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MICROLEPIDÓPTEROS DO BRASIL: DESCRIÇÃO DE TRÊS ESPÉCIES DE GRACILLARIIDAE, ELACHISTIDAE E HELIOZELIDAE

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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(a): Prof. Dr. Gilson R. P. Moreira

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“O que sabemos é uma gota; o que ignoramos é um oceano.”

Isaac Newton

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RESUMO

O termo Microlepidoptera diz respeito a um clado artificial que engloba as mariposas de pequeno porte, altamente especializadas quanto à morfologia do estágio larval, que se alimenta, geralmente, no interior de tecidos vegetais. São pouco conhecidas na região Neotropical e, as descrições de espécies correspondentes têm sido feitas, com frequência, utilizando-se apenas das características dos adultos, tais como coloração geral, associada à morfologia das asas e genitálias. As fases larvais costumam ser duradoras comparada ao adulto e apresentam hábitos alimentares variados (ex. minador, galhador ou brocador), podendo conter caracteres com valor taxonômico importante. Algumas espécies de minadores foliares são consideradas pragas, pois se alimentam de culturas com importância econômica, mas para a grande maioria desconhece-se o dano ocasionado às hospedeiras. O objetivo desse trabalho foi a descrição de três espécies de micromariposas pertencentes as famílias Gracillariidae (Lithocolletinae: *Porphyrosela arachisella* Moreira & Becker), Elachistidae (Elachistinae: *Elachista* **sp.**) e Heliozelidae (*Heliozela* **sp.**), associadas as seguintes plantas hospedeiras, respectivamente, das quais são minadores foliares: *Arachis pintoi* Krapov. & W.C. Greg. (Fabaceae), *Chusquea tenella* Nees (Poaceae) e *Eugenia uniflora* L. (Myrtaceae). As descrições abrangem adultos e imaturos, com ilustrações calcadas na microscopia óptica e eletrônica de varredura, e informações a respeito da história de vida. Além disso, são obtidas e comparadas sequências de DNA mitocondrial (COI), a fim de estabelecer de forma preliminar o status específico e as relações filogenéticas com espécies aparentadas para cada grupo. Esse trabalho possibilitou o registro inédito no Brasil da familia Elachistidae, da subfamilia Lithocolletinae e do gênero *Heliozela*.

Palavras-chave: Microlepidoptera, minadores foliares, região Neotropical, taxonomia.

ABSTRACT

Microlepidoptera refers to an artificial group that abridge small moths, which are highly specialized in larval morphology, most of endophytic feeding habit. They are little known in the Neotropical region, where original description of species have been performed mainly based upon adult characters such as body coloration associated with wing and genitalia morphology. The larval phases usually last longer than the adults and show variation in feeding habits (ex. miners, gallers, borers), an may contain characters with important taxonomic value. Some species of leaf-miners are considered agriculture pests, however for most of them the damage for host plants are unknown. The goal of this work was to describe in detail three new species of micromoths belonging to Gracillariidae (Lithocolletinae: *Porphyrosela arachisella* Moreira & Becker), Elachistidae (Elachistinae: *Elachista* **sp.**) e Heliozelidae (*Heliozela* **sp.**), associated as leaf-miners of the following hostplants, respectively: *Arachis pintoi* Krapov. & W.C. Greg. (Fabaceae), *Chusquea tenella* Nees (Poaceae), and *Eugenia uniflora* L. (Myrtaceae). Descriptions abridge the adults and immature stages. They are based on both optical and scanning electron microscopy, and information on life history. DNA mitochondrial (COI) sequences are also obtained and compared to obtain under preliminary base the taxonomic status at specific level, and corresponding relationship within phylogenetic related groups. In this study are recorded for the first time in Brazil, the family Elachistidae, subfamily Lithocolletinae, and the genus *Heliozela*.

Keywords: Microlepidoptera, leaf-miners, Neotropical region, taxonomy.

CAPÍTULO I

INTRODUÇÃO GERAL

Lepidoptera, uma das maiores e mais bem estudadas ordens de insetos, ocorre em praticamente todas as regiões do planeta, ocupando uma grande quantidade de hábitats (Kristensen *et al*. 2007; Regier *et al*. 2015a). Esse grupo abrange uma das maiores radiações de insetos herbívoros e polinizadores, além de contribuírem como modelos importantes para os estudos de genética, fisiologia, desenvolvimento, ecologia e biologia evolutiva (Mitter *et al*. 2017). Na atualidade, a ordem conta com aproximadamente 160 mil espécies, divididas em 43 superfamílias e 133 famílias, sendo o clado *Ditrysia* (duas aberturas genitais femininas) o mais abundante, abrangendo 98% das espécies e com maior quantidade de trabalhos explorando sua filogenia (Mitter *et al*. 2017; Kawahara *et al*. 2019). O restante dos lepidópteros fazem parte dos *Monotrysia* (uma abertura genital única na fêmea), um clado polifilético que contém 14 superfamílias e 21 famílias, de pequeno porte (<10mm de envergadura de asa) e cujas larvas, em sua maioria, se alimentam no interior de tecidos vegetais (Kristensen *et al*. 2007; Mitter *et al*. 2017). Kristensen (2003) propôs uma filogenia para a ordem, que apensar de conter controvérsias, foi bem aceita, na época. A filogenia dessas linhagens mais antigas de lepidópteros (i.e. *Monotrysia*) foi revista por Regier *et al*. (2015b) que resolveu algumas das questões deixadas anteriormente, revisando as principais características da morfologia de forma comparada, bem como em relação à história natural e evolução dos principais grupos.

Minas são canais de alimentação formadas por larvas de insetos dentro de tecidos superficiais das plantas, tais como parênquimas ou epiderme (Figura 1), os quais fornecem alimento e abrigo (Hering, 1951). Alguns autores sugerem que esse hábito de vida pode trazer vantagens, como a proteção contra predadores e às defesas físicas das plantas, bem como em relação a situações adversas de recursos naturais (ex. Impacto da precipitação e radiação ultravioleta). Outros apontam prováveis desvantagens, como a redução da mobilidade, o que pode tornar esses insetos mais suscetíveis ao ataque de parasitóides especializados (Connor & Tavener 1997; Sinclair & Hughes 2010). As minas podem ser construídas por pelo menos quatro ordens de insetos: Coleoptera, Diptera, Hymenoptera e Lepidoptera (Sinclair & Hugues, 2010) e esse hábito evoluiu independentemente diversas vezes (Auerbach *et al*. 1995). Elas podem ser classificadas quanto à forma (ex., serpenteantes, lineares, com forma de manchas ou blocos) e tendem a ser características, podendo auxiliar na identificação de espécimes tanto em

nível genérico quanto específico (Moore 1966). Os microlepidópteros, um grupo artificial que engloba as mariposas de pequeno porte, podem passar todo o estágio larval dentro de uma única mina, com alta especificidade quanto à planta hospedeira, mas com uso em termos de guilda extensivo a um grande número de angiospermas (De Prins & De Prins, 2018). Algumas espécies nesse caso estão associadas a hospedeiras de interesse econômico, tais como *Citrus* sp., *Coffea* sp., *Malus* sp., *Theobroma* sp., *Vitis* sp. (Sinclair & Hugues, 2010; Nieukerken *et al*. 2012; De Prins *et al*. 2019).

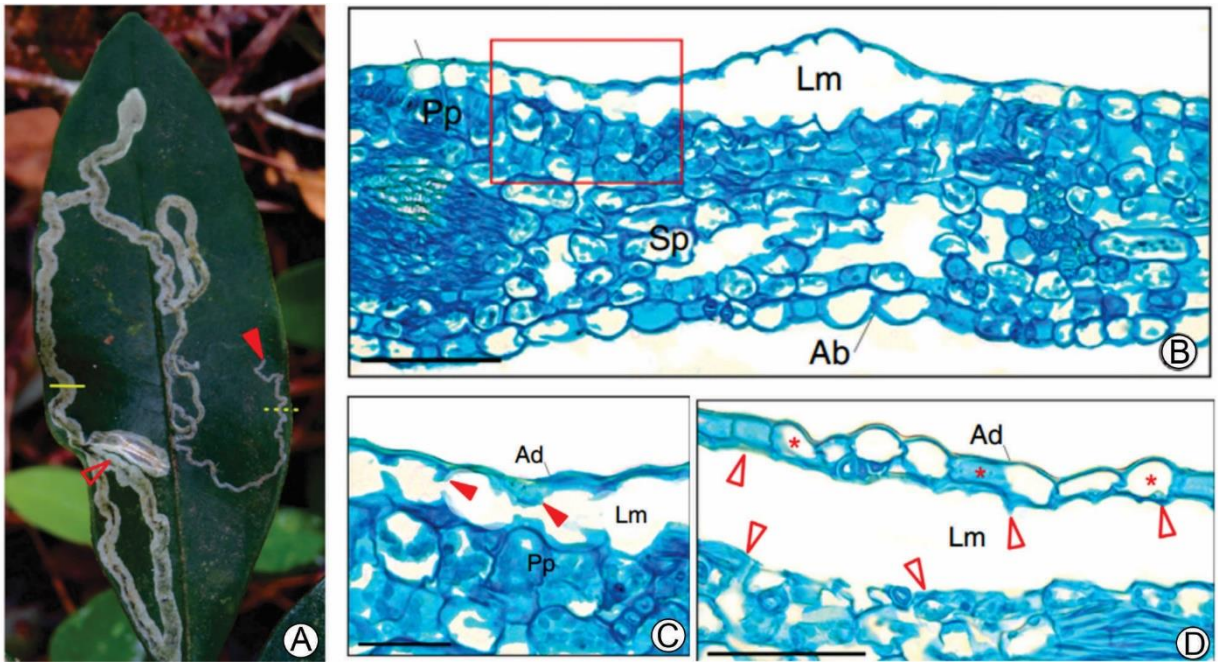

Figura 1. Minas de *Phyllocnistis hemera*.Brito & Fochezato (A) mina em uma folha de *Daphnopsis fasciculata* (Meisn); (B) corte histológico da mina construída por *P. hemera*; (C) detalhe da porção inicial da mina (indicado pelo retângulo em B); (D) detalhe da porção intermediária da mina. Fonte: Modificado de Fochezato *et al*. (2018).

GRACILLARIIDAE

É considerada a principal família de microlepidópteros minadores e apresenta aproximadamente 2000 espécies distribuídas por todas as regiões geográficas, com exceção da Antártica. Em torno de 180 foram descritas para a região Neotropical e, destas, apenas 40 para o Brasil (De Prins & De Prins, 2022). Segundo estimativas, no Neotrópico, o número de espécies pode ser até 20 vezes maior, o que reflete o baixo esforço amostral na região (Brito *et al*. 2016). São várias as dificuldades no estudo do grupo, sendo considerada a principal o tamanho reduzido dos adultos, que pode variar de 2-10 mm de envergadura de asa (Davis, 1987), além da escassez de profissionais capacitados na região. Kawahara *et al*. (2018) propuseram a divisão de Gracillariidae em oito subfamílias com base em caracteres moleculares:

Lithocolletinae Stainton, 1854, Acrocercopinae Kawahara & Ohshima, 2017, Phyllocnistinae Herrich-Schäffer, 1857, Marmarinae Kawahara & Ohshima, 2017, Oecophyllembiinae Réal & Balachowsky, 1966, Gracillariinae Stainton, 1854, Parornichinae Kawahara & Ohshima, 2017 e Ornixolinae Kuznetzov & Baryshnikova, 2001. Li *et al*. (2021) revisou a filogenia e adicionou uma nona subfamília: Callicercopinae Li, Oshima & Kawahara, 2021.

Os ovos dos gracilarídeos são depositados na superfície adaxial ou abaxial da folha na planta hospedeira (Sinclair & Hugues, 2010). Após a eclosão, a larva penetra pela epiderme dando início a alimentação e a construção da mina (Davis, 1987). A característica marcante da família é o fato de as larvas apresentarem hiper-metamorfose, ou seja, transformações morfológicas marcantes ao longo da ontogênese larval. Em consequência, podem ser divididas em três tipos morfológicos, dependendo da espécie em questão: 1) *sap-feeding* (típica dos primeiros ínstares), é caracterizada pela cabeça prognata com peças bucais adaptadas à dilaceração de tecidos, seguida da ingestão dos líquidos; cabeça e corpo achatados com pernas e pseudopódios reduzidos ou, na maioria dos casos, ausentes; 2) *tissue-feeding*, apresenta cabeça hipognata com peças bucais do tipo mastigadora; corpo cilíndrico com pernas e pseudopódios geralmente presentes do terceiro ao quinto e no décimo segmento abdominal; 3) *spinning*, também conhecida como pré-pupa, caracterizada pelo aparelho bucal atrofiado e o espinerete funcional, utilizado na construção do casulo (Kumata, 1978).

Entre as espécies da família há uma grande variação quanto a história natural e podem utilizar para a construção da mina e alimentação, não apenas tecidos foliares, mas também de pecíolos, flores e frutos (Davis, 1987). As espécies geralmente são monófagas, algumas oligófagas, e podem estar associadas a diversas famílias de plantas espalhadas nos diferentes continentes (Kawahara *et al*. 2018; De Prins & De Prins, 2022). O conhecimento da planta hospedeira é muito importante para identificação das espécies em campo quando as minas são encontradas, sendo os adultos de uma maneira geral pouco atraídos à luz (armadilhas luminosas), o que reflete em reduzida representatividade nas coleções entomológicas.

LITHOCOLLETINAE

É uma subfamília de Gracillariiedae monofilética com 12 gêneros e cerca de 550 espécies, sendo que apenas 21 dessas ocorrem no neotrópico (Kawahara *et al*. 2011; Kawahara *et al*. 2018; Li *et al*. 2021; De Prins & De Prins, 2022). A característica que separa os litocoletíneos dos demais gracilarídeos é a condição paralela da veia Rs com M_1 ou M_{1+2} (Kumata, 1993). As espécies da subfamília costumam apresentar três ínstares *sap-feeding*, seguidos por mais dois instares do morfotipo *tissue-feeding* (Figura 2), todos endófilos e se

alimentando de parênquima, formando uma mina, geralmente, em forma de mancha. Ao empupar, as espécies tentem a deformar a folha, causando uma dobra (Bentacourt & Scatoni 2007; Doorenweerd *et al*. 2014), resultando em mina conhecida como tentiforme. Algumas espécies são conhecidas como pragas agrícolas, como: *Cameraria ohridella* Deschka & Dimić, 1986 que se alimenta de *Aesculus hippocastanum* L., *Phyllonorycter blancardella* (Fabricius, 1781) em *Malus* spp. (De Prins & Kawahara 2012).

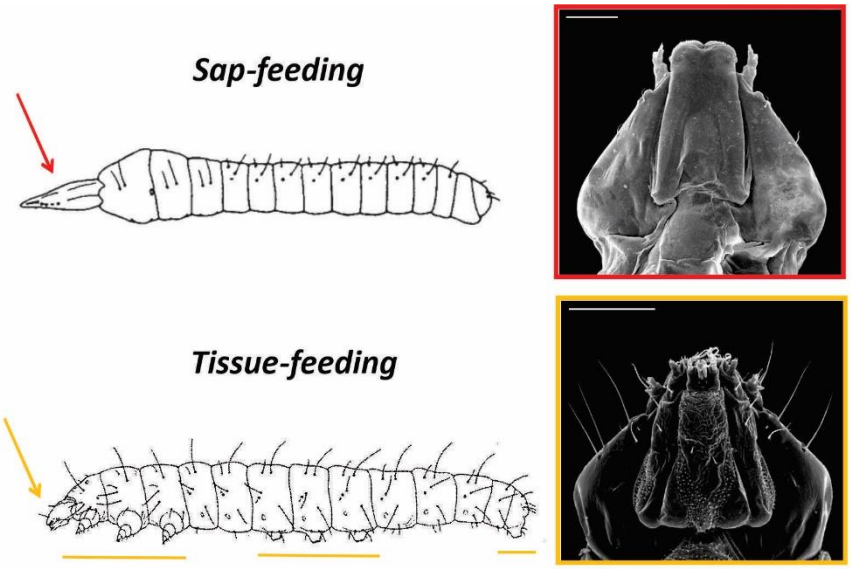

Figura 2: Morfotipos larvais encontrados em Lithocolletinae. Fonte: Modificado de Davis & De Prins (2011).

Porphyrosela Braun compreende doze espécies associadas a Fabaceae (de Prins & de Prins 2022). Duas dessas espécies, *P. desmodiella* (Clemens) e *P. minuta* Clarke são listadas para o neotrópico (nenhuma no Brasil), sendo a última com ocorrência conhecida no Uruguai e Argentina e, recentemente, encontrada nos Estados Unidos (Eiseman *et al*. 2017). Os adultos estão entre os menores lepidópteros conhecidos, com algumas espécies apresentando menos de três milímetros de envergadura de asa (Stonis *et al*. 2021). De Prins & Kawahara (2012) estabelecem que esse grupo em particular é pouco atraído por armadilhas luminosas. Portanto, as alternativas para conhecimento da diversidade geralmente envolvem a busca por minas em campo para criação ou o uso de armadilhas *Malaise*, por exemplo.

Os estágios imaturos de *Porphyrosela* são pouco conhecidos, provavelmente em função do tamanho diminuto, e a única espécie que inclui dados de morfologia sob microscopia ótica é *P. minuta* que é encontrada associada as folhas de *Trifolium repens* L. (Fabaceae) no Uruguai (Bentacourt & Scatoni 2007).

ELACHISTIDAE

Gelechioidea possui aproximadamente 18 mil espécies e é uma das superfamílias mais diversas de Lepidoptera. A maior abundância de espécies encontra-se nas regiões temperadas e boreais, embora esse fato talvez seja reflexo da baixa amostragem nos trópicos (Kaila *et al*. 2011). Durante os estágios larvais, a maior parte das espécies se alimenta externamente à planta, junto ao solo, e costumam se abrigar unindo folhas ou construindo casulos de seda portáteis (Powell *et al*. 1998). Porém, também podem ser verificadas espécies com hábito de alimentação interno a tecidos vegetais, como minadores, associados a famílias como Elachistidae.

Elachistidae se encontra atualmente subdividida em três subfamílias: Parametriotinae, Agonoxeninae e Elachistinae (Kaila, 2019). No Brasil, não há nenhum representante conhecido, visto que *Orthiostola* Meyrick, 1927, com quatro espécies registradas no Brasil por Moreira (2018), foi recentemente realocado para Acrolepiidae. Elachistinae contém 805 espécies predominantemente minadoras de folhas, pelo menos em algum estágio do desenvolvimento, e é o maior grupo de lepidópteros especializado em hospedeiras monocotiledôneas (Kaila *et al*. 2011). Entre seus 66 gêneros *Elachista* Treitschke é o mais diverso, e se alimenta majoritariamente de Poaceae, Cyperaceae ou Juncaceae. São conhecidas por minar folhas no outono, completando seu desenvolvimento somente no início da primavera (Braun, 1920; Braun, 1921; Braun, 1948; Kaila *et al*. 2011; Kaila, 2019). Na América do Sul, são reconhecidas por Kaila (2000) sete espécies, sendo três delas listadas com posição taxonômica incerta. Em adicional a estas, foram descritas mais cinco espécies por Struoga (2010) para o Equador.

O pequeno tamanho, o modo de vida críptico e o fato de os adultos apresentarem as asas frequentemente escuras e/ou cores discretas, fazem com que os elachistídios, assim como os microlepidópteros no geral, possuam poucos dados disponíveis quanto à morfologia e biologia (Baran & Buszko 2010). Algumas poucas espécies de *Elachista* possuem descrições dos imaturos, normalmente da larva de último instar e pupa, tais como: *Elachista irenae* Buszko, 1989; *Elachista zonulae* Struoga, 1992 e *Elachista synethes* Meyrick, 1897. Esta última, cuja localidade tipo é a Austrália, foi listada como espécie exótica, com ocorrência no Deserto do Atacama, Chile (Gonçalves *et al*. 2015; Vargas *et al*. 2015). As minas podem ser serpenteantes, em forma de mancha ou mesmo alterando entre um padrão e outro ao longo do desenvolvimento (Vargas *et al*. 2015). As pupas possuem características marcantes para identificação, como a presença de projeções latero-dorsais denominadas tubérculos, além de espinhos em forma de gancho nos últimos segmentos abdominais (Braun 1948; Vargas *et al*. 2015).

HELIOZELIDAE

Pertencente à Adeloidea, aonde se situa esta família, foi anteriormente tratada como Incurvarioidea (Davis 1986; Kristensen 2003b). Consta de uma superfamília primitiva (pertencente à *Monotrysia*) de mariposas que compreende também Adelidae, Incurvariidae, Cecidosidae e Prodoxidae, e a mais recentemente descrita, Tridentaformidae (Nielsen & Davis 1985; Nieukerken *et al*. 2011; Regier *et al*. 2015b). Em todas, observa-se a presença de um ovipositor do tipo "piercing", especializado na oviposição endofítica. Diversas estratégias de alimentação são adotadas pelos imaturos. Dentre elas, podemos citar brocadores de sementes, flores e caules, portadores de casulos móveis que se alimentam de folhas mortas no chão, minadores foliares e galhadores, sendo que cada família possui suas particularidades (Regier *et al*. 2015b).

Heliozelidae é cosmopolita e, geralmente, de hábitos diurnos; possui menos de 130 espécies divididas em 12 gêneros, sendo quatro deles os mais diversos, contendo 90% da diversidade: *Antispila* Hübner, *Coptodisca* Walsingham, *Heliozela* Herrich-Säffer e *Hoplophanes* Meyrick (Nieukerken *et al.* 2012; Liu & Wang 2017; Regier *et al*, 2015b; Milla *et al.* 2019). Abriga espécies consideradas praga, pois se alimentam de culturas de interesse econômico como mirtilo, videiras, nozes e castanha (Kuroko, 1982; Maier, 1988; Bernardo *et al*. 2015; Nieukerken & Eiseman 2020). Na região Neotropical há 14 espécies, duas delas no Brasil, descritas com base nos adultos, tendo sido a maioria delas designada ao gênero *Antispila* (Heppner, 1984). Porém, conforme sugerido por estudos filogenéticos recentes como os de Milla *et al*. (2018, 2019) várias dessas espécies podem pertencer a outros gêneros (Nieukerken & Eisman 2020). A maior diversidade de espécies é encontrada na América do Norte e Austrália (Milla *et al*. 2018; Milla *et al*. 2019). Porém, essa informação pode não condizer com a realidade devido ao baixo esforço amostral, que pode ser percebido devido à baixa quantidade de espécies descritas nos últimos anos (Brito *et al*. 2016; Milla *et al*. 2019). A quantidade escassa de informações acerca dos imaturos, juntamente com esse baixo esforço amostral, dificultam a identificação e a comparação das espécies sem a criação dos imaturos (Kristensen *et al*. 2007).

Ao contrário dos demais Adeloidea, a família possui predominantemente o hábito minador, podendo de alimentar de estruturas como folhas, pecíolos, caules (Davis, 1998). Entre as espécies Australianas, o hábito de minar flores e sementes parece ser predominante (Milla *et al*. 2018). Uma característica é marcante quanto ao desenvolvimento: as espécies cortam a folha em forma de casulo, que, com a larva em seu interior, cai no chão e é carregado por ela até empupar. Esse comportamento deixa um orifício ovalado nas folhas anteriormente minadas e o fato de carregar pelo solo esse casulo de folha dá o nome *shield-bearer* (Figura 3). A forma

desse casulo, bem como a mina foliar e a planta hospedeira, são de grande valia como caracteres que auxiliam a identificação das espécies (Kuroko, 1961; Nieukerken *et al*. 2012, Milla *et al*. 2018). As sinapomorfias da família incluem os braços tentatoriais anteriores curvados dorsalmente, a falta da veia M-Cua nas asas posteriores e as escamas lamelares achatadas sobrepostas sobre a cabeça (Nielsen & Davis 1985; Karshold & Kristensen, 2003; Milla *et al*. 2019).

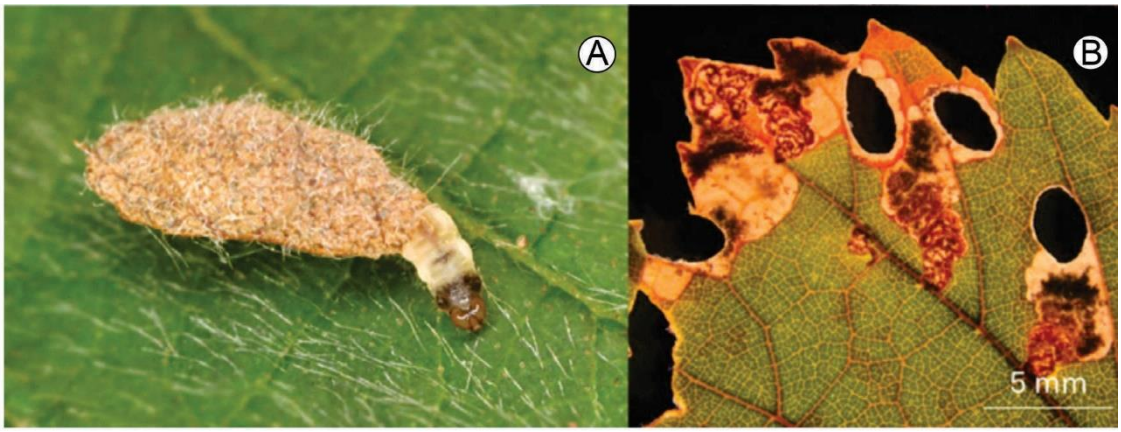

Figura 3. Larva e dano provocado pelo hábito de *Antispila hydrangaeella*. (A) larva em seu casulo portátil formado a partir do corte da face adaxial e abaxial da folha visto em (B). Fonte: Modificado de Milla *et al*. (2018).

Entre os gêneros da família, encontramos *Heliozela* Herrich-Schäffer, 1853 que possui cerca de 30 espécies no mundo e pode ser encontrado associado a várias plantas hospedeiras, especialmente Myrtaceae. As espécies são difíceis de serem diferenciadas pela morfologia externa dos adultos, já que costumam ser semelhantes, geralmente exibindo uma coloração escura (marrom ou cinza) com marcas transversais brancas (Simonsen, 2001; Lee *et al*. 2006; Milla *et al*. 2019).

As espécies descritas neste gênero, assim como a maior parte dos Heliozelidae, são feitas com base na morfologia externa dos adultos e das genitálias, não havendo muitas informações sobre estágios imaturos. Quando as coletas são decorrentes de minas, que posteriormente são criadas em laboratório, é possível encontrar uma breve caracterização das minas, casulo e planta hospedeira, como pode ser visto no trabalho de Mutanen *et al*. (2007).

O objetivo geral deste estudo foi descrever três espécies de microlepdópteros minadores de folhas pertencentes a Gracillariidae, Elachistidae e Heliozelidae associadas as plantas hospedeiras *Arachis pintoi* Krapov. & W.C. Greg. (Fabaceae), *Chusquea tenella* Nees (Poaceae) e *Eugenia uniflora* L. (Myrtaceae), respectivamente. A primeira ocorre no bioma Cerrado e, as duas últimas, em fragmentos de mata mais austrais da Mata Atlântica. Contempla-

se a morfologia geral externa dos adultos e imaturos sob microscopia ótica e eletrônica de varredura, suas histórias de vida, incluindo a caracterização externa das minas e planta hospedeira. Além disso, são apresentados dados moleculares com base em DNA mitocondrial (COI) para inferir relações filogenéticas dentro das respectivas famílias. Cada espécie é tratada em capítulos distintos, já formatados para publicação. Aquele referente a primeira espécie de gracilarídeo (*Porphyrosela arachisella* Moreira & Becker 2022) já se encontra publicado.

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CAPÍTULO II

Artigo **publicado** como:

Fochezato, J., Brito, R., Gonçalves, G. L., Specht, A., Becker, V.O., Moreira, G. R. P., 2018. Description of *Porphyrosela arachisella* sp. nov. (Lepidoptera: Gracillariidae), the first report of Lithocolletinae for Brazil. Zootaxa 5165, 387–404.

Description of *Porphyrosela arachisella* sp. nov. (Lepidoptera: Gracillariidae), the first report of Lithocolletinae for Brazil.

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Abstract

During a recent survey of leaf-mining microlepidoptera in the Cerrado biome, mines of an undescribed *Porphyrosela* Braun (Lepidoptera: Gracillariidae: Lithocolletinae) were found associated with the forage peanut, *Arachis pintoi* Krapov. & W.C. Greg. (Fabaceae). Consequently, adults, immature stages and the leaf mine of *Porphyrosela arachisella* **sp. nov.** are herein described based on light and scanning electron microscopy. A preliminary analysis of DNA barcode sequences including putative members of other lithocolletine species and all BINs (Barcode Index Numbers) available for *Porphyrosela* supports *P. arachisella* as an independent cluster, with 8 to 11% divergence. Its nearest neighbor was the cluster formed by three BINs (BOLD: ADT2137, BOLD: AAG1161 and BOLD: ADU9985) that includes specimens from Australia, Vietnam and Bangladesh. This is the first report of a lithocolletine gracillariid for Brazil, and the third species recognized for the genus in the Neotropical region.

Keywords

Leaf mining, *Arachis pintoi*, forage peanut, micromoths, Neotropical region

Introduction

Gracillariidae is one of the most important families of leaf-mining microlepidoptera, comprising ca. 2000 recognized species that are distributed worldwide, except for Antarctica (Davis 1984; De Prins & De Prins 2022). The family was divided into

eight subfamilies by Kawahara *et al*. (2017), with a ninth added by Li *et al*. (2021). One of these is Lithocolletinae Stainton, with twelve genera and ca. 550 species. Most of the described lithocolletines occur in the Palearctic region. For the Neotropics, 20 species are recorded (De Prins *et al*. 2019; De Prins & De Prins 2022). A synapomorphy for the subfamily is the parallel condition of Rs vein with either M_1 or M_{1+2} (Kumata 1993). The larvae have a hypermetamorphic development, with three sap-feeding and two tissue-feeding instars, all endophyllous. They feed on parenchyma, leaving both epidermises intact (Doorenweerd *et al*. 2014). At the end of development, mines are typically tentiform. Monophyly of Lithocolletinae has also been supported by molecular data (Kawahara *et al*. 2011; Kawahara *et al*. 2017; Li *et al*. 2021). The majority of lithocolletines are monophagous. A few species, particularly in the genera *Cameraria* Chapman and *Phyllonorycter* Hübner, are however oligophagous. Some, for example, *Cameraria ohridella* Deschka & Dimić that feeds upon *Aesculus hippocastanum* L. (Sapindaceae), and *Phyllonorycter blancardella* (Fabricius), associated with *Malus* spp. (Rosaceae), may cause severe damage to their host plants (De Prins & Kawahara 2012; De Prins & De Prins 2022).

Porphyrosela Braun comprises twelve species that are distributed worldwide, all associated with Fabaceae (De Prins & De Prins 2022). Only two of those, *P. desmodiella* (Clemens) and *P. minuta* Clarke, have been recorded in the Neotropics. The latter had been previously known only in southern South America (Uruguay and Argentina), but was recently found in the USA (Eiseman *et al*. 2017). Lithocolletinae has not been reported in Brazil yet (Brito & Duarte 2022). However, pictures of the leaf mine and adult of *P. minuta* were provided without collection data by Brito *et al*. (2016), from a population of Bagé municipality (Rio Grande do Sul state, Brazil) located close to the Uruguayan border. Adults of *Porphyrosela* are among the smallest Lepidoptera, some species measuring less than three millimeters in wingspan (Stonis *et al*. 2021). They are not attracted much to light traps and are rarely present in insect collections from the region. Thus, the distribution and diversity of the genus in the Neotropics are supposed to be much greater, the situation described above resulting from a scarcity of sampling, as already pointed out by Brito *et al*. (2016) for gracillariids in general. The immatures of *Porphyrosela* are also poorly known, except for *P. minuta*, which were described under light microscopy in association with *Trifolium repens* L. (Fabaceae) in Uruguay (Bentancourt & Scatoni 2007).

The lithocolletine described in the present study was first reared in 1976 by V. Becker, from leaf-mines associated with *Arachis pintoi* Krapov. & W.C. Greg. (Fabaceae) in a small garden in downtown Goiás, Goiás state, Brazil. Recently, a second population of the same

plant species, with a high density of leaf mines was found at the Brasilia urban area, Federal District, ca. 316 km apart. A comparison from a morphological perspective, including wing color pattern and genitalia of both sexes, showed that it does not conform to any other known *Porphyrosela* species. Thus, the adults, immature stages, and the leaf mine of the new species are herein described, based upon light and scanning electron microscopy. Data on the natural history of the new species are also provided. A DNA-based phylogenetic tree and associated genetic distances are also provided, to delimit the new species.

Material and Methods

Most of the material used in this study was obtained from leaf mines collected during September 2018 on a population of *Arachis pintoi* grown at the gardens of the National Congress, Brasilia, Federal District (DF), Brazil (15º47'53" S; 47º51'51" W; Fig. 7A). Branches containing leaves with active mines were brought to the Entomology Laboratory of Embrapa - Brazilian Agricultural Research Corporation, decentralized unit Embrapa Cerrados, located in Planaltina municipality (Federal District). The branches had their bases attached to moistened cotton and placed in plastic vials that were maintained at controlled abiotic conditions in a climatic chamber (14h light/10h dark; 25 ± 2ºC). They were checked daily for adult emergence. Adults were pinned and dried. Additional leaves and mines were dissected for obtaining eggs, larvae, and pupae that were fixed in Dietrich´s fluid and preserved in 70% ethanol, and used in the morphological descriptions. Some were kept apart in ethanol 100% at -20ºC for DNA extraction.

Morphological analyses were performed at Laboratório de Morfologia e Comportamento de Insetos (LMCI), Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre municipality, Rio Grande do Sul (RS), Brazil. For descriptions of adults, genitalia were dissected and cleared in a 10% potassium hydroxide (KOH) aqueous solution, stained with either eosin or Chlorazol black E, and slide-mounted in Canada balsam. Last instar larvae were prepared similarly for the chaetotaxy. Observations were performed with the aid of a Leica® M125 stereomicroscope. Structures selected to be drawn were previously photographed with a Sony® Cyber-shot DSC-H10 digital camera attached to the stereomicroscope. Vectorized line drawings were then made with the software Corel Draw® X9, using the corresponding digitalized images as a guide.

Specimens used for scanning electron microscope analyses were dehydrated in a Bal-tec® CPD030 critical-point dryer, mounted with double-sided tape on metal stubs, coated with gold in a Bal-tec® SCD050 sputter coater and then examined and photographed in a JEOL®

JSM6060 scanning electron microscope at the Centro de Microscopia e Microanálise (CMM) of UFRGS.

At least ten specimens of each stage were examined during the morphological analyses. The terminology used for descriptions followed Davis (1987), Patočka and Turčani (2005), and De Prins & Kawahara (2012) for the larvae, pupae, and adults, respectively.

Museum Collections

The material examined was deposited in the following entomological collections:
LMCI – Coll. Laboratório de Morfologia e Comportamento de Insetos, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil;
VOB – Coll. Vitor O. Becker, Reserva Serra Bonita, Camacan, BA, Brazil

Molecular analysis

DNA was extracted from larvae of four specimens (LMCI 344-47A, 344-47B, 344-47C, 344-47D) dissected from leaf mines of *A. pintoi* collected from the population already mentioned. Samples were amplified and sequenced using the barcoding primers LCO1490 and HCO2198 (Folmer *et al.* 1994), which resulted in 650-base pairs fragment of the Cytochrome Oxidase 'barcode' gene. These barcodes were aligned with 56 sequences of *Porphyrosela* represented by 10 Barcode Index Numbers (BINs) (Ratnasingham & Hebert 2013), as well as six private sequences identified as *Porphyrosela*, downloaded from Barcode of Life Data Systems (BOLD) (www.barcodinglife.org) (Table 2). Two other Lithocolletinae [*Phyllonorycter salicicolella* (Sircom) and *Cameraria guttifinitella* (Clemens) (Genbank accession HM874857.1 and KU380345.1, respectively)] were incorporated in the analysis as an outgroup, based on the molecular phylogeny proposed by Kawahara *et al*. (2017). MEGA X (Kumar *et al*. 2018) was used to estimate genetic distances (using Kimura-2 parameter model) and build a neighbor-joining (NJ) tree, with 1000 bootstrap replication.

Results

Porphyrosela arachisella Moreira & Becker sp. nov.

Figs. 1-8

Type material. MALE HOLOTYPE: BRAZIL: DF, Brasilia, gardens of Federal Senate, 13.ix.2018, pinned-dried, ex. larvae mining on *Arachis pintoi* leaflets, Julia Fochezato col., deposited in LMCI collection, under accession code 344-67. Paratypes: pinned-dried adults; same data collection as holotype: seven males (LMCI 344-49 to 51, 56, 58, 70 and 76); eight

females (LMCI 344-52, 55, 57, 59, 61, 63, 65, 69); all deposited in LMCI collection, except LMCI 344-56, 58, 63, 65 which were donated to VOB.

Dissected adults: same collection data as the holotype: genitalia on slides, 5 males (LMCI 344-34A, 34B, 36A, 71), 7 females (LMCI 344-34C, 36B, 36C, 72, 74, 75, 77). **Immature stages**: fixed in Dietrich´s fluid, preserved in 70% ethanol, same collection data as the holotype: three leaf fragments with attached eggs (LMCI 344-37); 70 sap-feeding larvae (LMCI 344-4), 60 tissue feeding larvae (LMCI 344-5), 15 pupae (LMCI 344-6).

Other adult specimens examined: *Pophyrosela arachisella* sp. nov - GO, Goiás, em. 15–25.vi.1976, 52 pinned dried, ex. larvae mining on *Arachis pintoi* leaflets (Becker 19924) (VOB). *Porphyrosela minuta* Clarke – EMBRAPA Pecuária Sul, Bagé, RS, pinned-dried, one male and one female (LMCI 304-03), with genitalia on slides, ex. larvae mining *Trifolium repens* leaflets, Gilson R.P. Moreira, 9.ix.2016, deposited in LMCI collection, under accession codes 304-01 and 304-03, respectively.

Diagnosis. *Porphyrosela arachisella* sp. nov. is morphologically similar to *P. desmodiella* and *P. minuta*, from which it can be separated by a conjunction of morphological characters. The adult of *P. arachisella* differs from both species by possessing forewings with four costal and three dorsal white strigulae. Rarely the basal strigulae are partially fused mesally, forming a transversal fascia as in *P. desmodiella* and *P. minuta*. When this is the case, such a transverse fascia is always followed in *P. arachisella* not by a second fascia but by a pair of well-separated strigulae slightly beyond the middle of the forewing. The second fascia appears either perpendicular to the wing margin or in opposite direction from the basal fascia in *P. desmodiella* and *P. minuta*, respectively (Betancourt & Scatoni 2007, Eiseman *et al*. 2017). In contrast, the forewing of *P. arachisella* has the two basal pairs of strigulae outwardly oblique, aligned parallel to each other, particularly in the females. The new species is distinguished further from *P. desmodiella* by the presence of a black terminal line on the forewing, also found in *P. minuta*. However, *P. minuta* and *P. desmodiella* lack the penultimate costal strigula (Clarke 1953; Betancourt & Scatoni 2007; Eiseman *et al*. 2017) that is found in *P. arachisella*, as already noted.

Male genitalia are similar to those of *P. minuta* by possessing a blunt distal outward margin of valvae, which is bumped in *P. desmodiella* (Vári 1961, Pl. 49, fig. 6). Also, by having cornutus in the vesica, which is absent in *P. desmodiella*. This structure consists of a slightly curved, lightly sclerotized hook in *P. arachisella*, but is much stronger, looking like a hammer

in *P. minuta*. The saccus is short and stout, with an acute apex in *P. arachisella*. To the contrary, it has a narrower basal portion and a dilated apex in *P. minuta*.

There is no detailed description available for larvae and pupae of *P. desmodiella* and *P. minuta*, which impedes the corresponding diagnosis at such stages. Notwithstanding, up to now mines of *P. arachisella* have only been found on *Arachis pintoi*, a plant not reported as a host for the other two species (De Prins & De Prins 2022). Contrary to those of *P. desmodiella* (see Eiseman *et al*. 2017), mines of *P. arachisella* are formed on the upper surface of the leaves. They are similar to those of *P. minuta*, but can be easily distinguished from them by the grouped, side by side attached eggs, either under development or represented by empty chorions, usually three per cluster in *P. arachisella*. They are laid isolated in the case of *P. minuta* (Betancourt & Scatoni 2007).

Description.

Male adult (Figs. 1A-B, D-G). Forewing length: 1.51–2.08 mm (n = 7). Head: Vertex tufted with short ochreous, distally furcated, piliform scales; frons smooth, covered with metallic shiny scales projecting anteriorly. Labial palpus as long as the diameter of eye, drooping, directed downwards. Haustellum short, pale beige. Antennae slightly shorter than forewing; basal part of antennae mostly light brown, distal flagellomeres pure white; scape shorter and thicker than pedicel, dorsally loosely covered with appressed, short, brownish, piliform scales, with 3–5 short, brownish hair-like pecten. Thorax: Basic color ochreous, with strong metallic gloss. Forewing ground color coppery, with four costal and three dorsal white, black-edged strigulae, and a conspicuous black-lined termen. Strigulae reach near the middle line of the wing. The first costal strigula at 1/3 forewing; the three basal strigulae transversally aligned with the dorsal ones; distal costal strigula on apical fifth. Cilia short, pale brownish at apex and termen, gradually getting longer, and dense, at tornus. Hind wing and cilia blackish fuscous. Forewing venation with 7 veins; Sc ending near 1/3 of costa; terminating apical part with three veins, R_4, R_5, M_1; M_1 and Cu_1 separate; CuP indistinct over entire length; 1A separate; cell closed. Hind wing lanceolate, narrow, venation reduced to four veins: Sc very short terminating near the base of costa; Rs very long, running almost to apex of hindwing; M1 unbranched, ending near 2/3 of dorsum; Cu1 strong, ending slightly before 1/2 of the dorsum. Frenulum - a single stout bristle. Legs slender, general color light brown; hind tibia coppery, with basal and distal margin white; distal tarsomere and pretarsus white. Abdomen: Dark brownish grey.

Male genitalia (Figs. 2B-E). Tegumen subconical, longer than valva, slightly rounded apically, with a pair of setae laterally on distal margin; basal parts of tegumen narrow and sclerotized, running parallel to each other with smooth anastomosis into ca. 2/3 part of the tegumen. Tuba analis slightly protruding beyond tegumen. Valvae symmetrical, bar-shaped, with cucullus blunt, curved medially, and bearing fine setae. Vinculum narrow, with short and stout saccus bearing acute distal end. Transtilla narrow for the whole length. Aedeagus tubular, slightly shorter than the valvae, broader basally; vesica tubular, weakly sclerotized, covered by sparse, fine setae, and bearing from one to two slightly sclerotized, hooked shaped cornuti at the apex.

Female adult (Fig. 1C, 2A). Similar to male in color, but larger in body size. Forewing length: 2.39–2.70 mm (n = 8). Contrary to the male forewing, where the first three costal and dorsal strigulae are transversally aligned, on the female they are outwards, obliquely aligned. Frenulum - 2 tightly appressed bristles.

Female genitalia (Figs. 2F, G). Papillae anales lobe-like, semi-rectangular in shape, almost connected dorsally, covered with long, slender setae of greater density on distal section. Anterior apophyses absent. Posterior apophyses long, thickened in ca. basal ¼; apical parts slender, straight and parallel to each other, apices reaching the middle of abdominal segment VII. Ostium bursae opening at the middle of posterior margin of segment VII, followed by short, weakly melanized sterigma. Ductus bursae not separated from the saculiform, membranous corpus bursae. Ductus bursae mostly membranous, except for the slightly sclerotized basal portion. Ductus spermathecae not observed.

Immature stages.

Egg (Figs. 4A-B; 7D). Dimensions (mean ± standard deviation; n = 3): length = 0.779 ± 0.010 mm; width = 1.004 ± 0.017 mm. Flat, slightly ellipsoid; chorion translucent (Fig. 7D), smooth, ornamented with ill-defined carinae (Fig. 4B); vitellum whitish during oviposition, seen by transparency. Aeropyles and micropylar area were not observed.

Larva

Leaf-miner, with hypermetamorphic development and five instars, all endophyllous as found for all lithocolletines. The first three instars are sap feeders, with vestigial thoracic legs, highly modified buccal apparatus, and depressed body; the last two instars have buccal apparatus of

chewing type, hypognathous, with the body more cylindrical in shape (Figs. 5A, B; 7E, F). Minimum and maximum length of larvae (n = 11) examined for both morphotypes = 0.87 and 4.76 mm, respectively. Within each morphotype, instars can be correctly identified through measurements of the head capsules, since there is no overlap between the head capsule widths of succeeding instars (Table 1). General body color light yellow in all instars.

Sap-feeding larva (Figs. 3A, 4C-M, 7E). Body covered with sparse microtrichia, particularly around thoracic and abdominal appendices; setae mostly reduced or absent. Head: flattened, prognathous. Labrum bi-lobed, with distally concave, serrated margin (Fig. 4D, G). Labium slightly bi-lobed (Figs. 4E, H); hypopharynx densely spinose. Maxillary and labial palpi absent. Spinneret is not differentiated (Fig. 4H). Antennae tri-segmented, with the distal segment having two globular and one filiform sensillum. Stemmata absent. Four pairs of median-sized setae, laterally, on the middle section of the head (Fig. 4C). Thorax: dorsal and ventral plates progressively more developed in later instars (Fig. 3A). Legs vestigial, reduced to small callosities (Figs.4J-K), each bearing three small setae. Four pairs of setae dorsally on prothorax, two pairs on anterior margin outside the dorsal plate, and two posteriorly located, internal to the plate. Similar setae on meso- and metathorax, the posterior ones located outside the plate, close to its margin. Two pairs of lateral setae with greater size, and unequal length, on each thoracic segment. A pair of dorsal calli on T1-3 next to the lateral margin (Fig. 4I). Abdomen: similar to thorax, plates of each segment are progressively more developed in the second and third instars on Ab1-9. Pairs of ventral calli on segments Ab3-5, each bearing three small setae (Fig. 4L-M). Three pairs of dorsal setae, lateral to the plate on Ab1-5; two pairs of dorsal setae, lateral to the plate on Ab 6-9, two pairs of lateral setae, the anterior larger than the posterior one on Ab1-5 and smaller than the posterior one on Ab6-9; one pair of ventral, short setae (Fig. 3A, 4L); twelve pairs of setae on Ab10; three pairs dorsally, the anterior ones longer than the posterior; four laterally, the anterior longer than the posterior ones, and five ventral (Fig. 3A).

Tissue-feeding larva (Figs. 3B, 5, 7F). Body covered with dense microtrichia. Head subcylindrical, hypognathous. Labrum with concave distal margin, bearing three pairs of setae on lateral margins, and two meso-laterally (Fig. 5D). Frontoclypeus ill-defined. Three pairs of ill-defined stemmata, latero-ventrally (Fig. 5B). Antennae three-segmented; distal segment narrower, with two stout sensilla, and one filiform sensillum on distal margin; middle segment with four stout sensilla, two of medium size and one short, and one longer, filiform (Fig. 5F). Maxillae well developed, with palpi bearing five pairs of stout sensilla (Fig. 5E). Spinneret

short, tubular (Fig. 5B). Chaetotaxy: C1 and F1, present, short; AF1, AF2, P1, P2, and L1, present, minute; A trisetose, short; S trisetose, long; SS trisetose, short (Figs.5A-D). Thorax with legs well developed; setae four, two, and four, on coxa, tibia and tarsi, respectively; tarsi with hooked, distal claw (Fig. 5H). Prothoracic plate well developed with four rounded lobes (Fig. 3B). Prothoracic and abdominal spiracle circular on Ab1-8, with peritreme slightly elevated (Fig. 5I). Abdomen with three pairs of pseudopodia on segments Ab3-5 (Fig. 5J), and one on Ab10. Crochets uniordinal, distributed on center and anterior margin (Figs. 5K, L). Chaetotaxy: thorax with XD group bisetose, with XD2 longer than XD1; SD2 absent on prothorax, but present on meso- and metathorax, and shorter than SD1; D-group bisetose, with D1 shorter than D2. MD1 present on meso- and metathorax. L-group bisetose on prothorax, with L1 shorter than L2; trisetose on meso- and metathorax, near equal in size. SV1 present on all segments, with smaller sizes on meso- and metathorax. V-group unisetose. Abdomen with SD-group bisetose, with SD2 short, located near the spiracle. MD1 near SD2. D-group bisetose, with D1 shorter than D2. L-group bisetose with near equal size on Ab3-5; only L3 present on Ab1-2 and Ab6-8. SV-group trisetose, near to pseudopodia on Ab3-5; only SV1 present on Ab1-2 and Ab6-8. V-group unisetose, present on Ab7 an Ab9. D2, SD1, L3, and SV1 present with near equal size on Ab9. Ten pairs of unnamed setae on Ab10; four pairs located dorsally, one pair laterally and five ventrally (Figs. 3B).

Pupa (Figs. 3C, 6 and 7G). Yellowish in general color; sub-cylindrical in shape, with head and thorax slightly wider than abdomen, which tapers gradually to posterior apex. Maximum diameter and length of specimens examined (n = 10) = 0.7 and 2.8 mm, respectively. Vertex with relatively short, broadly triangular, acute frontal process (cocoon-cutter) with serrate lateral margins (Figs. 6A-D). Lower frons with two pairs of short setae, with lateral setae half the length of mesal ones. Antenna long and straight, extending to 7th abdominal segment (Ab7); forewing reaching almost to Ab6; proboscis extending to middle Ab1; anterior, median, and posterior legs reaching abdominal segments Ab2, Ab4, and Ab7, respectively. One pair of dorsal setae anteriorly located on mesothorax; two pairs of dorsal setae in similar position on metathorax, and Ab1-4 (Fig. 6F); one pair of subspiracular setae on Ab4-6. Abdominal segments covered with microtrichia (Figs. 6G, H). Spiracle with elevated peritreme, open on Ab2-7 (Fig. 6G). Distal margin of the last segment with two pairs of flattened hooks, one dorsal and the other lateral (Figs. 6H-I).

Etymology. The specific epithet refers to *Arachis* L., the food plant, and is treated as a feminine Latin adjective.

Host plant and distribution. *Porphyrosela arachisella* is known only from cultivated plants of *Arachis pintoi* Kranov. & W.C. Gregory (Fabaceae), located in urban gardens of Goiás, Goiás State, and Brasilia, Federal District (National Congress of Brazil; Fig. 7A), from which adults were reared. The hostplant was originally described from the Cerrado, a biome within which such municipalities are located. However, it is unknown whether these plants were locally infested or transplanted with *P. arachisella* to the urban gardens. *Arachis pintoi* is an herbaceous, evergreen legume with prostrate growing habitat. It is drought-resistant and well adapted to heavy rain and is cultivated as a forage crop for cattle (Kerridge & Hardy, 1994).

Life history (Fig.7). *Porphyrosela arachisella* eggs are laid in clusters of three, placed side by side on the upper surface of the leaf, partially overlapping each other on the lateral margins (Fig. 4A, 7D). During eclosion, the sap-feeding larvae penetrate the leaf epidermis, leaving the empty chorion on the leaf surface. They promptly initiate the mine, which has a blotch aspect from the beginning, increasing in size during larval development. The sap-feeding larva uses the superficial layer of parenchyma, leaving the transparent, lustrous epidermis intact (Fig. 7). The tissue-feeding stage, however, extends feeding to lower layers, as evidenced by the feeding scars that can be seen by transparency on the abaxial surface on a mature mine (Fig. 7I). In the end, it is internally lined with silk by the larvae. In consequence, the leaf is folded upwards, the mine thus showing the tentiform aspect (Fig. 7H) typically found among lithocolletine gracillariids (Kawahara *et al*. 2017). Pupation occurs inside the mine. During adult emergence, the mine wall is ruptured by the frontal process of the pupa (cocoon-cutter). Generally, after the adult emergence, the anterior half of the pupal exuviae (head and thorax) protrudes outside, while the posterior half remains in the pupal cocoon (Fig. 7J).

Frequently, more than one egg cluster is found per leaf. In such cases, mines may coalesce into one, forming larger units that may cover most of the leaf. *Porphyrosela arachisella* mines are abundant on the Brasilia site (Fig. 7B), where plants are fertilized and watered periodically. There was no attempt to measure damage they cause to *A. pintoi* plants, if any.

Molecular analyses

Barcode sequences evidenced highly divergent lineages of *Porphyrosela*, with 9% mean genetic distance (Table 3). A total of 13 BINs were recovered, including three newly identified (BIN 1, BIN 2, BIN 3) considering the level of divergence to their nearest neighbor > 4%. The BIN 1 represents *Porphyrosela arachisella*, which was delimited based on monophyly, and the genetic distance of 8% to its nearest neighbor, a cluster formed by BOLD: ADT2137, BOLD: AAG1161, and BOLD: ADU9985. The two species assigned to the Neotropics, *P. minuta* (BOLD: ABV5485) and *P. desmodiella* (BOLD: ACQ3885, new BIN 2) were recovered.

Discussion

This study adds a third species for *Porphyrosela* in the Neotropical region, as well as the first record of a lithocolletine gracillariid in Brazil. *Porphyrosela arachisella* sp. nov. shows the characteristics that are unique for the genus within lithocolletines, such as two pairs of apical setae on the male tegumen and absence of anterior apophyses in the eighth abdominal segment of the female (Vári 1961, Kumata 1993, De Prins & Kawahara 2012). As suggested for congeners in the Afrotropical region (De Prins & Kawahara, 2012), they can be identified readily based on variation in forewing color patterns. As noted in the diagnosis section, in the adult stage *P. arachisella* shows differences from the other two Neotropical *Porphyrosela* not only in wing pattern but also in the genitalia of both sexes, egg-laying behavior and host-plant use. Unfortunately, the external morphology of *Porphyrosela* immature stages is poorly known in general from a fine structure perspective, *P. arachisella* being the first species to have them described with the aid of scanning electron microscopy. Thus, we prefer to wait for further comparison until similar information is available for other *Porphyrosela* species. This may lead to the discovery of morphological novelties for the genus. For example, vestigial thoracic legs and calli are for the first time herein recorded for the larval stage in *Porphyrosela*. The setae for the pupal stage are as well, and should thus be further explored.

From a molecular perspective, this analysis of *Porphyrosela* revealed a hidden diversity of lineages, evidently reflecting a lack of sampling. Given the number of host plants used by the species and the wide distribution of this genus, such variation is not surprising. The barcode tree showed that *P. arachisella* was distinct from its congeners, with the nearest group formed by specimens from Oriental and Australasian biogeographic regions, and not from the Neotropics. Three *Porphyrosela* species with forewings bearing four costal and three dorsal strigulae have been recorded from these biogeographic regions: *P. aglaozona* (Meyrick), *P. dorinda* (Meyrick), and *P. hardenbergiella* (Wise). Pictures of the former two species are presented by De Prins & De Prins (2022), showing a different forewing color pattern, without

a black-lined termen. The existence of such a conspicuous forewing distal lining is not mentioned in the original description of the latter, provided by Wise (1957). Corresponding affinities should be further explored with the increase in the number of DNA sequences available, particularly to the African region, where the genus is well represented (De Prins & Kawahara 2012), but from which molecular data are mostly lacking.

As also already mentioned, some of the smallest Lepidoptera are found within *Porphyrosela* (Stonis *et al*. 2021), and accordingly the adult size of *P. arachisella* is minute, especially the male. It is unclear how common the occurrence of size dimorphism is within the genus. Variation in adult size has been usually reported not separately but in conjunction for both sexes in *Porphyrosela*, and females are still unknown for some species (e.g. Vári 1961, Betancourt & Scatoni 2007, De Prins & Kawahara 2012), which makes difficult any generalization about this aspect. Sexual difference in size is conspicuous in *P. arachisella*, males being much smaller than females in the two populations studied. There was no attempt to quantify in this study variation in sex ratio among the three larvae in each shared mine. Corresponding implications for life history, ecology, and behavior may be important and should be explored, since larvae of *P. arachisella* are gregarious, and somatic investment to produce males is supposed to be lower in this case. As already described, three eggs are usually found per cluster and correspondingly three larvae inhabit the same mine. However, it is not uncommon to find two or even three egg clusters per leaf. With the development of larvae, increase in feeding activity and in size, and expansion of mines, in consequence, such units may merge to form a single larger mine that covers most of a given leaf.

Previously known Neotropical *Porphyrosela* species have been found associated with Fabaceae, such as *Desmodium* and *Lespedeza* (for *P. desmodiella)* and *Trifolium* Linnaeus (for *P. minuta*), among other genera (De Prins & De Prins 2022), but not with *Arachis.* In fact, this is the first record for this plant genus as a host for gracillariids in general. *Arachis pintoi* is one of the most important forages used for cattle feeding in Brazil. It is grown intercropped with pastures and other legumes in circa two million hectares in Central areas (Azêvedo *et al*. 2016), among other parts of the country including southern states (Nascimento 2006). Lately, the species has been widely used as an ornamental, and also as a ground cover in gardens and orchards in other parts of Brazil. Furthermore, the cultivation of *A. pintoi* has been expanded to other countries (Kerridge & Hardy, 1994). As a consequence, actual distribution of this new species is expected to be broader than that reported herein. It remains unknown whether leaf-mining activity represents significant damage for *A. pintoi* plants.

Finally, it is important to mention that *Arachis* originated in South America, comprising circa 80 recognized species (Krapovickasl & Gregory 1994; Valls & Simpson 2005), most of each are widely distributed in Brazil (Valls 2015). Thus, one should also search for a wider host plant range in *A. arachisella*, with particular attention to closely related species to *A. pintoi*, such as *A. repens* Handro, also cultivated as cattle forage, and from which there is still doubt about taxonomic separation on both morphological and molecular bases (Azêvedo *et al*. 2016).

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Figure Legends:

Fig. 1. Adults of *Porphyrosela arachisella*: (A, C) wings spread, pinned and dried, dorsal view, male (LMCI 344-67, holotype) and female (LMCI 344-69, paratype), respectively; (B) live, with wings folded, on *Arachis pintoi* leaf surface; (D) distal portion of female forewing in detail, dorsal; (E, F) head in dorsal and lateral views, respectively; (G) hind leg, lateral. Scale bars: 1 mm (A, C), 0.5 mm (B), 0.25 mm (D–G).

Fig. 2. *Porphyrosela arachisella* adult morphology: (A) venation of female right wings, dorsal view; (B, C) male genitalia, in ventral and lateral views, respectively; (D) aedeagus, lateral; (E) distal portion of aedeagus in detail (enlarged area delimited by rectangle in D), open red arrow points to cornutus; (F, G) female genitalia under ventral and lateral views, respectively. Closed red arrow and asterisk indicate the posteriorly displaced basal portion of ductus bursae and protruded rectum, provoked by internal hydrostatic pressure. Scale bars: 0.5 mm (A), 0.1 mm (B–C, F–G), 0.05 mm (D–E).

Fig. 3. Immature stages of *Porphyrosela arachisella* under light microscopy: (A) sap-feeding larva; (B) tissue-feeding larva; (C) pupa. All in dorsal and ventral view, respectively. Scale bars: 250 μm.

Fig. 4. Scanning electron micrographs of *Porphyrosela arachisella* egg (A, B) and sap-feeding larva (C–M): (A) egg-cluster, dorsal view; (B) detail of chorion, dorsal; (C–E) head, lateral, dorsal and ventral, respectively; (F) antenna, dorsal; (G) labrum, dorsal; (H) labium, ventral; (I) mesothoracic callus, dorsal; (J) mesothorax, lateral (arrow indicates dorsal callus); (K) vestigial thoracic legs, lateral (indicated by the red rectangle in J); (L) abdominal segment Ab3, lateral; (M) abdominal callus, lateral (indicated by the red rectangle in L). Scale bars: 100 μm (A), 20 μm (B, K, M), 50 μm (C, D, E, J, L), 10 μm (F, G, H, I).

Fig. 5. Scanning electron micrographs of *Porphyrosela arachisella* tissue-feeding larva: (A–C) head, in dorsal, ventral and lateral view, respectively (closed and open arrows point to spinneret and stemma, respectively); (D) labrum and frontoclypeus, dorsal; (E) maxila, mesal; (F) left antenna, dorsal, (G) mesothorax, lateral, (H) mesothoracic leg, ventral; (I) prothoracic spiracle, lateral; (J) abdominal segment Ab3, lateral; (K) proleg of abdominal segment Ab5, latero-ventral; (L) crochets in detail, latero-ventral. Scale bars: 50 μm (A, B), 100 μm (C, G, J), 10 μm (D, E, F), 25 μm (H, K), 5 μm (E, I, L).

Fig. 6. Scanning electron micrographs of *Porphyrosela arachisella* pupa: (A–B) head, under ventral and lateral views, respectively; (C–D) cocoon-cutter in detail, anterior and ventral views, respectively; (E) abdominal segments Ab1–2, lateral; (F) dorsal setae on abdominal segment Ab1, lateral (area delimited by rectangle in E); (G) spiracle on abdominal segment Ab6, lateral; (H) last abdominal segments, dorsal; (I) corresponding flattened hooks in detail, dorsal (indicated by the red rectangle in H). Scale bars: 100 μm (A, B, E), 25 μm (C), 20 μm (D, F), 10 μm (G), 50 μm (H), 5 μm (I).

Fig. 7. Natural history of *Porphyrosela arachisella* at the garden of Federal State Senate, Brasília, DF: (A) general view of the garden; (B) *Arachis pintoi* plant stand; (C) mines on *A. pintoi* leaves in detail; (D) eggs on adaxial leaf surface; (E) sap-feeding larva, dorsal; (F) tissue-feeding larva, dorsal; (G) pupa, dorsal; (H, I) mature mines viewed from adaxial and abaxial surfaces, respectively; (J) pupal exuviae protruding from cocoon after adult emergence. Scale bars: 3 cm (C); 100 μm (D), 400 μm (E), 500 μm (F, G, J), 1 cm (H, I).

Fig. 8. A Neighbor-Joining tree, based on COI barcode fragment generated under the K2P nucleotide substitution model, of *Porphyrosela arachisella* and its congeners. Each specimen is identified by its Process ID code (see Tab. 2). Branch lengths represent the number of substitutions per site. BIN numbers from BOLD Systems are given on the right bars for all clusters; black bars indicate new BINs. Bootstrap support higher than 50% is presented in the branches.

Table 1. Variation in head capsule width among instars of *Porphyrosela arachisella* (n = 10 per instar).

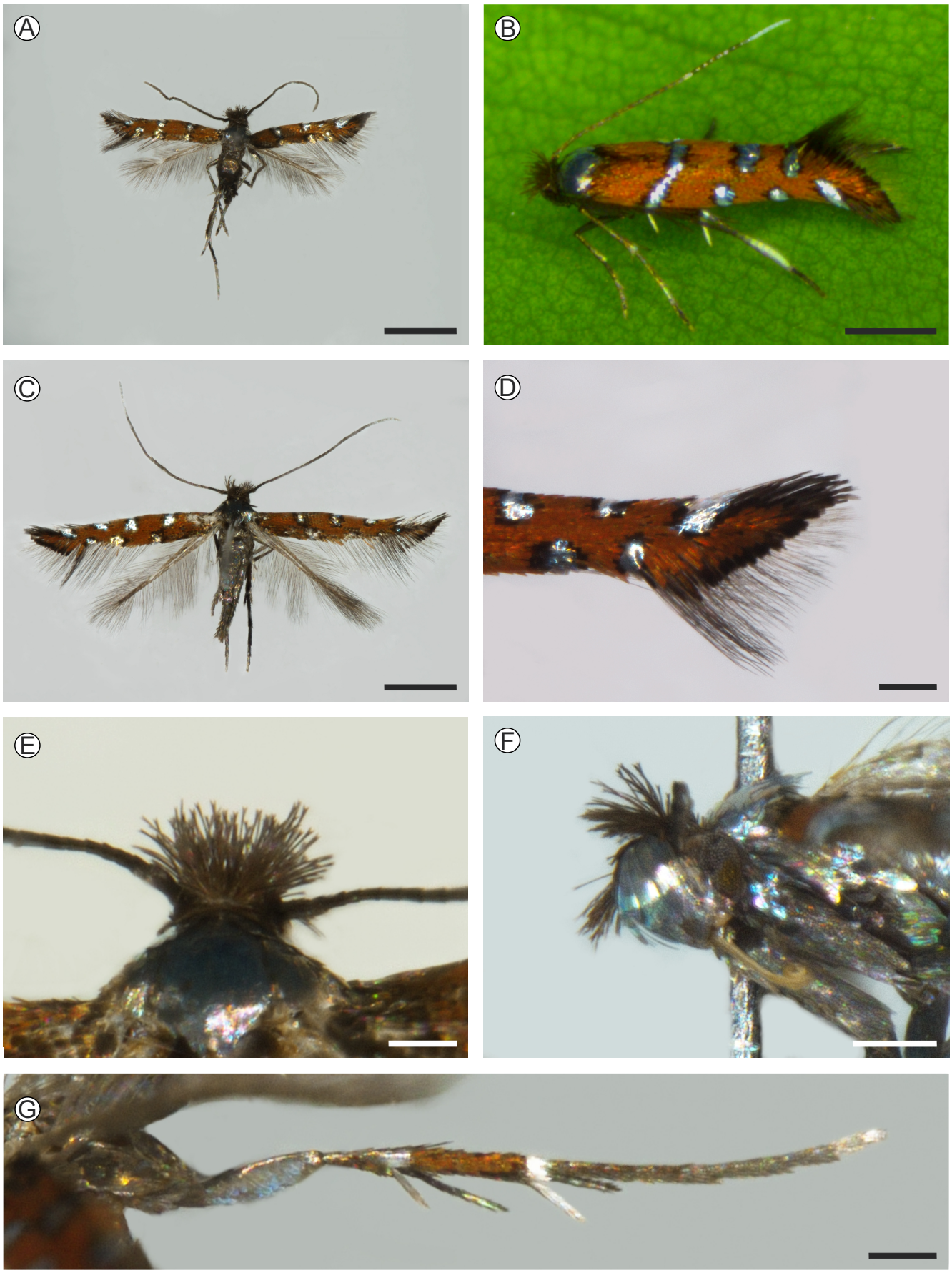
Instar	Head capsule width (mm)		
	Mean ± standard error	Range	Growth rate
I	0.158 ± 0.004	0.166-0.146	-
II	0.201 ± 0.003	0.187-0.208	1.27
III	0.294 ± 0.003	0.271-0.312	1.46
IV	0.238 ± 0.003	0.229-0.249	-
V	0.327 ± 0.004	0.317-0.348	1.37

Table 2. Specimens used for molecular analyses of *Porphyrosela* species. The clusters refer to 10 BINs (Barcode Index Number; Ratnasingham and Hebert 2013) identified for *Porphyrosela* by BOLD Systems v4 and three new BINs not assigned yet in the database, which includes the new species (new BIN 1). Process ID codes are unique identifiers linking the record in the BOLD database. Sample ID of *P. arachisella* links to the voucher specimen from which the sequence is derived. The species name follows data recorded for each Process ID, as well as their host plant and locality.

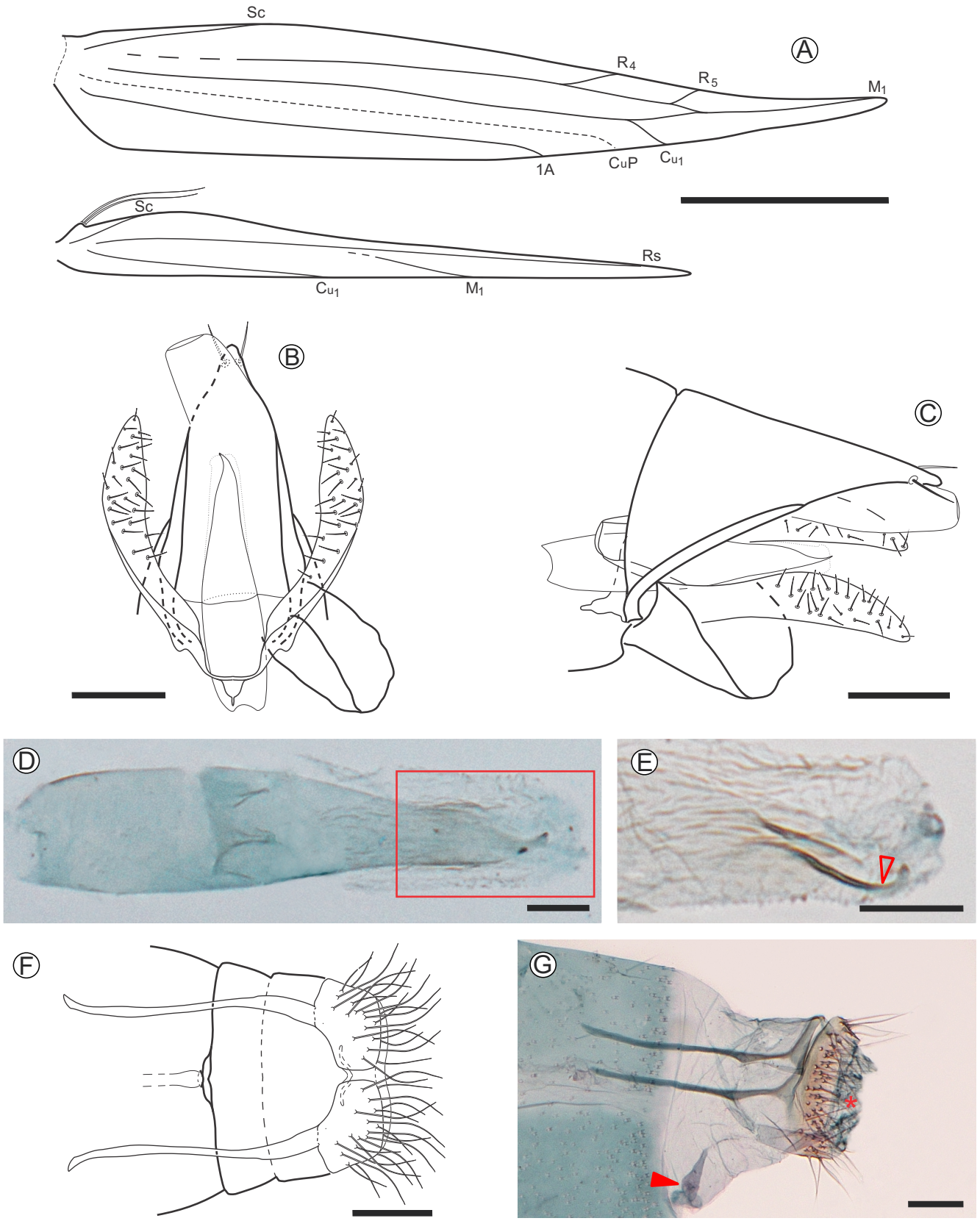
#	Cluster	Process ID (sample ID)	Species	Host plant	Locality
1	New BIN 1	GRABR013-21 (LMCI344-47A); GRABR014-21 (LMCI 344-47B); GRABR015-21 (LMCI 344-47C); GRABR016-21 (LMCI 344-47D)	*Porphyrosela arachisella* sp.nov.	*Arachis pintoi*	Brazil: Brasilia
2	New BIN 2	CNCLA3613-13	*Porphyrosela desmodiella*		Canada: Ontario
3	New BIN 3	GMAFD139-15; GMAFJ910-15;	*Porphyrosela*	-	Argentina: Formosa
4	BOLD:AAG1161	GCQT2493-18; GCQT617-17; GMAHA119-18; GMAHA473-18; GMAHA586-18; GMAHG298-18; GMAHK665-18; GMAHK998-18; LOQTE507-09	*Porphyrosela*	*Desmodium*	Australia: Queensland
5	BOLD:ABV2088	GMACG1157-15; GMACM779-15; GMACM787-15	*Porphyrosela*	-	Costa Rica: Guanacaste
6	BOLD:ABV5485	LNOUD806-12; GMGSX014-13; GMRDM044-18; LNOUD830-12; MNAM1114-16; MNAM1115-16	*Porphyrosela minuta*	*Lespedeza*? (Faboideae)	French Guiana; USA: Tennessee; USA: California; Uruguay: Montevideo; Mexico: Jalisco; USA: North Carolina; USA: Oklahoma
7	BOLD:ACG4759	GMCRH324-13; GMCYY1845-15	*Porphyrosela*	-	Costa Rica: Guanacaste
8	BOLD:ACM5075	GMAFV343-15; GMAFZ377-15; GMAGF616-15; GMAGH921-15; GMARC858-14; GMARS864-14	*Porphyrosela*	-	Argentina: Formosa; Argentina: Misiones
9	BOLD:ACQ3885	GBGL16075-14; GMARA531-14	*Porphyrosela desmodiella; Porphyrosela*	-	Argentina: Misiones
10	BOLD:ACU1471	PLABY202-19; GMMAE770-15; GMMAE945-15; GMMCV088-15; GMMCW028-15; GMMCW054-15; GMMCW065-15; GMMCX431-15; GMMCZ477-15; GMMCZ478-15; GMMCZ493-15; PLABU801-19; GMMAQ136-15; GMMCY715-15	*Porphyrosela*	-	Costa Rica: Guanacaste; Mexico: Jalisco
11	BOLD:ACY4113	WOGRA414-15; WOGRA415-15	*Porphyrosela*	Fabaceae	Suriname: Wanica
12	BOLD:ADT2137	NSWHO3192-18	*Porphyrosela*	-	Australia: New South Wales
13	BOLD:ADU9985	WOGRA320-15; GMBRR456-18; GCQT365-17; GMAHB301-18; GMAHD325-18; GMAHQ879-18; GMAHR1081-18; GMAHR1167-18; GMAHR861-18;	*Porphyrosela*	*Desmodium*	Vietnam: Ninh Binh; Bangladesh: Rajshahi; Australia: Queensland

Table 3. Genetic distance between *Porphyrosela arachisella* (new BIN 1, in bold) and other clusters (BINs) of *Porphyrosela* both mined from BOLD and putatively new (given the level of divergence to the nearest neighbor > 4%). Analysis used 658 base pairs sequences of the Cytochrome oxidase c gene under the Kimura 2-parameter model. Specimens present in each BIN are found in Table 2. The outgroup is composed of two species of Lithocolletinae (*Phyllonorycter salicicolella* and *Cameraria guttifinitella*) obtained from Genbank (HM874857.1 and KU380345.1, respectively).

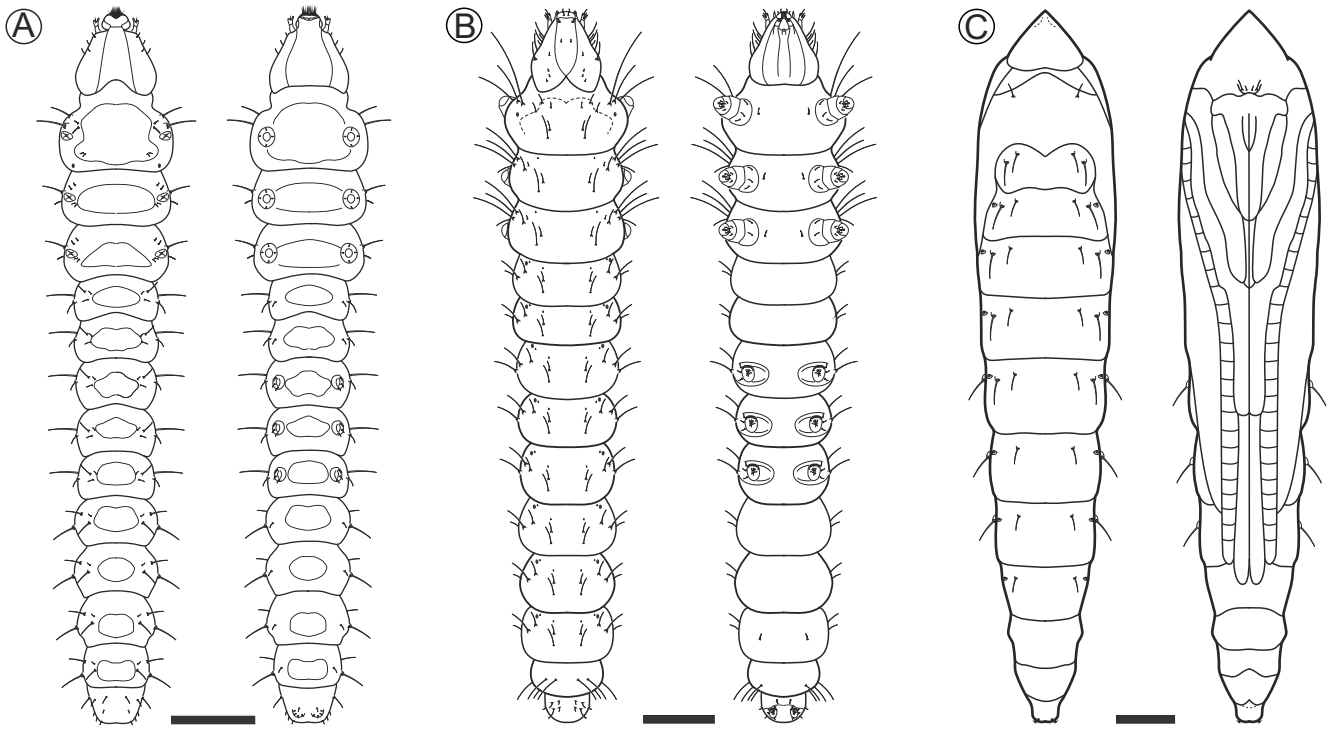
#	Cluster	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
1.	New BIN 1													
2.	New BIN 2	**0.09**												
3.	New BIN 3	**0.09**	0.09											
4.	BOLD:AAG1161	**0.10**	0.10	0.14										
5.	BOLD:ABV2088	**0.11**	0.09	0.06	0.14									
6.	BOLD:ABV5485	**0.11**	0.09	0.11	0.12	0.10								
7.	BOLD:ACG4759	**0.11**	0.09	0.05	0.12	0.06	0.12							
8.	BOLD:ACM5075	**0.10**	0.09	0.05	0.13	0.05	0.11	0.06						
9.	BOLD:ACQ3885	**0.11**	0.06	0.10	0.15	0.10	0.13	0.12	0.11					
10	BOLD:ACU1471	**0.09**	0.09	0.04	0.13	0.06	0.12	0.02	0.06	0.11				
11.	BOLD:ACY4113	**0.09**	0.09	0.04	0.14	0.04	0.08	0.05	0.05	0.09	0.04			
12.	BOLD:ADT2137	**0.09**	0.08	0.13	0.02	0.13	0.11	0.11	0.12	0.14	0.12	0.12		
13.	BOLD:ADU9985	**0.08**	0.09	0.11	0.05	0.12	0.12	0.11	0.11	0.12	0.10	0.11	0.04	
14.	*Outgroup*	**0.16**	0.18	0.16	0.20	0.16	0.19	0.17	0.15	0.18	0.17	0.17	0.18	0.18



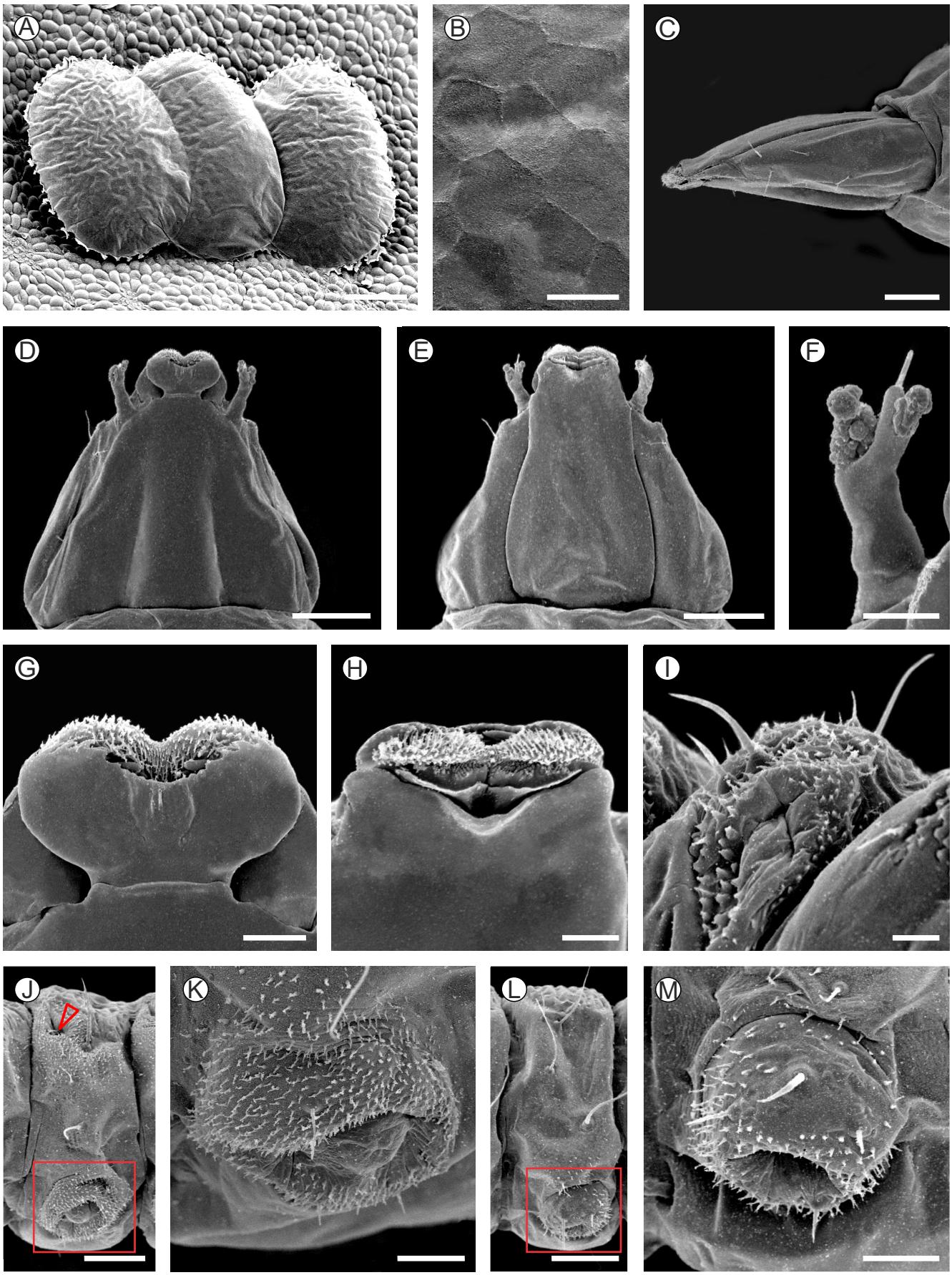
Fochezato *et al*. - Fig.01



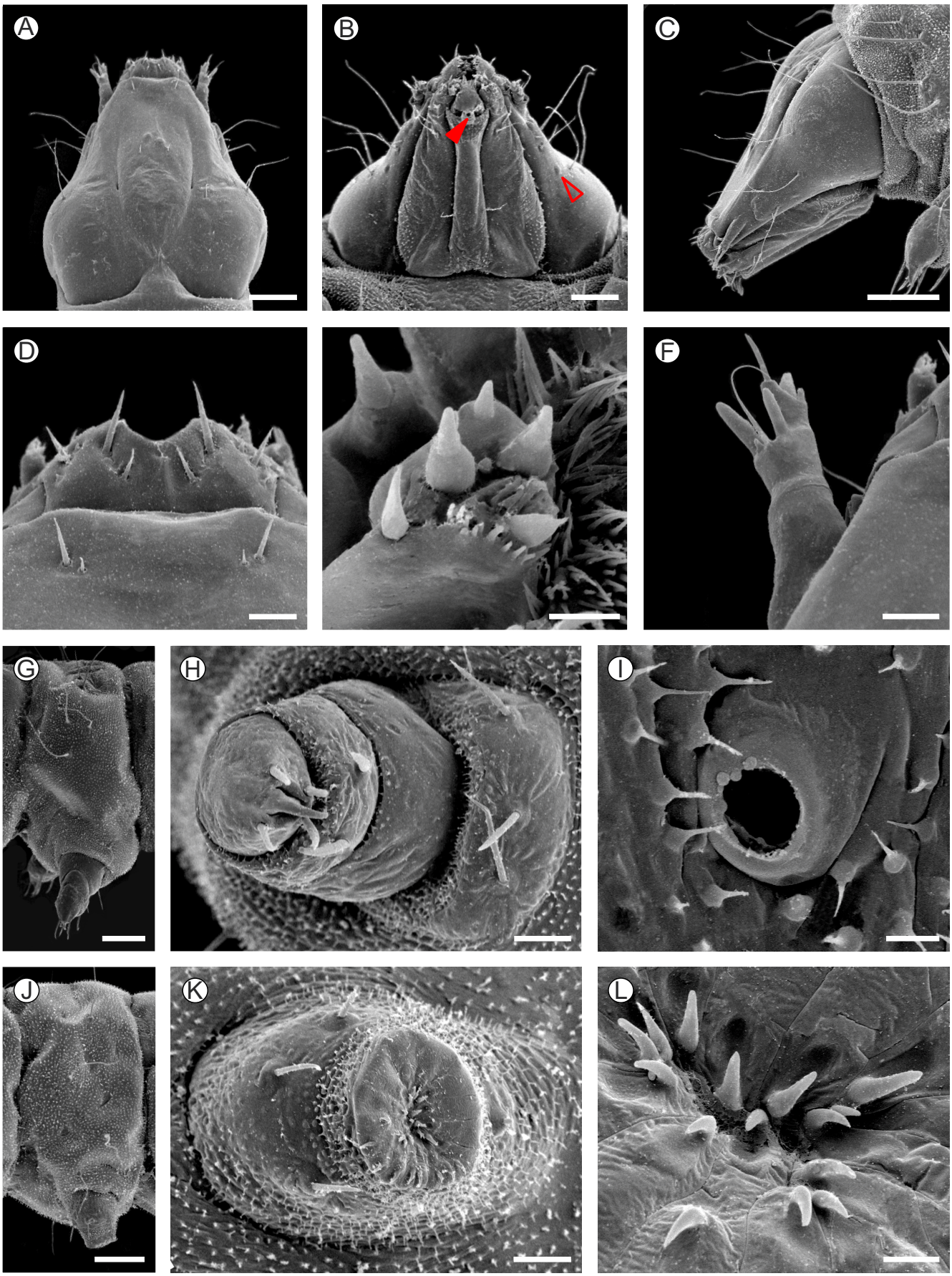
Fochezato *et al*. - Fig.02



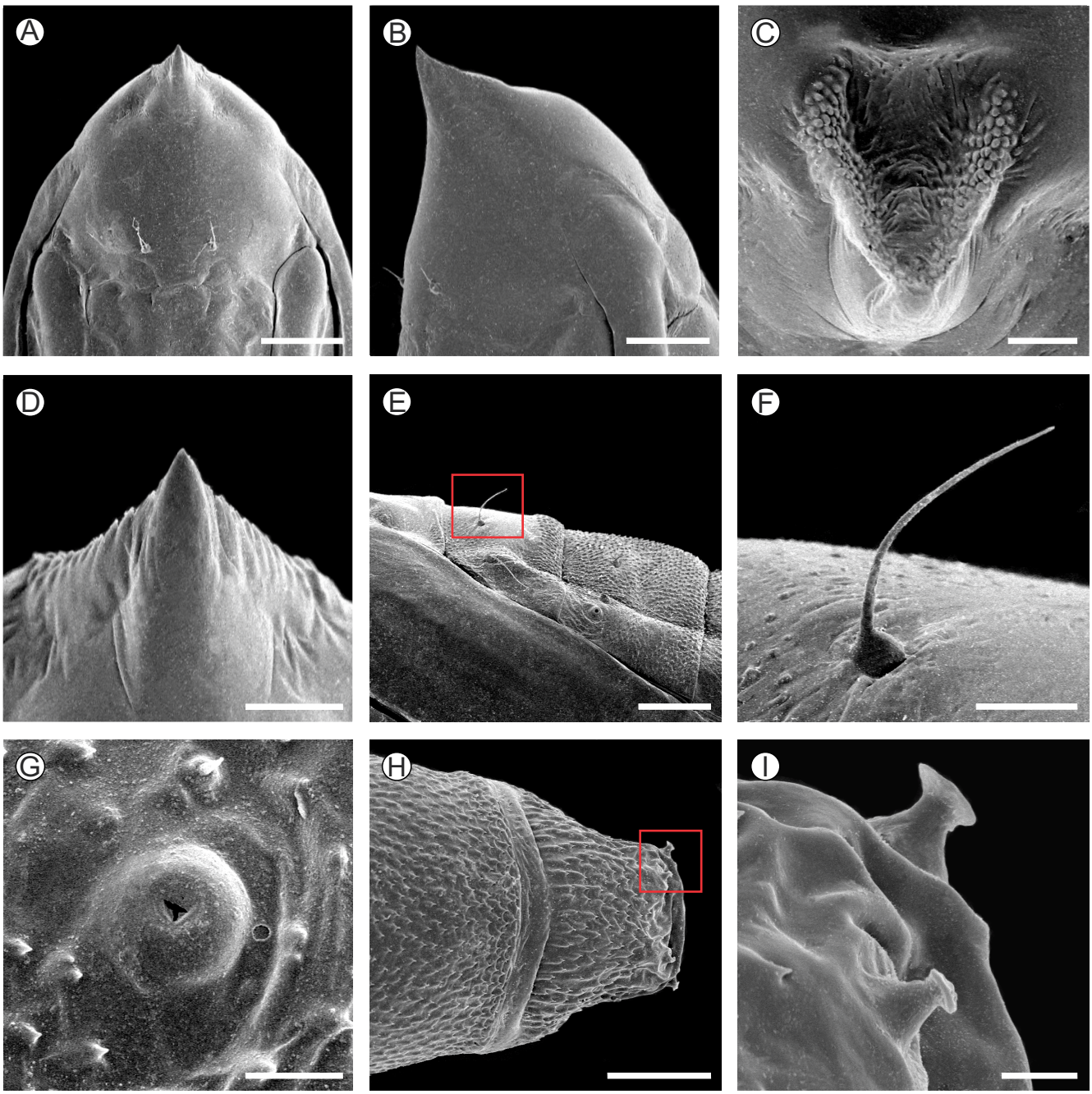
Fochezato *et al*. - Fig.03



Fochezato *et al*. - Fig.04

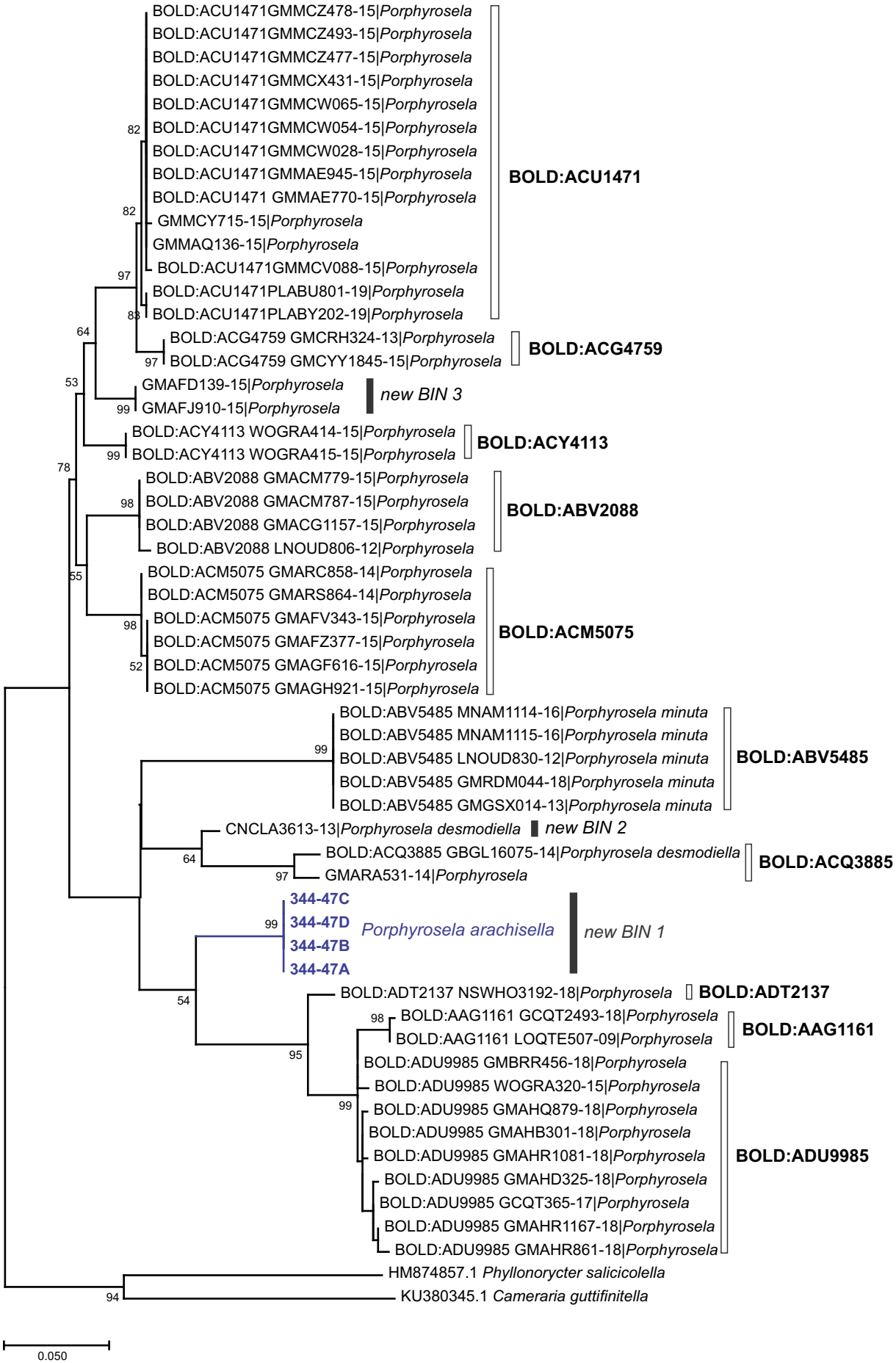


Fochezato *et al*. - Fig.05



Fochezato *et al*. - Fig.06

Fochezato *et al*. - Fig.07



Fochezato *et al*. - Fig.08

CONCLUSÕES

Taxonomia, a prática de classificar os organismos, é um ramo de pesquisa essencial desde a ciências básicas até as aplicadas e, sem ela, a comunicação entre os cientistas seria comprometida (Moraes, 1986). Por muito tempo a descrição de espécies foi simples e sucinta, restrita pelo pequeno espaço de publicação e destinada somente a especialistas de cada área. No entanto, com o avanço do conhecimento filogenético e molecular, e advento das publicações *online*, as descrições estão se tornando mais abrangentes, incluindo ilustrações, biologia e dados genéticos o que abre portas para a universalização do conhecimento e sua aplicação em diversas áreas, inclusive na criação de políticas de conservação (Godfray, 2002; Dubois, 2003; Carvalho *et al*. 2007).

Neste trabalho, foram descritas três espécies de mariposas minadores de folhas pertencentes a grupos diferentes: *Porphyrosela* (Gracillariidae: Lithocolletinae), *Elachista* (Gelechiodea: Elachistidae) e *Heliozela* (Adeloidea: Heliozelidae), encontradas no Brasil associadas às seguintes plantas hospedeiras *Arachis pintoi* Krapov. & W.C. Greg (Fabaceae), *Chusquea tenella* Nees (Poaceae) e *Eugenia uniflora* L. (Myrtaceae), respectivamente. As descrições foram realizadas com base em caracterização da morfologia sob microscopia ótica e eletrônica de varredura e são disponibilizadas ilustrações, dados de história de vida, além de sequências de DNA mitocondrial (COI) de forma comparativa. Os registros são inéditos respectivamente ao nível de gênero (*Heliozela*), subfamília (Lithocolletinae) e família (Elachistidae). Da mesma forma, com relação as plantas hospedeiras para os três taxa descritos.

Sabe-se que a diversidade de microlepidópteros na região Neotropical ainda é pouco conhecida. Este trabalho possibilitou, assim, o conhecimento de taxa que até então não haviam sido registrados no país e forneceu caracteres passíveis de comparações não só a partir dos adultos, mas incluindo os estágios imaturos, biologia e plantas hospedeiras, podendo contribuir para o enriquecimento do conhecimento acerca desses grupos, ainda pouco explorados, e estimulando a realização de novos estudos a respeito.

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Apêndice 1

Normas para publicação na Revista Zootaxa:

Preparation of manuscripts

1) General. All papers must be in English. Authors whose native language is not English are encouraged to have their manuscripts read by a native English-speaking colleague before submission. Nomenclature must be in agreement with the International Code of Zoological Nomenclature (4th edition 1999), which came into force on 1 January 2000. Author(s) of species name must be provided when the scientific name of any animal species is first mentioned (the year of publication needs not be given; if you give it, then provide a full reference of this in the reference list). Authors of plant species names need not be given. Metric systems should be used. If possible, use the common font Times New Roman and use as little formatting as possible (use only bold and italics where necessary and indentions of paragraphs except the first). Special symbols (e.g. male or female sign) should be avoided because they are likely to be altered when files are read on different machines (Mac versus PC with different language systems). You can code them as m# and f#, which can be replaced during page setting. The style of each author is generally respected but they must follow the following general guidelines.

2) The title should be concise and informative. The higher taxa containing the taxa dealt with in the paper should be indicated in parentheses: e.g. A taxonomic revision of the genus Aus (Order: family).

3) The name(s) of all authors of the paper must be given and should be typed in the upper case (e.g. ADAM SMITH, BRIAN SMITH & CAROL SMITH). The address of each author should be given in italics each starting a separate line. E-mail address(es) should be provided if available.

4) The abstract should be concise and informative. Any new names or new combinations proposed in the paper should be mentioned. Abstracts in other languages may also be included in addition to English abstract. The abstract should be followed by a list ofkey wordsthat are not present in the title. Abstract and key words are not needed in short correspondence.

5) The arrangement of the main text varies with different types of papers (a taxonomic revision, an analysis of characters and phylogeny, a catalogue etc.), but should usually start with an introduction and end with a list of references. References should be cited in the text as Smith (1999), Smith & Smith (2000) or Smith et al. (2001) (3 or more authors), or alternatively in a parenthesis (Smith 1999; Smith & Smith 2000; Smith et al. 2001). All literature cited in the text must be listed in the references in the following format (see a sample page here in PDF).

A) Journal paper:

Smith, A. (1999) Title of the paper. Title of the journal in full, volume number, issue number if possible & page range.

B) Book chapter:

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.

D) Internet resources

Author (2002) Title of website, database or other resources, Publisher name and location (if indicated), number of pages (if known). Available from: http://xxx.xxx.xxx/ (Date of access).

Dissertations resulting from graduate studies and non-serial proceedings of conferences/symposia are to be treated as books and cited as such. Papers not cited must not be listed in the references.

Please note that:

(1) journal titles must be written in full (not abbreviated)

(2) journal titles and volume numbers are followed by a ","

(3) page ranges are connected by "n dash", not hyphen "-", which is used to connect two words.

For websites, it is important to include the last date when you see that site, as it can be moved or deleted from that address in the future.

On the use of dashes: (1) Hyphens are used to link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use). (2) En-dash or en-rule (the length of an ‘n’) is used to link spans. In the context of our journal that means numerals mainly, most frequently sizes, dates and page numbers (e.g. 1977–1981; figs 5–7) and also geographic or name associations (Murray–Darling River; a Federal–State

agreement). (3) Em-dash or em-rule (the length of an ‘m’) are used far more infrequently, and are used for breaks in the text or subject, often used much as we used parentheses. In contrast to parentheses an em-dash can be used alone; e.g. What could these results mean—that Niel had discovered the meaning of life? En-dashes and em-dashes should not be spaced.

6) Legends of illustrations should be listed after the list of references. Small illustrations should be grouped into plates. When preparing illustrations, authors should bear in mind that the journal has a matter size of 25 cm by 17 cm and is printed on A4 paper. For species illustration, line drawings are preferred, although good quality B&W or colour photographs are also acceptable. See a guide here for detailed information on preparing plates for publication.

7) Tables, if any, should be given at the end of the manuscript. Please use the table function in your word processor to build tables so that the cells, rows and columns can remain aligned when font size and width of the table are changed. Please do not use Tab key or space bar to type tables.

8) Keys are not easy to typeset. In a typical dichotomous key, each lead of a couplet should be typed simply as a paragraph as in the box below:
1 Seven setae present on tarsus I ; four setae present on tibia I; leg I longer than the body; legs black in color ... Genus A
- Six setae present on tarsus I; three setae present on tibia I; leg I shorter than the body; legs brown in color ... 2
2 Leg II longer than leg I ... Genus B
- Leg II shorter than leg I ... Genus C

Our typesetters can easily convert this to a proper format as in this PDF file.

Apêndice 2

Normas para publicação na Revista Zoologia:

The copyediting instructions below represent a concise summary of the journal's formatting requirements. The instructions are intended for use by the authors during preparation of the final revised versions of their manuscripts, technical editors, copy editors and typesetters.

Author names

- Omit titles, degrees, etc.
- Provide ORCID if available

Affiliation (Department,) Institution, City, Country

Article title

Title of article: Subtitle of article

- Title: Sentence case
- Colon between title and subtitle (if any)
- No footnotes
- No bold (use when needed sub-/superscript, and/or italics only for the terms in Latin)
- Higher taxa within the title should be separated with commas and not with a semicolon

Running head

- A short version of title up to 50 characters (including spaces); normally the short title should have been suggested by the authors and checked for clarity by the copy editor

Abstract

- No references to tables, figures, etc., no footnotes
- No citations (preferably) o If citations unavoidable: Complete citations, allowing unambiguous identification of cited publication!
- Should be written consistently in either third or first person
- Note: The abstract has to be a stand-alone entity, to present a really well written and concise summary of the article! A special care for copy editors to check!
- Designations of nomenclatural novelties should be in bold and spelled in the way suggested (sp. nov., gen. nov., comb. nov.)

Keywords (up to 8 words)

keyword a, keyword b, keyword n

- Do not repeat words from the title

- Listed in alphabetical order and separated by commas
- Lowercase letters, except proper names
- No bold font
- Without any punctuation marks after last keyword

Tables

- Table caption: Start with label "Table N." in bold. Sentence case, i.e.:
  - Table 2. Table caption text.
- Numbered consecutively with Arabic numerals
- Heading for every column (including the leftmost!)
- No shading of cells, rows, columns; no colored fonts
- No horizontal or vertical lines in table body
- Same number of decimal places for same statistics (usually within same column)
- Text formatting in the cell without paragraph and line break
- Table must be in an editable format (.docx, .xlsx, etc., not as images)
- Caption and footnotes as texts (not as part of a table)

Figures

- Figure caption: Start with label "Figure N." in bold. Sentence case, i.e.:
  - Figure 6. Figure caption text.
- Numbered consecutively with Arabic numerals
- Figure parts: Use capital letters in bold. No punctuation separator, i.e.:
  - Figure 1. Figure general caption text. A part caption text B part caption text N part caption text.
- If abbreviations are used, these are placed after the parts with a colon, i.e.:

Abbreviations: xxxx

- If there are scale bars on the figure parts, reference to them is last and in the format: Scale bars: 20 μm (D, N, O, Q); 50 μm (F, K); 10 μm (G, P); 5 μm (H); 100 μm (M).
- High quality (at least 300 dpi)
- Text sharp and readable (e.g., no overlap of text and graphical elements like lines)
- White or transparent background
- No image border
- Caption as text (not as part of the image)

Capitalization

- Article title: Sentence case

- Running head: Sentence case
- Section and subsection titles:

  - For separated titles (usually H1-H3): Sentence case o For paragraph titles (usually H4): Sentence case
- Table captions: Sentence case
- Headings of table rows and columns:

  - Sentence case or lower case (check for consistency only!)
- Figure captions: Sentence case
- In text body: Nouns followed by numerals/letters (citations of figures, tables, appendices and supplementary files) e.g.: o Fig. 4; Figs 1, 2; Table 2; Appendix 1
- In text body: Titles of articles, book chapters, books, tests
- In references: Sentence case

Equations and statistical symbols

- Typeface

  - standard typeface for Greek letters, sub-/superscripts, and abbreviations that are not variables

  - italic typeface for all other statistical symbols
- Space before and after equal/inequality signs
- Same number of decimal places for decimal values
- Omit the zero before a decimal fraction, when the statistic cannot exceed 1, e.g., p = .34

  - Alternative A: Omit the zero before a decimal fraction only for the following statistics: p, r, R (and R 2), α (Cronbach's α), η 2 (Eta-Square, also ηp 2).

  - Alternative B: If zero is omitted before a decimal fraction, this should be done consistently for the respective statistic.
- Standard formats for common statistics, e.g., t(23) = 3.51, p = .002

  - commas (not semicolons!) between test statistics and p values

  - exact p values, if p not less than .001

Text body

- Regular font usage:

  - Main text

  - Abbreviations e.g., i.e., et al., etc., cf., vs.

  - Greek letter e.g., α, β, γ, δ, ε, σ, φ, χ, ω
- Italic font usage:

- Scientific names of taxa of species and genera (authorities in regular font, not in italics)

- Long direct quotations

- Symbols for variables and constants, such as p, F, U, T, N, r, but not for SD (standard deviation), SE (standard error), DF (degrees of freedom), and NS (non significant). These symbols in illustrations and equations should be in italics to match the text.

- Do not use italics for emphasis

- No underlining
- Bold font usage:

- Subheadings, sections and subsections

- Figure captions – For the label and designation of figure's parts:

- Figure 1. Figure general caption text. A part caption text B part caption text N part caption text.

- Table captions – For the label:

- Table 1. Table caption text.

- In systematic sections for specimen designation such us: holotype, paratype, syntype, lectotype, isotype, etc.

- Abbreviations of institutions or morphological characters or indices listed alphabetically in the section Materials and methods, i.e.:

- NHML Natural History Museum, London
- MW Naturhistorisches Museum, Vienna
- EL length of elytra
- EW maximum width of elytra
- TL total length (PL+EL)

- In species descriptions – designation of main anatomical structures followed by a colon mark, i.e. Head:…, Thorax:…, Legs:…, Abdomen:…, etc., in this case these should be followed by a section describing other anatomical organs and structures attached to these. o Subsection "Specimens examined" - the preferred order is as follows, HOWEVER THESE FINE-GRAINED FORMATTING GUIDELINES ARE NOT COMPULSORY. Authors who follow the guidelines will benefit from the submission of their specimen records to GBIF after publication. The records on GBIF will bear the article citation details contributiing to a wider dissemination and re-use of the published data.

- COUNTRY • specimens [e.g. 1 ♂, size]; geographic/locality data [from largest to smallest]; coordinates; altitude/elevation/depth [using alt./m a.s.l. etc.]; date [format: 16

Jan. 1998]; collector [followed by "leg."]; other collecting data [e.g. micro habitat/host/method of collecting]; barcodes/identifiers [e.g. GenBank: MG779236]; institution code and specimen code [e.g. CBF 06023]. For Example: Holotype: CHINA • ♀; Sichuan, Kangding; 30.04°N, 101.57°E; 15.VI.2017; Yanzhou Zhang leg.; Hyp-2018-06, original number ZYZ-2017- 28. Paratypes: CHINA • 1♀1♂; Sichuan, Kangding; 29.VI.2017; Yanzhou Zhang leg.; Hyp-2018-01, Hyp-2018-02, original number ZYZ-2017-08 • 1♀; Sichuan: Kangding; 2.VIII.2017; Yanzhou Zhang leg.; Hyp-2018-03, original number ZYZ-2017-20 • 1♂, Sichuan: Kangding; 29.VI.2017; Yanzhou Zhang leg.; Hyp-2018-08, original number ZYZ-2017-029.

- Punctuation:

A bullet point "•" (unicode: 2022) is used to signify the beginning of a material citation. Within each citation, the different fields are delimited by a semicolon. A single field can be composed of several elements, which are separated by commas (e.g. the details region, area, town and street for the ‘locality’ field). Semicolons should not be used elsewhere in a material citation.

- Repetitive data: Authors can indicate repetitive data with indications such as "same data as for holotype", "same data as for preceding", "same locality", "ibid", etc. as long as the same method and wording are used consistently throughout the paper.
- ‘Missing’ elements: It is not necessary to include information such as "no date" or "no locality data"; just list the elements that are available.
- see more details here
- Quotation marks

- Avoid quotation marks except for direct quotations, words defined by the author, and words used in unusual contexts.

- Short quotations should be embedded in the text and enclosed in double quotation marks ("). Long quotations should be on a separate line, italicized, but without quotation marks.

- Single quotation marks are to be used only for a quotation that occurs within another quotation.

- Hyphen and dash characters

  - Consistent use of (-, –, —).

  - In contrast to parentheses an em-dash can be used alone.

  - En-dashes and em-dashes should not be spaced.

- Hyphens (-) are used to:

- link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use)
- En-dash (–) or en-rule (the length of an 'n') is used to:
- link spans.
- link numerals, sizes, dates and page numbers (e.g., 1977–1981; figs 5–7; pp. 237–258)
- geographic or name associations (e.g., Murray–Darling River; a Federal–State agreement)
- character states combinations (e.g., long–pubescent or red–purple).
- Em-dash (—) or em-rule (the length of an 'm') should be used rarely:
- only for introducing a subordinate clause in the text that is often used much as we use parentheses.

Section hierarchy

- No more than 4 levels, from hierarchical level 1 (H1) to hierarchical level 4 (H4)
- Unambiguous hierarchy levels
- No numbering of hierarchical levels

Section titles

- Capitalization:

  - For separated titles (usually H1-H3): Sentence case

  - For paragraph titles (usually H4): Sentence case

Mandatory statements

- Funding

o If missing, add the following statement (depending on the number of authors):

- The author has no funding to report.
- The authors have no funding to report.
- Competing interests

  - If missing, add the following statement (depending on the number of authors):

- The author has declared that no competing interests exist.
- The authors have declared that no competing interests exist.
- Acknowledgements (= non-financial support)

  - If missing, add the following statement (depending on the number of authors):

- The author has no support to report.
- The authors have no support to report.
- Data Resources (mandatory for empirical articles)

Geographical coordinates

One of the following formats should be used:

- Degrees, Minutes and Seconds (DMS), i.e.: o 36°31'21"N; 114°09'50"W
- Degrees and Decimal Minutes (DDM), i.e.: o 36°31.46'N; 114°09.84'W
- Decimal Degrees (DD), i.e.: 36.5243°S; 114.1641°W

  −36.5243; −114.1641 (using minus to indicate southern and western hemispheres)

In-Text Citations

- References

o 1-2 authors

- Jackson and Miller (2012) found out that...
- A recent study (Jackson and Miller 2012) confirmed that...

o 3 or more authors

- Jackson et al. (2012) found out that...
- A recent study (Jackson et al. 2012) confirmed that...

  - Multiple sources in chronological order:
- same authors different years - separated by a comma:
- Jackson and Miller (2012, 2015) found out that...
- Recent studies (Jackson et al. 2012, 2015) confirmed that...
- different authors - separated by a semicolon:
- (Smith et al. 1998, 2000, 2016; Brock and Gunderson 2001; Felt 2006)
- two or more fully identical citations (the same authors and years) are distinguished by adding the letters 'a', 'b', 'c', etc. after the year:
- Jackson 2008a, 2008b
- Jackson and Miller 2014a, 2014b
- Reyes-Velasco et al. 2018a, 2018b

  - Sources with page numbers
- Jackson and Miller (2012: 120–121) found out that
- A recent study (Jackson and Miller 2012: 120) confirmed that
- Figures:

  - Fig. 1
  - Fig. 1A, B
  - Fig. 1A–D
  - Figs 1, 2

- Figs 1–3
- Figs 1A, B, 3F, G, 7A
- Tables:
- Table 1
- Tables 1, 2
- Tables 1–3

- Appendixes:
- Appendix 1
- Appendices 1, 2
- Appendices 1–4

- Referenced materials from other sources:
- All figures, tables, etc., from other sources should be written with small letters i.e.: see fig. 2 in Author (Year) ...

References

- Author names: surname first; all given names abbreviated, no full stops, commas or spaces, i.e.:
  - Lyal CHC
  - van Tol J
  - de Albuquerque PRA
- Different authors separated by comma
- Year in brackets; no comma or full stop after it
- No italics (except for Latin terms)

Published papers:

Polaszek A, Alonso-Zarazaga M, Bouchet P, Brothers DJ, Evenhuis NL, Krell FT, Lyal CHC, Minelli A, Pyle RL, Robinson N, Thompson FC, van Tol J (2005) ZooBank: The openaccess register for zoological taxonomy: Technical Discussion Paper. Bulletin of Zoological Nomenclature 62: 210–220.

Accepted papers:

Same as above, but "in press" appears instead of the year in parentheses.

Electronic journal articles:

Mallet J, Willmott K (2002) Taxonomy: Renaissance or Tower of Babel? Trends in Ecology and Evolution 18(2): 57–59. https://doi.org/10.1016/S0169-5347(02)00061-7

Paper within conference proceedings:

Orr AG (2006) Odonata in Bornean tropical rain forest formations: Diversity, endemicity and applications for conservation management. In: Cordero Rivera A (Ed.) Forest and Dragonflies. Fourth WDA International Symposium of Odonatology, Pontevedra (Spain), July 2005. Pensoft Publishers, Sofia-Moscow, 51–78.

Book chapters:

Mayr E (2000) The biological species concept. In: Wheeler QD, Meier R (Eds) Species concepts and phylogenetic theory: A debate. Columbia University Press, New York, 17–29.

Books:

Goix N, Klimaszewski J (2007) Catalogue of Aleocharine Rove Beetles of Canada and Alaska. Pensoft Publishers, Sofia-Moscow, 166 pp.

Book with institutional author:

ICZN [International Commission on Zoological Nomenclature] (1999) International code of zoological nomenclature. Fourth Edition. The International Trust for Zoological Nomenclature, London.

PhD thesis:

Dalebout ML (2002) Species identity, genetic diversity and molecular systematic relationships among the Ziphiidae (beaked whales). PhD Thesis, University of Auckland, Auckland, ## pp.

Link/URL:

BBC News (2012) Island leopard deemed new species http://news.bbc.co.uk/ [Accessed on dd.mm.yyyy]

Apêndice 3

Normas para publicação na Revista Brasileira de Entomologia:

Manuscript length should not exceed 80 pages, including figures. In the case of longer manuscripts, authors should consult the Editorial Board previous to submission.

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Divide your article into clearly defined sections. Each subsection is given a brief heading. Each heading should appear on its own separate line. Subsections should be used as much as possible when crossreferencing text: refer to the subsection by heading as opposed to simply 'the text'.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Material and methods

Provide sufficient details to allow the work to be reproduced by an independent researcher. Methods that are already published should be summarized, and indicated by a reference. If quoting directly from a previously published method, use quotation marks and also cite the source. Any modifications to existing methods should also be described.

Results

Results should be clear and concise.

Discussion

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

Conclusions

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

Essential title page information

• **Title**. Concise and informative. Titles are often used in information-retrieval systems. Avoid abbreviations and formulae where possible.

• **Author names and affiliations, including ORCID ID**. Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and in front

of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author. Author affiliations should be presented in decreasing hierarchical order (e.g. Harvard University, Harvard Business School, Boston, USA) and should be written as established in its own language (e.g. Universit Paris-Sorbonne; Harvard University, Universidade de São Paulo). The ORCID ID must be inserted in all authors' profile. To do that go to Update your details , ORCID field; if any of the authors does not have an ORCID ID, it can be registered at https://orcid.org/register.

• **Corresponding author**. Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.

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A concise and factual abstract is required. The abstract should be presented in a maximum of 250 words and state briefly the purpose of the research, the principal results and major conclusions. Na abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, nonstandard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself. Immediately after the abstract, provide a maximum of 5 keywords in alphabetical order. Words already included in the title and abstract should not be used as keywords.

Abbreviations

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:
Funding: This work was supported by the National Institutes of Health [grant numbers xxxx, yyyy]; the Bill & Melinda Gates Foundation, Seattle, WA [grant number zzzz]; and the United States Institutes of Peace [grant number aaaa].
It is not necessary to include detailed descriptions on the program or type of grants and awards. When funding is from a block grant or other resources available to a university, college, or other research institution, submit the name of the institute or organization that provided the funding.
If no funding has been provided for the research, please include the following sentence:
This research did not receive any specific grant from funding agencies in the public, commercial, or
not-for-profit sectors.

Units

Follow internationally accepted rules and conventions: use the international system of units (SI). If other units are mentioned, please give their equivalent in SI.

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Image manipulation

Whilst it is accepted that authors sometimes need to manipulate images for clarity, manipulation for purposes of deception or fraud will be seen as scientific ethical abuse and will be dealt with accordingly.

For graphical images, this journal is applying the following policy: no specific feature within an image may be enhanced, obscured, moved, removed, or introduced. Adjustments of brightness, contrast, or color balance are acceptable if and as long as they do not obscure or eliminate any information present in the original. Nonlinear adjustments (e.g. changes to gamma settings) must be disclosed in the figure legend.

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• Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.

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TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.
TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.
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References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either 'Unpublished results' or 'Personal communication'. Citation of a reference as 'in press' implies that the item has been accepted for publication.

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As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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2. Two authors: both authors' names and the year of publication;
3. Three or more authors: first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'

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Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

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Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 9, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in: Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:

Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).

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[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

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Supplementary material

Supplementary material such as applications, images and sound clips, can be published with your
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