalis not coiled, as long as the processus vesicae. Length of vesica and conjunctiva equal to half the length of phalloteca.

Measurements: (n=3) Body length 7.3 (7.1-7.5); abdominal width 2.84 (2.71-3.09); head length 1.68 (1.6-1,8); length before eyes 0.96 (0.92-1.00); head width 1.76 (1.72-1.80); interocular distance 1.04 (1.00-1.12); antennal segments length: I 0.53 (0.46-0.53); II 0.77 (0.71-0.77); III 0.71 (0.59-0.71); IV 0.96 (0.84-1.02); V 1.36 (1.18-1.40); pronotum length 1.74 (1.70-1.80); pronotum width 5.48 (5.23-5.61); scutellum length 2.46 (2.46-2.46); scutellum width 2.77 (2.71-2.83).

Female Genitalia: (Figs. 16-18) Gonocoxites 8 with sutural margins sinuous, overlapping at base, posterior margin convex. Gonocoxites 9 trapezoidal, obtuse angles. Laterotergites 8 acute at apex, posterolateral and posterior margins almost straight. Laterotergites 9 obtuse at apex, exceeding the band that unites laterotergites 8 dorsally. Gonapophyses 9 lightly sclerotized on disk. Chitinelipsen elliptical. Posterior portion of the thickening of vaginal intima ovoid; anterior portion rounded. Ductus receptaculi before vesicular area slightly longer than the vesicular area, dilated near that area. Ductus receptaculi after vesicular area uniform. Anterior and posterior annular flanges convergent, posterior annular flange larger than the anterior. Pars intermedialis slightly longer than adjacent ductus receptaculi. Capsula seminalis digitiform, longer than pars intermedialis.

Measurements: (n=5) Body length 8.5 (8.3-8.6); abdominal width 3.60 (3.34-3.72); head length 1.84 (1.72-1.88); length before eyes 1.00 (1.00-1.04); head width 1.84 (1.76-1.88); interocular distance 1.12 (1.12-1.20); length of antennal segments: I 0.49 (0.43-0.50); II 0.77 (0.74-0.84); III 0.65 (0.62-0.71); IV 0.90 (0.87-0.93); V 1.27 (1.05-1.30); pronotum length 1.86 (1.77-1.90); pronotum width 5.86 (5.73-5.92); scutellum length 2.77 (2.65-2.83); scutellum width 3.02 (2.96-3.15).

Comments: The male genitalia suggests that *Euschistus* (*Mitripus*) *saramagoi* is closely related to species of *E.* (*M.*) *anticus* group (*E.* (*M.*) *paranticus, E.* (*M.*) *hansi* and *E.* (*M.*) *irroratus*. *E.* (*M.*) *saramagoi* can be distinguished from *E.* (*M.*) *irroratus* and *E.* (*M.*) *anticus* by the straight median third of the ventral rim of pygophore; from *E.* (*M.*) *paranticus* by the deeper excavation and *E.* (*M.*) *hansi* by the parallel lateral margins of the excavation. The general morphology is similar to *E.* (*M.*) *hansi* but it can be easily distinguished by the striped antennae.

Ladeaschistus borgesi **sp. nov. Bianchi, Cioato & Grazia**

Etymology: Named in honor of Jorge Luis Borges, one of the most important writers from South-America. This late Argentinean writer is a milestone of literary fiction with his metaphysical tales, essays, and poetry.

Distribution: Argentina: Salta, Tucumán; Bolivia: Santa Cruz; Brazil: Mato Grosso, São Paulo, Santa Catarina; Paraguay: Central.

Type locality.São José do Rio Preto, São Paulo, Brazil.

Type material. Holotype ♂. BRAZIL, *São Paulo*: São José do Rio Preto (IBILCE-UNESP), 12.I.2009, Souza, H. V. col. [-20.7869, -49.3606] (UFRG).

Paratypes. ARGENTINA, *Chaco:* 9 de julio. 1♂2♀, ?.II.1996, O. E. Gonzalez col. (FSCA). *Formosa*. 1♂ (without abdomen), Martines, E. (MACN). *Salta:* Urundel. 2♀ (one without abdomen), 25.I.1950, Golbach col. (IFML). *Tucumán:* El Cadillal. 1♂ (without pygophore), 26.II.1966, Barrera, M. col. (IFML). BOLIVIA, *Santa Cruz*: Sara (Nueva Moka). 1♂ (without pygophore) I.1963, Martinez, A. col. (MZSP). 2♀ (one without abdomen), I.1963, Martinez, A. col. (MZSP). BRAZIL, *Mato Grosso*: Cáceres. 1♂ (without pygophore), 10.III.1985, Elias, C. col. (MZSP). Riacho do Herval (Rio Paraná). 3♀ (three without abdomen), (MZSP). Ribeirãozinho. 1♂, 12.II.1962, Bechyné, J. col. (MZSP). Três Lagoas, 2♂, 15.V.1964 (MZSP). *Santa Catarina:* Guatambú (FLONA de Chapecó). 1♀, 18.IX.2004, Bianchi, F. M. col. [-27.0972, -52.7778] (UFRG). *São Paulo*: São José do Rio Preto (IBILCE-UNESP) 4♂ (three specimens without pygophore), 12.I.2009, Souza, H. V. col. [-20.7869, -49.3606] (UFRG). 3♀ (two without abdomen), 12.I.2009, Souza, H. V. col. [-20.7869, -49.3606] (UFRG). PARAGUAY, *Central:* Areguá. 1♂1♀, 10.V.2006, C. Aguilar col. (JEEC). Central: *Asunción.* 1♂, 11-30.X.1990, G. Arrigada col. (JEEC). Central: Capiatá. 1♀, 21.II.1994, G. Arrigada col. (JEEC).

Description: (Fig. 3) Dorsal surface light brown to dark brown; punctures darker than dorsal surface, sparse on hemelytra. Humeri varying from obtuse to acutely projected. Ventral surface pale yellow to pale orange, with brown punctures, sparse and shallow, however those on abdomen concolorous with the ventral surface. Antennae varying from uniformly pale yellow to pale yellow at base darkening toward apex.

Head: Longer than wide. Mandibular plates slightly surpassing clypeus, apices acuminate. Antennae variable, usually pale yellow, but presenting patterns of apex darker than base, in some specimens presenting dark bands at apex of III, IV and V segments. Bucculae evanescent at base of head. Rostrum slightly surpassing metacoxae.

Thorax: Anterolateral margins denticulate at least along anterior half, denticles pale yellow; Humeri varying from obtuse when undeveloped to acutely projected, directed laterad, but not forming a sharp spine; cicatrices of pronotum bearing callus at basal angles, concolorous. Scutellum with small fovea present at basal angles, sometimes flanked by whitish spot; medial whitish spot present on basal margin. Membrane of hemelytra fumose. Ventrally with dark spot at supracoxal cleft of each pleurite, plus at middle metaepimeron. Evaporatorium unpunctures, not reaching halfway between the ostiole and the lateral margin of metapleuron; peritreme spout-shaped. Metasternum carinate. Legs concolorous with ventral surface of thorax; femora armed with spine, small dark spot at base of each spine; pro- and mesofemora bearing two pairs of dark preapical tubercles, the apical pair bigger than basal.

Abdomen: Conexivum dark brown, bearing a pale yellow spot between anterior and posterior angles. Spiracles concolorous with ventral surface of abdomen.

Male Genitalia. (Figs. 19-24) Ventral rim of pygophore deep-concave, middle third flat. Dorsal rim with median third and lateral margins straight, diverging. Superior processes of dorsal rim of pygophore in a large subquadrangular tubercles, located near the base of the paramere. A pair of acute denticles, placed at inner margin of posterolateral angles of pygophore. (Fig. 25) Parameres with ample head, flat dorsoventrally, curved at a 60 ° angle. Phallus. (Figs. 26-28) Processes of the phallotheca curved dorsally, slightly divergent, narrowing towards apex. Conjunctiva with tapered lateral lobes, median lobe sclerotized; ductus seminis distalis not coiled, not reaching the processus vesicae; length of conjunctiva and vesica together corresponds to one third the length of phalloteca.

Measurements: (n=10) Body length 7.8 (7.30-8.50) abdominal width 2,96 (2.77-3.09) head length 1.72 (1.64-1.84) length before eyes 0.94 (0.84-1.08); head width 1.8 (1.68-1.84); interocular distance 0.98 (0.96-1.00); length of antennal segments: I 0.53 (0.50-0.62); II 0.77 (0.67-0.84); III 0.91 (0.74-0.99); IV 1.39 (1.21-1.43); V 1.55 (1.46-1.61); pronotum length 1.75 (1.55-1.86); pronotum width 5.29 (4.78-5.61); scutellum length 2,89 (2.58-2.96); scutellum width 3.02 (2.58-2.96).

Female Genitalia: (Figs. 29-31) Gonocoxites 8 with sutural margin slightly sinuous, sutural angle acute, projected over gonocoxites 9, posterior margin concave. Gonocoxites 9 with obtuse posterolateral angles, surface biconcave. Laterotergites 8 acute, forming a black spine at apex. Laterotergites 9 obtuse at apex, exceeding the band that unites laterotergites 8 dorsally. Gonapophyses 9 with secondary thickening. Chitinelipsen elliptical. Thickening of vaginal intima with anterior and posterior regions subtriangular. Ductus receptaculi before vesicular area, shorter than this area. Ductus receptaculi after vesicular area shorter than pars intermedialis, dilated near the anterior annular flanges. Annular flanges convergent. Pars intermedialis subequal in length to capsula seminalis. Capsula seminalis elongated, digitiform.

Measurements: (n=10) Body length 8.95 (8.1-9.3); abdominal width 3.34 (2.90-4.46); head length 1.84 (1.68-2.12); length before eyes 1.00 (0.88-1.00); head width 1.92 (1.80-2.00); interocular distance 1.1 (1.00±1.12); antennal segments length: I 0.54 (0.50-0.62); II 0.81

(0.65-0.90); III 0.98 (0.90-1.36); IV 1.41 (0.93-1.58); V 1.52 (1.46-1.67); pronotum length 1.97 (1.83-2.01); pronotum width 5.80 (5.04-5.92); scutellum length 3.18 (3.02-3.21); scutellum width 3.34 (3.15-3.46).

Comments: *Ladeaschistus borgesi, L. boliviensis* and *L. armipes* present the same pattern of male genitalia, with the ventral rim broadly with excavation deep-concave, middle third flat. The "facies" of *L. borgesi* resembles that of *L. bilobus* and *L. trilobus*, but the non-emarginated ventral rim of pygophore will distinguish *L. borgesi.*

Acknowledgments:

We thank CNPq for fellowships to the first and third authors, and to CAPES for fellowship to the second author. To Dra. Mary Massumi Itoyama for providing the specimens of *Ladeaschistus borgesi* sp. nov. from São José do Rio Preto, São Paulo.

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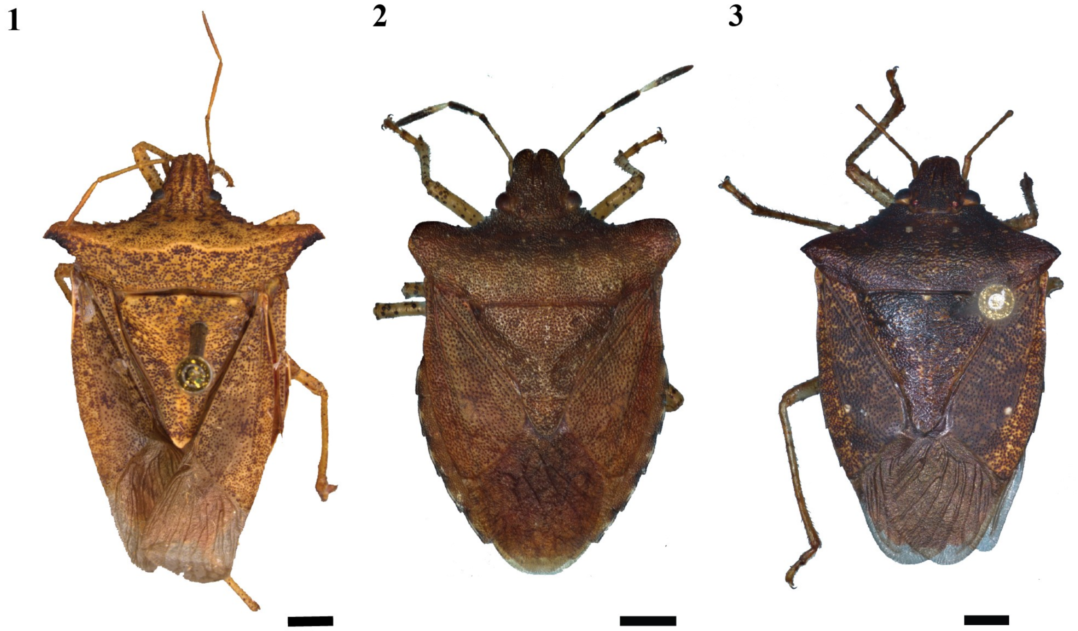
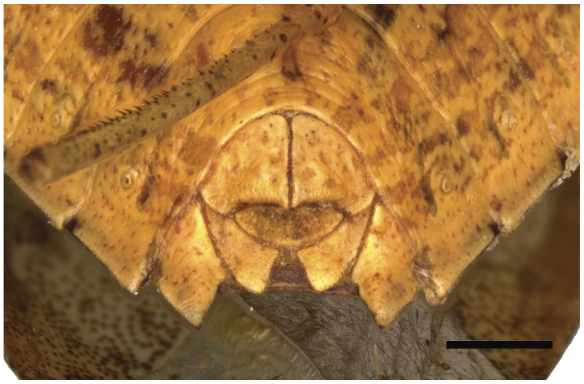
Figures and Captions



FIGURES 1–3. Dorsal view: 1: *Euschistus (Euschistus) baranowskii* sp. nov.; 2: *Euschistus (Mitripus) saramagoi* sp. nov.; 3- *Ladeaschistus borgesi sp nov.* (Scales = 1 mm).

4



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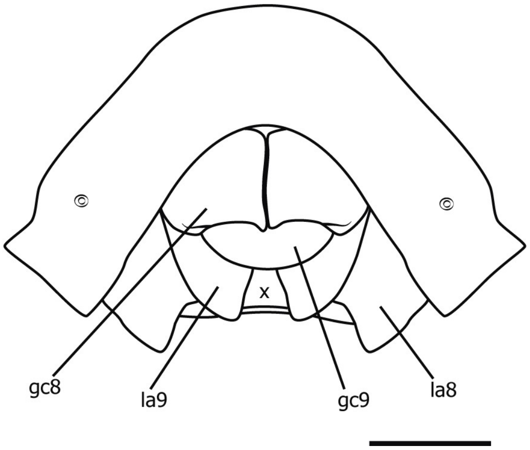
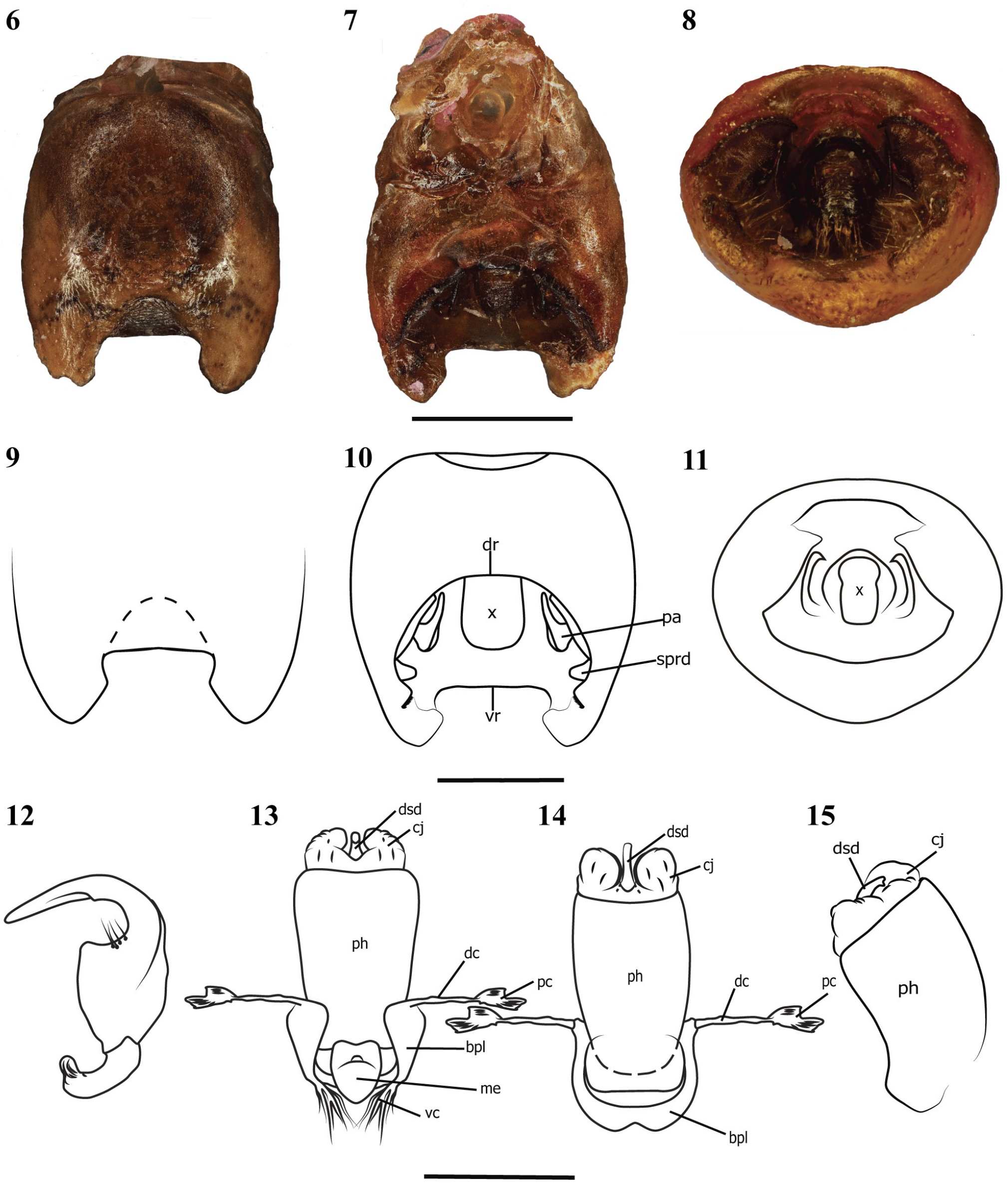
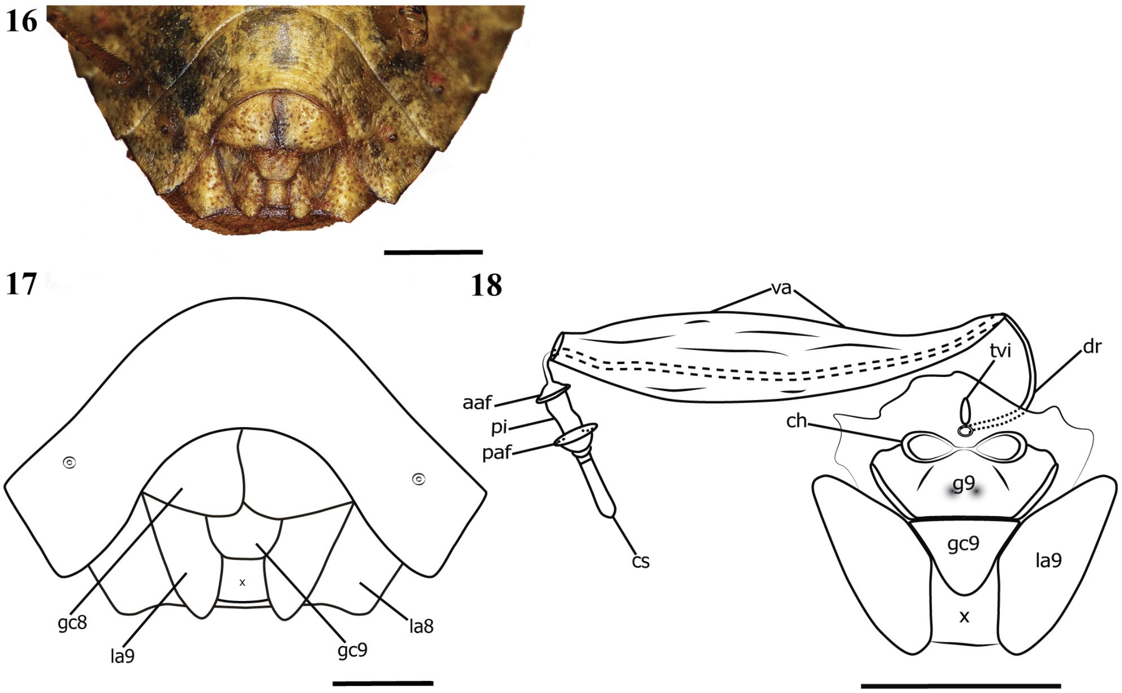


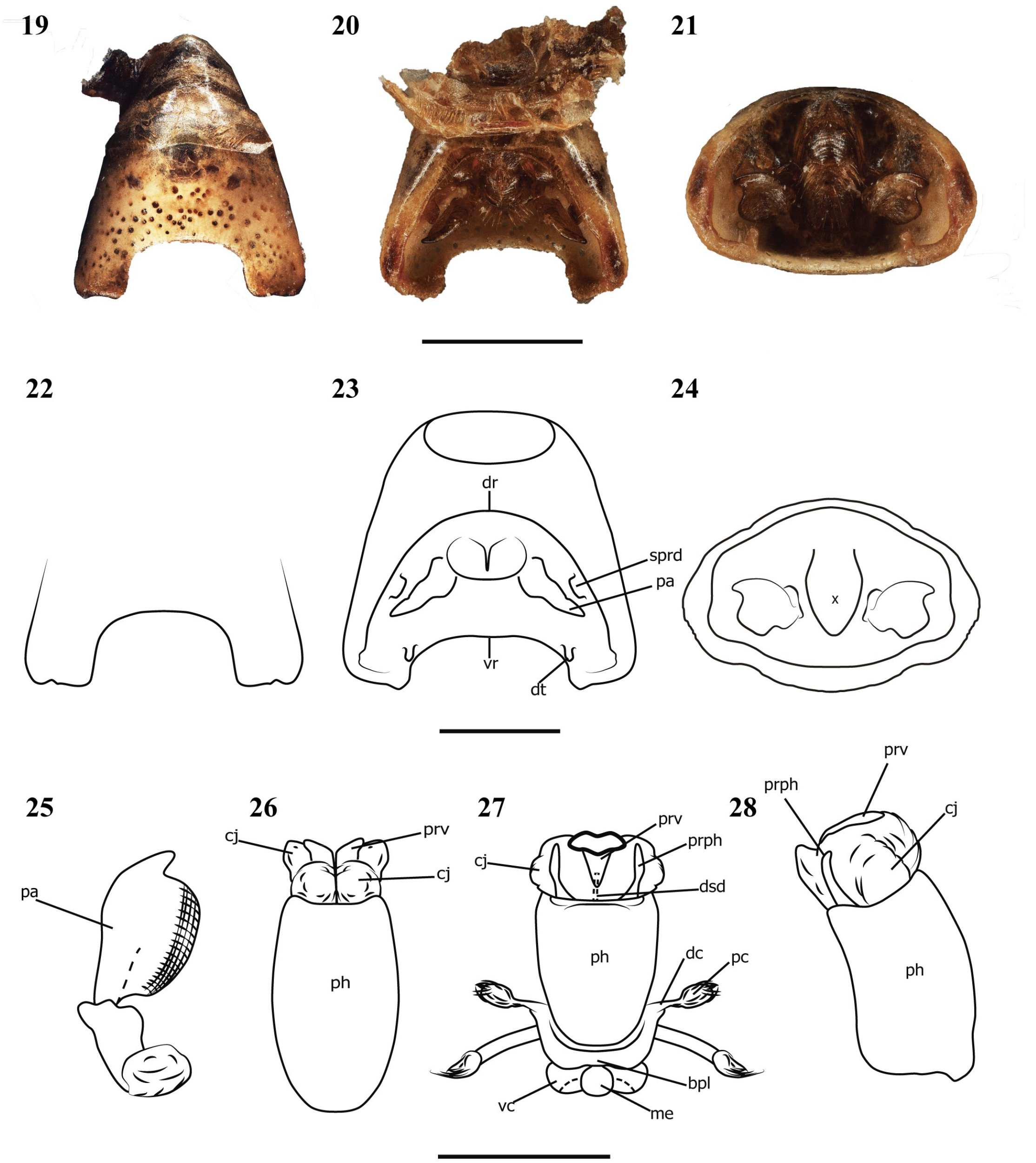
FIGURES 4-5. *Euschistus (Euschistus) baranowskii* sp. nov.; female, genital plates, posteroventral view. (gc8, gonocoxites 8; gc9, gonocoxites 9; la8, laterotergite 8; la9, laterotergite 9; X, proctiger). (Scales = 1 mm)



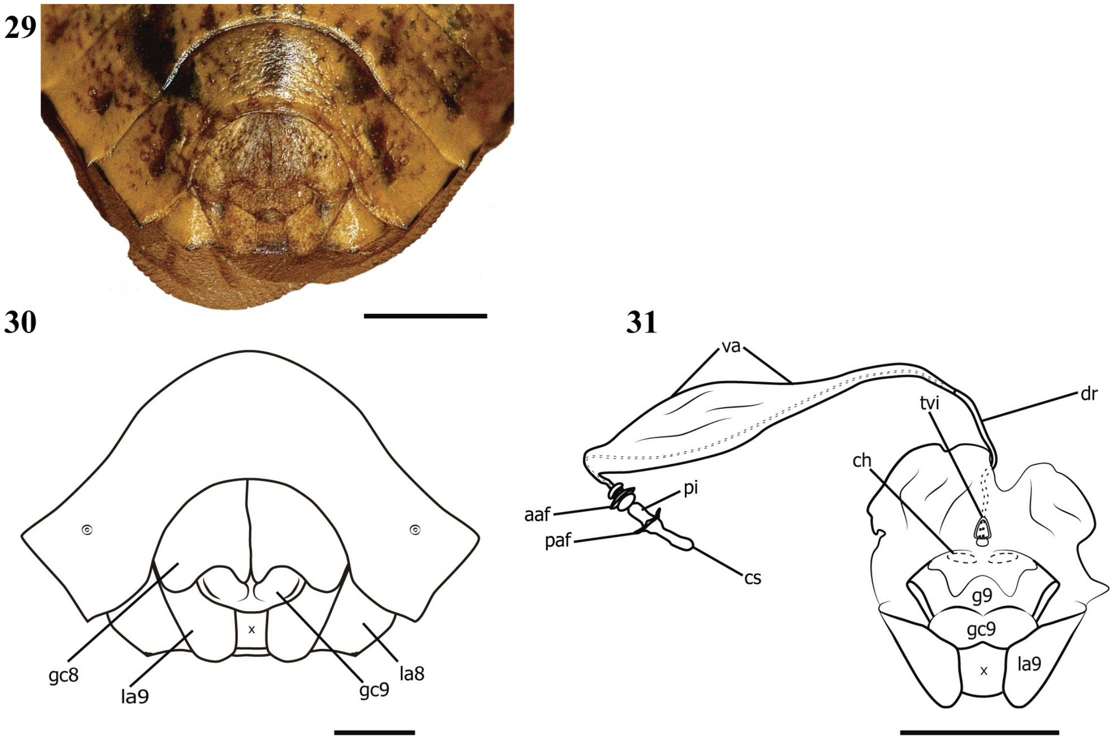
FIGURES 6-15. *Euschistus (Mitripus) saramagoi* sp. nov. 6-11: Pygophore, respectively ventral, dorsal and posterior views. 12: Paramere, left. 13-15: Phallus, respectively ventral, dorsal and lateral views. (bpl, basal plate; cj, conjunctiva; dc, connective dorsal; dr, dorsal rim; dsd, ductus seminis distalis; pa, paramere; pc, processus capittati; ph, phallotheca; vc, connective ventral; vr, ventral rim; X, proctiger). (Scales = 1 mm).



FIGURES 16-18. *Euschistus (Mitripus) saramagoi* sp. nov. 16-17: Female, genital plates, posteroventral view. 18: Laterotergites, gonapophyses and gonocoxites of ninth segment, and ectodermical genital ducts, ventral view. (aaf, anterior annular flange; ch, chitinellipsen; cs, capsula seminalis; dr, ductus receptaculi; gc8, gonocoxites 8; g9, gonapophyses 9; gc9, gonocoxites 9; la8, laterotergite 8; la9, laterotergite 9; paf, posterior annular flange; pi, pars intermedialis; tvi, thickening of vaginal intima; va, vesicular area; X, proctiger). (Scales = 1 mm).



FIGURES 19-28. *Ladeaschistus borgesi* sp. nov. 19-24: Male, pygophore, respectively ventral, dorsal and posterior views. 25: Paramere, left. 26-28: Phallus, respectively ventral, dorsal and lateral views. (bpl, basal plate; cj, conjunctiva; dc, connective dorsal; dr, dorsal rim; dsd, ductus seminis distalis; dt, denticle; me, membroblásica membrane; pa, paramere; pc, processus capittati; ph, phallotheca; prph, processus phallothecae; prv, processus vesicae; spdr, superior process of dorsal rim; vc, connective ventral; vr, ventral rim; X, proctiger). (Scales = 1 mm).



FIGURES 29-31. *Ladeaschistus borgesi* sp. nov. 29-30: Female, genital plates, posteroventral view. 31: Laterotergites, gonapophyses and gonocoxites of ninth segment, and ectodermicalgenital ducts, ventral view. (aaf, anterior annular flange; ch, chitinellipsen; cs, capsula seminalis; dr, ductus receptaculi; gc8, gonocoxites 8; g9, gonapophyses 9; gc9, gonocoxites 9; la8, laterotergite 8; la9, laterotergite 9; paf, posterior annular flange; pi, pars intermedialis; tvi, thickening of vaginal intima; va, vesicular area; X, proctiger). (Scales = 1 mm).

O erro, na verdade, não é ter um certo ponto de vista, mas absolutizá-lo e desconhecer que, mesmo do acerto do seu ponto de vista, é possível a razão ética nem sempre esteja com ele.

Paulo Freire

Capítulo II

Submetido na Revista Systematic Entomology

Total evidence phylogenetic analysis and reclassification of *Euschistus* Dallas within Carpocorini (Hemiptera: Pentatomidae: Pentatominae)

Bianchi F. M., Deprá M., Ferrari A., Grazia J.,Valente V. L. S., Campos L. A.

Abstract: Robust phylogenetic hypotheses have become key for studies addressing the evolutionary biology and ecology of various groups of organisms. In the species-rich heteropteran superfamily Pentatomoidea, phylogenies at lower taxonomic levels are still scarce and mostly employ exclusively morphological data. We here conducted a total evidence phylogeny focusing on the tribe Carpocorini (Pentatomidae), using morphological data and four DNA markers (COI, Cytb, 16S and 28S rDNA; ~ 2330 bp; 32 taxa) in order to investigate the relationships within *Euschistus* Dallas, one of the most speciose pentatomid genera, and between *Euschistus* and related genera. Our hypotheses generated by maximum-likelihood and Bayesian inference show that the current taxonomic composition and classification of *Euschistus* and allied genera are in need of revision. *Euschistus* was recovered as non-monophyletic, with the subgenera forming four independent lineages: *Euschistus* (*Euschistus*) and *Euschistus* (*Lycipta*) Stål are sister groups; *Euschistus* (*Euschistomorphus*) Jensen-Haarup is more closely related to *Dichelops* Spinola and *Agroecus* Dallas; and *Mitripus* Rolston is divided into two clades closely related to *Sibaria* Stål and *Ladeaschistus* Rolston. We choose not changing the classification of *E.* (*Euschistomorphus*) until further date become available, and propose to split *Euschistus* into three genera with the exclusion of the *Euschistus* (*Mitripus*) and all of its species. We here elevate *Mitripus* to genus rank to include *M. acutus* **comb. n.**, *M. convergens* **comb. n.**, and *M. legionarius* **comb. n.**, and propose *Adustonotus* Bianchi **gen. n.** to include *A. anticus* **comb. n.,** *A. latus* **comb. n.**, *A. tauricornis* **comb. n.**, *A. grandis* **comb. n.**, *A. hansi* **comb. n.**, *A. paranticus* **comb. n.**, *A. irroratus* **comb. n.**, and *A. saramagoi* **comb. n.**. We also provide identification keys to the genera *Adustonotus* **gen. n.**, *Ladeaschistus*, *Mitripus* **n. rank** and *Sibaria*, here defined as the *Mitripus* genus group, and to the species of *Mitripus* and *Adustonotus* **gen. n.** Our results provide insights into the current status of the classification of the Pentatomidae, suggesting the need for phylogenetic analyses at different taxonomic levels within stink bugs.

This published work has been registered in ZooBank,
http://zoobank.org/urn:lsid:zoobank.org:act:1B8A3CA9-2F60-4DFC-BA73-6284D7FA6037

Introduction:

Robust phylogenetic hypotheses are key to the advancement of comparative studies addressing questions in different fields of life sciences, such as evolutionary biology and ecology. The first hypothesis of phylogenetic relationships for the Heteroptera (Hemiptera) explicitly employing cladistic methods was developed by Schuh (1979) (for a historical overview see Weirauch & Schuh, 2011; and Ferrari *et al.*, 2015). Since then, phylogenetic hypotheses have been produced for higher-level taxa of Heteroptera based either on morphological (Mahner, 1993) or molecular data (Xie *et al.* 2008), or both data sources combined (Wheeler *et al.*, 1993). While molecular phylogenies are becoming more common for analyses at the family-level and above (e.g. Wu *et al.*, 2016; Zhang *et al.*, 2016), phylogenetic analyses for genus- or tribal-level taxa are usually still generated using only morphological data (e.g., Garbelotto *et al.*, 2013; Namyatova & Cassis, 2016), yet phylogenies using combined datasets are have been published (e.g., Menard *et al.*, 2014; Forero & Weirauch, 2016). However, the high degree of morphological specializations observed in some true bugs, and character convergence between taxa, may negatively impact the reconstruction of phylogenetic hypotheses based on morphology alone (Wu *et al.*, 2016). Modern approaches combining morphological and molecular data have been able, in some cases, to overcome these confounding factors (Abrams *et al.*, 2012).

Pentatomidae, the stink bugs, are the largest family within the Pentatomoidea (Heteroptera), include more than 4720 nominal species in almost 900 genera (Rider, 2016), and present remarkable morphological diversity. The family is found in all zoogeographical regions, but the tropical and subtropical faunas are more diverse than those of temperate regions (Grazia *et al.*, 2015a). The majority of species are phytophagous, but one out of the nine subfamilies is characterized by predacious habits (Grazia *et al.*, 2015b). The monophyly of the Pentatomidae is still under dispute, with analyses based on datasets of morphological (e.g. Gapud, 1991; Henry, 1997), molecular (e.g. Li *et al.*, 2005; Wu *et al.*, 2016), or combined morphological and molecular data (Grazia *et al.*, 2008) resulting in different hypotheses. Phylogenetic hypotheses for subordinate groups within Pentatomidae are scarce (Grazia *et al.*, 2008), and there is no generally accepted phylogenetic framework relationships of the nine subfamilies (*sensu* Rider, 2016). Tribal classifications of the nominal subfamily recognize from eight (Schuh & Slater, 1995) or nine (Grazia *et al.*, 1999) to 42 tribes (Rider, 2016). None of these classifications have been phylogenetically tested, with the Procleticini being the only tribe that has been subjected to a phylogenetic study (Schwertner & Grazia, 2012).

The Carpocorini, whose members occur worldwide, is the largest tribe within Pentatominae, containing 100 genera and approximately 450 species (Rider, 2016). *Euschistus* Dallas occurs exclusively in the New World, and is the most speciose genus within the tribe with 77 species. The species of *Euschistus* are considered largely polyphagous, and some are amongst the most injurious species to agriculture, including *Euschistus* (*Euschistus*) *servus* (Say)*, E.* (*E.*) *heros* (Fabricius)*,* and *E.* (*E.*) *tristigmus* (Say) (Panizzi *et al*., 2000).

Euschistus is subdivided into four subgenera: the nominal subgenus (53 species); *Euschistus* (*Lycipta*) Stål (12 species); the monotypic *Euschistus* (*Euschistomorphus*) Jensen-Haarup; and *Euschistus* (*Mitripus*) Rolston (11 species). The subgenera were proposed based upon diagnostic characteristics mainly from the general habitus and external and internal genitalic morphology. However, the classification of the genus is unsatisfactory. *Euschistus* shows a broad morphological diversity and has been considered a taxonomic dumping ground (Cioato *et al*., 2015). Species-level relationships within *Euschistus* have not been investigated and relationships between *Euschistus* and genera that are treated as similar and potentially related in the taxonomic literature (e.g. *Sibaria* Stål and *Ladeaschistus* Rolston) have not yet been analysed.

In order to investigate the relationship of the subgenera of *Euschistus* and their relationships to *Ladeaschistus* and *Sibaria*, we conducted a total evidence phylogenetic analysis, including nuclear and mitochondrial DNA sequence markers, and morphological characters. We focused on the subgenus *Mitripus* as a case study within the genus, but aimed on a broad taxon sampling to also test the monophyly of both *Ladeaschistus* and *Sibaria*. A new classification scheme for *Euschistus* is proposed based on the phylogenetic results and new generic diagnoses and keys are provided. We believe that this analysis is the first step towards shedding light on relationships within Carpocorini and will provide a model for future analyses that are aimed on generating data toward a robust classification of the Pentatomidae

Material and Methods:

Taxon sampling

Thirty-two terminals were included in datasets herein analysed (Tab. 1). The ingroup comprises 27 species: the 11 described species of *Euschistus* (*Mitripus*); four species of *Euschistus* (*Euschistus*), three of *Euschistus* (*Lypicta*) (selected upon availability), the monotypic *Euschistus* (*Euschistomorphus*), all three species of *Sibaria* and all five species of *Ladeaschistus*. The outgroup comprises representatives of *Dichelops* Spinola (two species), *Agroecus* Dallas, *Caonabo* Rolston, and *Glyphepomis* Berg (one species each). We selected *Glyphepomis spinosa* Campos & Grazia to root the obtained topologies. Specimens used in this study were borrowed from the following collections: USA, New York, American Museum of Natural History (AMNH); Brazil, São Paulo, Museu de Zoologia da Universidade de São Paulo (MZSP); Brazil, Porto Alegre, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRG); Brazil, Curitiba, Universidade Federal do Paraná, Museu de Entomologia Pe. Jesus Santiago Moure (DZUP); Argentina, Tucumán, Instituto Fundación Miguel Lillo (IFML); Brazil, Rio de Janeiro, Fundacão Instituto Oswaldo Cruz (FIOC); Argentina, Buenos Aires, Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN); Italy, Torino, Museo Regionale di Scienze Naturali (MRSN).

Molecular data were not available for nine species of the ingroup (Tab. 1) because they are either rarely collected or no ethanol-preserved specimens were available. Therefore, analyses for two data matrices were carried out, one with the complete taxon sampling (“Full matrix”) and the second that includes only species with at least one DNA marker sequenced (“Reduced matrix”). The latter included 23 terminals representing all relevant supraspecific taxa except the subgenus *Euschistomorphus*.

Morphological characters

The morphological partition is composed of 85 characters (File S1), of which 43 pertain to non-genitalic morphology and 42 to genitalic morphology (15 female-based and 27 male-based characters). Genitalia were dissected and prepared using potassium hydroxide (10% or supersaturated heated solution) and stained with Congo red aqueous solution. The terminology follows Baker (1931), Dupuis (1970), and Schaefer (1977) for genitalic characters, and Kment & Vilímová (2010) for external scent efferent system of the metathoracic glands. Character statements follow the format proposed by Sereno (2007). Morphological data were coded in Mesquite 3.04 (Maddison & Maddison, 2011) and the matrix was exported as a NEXUS file for phylogenetic analyses. Unobserved states were scored with ‘?’ and inapplicable states with ‘-’. All characters were treated as nonadditive.

The morphological matrix is provided in File S2. Morphological characters analysed using probabilistic methods were treated under the Mkv evolutionary model (Lewis, 2001).

DNA extraction, amplification and sequencing

DNA was extracted from ethanol-preserved and pinned specimens. For ethanol-preserved specimens, we preferably removed the right foreleg from the coxal cavity. Pinned specimens had the right pleural sclerites removed to access thoracic muscles. Genomic DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, eluting to a final volume of 100µl when DNA was extracted from ethanol-preserved specimens or 50µl from pinned specimens.

Four DNA markers were amplified, the nuclear D3-D5 region of 28S rDNA (28S) and three mitochondrial gene regions, cytochrome oxidase subunit I (COI); cytochrome b (Cyt b); and 16S rRNA (16S). DNA markers were amplified using Polymerase Chain Reactions (PCR). Primers and PCR annealing temperatures are listed in Tab. S3.

PCR products were purified using Exonuclease I and shrimp alkaline phosphatase (Affymetrix, Inc. USB® Products). Both DNA strands for all PCR products were sequenced by Macrogen, Inc. (Seoul, South Korea). Sequence chromatograms were visually inspected, verified, and manually edited using the STADEN package (Staden *et al.*, 2000). Sequences were verified using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) confirming high similarity of our submitted sequences to pentatomid species. GenBank accession numbers are provided in Tab. 1.

Multiple sequence alignments

Alignments of individual gene regions were performed using MAFFT 7 (Katoh & Standley, 2013) on-line version (http://mafft.cbrc.jp/alignment/server/index.html), applying the G-INS-i strategy to mitochondrial fragments and the Q-INS-i algorithm to ribosomal fragments. Reading frames for each protein-coding gene alignment was checked using the ORF Finder (Open Read Frame Finder) available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/gorf/gorf.html).

The substitution saturation index (Iss) (Xia *et al.*, 2003) was estimated in DAMBE 5 (Xia, 2013) to evaluate the occurrence of substitution saturation in the protein-coding markers. 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) and NEXUS (for MrBayes analyses) formats.

Molecular and morphological datasets were merged using Mesquite 3.04 (Maddison & Maddison, 2011). The concatenated molecular dataset was composed of 23 taxa and 2327 bp, including 528 bp from 28S (available for 15 taxa out of 23), 818 bp from COI (19/23), 450 bp from Cytb (19/23) and 531 bp from 16S (19/23).

Phylogenetic analyses

Exploratory preliminary phylogenetic analyses were conducted individually for each molecular marker (File S4). Separate analyses for the morphological and the concatenated molecular dataset were performed using maximum-likelihood as specified below.
Maximum-Likelihood analyses were performed on the total evidence matrix (four markers + morphological data) using RAxML-HPC2 at CIPRES Science Gateway (Miller *et al*., 2011) (www.phylo.org/portal2/). The GTR-CAT model optimizes site-specific evolutionary rates and was employed for each molecular marker, while the Multi-state-CAT+MK was used for the morphological partition. Nodal support was assessed with autoMRE (automatic Majority Rule Criterion) that automatically stops bootstrapping.
Bayesian inference of the total evidence dataset was performed in the multithreading version of the program MrBayes 3.2.0 (Ronquist & Huelsenbeck, 2003) setting nst=1 rates=equal for morphological partition and nst=6 rates=invgamma for each molecular marker; 4 millions of generation (nruns=2 nchains=4) with trees sampled every 1000 generations. Tracer v.1.6.0 (Rambaut et al. 2014) was used to inspect the convergence to the stationary distribution of the chains. The first 10% of the generations were discarded as “burn-in” and then the chains were combined, the combined ESS values for each parameter were higher than 200. The posterior probability was estimated for the remaining generations. Analyses were conducted for both full and reduced matrices. Phylogenetic trees were visualized and edited using FigTree v1.4.0 (Rambaut *et al.*, 2014) (http://tree.bio.ed.ac.uk/software/figtree/).

Results:

The topologies resulting from the molecular and morphological dataset are partially incongruent, but both trees recover *Euschistus* as non-monophyletic and both recover *E.* (*Mitripus*) + *Ladeaschistus* + *Sibaria* as an independent lineage from the remainder of *Euschistus* (File S4). The maximum-likelihood (Ln score = -14404.743445) (File S5) and Bayesian inference (Fig. 1) phylogenies derived from the total evidence matrix resulted in a similar topology, and nodes were strongly supported by posterior probability (PP) and bootstrap (BS) support values. Topologies obtained from the reduced matrix were fully congruent between the two optimality criteria (Fig. 2; ML tree in File S5). The same relationships between genera and subgenera were recovered in both the reduced (Fig. 2) and full matrices (Fig. 1) analyses, although several clades are better supported in the reduced matrix (see below).

Euschistus (*Mitripus*) was recovered as polyphyletic in the full matrix analyses (Fig. 1). The genus *Euschistus* was not recovered as monophyletic either, with species of *Euschistus* being distributed in four independent lineages. Species of *E.* (*Euschistus*) and *E.* (*Lycipta*) represented in the analyses were each recovered as monophyletic and form sister clades (clade 1, PP= 0.95; BS= 77). *E.* (*Euschistomorphus*) *longiceps* Berg was recovered as more closely related to *Agroecus scabricornis* (Herrich-Schäffer) and *Dichelops* (*Diceraeus*) *furcatus* (Fabricius) than to other species of *Euschistus*, but with low clade support (PP= 0.72; BS= 51).

The clade including the type species of *E.* (*Mitripus*), *E.* (*M.*) *acutus* Dallas (clade 5 PP= 0.70; BS= 36), is sister to *Ladeaschistus* (clade 4 PP= 1; BS= 89), while relationships between that clade, *Sibaria*, (clade 3 PP= 1; BS= 100) and the remaining *E.* (*Mitripus*) spp. (clade 2 PP= 0.87; BS= 66) are unresolved (clade 6 PP= 0.99; BS= 98).

The main differences between trees obtained from the reduced matrix to those from the full matrix are the well-resolved relationships within clade 6. *Sibaria* (clade 3 PP= 1; BS= 100) was recovered as sister to *Ladeaschistus* (clade 4 PP= 1; BS= 100) + (*E.* (*M.*) *acutus* + (*E. (M.) convergens* (Herrich-Schäffer)) (clade 5 PP= 0.82; BS= 78). The clade containing the remaining *E.* (*Mitripus*), clade 2 (PP= 1; BS= 100), was fully resolved and recovered with high support, with *E. (M.) grandis* Rolston as the sister taxon to (*E.* (*M.*) *hansi* Grazia + *E.* (*M.*) *saramagoi* Bianchi, Cioato & Grazia) + (*E.* (*M.*) *paranticus* Grazia + *E.* (*M.*) *irroratus* Bunde, Mendonça & Grazia).

Discussion:

We performed for the first time in Pentatomidae total evidence analyses combining morphological and molecular data. Our results question the current classification and species composition of *Euschistus* and allied genera. Analyses were congruent in reconstructing the genus *Euschistus* to be non-monophyly, placing its *E.* (*Euschistomorphus*) and *E.* (*Mitripus*) in different clades, while combining species currently classified in the subgenera *E.* (*Euschistus*) and *E.* (*Lycipta*) in a separate clade.

Monophyly and phylogenetic relationship of the subgenus Mitripus

Rolston (1973) raised the genus *Ladeaschistus* to include some species originally described in *Euschistus*, based on a set of distinctive female genitalic characters and armed femora. Rolston (1975, 1978) found similar genitalic structures to those in *Sibaria* and *E.* (*Mitripus*), suggesting phylogenetic affinities to these species (for more details on the taxonomic history of these genera see the taxonomy section below). The placement of *Sibaria* (clade 3) and *Ladeaschistus* (clade 4) as closely related to *E.* (*Mitripus*) (clades 2 and 5) recovered herein corroborates Rolston's postulation of a close affinity between *E.* (*Mitripus*) and those genera. Weiler *et al.* (2016) in their cladistic morphological analysis focusing on *Euschistus (Lycipta)*, also always recovered *E.* (*Mitripus*) as being more closely related to *Ladeaschistus* than to other subgenera of *Euschistus*. However, the authors found *E.* (*Mitripus*) either monophyletic or paraphyletic under different K-values (following Mirande, 2009). Weiler *et al.* (2016) did not include either *E.* (*Euschistomorphus*) or any species of *Sibaria* in their analyses.

The two clades containing the species of *E.* (*Mitripus*) (clades 2 and 5) are strongly supported and members can be distinguished by morphological features (Fig. 2). Because the type-species of *E.* (*Mitripus*), *E. (M.) acutus*, was recovered in clade 5 we consider this clade as *Mitripus* **new rank** (see taxonomy section). The species within clade 5 share a few exclusive morphological characters (e.g., mesial portion of ventral rim of the pygophore concave and the coiled pars intermedialis).

The clade that includes the remaining species of *E.* (*Mitripus*) (clade 2) is consistent with relationships previously proposed in the taxonomic literature. Grazia (1987) compared *E.* (*M.*) *hansi* and *E.* (*M.*) *paranticus* to *E.* (*M.*) *anticus* Stål, indicating the three were similar with regard to their general and genitalic morphology, and proposed that they might form a group of species within the subgenus. Bunde *et al.* (2006) tentatively added *E.* (*M.*) *irroratus* to that group, and Cioato *et al.* (2015) also suggested *E.* (*M.*) *saramagoi* as a related species. Although the close relationship of *E.* (*M.*) *anticus* with clade 7 was not recovered, those species bear a singular male genitalic morphology, i.e. the excavation of the ventral rim of pygophore. A well-supported grouping in clade 2 is *E.* (*M.*) *grandis* + *E.* (*M.*) *latus* (Dallas).

Rolston (1978) called attention to the somewhat globose *capsula seminalis* (treated as spermathecal bulb) found in both *E.* (*M.*) *grandis* and *E.* (*M.*) *latus*, rather than the usually finger-like *capsula seminalis* in the remainder *E.* (*Mitripus*). The reduced matrix recovered *E.* (*M.*) *grandis* as sister group to the remaining *E.* (*Mitripus*) of clade 2. Due to the lack of molecular data for *E.* (*M.*) *anticus* and *E. (M.) tauricornis* Stål we cannot be conclusive about the placement of these species in clade 2.

Our phylogenetic results are incompatible with the current classification of *Euschistus*. Not only do clades 2 and 5 represent phylogenetically independent lineages, asking for a taxonomic revision (see taxonomy section), but they were also shown to be only distantly related with the remaining species of *Euschistus*, and thus should be removed from the genus.

Euschistus *(*Euschistomorphus*)* longiceps

The male of *E.* (*Euschistomorphus*) *longiceps* and the female genitalia had not been described in the literature previous to this analysis. This species has a southern distribution in the Neotropical region and is poorly represented in scientific collections. Examination of the female genitalia allowed us to recognize an unusual set of features within the genus *Euschistus.* The internal and external male and female genitalic morphology is similar to *D.* (*Diceraeus*) *furcatus*, *D.* (*Diceraeus*) *lobatus* Grazia, and *Prionotocoris suilari* Kormilev in sharing robust parameres, the overall shape of the genital cup, *pars intermedialis* coiled, *capsula seminalis* globose, and shape of the genital plates.

Our analyses recovered the monotypic subgenus to be more closely related to *A. scabricornis* and *D.* (*Diceraeus*) *furcatus* than to any included species of *Euschistus.* The lack of molecular data for *E.* (*Euschistomorphus*) *longiceps* has likely contributed to the only moderate support of this clade. Until analyses that include additional terminals of *Dichelops, Agroecus*, and closely related genera are available, we cannot precisely determine the position of *E.* (*Euschistomorphus*) *longiceps*. We choose not to change the classification of *E.* (*Euschistomorphus*) until further data become available.

Euschistus *stricto sensu*

Euschistus (*Euschistus*) and *E.* (*Lycipta*) were recovered as reciprocally monophyletic sister clades. The same relationship was found by Weiler *et al.* (2016) while investigating the phylogeny of *E.* (*Lycipta*). Although the species of *E.* (*Euschistus*) included in our analysis are grouped, we do not suggest that the entire subgenus is monophyletic, because our sampling represents only a small number of the species in the subgenus. *Euschistus* (*Euschistus*) is broadly heterogeneous regarding the male genitalic and general morphology, and in the pertinent literature (e.g. Rolston, 1971; 1974; McPherson & Ahmad, 2012) clusters of species are highlighted based on comparative morphology. Rolston (1974), for instance, assigned groups of species with what he considered conspicuous morphological similarities in *Euschistus* (e.g., *E.* (*Euschistus*) *crenator* group, *E.* (*Euschistus*) *strenuus* group, and *E.* (*Euschistus*) *sulcacitus* group). These groups of species may serve as starting points for future phylogenetic studies of *E.* (*Euschistus*).

Conclusions:

This is the first published phylogeny within the Pentatomidae using a total evidence approach that combines morphological data with DNA sequences. Our results provide strong evidence to refute the current classification of *Euschistus*. Analyses based on the combined datasets provided good resolution for relationships at the generic and specific levels. The relationships among supraspecific taxa and among nearly all clades within genera and subgenera were not affected by the use of terminals only represented by morphological data. The bootstrap and posterior probability of their nodes neither substantially changed, as evidenced when comparing the trees from the full and the reduced matrices (Figs. 1 and 2).

We propose to split *Euschistus* into three genera (see the taxonomy section), with the exclusion of *E.* (*Mitripus*) and all of its species. Although it is difficult to obtain fresh specimens of most species of *Euschistus*, we strongly encourage employing total evidence analyses in future studies, at least for the nominal subgenus, because it is the most diverse within *Euschistus* and it has not been tested phylogenetically.

The hypothesis in Rolston's papers based on morphological similarities and the close phylogenetic relationships of *Ladeaschistus*, *Sibaria*, and *E. (Mitripus)* was confirmed here. *Euschistus (Mitripus)* was divided into clades 2 and 5, and both clades are phylogenetically closer to *Ladeaschistus* and *Sibaria* than to other taxa. Clades 2 (*Adustonotus* **gen. n.**) and 5 (*Mitripus* **new rank**), and *Euschistus* sensu stricto represent therefore separate phylogenetic lineages.

Taxonomy:

Conspectus of subgenus Mitripus *and related genera*

Ladeaschistus was originally proposed to include three species of *Euschistus* and a new species, all from South America. They all have femora with conspicuous tubercles on the inferior surfaces, the superior ridge of the pygophore is tectiform, and the parameres are inversely bent, when compared to *Euschistus.* Rolston (1973) compared the genitalic morphology of *Ladeaschistus* species with *E. (E.) tristigmus* and *E. (M.) hansi* (as *E. anticus*), and concluded that "the genital morphology of both sexes demonstrates that *Ladeaschistus* species form a homogeneous group whose phylogenetic relationship is much closer to a group of South American *Euschistus* of which *E. anticus* is representative than it is to the type species *E. tristigmus.*" (p. 104). Concerning the same research, Rolston also suggested a close relationship between *Ladeaschistus* and *Sibaria*, due to both possessing well-developed preapical tubercles on the femora. In a review of *Sibaria*, Roston (1975) highlighted other similarities between *Ladeaschistus* and *Sibaria* in addition to those of external morphology. Rolston (1978) described the *E.* (*Mitripus*) based on male genitalic morphology and the length and orientation of the mandibular plates. Rolston (1978) included within *E.* (*Mitripus*) six species transferred from the nominal subgenus; *E.* (*Mitripus*) *acutus*, *E. (M.) anticus*, *E. (M.) convergens*, *E. (M.) latus*, *E. (M.) legionarius* (Breddin), *E. (M.) tauricornis*; and a new species *E. (M.) grandis*. Four more species were subsequently described in *Mitripus*: *E. (M.) hansi*, *E. (M.) paranticus*, *E. (M.) irroratus*, and *E. (M.) saramagoi*. *Euschistus* (*Mitripus*) is restricted to the Neotropics, its species occurring mainly in South America. Breddin (1914), when describing *E. (M.) legionarius*, indicated similarities with *E. (M.) convergens* regarding the shape of the humeral angles. In the diagnosis of *E. (Mitripus)*, Rolston (1978) called attention to two characters that made *E. (M.) convergens* an exception within the subgenus: the mandibular plates surpass the clypeus (referred to as juga and tylus, respectively), and the dorsal rim of the pygophore bears a diagonal depression. However, our observations showed that *E. (M.) convergens* has the mandibular plates and clypeus subequal in length, and the dorsal rim of the pygophore straight.

Our decisions on raising *Mitripus* **new rank** and describing *Adustonotus* **gen. n.** are supported by the phylogenetic hypothesis here presented, highlighting the common ancestry of the included species. In addition, we stress morphological distinctness while emphasizing affinities among groups of species (Winston, 1999) in an attempt to preserve the diagnosability of these supraspecific taxa.

***Mitripus* Roston, 1978, new rank**

Type species. *Euschistus acutus* Dallas, 1851, by original designation.

Included species. *Mitripus acutus* (Dallas, 1851), **comb. n.**; *M. convergens* (Herrich-Schaffer, 1842) **comb. n.**; *M. legionarius* (Breddin, 1914) **comb. n.**
(File S6 for habitus images)

Distribution. South America

Diagnosis. *Mitripus* is phylogenetically related to *Adustonotus* **gen. n.**, *Ladeaschistus* and *Sibaria*. It can be distinguished from other genera by the unarmed femora, rostrum never surpassing the posterior limit of metacoxae, mandibular plates usually subequal in length to clypeus, and ivory maculae present at apex of the radial vein.

Description. Brown or dark brown; ventrally light brown or orange light brown; legs pale yellow with black spots at bases of setae. **Head:** longer than wide; mandibular plates tapering toward the apex but not spinose, convergent, equal in length to slightly longer than clypeus. Anterior margins of buculae rectilinear or rounded, posterior margins evanescent. Rostrum reaching the metacoxae. Antennae five-segmented. Ocelli reddish, well-developed. **Thorax:** pronotum with 1+1 callus at inner posterior margin of each scar; anterolateral margins toothed; humeral angle projected laterad, varying in size and shape, apex slightly rounded or acute. Scutellum foveate in basal angles. Metasternal gland bearing short spout-like peritreme; evaporatorium not contrasting with ventral color, on metepisternum extending to the lateral margin of thorax. Apex of radial vein presenting small ivory spot. Membrane of hemelytra brownish, translucent. **Abdomen:** connexivum segments brown to dark brown with a pale semicircular spot between the anterior and posterior angle. Spiracles varying from light brown to black. **Female genitalia:** sutural margins of gonocoxites 8 overlapping along basal half. *Ductus receptaculi proximalis* about twice the length of vesicular area, *ductus receptaculi distalis* tubular to dilated. *Pars intermedialis* coiled. **Male genitalia:** Ventral rim of pygophore concave at middle. Segment X entirely convex, presenting bumps near base. *Ductus seminis distalis* short.

***Adustonotus* Bianchi, gen. n.**

http://zoobank.org/urn:lsid:zoobank.org:act:1B8A3CA9-2F60-4DFC-BA73-6284D7FA6037

Etymology. From *adustus* (Latin) burned by the sun, tanned, brown and Greek *noton*, neuter; *notos*, masculine back, ridge; referring to the darker dorsal color.

Type species. *Euschistus anticus* Stål, 1860, here designed.

Included species. *Adustonotus anticus* (Stål, 1860) **comb. n.**; *A. latus* (Dallas, 1852) **comb. n.**; *A. tauricornis* (Stål, 1872) **comb. n.**; *A. grandis* (Rolston, 1978) **comb. n.**; *A. hansi* (Grazia, 1987) **comb. n.**; *A. paranticus* (Grazia, 1987) **comb. n.**; *A. irroratus* (Bunde *et al.* 2006) **comb. n.**; *A. saramagoi* (Bianchi *et al.* 2015) **comb. n.**
(File S7 for habitus images)

Distribution. Argentina, Brazil and Uruguay.

Diagnosis. *Adustonotus* **gen. n.** is phylogenetically related to *Ladeaschistus*, *Mitripus* **new rank**, and *Sibaria*. It can be distinguished from other genera by the unarmed femora, rostrum at least reaching metacoxae, mandibular plates rounded at apex, convergent, and at least slightly longer than clypeus, and spiracles dark brown to black.

Description. Brown or light brown, darker on anterior portion of pronotum and head; ventrally yellowish light brown; legs pale yellow with black spots at bases of setae. **Head:** longer than wide; mandibular plate rounded at apex, convergent, and at least slightly longer than clypeus. Anterior margins of buculae rectilinear or rounded, posterior margins evanescent. Rostrum at least reaching metacoxae. Antennae five segmented. Ocelli reddish, well developed. **Thorax:** pronotum with 1+1 callus at inner posterior margins of scars; anterolateral margins toothed; humeral angles varying in size and shape, apices slightly rounded, acute, or spatulate. Scutellum foveate at basal angles. Metasternal gland bearing a short spout-like peritreme; evaporatorium not contrasting with ventral color, on metepisternum extending to the lateral margin of thorax. Apex of radial vein immaculate, except in *A. latus* **comb. n.**. Membrane of hemelytra somewhat brown, translucent. **Abdomen:** Connexivum segments brown to dark brown with a pale semicircular spot between the anterior and posterior angles of each. Spiracles dark brown to black. **Female genitalia:** Sutural margins of gonocoxites 8 overlapping along the basal half. *Ductus receptaculi proximalis* varying in length, *ductus receptaculi distalis* dilated near anterior flange. *Pars intermedialis* straight. **Male genitalia:** Superior rim of phygophore interrupted by diagonal depression. Segment X entirely convex, presenting bumps near base. *Ductus seminis distalis* short.

Key to *Mitripus* **and related genera**

1. Apex of femora armed … 2

1'. Apex of femora unarmed … 3

2. Rostrum not extending posteriorly to mesocoxae … *Sibaria* Stål

2'. Rostrum reaching or extending posteriorly beyond metacoxae … *Ladeaschistus* Rolston

3. Apex of radial vein bearing ivory spot; anterolateral angles of pronotum never metallic in color … *Mitripus* Rolston **new rank**
3'. Apex of radial vein immaculate; if spot present, then anterolateral angles of pronotum metallic … *Adustonotus* Bianchi **gen. n.**

Key to the species of *Mitripus* Roston, 1978 **new rank** (clade 5)

1. Humeral angles spinose … *acutus* (Dallas) **comb. n.**

1'. Humeral angles rounded to truncate, but never spinose … 2

2. Ventral rim of pygophore with distinct V-shape excavation; posterior margins of gonocoxites 8 concave laterally… *convergens* (Herrich-Schaffer) **comb. n.**

2'. Ventral rim of pygophore slightly excavated in a shallow U-shape; posterior margin of gonocoxites 8 straight … *M. legionarius* (Breddin, 1914) **comb.n.**

Key to the species of *Adustonotus* **Bianchi gen. n.** (clade 2)

1. Humeral angles spinose … *tauricornis* (Stål) **comb. n.**

1'. Humeral angles rounded to truncate, but never spinose … 2

2. Scutellum with 1+1 patches of punctures near base; humeral angles developed into short, rounded projection, if humeral angles spatulate the body length is less than 10 mm … 4

2’. Scutellum uniformly punctured; humeral angles spatulate; body length greater than 11mm … 3

3. Posterior angles of humeri dark; head with anterior margin and base metallic green … *latus* (Dallas) **comb. n.**

3’. Posterior angles of humeri pale; head with anterior margin and base dark or fuscous … *grandis* (Rolston) **comb. n.**

4. Humeral angles broadly spatulate, each subquadrangular at apex … *anticus* (Stål) **comb. n.**

4’. Humeral angles short and rounded, if somewhat developed, apices are never subquadrangular … 5

5. Antennal segments ringed with white and black … *saramagoi* (Bianchi, Cioato & Grazia) **comb. n.**

5’. Antennal segments light brown, sometimes reddish, without rings … 6

6. Ventral rim of pygophore biconvex medially … *irroratus* (Bunde, Mendonça & Grazia) **comb. n.**

6’. Ventral rim of pygophore straight excavated medially… 7

7. Posterolateral angles of pygophore triangular in ventral view, each without lateral incision …*hansi* (Grazia) **comb. n.**

7’. Posterolateral angles of pygophore rectilinear in ventral view, each with lateral incision …*paranticus* (Grazia) **comb. n.**

Figure 1: Bayesian inference consensus tree based on the analysis of four molecular markers and morphological characters for 32 species of *Euschistus* and related carpocorines. Numbers in circles above branches refer to clade numbers discussed in the text. Numbers close to nodes are Bayesian posterior probabilities / Maximum-likelihood bootstrap support, respectively. Only nodal support above PP = 0.5 or BS = 50 are displayed (“--” indicates no support for

that clade). Habitus photos of representative species of *Euschistus* Dallas and other Carpocorini’s genera.

Figure 2: Bayesian Inference consensus tree based on the analysis of four molecular markers and morphological characters for 23 species of *Euschistus* and related carpocorines. Numbers in circles near branches refer to clades discussed in the text. Numbers close to nodes are Bayesian posterior probabilities and maximum-likelihood bootstrap support, respectively. Only nodal support above PP = 0.5 or BS = 50 are displayed (“--” indicates no support). The fast track depicts morphological features for ease recognition of species within the specified clades.

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Table 1: Taxon sampling for the phylogenetic analysis of *Mitripus* and related carpocorines, including collection where DNA voucher specimen is deposited and Genbank accession number for respective DNA fragment. Fragment not sequenced marked with "-".

Species	COI	Cyt b	16S	28S
Agroecus scabricornis (Herrich-Schäffer, 1840)	KU892539	KU853783	KU853764	-
Caonabo pseudoscylax (Bergroth, 1891)	KU892540	KU853784	KU853765	KU853749
Dichelops (*Diceraeus*) *furcatus* (Fabricius, 1775)	KU892541	KU853785	KU853766	KU853750
Dichelops (*Dichelops*) *leucostigmus* (Dallas, 1851)	KU892542	KU853786	KU853767	KU853751
Euschistus (*Euschistomorphus*) *longiceps* Berg, 1891	-	-	-	-
Euschistus (*Euschistus*) *crenator* (Fabricius, 1794)	-	KU853787	KU853768	KU853752
Euschistus (*Euschistus*) *heros* (Fabricius, 1798)	KU892543	KU853788	KU853769	KU853753
Euschistus (*Euschistus*) *rufimanus* Stål, 1872	KU892544	-	-	-
Euschistus (*Euschistus*) *taurulus* Berg, 1878	KU892545	KU853789	KU853770	KU853754
Euschistus (*Lycipta*) *circumfusus* Berg, 1883	-	KU853790	KU853771	KU853755
Euschistus (*Lycipta*) *cornutus* (Dallas, 1851)	KU892546	KU853791	KU853772	KU853756
Euschistus (*Lycipta*) *triangulator* (Herrich-Schäffer, 1842)	-	KU853792	KU853773	KU853757
Euschistus (*Mitripus*) *acutus* Dallas, 1851	KU892547	KU853793	KU853774	KU853758
Euschistus (*Mitripus*) *anticus* Stål, 1860	-	-	-	-
Euschistus (*Mitripus*) *convergens* (Herrich-Schäffer, 1842)	KU892548	KU853794	-	-
Euschistus (*Mitripus*) *grandis* Rolston, 1978	KU892549	KU853795	KU853775	KU853759
Euschistus (*Mitripus*) *hansi* Grazia, 1987	KU892550	KU853796		KU853760
Euschistus (*Mitripus*) *irroratus* Bunde, Grazia & Mendonça, 2006	-	-	KU853776	-
Euschistus (*Mitripus*) *latus* (Dallas, 1852)	-	-	-	-
Euschistus (*Mitripus*) *legionarius* Breddin, 1914	-	-	-	-
Euschistus (*Mitripus*) *paranticus* Grazia, 1987	KU892551	KU853797	KU853777	KU853761
Euschistus (*Mitripus*) *saramagoi* Bianchi, Cioato & Grazia 2015	KU892552	KU853798	KU853778	-
Euschistus (*Mitripus*) *tauricornis* Stål, 1872	-	-	-	-
Glyphepomis spinosa Campos & Grazia, 1998	KU892553	KU853799	-	-
Ladeaschistus armipes (Stål, 1872)	-	-	-	-
Ladeaschistus bilobus (Stål, 1872)	KU892554	KU853800	KU853779	KU853762
Ladeaschistus boliviensis Rolston, 1973	-	-	-	
Ladeaschistus borgesi Bianchi, Cioato & Grazia, 2015	KU892555	-	KU853780	
Ladeaschistus trilobus (Stål, 1872)	-	-	-	
Sibaria andicola Breddin, 1904	-	-	-	-
Sibaria armata (Dallas, 1851)	KU892556	KU853801	KU853781	KU853763
Sibaria englemani Rolston, 1975	KU892557	-	KU853782	-

Figure 1. Bayesian inference consensus tree based on the analysis of four molecular markers and morphological characters for 32 species of Euschistus and related carpocorines. Numbers in circles above branches refer to clade numbers discussed in the text. Numbers close to nodes are Bayesian posterior probabilities / Maximum-likelihood bootstrap support, respectively. Only nodal support above PP = 0.5 or BS = 50 are displayed ("--" indicates no support for that clade). Habitus photos of representative species of Euschistus Dallas and other Carpocorini's genera.

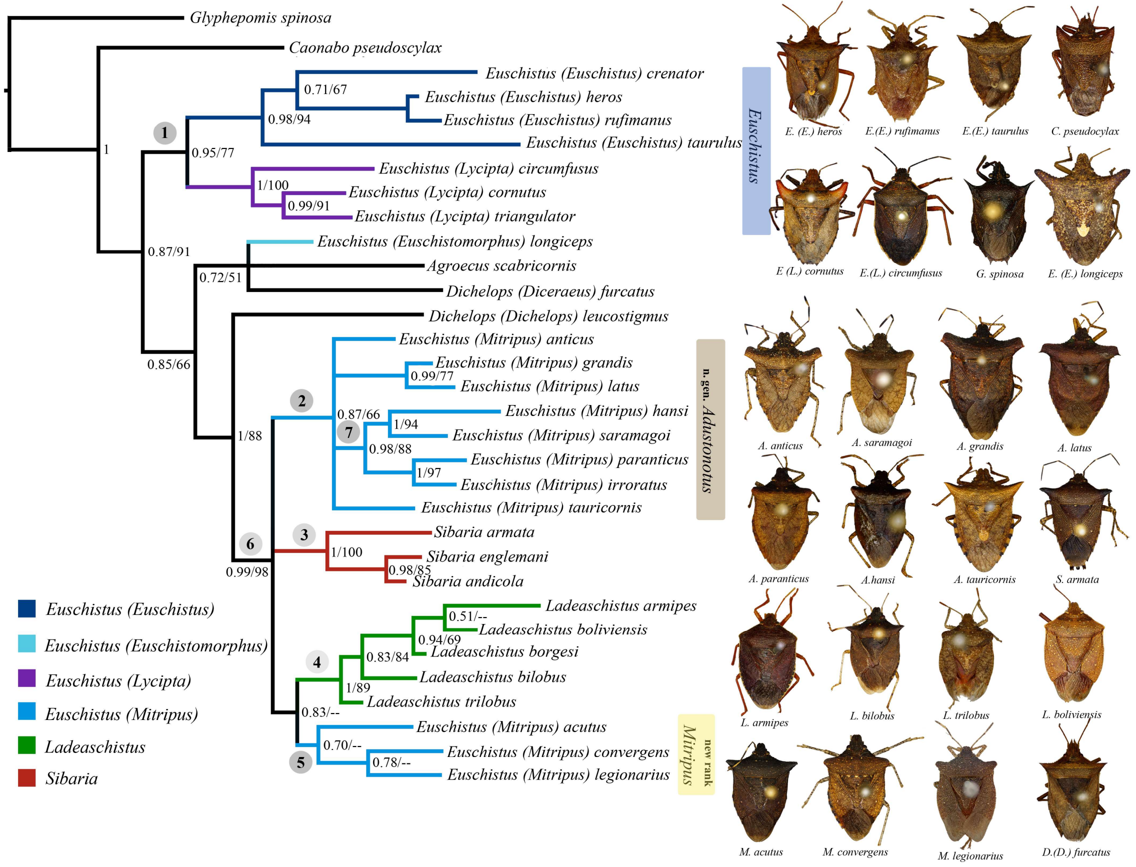
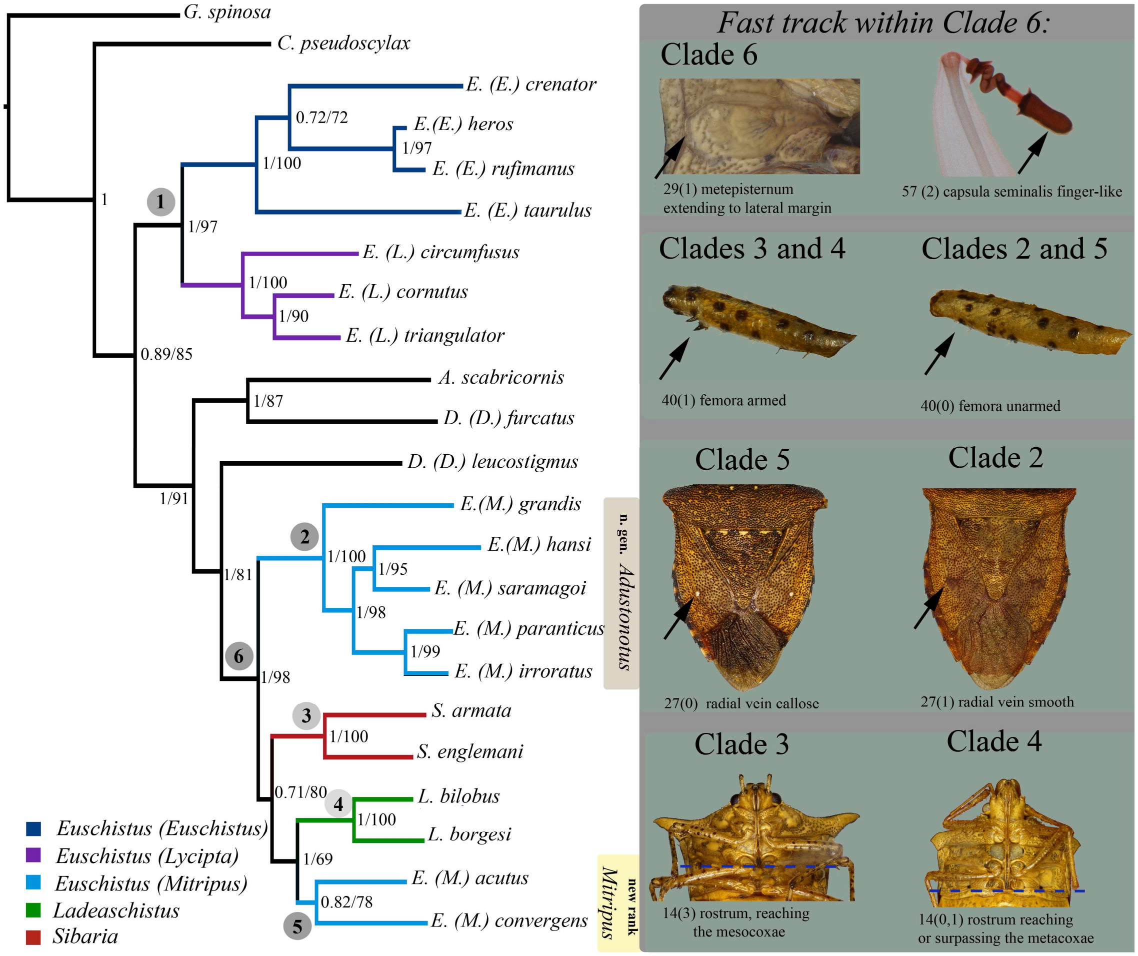


Figure 2. Bayesian Inference consensus tree based on the analysis of four molecular markers and morphological characters for 23 species of Euschistus and related carpocorines. Numbers in circles near branches refer to clades discussed in the text. Numbers close to nodes are Bayesian posterior probabilities and maximum-likelihood bootstrap support, respectively. Only nodal support above PP = 0.5 or BS = 50 are displayed ("--" indicates no support). The fast track depicts morphological features for ease recognition of species within the specified clades.



Supplementary Files.

File Supplementary 1: List of morphological characters examined and coded for the 32 analysed terminals. Data matrix in File S2. Character statements were elaborated following Sereno (2007).

1. Head, mandibular plates, length in relation to clypeus
    - 0 subequal
    - 1 longer
    - 2 shorter
2. Head, mandibular plates, apex, shape
    - 0 rhombus
    - 1 acute
    - 2 pointed
3. Head, apex of mandibular plates, position related to each other
    - 0 convergents
    - 1 parallels
    - 2 divergents
4. Head, mandibular plates, surface
    - 0 flat
    - 1 deflected at lateral margins
5. Head, mandibular plates, internal margin, orientation
    - 0 straight
    - 1 sinuose near to base
6. Head, discal surface
    - 0 flattened
    - 1 convex
7. Clypeus, insertion related to an imaginary line through anterior margin of the eye
    - 0 posterior
    - 1 anterior
8. Head, clypeus, apex, form
    - 0 rhombus
    - 1 acuminate
    - 2 sharp
9. Head, apex, relation between clypeus and mandibular plates
    - 0 contiguous
    - 1 with an incision
10. Head, mandibular plate, position in relation to clypeus, in lateral view
    - 0 equal
    - 1 superior
11. Head, bucculae, anterior margin, form
    - 0 convex
    - 1 rectilinear
12. Head, bucculae, posterior margin, form
    - 0 truncate
    - 1 evanescent
13. Head, gena, form
    - 0 flat

  1 convex
14. Head, rostrum, length
  0 reaching the metacoxae
  1 surpassing the metacoxae
  2 surpassing the mesocoxae
  3 reaching the mesocaxae
15. Thorax, pronotum, posterior margin of scars, inner third, callus
  0 absent
  1 present
16. Thorax, pronotum, anterolateral margins, ornamentation
  0 absent
  1 present
17. Thorax, pronotum, anterolateral margins, ornamentation, form
  0 serrated
  1 crenulated
  2 toothed
18. Thorax, pronotum, anterior half, color related to the disc
  0 lighter
  1 darker
  2 same color
19. Thorax, pronotum, humeral angles, form
  0 rounded
  1 pointed
  2 in right angle
  3 spatulate
20. Thorax, pronotum, humeral angles, position related to pronotal disc in frontal view
  0 same level
  1 higher level
21. Thorax, pronotum, humeral angles, length related to eye diameter
  0 not exceeding
  1 exceeding until twice
  2 exceeding twice
22. Thorax, pronotum, humeral angles, orientation
  0 anteriorly
  1 laterally
23. Thorax, scutellum, apex, callus or calloused stain
  0 absent
  1 present
24. Thorax, scutellum disc, pairs of clustered punctures
  0 absent
  1 present
25. Thorax, scutellum, foveas
  0 absent
  1 present
26. Thorax, hemelytrum, membrane, venation
  0 parallel
  1 reticulate
27. Thorax, hemelytrum, radial vein apex, callus

0 absent
1 present

28. Thorax, hemelytrum, radial vein apex, callus color
    0 ivory
    1 darkened
29. Thorax, mesopleura, metaepisternum, length
    0 not extending to lateral margin
    1 extending to lateral margin
30. Thorax, metapleura, external scent efferent system, evaporatorium, length
    0 not surpassing the middle of distance between ostiole and suture
    1 at least reaching the middle of distance between ostiole and suture
31. Thorax, metapleura, external scent efferent system, evaporatorium, lateral fold
    0 absent
    1 present
32. Thorax, metapleura, external scent efferent system, evaporatorium, gyrification, extension
    0 near to ostiole
    1 covering all surface
33. Thorax, metapleura, external scent efferent system, evaporatorium, lateral limit
    0 convex
    1 straight
    2 sinuose
    3 concave
34. Thorax, external scent efferent system, ostiole, form
    0 guttiform
    1 rounded
35. Thorax, external scent efferent system, evaporatorium, punctures
    0 absent
    1 present
36. Thorax, external scent efferent system, peritreme, median furrow
    0 absent
    1 present
37. Thorax, external scent efferent system, peritreme length
    0 not exceeding twice ostiole diameter
    1 longer than twice ostiole diameter
38. Thorax, legs, pigmentation at bristles base
    0 absent
    1 present
39. Thorax, legs, area of pigmentation at bristles base
    0 restricted to insertion area
    1 broad
40. Thorax, legs, femora
    0 unarmed
    1 armed
41. Abdomen, spiracle, color
    0 luteous
    1 black
    2 concolor
42. Abdomen, Urosternite VII in males, posterolateral angles, form

    0 slightly projected
    1 strongly projected, spine like
43. Abdomen, Urosternite VII in males, anterior margin, concavity
    0 shallow
    1 deep
44. Genital plates, gonocoxites 8, disc, form
    0 convex
    1 concave
    2 flat
45. Genital plates, gonocoxites 8, sutural margins, form
    0 parallel
    1 overlapping along the basal half
46. Genital plates, gonocoxites 8, posterior margin, median third, hairiness density
    0 sparse
    1 concentrated
47. Genital plates, gonocoxites 9, position in relation to segment X
    0 on the same level
    1 oblique, in an obtuse angle
48. Genital plates, gonocoxites 9, surface
    0 convex
    1 depressed
    2 1+1 concave areas
49. Genital plates, gonocoxites 9, median longitudinal furrow
    0 absent
    1 present
50. Genital plates, laterotergites 8, apex, tapered shape
    0 absent
    1 present
51. Genital plates, laterotergites 9, disc, form
    0 concave
    1 flat
    2 convex
52. Genital plates, laterotergite 9, length in relation to the band uniting laterotergites 8
    0 nor or just surpassing
    1 well surpassing
53. Ectodermal ducts, proximal ductus receptaculi, length in relation to the vesicular area
    0 shorter
    1 equal
    2 around twice
54. Ectodermal ducts, proximal ductus receptaculi, basal sclerotization of vesicular area
    0 absent
    1 present
55. Ectodermal ducts, distal ductus receptaculi, next anterior flange, form
    0 tubular
    1 dilated
    2 conic
    3 globose
56. Ectodermal ducts, pars intermedialis, form

  0 rectilinear
  1 coiled
57. Ectodermal ducts, capsula seminalis, form
  0 globose
  1 ovate
  2 fingerlike
58. Pygophore, superior process of dorsal rim, texture
  0 sclerotized
  1 membranous
59. Pygophore, superior process of dorsal rim, form
  0 blade-like
  1 tumescent
60. Pygophore, superior process of dorsal rim, placement
  0 restricted to dorsal rim
  1 projected inward, into genital cup
  2 extending along dorsal rim, reaching posterolateral angles
61. Pygophore, superior ridge, form
  0 contiguous with dorsal rim, not extending over segment X
  1 extending over segment X
62. Pygophore, dorsal rim, diagonal depression
  0 absent
  1 present
63. Pygophore, transverse ridge
  0 inconspicuous
  1 conspicuously
64. Pygophore, transverse ridge, form
  0 restricted to lateral areas of ventral rim
  1 depressed at middle
  2 entire
65. Pygophore, ventral rim, posterolateral angles, bristles tufts
  0 absent
  1 scarcely present
66. Pygophore, ventral rim, posterolateral angles, bristles density
  0 scarce
  1 dense
67. Pygophore, ventral rim at middle, form (ventral view)
  0 slited
  1 biconvex
  2 convex
  3 concave
  4 rectilinear
68. Pygophore, ventral rim at middle, bristles tufts
  0 absent
  1 present
69. Pygophore, segment X, form
  0 entirely convex
  1 flat at apex
  2 entirely concave

70. Pygophore, segment X, size in relation the pygophore diameter
    0 less than 1/3
    1 1/3
71. Pygophore, segment X, bumps
    0 absent
    1 present
72. Pygophore, segment X, bumps, placement
    0 near base
    1 near apex
73. Pygophore, parameres, lateral surface, density of hairy area
    0 scarce
    1 dense
74. Phallus, ductus seminis distalis, length in relation the phallotheca diameter
    0 longer
    1 shorter
75. Phallus, ductus seminis distalis,
    0 straight
    1 coiled
76. Phallus, phallotheca, ventral processes
    0 absent
    1 present
77. Phallus, phallotheca, ventral processes, distance between processes in relation to the processes width at base
    0 subequal
    1 unitil twice
    2 lesser
    3 more than three times
78. Phallus, phallotheca, ventral processes, width
    0 uniform
    1 tapering toward the apex
    2 abruptly tapered at apex 2/3
    3 spatulate
79. Phallus, phallotheca, ventral processes, shape
    0 straight
    1 arched
80. Phallus, vesica, processus vesicae
    0 absent
    1 present
81. Phallus, vesica, processus vesicae, form
    0 lip-like
    1 gutter-like
82. Phallus, secondary gonopore, processus
    0 absent
    1 present
83. Phallus, secondary gonopore processus, form
    0 guetterlike
    1 tumescent
    2 spatulate

84. Phallus, phallotheca, posterolateral angles, rounded projections
    0 absent
    1 present
85. Phallus, phallotheca, insertion of the ductus seminis proximalis in ejaculatory reservoir
    0 near to the base of phallotheca
    1 beyond the middle of the ejaculatory reservoir

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
em acutus	2	0	1	0	0	0	0	0	1	0	0	1	1	0	1	1	2	2	1	0	1	1	0	0	1	0	1	0	1	1	1	0	1	0	0	1	1	1	0	0	2	0	0	0
em anticus	0	1	0	0	0	0	0	0	1	0	1	1	1	0	1	1	2	1	3	1	2	1	0	1	1	0	0	-	1	1	1	0	1	1	0	1	1	1	0	0	1	0	1	0
em convergens	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	1	2	1	0	0	0	1	0	0	1	0	1	0	1	1	1	0	1	0	0	1	0	1	0	0	2	0	1	2
em grandis	0	0	1	1	0	0	0	0	1	0	1	1	1	1	1	1	2	1	3	1	2	0	0	0	1	0	0	-	1	1	1	1	1	1	0	1	0	1	0	0	1	0	1	0
em hansi	0	0	0	1	0	1	0	0	1	0	0	1	0	1	1	1	2	1	0	0	1	0	0	1	1	0	0	-	1	1	1	1	1	0	0	0	1	1	0	0	1	0	1	2
em latus	0	0	1	1	0	0	0	0	1	1	1	1	1	1	1	1	2	1	3	1	2	1	0	0	1	0	1	0	1	0	1	1	1	1	0	0	0	1	0	0	1	0	0	0
em paranticus	0	0	0	1	0	0	0	0	1	0	0	1	1	1	1	1	2	1	0	0	0	1	0	1	1	0	0	-	1	1	1	1	1	1	0	1	1	1	0	0	1	0	1	0
em tauricornis	2	0	1	0	0	0	0	0	1	0	1	1	1	0	?	1	2	2	1	1	2	1	1	1	1	0	1	1	1	0	1	1	2	1	1	1	1	1	0	0	1	0	1	0
em irrotarus	0	0	0	1	0	0	0	0	1	0	0	1	1	0	1	1	2	1	0	0	0	1	0	1	1	1	0	-	1	1	1	1	1	1	0	1	1	1	0	0	1	0	1	?
em legionarius	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	2	1	3	0	1	1	0	0	1	0	0	-	1	1	1	0	1	1	1	1	1	1	0	0	1	0	1	2
em saramagoi	0	0	1	1	0	0	0	0	1	1	0	1	0	1	1	1	2	1	0	0	1	0	0	1	1	0	0	-	1	1	1	1	2	1	1	0	0	1	0	0	1	0	1	2
ee crenator	2	1	0	1	0	1	0	0	2	1	0	1	1	0	0	1	0	2	1	0	1	1	1	0	1	0	0	-	0	1	0	0	2	1	0	0	1	0	-	0	2	0	1	0
ee heros	2	1	0	1	0	1	1	0	2	1	0	1	1	1	0	1	0	2	1	1	2	0	1	0	1	0	0	-	0	1	0	0	3	1	1	1	1	0	-	0	2	0	0	2
ee rufimanus	2	1	0	0	0	1	2	0	2	1	0	1	1	0	0	1	2	2	1	0	1	0	1	0	1	0	0	-	0	0	0	1	0	1	1	0	0	1	0	0	1	1	1	2
ee taurulus	2	1	0	1	0	1	0	1	0	0	1	1	1	0	0	1	2	2	1	0	2	0	1	0	1	0	0	-	0	1	0	0	1	1	0	0	0	0	-	0	2	1	1	2
el circunfusus	2	1	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	2	2	0	0	1	0	0	1	0	1	0	0	1	0	?	0	1	1	0	1	1	0	0	1	0	0	1
el cornutus	1	1	0	1	0	1	1	1	0	0	0	1	0	1	0	1	2	2	3	0	2	0	1	1	1	0	0	-	0	0	1	1	0	1	1	0	0	1	1	0	0	1	0	1
el triangulator	2	1	1	1	0	1	1	1	2	0	0	1	1	0	0	1	2	2	1	0	2	0	1	0	1	1	1	0	0	0	1	1	0	1	1	0	0	1	1	0	0	1	0	2
eem longiceps	2	0	0	0	1	0	0	0	1	1	0	1	1	0	0	1	2	2	1	0	2	0	1	0	1	0	0	-	0	0	?	1	0	1	1	0	0	0	-	0	2	0	0	0
l armipes	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	1	2	2	0	0	0	0	0	1	0	0	-	1	1	1	0	2	0	0	0	1	0	-	1	2	0	1	0
l bilobus	2	1	0	0	0	0	0	1	1	0	1	1	1	0	1	1	?	2	0	0	1	1	0	0	1	0	0	-	1	1	1	0	2	1	0	0	1	1	0	1	2	0	1	2
l trilobus	2	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	2	1	1	0	1	1	0	1	1	0	0	-	1	1	1	0	1	1	0	0	1	1	0	1	2	0	1	?
l boliviensis	2	0	0	1	0	0	0	1	1	0	1	1	1	1	1	1	0	2	2	0	0	1	0	0	1	0	1	0	1	1	1	0	2	0	0	0	1	0	-	1	2	0	0	2
l borgesi	2	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	2	2	1	0	0	1	0	1	1	0	1	0	1	1	1	0	2	0	0	0	1	1	0	1	2	0	0	2
c pseudocylax	0	1	1	1	0	1	1	1	1	1	0	0	1	2	1	0	-	2	1	0	1	0	0	0	0	0	0	-	1	1	0	1	3	1	1	0	0	1	0	0	2	1	0	0
a scabricornis	2	0	1	1	0	0	0	0	1	0	1	0	1	2	1	1	2	2	1	0	1	0	1	0	1	0	0	-	0	0	1	0	0	1	1	0	0	0	-	1	1	0	0	0
d leucostigmus	1	2	0	1	0	0	1	1	1	1	1	0	1	1	1	1	0	2	1	0	1	0	0	0	1	0	0	-	0	1	0	1	1	1	1	0	1	0	-	0	1	1	1	0
d furcatus	1	2	0	1	0	1	0	0	1	0	0	1	1	1	0	1	0	0	1	1	2	1	0	0	1	0	0	-	0	0	0	0	1	1	0	0	1	1	0	0	0	1	1	0
g spinosa	1	1	0	1	1	1	1	0	2	1	0	1	0	1	0	0	-	2	1	0	2	0	0	0	0	0	0	-	1	0	0	1	0	1	1	1	0	0	-	0	1	1	0	0
s armata	2	0	1	0	0	0	0	0	0	1	1	1	1	3	1	1	1	2	1	0	1	1	0	1	1	0	1	0	1	1	1	0	1	0	0	0	1	1	0	1	1	0	0	2
s englemani	2	0	0	0	0	0	0	0	0	1	1	1	1	3	1	1	1	2	1	0	1	1	1	1	1	0	1	0	1	1	1	0	0	0	0	0	1	1	0	1	2	0	0	0
s andicola	2	0	0	1	0	1	0	0	0	1	1	1	1	2	1	1	1	2	1	0	1	1	1	1	1	0	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	0	0	0

	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
em acutus	1	0	0	1	0	0	0	1	0	2	0	1	1	2	0	1	0	0	1	1	0	0	-	3	0	0	0	1	0	0	1	-	2	1	1	1	0	0	-	1	1
em anticus	1	0	0	0	0	0	0	1	0	2	1	1	0	2	0	1	0	0	1	1	0	1	0	3	0	0	0	1	0	0	1	-	0	1	1	1	0	?	?	1	1
em convergens	1	0	0	1	0	0	0	0	0	2	0	1	1	2	0	0	0	1	0	1	0	0	-	3	0	0	0	1	0	0	1	-	1	1	1	1	1	1	-	1	1
em grandis	1	0	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	1	1	1	0	1	0	2	0	0	0	1	0	0	1	-	1	1	1	1	1	?	-	1	1
em hansi	1	0	0	0	0	0	0	0	0	2	0	1	0	2	0	1	2	1	1	0	0	0	-	4	0	0	0	1	0	0	1	-	0	1	1	1	1	1	-	1	1
em latus	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	0	1	0	1	0	0	0	1	0	0	1	-	1	1	1	1	1	?	-	1	1
em paranticus	1	0	0	1	0	0	0	0	0	0	0	1	0	2	0	1	0	1	1	1	0	0	-	4	0	0	0	1	0	0	1	-	3	1	0	1	1	?	-	1	1
em tauricornis	1	0	0	0	0	0	0	0	0	1	0	1	0	2	0	1	2	1	1	1	0	1	0	4	0	0	0	1	0	0	1	-	0	1	1	1	1	1	-	1	1
em irrotarus	1	?	0	0	0	0	0	1	0	1	0	1	0	2	0	1	?	1	1	1	0	0	-	1	0	0	0	1	0	0	1	-	3	1	0	1	1	1	?	1	1
em legionarius	1	0	0	0	0	0	0	1	0	2	0	0	1	2	0	0	?	0	0	1	0	0	-	3	0	0	0	1	0	0	1	-	1	1	1	1	1	1	2	1	0
em saramagoi	1	0	0	0	0	0	0	0	0	1	0	1	0	2	0	1	2	1	1	0	0	1	0	4	0	0	0	1	0	0	1	-	3	1	0	1	1	1	2	1	1
ee crenator	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1	2	1	1	1	1	0	-	3	0	1	0	1	1	0	0	1	2	0	0	1	1	0	-	0	0
ee heros	0	1	1	0	1	1	0	2	0	1	0	1	0	0	0	0	0	1	0	1	1	0	-	1	0	1	0	1	1	1	0	1	2	1	1	1	1	0	-	0	1
ee rufimanus	0	1	1	0	1	1	0	2	0	2	0	2	0	0	0	0	0	1	0	1	0	1	0	3	0	1	1	1	1	1	0	1	1	2	0	1	1	1	1	1	1
ee taurulus	0	0	1	0	0	0	0	2	0	1	0	2	0	1	0	0	0	0	0	1	1	0	-	2	0	1	0	1	1	0	0	1	2	1	1	1	1	0	-	0	0
el circunfusus	1	1	1	0	1	0	0	1	0	1	0	1	0	0	1	1	0	0	0	1	1	1	0	3	0	1	1	1	1	1	0	1	2	2	1	1	1	1	2	1	1
el cornutus	1	1	0	0	1	0	0	2	0	1	0	3	0	0	1	1	2	1	0	1	1	1	0	1	0	1	1	1	1	1	0	1	2	2	1	1	1	1	2	1	1
el triangulator	1	1	1	0	1	1	0	2	0	1	0	3	0	0	1	1	2	0	0	1	1	1	0	2	0	1	1	1	1	0	0	1	2	2	1	1	1	1	2	1	1
eem longiceps	1	1	1	?	0	0	0	2	0	0	0	1	1	0	0	1	1	1	0	1	1	1	0	1	0	1	0	1	0	0	1	-	1	1	1	1	0	0	-	0	1
l armipes	1	0	0	2	0	0	0	0	0	1	0	1	0	2	0	0	0	1	1	0	0	1	0	3	1	0	0	1	0	0	1	-	3	1	0	0	-	0	-	1	1
l bilobus	1	0	0	2	0	0	0	0	0	1	0	1	1	2	0	0	0	1	0	0	0	1	0	2	1	0	0	1	0	0	1	-	0	1	0	0	-	0	-	1	1
l trilobus	?	?	0	2	0	?	0	0	0	1	0	1	1	2	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	-	1	1	1	0	-	0	?	1	1
l boliviensis	1	0	0	2	0	0	0	0	0	1	0	1	?	?	0	0	0	1	0	0	0	1	0	3	1	0	0	1	0	0	1	-	0	1	1	0	-	0	?	1	1
l borgesi	1	0	0	2	0	0	0	0	0	1	0	1	1	2	0	0	0	1	0	0	0	1	0	3	1	0	0	1	0	0	1	-	1	1	1	0	-	0	?	1	1
c pseudocylax	1	0	1	?	1	1	0	0	0	0	0	2	1	0	0	0	0	1	0	1	1	0	?	2	0	2	0	0	-	0	0	0	0	3	1	1	1	1	0	1	0
a scabricornis	0	0	?	?	?	0	0	1	0	0	0	?	?	?	1	1	2	1	0	1	0	1	0	0	0	1	1	1	1	0	1	-	1	1	0	0	0	0	?	1	0
d leucostigmus	0	0	?	?	?	1	1	1	0	0	0	1	0	2	0	0	1	1	0	1	2	1	1	3	0	0	0	1	1	0	1	-	1	2	1	1	?	?	?	0	1
d furcatus	1	1	1	0	0	1	0	1	1	0	0	1	1	0	0	0	1	1	0	1	2	1	0	1	0	0	0	1	1	0	1	-	0	1	1	?	?	0	-	1	1
g spinosa	0	0	0	0	0	0	0	1	0	1	0	3	0	0	0	0	1	1	0	1	1	0	?	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	?	0	0
s armata	1	0	0	0	0	1	0	0	0	0	1	1	1	2	0	0	0	1	0	0	0	1	0	4	0	0	0	1	0	0	1	-	1	1	1	1	1	1	2	0	1
s englemani	1	0	0	0	0	1	0	0	0	0	1	1	1	2	0	0	0	1	0	0	0	1	0	3	0	0	0	1	0	0	1	-	1	1	1	1	1	1	2	0	1
s andicola	1	0	0	0	0	1	0	0	0	0	1	1	1	2	0	0	0	1	0	0	0	1	0	3	0	0	0	1	0	0	1	-	1	1	1	1	1	1	2	0	1

Table Supplementary 3. Genes included in study, primer sequences and sources and annealing temperatures (TA).

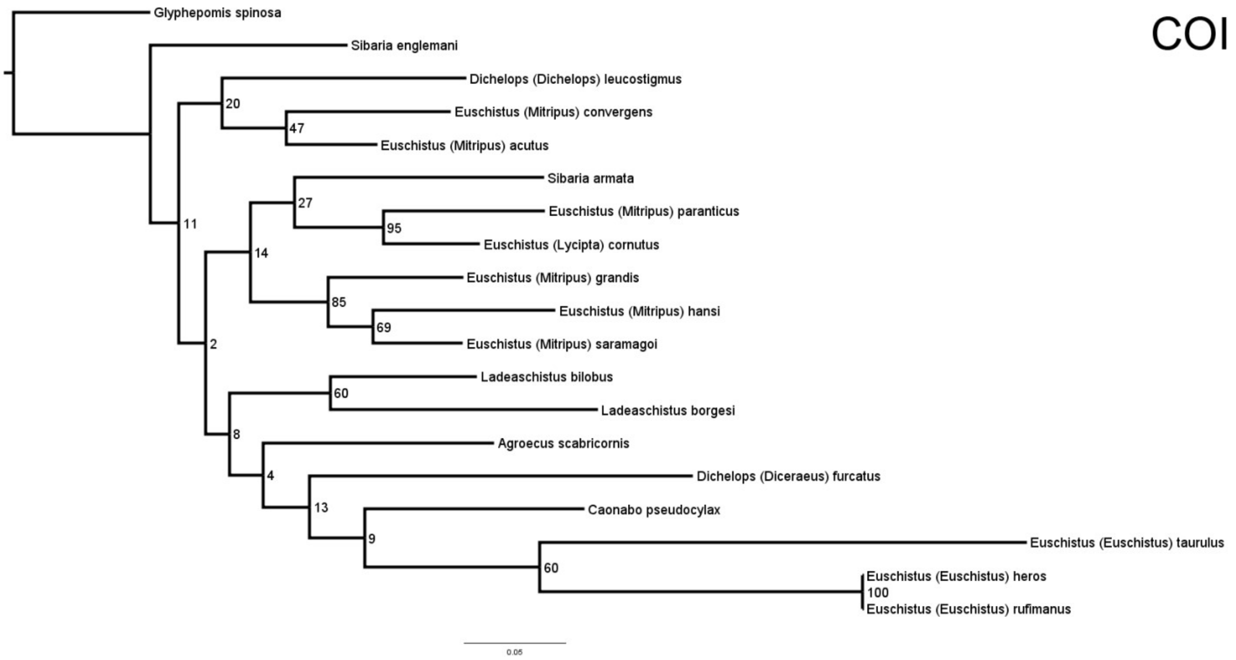
Gene	**Primer name and sequence(5’-3’)**	**Primer source**	**TA**
16S rDNA	16S: CCG GTT TGA ACT CAG ATC ATG T	Simon *et al.*, 1994	48°C
	16S: CGC CTG TTT AAC AAA AAC AT	Simon *et al.*, 1994	
Cyt b	Cytb: TAG GAT ATG TTT TAC CTT GAG GAC A	Mujari *et al.*, 2000	45-50°C
	Cytb: TCC TCC TAA TTT ATT AGG AAT TG	Mujari *et al.*, 2000	
COI	COI: GGA ACA GGA TGA ACA GTT TAC CCT CC	Simon *et al.*, 1994	45-50°C
	COI: TCT GAA TAT CGT CGA GGT ATT CC	Simon *et al.*, 1994	
28S D3-5	28S: ACC CSC TGA AYT TTA AGC CAT	Yu *et al.*, 2013 modified	52°C
	28S: AAC TCT CC MTT CAR AGT TC	Yu *et al.*, 2013 modified	

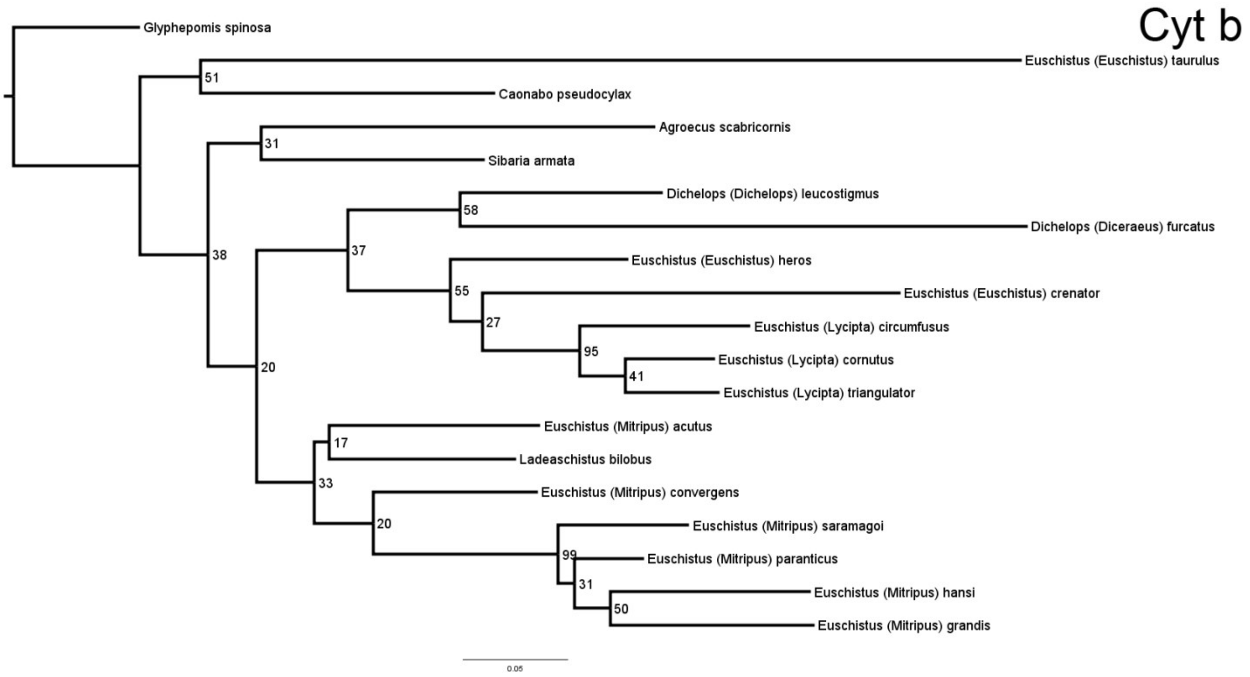
Muraji, M., Kawasaki, K. & Shimizu, T. (2000) Phylogenetic utility of nucleotide sequences of mitochondrial 16S ribosomal RNA and cytochrome b genes in anthocorid bugs (Heteroptera: Anthocoridae). *Applied Entomology and Zoology*, 35, 293–300.

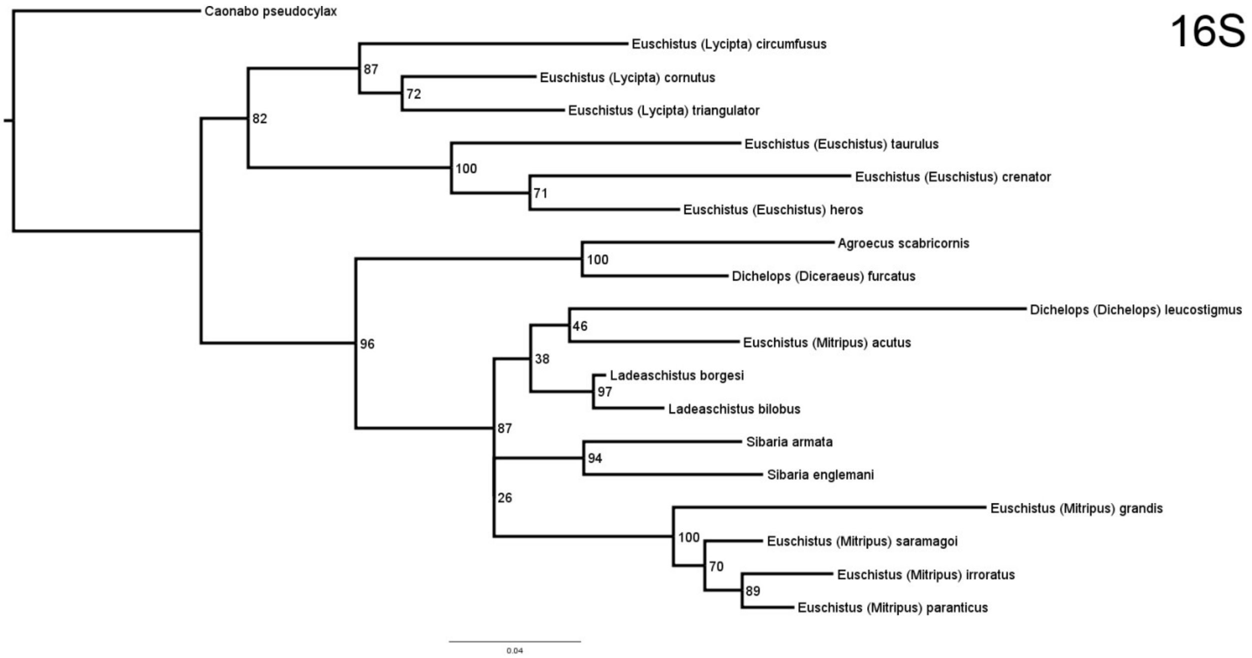
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America*, *87*(6), 651-701.

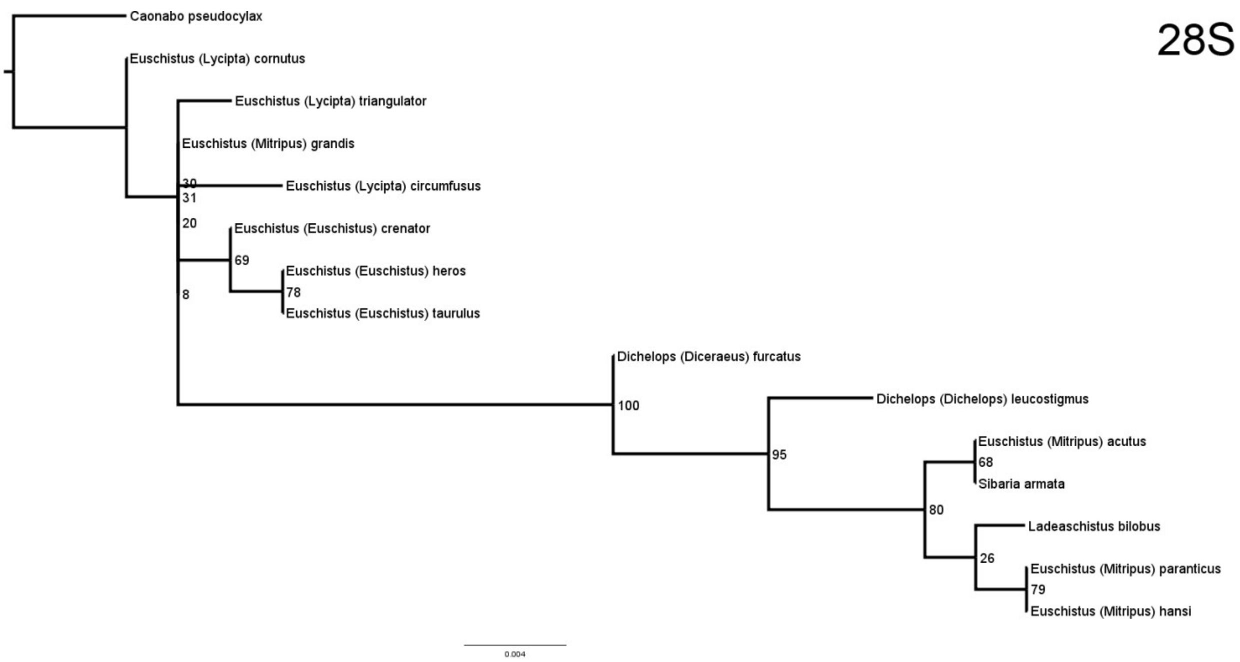
Yu, S., Wang, Y., Rédei, D., Xie, Q., & Bu, W. (2013). Secondary structure models of 18S and 28S rRNAs of the true bugs based on complete rDNA sequences of Eurydema maracandica Oshanin, 1871 (Heteroptera, Pentatomidae). *ZooKeys*, 319, 363-377.

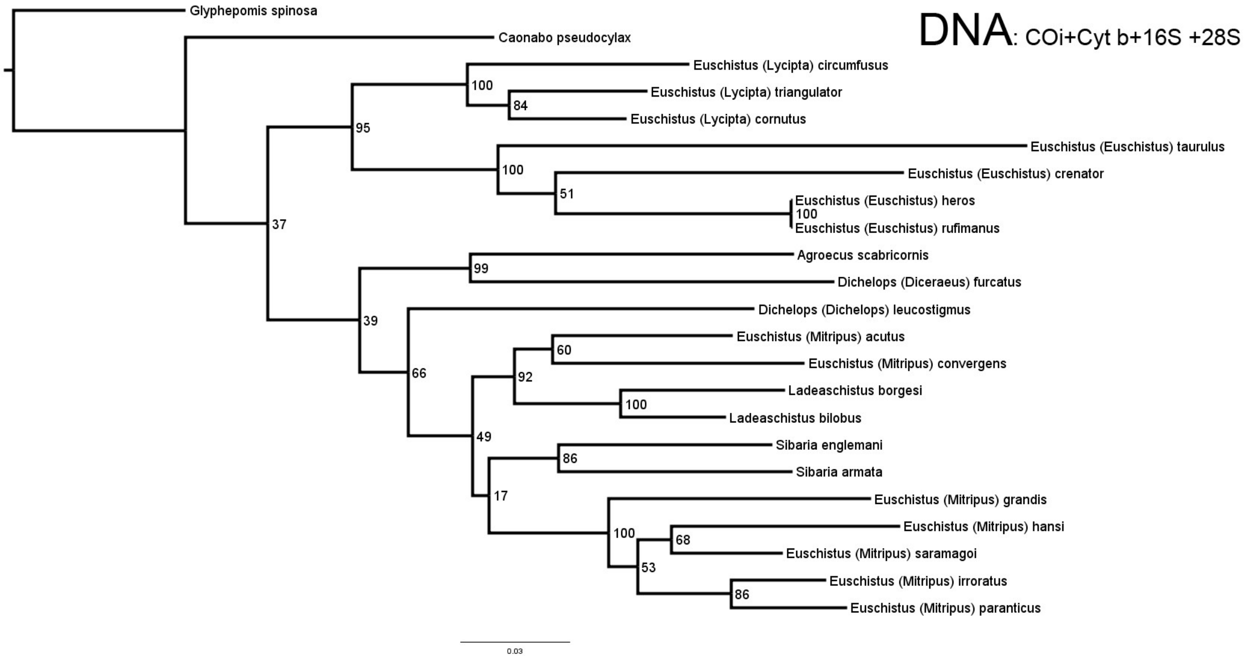
File Supplementary 4: Phylogenetic reconstruction for each partition separately [*cytochrome oxidase subunit I* (COI), *cytochrome b* (Cyt b), *16S* rRNA (16S) and, *28S* D3-D5 rDNA (28S)], morphological and DNA concatenated datasets. All presented reconstructions were conducted at CIPRES Science Gateway (Miller *et al*., 2011) (www.phylo.org/portal2/) using RAxML-HPC2, GTR-CAT model for each molecular marker, while the Multi-state-CAT+MK for the morphological partition. Nodal support was assessed with automatic Stop Bootstrapping Automatically with Majority Rule Criterion (autoMRE).

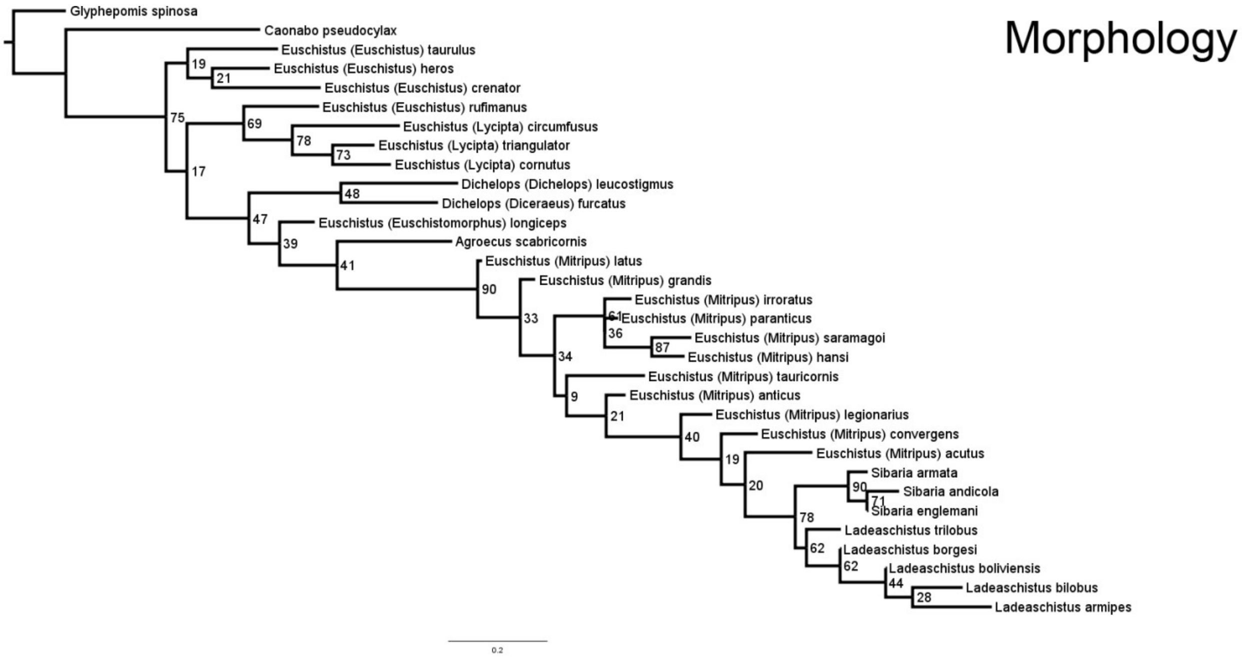



Cyt b
Glyphepomis spinosa
Euschistus (Euschistus) taurulus
51
Caonabo pseudocylax
Agroecus scabricornis
31
Sibaria armata
Dichelops (Dichelops) leucostigmus
58
Dichelops (Diceraeus) furcatus
37
38
Euschistus (Euschistus) heros
55
Euschistus (Euschistus) crenator
27
Euschistus (Lycipta) circumfusus
95
Euschistus (Lycipta) cornutus
20
41
Euschistus (Lycipta) triangulator
Euschistus (Mitripus) acutus
17
Ladeaschistus bilobus
33
Euschistus (Mitripus) convergens
20
Euschistus (Mitripus) saramagoi
99
Euschistus (Mitripus) paranticus
31
Euschistus (Mitripus) hansi
50
Euschistus (Mitripus) grandis
0.05

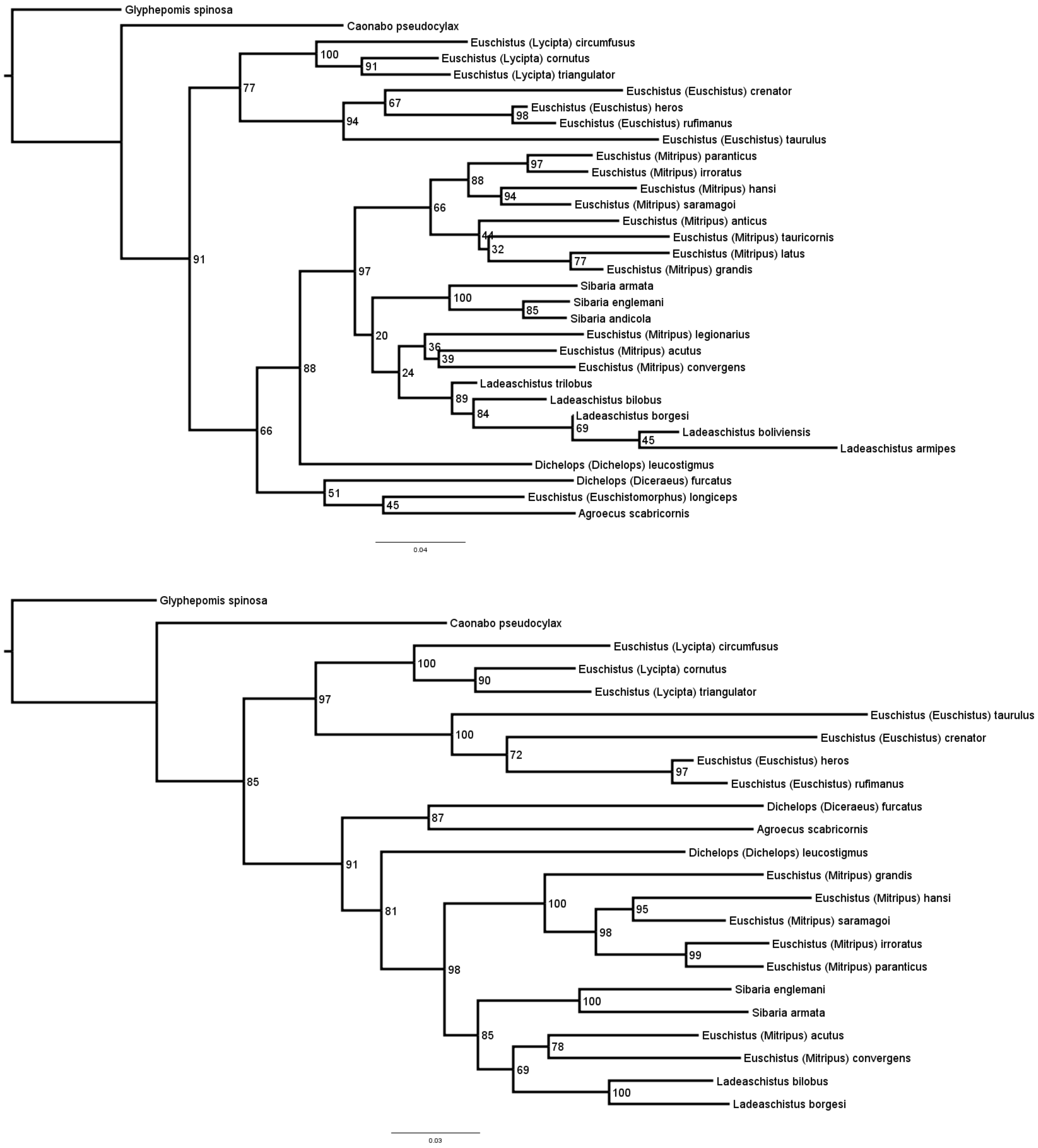

Caonabo pseudocylax
16S
Euschistus (Lycipta) circumfusus
87
Euschistus (Lycipta) cornutus
72
82
Euschistus (Lycipta) triangulator
Euschistus (Euschistus) taurulus
100
Euschistus (Euschistus) crenator
71
Euschistus (Euschistus) heros
Agroecus scabricornis
100
Dichelops (Diceraeus) furcatus
Dichelops (Dichelops) leucostigmus
46
96
Euschistus (Mitripus) acutus
38
Ladeaschistus borgesi
97
Ladeaschistus bilobus
87
Sibaria armata
94
Sibaria englemani
26
Euschistus (Mitripus) grandis
100
Euschistus (Mitripus) saramagoi
70
Euschistus (Mitripus) irroratus
89
Euschistus (Mitripus) paranticus
0.04


28S
Caonabo pseudocylax
Euschistus (Lycipta) cornutus
Euschistus (Lycipta) triangulator
Euschistus (Mitripus) grandis
30
31
Euschistus (Lycipta) circumfusus
20
Euschistus (Euschistus) crenator
69
Euschistus (Euschistus) heros
78
Euschistus (Euschistus) taurulus
8
Dichelops (Diceraeus) furcatus
100
Dichelops (Dichelops) leucostigmus
95
Euschistus (Mitripus) acutus
68
Sibaria armata
80
Ladeaschistus bilobus
26
Euschistus (Mitripus) paranticus
79
Euschistus (Mitripus) hansi
0.004


DNA: COi+Cyt b+16S +28S
Glyphepomis spinosa
Caonabo pseudocylax
Euschistus (Lycipta) circumfusus
100
Euschistus (Lycipta) triangulator
84
Euschistus (Lycipta) cornutus
95
Euschistus (Euschistus) taurulus
100
Euschistus (Euschistus) crenator
51
Euschistus (Euschistus) heros
100
Euschistus (Euschistus) rufimanus
37
Agroecus scabricornis
99
Dichelops (Diceraeus) furcatus
39
Dichelops (Dichelops) leucostigmus
Euschistus (Mitripus) acutus
60
Euschistus (Mitripus) convergens
66
92
Ladeaschistus borgesi
100
Ladeaschistus bilobus
49
Sibaria englemani
86
Sibaria armata
17
Euschistus (Mitripus) grandis
Euschistus (Mitripus) hansi
100
68
Euschistus (Mitripus) saramagoi
53
Euschistus (Mitripus) irroratus
86
Euschistus (Mitripus) paranticus
0.03


Morphology
Glyphepomis spinosa
Caonabo pseudocylax
Euschistus (Euschistus) taurulus
Euschistus (Euschistus) heros
Euschistus (Euschistus) crenator
Euschistus (Euschistus) rufimanus
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) triangulator
Euschistus (Lycipta) cornutus
Dichelops (Dichelops) leucostigmus
Dichelops (Diceraeus) furcatus
Euschistus (Euschistomorphus) longiceps
Agroecus scabricornis
Euschistus (Mitripus) latus
Euschistus (Mitripus) grandis
Euschistus (Mitripus) irroratus
Euschistus (Mitripus) paranticus
Euschistus (Mitripus) saramagoi
Euschistus (Mitripus) hansi
Euschistus (Mitripus) tauricornis
Euschistus (Mitripus) anticus
Euschistus (Mitripus) legionarius
Euschistus (Mitripus) convergens
Euschistus (Mitripus) acutus
Sibaria armata
Sibaria andicola
Sibaria englemani
Ladeaschistus trilobus
Ladeaschistus borgesi
Ladeaschistus boliviensis
Ladeaschistus bilobus
Ladeaschistus armipes
19
21
75
69
78
73
17
48
47
39
41
90
33
61
36
87
34
9
21
40
19
20
90
71
78
62
62
44
28
0.2

File Supplementary 5: Maximum-likelihood phylogeny based on the analysis of four molecular markers and 85 morphological characters for 32 and 23 species, respectively, of *Mitripus* and related carpocorines. Numbers close to nodes are ML bootstrap support.



Mitripus acutus (Dallas)

Mitripus convergens (Herrich-Schaffer)

Mitripus legionarius (Breddin)

Adustonotus anticus (Stål)

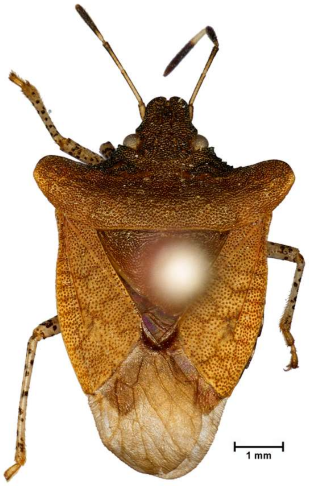
Adustonotus grandis (Rolston)

Adustonotus hansi (Grazia)

Adustonotus irroratus (Bunde et al.)

Adustonotus latus (Dallas)

Adustonodus paranticus (Grazia)



Adustonotus saramagoi (Bianchi et al.)

Adustonotus tauricornis (Stål)

Além disso, assim como é importante reconhecer a presença do significado, é igualmente importante não extrair significado de onde ele não existe.

Leonard Mlodinow

Capítulo III

Genes enlightening over neglected structures: Exploring the female genital evolution of *Euschistus* (Heteroptera:Pentatomidae)

Abstract: The insect spermatheca is a female reproductive accessory organ enrolled mainly on sperm storage. In conflicts over the mating decision, elaboration of genital and secondary sexual structures may be one outcome of the coevolutionary arms race. In Heteroptera (Hemiptera), the spermatheca is a complex organ with great interspecific variation in shape and extension. *Euschistus* (Pentatomidae) is a speciose genus taxonomically related to other 19 Carpocorini genera. We conducted a molecular phylogeny based on six molecular markers from 53 species intending to provide phylogenetic insights for the *Euschistus*-group within Carpocorini .The ancestral states were also reconstructed to furnish an overview of the evolution of the spermatheca. Both the Bayesian inference and Maximum Likelihood recovered *Euschistus*-group as non-monophyletic; and *Euschistus*, as well. The ancestral state reconstruction showed convergent evolution to many characters. Statistical test for correlated evolution showed dependent evolution for only two spermathecal structures. The spermathecal structures seems to evolve mostly uncorrelated, working in distinct phases and probably able to bar the male reproductive success.

Introduction

The reproductive behavior of insects likely followed a path from external fertilization to internal fertilization with spermatophores, just later it was evolved the copulation with the spermatophore deposited inside the reproductive tract of the female evolve (Parker, 1970). It had been claimed that a next strategic step has been reached in some groups (e.g. Diptera, Coleoptera, Mecoptera) with the introduction of free sperm transfer (Parker 1970). An alternative and rarer behavior is the hypodermic insemination, also called traumatic or hoemocelic insemination, present in at least three insect orders, Strepsiptera, Diptera and Hemiptera (Tatarnic et al. 2014). This variety of mating mechanisms and sperm transfer evolved along different lineages within Insecta, resulting in a high morphological genital diversity in both sexes (Chapman, 1971).

The mate choice occurs in males and females, and this sexual conflict between the sexes can add further selection pressures on preferred traits (Parker 1970). This relationship of selection is often treated as a female choice of male traits (Anderson and Simmons, 2006). In conflicts over the mating decision, elaboration of genital and secondary sexual structures may be one outcome of the coevolutionary arms race (Eberhard, 1985). In polyandrous species some mechanisms have been interpreted as male adaptations to avoid or reduce sperm competition (Anderson and Simmons, 2006), i.e. the rivalry between the sperm of different males to fertilize a given female (Parker, 1970). Male characteristics evolved and sometimes coerce in some level the female postcopulatory choice, or the cryptic female choice (Eberhard, 1985; 1996), such as mating plugs, seminal fluid peptides that influence the female reproductive physiology, and postcopulatory mate guarding (Alcock, 1994; Anderson and Simmons, 2006; Tsai *et al.* 2015). The cryptic female choice is the biasing of paternity by females towards some males over others; the sperm stored might reflect the female choice imposed during the travel of sperm within her reproductive tract until be kept in the spermatheca (Eberhard, 1996).

The insect spermatheca is a female reproductive accessory organ of ectodermal origin (Chapman, 1971; Matsuda, 1976), and enrolled on important reproductive functions such as copulation, fertilization, and oviposition, but its main attribute is sperm storage (Gschwentner and Tadler 2000). In Heteroptera (Hemiptera), the spermatheca is a complex organ with great interspecific variation in shape and extension, its conformation regarded an important source of useful characters for phylogenetic, taxonomic, and classification (Pendergrast, 1957; Servadei, 1964). The causes of this interspecific divergence in sperm-storage, number and shape of spermathecae are not well understood (Pitnick et al. 1999), but the female reproductive organs can be highly variable across closely related species (Pitnick et al., 1999; Eberhard, 2010; Yassin, 2016).

The spermatheca in the Pentatomoidea (Heteroptera) usually consists of a spermathecal duct (*ductus receptaculi*) leading from the vagina (*pars communis*) to an enlarged spermathecal bulb (*capsula seminalis*). It is also characterized by a conspicuous pumping region (*pars intermedialis*) flanked by proximal and distal flanges (annular flanges) (Pendergrast, 1957;

McDonald, 1966; Dupuis, 1970; Pluot-Sigwalt and Lis 2008). Major contributions to the current knowledge on the morphology of spermatheca in Pentatomoidea were made by Kumar (1962), Servadei (1964) and McDonald (1966). After these, some descriptive and comparative studies were published with a more or less restricted taxonomic coverage (e.g, Kocorek and Danielczok-Demska 2002; Candan et al. 2010, 2012, 2014; Stacconi and Romani 2011).

One of the most speciose genus within the Pentatomidae, *Euschistus* Dallas, 1851 occurs in the Neotropical and Nearctic regions, and some of its species such as *Euschistus* (*Euschistus*) *servus* (Say), *E.* (*E.*) *heros* (Fabricius), and *E.* (*E.*) *tristigmus* (Say) cause important damage to crops (Panizzi et al., 2000). Notwithstanding its economic importance (e. g. Moraes et al., 2005; Cullen and Zalom, 2005; Borges et al, 2015) the systematics of *Euschistus* was grounded exclusively on taxonomic studies (but see Weiler *et al.*, *in press*) until a recent phylogeny inferring paraphyly of *Euschistus* (Bianchi *et al.*, *in prep*). Beyond *Euschistus*, Rolston (1974a) suggested a grouping of ten genera based on external morphology (e.g. color and size). Since then, other genera were taxonomically related to *Euschistus*-group or erected from the genera previously considered *Euschistus*-group (e.g. *Coenus* Dallas, *Caonabo* Rolston, *Acledra*, Signoret). Currently, *Euschistus*-group includes 20 genera (Table 1).

The spermatheca of most species of *Euschistus* remain undescribed, being considered of little taxonomic importance and presenting only subtle differences (Rolston, 1974a). Bianchi et al. (*in prep*) highlighted the importance of spermathecal traits to recognize *Mitripus* Rolston and *Adustonotus* Bianchi as independent lineages of *Euschistus*. Reconstructing the history of structures and recognizing patterns within phylogenetic lineages can provide insights into the evolution of traits (Gamble et al., 2015).

We explored the genital morphology and conducted a molecular phylogeny intending to (i) discuss the phylogenetic relationships between *Euschistus* and taxonomically related genera, providing a phylogenetic insights for the *Euschistus*-group within Carpocorini; (ii) furnish an overview of the evolution of the internal female genitalia of the studied genera. Based on the assumption that female reproductive traits, particularly the spermatheca, are highly variable across closely related species, and its structures allow the female to be active and more effective on the post-copula choice, our evolutionary hypothesis here is: the internal female genitalia acts in different levels, which the structures evolve independently to each other, working in different phases during the copula and post-copula, building a complex way to the male fertilization. For this reason we selected relevant structures of spermatheca, and track the evolution of six characters of *capsula seminalis*, *pars intermedialis* and vesicular area.

Material and methods

Taxa sampling

A total of 53 taxa were sampled including 22 ingroup (*Euschistus*), 29 outgroup taxa belonging to Carpocorini, and *Diploxys* sp. (Diploxyini) and *Banasa euchlora* Stål, 1872 (Pentatomini) (Table 1). The outgroup comprises 19 representative genera of the *Euschistus* group, the larger genus group in number of species within Carpicorini. The ingroup sample comprises 10 species representing species-groups within *Euschistus* (*Euschistus*) as proposed by Rolston (1974; 1984) (i.e. groups *crenator*, *heros*, *strenuus* and *sulcacitus*), other six species of *Euschistus* (*Euschistus*) not included within any group proposed by Rolston; and six species of *Euschistus* (*Lycipta*). This is the best sample of *Euschistus* ever considered in a phylogenetic analysis, allowing its first and most comprehensive test of monophyly.

Dissection of female genitalia

Females had the whole abdomen removed and macerated in a supersaturated and heated solution of potassium hydroxide (KOH) for 5–15 min until no traces of fat bodies or muscles were remained. The genital structures were detached from abdominal sclerites and stained with Congo red solution. Pictures were taken using a Nikon DS-Fi2 camera coupled to a stereomicroscope Nikon AZ 100M in multiple focal planes and stacked with the software NIS— Elements Advanced Research. The terminology follows Dupuis (1970).

Molecular markers, primers, DNA extraction, PCR amplifications, purification and sequencing

Six molecular markers were amplified and sequenced, including three mitochondrial protein-coding genes [*Cytochrome Oxidase subunit I* (COI) and *II* (COII), *Cytochrome b* (Cyt b)], two ribosomal [*16S* (16S) and *28S D3-5D rDNA* (28S)] and nuclear protein-coding [*Deformed*

(Dfd)]. The markers were amplified using Polymerase Chain Reactions (PCR). Primers and PCR annealing temperature are listed in Tab. S1.

The DNA was extracted from 99% ethanol-preserved or pinned specimens. For ethanol preserved specimens, the right foreleg was removed from the coxal cavity. Pinned specimens had the right pleural sclerites removed to access the thoracic muscles. The samples were digested in Proteinase-K solution for 12 – 20 hours at 50 –55 °C in an incubator and then used for DNA extraction. Genomic DNA was extracted using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, eluting to a final volume of 100µl when DNA was extracted from ethanol-preserved specimens or 50µl from pinned specimens.

The PCR amplifications were conducted using GoTaq Hot Start Green Master Mix (Promega, Madison, USA). All PCR products were sequenced in both forward and reverse direction at Macrogen, Inc., Sequencing Center (New York, United States) and ACTGen (Porto Alegre, Brazil). Sequence chromatograms were visually inspected, verified and manually edited using STADEN package (Staden et al., 2000). The sequences were verified using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) confirming the high similarity of our submitted sequences to pentatomidean species. Genbank access numbers provided in Tab 2.

Sequence Alignment and Phylogenetic Analyses

Alignments of individual genes were performed using MAFFT 7 (Katoh & Standley, 2013) on-line version (http://mafft.cbrc.jp/alignment/server/index.html), applying the strategy G-INS-i to mithochondrial fragments, and Q-INS-i algorithm to ribosomal and nuclear markers. Reading frame for each protein-coding gene alignment was checked using the ORF Finder (Open Read Frame Finder) available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). Phylogenetic inferences were first performed on the single-genes, mitochondrial and nuclear-genes grouped in order to evaluate each signal and to detect any incongruence. These analyses were evaluated under the Maximum Likelihood performed by RAxML-HPC2 at CIPRES Science Gateway (Miller et al., 2011) (www.phylo.org/portal2/) applying the GTR-CAT model (Suplementary File S3).

The individual gene alignments were concatenated in a single matrix using SequenceMatrix 1.8 (Vaidya et al., 2011), recoding external gaps as question marks. The final matrix was exported in both Phylip (for RAxML analyses on CIPRES) and NEXUS (for MrBayes analyses) formats. The matrix was divided into six partitions (16S, COI, COII, Cyt b, 28S and Dfd). The appropriate substitution model for each marker individually was chosen using jModelTest v2.1.6 (Guindon and Gascuel, 2003; Darriba et al., 2012) via the Bayesian information criterion (BIC) as suggested by Luo et al. (2010). The GTR+I+G was selected for 16S, COI and COII partitions; GTR+G for Cyt b; JC+I+G for 28S and HKY+G for Dfd.

The Bayesian inference of the concatenated dataset was performed in the multithreading version of the program MrBayes 3.2.0 (Ronquist & Huelsenbeck, 2003). Two runs with four chains each for 14,000,000 generations and tree sampled every 1000 generations. Tracer v.1.6.0 (Rambaut et al. 2014) was used to inspect the convergence to the stationary distribution of the chains. The first 2,400,000 generations were discarded as "burn-in" and the posterior probability estimated for the remaining generations.

Additionally, Maximum Likelihood analyses were performed on the concatenated matrix using RAxML-HPC2 at CIPRES Science Gateway (Miller et al., 2011) (www.phylo.org/portal2/). The GTR-CAT model, that optimizes site-specific evolutionary rates, was individually used for each partition. Nodal support was assessed with automatic Stop Bootstrapping Automatically with Majority Rule Criterion (autoMRE).

Reconstruction of ancestral traits

To investigate the evolutionary history of the selected spermathecal structures of the *Euschistus* group we estimated the ancestral character states by Parsimony and Maximum Likelihood reconstruction (Mk1 model) using the software Mesquite 3.02 (Maddison & Maddison, 2011). Morphological character data were obtained by examining the voucher specimens or additional pinned-dry specimens. When necessary the states were retrieved from the literature. Ancestral state reconstructions were made on the topology of the tree from the Bayesian analysis. The characters were treated as unordered, and comprise structures of the *capsula seminalis*, *pars intermedialis* and vesicular area.

For pairwise correlations between the discrete variables, we used Pagel's (1994) test for correlation of binary characters with 500 simulations and 10 replications to evaluate the p-value. This analysis is used to identify biological traits that do not evolve independently, which can reveal an underlying dependence. Once our intention is to test if the structures are evolving in dependence to one another, but not what character states are specifically evolving together, we transform non-binary characters to binary, assuming the more ancestral character as state "0" and alternatives to it as state "1".

The following characters and states were evaluated: 1 *Capsula seminalis*, process: (0) absent, (1) present; 2 *Capsula seminalis*, shape: (0) globose, (1) obovate, (2) finger-like; 3 *Pars intermedialis*, shape: (0) coiled, (1) conical, (2) convoluted, (3) 'cup-like', (4) fusiform, (5) cylindrical; 4 Vesicular area, distal portion of *ductus receptaculi proximalis*, shape: (0) tapering, (1) globose, (2) elongated (3) coiled; 5 Vesicular area, median tube: (0) dilated (1) linear; 6 *Pars intermedialis*, anterior annular flange: (0) exended laterally, (1) folded over the pars intermedialis, (2) folded covering the distal ductus *receptaculi*. (Fig. 1 for the matrix Tab. S2)

Results

Phylogenetic analyses

The final alignment comprises 3300 sites from the six markers: 16S (541 bp), 28S (523 bp), COI (821 bp), COII (596 bp), Cyt b (534 bp) and Dfd (315 bp). The majority-rule consensus from the Bayesian inference (BI) and the best tree found in the Maximum likelihood (ML) (Ln = -36678.405567) analyses are shown in Fig.2. The results of the the BI and ML analyses of the combined dataset yielded almost identical branching patterns. Incongruences between the BI and ML analyses are in nodes which the ML-tree presents low bootstrap support.

Euschistus was not recovered as monophyletic in both the BI and ML analyses. Although a single low supported node (BS = 53; PP = 0.68) clusters all the *Euschistus* (clade 5), this clade includes *Berecynthus delirator* Fabricius and *Hymenarcys crassa* Uhler. The subgenera *E.* (*Euschistus*) is recovered as polyphyletic. The grouping between species of the nominal subgenus + *H. crassa* (clade 7) is well supported (BS = 100; PP = 0.84), and the internal relationships of this clade are strongly supported by PP but not completely by ML (Fig. 2-B). Regarding the species groups within *Euschistus* (*Euschistus*), the *crenator* group was the best sampled and recovered polyphyletic. Both the *sulcacitus* and *heros* groups were recovered as monophyletic though represented by two species each. The *strenuus* group was represented only by one species.

Our analyses indicated the paraphyly of *Euschistus* (*Lycipta*), once it includes *E.* (*Euschistus*) *comptus* Walker, and *Berecynthus delirator* is sister to *Euschistus* (*Lycipta*)+ *E.* (*Euschistus*) *comptus* (clade 6) (BS = 89; PP = 0.84) (Fig. 2 A and B).

The result of BI shows *Adustonotus* + *Sibaria* + *Ladeaschistus* + *Mitripus* a strongly supported clade (PP = 0.94) and sister to *Dolycoris baccarum* Linnaeus (clade 2), while the ML analysis recovered a low supported clade 2 (BS = 60) with *D. baccarum* sister to *Sibaria*, and *Mitripus acutus* Dallas sister to *Mitripus convergens* + *Ladeaschistus* (BS = 88). The sister-group relationship between *Padaeus* + *Proxys* and *Spinalanx* + *Dichelops* (*Dichelops*) (clade 1) was highly supported (BS = 95; PP = 1) sister to clade 2.

The remaining taxa included in our sample were inferred as independent lineages within clade 8 in both analyses: *Acledra* + *Agroecus* + *Dichelops* (*Diceraeus*) (clade 3) (BS = 96 ; PP =1); and *Caonabo* + *Oebalus* + *Mormidea* + *Stysiana* + *Hypatrops* (clade 4) (BS = 62 ; PP = 0.78). Notably, the *Euschistus*-group originally proposed by Rolston (1974) was not recovered as monophyletic. Excepting *Caonabo*, the taxa within the clade 4 were never considered part of *Euschistus*-group, neither were *Prionosoma podopinoides* (recovered at the base of clade 5), and *Dolycoris baccarum* (associated to clade 2).

Characters evolution

The ancestral states reconstructions were consistent under parsimony and ML (Fig 3 and Fig. S4). The analyses produced scenarios a little different to the evolutionary history of the shape of the *capsula seminalis* (see below). The Pagel's pairwise test found correlation between the shape of *pars intermedialis* and the shape of the apex of *ductus receptaculi proximalis* (p=0.006); and between the orientation of anterior annular flange and the shape of the apex of *ductus receptaculi proximalis* (p=0.03). No significant correlation was found for any other pair of characters (Table S4).

Capsula seminalis

The *capsula seminalis* was evaluated for two characters, its shape and the presence/absence of processes. The latter are present in few species in our sample, with three independent origins of processes in the sampled species: in the node of *Dichelops* (*Dichelops*) *leucostigmus* and *Spinalanx rolstoni* within clade 1 with moderate proportional likelihood (0.71); in the node of *Mormidea* within clade 4 with high proportional likelihood (0.92); and in the node of *Oebalus ypsilongriseus* and *Caonabo pseudocylax*, or in the node grouping *Caonabo* + *Oebalus* and *Mormidea* both with ambiguous proportional likelihood (absence: 0.51; presence: 0.49).

The globose *capsula seminalis* is the most common shape in our sample, optimized as the ancestral state of clade 5, changing to obovate one time within clade 6 and twice within clade 7. The obovate *capsula seminalis* has raised independently at least six times from the globose shape in the clade 8. The finger-like *capsula seminalis* evolved once within clade 2, in the node common to *Adustonotus* and its sister taxa, presenting a high proportional likelihood (0.91) though ambiguous under parsimony.

Pars intermedialis

The *pars intermedialis* is the most morphologically variable structure evaluated. Cylindrical *pars intermedialis* has high proportional likelihood (0.96) in the common ancestor of the clade 8, evolving to conical at least five times. Under ML, *Dichelops* (*D.*) *leucostigmus* + *S. rolstoni* have a hypothetical ancestor bearing a fusiform (0.67) *pars intermedialis*. Within the clade 2 the ancestor of *Sibaria*+ (*Ladeaschistus*+*Mitripus*) presents the *pars intermedialis* coiled (0.86). *Euschistus* had an ancestor with a conical *pars intermedialis* (0.95) conserved in almost all clade 6 but *E.* (*E.*) *comptus.* Within clade 7 the shape reverted from conical to cylindrical at least three times, and evolved once to cup-like.

The orientation of the anterior annular flange changed thirteen independent times within the phylogenetic tree. The changes from extended laterally to folded over the *pars intermedialis* occurred four times. The latter state is present in *Sibaria*, *Dichelops* (*Diceraeus*) *furcatus*, *Stysiana acarinatis*, and in clade 6 but reverting in *E.* (*E.*) *comptus.* The anterior annular flange folded toward the *ductus seminis distalis* evolved in clades 2, 3, 4, and three times in clade 7.

Vesicular area

The distal portion of the *ductus receptaculi proximalis* presents three different shapes, the tapering *ductus receptaculi* with a high proportional likelihood (0.99) in the basal most node of the clade 8, changing to elongated nine times through the phylogeny. The reconstruction suggests elongated *ductus receptaculi* as the ancestral state of clade 6 (0.87). The globose shape arises in *D.*(*D.*) *leucostigmus* and four independent times in clade 5.

A linear median wall is optimized as the probable ancestral state of the clade 8 (0.70), changing to dilated eight times and reverting four times after that. In clade 2, the ancestor of *Adustonotus* + sister genera has moderated proportional likelihood (0.55) for dilated median wall, reverting in *Adustonotus irroratus* + *Adustonotus paranticus* and in *Ladeaschistus*.

Discussion

Phylogenetic relationships of Euschistus

This is the most comprehensive sample of the *Euschistus*-group evaluated under a phylogenetic framework. Rolston (1974a) proposed the *Euschistus*-group suggesting a phylogenetic affinity among some genera within Carpocorini (Pentatominae). All genera in the *Euschistus*-group possess a pair of movable processes of phallotheca (referred to as dorsal thecal appendages in Rider, 1995), however putative homologous structures are found in the genera of the *Mecocephala*-group, e.g. *Mecocephala, Stysiana*, and *Glyphepomis* (see respectively Schwertner *et al.* 2002; Grazia *et al.* 1999; and Bianchi *et al.* 2016). Within this group are the genera *Hypatropis* and *Stysiana* sampled here and recovered in clade 4. The hypothesis of phylogenetic relationship of the *Euschistus*-group genera was rendered non-monophyletic.

The former subgenus *Euschistus* (*Mitripus*) was splitted into *Adustonotus* and *Mitripus* after a phylogenetic analysis by Bianchi *et al.* (*in prep.*), whose inferred relationships with *Sibaria* and *Ladeaschistus* were also recovered here with high posterior probability. The generic groupings in clades 1 and 3 are new relationship hypotheses in contrast to previously proposed ones e. g. between *Spinalanx* and *Agroecus* (Rolston & Rider, 1988), and *Caonabo* and *Euschistus* (Rolston, 1974). Also, the monophyly of *Dichelops* proposed by Grazia (1978) was not recovered here as already indicated by *Bianchi et al.* (*in prep*). On the other hand the close relationship between *Mormidea* and *Oebalus* in clade 4 corroborates past hypotheses based on morphological similarities (Rolston 1978b).

Euschistus is currently composed of three subgenera: the nominate (53 species); *Lycipta* Stål (12 species); and *Euschistomorphus* Jensen-Haarup (monotypic). Formal systematic knowledge or tested hypotheses within *Euschistus* and its allied genera are scarce. Our results partially recover relationships proposed in previous studies focusing different subgenera of *Euschistus* and comprising smaller samples of species and genera: Weiler *et al.* (2016) recovered the monophyly of *Euschistus* (*Lycipta*) supported by morphological characters;. and Bianchi *et al.* (*in prep*) combining morphological and molecular data suggested the monophyly of *Euschistus* sorting it into two clades representing the subgenera *Lycipta* and *Euschistus*. However, the monophyly of both subgenera is not recovered in our study: the clade 6 comprising *Lycipta* species, *B. delirator*, and *E.* (*E.*) *comptus* is sister to all the remain sampled *Euschistus* (*Euschistus*) and *H. crassa*.

Besides the non-monophyly of *Euschistus*, our results also suggest the groups of species within *E.* (*Euschistus*) proposed by Rolston (1974a; 1984a) should be better explored before treated as natural lineages within the genus. Mainly the *crenator* group, which includes 13 out of 53 species of the subgenus, whose was recovered polyphyletic. The propositions of species groups were based on external features but mostly on male genitalia. The morphology of both genital and nongenital structures of *Euschistus* is widely variable (Rolston, 1974a; Cioato *et al.* 2015), and based on published phylogenetic analyses including *Euschistus* and Carpocorini as well we may infer a high plasticity and convergence of some characters (see below). Although morphology has been the main source to infer species relationships over the last century, undoubtedly still a powerful tool, morphological approaches may be tricked by convergent, adaptive features, resulting from strong and sometimes unknown adaptive pressures (Wiens et al. 2003; Daniels et al. 2006; Abrams et al 2012). Indeed, several morphological specializations are apparently homoplasious within the Pentatomoidea (Wu *et al.* 2016), the current molecular approaches may be combined with morphological data intending to overcome dubious factors inherent from interpretation of structures and their homologies (Abrams et al 2012, Bianchi et al. *in prep*).

The tricks of female internal genitalia

We described an evolutionary hypothesis of the internal female genital structures and tested their mutual evolutive correlation in a group of stink bugs belonging to the Pentatomidae. Our results track multiple changes of states for the most part of the analyzed characters, and in some of them parallel evolution and reversions were observed. Beyond that, our initial hypothesis was broadly supported by the Pagel's test, i.e. the individual structures evolves independently to each other, being only two out of fifteen tested pairs correlated. This result suggests different female traits not only evolve independently but work in distinct phases and perform different functions during the copula, resulting in a mechanism of multiple barriers to the male success in egg fertilization (Gschwenthner & Tadler 2000; Stacconi & Romani, 2011). In other words, in Pentatomidae the female internal genitalia may play different roles in the post-copula female choice. It is becoming accepted that different genital features may evolve independently as result of distinct pressure processes (Song and Wenzel 2008; Rabieh et al. 2015).

The spermathecal structures and functioning must provide reception for the spermatozoa during copula, maintenance during storage, and release during fertilization (Huebner, 1980). For the establishment and maintenance of spermatozoa, a microenvironment to the spermathecal epithelium is necessary, providing appropriate nutrient, ionic, pH, and other factors to ensure sperm survival (Parker, 1970). The *capsula seminalis* in insects is the distal portion of the spermatheca responsible for storage of the spermatozoa (Parker, 1970). This structure is surrounded by glandular tissues (Pendergrast, 1957; Stacconi and Romani, 2011) which provide the secretion accountable to the maintenance of spermatozoa for long periods of time for an efficient use of them during fertilization (Parker, 1970). The function and evolutionary impact conferred upon the shape and the presence of processes on the *capsula seminalis* are still not elucidated to Pentatomoidea, although the shape can be directly related to the volume of content the structure is able to store. There are no differences in the external texture and sculpturing between the *capsula seminalis* and the processes (Candan et al. 2014; 2015), suggesting the development of the processes can be at least related to increasing the storage volume.

The finger-like shape of *capsula seminalis* (digitiform sensu Rolston 1973; 1975, 1978a) is shared by almost all species within clade 2, and was already used to diagnose the genera belonging to this clade except *Dolycoris* (Rolston 1973; 1975, Bianchi *et al. in prep*). Under parsimony reconstruction the finger-like state is ambiguous, providing a scenario of two arisings while under ML optimization it occurred just once, reverting to globose in *A. grandis*. Furthermore, *Adustonotus latus* (Dallas) recovered by Bianchi *et al.* (*in prep*) as sister to *A. grandis* also presents a globose *capsula seminalis*.

The *pars intermedialis* works as a pump pushing the sperm in and expelling it out from the *capsula seminalis*. In Pentatomidae there are two internal layers of endocuticle at the same level of the anterior and posterior annular flanges, those layers operate like valves (Stacconi and Romani, 2011). External to the *pars intermedialis* longitudinal muscles are attached to both annular flanges (Pendergrast, 1957) connecting them. The contraction of the longitudinal muscles probably compresses a thinner portion of the *pars intermedialis* resulting in internal pressure to move fluids to or from the *capsula seminalis*.

Gschwenthner and Tadler (2000) studied the spermathecal functional anatomy of the seed bug *Lygaeus simulans*, concluding that the female has control, by means of valve and spermathecal musculature, on the entry of the male *ductus seminis* at the spermatheca; and to keep or eject sperm previously acquired using the muscles associated with the spermatheca and valves. We suppose this control on the seminal fluids by the female can also happen in Pentatomidae. Some pentatomid males have extremely long *ductus seminis* (e.g. species of *Glyphepomis* and *Mecocephala*), but there is no record of the *ductus seminis* reaching the valve of the *pars intermedialis*, or even penetrating through the ectodermal ducts. Dissections of mating pairs of *Perillus bioculatus* (Fabricius) (Pentatomidae, Asopinae) revealed the *ductus seminis* in direct contact with the *orificium receptaculi* (Adams, 2001). Parker (1970) infers an advantage upon males able to inject sperm nearer the *capsula seminalis*, and fostering on males to protect their sperm from the displacement by other male's sperm. The length of the ectodermal ducts, in different taxa, correlates with the length of the male intromittent organ (Yassin, 2016). Few studies in Pentatomomorpha goal the interaction of male and female genital organs (e.g. Adams, 2001) and their evolution (e.g. Genevcius *et al.*, *in prep*), however Genevcius *et al.* (*in prep*) show evolutionary correlation in sexual structures between both sexes.

In his functional study of *Perillus bioculatus*, Adams (2001) refers as “calyx” to the distal portion of the female *ductus receptaculi proximalis*, and as “duct assembly” to the median wall of vesicular area, assigning to these structures the function of a valve. In this portion of the duct, the median wall can be displaced anteriorly (= distally), opening a space that leads to the vesicular area (spermathecal pouch sensu Adams, 2001). In fact, Candan *et al.* (2015) using scanning electron micrographs suggest there are muscles associated to the apex of *ductus receptaculi proximalis* and the base of vesicular area, what could corroborate a gate action between the duct and the vesicular area. The calyx, when not displaced, is in contact to the base of the median wall, forming a straight way from the *ductus receptaculi proximalis* to the *ductus receptaculi distalis*, and then to both *pars intermedialis* and *capsula seminalis*.

The dilation of the median wall, when present, varies between species in shape and position along the median wall. When the dilatation is posterior (= proximal) it may assist the coupling of the calyx with the base of the median wall, acting as a plug increasing the capability to seal the sperm way through the *capsula seminalis*. Adams (2001) dissected couples in different mating times and found sperm always associated to the *capsula seminalis* and the inner tube of the *ductus receptaculi*, while the vesicular area (outer tube) was filled with glycoproteins transferred by the male, probably an oviposition enhancer (e. g. Fig1-D). The transfer of nongametic components is often associated to prolonged copulation, a behaviour offering different fitness benefits to both sexes (Alcock, 1994). The coupling time is an important aspect to the male actually inseminate the female (Adams, 2001). In spite of long coupling times, less than half of the copulations do not result in the insemination; a possible reason for the high rate of failure may be the active closure of the ducts by contractions of spermathecal musculature (Gschwertner & Tandler 2000).

Although it seems to be a fruitful source of information to systematic and phylogenies, the studies on genitalia of Pentatomoidea are not different to other animal taxa, where the female genital traits are understudied compared to male genitalia (e.g. evolution, structures, mechanics, and behavior) (Ah-King et al, 2014). This male bias historically characterizes the research field and has resulted in much less reports with detailed investigations of the female genitalia, and so its role in evolution may be underestimated (Genevcius et al., *in prep*). Elucidation of the functional morphology of any such traits in the female is a key to understanding the evolutionary dynamics of sexual conflicts (Sakaluk et al. 1995). Also, knowledge about the operation of different structures and their evolution provides insights in understanding the mate choice, copula, and post-copular behaviors.

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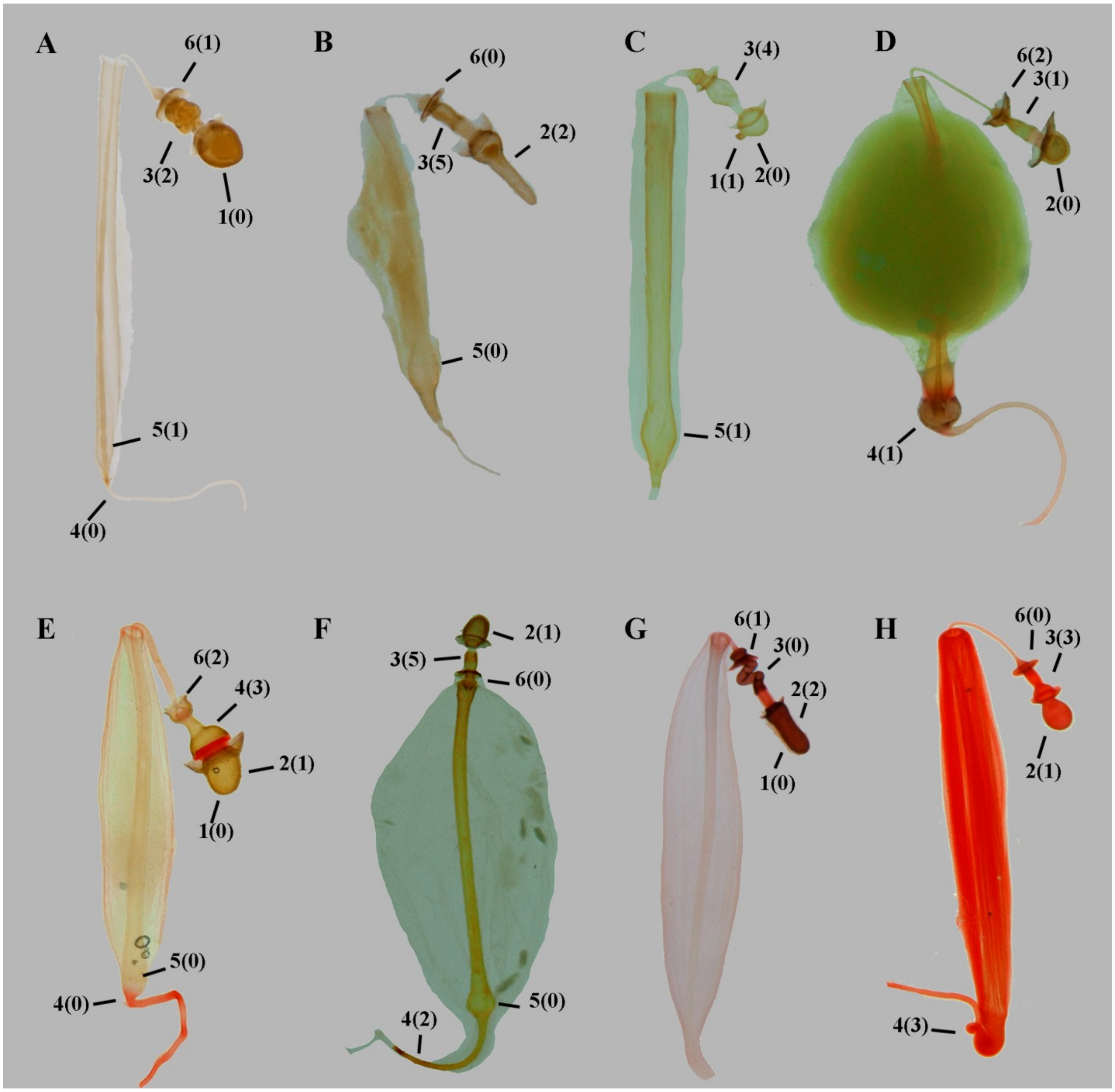


Figure 1: Spermatheca of Pentatomidae. A: *Dichelops* (*Diceraeus*) *furcatus*, B: *Adustonotus irroratus*, C: *Padaeus* sp., D: *Euschistus* (*Euschistus*) *biformis*, E: *Euschistus* (*Euschistus*) *obscurus*, F: *Spinalanx rolstoni*, G: *Ladeaschustus engleman*i, H: *Banasa euchlora*. Morphological characters and states evaluated to reconstruction of ancestral traits: 1 *Capsula seminalis*, process: (0) absent, (1) present; 2 *Capsula seminalis*, shape: (0) globose, (1) obovate, (2) finger-like; 3 *Pars intermedialis*, shape: (0) coiled, (1) conical, (2) convoluted, (3) 'cup-like', (4) fusiform, (5) cylindrical; 4 Vesicular area, distal portion of *ductus receptaculi proximalis*, shape: (0) tapering, (1) globose, (2) elongated (3) coiled; 5 Vesicular area, median tube: (0) dilated (1) linear; 6 *Pars intermedialis*, anterior annular flange: (0) exended laterally, (1) folded over the pars intermedialis, (2) folded covering the distal ductus *receptaculi*.

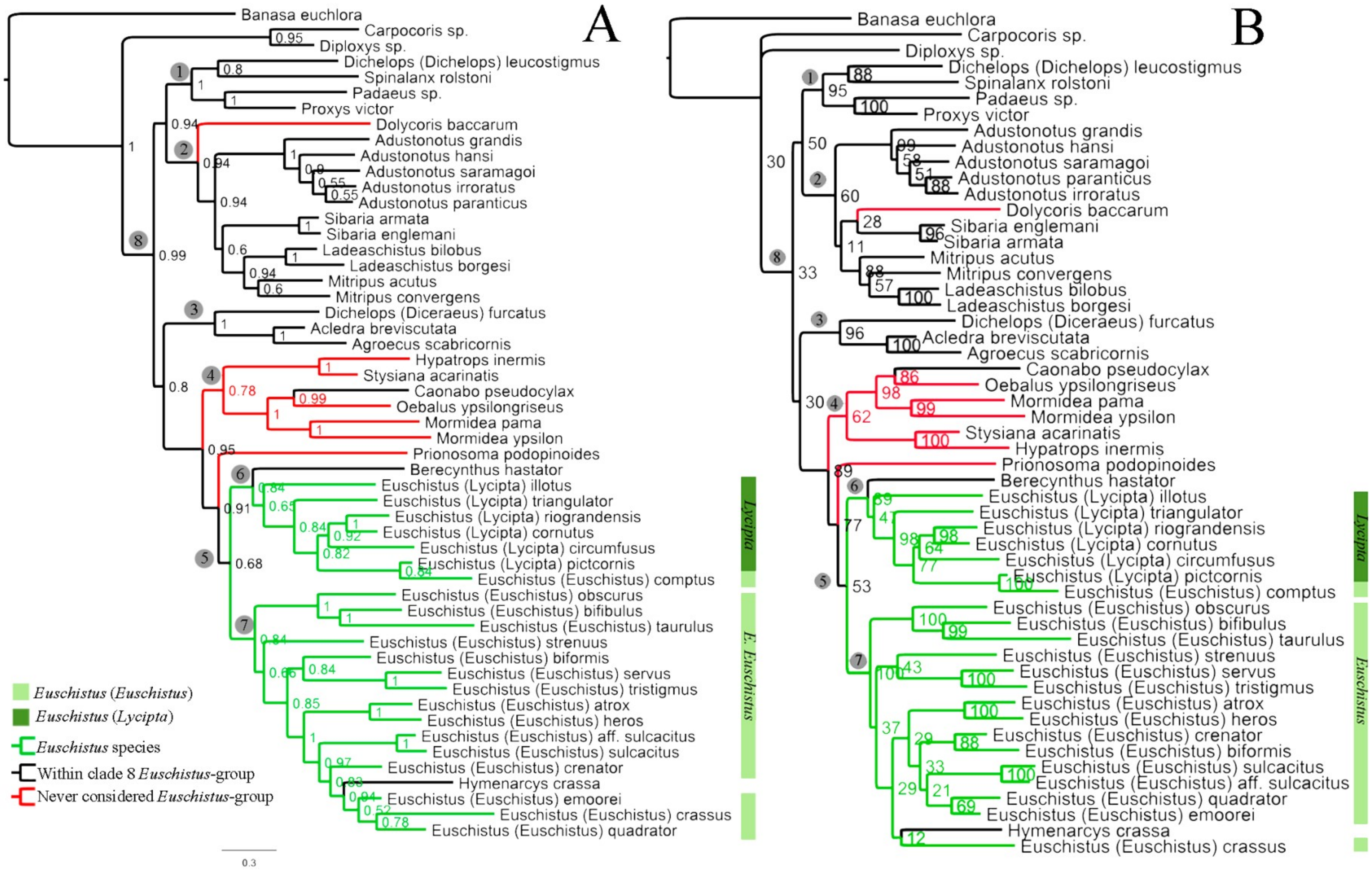


Figure 2. Bayesian Inference consensus tree (A) and best tree found in the Maximum likelihood (B) based on the analysis of six molecular markers (16S, 28S, COI, COII, Cytb, Dfd) for 53 species of *Euschistus* and related carpocorines. Numbers in circles over branches refer to clade numbers further discussed in the text. Numbers close to nodes are respectively Bayesian posterior probability / ML bootstrap support.

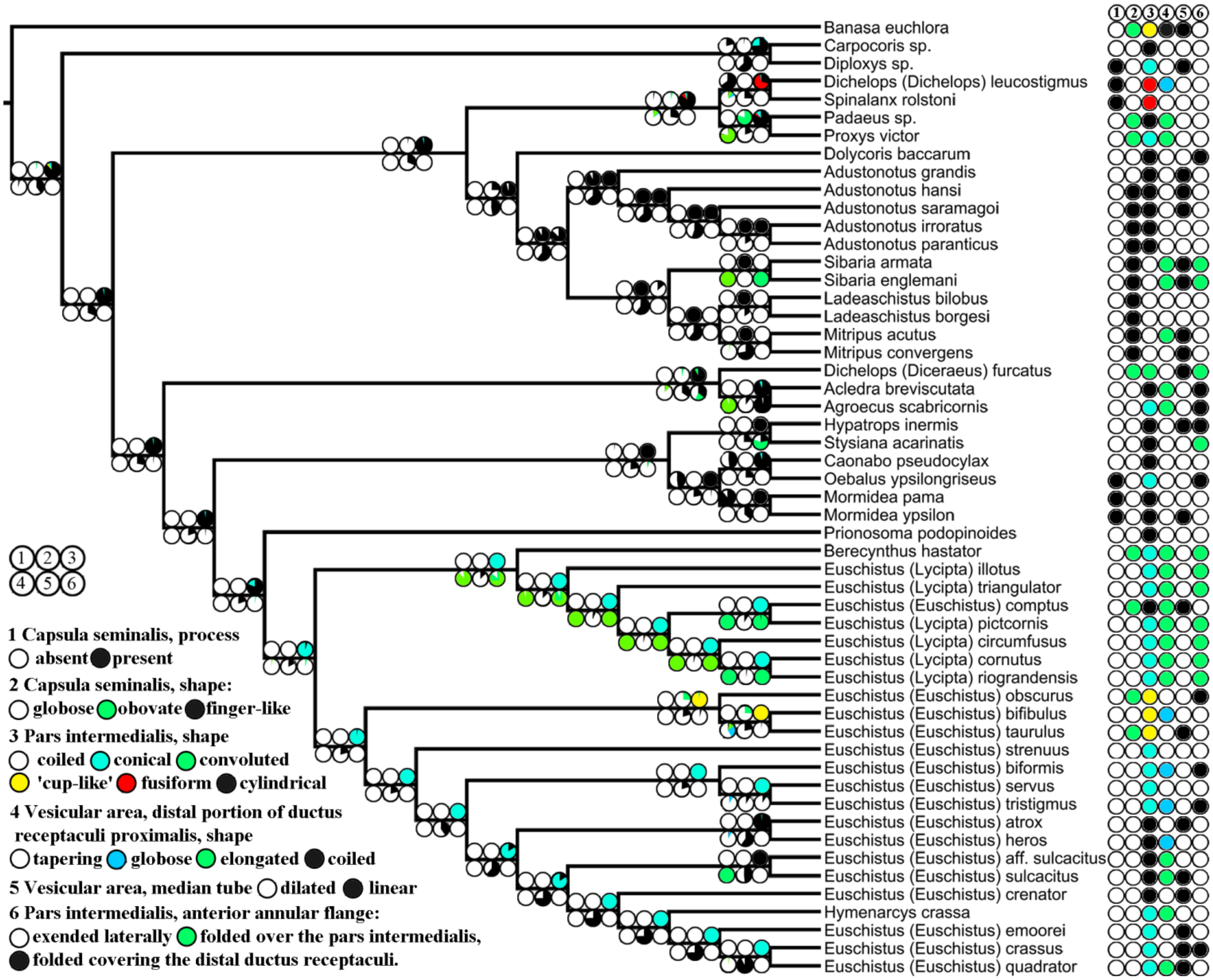


Figure 3. Summary of the maximum likelihood ancestral state reconstructions for spermathecal structures. The tree shown is the majority-rule consensus of Bayesian Inference analyses. The pie charts along the branches illustrate the likelihood for the ancestral state reconstructed in Mesquite3.02. States of each spermathecal character for taxa is indicated in the circles after taxon names.

Table 1: Composition genera considered phylogenetically related to *Euschistus* Dallas after the proposition of *Euschistus*-group by Rolston (1974a).

Genus	Proposition of relationship with *Euschistus*
Acledra Signoret, 1864	Fernández-Aldea *et al.* 2014
Adustonotus Bianchi *in prep*	Bianchi *et al.* (*in prep*)
Agroecus Dallas, 1851	Rolston 1974a
Berecynthus Stål, 1862	Rolston 1974a
Caonabo Rolston, 1974	Rolston 1974b
Coenus Dallas, 1851	Rider 1995
Dichelops Spinola 1837	Rolston 1974a
Euschistus Dallas 1851	-
Galedanta Amyot & Serville, 1843	Rolston 1974a
Hymenarcys Amyot & Serville, 1843	Rolston 1974a
Ladeaschistus Rolston, 1973	Rolston 1973
Mcphersonarcys Thomas 2012	Thomas 2012
Menecles Stål, 1867	Rolston 1974a
Mitripus Rolston 1878	Bianchi *et al. (in prep)*
Oenopiella Bergroth, 1891	Fernández-Aldea *et al.* 2014
Padaeus Stål, 1862	Rolston 1974a
Parentheca Berg, 1891	Campos & Grazia, 1999
Proxys Spinola, 1837,	Rolston 1974a
Sibaria Stål, 1867	Rolston 1974a
Spinalanx Rolston & Rider, 1988	Rolston & Rider, 1988

Table 2: Taxon sampling for the phylogenetic analysis of *Euschistus* and related carpocorines, including voucher specimen number and Genbank accession number for respective DNA fragment. Fragments generated here marked with “x”, while fragment not sequenced marked with “-”.

	Voucher ID	**16S**	**28S_D3-5**	**COI**	**COII**	**Cytb**	**Def**
Acledra breviscutata **Breddin, 1914**	PTM0273	x	x	x	x	x	x
Agroecus scabricornis **(Herrich-Schäffer, 1840)**	-	KU853764	-	KU892539	-	KU853783	-
Banasa euchlora **Stål, 1872**	PTM0121	x	x	x	x	x	x
Berecynthus hastator **Fabricius, 1798**	PTM0084	x	x	x	x	x	x
Caonabo pseudocylax **(Bergroth, 1891)**	-	KU853765	KU853749	KU892540	-	KU853784	-
Carpocoris **sp.**	PTM0104	x	x	KM021778	-	-	
Dichelops (Diceraeus) furcatus **(Fabricius, 1775)**	PTM0406	x	x	x	x	x	x
Dichelops (Dichelops) **leucostigmus (Dallas, 1851)**	PTM0065	x	x	x	x	x	x
Diploxys sp.	PTM00409	x	-	x	x	-	x
Dolycoris baccarum **(Linnaeus, 1758)**	PTM0272	x	-	x	x	NC_020373	x
Euschistus **(*Euschistus*)** ***atrox*** **(Westwood, 1837)**	PTM0296	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***bifibulus*** **(Palisot de Beauvois, 1805)**	PTM0303	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***biformis*** **Stål, 1862**	PTM0355	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***comptus*** **Walker, 1868**	PTM0353	x	-	-	-	x	-
Euschistus **(*Euschistus*)** ***crassus*** **Dallas, 1851**	PTM0327	x	x	KJ642003	x	x	x
Euschistus (Euschistus) crenator **(Fabricius, 1794)**	PTM0340	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***emoorei*** **Rolston, 1972**	PTM0308	x	x		x	x	x
Euschistus (Euschistus) heros **(Fabricius, 1798)**	PTM0302	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***obscurus*** **(Palisot de Beauvois, 1805)**	PTM0317	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***quadrator*** **Rolston, 1974**	PTM0314	x	x	x	-	x	x
Euschistus **(*Euschistus*)** ***servus*** **(Say, 1832)**	PTM0334	x	x	KR045133	-	x	
EuschistusE aff sulcacitus	PTM0286	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***sulcacitus*** **Rolston, 1971**	PTM0283	x	x	x	x		x
Euschistus **(*Euschistus*)** ***strenuus*** **Stål, 1862**	PTM0307	x	x	x	x	x	x
Euschistus (Euschistus) taurulus **Berg, 1878**	PTM0311	x	x	x	x	x	x
Euschistus **(*Euschistus*)** ***tristigmus*** **(Say, 1831)**	PTM0300	x	x	x	-	x	x
Euschistus (Lycipta) circumfusus **Berg, 1883**	PTM0399	x	x	-	x	x	x
Euschistus (Lycipta) cornutus **(Dallas, 1851)**	PTM0396	x	x	x	x	x	x
Euschistus **(*Lycipta*)** ***illotus*** **Stål, 1860**	PTM0341	x	x	x	x	x	x
Euschistus **(*Lycipta*)** ***pictcornis*** **Stål, 1872**	PTM0354 PTM0397	x	x	x	x	x	x
Euschistus **(*Lycipta*)** ***riograndensis*** **Weiler & Grazia, 2011**	PTM0402	x	x	-	x	x	x

***Euschistus (Lycipta) triangulator* (Herrich-Schäffer, 1842)**	PTM0398	x	x	-	x	x	x
***Mitripus acutus* (Dallas, 1851)**	PTM0293	x	x	x	x	x	x
***Mitripus convergens* (Herrich-Schäffer,1842)**	-	-	-	KU892548	-	KU853794	
***Adustonotus grandis* (Rolston, 1978)**	PTM0401	x	x	x	x	x	x
***Adustonotus hansi* (Grazia, 1987)**	PTM0400	-	x	x	x	x	-
***Adustonotus irroratus* (Bunde, Grazia & Mendonça, 2006)**	PTM0349	x	-	-	-	-	-
***Adustonotus paranticus* (Grazia, 1987)**	PTM0404	x	x	x	x	x	x
***Adustonotus saramagoi* (Bianchi, Cioato & Grazia 2015)**	-	KU853778	-	KU892552		KU853798	
***Hymenarcys crassa* Uhler, 1897**	PTM0319	x	x	x	x	x	x
***Hypatrops inermis* (Jensen-Haarup, 1928)**	PTM0112	x	x	x	x	x	x
***Ladeaschistus bilobus* (Stål, 1872)**	-	KU853779	KU853762	KU892554	-	KU853800	-
***Ladeaschistus borgesi* Bianchi, Cioato & Grazia, 2015**	PTM0350	x	-	x	-	-	-
***Mormidea pama* Rolston, 1978**	PTM0093	x	x	-	x	x	x
***Mormidea ypsilon* (Linnaeus, 1758)**	PTM0075	x	x	x	x	x	x
***Oebalus ypsilongriseus* (DeGeer, 1773)**	PTM0068	x	x	x	x	x	x
Padaeus sp.	PTM0110	x		x	x	x	-
***Prionosoma podopinoides* Uhler 1863**	PTM0094	x	x	x			x
***Proxys victor* (Fabricius, 1775)**	PTM0073	x	x	x	x	x	-
***Sibaria armata* (Dallas, 1851)**	PTM0050	x	x	x	x	x	x
***Sibaria englemani* Rolston, 1975**	PTM00407	x	-	x		-	-
***Spinalanx rolstoni* Thomas, 1995**	PTM0049	x	x	x	x	x	x
***Stysiana acarinatis* Grazia, Fernades & Schewertner, 1999**	PTM0045	-	x	x	-	-	x

Supplementary Files

Table Supplementary 1. Genes included in study, primer sequences and sources and annealing temperatures (AT).

Gene	**Primer name and sequence(5'-3')**	**Primer source**	**AT**
16S rDNA	16S: CGC CTG TTT ATC AAA AAC AT	Weirauch & Munro 2009	48°C
	16S: CGC CTG TTT AAC AAA AAC AT	Simon *et al.*, 1994	
Cyt b	Cytb: GGA CGW GGW ATT TAT TAT GGA TC	Monteiro *et al.* 2003	45-50°C
	Cytb: GCW CCA ATT CAR GTT ART AA	Monteiro *et al.* 2003	
COI	COI: GGA ACA GGA TGA ACA GTT TAC CCT CC	Simon *et al.*, 1994	45-50°C
	COI: TCT GAA TAT CGT CGA GGT ATT CC	Simon *et al.*, 1994	
28S D3-5	28S:TTG AAA CAC GGA CCA AGG AG	Weirauch & Munro 2009	52°C
	28S: CGC CAG TTC TGC TTA CCA	Weirauch & Munro 2009	
Dfd	Dfd:C GTC GAY CCN AAR TTY CCN CC	Tian *et al.* 2011	48°C
	Dfd:TGT ATY TTN CGC ATC CAN GGR TA	Tian *et al.* 2011	

Monteiro, F.A., Barrett, T.V., Fitzpatrick, S., Cordon-Rosales, C., Feliciangeli, D., Beard, C.B., 2003. Molecular phylogeography of the Amazonian Chagas disease vectors *Rhodnius prolixus* and R. robustus. *Molecular Ecology*, 12, 997–1006.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the entomological Society of America*, *87*(6), 651-701.

Tian, X., Xie, Q., Li, M., Gao, C., Cui, Y., Xi, L., & Bu, W. (2011). Phylogeny of pentatomomorphan bugs (Hemiptera-Heteroptera: Pentatomomorpha) based on six Hox gene fragments. *Zootaxa*, *2888*, 57-68.

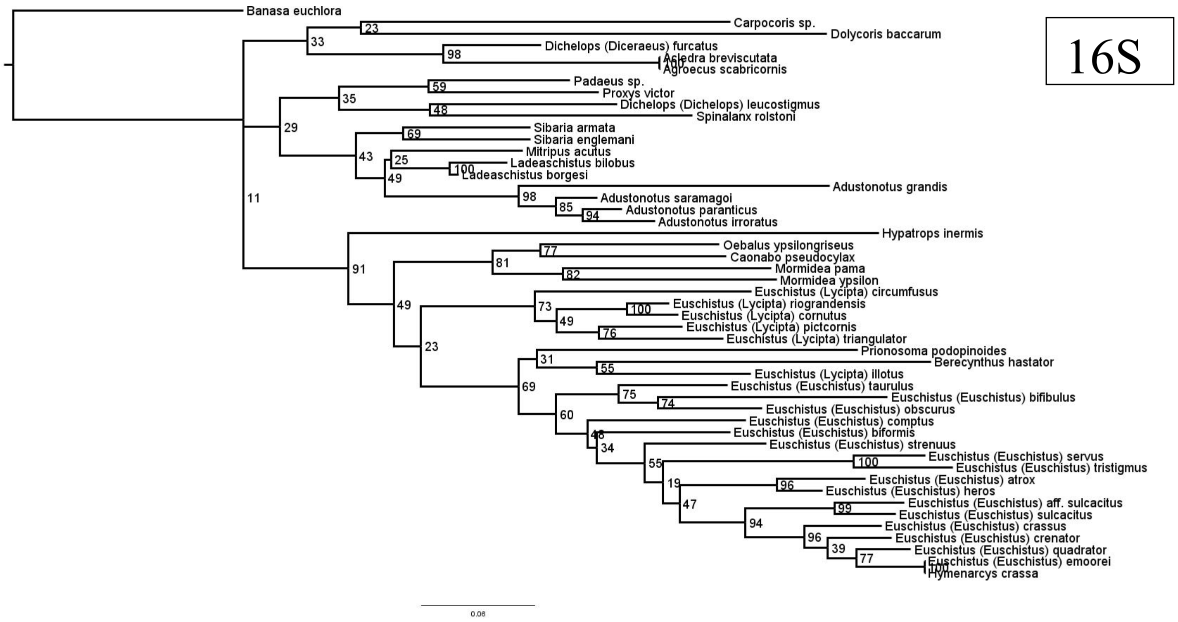
Weirauch, C., & Munro, J. B. (2009). Molecular phylogeny of the assassin bugs (Hemiptera: Reduviidae), based on mitochondrial and nuclear ribosomal genes. *Molecular Phylogenetics and Evolution*, *53*(1), 287-299

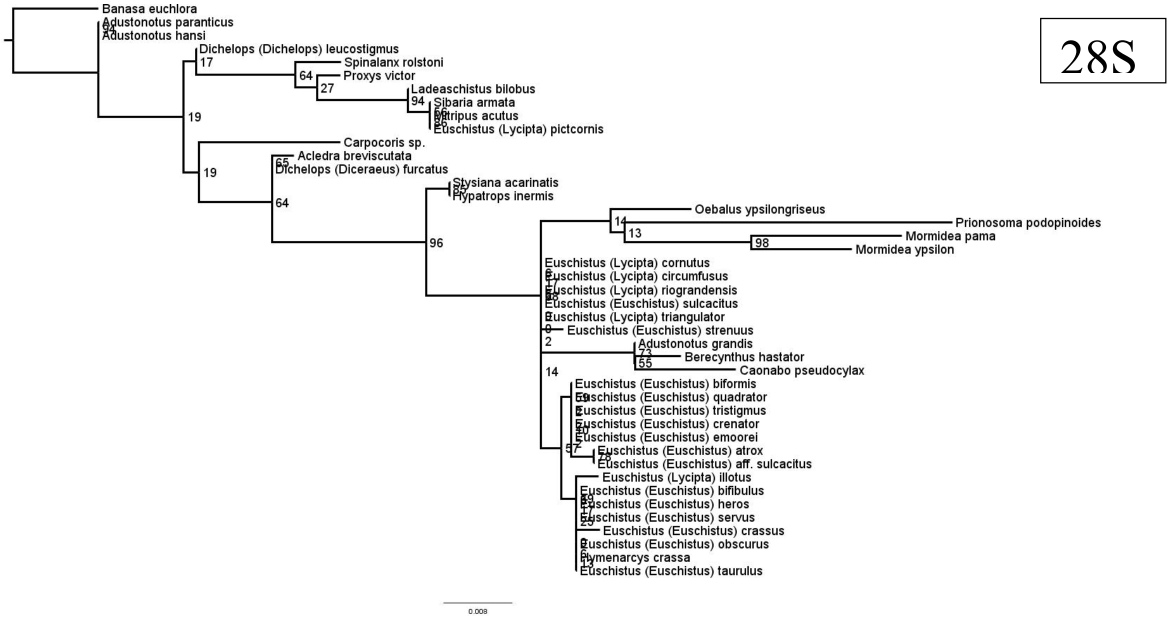
Table Supplementary 2. Matrix of morphological characters and states built to reconstruction of ancestral traits.

	Capsula seminalis, shape	**Pars intermedialis, shape**	**Vesicular area, apex of ductus receptaculi proximalis, shape**	**Capsula seminalis . process**	**Vesicular area, median tube**	**Pars intermedialis, anterior anular flange**
Acledra breviscutata **Breddin, 1914**	0	5	2	0	0	2
Adustonotus grandis **(Rolston, 1978)**	0	5	0	0	1	0
Adustonotus hansi **(Grazia, 1987)**	2	5	0	0	1	0
Adustonotus irroratus **(Bunde, Grazia & Mendonça, 2006)**	2	5	0	0	0	0
Adustonotus paranticus **(Grazia, 1987)**	2	5	0	0	0	0
Adustonotus saramagoi **(Bianchi, Cioato &Grazia 2015)**	2	5	0	0	1	0
Agroecus scabricornis **(Herrich-Schäffer, 1840)**	0	1	2	0	0	2
Banasa euchlora **Stål, 1872**	1	3	3	0	1	0
Berecynthus hastator **Fabricius, 1798**	1	1	2	0	0	1
Caonabo pseudocylax **(Bergroth, 1891)**	0	5	0	0	0	0
Carpocoris **sp.**	0	5	0	0	0	0
Dichelops (Diceraeus) furcatus **(Fabricius, 1775)**	1	2	0	0	1	1
Dichelops (Dichelops) **leucostigmus (Dallas, 1851)**	0	4	1	1	0	0
Diploxys_sp.	0	1	0	1	1	0
Dolycoris baccarum **(Linnaeus, 1758)**	0	5	0	0	0	2
Euschistus **(*Euschistus*)** ***aff. sulcacitus***	0	5	2	0	0	0
Euschistus **(*Euschistus*)** ***atrox*** **(Westwood, 1837)**	0	5	0	0	1	0
Euschistus **(*Euschistus*)** ***bifibulus*** **(Palisot de Beauvois, 1805)**	0	3	1	0	0	0
Euschistus **(*Euschistus*)** ***biformis*** **Stål, 1862**	0	1	1	0	0	2
Euschistus **(*Euschistus*)** ***comptus*** **Walker, 1868**	1	5	2	0	1	0
Euschistus **(*Euschistus*)** ***crassus*** **Dallas, 1851**	0	1	0	0	1	2
Euschistus **(*Euschistus*)** ***crenator*** **(Fabricius, 1794)**	0	5	0	0	1	0
Euschistus **(*Euschistus*)** ***emoorei*** **Rolston, 1972**	0	1	0	0	1	0
Euschistus (Euschistus) heros **(Fabricius, 1798)**	0	5	1	0	0	0
Euschistus **(*Euschistus*)** ***obscurus*** **(Palisot de Beauvois, 1805)**	1	3	0	0	0	2
Euschistus **(*Euschistus*)** ***quadrator*** **Rolston, 1974**	0	1	2	0	1	0

***Euschistus* (*Euschistus*) *servus* (Say, 1832)**	0	1	0	0	0	0
***Euschistus* (*Euschistus*) *strenuus* Stål, 1862**	0	1	0	0	0	0
***Euschistus* (*Euschistus*) *sulcacitus* Rolston, 1971**	0	5	2	0	1	0
***Euschistus* (*Euschistus*) *taurulus* Berg, 1878**	1	3	2	0	1	0
***Euschistus* (*Euschistus*) *tristigmus* (Say, 1831)**	0	1	1	0	0	2
***Euschistus* (*Lycipta*) *circumfusus* Berg, 1883**	0	1	2	0	0	1
***Euschistus* (*Lycipta*) *cornutus* (Dallas, 1851)**	0	1	2	0	0	1
***Euschistus* (*Lycipta*) *illotus* Stål, 1860**	0	1	2	0	0	1
***Euschistus* (*Lycipta*) *pictcornis* Stål, 1872**	0	1	2	0	0	1
***Euschistus* (*Lycipta*) *riograndensis* Weiler & Grazia, 2011**	0	1	2	0	0	1
***Euschistus* (*Lycipta*) *triangulator* (Herrich-Schäffer, 1842)**	0	1	2	0	0	1
***Hymenarcys crassa* Uhler, 1897**	0	1	2	0	0	0
***Hypatrops inermis* (Jensen-Haarup, 1928)**	0	5	0	0	1	2
***Ladeaschistus bilobus* (Stål, 1872)**	2	0	0	0	0	0
***Ladeaschistus borgesi* Bianchi, Cioato & Grazia, 2015**	2	0	0	0	0	0
***Mitripus acutus* (Dallas, 1851)**	2	0	2	0	1	0
***Mitripus convergens* (Herrich-Schäffer,1842)**	2	0	0	0	1	0
***Mormidea pama* Rolston, 1978**	0	5	0	1	0	0
***Mormidea ypsilon* (Linnaeus, 1758)**	0	5	0	1	1	0
***Oebalus ypsilongriseus* (DeGeer, 1773)**	0	1	0	1	0	2
Padaeus sp.	1	5	2	0	0	0
***Prionosoma podopinoides* Uhler 1863**	0	5	0	0	0	0
***Proxys victor* (Fabricius, 1775)**	1	1	2	0	0	0
***Sibaria armata* (Dallas, 1851)**	2	0	2	0	1	1
***Sibaria englemani* Rolston, 1975**	2	0	2	0	1	1
***Spinalanx rolstoni* Thomas, 1995**	0	4	0	1	0	0
***Stysiana acarinatis* Grazia, Fernades & Schewertner, 1999**	0	5	0	0	0	1

File Supplementary 3: Phylogenetic reconstruction for each partition separately [*16S* rRNA (16S), *28S* D3-D5 rDNA (28S), *cytochrome b* (Cyt b), *cytochrome oxidase subunit I* (COI), *cytochrome oxidase subunit II* (COII), *Deformed* (Dfd), Mitochondrial markers concatenated (16S+Cytb+COI+COII) (Mit) and nuclear markers concatenated (28S+Dfd) (Nuc) datasets. All presented reconstructions were conducted at CIPRES Science Gateway (Miller *et al.*, 2011) (www.phylo.org/portal2/) using RAxML-HPC2, GTR-CAT model for each molecular marker. Nodal support was assessed with automatic Stop Bootstrapping Automatically with Majority Rule Criterion (autoMRE).

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Adustonotus hansi
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Spinalanx rolstoni
Proxys victor
Ladeaschistus bilobus
Sibaria armata
Mitripus acutus
Euschistus (Lycipta) pictcornis
Carpocoris sp.
Acledra breviscutata
Dichelops (Diceraeus) furcatus
Stysiana acarinatis
Hypatrops inermis
Oebalus ypsilongriseus
Prionosoma podopinoides
Mormidea pama
Mormidea ypsilon
Euschistus (Lycipta) cornutus
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) riograndensis
Euschistus (Euschistus) sulcacitus
Euschistus (Lycipta) triangulator
Euschistus (Euschistus) strenuus
Adustonotus grandis
Berecynthus hastator
Caonabo pseudocylax
Euschistus (Euschistus) biformis
Euschistus (Euschistus) quadrator
Euschistus (Euschistus) tristigmus
Euschistus (Euschistus) crenator
Euschistus (Euschistus) emoorei
Euschistus (Euschistus) atrox
Euschistus (Euschistus) aff. sulcacitus
Euschistus (Lycipta) illotus
Euschistus (Euschistus) bifibulus
Euschistus (Euschistus) heros
Euschistus (Euschistus) servus
Euschistus (Euschistus) crassus
Euschistus (Euschistus) obscurus
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Euschistus (Euschistus) taurulus
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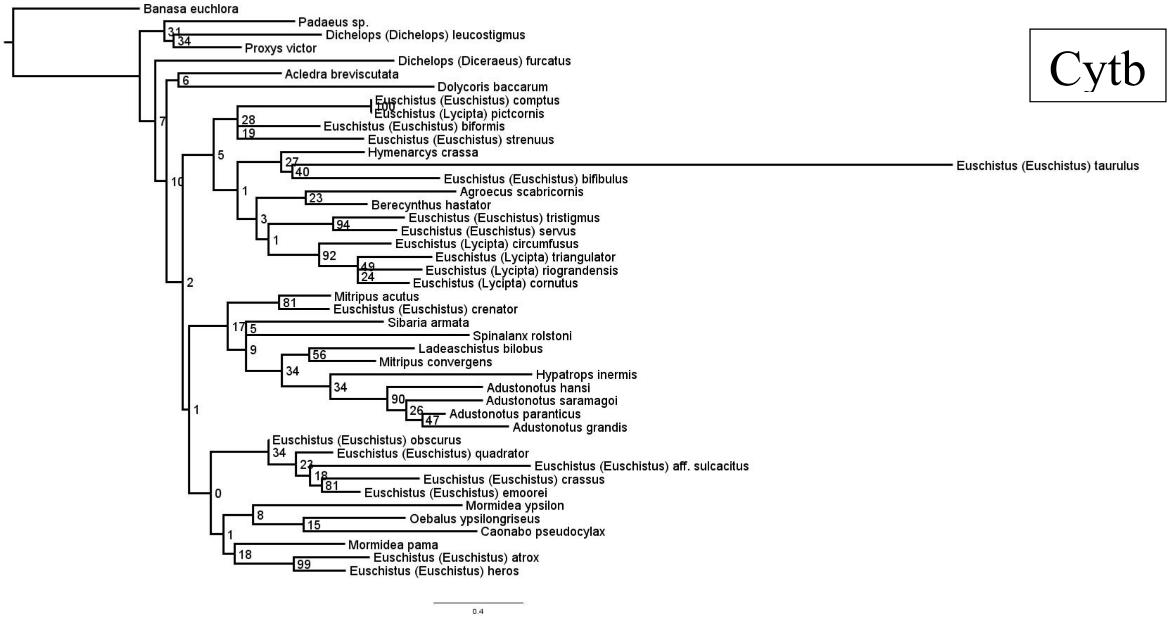
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Proxys victor
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Acledra breviscutata
Dolycoris baccarum
Euschistus (Euschistus) comptus
Euschistus (Lycipta) pictcornis
Euschistus (Euschistus) biformis
Euschistus (Euschistus) strenuus
Hymenarcys crassa
Euschistus (Euschistus) taurulus
Euschistus (Euschistus) bifibulus
Agroecus scabricornis
Berecynthus hastator
Euschistus (Euschistus) tristigmus
Euschistus (Euschistus) servus
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) triangulator
Euschistus (Lycipta) riograndensis
Euschistus (Lycipta) cornutus
Mitripus acutus
Euschistus (Euschistus) crenator
Sibaria armata
Spinalanx rolstoni
Ladeaschistus bilobus
Mitripus convergens
Hypatrops inermis
Adustonotus hansi
Adustonotus saramagoi
Adustonotus paranticus
Adustonotus grandis
Euschistus (Euschistus) obscurus
Euschistus (Euschistus) quadrator
Euschistus (Euschistus) aff. sulcacitus
Euschistus (Euschistus) crassus
Euschistus (Euschistus) emoorei
Mormidea ypsilon
Oebalus ypsilongriseus
Caonabo pseudocylax
Mormidea pama
Euschistus (Euschistus) atrox
Euschistus (Euschistus) heros
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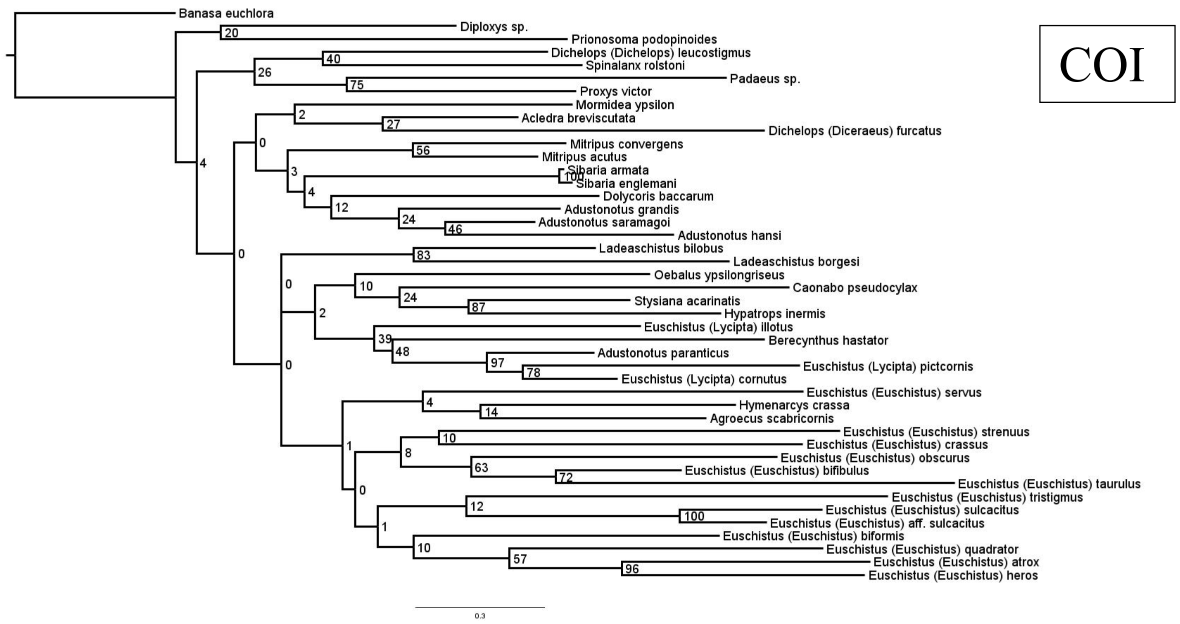
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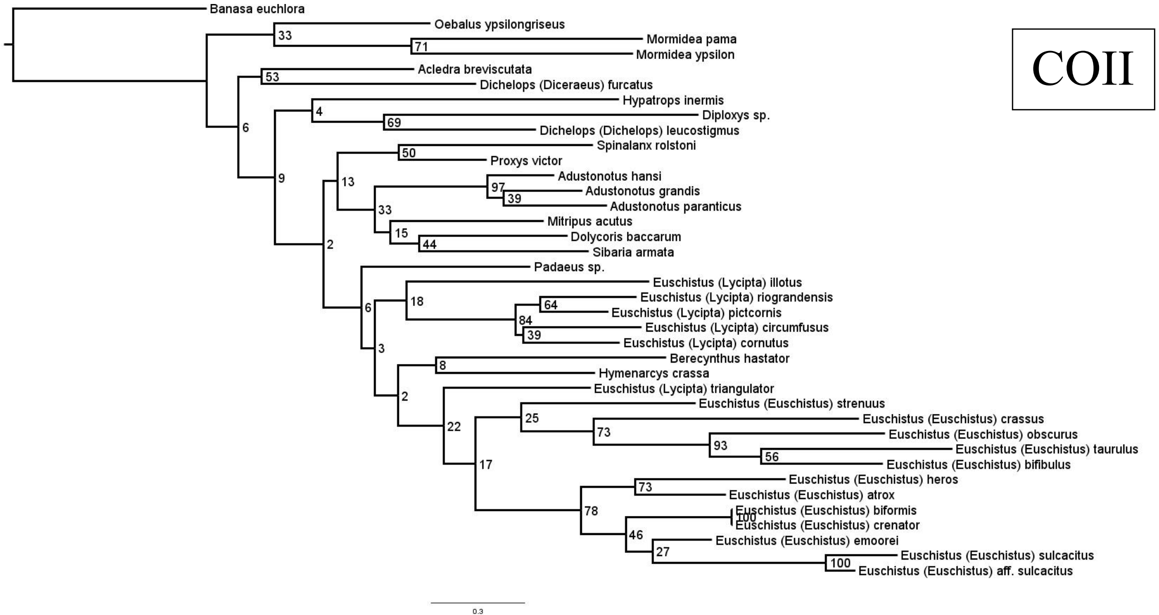
Prionosoma podopinoides
40
Dichelops (Dichelops) leucostigmus
26
Spinalanx rolstoni
75
Padaeus sp.
Proxys victor
4
2
Mormidea ypsilon
27
Acledra breviscutata
Dichelops (Diceraeus) furcatus
0
56
Mitripus convergens
Mitripus acutus
3
100
Sibaria armata
Sibaria englemani
4
Dolycoris baccarum
12
24
Adustonotus grandis
46
Adustonotus saramagoi
Adustonotus hansi
0
83
Ladeaschistus bilobus
Ladeaschistus borgesi
0
10
Oebalus ypsilongriseus
Caonabo pseudocylax
24
87
Stysiana acarinatis
Hypatrops inermis
2
39
Euschistus (Lycipta) illotus
Berecynthus hastator
48
97
Adustonotus paranticus
0
78
Euschistus (Lycipta) pictcornis
Euschistus (Lycipta) cornutus
4
Euschistus (Euschistus) servus
14
Hymenarcys crassa
Agroecus scabricornis
1
10
Euschistus (Euschistus) strenuus
Euschistus (Euschistus) crassus
8
63
Euschistus (Euschistus) obscurus
72
Euschistus (Euschistus) bifibulus
Euschistus (Euschistus) taurulus
0
12
Euschistus (Euschistus) tristigmus
100
Euschistus (Euschistus) sulcacitus
Euschistus (Euschistus) aff. sulcacitus
1
10
Euschistus (Euschistus) biformis
57
Euschistus (Euschistus) quadrator
96
Euschistus (Euschistus) atrox
Euschistus (Euschistus) heros
0.3


COII
Banasa euchlora
Oebalus ypsilongriseus
Mormidea pama
Mormidea ypsilon
Acledra breviscutata
Dichelops (Diceraeus) furcatus
Hypatrops inermis
Diploxys sp.
Dichelops (Dichelops) leucostigmus
Spinalanx rolstoni
Proxys victor
Adustonotus hansi
Adustonotus grandis
Adustonotus paranticus
Mitripus acutus
Dolycoris baccarum
Sibaria armata
Padaeus sp.
Euschistus (Lycipta) illotus
Euschistus (Lycipta) riograndensis
Euschistus (Lycipta) pictcornis
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) cornutus
Berecynthus hastator
Hymenarcys crassa
Euschistus (Lycipta) triangulator
Euschistus (Euschistus) strenuus
Euschistus (Euschistus) crassus
Euschistus (Euschistus) obscurus
Euschistus (Euschistus) taurulus
Euschistus (Euschistus) bifibulus
Euschistus (Euschistus) heros
Euschistus (Euschistus) atrox
Euschistus (Euschistus) biformis
Euschistus (Euschistus) crenator
Euschistus (Euschistus) emoorei
Euschistus (Euschistus) sulcacitus
Euschistus (Euschistus) aff. sulcacitus
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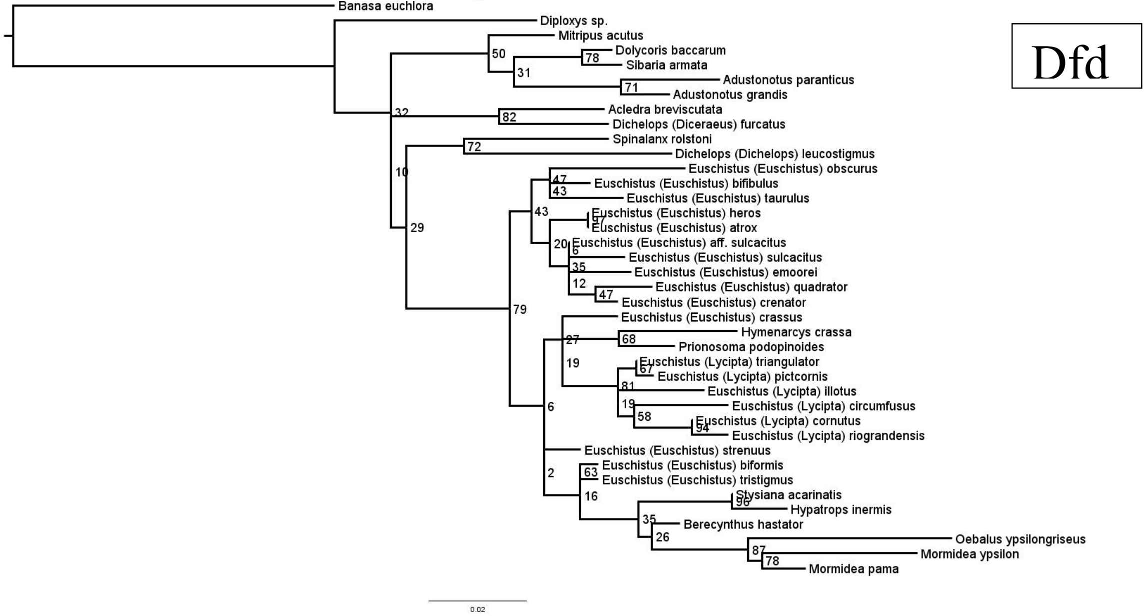
Dfd
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Acledra breviscutata
Dichelops (Diceraeus) furcatus
Spinalanx rolstoni
Dichelops (Dichelops) leucostigmus
Euschistus (Euschistus) obscurus
Euschistus (Euschistus) bifibulus
Euschistus (Euschistus) taurulus
Euschistus (Euschistus) heros
Euschistus (Euschistus) atrox
Euschistus (Euschistus) aff. sulcacitus
Euschistus (Euschistus) sulcacitus
Euschistus (Euschistus) emoorei
Euschistus (Euschistus) quadrator
Euschistus (Euschistus) crenator
Euschistus (Euschistus) crassus
Hymenarcys crassa
Prionosoma podopinoides
Euschistus (Lycipta) triangulator
Euschistus (Lycipta) pictcornis
Euschistus (Lycipta) illotus
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) cornutus
Euschistus (Lycipta) riograndensis
Euschistus (Euschistus) strenuus
Euschistus (Euschistus) biformis
Euschistus (Euschistus) tristigmus
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Hypatrops inermis
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Mormidea ypsilon
Mormidea pama
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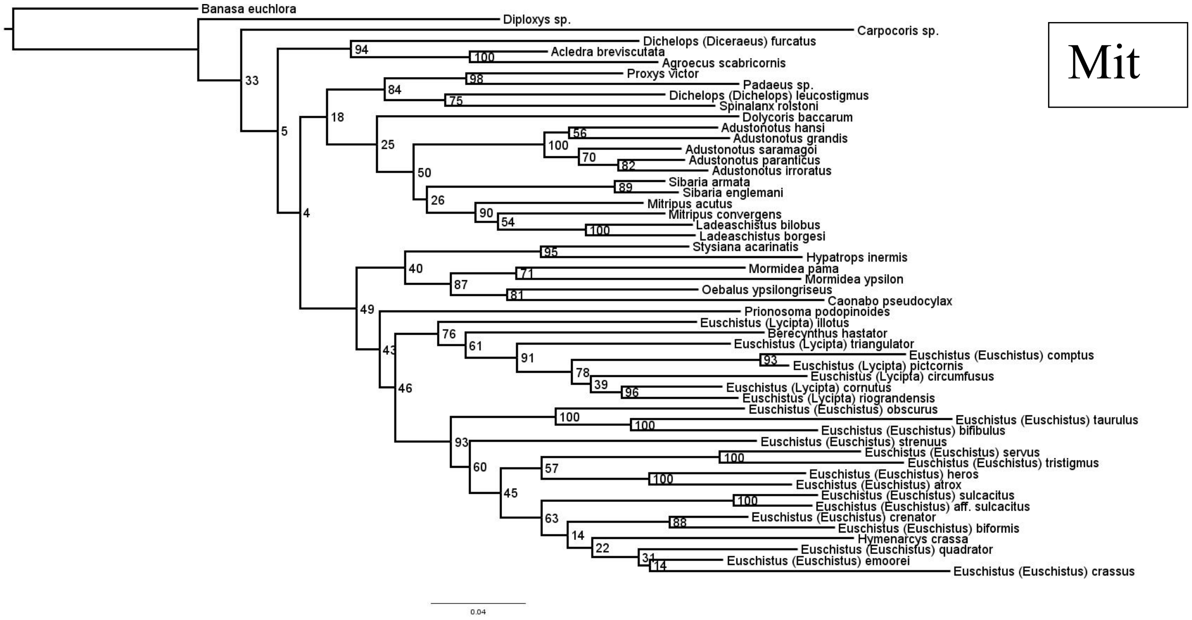
Mit
Banasa euchlora
Diploxys sp.
Carpocoris sp.
Dichelops (Diceraeus) furcatus
Acledra breviscutata
Agroecus scabricornis
Proxys victor
Padaeus sp.
Dichelops (Dichelops) leucostigmus
Spinalanx rolstoni
Dolycoris baccarum
Adustonotus hansi
Adustonotus grandis
Adustonotus saramagoi
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Adustonotus irroratus
Sibaria armata
Sibaria englemani
Mitripus acutus
Mitripus convergens
Ladeaschistus bilobus
Ladeaschistus borgesi
Stysiana acarinatis
Hypatrops inermis
Mormidea pama
Mormidea ypsilon
Oebalus ypsilongriseus
Caonabo pseudocylax
Prionosoma podopinoides
Euschistus (Lycipta) illotus
Berecynthus hastator
Euschistus (Lycipta) triangulator
Euschistus (Euschistus) comptus
Euschistus (Lycipta) pictcornis
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) cornutus
Euschistus (Lycipta) riograndensis
Euschistus (Euschistus) obscurus
Euschistus (Euschistus) taurulus
Euschistus (Euschistus) bifibulus
Euschistus (Euschistus) strenuus
Euschistus (Euschistus) servus
Euschistus (Euschistus) tristigmus
Euschistus (Euschistus) heros
Euschistus (Euschistus) atrox
Euschistus (Euschistus) sulcacitus
Euschistus (Euschistus) aff. sulcacitus
Euschistus (Euschistus) crenator
Euschistus (Euschistus) biformis
Hymenarcys crassa
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Euschistus (Euschistus) emoorei
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0.04

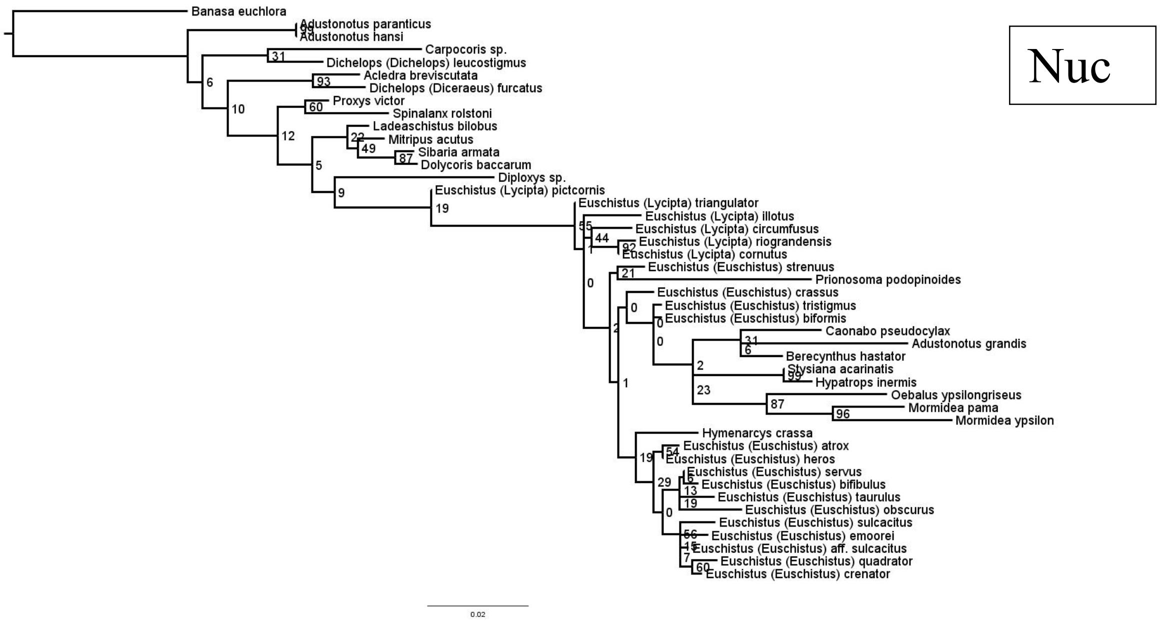
Nuc
Banasa euchlora
Adustonotus paranticus
Adustonotus hansi
Carpocoris sp.
Dichelops (Dichelops) leucostigmus
Acledra breviscutata
Dichelops (Diceraeus) furcatus
Proxys victor
Spinalanx rolstoni
Ladeaschistus bilobus
Mitripus acutus
Sibaria armata
Dolycoris baccarum
Diploxys sp.
Euschistus (Lycipta) pictcornis
Euschistus (Lycipta) triangulator
Euschistus (Lycipta) illotus
Euschistus (Lycipta) circumfusus
Euschistus (Lycipta) riograndensis
Euschistus (Lycipta) cornutus
Euschistus (Euschistus) strenuus
Prionosoma podopinoides
Euschistus (Euschistus) crassus
Euschistus (Euschistus) tristigmus
Euschistus (Euschistus) biformis
Caonabo pseudocylax
Adustonotus grandis
Berecynthus hastator
Stysiana acarinatis
Hypatrops inermis
Oebalus ypsilongriseus
Mormidea pama
Mormidea ypsilon
Hymenarcys crassa
Euschistus (Euschistus) atrox
Euschistus (Euschistus) heros
Euschistus (Euschistus) servus
Euschistus (Euschistus) bifibulus
Euschistus (Euschistus) taurulus
Euschistus (Euschistus) obscurus
Euschistus (Euschistus) sulcacitus
Euschistus (Euschistus) emoorei
Euschistus (Euschistus) aff. sulcacitus
Euschistus (Euschistus) quadrator
Euschistus (Euschistus) crenator
0.02

Figure S4.Summary of the parsimony ancestral state reconstructions for spermathecal structures. The tree shown is the majority-rule consensus of Bayesian Inference analyses. The pie charts along the branches illustrate the likelihood for the ancestral state reconstructed in Mesquite3.02. States of each spermathecal character for taxa is indicated in the circles after taxon names.

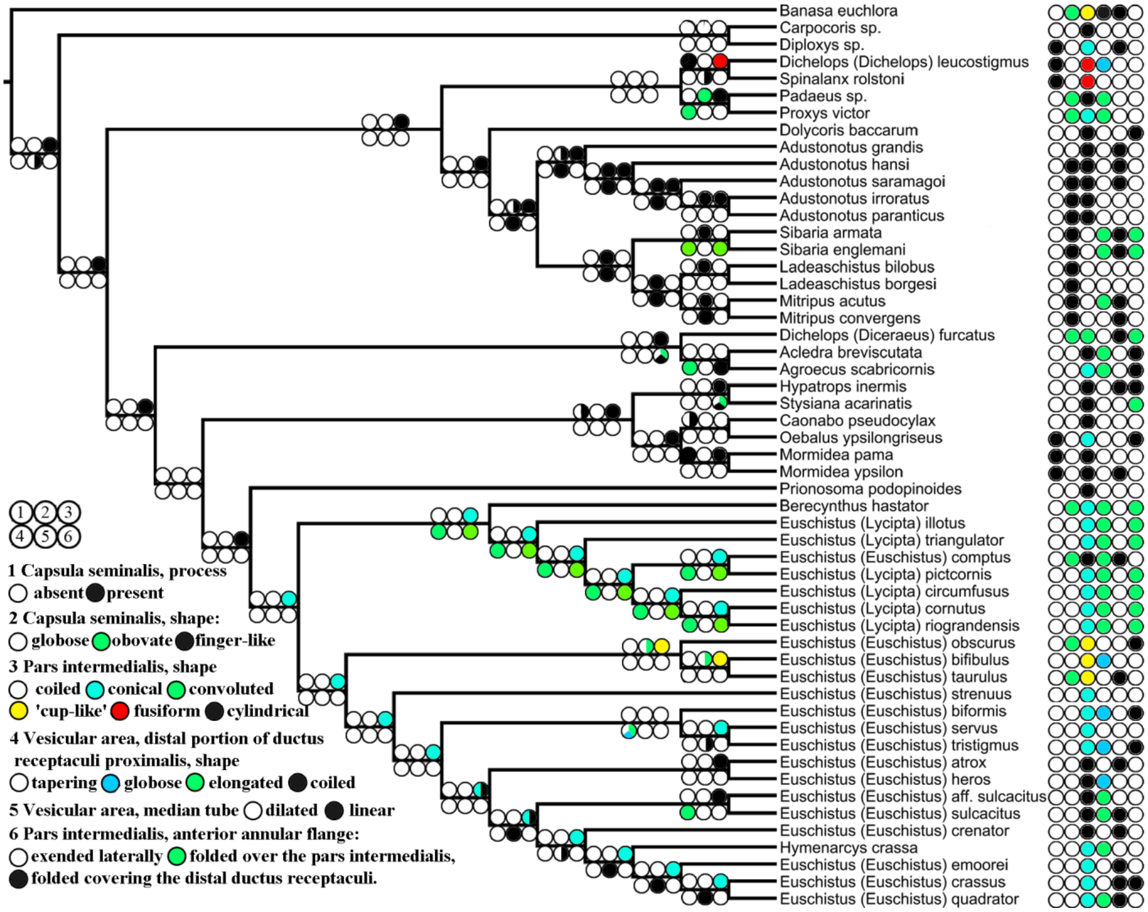

Table S4: Pagel's (1994) pairwise correlation test between six discrete variables of Carpocorini's (Pentatomidae) internal female genitalia. Values in bold indicate statistically significant correlations at a P-value of 0.05.

	***Capsula seminalis*, shape**	***Capsula seminalis*, process**	***Pars intermedialis*, shape**	***Pars intermedialis*, anterior annular flange**	**Vesicular area, apex of *ductus receptaculi proximalis*, shape**	**Vesicular area, median tube**
***Capsula seminalis*, shape**	x					
***Capsula seminalis*, process**	0.13	x				
***Pars intermedialis*, shape**	0.42	0.47	x			
***Pars intermedialis*, anterior annular flange**	0.26	0.39	0.28	x		
Vesicular area, apex of *ductus receptaculi proximalis*, shape	0.53	0.36	**0.006**	**0.03**	x	
Vesicular area, median tube	0.15	0.83	0.15	0.17	0.36	x

6 Considerações Finais

No capítulo I foi realizado um trabalho de taxonomia clássica, onde são descritas três espécies de Carpocorini (Pentatomidae). Aspéctos morfológicos dos gêneros de Carpocorini, principalmente gêneros relacionados à *Euschistus* são avaliados e considerados para o posicionamento das novas espécies dentro dos gêneros. *Euschistus* (*Euschistus*) *baranowskii* Eger & Bianchi é descrito a partir de uma fêmea coletada na Jamaica. Esta espécie de grande porte, considerando as outras espécies do gênero, se assemelha a *Euschistus* (*Euschistus*) *servus* (Say, 1832), *Euschistus* (*Euschistus*) *heros* (Fabricius, 1798) e *Euschistus* (*Euschistus*) *rufiger* Stål, 1872. Os ângulos humerais desenvolvidos em forma de foice distingue este dos outros *Euschistus* que ocorrem no Caribe. *Adustonotus saramagoi* (Bianchi, Cioato & Grazia) foi descrito no subgênero *Euschistus* (*Mitripus*). Esta espécie está distribuida no sul do Brasil. Pela morfologia externa do pigóforo é sugerida como parte do complexo de espécie formado por: *Euschistus* (*Mitripus*) *anticus* Stål, 1879, *Euschistus* (*Mitripus*) *paranticus* Grazia, 1987, *Euschistus* (*Mitripus*) *hansi* Grazia, 1987, e *Euschistus* (*Mitripus*) *irroratus* Bunde, Grazia & Mendonça, 2005. Na análise filogenética de *Euschistus* (capítulo II desta tese), o relacionamento filogenético deste complexo de espécies é recuperado, no entanto, o subgênero *E.* (*Mitripus*) é considerado linhagem filogenética independente de *Euschistus*. Uma nova combinação é proposta para o táxon, sendo o táxon aqui descrito nomeado *Adustonotus saramagoii.* A terceira espécie descrita no capítulo I é *Ladeaschistus borgesi* Bianchi, Cioato & Grazia, 2015, a qual se distribui pela Argentina, Bolívia, Brasil e Paraguai. O padrão de genitália masculina sugere proximidade filogenética com *Ladeaschistus boliviensis* Rolston 1973 e *Ladeaschistus armipes* (Stål, 1872), contudo a morfologia externa é semelhante a *Ladeaschistus bilobus* (Stål, 1872) e *Ladeaschistus trilobus* (Stål, 1872). Explorar caracteres morfológicos contribui para as inferências feitas para Carpocorini, sendo que o reconhecimento de padrões dentro dos taxa um confiável ponto de partida para explorações filogenéticas.

No capítulo II é realizada a primeira filogenia dentro de Pentatomidae usando evidências morfológicas e moleculares combinadas para testar uma hipótese filogenética. Neste trabalho foi testada a hipótese filogenética entre os subgêneros de *Euschistus*. Este gênero é o mais especioso dentro de Carpocorini e está entre os mais numerosos dentre Pentatomidae. Para tal, utilizamos dados morfológicos (85 caracteres) e moleculares (2.327 pb provenientes de 4 marcadores) para uma maior robustez analítica. A amostragem abrangeu os quatro subgêneros de *Euschistus* e também gêneros com morfologia similares, hipoteticamente relacionados. *Euschistus* foi recuperado como não monofilético em todas as análises. Tal resultado nos permitiu tomar as seguintes decisões taxonômicas: elevar *Euschistus* (*Mitripus*) ao rank de gênero, e ainda a descrição de um novo gênero, *Adustonotus* Bianchi, para conter parte das espécies anteriormente pertencentes a *Euschistus* (*Mitripus*). Estes gêneros estão relacionados com *Ladeaschistus* Rolston 1973, e *Sibaria* Stål, 1872. Ainda neste trabalho, foram contruidas chaves de identificação para estes gêneros relacionados, e espécies de ambos os novos gêneros. Estes resultados fornecem ideias sobre a atual classificação de Pentatomidae, ressaltando a necessidade de estudos focando as relações filogenéticas em diferentes níveis dentro da família.

No capítulo III é abordado um agrupamento de gêneros proposto inicialmente por L. Rolston, que relaciona *Euschistus* a outros dez gêneros baseado em foram e coloração do corpo. Posteriormente, outros gêneros foram relacionados a este agrupamento (e. g. *Coenus*, *Acledra*) e, também, taxa foram elevados de dentro deste grupo (e. g. *Mitripus, Ladeaschistus*). Atualmente o grupo *Euschistus* é composto por 20 gêneros. Apesar de os trabalhos taxonômicos terem crescentemente trazido mais informações na descrição e nas revisões dos taxa, a descrição da genitália de fêmea em Pentatomidae é por muitas vezes negligenciada. Explorar a morfologia da genitália interna feminina nos possibilitou detectar semelhanças e diferenças entre estruturas-chave para o processo de reprodução. Para inferir a evolução de caracteres de genitália interna de fêmea, nós realizamos análises filogenéticas baseadas em seis marcadores moleculares (3.300 pb) para 53 espécies, focando o grupo-*Euschistus*. Nesta análise o grupo *Euschistus* não resultou como um clado. As relações entre os gêneros, em parte, diferiram das propostas pela literatura. Estas hipóteses de relacionamentos aqui apresentadas, em parte são o primeiro teste filogenético que incluem determinados gêneros de Carpocorini. As estruturas das espermateca resultaram amplamente em estruturas que variam sua forma múltiplas vezes dentro da filogenia. Para a maior parte das estruturas não foi encontrado correlação entre a evolução das estruturas. Este resultado traz uma nova visão sobre os caracteres de genitália feminina e sua evolução. Elucidações sobre estruturas genitais podem trazer avanços para a relação de escolha de parceiros, viés sobre a prole, além de outros campos mais aplicados da entomologia.

O uso de moléculas para a inferência filogenética ainda é escasso em Heteroptera. Contudo, esta ferramenta é uma abordagem promissora, que juntamente com aspectos morfológicos podem progressivamente contribuir para as hipóteses de relacionamento filogenético em Pentatomidae. Contribuições futuras, tanto para níveis genéricos quanto de superfamília, devem ter em vista a avaliação outros marcadores moleculares. Assim então, padrões da evolução de cada gene poderão ser ultizados de maneira mais pontal para determinada questão científica.

7 Anexos

Normas para a submissão da Revista Zootaxa

ZOOTAXA

ISSN 1175-5326 (Print Edition) & ISSN 1175-5334 (Online Edition)

A mega-journal for zoological taxonomists in the world

Information for authors

- Aim and scope
- Research article
- Correspondence
- Special issues with collected papers (e.g. Festschrift)
- Preparation of manuscripts
- Submission of manuscripts
- Review process
- Publication
- Page charge and colour plates
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Aim and scope

Zootaxa is a peer-reviewed international journal for rapid publication of high quality papers on any aspect of systematic zoology, with a preference for large taxonomic works such as monographs and revisions. *Zootaxa* considers papers on all animal taxa, both living and fossil, and especially encourages descriptions of new taxa. All types of taxonomic papers are considered, including theories and methods of systematics and phylogeny, taxonomic monographs, revisions and reviews, catalogues/checklists, biographies and bibliographies, identification guides, analysis of characters, phylogenetic relationships and zoogeographical patterns of distribution, descriptions of taxa, and nomenclature. Open access publishing option is strongly encouraged for authors with research grants and other funds. For those without grants/funds, all accepted manuscripts will be published but access is secured for subscribers only. All manuscripts will be subjected to peer review before acceptance. *Zootaxa* aims to publish each paper within one month after the acceptance by editors.

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- taxonomic/nomenclatural notes of importance
- book reviews meant to introduce readers to new or rare taxonomic monographs (interested authors/publishers must write to subject editors before submitting books for review; editors then prepare the book review or invite colleagues to prepare the review; unsolicited reviews are not published)
- and short papers converted from manuscripts submitted as research articles but are too short to qualify as formal research articles.

These short contributions should have no more than **20 references** and its **total length should not exceed four printed pages (except editorials).** Neither an abstract nor a list of key words is needed; major headings (Introduction, Material and methods...) should NOT be used, except for new taxon heading and references. A typical correspondence should consist of (1) a short and concise title, (2) author name and address (email address), (3) a series of paragraphs of the main text,and (4) a list of references if any. For correspondence of 3 or 4 pages, the first or last paragraph may be a summary.

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Smith, A. (1999) Title of the paper. *Title of the journal in full*, volume number, page range.

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Smith, A. & Smith, B. (2000) Title of the Chapter. *In*: Smith, A, Smith, B. & Smith, C. (Eds), *Title of Book*. Publisher name and location, pp. x–y.

C) **Book**:
Smith, A., Smith, B. & Smith, C. (2001) *Title of Book*. Publisher name and location, xyz pp.

C) **Internet resources**
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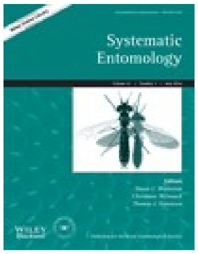

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Pachygnatha atromarginata sp. n. (Figs 100-111)

Holotype. m, CAMEROON, Mount Koupé, 1600 m, rain forest, 8 February 1983, Bosmans & Bosselaers (sweep net), (MRAC).

Paratypes. Same data as for holotype, 4 mm 8ff W (MRAC).

Other material. Mount Koupé, 1300 m, rain forest, 1f, 31 January 1983 (pitfall trap); 1f, 2 February 1983 (sweep net) (MRAC Mount Koupé, 900 m, rain forest, 1 m (subadult) 2ff, 31 January 1983 (pitfall trap), Bosmans & Bosselaers (all MRAC).

Etymology. The name refers to the dark, reticulated marginal zone of the carapace.

Diagnosis. This species can be distinguished by the shape of the paracymbium (Fig. 103) and the vulvar morphology (Fig. 111) in the female.

Description

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Family PHYLLODOCIDAE

Genus *Phyllodoce* Lamarck, 1818

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Phyllodoce badia Malmgren, 1867: 22, pl. II, fig. 6.

Anaitides citrina Bergström 1914: 140-141, fig. 41; Eliason 1962a: 18; Hartmann-Schröder 1971: 105-107, fig. 33D-F; Uschakov 1972: 136-137, pl. V, figs 5, 6.

Material examined. Phyllodoce citrina: 3 syntypes from Spitsbergen (SMNH type collection 2419 and 2420); 1 syntype from Spitsbergen (BMNH 1865.9.23.3); about 30 specimens from Wales, Shetland, western Norway, Spitsbergen, and Greenland (SMNH, MZB); about 10 specimens from the Arctic, Bering Sea and the Sea of Okhotsk (ZIL). *Phyllodoce badia*: several syntypes (at least 4 specimens and some fragments) from Bohuslän, Sweden (SMNH type collection 2423).

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