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MICROLEPIDÓPTEROS DE HÁBITO MINADOR E GALHADOR: ESTÁGIOS IMATUROS, HISTÓRIAS DE VIDA, E DESCRIÇÃO DE NOVAS ESPÉCIES PARA A REGIÃO NEOTROPICAL

PORTO ALEGRE
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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 à obtenção do título de Doutor em Biologia Animal.

Área de concentração: Biodiversidade

Orientador: Dr. Gilson R. P. Moreira

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Tese aprovada como requisito para obtenção do grau de "Doutor em Biologia Animal", no Programa de Pós-graduação em Ciências Biológicas, Área de Concentração Biodiversidade, da Universidade Federal do Rio Grande do Sul, pela Comissão formada pelos professores:

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BANCA EXAMINADORA

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Dr. Luiz Alexandre Campos

EPÍGRAFE

"Em algum lugar, alguma coisa incrível está esperando para ser descoberta".

Carl Sagan

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APRESENTAÇÃO

Esta tese é apresentada de acordo com as normas do Programa de Pós-graduação em Biologia Animal, resolução N° 37/2018, estando estruturada em seis capítulos.

O capítulo I é uma introdução geral a respeito dos assuntos tratados. Os capítulos II ao V estão sob a forma de artigos científicos, cada qual seguindo a formatação especifica da revista para o qual foi submetido/publicado. O capítulo VI traz as considerações finais a respeito dos temas abordados.

O capítulo II foi publicado na revista Zoologia, em outubro de 2018. O capítulo III foi publicado na Revista Brasileira de Entomologia, em novembro de 2016. O capítulo IV foi submetido à revista Zootaxa em novembro de 2018, aceito para publicação em março de 2019. O capítulo V foi submetido para publicação na revista Zoologia, em março de 2019.

SUMÁRIO

RESUMO

Nomes de organismos são essenciais para estudos na área biológica, e diversas ferramentas vem sendo incorporadas na busca de identificações mais facilitadas e precisas. Isto inclui a taxonomia integrativa, que se baseia na utilização de todas as técnicas e fontes de características diagnósticas disponíveis, tais como aspectos morfológicos, ecológicos e moleculares. A região Neotropical é composta por uma rica e ainda não descrita fauna de microlepidópteros, muitos deles associados as guildas com hábitos minador e galhador. Descrever esta diversidade é necessário tanto do ponto de vista econômico, quanto conservacionista. Ao longo deste trabalho são conduzidos estudos envolvendo três famílias de microlepidoptera: Nepticulidae, Gracillariidae e Alucitidae. Um novo gênero e três novas espécies são descritos e também são apresentados dados referentes à morfologia dos imaturos, história natural, plantas hospedeiras e danos histológicos causados. Estas informações são igualmente levantadas para *Stigmella schinivora* van Nieukerken, 2016 (Nepticulidae), uma das linhagens mais ancestrais de lepidópteros minadores foliares. Inferências moleculares também são feitas com relação à posição filogenética dos grupos descritos. Dentre as espécies novas *Leurocephala chilensis* Vargas & Moreira, 2016 (Gracillariidae) é encontrada em Arica – Chile, no Deserto do Atacama, e possui hábito minador foliar, tendo como planta hospedeira a aroeira-salsa, *Schinus molle* (Anacardiaceae). O gênero *Valissiana* (Gracillariidae) foi criado para alocar uma nova espécie, *Valissiana universitaria,* sendo encontrada na região de Porto Alegre-RS minando folhas de *Erythroxylum argentinum* (Erythroxylaceae). Uma nova espécie de *Prymnotomis* Meyrick, 1931 é descrita, encontrada induzindo galhas em frutos de *Cordiera elliptica* (Rubiaceae), no Cerrado brasileiro. Os dados aqui levantados contribuíram para o aumento do conhecimento taxonômico e também da biodiversidade de microlepidópteros neotropicais.

Palavras chave: Biodiversidade; Lepidoptera; taxonomia integrativa.

ABSTRACT

Organism names are essential to all studies in the biological area, and several tools has been incorporated in the search for more easy and precise identifications. These include the integrative taxonomy which is based on the use of all the techniques and sources of available diagnostic features, such as morphological, ecological, and molecular aspects. The Neotropical region is composed of a rich and not yet described fauna of microlepidoptera, many of them associated with leaf-miner and gall-inducing guild habits. To describe this diversity is necessary under both economically and conservationist perspectives. Throughout this work are conducted studies involving three families of microlepidoptera: Nepticulidae, Gracillariidae and Alucitidae. A new genus and three new species are described and information about the morphology of immatures, natural history, host plants and histological damage are also presented. This information is also pointed for *Stigmella schinivora* van Nieukerken, 2016 (Nepticulidae), one of the most ancestral lepidopteran leaf-miner. Molecular inferences are also made regarding the phylogenetic position of the groups described. The new species *Leurocephala chilensis* Vargas & Moreira, 2016 (Gracillariidae) is found in Arica - Chile, at the Atacama Desert, and has a leaf-miner habit, with host plant aroeira-salsa, *Schinus molle* (Anacardiaceae). The genus *Valissiana* (Gracillariidae) was created to allocate a new species, *Valissiana universitaria*, found in the region of Porto Alegre-RS, mining leaves of *Erythroxylum argentinum* (Erythroxylaceae). A new species of *Prymnotomis* Meyrick, 1931 is described, found inducing galls in fruits of *Cordiera elliptica* (Rubiaceae), at the Brazilian Savana. The informations collected here contributed to the increase of taxonomic knowledge and also the biodiversity of Neotropical microlepidoptera.

Keywords: Biodiversity; Lepidoptera; integrative taxonomy.

CAPÍTULO 1

Introdução Geral

Interações Inseto-Planta: Lepidópteros Minadores e Galhadores

Interações ecológicas ocorrem de maneira constante no meio ambiente, tendo variadas formas, diferentes efeitos e distintas complexidades. Geralmente envolvem uma ampla gama de fatores, abióticos e bióticos, e podem trazer benefícios para ambas as partes, ou serem prejudiciais para um ou mais grupos envolvidos; neste segundo cenário, pode-se inserir a herbivoria (Zhang 1997, Townsend et al. 2006).

Dentre as diversas interações existentes entre herbívoros e plantas, destacam-se as ocorrentes com a ordem Lepidoptera, a qual é megadiversa, com mais de 160.000 espécies descritas e altamente variável em comportamentos de herbivoria. Dentre estes, as guildas com hábito minador e galhador são compartilhados por milhares de espécies, e em diversas famílias em Lepidoptera. São insetos holometábolos, e a ação é efetuada pelo estágio larval, que ali encontram alimento e proteção durante esta fase de desenvolvimento (Heppner 1991). Estes grupos interagem a milhares de anos com suas plantas hospedeiras, criando assim inúmeros e complexos mecanismos, com uma grande riqueza de mecanismos, e com potencial para incontáveis sistemas de estudo no campo das interações inseto-planta (Hering, 1951, Fernandes et al. 2010).

Via de regra, as guildas de minadores e galhadores em Lepidoptera estão associadas a tamanhos diminutos, podendo ser chamados de microlepidópteros. Este termo é comumente empregado para designar espécies que apresentam tamanho e envergadura diminuta, normalmente abaixo de dois centímetros. Tal definição, no entanto não constitui uma categoria taxonômica formal, visto que os microlepidópteros não formam um grupo natural, pois agrupa diversas famílias que não tem necessariamente uma relação filogenética próxima (Robinson et al. 1994, van Nieukerken et al. 2011, Walhberg et al. 2013, Regier et al. 2009, 2015).

Apesar do grupo dos macrolepidópteros constituírem o maior número de espécies descritas, estima-se que a maior diversidade de espécies existentes seja a de microlepidópteros, sendo a maioria ainda desconhecida pela ciência (Krinstensen et al. 2007, van Nieukerken et al. 2011, Brito et al. 2016, De Prins et al. 2016). Estes organismos estão presentes na maioria das regiões biogeográficas, principalmente na região Neotropical, a qual é ainda pouco explorada com relação a sua

microlepidopterofauna. Este cenário não é diferente para o Brasil, e dentre os principais motivos, pode-se destacar o negligenciamento da necessidade do estudo destes pela grande maioria dos pesquisadores, e a falta de incentivo e investimento na formação e capacitação de pessoal na área.

Minadores Foliares

Caracterizam-se pelo consumo de estruturas foliares, como parênquima e epiderme de maneira endofágica, formando canais chamados de minas (Hering 1951, Hespenheide 1991). Minas foliares são interessantes objetos de estudo, pois guardam em seu interior importantes vestígios, que podem possibilitar a reconstrução da história de vida e aspectos das interações com outros organismos, bem como da ontogenia das espécies (Connor e Tavener 1997, Storey-Palma et al. 2012).

Todas as espécies de lepidópteros minadores foliares conhecidas pertencem ao grupo das mariposas, e apesar de cada qual possuir suas peculiaridades, apresentam características comuns ao hábito minador, tais como: tamanho diminuto em todos os estágios de desenvolvimento, alta especificidade quanto à planta hospedeira e tipo de tecido minado, tendência à monofagia ou oligofagia, ciclo de vida com predomínio do estágio larval, e um comportamento estereotipado no modo como constroem suas minas, o qual é inclusive um bom indicativo na determinação da espécie de minador (Hering 1951, Connor e Tavener 1997).

Família Nepticulidae

Nepticulidae possui os menores indivíduos e está entre as mais antigas linhagens de Lepidoptera. Com relação a morfologia da genitália feminina é do tipo monotrysia, com somente uma abertura genital, relacionada tanto a copula quanto a oviposição, sendo esta característica considerada ancestral (Dudgale 1974, Torre-Bueno 1989, Davis 1998). Do ponto de vista ontogenético, o desenvolvimento das peças bucais pode ser diferenciado em certas linhagens de Lepidoptera; este não é o caso de Nepticulidae, que não apresentam diferenças morfológicas no desenvolvimento da mandíbula, apresentando estruturas relacionadas à trituração do alimento (= tissue feeding) desde o início do desenvolvimento larval (Davis 1987a).

A família Nepticulidae é cosmopolita, com aproximadamente 884 espécies descritas, divididas em 29 gêneros (van Nieukerken et al. 2016a, van Nieukerken 2018). São em sua maioria minadores foliares, e sua taxonomia é ainda pouco estudada, sendo as espécies quase que em totalidade descritas apenas por características morfológicas do estágio adulto, sendo raros os trabalhos que levem em consideração a morfologia de imaturos correspondentes (van Nieukerken et al. 2004).

Com cerca de 420 espécies descritas, *Stigmella* é o gênero mais diverso de Nepticulidae, apresentando uma distribuição global, com 60 espécies apontadas para a região Neotropical (van Nieukerken 2018). Curiosamente, apesar desta grande diversidade, apenas 1 espécie tinha até então sido assinalada para o Brasil, sendo este fator provavelmente associado à dificuldade na obtenção e preparação de material, e principalmente pela ausência de pesquisadores relacionados a este grupo, atuando em pesquisas de cunho taxonômico.

A identificação das espécies de *Stigmella* pode se tornar uma tarefa difícil, principalmente pela grande quantidade e pela existência de complexos de espécies onde os adultos são similares, tanto externamente quanto no padrão morfológico das genitálias (van Nieukerken et al. 2016a, van Nieukerken 2018). Deste modo, a utilização de características morfológicas presentes em imaturos, pode vir a enriquecer, e facilitar na correta identificação das espécies.

Família Gracillariidae

Gracillariidae é a mais diversa família de lepidópteros com hábito minador foliar; a fauna mundial conta atualmente com 106 gêneros e 1966 espécies reconhecidas (Davis 1987b, De Prins e De Prins 2018). Deste total, 27 gêneros e 187 espécies foram descritos para a região Neotropical (De Prins e De Prins 2018), sendo que 10 gêneros e 38 espécies são assinaladas para o Brasil (Brito e Duarte 2018). Tanto em nível global, quanto local, o número de espécies descritas é considerado baixo, principalmente quando considera-se a abrangência do bioma Mata Atlântica, e Floresta Amazônica, os quais abrigam os mais elevados níveis de diversidade animal e vegetal do planeta, assim como altos índices de endemismo de espécies (Myers et al. 2000, Stehmann et al. 2009).

Avanços tem sido feitos quanto à taxonomia de Gracillariidae, tanto em relação a melhor compreensão dos padrões morfológicos e filogenéticas entre os grupos (De Prins e Kawahara 2012, Regier et al. 2009, Kawahara et al. 2016), quanto no

compilamento de informações, como bancos de dados para pesquisa de dados taxonômicos (De Prins e De Prins 2018). Apesar disto, muitos grupos ainda possuem uma história taxonômica confusa e que necessitam de análises e revisões, tanto do ponto de vista morfológico, quanto filogenético.

A morfologia da genitália feminina de Gracillariidae a coloca no grupo Ditrysia, onde se apresentam duas aberturas genitais, uma utilizada na cópula e outra na oviposição (Dudgale 1974, Torre-Bueno 1989). Tal característica é considerada derivada, dando a família um status evolutivo recente quando comparada com os nepticulídeos (Kristensen 1998). Outra característica derivada e exclusiva da família é a morfogênese larval caracterizada por uma notável hipermetamorfose, na morfologia e no hábito alimentar (Kumata 1978, Davis 1987b).

Via de regra, Gracillaridae apresenta uma transição de uma forma com mandíbulas dilaceradoras "*sap-feeding*" para mandíbulas trituradoras "*tissue feeding*". A forma dilaceradora ocorre nos primeiros ínstares do desenvolvimento, sendo ápodas, e com o corpo, cápsula cefálica e mandíbulas achatadas dorso-ventralmente. São também as mandíbulas que dão a esta forma larval uma característica marcante, que é a de cortar/dilacerar o tecido da planta, e absorver o conteúdo interno de células rompidas (Kumata 1978, Davis 1987). A forma larval trituradora, normalmente subsequente na ontogênese apresenta cabeça e corpo cilíndrico ou subcilíndrico, três pares de pernas torácicas, e pseudopódios bem diferenciados nos segmentos abdominais A3-A5 e A10, geralmente. As peças bucais permitem a alimentação de tecidos mais rígidos, como células do parênquima foliar. A forma "*spinning*", ou pre-pupa, pode também estar presente, e difere das demais por não se alimentar e apresentar estruturas locomotoras e peças bucais reduzidas ou atrofiadas, com exceção ao espinerete, necessário para a construção do casulo (Davis e Robinson 1998).

As larvas de Gracillariidae tem um importante papel no ecossistema e na teia de interações ecológicas. A estreita relação que tem com seus hospedeiros torna os gracilarídeos minadores foliares particularmente adequados para estudos de relação inseto-planta, tanto do ponto de vista evolutivo quanto da conservação, pois permitem compreender os processos que determinam os padrões de diversidade observados na natureza (Lopez-Vaamonde 2003, Forister et al. 2008, Oshima 2008, Avise 2009).

Galhadores

Também chamados de cecidógenos, esta guilda possui as espécies mais intimamente relacionados às suas plantas hospedeiras, desenvolvendo complexas interações. Insetos galhadores são capazes de redirecionar e modificar os padrões de crescimento vegetal, causando anomalias nos mais diversos tecidos, tanto por hiperplasia, quanto por hipertrofia das regiões galhadas (Abrahamson et al. 1998).

Galhas podem ser induzidas em qualquer região da planta, vegetativo ou reprodutivo, e apresentam uma grande diversidade fenotípica. O mecanismo de ação do início e desenvolvimento da galha é complexo, variável, e ainda não totalmente compreendido. Sabe-se, entretanto, que se inicia pelo contato da planta com substâncias cecidogênicas produzidas pelo inseto, tais como aminoácidos, hormônios reguladores de crescimento e compostos fenólicos (Mathur e Rajamani 1984, Aljbory e Chen 2018).

Para o inseto galhador, a galha representa uma fonte contínua de alimento, além de um local relativamente seguro e protegido do ambiente externo, no qual a fase larval (e pupal, dependendo da espécie) possui melhores condições para desenvolvimento e por consequência, maiores chances de sobrevivência (Mani 1964, Price 1986, Fernandes 1987, Dreger-Jauffret e Shorthouse 1992, Shorthouse et al. 2005).

Espécies galhadoras são conhecidas como “engenheiras de ecossistemas”, pois acabam sendo usadas não apenas pelo organismo cecidógeno, mas como recurso por diversas espécies, e para uma ampla gama de interações interespecíficas. Como exemplos pode-se citar a cecidofagia, o inquilinismo, parasitismo e cleptoparasitismo, bem como insetos sucessores, usam as galhas vazias como abrigo e nidificação (Mani 1964, Sanver and Hawkins 2000, Luz et al. 2014 , Moreira et al. 2017).

Sabe-se da existência da ação cecidogênica em pelo menos 20 famílias de Lepidoptera. Muitas delas são importantes do ponto de vista agrícola, mas a grande maioria tem sua importância relacionada às interações ecológicas em ambientes naturais. Estima-se a existência de milhares de espécies ainda não descritas de lepidópteros galhadores, principalmente para a região Neotropical (Brito et al. 2016, De Prins et al. 2016) e os poucos registros existentes referem-se normalmente a morfotipos e relações com a planta hospedeira (Miller 2005, Espírito-Santo e Fernandes 2007, Coelho et al. 2009, Hanson et al. 2014, Luz et al. 2014, Araújo 2018).

Família Alucitidae

Indivíduos adultos de espécies pertencentes à família Alucitidae possuem as asas anteriores e posteriores divididas em cinco ou seis lóbulos, e por esta característica, são popularmente conhecidas como mariposas-de-muitas-plumas "many-plumed moths." A família possui 130 espécies descritas, alocadas em nove gêneros. Pouco se conhece a respeito da biologia do grupo, bem como em relação aos estágios imaturos, com algumas citações relacionando-os ao hábito principalmente brocador e também galhador (Viette 1956, Heppner 1987).

A fauna de Alucitidae conhecida para o Brasil conta atualmente com sete espécies, alocadas em quatro gêneros, três deles monotípicos (Moreira e Duarte, 2019). O gênero *Prymnotomis* Meyrick 1931 é um deles, e foi criado para abrigar a espécie *P. crypsicroca*, assim permanecendo até então. Em situação similar a da família, a descrição da espécie foi baseada na morfologia do adulto, e informações a respeito da biologia, bem como de estágios imaturos, são desconhecidas.

Taxonomia em microlepidoptera: importância do estudo de imaturos e de uma abordagem integrativa

Nomes de organismos são a chave para toda a literatura e estudos na área biológica. Porém, mesmo com a existência de diversas ferramentas digitais e progressos feitos com relação ao número de espécies descritas e a compilação destes dados, encontrar e aplicar um nome científico de modo correto pode se tornar um grande desafio (Nieukerken et al. 2016). Esta tarefa é ainda mais difícil e complexa quando se buscam informações relacionadas a grupos com grande diversidade ainda não descrita, e com escassez de especialistas, como no caso da taxonomia de microlepidópteros minadores e galhadores no Brasil (Brito et al. 2016).

A abordagem taxonômica integrativa pode ser definida como a utilização de todas as técnicas e fontes de características diagnósticas, tais como morfológicas, ecológicas e moleculares, na busca da melhor e mais correta diferenciação de espécies. (Dayrat 2005, Pires e Marinoni 2010, Padial et al. 2010, Misof et al. 2014).

A taxonomia integrativa é uma importante ferramenta que vem sendo utilizada na resolução de questões taxonômicas. Em insetos holometábolos, como é o caso dos

lepidópteros minadores foliares e galhadores, outra importante fonte de informação para fins taxonômicos surge do estudo e utilização da morfologia dos estágios imaturos (ovo, larva e pupa).

Devido a grande especialização desse grupo na utilização da planta hospedeira e vida endógena, diversas estruturas relacionas a tais adaptações podem estar presentes, apresentando variações na forma, possivelmente relacionada ao modo de vida e função. Da mesma maneira, a análise do formato da galha e da mina foliar de cada espécie pode mostrar um padrão estereotipado, trazendo características que auxiliam na sua identificação (Hering 1951, Miller 2005, Shorthouse et al. 2005, Moreira et al. 2007). Em resumo, questões presentes nos imaturos, na história de vida e nos adultos também devem ser levadas em consideração quando se busca trabalhar com a taxonomia de microlepidópteros minadores e galhadores.

Justificativa e Objetivos

A principal justificativa para este trabalho é a necessidade de descrever a diversidade de espécies e compilar dados a respeito da história de vida e estágios imaturos de minadores e galhadores que compõem a lepidopterofauna Neotropical. Isto se faz necessário para que então se possa buscar a representatividade necessária quanto à importância de se traçar estratégias para a preservação desta rica e ainda desconhecida e negligenciada fauna.

Objetivo Geral

Contribuir para o conhecimento da diversidade de espécies de microlepidoptera minadores e galhadores existentes na região Neotropical, utilizando uma abordagem integrativa.

Objetivos Específicos

Descrever um novo gênero de Gracillariidae Neotropical;

Descrever duas novas espécies de Gracillariidae minadores foliares, e uma nova espécie de Alucitidae, de hábito galhador;

Apresentar em detalhes a morfologia dos estágios imaturos das espécies aqui descritas, bem como de uma espécie já conhecida de minador foliar (*Stigmella schinivora* van Nieukerken, 2016) - Nepticulidae;

Estabelecer relações filogenéticas entre as novas espécies aqui descritas com as já conhecidas na literatura;

Fornecer dados relacionados à história de vida, plantas hospedeiras, danos histológicos causados, e distribuição para as espécies citadas.

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CAPÍTULO II

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RESEARCH ARTICLE

Description of immature stages and natural history of *Stigmella schinivora* (Lepidoptera: Nepticulidae), a leaf-miner associated with the Brazilian peppertree

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ABSTRACT. *Stigmella schinivora* van Nieukerken, 2016 was described from Cataratas de Iguazú, Misiones, Argentina, based on adults reared from *Schinus terebinthifolius* Raddi (Anacardiaceae) leaf mines. The aim of this study is to describe for the first time the external morphology of the immature stages of *S. schinivora* with the aid of light and scanning electron microscopy, based on mines collected on the same host plant, but in Laranjeiras do Sul, Paraná, Brazil. Data on natural history, including histology of the mines, are also provided. The larva passes through four instars, all endophytic, having chewing mouth parts and feeding on the palisade parenchyma. The first three instars are apodous and have a subcylindrical body, bearing only one pair of setae on the tenth abdominal segment; the fourth instar is eruciform, with well-developed ambulatory calli on thorax and abdomen and setae on all tagmata. A serpentine mine is constructed on the adaxial surface, progressively increasing in width during larval development. With the exception of the widened, terminal section, the mine is left filled with larval feces. The fully developed larva of last instar exits through a slit made at the distal end of the mine, building a silk cocoon on the leaf abaxial surface where pupation occurs. This is the first record of *S. schinivora* from Brazil, which was only known from the type locality in Argentina.

KEY WORDS. Atlantic forest, leaf-mining moths, microlepidoptera, nepticulids, *Schinus terebinthifolius*.

INTRODUCTION

The Nepticulidae is one of the most ancient lineages of Lepidoptera, with a global distribution and approximately 884 described species that are divided into 29 genera (van Nieukerken et al. 2016a, van Nieukerken 2018). They are among the smallest extant lepidopterans, having predominantly leaf miner habits and being associated with several plant families (Braun 1917, van Nieukerken et al. 2016a). Their minute size and the scarcity of the material available in collections has led their taxonomy to be mainly based on the morphology of adults, the immature stages rarely being taken into account (e.g., van Nieukerken et al. 2004). Studies that include the general appearance of their mines are not uncommon (e.g., Braun 1917, Stonis et al. 2013, 2014, van Nieukerken et al. 2016b), but specialization of larval feeding on tissues, if any, is largely unknown.

The worldwide distributed genus *Stigmella* Schrank, 1802 currently with ca. 420 species, is the largest genus of Nepticulidae (van Nieukerken 2018). Species identification in this genus can be difficult, since species complexes are common whose adults have similarities in external appearance; in these cases, only subtle differences in the genitalia morphology can be detected among species (Stonis and Remeikis 2016). There are at least 61 species of *Stigmella* recognized for the Neotropical Region (van Nieukerken et al. 2016a); however, this genus is still little studied in this region; thus, this number may not reflect its real diversity in the Neotropics (Puplesis and Robinson 2000, Šimkevičiūtė et al. 2009, Stonis et al. 2014, van Nieukerken et al. 2016a, Stonis and Remeikis 2017). This aspect is even more relevant in Brazil, where there are no records of *Stigmella* yet. This is unexpected since this is a megadiverse country, including biomes such as the Atlantic Forest, known

for the great diversity of plants and animals and high endemism indexes (Myers et al. 2000).

Stigmella schinivora van Nieukerken, 2016 was recently described as a leaf miner of the Brazilian peppertree, *Schinus terebinthifolius* Raddi (Anacardiaceae) from the region of Misiones, Argentina (van Nieukerken et al. 2016b). Its description relied on morphology of the male and female genitalia. In the present study, using material collected in southwest Paraná state, Brazil, we present a detailed description of the immature stages of *S. schinivora*, based on light and scanning electron microscopy. We also provide additional information about its natural history, including the histology of its mines on leaves of *S. terebinthifolius*.

MATERIAL AND METHODS

Specimens used in this study came from leaf mines of *S. terebinthifolius* collected in Laranjeiras do Sul municipality, Paraná, Brazil, in 2016 and 2017. They were brought to the Laboratório de Morfologia e Comportamento de Insetos (LMCI), Zoology Department of Federal University of Rio Grande do Sul (UFRGS), Porto Alegre city, and then they were either dissected or kept at room temperature in plastic pots containing moistened cotton for emergence of adults. The adults obtained in the laboratory were identified as *S. schinivora* based on comparison with original descriptions and illustrations of the adult stage, including female and male genitalia, provided by van Nieukerken et al. (2016b).

Adults were pinned and dried. Immature stages were fixed in Dietrich's fluid and preserved in 75% ethanol. For descriptions of the gross morphology, the specimens were cleared in a 10% potassium hydroxide (KOH) solution and slide-mounted in either glycerin jelly or Canada balsam. Observations were performed with the aid of a Leica M125 stereomicroscope, and measurements were performed using an attached ocular micrometer (precision = 0.01 mm). Structures selected to be drawn were previously photographed with a Sony Cyber-shot DSC-H10 digital camera attached to the stereomicroscope, and also by using a Nikon AZ 100M stereomicroscope. Vectorized line drawings were then made with the software Corel Photo-Paint X7, using the corresponding digitalized images as a guide. At least five specimens were used for the descriptions of each morphotype.

For scanning electron microscope analyses, additional specimens were dehydrated in a Bal-tec CPD030 critical-point dryer, mounted with double-sided tape on metal stubs and coated with gold in a Bal-tec SCD050 sputter coater. They were examined and photographed in a JEOL JSM6060 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of UFRGS.

For plant anatomical descriptions, field-collected leaf parts of *S. terebinthifolius* containing mines of *S. schinivora* were preserved in Dietrich's fluid. Leaf parts containing the different larval instar morphotypes were selected under a stereomicroscope, and freehand cross sections were cut with a razor blade. They were then stained for five seconds with safranin and photographed with a Nikon AZ 100M stereomicroscope.

Vouchers of specimens used in this study were deposited in the insect collection of the Laboratório de Morfologia e Comportamento de Insetos (LMCI), Zoology Department (UFRGS), as follows (all coming from *S. terebinthifolius* leaf-mines collected by the senior author at Laranjeiras do Sul, Paraná, Brazil): 16-23.VII.2016, pinned, dried adults, two females (LMCI 309-10 and 11, with genitalia on slides GRPM 50-151 and 152, respectively), two males (LMCI 309-12 and 13, with genitalia on slides GRPM 50-153 and 154, respectively); 29.XI.2017, immature stages, fixed in Dietrich's fluid, preserved in 70% ethanol, 3 first instar larvae (LMCI 323-2), 3 second instar larvae (LMCI 323-3), 4 third instar larvae (LMCI 323-4), 8 fourth instar larvae (LMCI 323-5) and 3 pupae (LMCI 323-7).

RESULTS

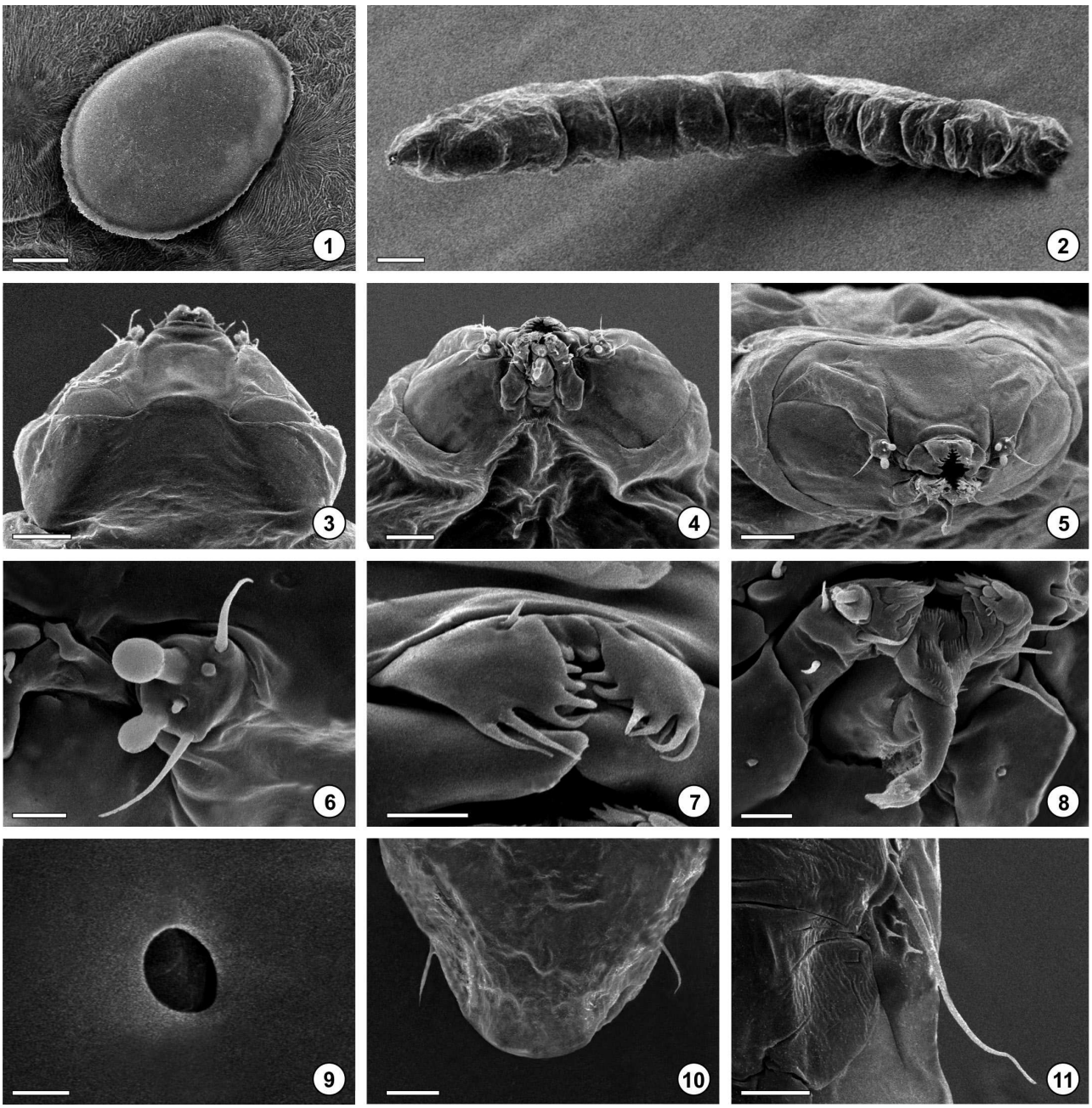
Egg. Flat and oval, firmly adhered to the leaf surface by a glistening substance (Figs 1, 44); average diameter + standard deviation = 0.16 ± 0.002 mm, n = 5. It is covered by a solid, smooth, transparent layer, forming a cap; micropyles and aeropyles were not found.

Larva. Prognathous, with buccal apparatus of chewing type. There are four instars and two morphotypes; the first form corresponds to the first three instars and the second to the last instar. The first morphotype has a subcylindrical, smooth body, without specialized locomotor structures (Fig. 2). The second morphotype has well-developed calli on thorax and abdomen, and setae of variable sizes distributed throughout the body (Fig. 19). We could not find major morphological differences among instars of the first morphotype. However, they can be identified by their size, since corresponding head capsule widths do not overlap (Table 1). The following exponential growth equation was adjusted for the head capsule width: $y = 0.049e^{0.400x}$; n = 37; r = 0.99; p < 0.0001.

Penultimate instar. Except for the absence of stemmata, the head of the first morphotype is similar to that of the second one in general color, shape (Figs 3–5), antennae (Fig. 6) and mouth parts (Figs 4, 5, 7, 8), which are described in detail below. The same occurs in relation to thorax and abdomen, including spiracles (Fig. 9). No evident setae were found on the thorax or abdomen of the first morphotype, except for the tenth segment where a pair of conspicuous setae appear dorsolaterally (Figs 10, 11).

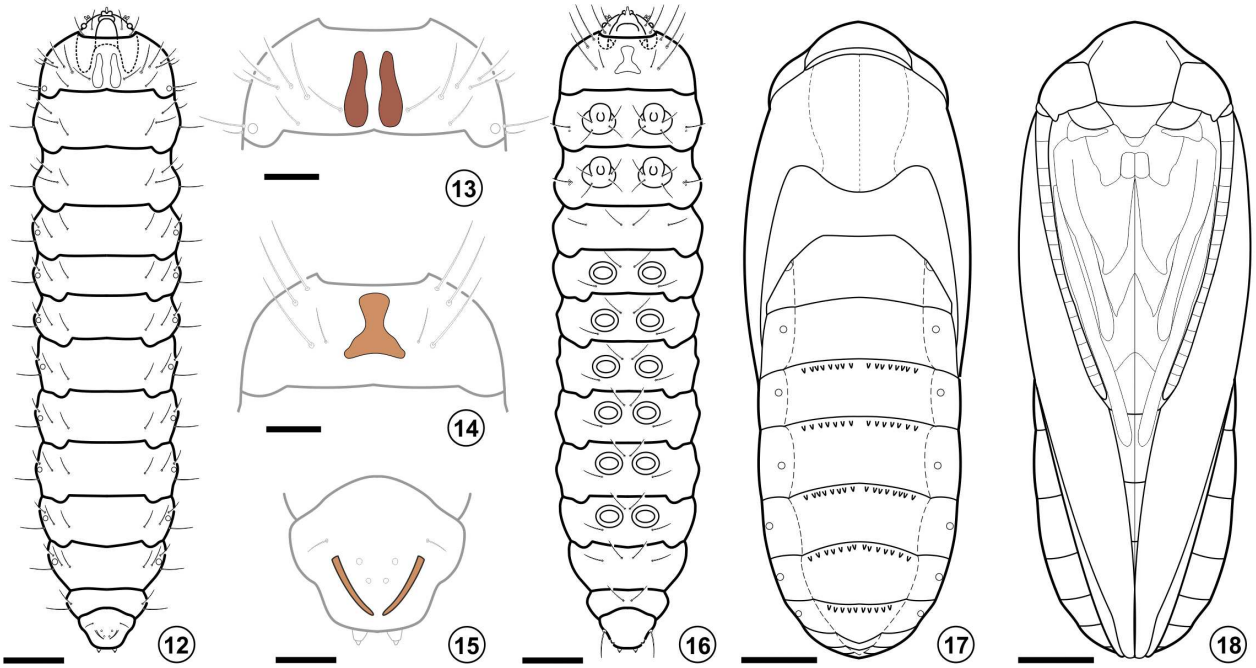
Table 1. Variation in size of head capsule width among instars of *Stigmella schinivora* reared on *Schinus terebinthifolius*.

Instar	N	Head capsule width (mm)		
		Mean ± standard error	Range	Growth rate
I	4	0.087 ± 0.003	0.084–0.095	–
II	5	0.116 ± 0.003	0.105–0.126	1.33
III	15	0.154 ± 0.002	0.147–0.168	1.33
IV	15	0.246 ± 0.002	0.231–0.263	1.60



Figures 1–11. Egg and third instar of *Stigmella schinivora* under scanning electron microscopy: (1) egg; (2) general view of larva, lateral; (3–5) head, under dorsal, ventral and anterior views, respectively; (6) antenna, anterior; (7) labrum, anteroventral; (8) labium, showing spinneret in detail, ventral; (9) spiracle of fourth abdominal segment, lateral; (10) last abdominal segment, dorsal; (11) seta of last abdominal segment in detail, dorsal. Scale bars: 50, 100, 20, 20, 20, 20, 5, 5, 1, 25, and 10 µm, respectively.

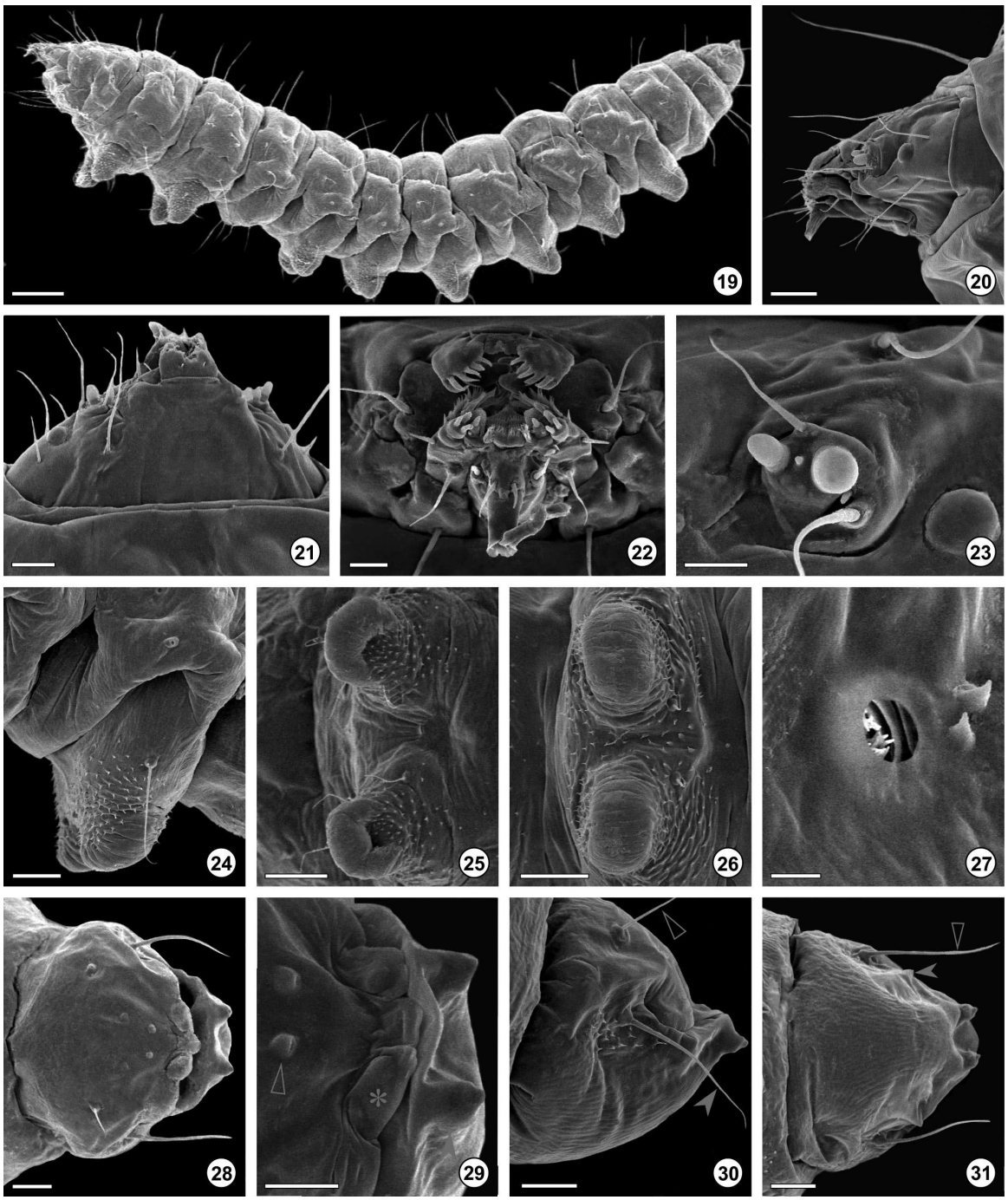
Last instar. Average length ± standard deviation = 1.42 ± 0.21 mm; n = 5. Head light brown, flattened dorsoventrally, partially concealed within the prothorax, with deep epicranial notch. Frontoclypeus rectangular, longer than wide. Labrum bilobed, with lobes having distal serrated edge, and bearing one pair of short setae mesally (Figs 21, 22); mandibles with well-developed cusps; one long seta on proximal base. Maxilla with well-developed galea and palpi. Labium with tubular spin-



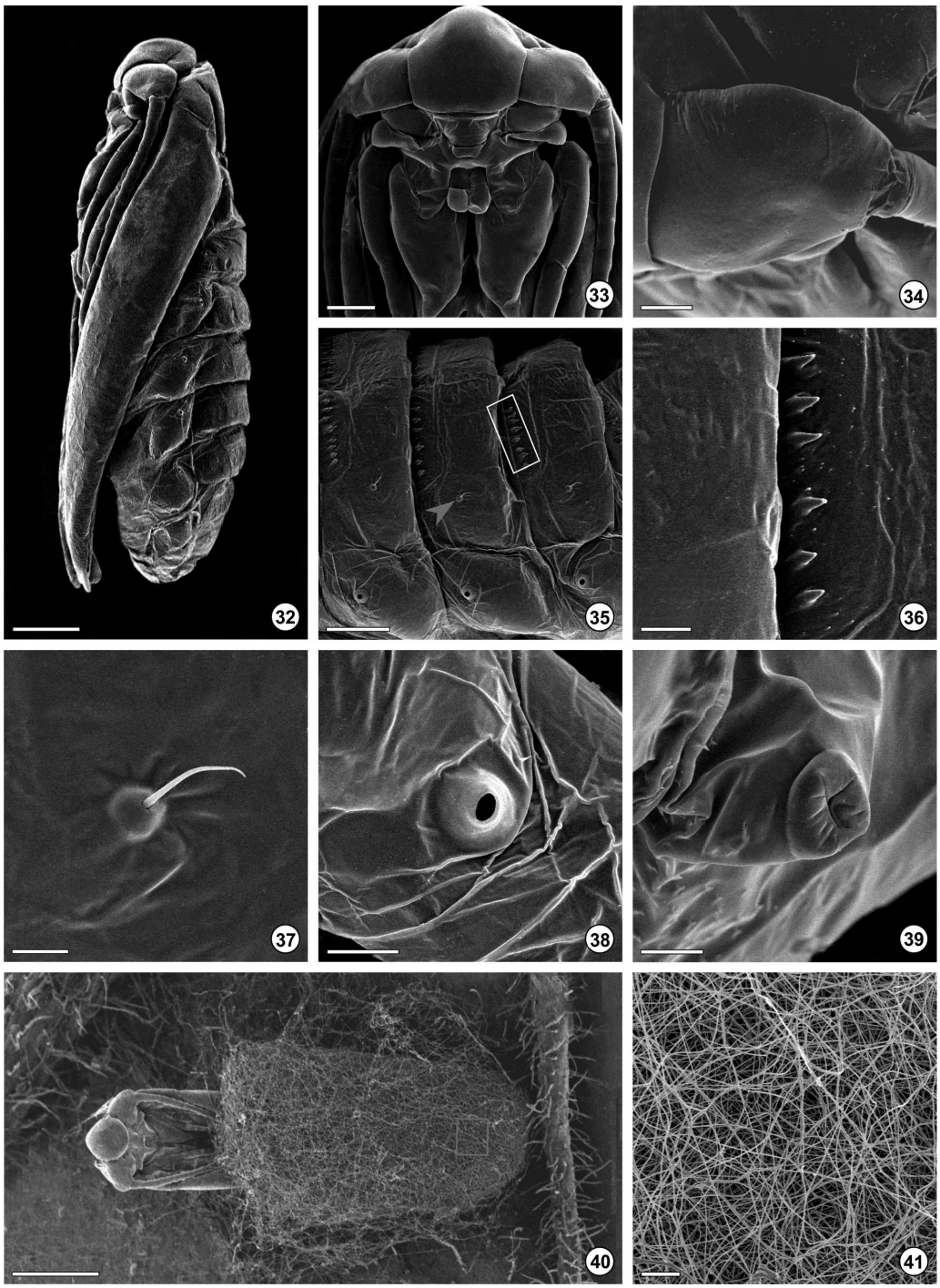
Figures 12–18. Last larval instar and pupal morphology of *Stigmella schinivora* under light microscopy: (12, 16) larva general, dorsal and ventral views, respectively; (13, 14) detail of tergal and sternal prothoracic plates seen through transparency, dorsal and ventral views, respectively; (15) anal rods of last abdominal segment, dorsal. (17, 18) pupa, dorsal and ventral, respectively. Scale bars: 300, 150, 150, 150, 300, 200, and 200 µm, respectively.

neret (Figs 20, 22), with a pair of setae on proximal base; labial palpi unisegmented, bearing a distal seta. Antenna unisegmented, with six apical sensilla; two minute in size, two stout and rounded, and two long and filiform (Fig. 23). A single, circular stemma, posterior to the antenna (Fig. 20). Thorax and abdomen cylindrical, creamy white in preserved material, bearing well-developed filiform setae. Thorax with integument smooth on T1 and sculptured with microtrichia ventrally in T2,3. T1 bearing a light brown shield on the tergum, divided into two elongated, meso-longitudinally arranged plates (Figs 12, 13); a light brown, cup-shaped plate on center of ventral prothoracic sternum (Figs 14, 16). A pair of lateral spiracles without elevated peritreme laterally on prothorax; legs and ambulatory calli absent. T2,3: Dorsal surface smooth; well-developed ambulatory calli ventrally, with the base wider than the transversally rounded apex, bearing an invagination on middle of the posterior wall (Figs 16, 24, 25). Each callus has the base sculptured with microtrichia and the distal edge smooth (Figs 24, 25). Abdominal segments similar in size from A1 to 8; A9 narrower; A10 smaller and subtriangular in shape. Integument mostly smooth on A1 and A10; partially sculptured with microtrichia dorsally on A3-8, laterally on A8-10 and ventrally on A2-9. Spiracles circular, without elevated peritreme (Fig. 27), laterally, from A1 to 8. Pairs of ambulatory calli present on A2-7, differing from the thoracic ones mainly by not having invagination (Fig. 26). A10 smooth, with a pair of light brown, longitudinally arranged, distally converging anal rods that are seen by transparency (Fig. 15); two pairs of triangular-shaped projections, one ventrolaterally (Fig. 31), the other on the distal edge of the segment (Figs 29, 31).

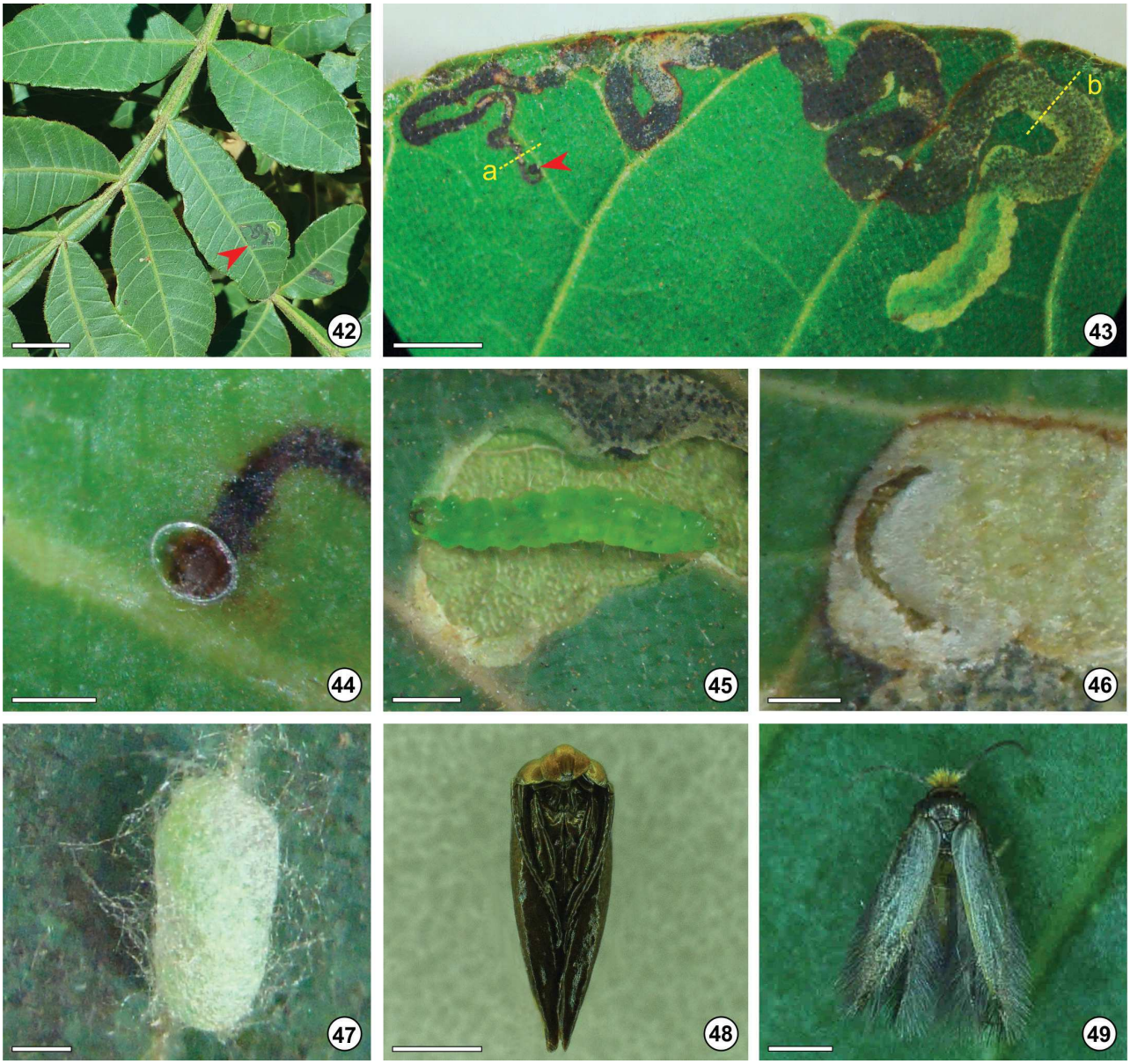
Chaetotaxy of the last instar larva. Head: Most of the setae are absent. Anterior group (A) and stemmatal group (S) unisetose. Substemmatal group (SS) bisetose. Thorax: T1 with thirteen pairs of setae. Dorsal group (D) bisetose; D1 near the lateral margin of the dorsal plate, D2 between D1 and spiracle. Extra dorsal group (XD) bisetose. Subdorsal group (SD) bisetose. Lateral group (L) trisetose; L1 and L3 ventral to spiracle, L3 between spiracle and L1, and L2 anterior to the spiracle, ventral to SD1. Subventral group (SV) trisetose; SV1 near the ventral plate, SV2 near the head capsule, and SV3 between SV1 and SV2. Ventral group (V) unisetose in the ventral plate margin. T2-3 with ten pairs of setae. Dorsal group (D) unisetose. Subdorsal group (SD) and lateral group (L) bisetose; L2 half of the length of L1. Subventral group (SV) trisetose; SV1 and SV3 in the callus, SV2 lateral to the callus. Ventral group (V) bisetose; V1 and V2 on the callus. Abdomen: A1–8 with six pairs of setae. Dorsal group (D) unisetose. Subdorsal group (SD) bisetose; SD1 between D2 and spiracle, SD2 near and anterior to spiracle. Lateral group (L) unisetose; L1 posteroventral to the spiracle. Subventral group (SV) unisetose. Ventral group (V) unisetose, near the ventral medial line. A9 with chaetotaxy similar to the



Figures 19–31. Last larval instar of *Stigmella schinivora* under scanning electron microscopy: (19) general view, lateral; (20, 21) head, lateral and dorsal views, respectively; (22) mouthparts, anterior; (23) antenna, anterior; (24, 25) mesothoracic ambulatory calli, lateral and ventral views, respectively; (26) ambulatory calli of sixth abdominal segment, ventral; (27) spiracle of eighth abdominal segment, lateral; (28) last abdominal segment, dorsal; (29) detail of tenth abdominal segment showing distal portion of anal rods (indicated by asterisk), posterior projections (indicated by closed arrow) and vestigial setae (indicated by open arrow), dorsal; (30) D2 and L1 setae, indicated by open and closed arrows, respectively, lateral; (31) last abdominal segment showing lateral projection (indicated by closed arrow) and L1 setae (indicated by open arrow). Scale bars: 200, 20, 150, 10, 10, 50, 50, 50, 5, 20, 20, 20, and 20 µm, respectively.



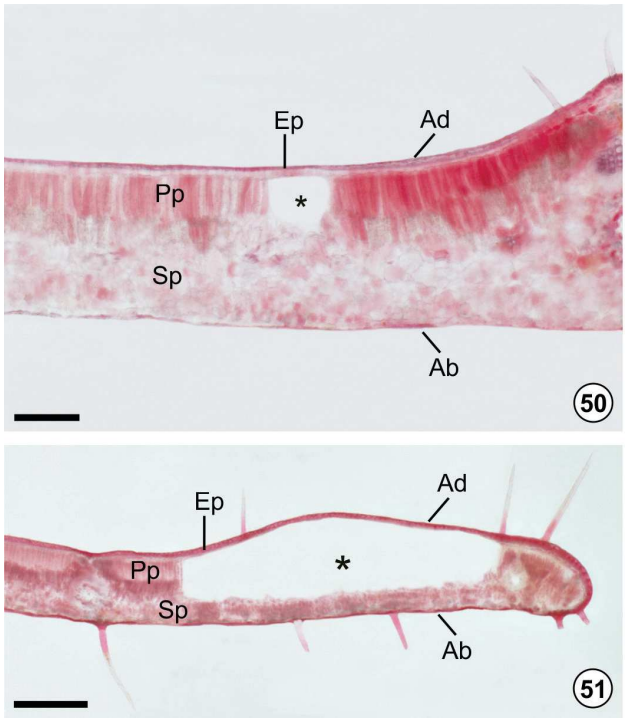
Figures 32–41. Pupa of *Stigmella schinivora* under scanning electron microscopy: (32) general view, lateral; (33) head and mouthparts, ventral; (34) eye-cap in detail, anteroventral; (35) third, fourth and fifth abdominal segments, laterodorsal; (36) detail of dorsal spines of fifth abdominal segment (indicated by rectangular area marked in Fig. 35), laterodorsal; (37) detail of fourth abdominal segment seta (indicated by arrow in Fig. 35), laterodorsal; (38) spiracles of third abdominal segment, laterodorsal; (39) spiracle (apparently closed) of eighth abdominal segment, lateral; (40) cocoon, with pupal exuvium extruded, ventral; (41) weaving pattern of the pupal cocoon surface (enlarged area marked by rectangle in Fig. 40). Scale bars: 200, 100, 40, 100, 30, 10, 20, 10, 500, and 50 µm, respectively.



Figures 42–49. Natural history of *Stigmella schinivora* on *Schinus terebinthifolius*: (42) host-plant leaf bearing leaf mine on the adaxial surface of a foliole (indicated by closed arrow); (43) general view of leaf mine on foliole, showing last instar larva seen through transparence (arrow points to empty egg, and letters indicate position of histological sectioning (treated in Figs 50, 51); (44) empty egg chorium in detail; (45) dissected mine at the final portion, showing last instar larva; (46) exit hole, used by a last instar larva to leave the mine; (47) pupal cocoon adhered to abaxial leaf surface; (48) pupa in detail, after removal of the cocoon, ventral; (49) adult on the leaf, dorsal view. Scale bars: 10, 1, 0.2, 0.5, 0.15, 0.5, 0.5, and 0.5 mm, respectively.

anterior segments, SD and SV group absent. A10 with four pairs of setae. Dorsal group (D) unisetose (represented by D2); two pairs of apparently rudimentary setae (D1 and SD2) (Figs 15, 28, 29). Lateral group (L) unisetose, represented by L1 (Figs 12, 30, 31), longer than D2.

Pupa. Average length ± standard deviation = 1.56 ± 0.03 mm, n = 5. Partially exarate, with distal portion of the wings slightly distant from the abdomen (Fig. 32). Body brownish during early development (darkening later in ontogeny) and flattened dorsoventrally (average maximum width ± standard



Figures 50–51. Transverse histological sections of *Schinus terebinthifolius* leaf (indicated by dashed lines "a" and "b" in Fig. 43), showing the organization of *Stigmella schinivora* mine during larval ontogeny. (50) First instar, initial linear section of mine (position indicated by letter "a" in Fig. 43); (51) last instar, final section of mine (position indicated by letter "b" in Fig. 43). Asterisks indicate leaf mine. (Ab) Abaxial surface of epidermis, (Ad) adaxial surface of epidermis, (Ep) epidermis, (Pp) palisade parenchyma, (Sp) spongy parenchyma. Scale bars: 0.1 and 0.2 mm, respectively.

deviation = 0.52 ± 0.007 mm, n = 5). Head vertex dome-shaped (Fig. 33), without projections or setae; front and clypeus also smooth, subtrapezoidal; labrum subtriangular and narrow; maxillae well-developed, positioned ventrally to maxillary palpi that are cuneiform and projecting mesally beyond the eyes; labial palpi short, located in between the maxillae. Antenna not reaching the apex of the mesothoracic legs (Fig. 18); scape enlarged, partially covering the eye (Fig. 34). Pronotum as a narrow stripe dorsally (Fig. 17); forewings covering the mesal portion of abdominal segments in ventral view (Fig. 18); hindwings mostly concealed by the forewings; prothoracic and mesothoracic legs visible ventrally, extending respectively to second and fourth abdominal segments; metathoracic legs mostly covered by the wings, extending to distal limit of the abdomen. Abdominal segments A3-7 with rows of posteriorly directed spines, located dorsally on the anterior margin (Figs 17, 35, 36). A pair of dorsal setae, positioned laterally, in the segments T2,3 and A1-7 (Figs 35, 37). Abdominal spiracles with elevated peritreme, opened in A1-7 (Fig. 38), partially closed in A8 (Fig. 39). Cremaster absent.

Life history. The egg is usually laid near a lateral vein on the adaxial leaf surface (Fig. 43). After eclosion the first instar larva bores into the leaf and begins to feed on the parenchyma, filling the empty egg with feces (Fig. 44). The serpentine mine is small and narrow, slowly widening throughout larval ontogeny (Figs 42, 43). From the beginning to the end of the mine, the larva feeds only on the palisade parenchyma (Figs 50, 51), which is formed by two layers of overlapping cells in the leaves of *S. terebinthifolius* (Grisi et al. 2011). The mine is filled fully with feces, which gives it a characteristic blackish appearance (Fig. 43). After completion of development, the last instar larva opens a hole (Figs 45, 46) in distal section of the mine through which it leaves, searching for a pupation site on the abaxial surface either of the same or an adjacent leaf. Cocoon yellowish, cylindrical and silk-made (Figs 40, 41, 47), having flimsy threads, some on the external surface used for attachment to the substrate. It bears a slit on anterior edge through which the pupa projects itself to the outside prior to adult emergence, leaving the exuvium partly protruded from the cocoon (Figs 40, 48, 49).

Densities of *S. schinivora* are generally low in Laranjeiras do Sul populations of *S. terebinthifolius*, and in most cases only one mine occurs per leaf and foliole. Mines with mining larvae of *S. schinivora* were collected mostly during the spring. Apparently, more than one generation occurs per year, which should be further explored.

DISCUSSION

Morphology of nepticulid eggs is still controversial, and has not yet been the subject of any detailed study (Davis 1998). According to Johansson et al. (1990), they are covered by a smooth helmet-shaped egg case, supposedly formed by secretion coming from the female colleterial glands. However, Kobayashi (1996) mentioned the existence of a chorion and presence of micropyle canals on the surface of *Stigmella castanopsiella* (Kuroko, 1978). That of *S. schinivora* is covered by a dome-shaped cap, which can be easily pulled off by physical pressure, thus being detached freely from the remaining egg contents in preserved material. Furthermore, neither micropyles nor aeropyles were found. To better resolve this question, we suggest that oogenesis should be explored in detail for *S. schinivora*, to test whether or not a true chorion is formed in this species.

The four larval instars found here for *S. schinivora* follow the general pattern recorded for nepticulids in general (Johansson et al. 1990). Although barely mentioned in the recent literature, the existence of two larval morphotypes has been known for a long time in other nepticulids, for example *Enteucha acetosae* (Stainton, 1854) (Sich 1908, 1909) and *Trifurcula immundella* (Zeller, 1939) (Sich 1917). The scarcity of setae on early instars was mentioned by van Nieukerken (2007) for *Acalyptris* Meyrick, 1921. The absence of true thoracic legs and

also of abdominal prolegs bearing chochets, but prominent thoracic and abdominal ambulatory calli instead follows the general pattern found for the last instar of nepticulids (Davis 1987). We associated the existence of these structures with a need for locomotion outside the mine in search for the place to spin the cocoon and pupate.

The prothoracic dorsal shield found in the last instar of *S. schinivora* is similar to that described for other species of *Stigmella* by Gustafsson (1985); however, the ventral prothoracic plate shows differences compared to those present in other congeneric species described by him. Thus, this structure may provide diagnosable taxonomic characters, and should be explored further regarding its use in identification of *Stigmella* species.

The dorsal sclerotized structures seen by transparency on the last abdominal segment of *S. schinivora* have received different names, such "brace rods" (Stehr 1987), "bar-like" (Gustafsson 1981) and "anal rods" (van Nieukerken 2007, van Nieukerken et al. 2011). We opted for anal rods, since given their position it is very likely that these structures are functionally related to the anus. van Nieukerken et al. (2011) reported that the anal rods may be important in the taxonomy of larvae belonging to *Acalyptris* which should also be further explored in *Stigmella*.

An interesting characteristic of the first morphotype of *S. schinivora* is the presence of a single pair of setae in the tenth abdominal segment. van Nieukerken (2007) reported the presence of similar setae, but in A8 (three pairs) in earlier instars in *Acalyptris*. The large number and size of setae present in the last instar in *S. schinivora* suggest that these structures may be important from a sensorial perspective when outside the mine prior pupation. They have probably not arisen in this instar in particular, but instead were lost in the previous ones in association with their endophytic habit.

The comparison of chaetotaxy in Nepticulidae, particularly in *Stigmella*, showed little variation, suggesting a conserved pattern. Compared to the chaetotaxy described by Gustafsson (1981) for *Stigmella auromarginella* (Richardson, 1890), *S. schinivora* has in T2-3 absence of L3; in A8 presence of L1 and absence of SV2; and in A9 presence of SV2. In comparison to the chaetotaxy described by Gustafsson (1985) for *Stigmella rhomboivora* Gustafsson, 1985, *S. schinivora* has in T2 absence of L3 and in A9 absence of SV1 and presence of L1. Gustafsson (1981) also states that SV3 is absent in *Stigmella plagicolella* (Stainton, 1854) and *Stigmella paradoxa* (Frey, 1858), whereas it is present in *S. schinivora*.

Two setae have been described in the literature for the last abdominal segment of nepticulid larvae (e.g., Gustafsson 1981, 1985), but they have not been named. The designation of D2 and L1 in this study were inferred by comparing locations of setae in previous abdominal segments. We presume the two pairs of vestigial setae found dorsally in the last abdominal segment of *S. schinivora* had not been noticed in previous studies due to their reduced size. They are herein tentatively nominated according to Hinton's system (Stehr 1987), and thus corresponding homologies should be explored further in comparison to other nepticulids.

We are not aware of scanning electron microscopy studies on the pupal morphology of Nepticulidae. The enlarged first antennal segment of *S. schinivora* stands out, associated with the eye cap in the adult, as well as the absence of any trace of a differentiated process on the head dorsum (= cocoon cutter) and a cremaster on the last abdominal segment. These absences are generally found in the family, as there is no need for the cocoon cutter and cremaster, since, as in *S. schinivora*, there is usually a slit anteriorly on nepticulid cocoons through which the pupa projects partially to the outside prior to adult emergence (van Nieukerken et al. 2004). Line drawings and description of the pupa of *S. plagicolella* provided by Patočka and Turčani (2005) are similar to those shown here for *S. schinivora*. However, these authors do not mention the existence of eight closed abdominal spiracles in *S. plagicolella*, which occurs in *S. schinivora*. Compared to other nepticulid genera such as *Trifurcula* Zeller, 1848 (van Nieukerken et al. 2004), *Roscidotoga* Hoare, 2000 (van Nieukerken et al. 2011) and *Acalyptris* (van Nieukerken 2007), differences are found in the arrangement of posteriorly directed abdominal spines in A3-7; they form only one row in the anterior margin in *S. schinivora*, contrary to what is found in these genera in which four to five lines of these spines can be found.

The leaf mine of *S. schinivora* is similar in general shape to congeneric species (e.g. van Nieukerken et al. 2006, Stonis et al. 2013, 2016, Stonis and Remeikis 2017) and to others described for different genera within the Nepticulidae (e.g. van Nieukerken et al. 2011), demonstrating a uniform pattern of the family, even though they may use different host plants. Unfortunately, we did not find other studies addressing histology of mines in Nepticulidae, which precludes comparison with results reported here. Pereira et al. (2017) demonstrated that the damage caused by the gracillariid *Leurocephala chilensis* Vargas & Moreira, 2016 to the leaves of a plant in the same genus, *Schinus molle* L., is different from that caused by *S. schinivora*. Most gracillariids show two different kinds of mandibles during development, which may be used initially for slicing and eating only the adaxial epidermis, as is the case of *L. chilensis*. Chewing mandibles, as in *S. schinivora*, appear only in latter ontogeny for that species, and are also used to eat the palisade parenchyma until the end of larval development. The different adaptations observed in these two species using closely related hostplants reflect different evolutionary patterns of the families in resource usage (Hering 1951, Menken et al. 2010, Doorenweerd et al. 2016).

Finally, it is important to emphasize that morphology of the immature stages in particular has been increasingly taken into account as an aid in species identification among leaf-miner moths, as for example among gracillariids (Davis and Wagner 2011, Kobayashi et al. 2013, Brito et al. 2017). Information on immature stage morphology is also a precondition for understanding interactions of these stages with host plants, particularly when damage on tissues and histology of the mines are explored in conjunction with ontogeny (e.g., Brito et al. 2012, 2013, Vargas et al. 2015, Pereira et al. 2017). Thus, our

results not only clarify the morphology of *S. schinivora* immature stages, but also could be used as an integrative framework for characterizing and comparing variation of immature stage morphology and associated host-plant interactions among other nepticulids and beyond.

This is the first report of *S. schinivora* in Brazil, expanding its geographical distribution that was restricted to the type locality in Argentina. *Schinus terebinthifolius* is widely distributed in southern South America (see Davis et al. 2011), and thus the range of *S. schinivora* may be much broader, and should be further explored.

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A new species of *Leurocephala* Davis & Mc Kay (Lepidoptera, Gracillariidae) from the Azapa Valley, northern Chilean Atacama Desert, with notes on life-history

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ABSTRACT

The Neotropical micromoth genus *Leurocephala* Davis & Mc Kay, 2011 (Lepidoptera, Gracillariidae) was originally described to include only the type species, *L. schinusae* Davis & Mc Kay, 2011, whose leaf miner larvae are associated with Anacardiaceae in Argentina, Brazil and Paraguay. An integrative analysis including morphology, life history and DNA barcode sequences revealed that specimens collected on *Schinus molle* L. (Anacardiaceae) in the coastal valleys of the Atacama Desert of northern Chile belong to a second species of this formerly monotypic genus. Adults of *Leurocephala chilensis* Vargas & Moreira **sp. nov.** are herein described and illustrated in association with the immature stages and life history, and corresponding phylogenetic relationships are assessed based on DNA barcode sequences. This finding provides the first record of *Leurocephala* from west of the Andes Range, expanding remarkably its geographic range. It is suggested that the extent of diversity within *Leurocephala* is much greater and that variation in geographic factors and host plant use may have modeled it, an evolutionary hypothesis that should be assessed in further studies.

Introduction

Gracillariidae is a highly diverse lineage of leaf-mining Lepidoptera, with 105 recognized genera and 1952 species distributed worldwide; over 180 taxa have been recorded in the Neotropical region (De Prins and De Prins, 2016; De Prins et al., 2016). Recent studies have suggested that the comparatively low diversity of the Neotropical fauna of Gracillariidae is an artifact due to the low sampling efforts in this geographic area; accordingly, further surveys should render many additional species (Lees et al., 2014; Brito et al., 2016).

The use in conjunction of distinct characters and methodologies to study taxonomical problems, since 2005 defined as integrative taxonomy, is the modern basis of delimitation and discovery of species (Dayrat, 2005). The usefulness of this approach has been widely recognized, especially in cases involving species with closely similar morphology (e.g., Schlick-Steiner et al., 2010; Barão et al., 2014; Kergoat et al., 2015; Kirichenko et al., 2015), a pattern probably widespread among genera of Neotropical Gracillariidae (Davis and Wagner, 2011).

The Neotropical micromoth genus *Leurocephala* Davis & Mc Kay, 2011 (Lepidoptera, Gracillariidae) originally included only the type species, *L. schinusae*, whose leaf miner larvae are associated with Anacardiaceae in Argentina, Brazil and Paraguay (Davis & McKay, 2011). Molecular phylogenetic analyses based on sequences of 21 nuclear protein-coding genes placed *Leurocephala* within the *Parectopa* group of Gracillariinae (Kawahara et al., 2011). This group of lineages is characterized by the placement of the ostium bursae on the VII sternum of the female, which is supposedly a highly distinct morphological apomorphy (Kumata et al., 1988; Kawakita et al., 2010).

Leurocephala remained as a monotypic genus until now. However, as part of a study of the Lepidoptera associated with native plants in the coastal valleys of the Atacama Desert of northern Chile, adults of *Leurocephala* were recently reared from leaf mines occurring on *Schinus molle* L. (Anacardiaceae). Thus, represents a novel record in terms of both geographic distribution and host plant use for such a micromoth genus. Furthermore, a preliminary analysis of the morphology of the male and female genitalia enabled us to hypothesize that these specimens were not conspecific with the

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type species. This hypothesis was subsequently supported by an integrative analysis of morphology, life history, and DNA barcode sequences.

Accordingly, the aim of this article is to provide descriptions of all the life stages and the life history of a new species of *Leurocephala* from the Atacama Desert. In addition, the first assessment of the phylogenetic relationships for the two species of this formerly monotypic genus is provided based on sequences of mitochondrial DNA.

Material and methods

Specimens used for description in this study were either dissected or reared from leaf mines collected on *S. molle* plants in the Azapa valley, Atacama Desert, northern Chile, between 2008 and 2016. They were brought to the entomology laboratory of the Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, where they were either dissected or reared in small plastic vials. These were maintained at room temperature and periodically inspected for emerged adults, which were pinned and dried.

Morphological analysis

Immature stages were fixed in Dietrich's fluid and preserved in 75% ethanol. For descriptions of the gross morphology, the specimens were cleared in a 10% potassium hydroxide (KOH) solution and slide-mounted in either glycerin jelly or Canada balsam.

Observations were performed with the aid of a Leica® M125 stereomicroscope, and measurements were performed using an attached ocular micrometer (precision = 0.01 mm). 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 Photo-Paint® X7, using the corresponding digitalized images as a guide. At least five specimens were used for the descriptions of each life stage or instar.

For scanning electron microscope analyses, additional specimens were dehydrated in a Bal-tec® CPD030 critical-point dryer, mounted with double-sided tape on metal stubs and coated with gold in a Bal-tec® SCD050 sputter coater. They were examined and photographed in a JEOL® JSM6060 scanning electron microscope at the Centro de Microscopia Eletrônica of Universidade Federal do Rio Grande do Sul (UFRGS).

For plant anatomical descriptions, field-collected leaf portions of *S. molle* containing mines of *Leurocephala chilensis* were fixed in FAA (37% formaldehyde, glacial acetic acid and 50% ethanol, 1:1:18, v/v), and preserved in 70% ethanol. Leaf portions containing the different larval instar morphotypes were selected under a stereomicroscope in the laboratory. They were then progressively hydrated, immersed in 10% potassium hydroxide for 20 min, stained for 24 h with toluidine blue (aqueous solution: 200 mg/L) and then mounted whole in glycerine on slides. Semi-permanent slides were also prepared with freehand cross sections cut with a razor blade, using additional mines containing larvae of different ages and prepared similarly. Head-capsule exuvia were located by transparency in the slide-mounted mines and measured under the stereomicroscope with an attached ocular micrometer.

Museum collections

Abbreviations of the institutions from which specimens were examined are as follows: IDEA, Colección Entomológica de la Universidad de Tarapacá, Arica, Chile; LMCI, Laboratório de Morfologia e Comportamento de Insetos, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil; MNNC, Museo Nacional de Historia Natural de Santiago, Santiago, Chile.

Molecular analysis

Total genomic DNA was extracted from fresh larval tissue using the PureLink kit (Life, Invitrogen, USA) following manufacturer's instructions. Specimens from the type locality of the new taxon *L. chilensis* ($n = 2$; Azapa) and of the only species recognized in the genus, *L. schinusae* ($n = 4$; Paraná and Rio Grande do Sul, Brazil) were surveyed to generate original data (Table 1). We also incorporated from the BOLD System and Genbank databases three individuals of *L. schinusae* from Misiones, Argentina. This dataset was used to assess the monophyletic status of *L. chilensis*. Gracillarid species of the *Spinivalva* Moreira & Vargas, likely the sister lineage of *Leurocephala*, and also of *Parectopa* Clemens and *Epicephala* Meyrick, all belonging to the '*Parectopa* group' (Brito et al., 2013), were used as outgroup, the corresponding sequences being downloaded from GenBank (Table 1). We amplified the DNA barcode region (part of the mitochondrial cytochrome oxidase I) including 658 base pairs, using primers and conditions described by Folmer et al. (1994). PCR products were purified using exonuclease (GE Healthcare Inc.) and Shrimp Alkaline Phosphatase (SAP), sequenced with BigDye chemistry and analyzed in an ABI3730XL (Applied Biosystems Inc.). Chromatograms obtained from the automatic sequencer were read and sequences were assembled using the software CodonCode Aligner (CodonCode Corporation). Sequences generated in this study were deposited in the databases GenBank and BOLD System (Table 1).

Corresponding tree was constructed using maximum likelihood (ML) method in the software PHYML 3.0 (Guindon et al., 2010). The program jModelTest 2 (Darriba et al., 2012) was used to estimate the substitution model (General Time-Reversible; Rodriguez et al., 1990), following the Akaike Information Criterion. Monophyly confidence limits were assessed with the bootstrap method (Felsenstein, 1985) at 50% cut-off after 1000 bootstrap iterations. We also analyzed the pairwise genetic distance using the Kimura 2-parameter model (Kimura, 1980) procedure, with 1000 bootstrap replications, between clusters defined in the phylogeny and outgroups.

Results

Leurocephala chilensis Vargas & Moreira **sp. nov.**

Type material. *Male holotype*: Azapa, Arica, Chile, August 2015, ex leaf miner larva on *S. molle*, July 2015, H.A. Vargas coll. (MNNC).

Paratypes: Two males, two females, same data as holotype (MNNC); two males and three females, same data as holotype (IDEA); one male and one female Azapa, Arica, Chile, December 2010, ex leaf miner larva on *S. molle*, July 2010, H.A. Vargas coll. (IDEA); three males Azapa, Arica, Chile, November 2011, ex leaf miner larva on *S. molle*, October 2011, H.A. Vargas coll (IDEA).

Genitalia dissected by H.A. Vargas (HAV) were deposited in IDEA, under accession numbers as follows, all from Azapa, Arica, Chile, ex leaf miner larva on *S. molle*: HAV276, one male, December 2010; HAV358, 359 and 402, three males, November 2011; HAV108, one female, December 2010; HAV1020 and 1024, two males, August 2015; HAV1021 and 1023, two females, August 2015. H.A. Vargas coll. Immature specimens of *L. chilensis* were deposited in LMCI, dissected from leaf mines on *S. molle* from Azapa, Arica, Chile, August 2012, H.A. Vargas and G.R.P. Moreira coll. as follows: preserved in 100% alcohol below −10 °C, used for DNA extraction (LMCI 191-3); preserved in 75% alcohol, used for microscopy studies, seven eggs (LMCI 191-37), six first instar larvae (LMCI 191-41), twelve last instar larvae (LMCI 43), seven pupae (LMCI 191-44).

Table 1
Gracillarid specimens used in this study to reconstruct the phylogenetic status and evolutionary relationships of *Leurocephala chilensis* based on 658 base pairs of DNA barcode (cytochrome oxidase subunit I gene) sequences.

Group	Genus	Species	Voucher	Locality	Accession number	
					Genbank	BOLD system
Ingroup						
	Leurocephala					
		chilensis	LMCI 191-3-1	Chile, Azapa	KY006921	MISA007-16.COI-5
			LMCI 191-3-5	Chile, Azapa	KY006922	MISA008-16.COI-5
		schinusae				
			DDAV-D546	Argentina, Misiones	HM382092	RDOPO 384-10.COI-5
			DDAV-D547	Argentina, Misiones	HM382093	RDOPO 385-10.COI-5
			DDAV-D576	Argentina, Misiones	HM382112	RDOPO 414-10.COI-5
			LMCI 295-19B	Brazil, Rio Grande do Sul	KY006923	MISA009-16.COI-5
			LMCI 295-19C	Brazil, Rio Grande do Sul	KY006924	MISA010-16.COI-5
			LMCI 309-01-10A	Brazil, Paraná	KY006925	MISA011-16.COI-5
			LMCI 309-01-10B	Brazil, Paraná	KY006926	MISA012-16.COI-5
Outgroup						
	Spinivalva					
		gaucha	LMCI 164-15	–	KC512112	GBGL13506-14.COI-5P
		sp.	LMCI 169-A1	–	KC512114	GBGL13508-14.COI-5P
	Parectopa	*ononidis*	CLV2269	–	KP845416	GRSLO654-11.COI-5P
	Epicephala	sp.	E312AK	–	FJ235388	–

Specimens of *L. schinusae* used for comparison were either dissected (immatures) or reared (adults) from leaf mines collected on *Schinus terebinthifolius* by C. M. Pereira (CMP) in Laranjeiras do Sul, PR, Brazil, as follows: LCMI 309-1, five larvae preserved in 100% alcohol below −10 °C, used for DNA extraction, 25.VII.2015; LMCI 309-3, one female, preserved in 70% alcohol, with genitalia in slide preparation (CMP 001-16F), 07.XI.2015; LMCI 309-4, one male, pinned, with genitalia in slide (CMP 001-18M), 07.XI.2015; LMCI 309-5, one male, in 75% alcohol, with genitalia in slide (CMP-22M), 16.VI.2016; LMCI 309-6, one female, pinned, with genitalia in slide (CMP-31F), 16.VI.2016. Also, five last instar larvae, preserved in 100% alcohol below −10 °C, used for DNA extraction, dissected from leaf mines on *Schinus* aff. *polygamus*, Coxilha das Lombas, Santo Antonio da Patrulha, Rio Grande do Sul, Brazil, April 2015, G.R.P. Moreira & S. L. Bordignon coll. (LMCI 295-19).

Diagnosis

Despite their morphological and life history resemblance, the two species of *Leurocephala* can be differentiated based on morphology of the adult and larval stages, and by the shape of the mine. The apex of the sacculus of male genitalia in *L. chilensis* is provided with a short, spine-like process that projects upwards, which is absent in *L. schinusae*; the dorsal surface of the aedeagus of *L. chilensis* is sculptured with several small tooth-like projections on the concave area, which are absent in *L. schinusae*. In the female genitalia, two signa are found on the corpus bursae of *L. chilensis*, while only one signum is found in *L. schinusae*; furthermore, the horn-like lateral extensions of the antrum are laterally projected in *L. chilensis*, while these structures are apically projected in *L. schinusae* At the larval stage, the ventral plate of the prothorax of the last instar of *L. schinusae* is uniformly sculptured by a great number of granular projections of similar size, about 12 of which are at the posterior margin of the plate, almost touching laterally, while in *L. chilensis* the greatest granular projections, almost 1.5 times the length of the smallest ones, are restricted to the posterior third of the ventral plate, with only four at the posterior margin, clearly separated from each another by a distance similar to the diameter of the respective projection. The serpentine mine constructed by the second instar of *L. schinusae* has a little blotch-like broadening a short distance from the empty chorion, while broadening is absent in the serpentine mine of the second instar of *L. chilensis*.

Description

Adult (Fig. 1)

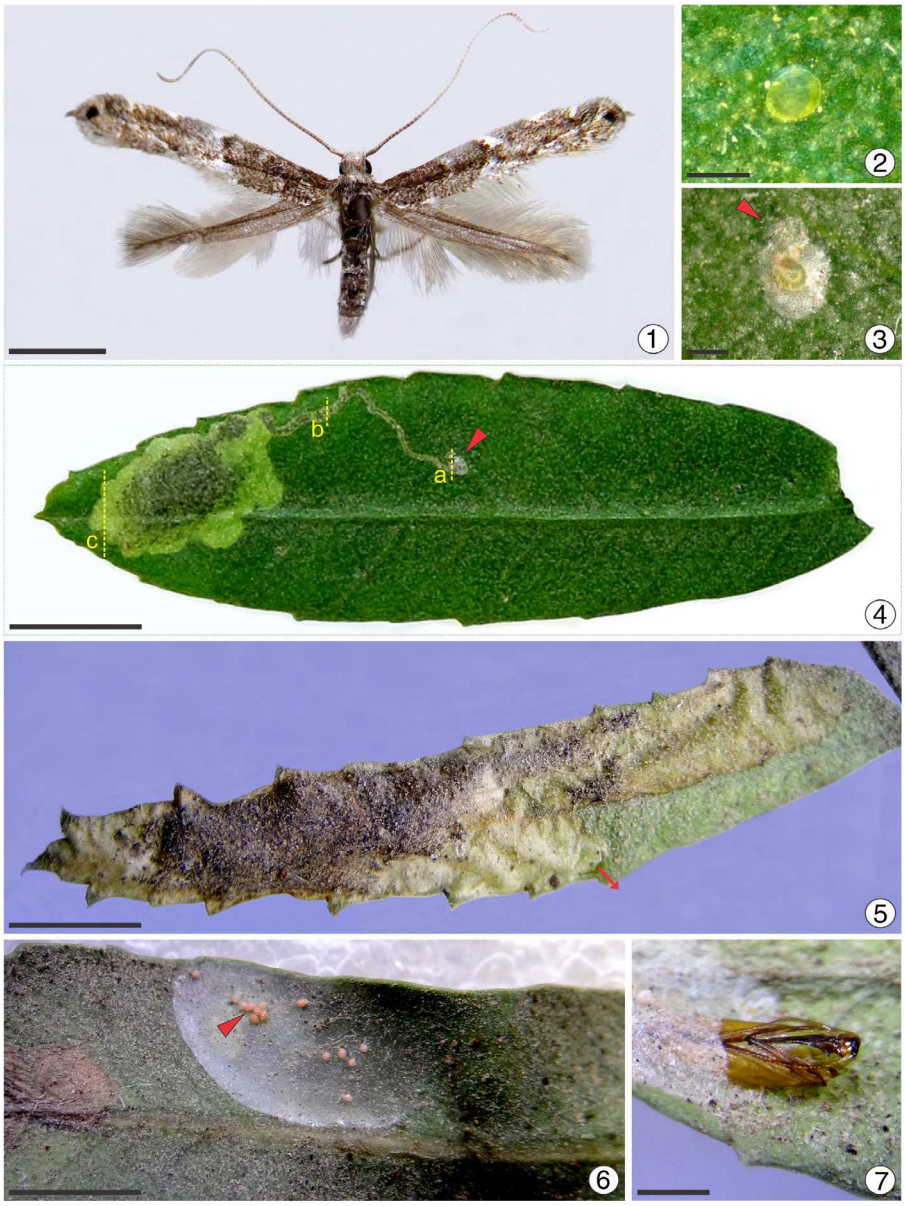
Male. Head. Front mostly whitish gray with brownish gray spots close to the compound eyes; vertex whitish gray; maxillary palpus whitish gray; labial palpus mostly whitish gray, second segment with brownish-gray spots distally; proboscis short, naked; antenna filiform, slightly shorter than forewing; scape elongated, brownish gray dorsally, whitish gray ventrally; with two narrow longitudinal stripes on the medial surface, one brownish gray in contact with the ventral area, the other whitish gray in contact with the dorsal area; pedicel and two first flagellomeres with coloration similar to the scape, remaining flagellomeres brownish gray.

Thorax. Mostly brownish gray dorsally with a few whitish gray scales; tegula dorsally brownish, ventrally whitish gray with a tuft of long scales at tip; lateral and ventral surfaces whitish gray. Foreleg mostly whitish gray, medial surface of femur and tibia brownish gray, tibial epiphysis whitish gray, a brownish gray ring at base of each tarsomere. Middle leg mostly whitish gray, tibia with two brownish gray rings, tibial spurs whitish gray, tarsomeres similar to those of the foreleg. Hindleg mostly whitish gray, a brownish gray ring at base of the femur, two brownish gray rings on the tibia, proximal tibial spurs brownish gray, distal tibial spurs mostly whitish gray with a brownish gray ring at middle, long whitish gray hair-like scales on the anterior and posterior surface of the tibia, tarsomeres whitish gray.

Forewing. Length: 4.0–4.5 mm ($n = 10$). Mostly brownish gray; a distinctive white transverse stripe arises in the middle of the coastal and hind margins, slightly projected apically at the longitudinal axis of the wing, sometimes interrupted by ground color scales; a short oblique, apically projected stripe arises from 3/4 of the hind margin reaching the longitudinal axis of the wing; a distinctive blackish gray dot subapically; fringe around apex short, concolor with the wing, a small apical tuft of plain scales; fringe on hind margin with long hair-like brownish gray scales. Venation as described by Davis et al. (2011) for *L. schinusae*.

Hindwing. Length: 3.2–3.4 mm ($n = 10$). Uniformly brownish gray with concolorous fringe of long hair-like scales. Venation as described by Davis et al. (2011) for *L. schinusae*.

Abdomen. Mostly brownish gray dorsally, with oblique segmental stripes of whitish gray ventrally until segment VI, completely whitish gray ventrally at apex. Segment VII with tergum and sternum reduced to fine transversal stripes. Segment VIII with sternum as a hood-like slightly sclerotized plate; sternum VIII as a slightly



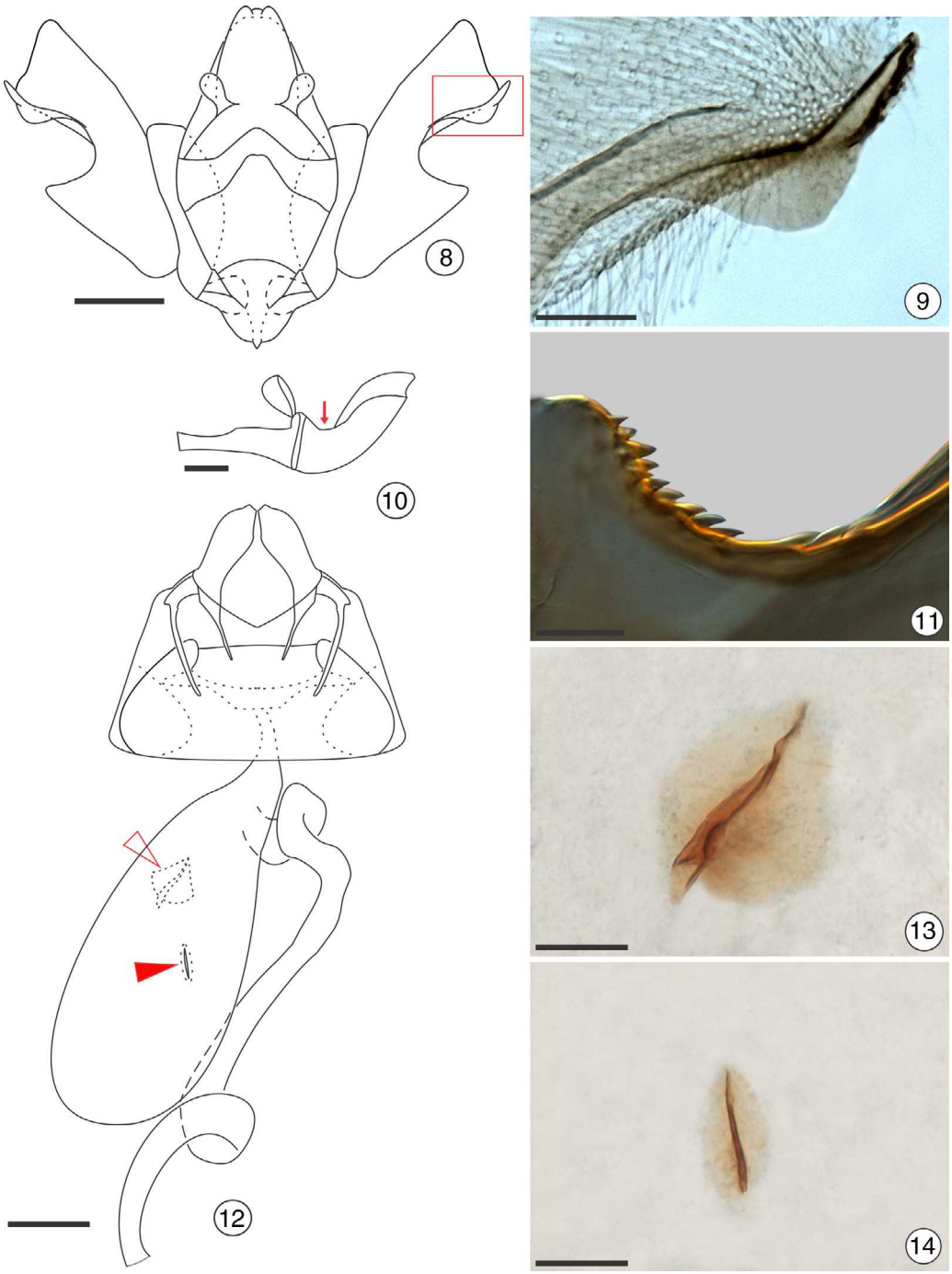
Figs. 1–7. Adult and life-history of *Leurocephala chilensis* on the abaxial surface of *Schinus molle* leaves: (1) pinned-dried male, dorsal view; (2) egg; (3) freshly hatched first instar larva, seen by transparence within the mine (empty chorium is indicated by arrow aside); (4) middle age mine (arrow indicates the egg-chorium at the beginning of the mine; letters and associated dashed-lines correspond to the locations of tissue sections, presented in transversal view in Figs. 33–35); (5) old, empty mine (seta indicates exit of last instar larva); (6) pupal cocoon, ornamented with bubbles (arrow); (7) pupal exuvium, partially protruding from the cocoon. Scale bars = 1, 0.5, 0.5, 5, 5, 3 and 1 mm, respectively.

sclerotized fine transversal stripe. Membranous area between segment VII and VIII laterally with a pair of hair-like coremata at the apex of a finger-like mostly membranose lobe provided with a rod-like sclerite. Segment VIII with a second pair of hair-like coremata on the lateral apex of the sternum.

Male genitalia (Figs. 8–11). Uncus absent. Tegumen as two fine stripes touching dorsally. Saccus U-shaped in posterior view, ventral area with the anterior margin slightly projected forward, posterior margin slightly convex. Gnathos (Fig. 8) as two short, slightly sclerotized finger-like lobes. Valva (Fig. 8) broadly joined basally to the posterior margin of the lateral arms of the saccus; costal margin straight; cucullus mostly membranous, ventral margin parallel to the costa; sacculus well sclerotized, basal part broad, triangle-like, delimited by a broad concavity on the ventral margin, with distal part straight, down-curved, slightly dilated subapically, bearing a short spine-like projection at apex (Fig. 9). Transtilla as a slightly sclerotized transversal band joining the base of the costal margin of the right and left valvae. Juxta absent. Aedeagus (Fig. 10) a bit shorter than valva, with insertion of the ductus ejaculatorius dorsally, close to the middle; basal half forward directed, tip blunt, diameter increasing toward the middle; distal half upcurved, with lateral sides slightly asymmetrical at apex, dorsal surface sculptured by several small tooth-like projections on the concave portion (Fig. 11). A small sclerite joined dorsally on the middle of the aedeagus. Cornuti absent.

Female. Similar to male in size and color.

Female genitalia (Figs. 12–14). Anterior and posterior apophyses well sclerotized, with length similar to the sternum VII. Ostium bursae broad, covering completely the posterior margin of the sternum VII. Antrum broad, sclerotized, trapezoid-like in ventral view, length about half of the sternum VII, cephalic side about half of the posterior side, with two laterally directed horn-like extensions



Figs. 8–14. Genitalia morphology of *Leurocephala chilensis* under light microscopy: (8) male, posterior view; (9) distal end of valva, in detail (enlarged, rectangular area marked in Fig. 8); (10) aedeagus, lateral view; (11) dorsal spines of aedeagus, in detail (enlarged area indicated by seta in Fig. 10); (12) female, ventral view; (13, 14) signa, in detail (indicated by open and closed arrows in Fig. 12). Scale bars = 200, 50, 100, 20, 200, 50 and 50 μm, respectively.

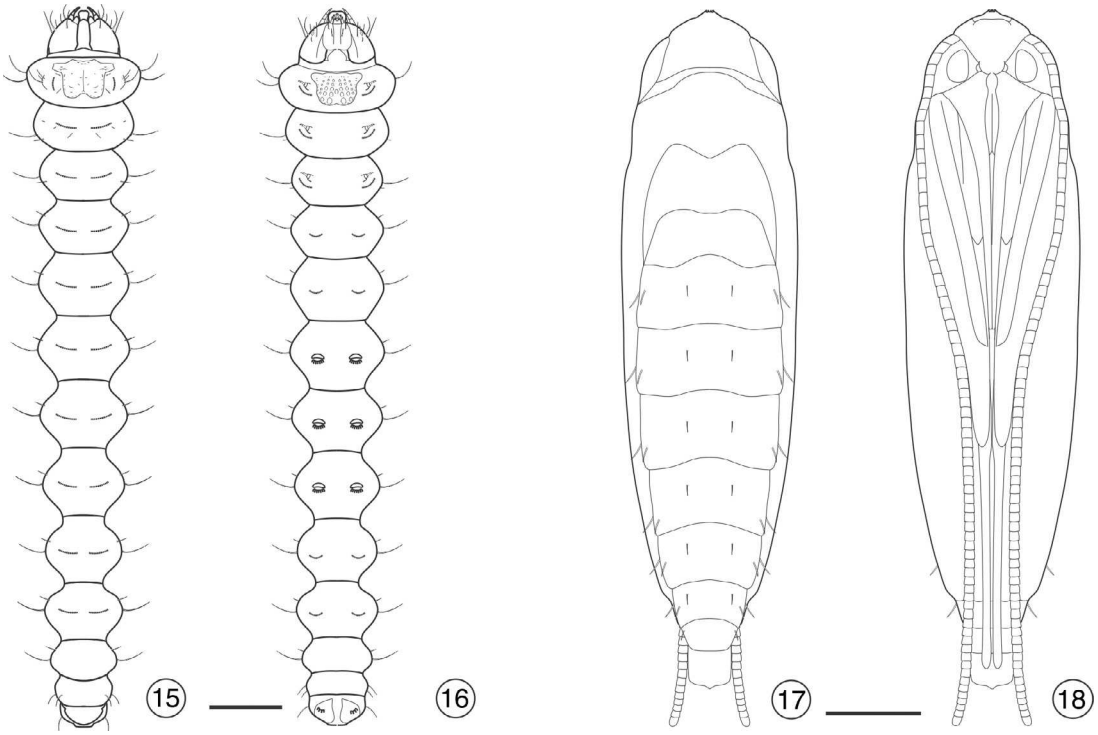
on the middle of the lateral sides. Ductus bursae membranous, with similar length to the antrum. Corpus bursae elliptical, elongated, mostly membranous, with two signa (Fig. 12); the larger (Fig. 13) one on the ventral surface, triangle-like, with a narrow sclerotized longitudinal stripe with the cephalic tip projected forward as a short spine into the corpus bursae; the smaller (Fig. 14) one on the dorsal surface, elliptical, elongated, length about 3/4 of the larger, with a longitudinal sclerotized stripe. Ductus seminalis basally inserted on the ventral surface of the corpus bursae (Fig. 12).

Egg (Fig. 2).

Round and flat, with a translucent chorion, allowing by transparence visualization of the embryo under development within.

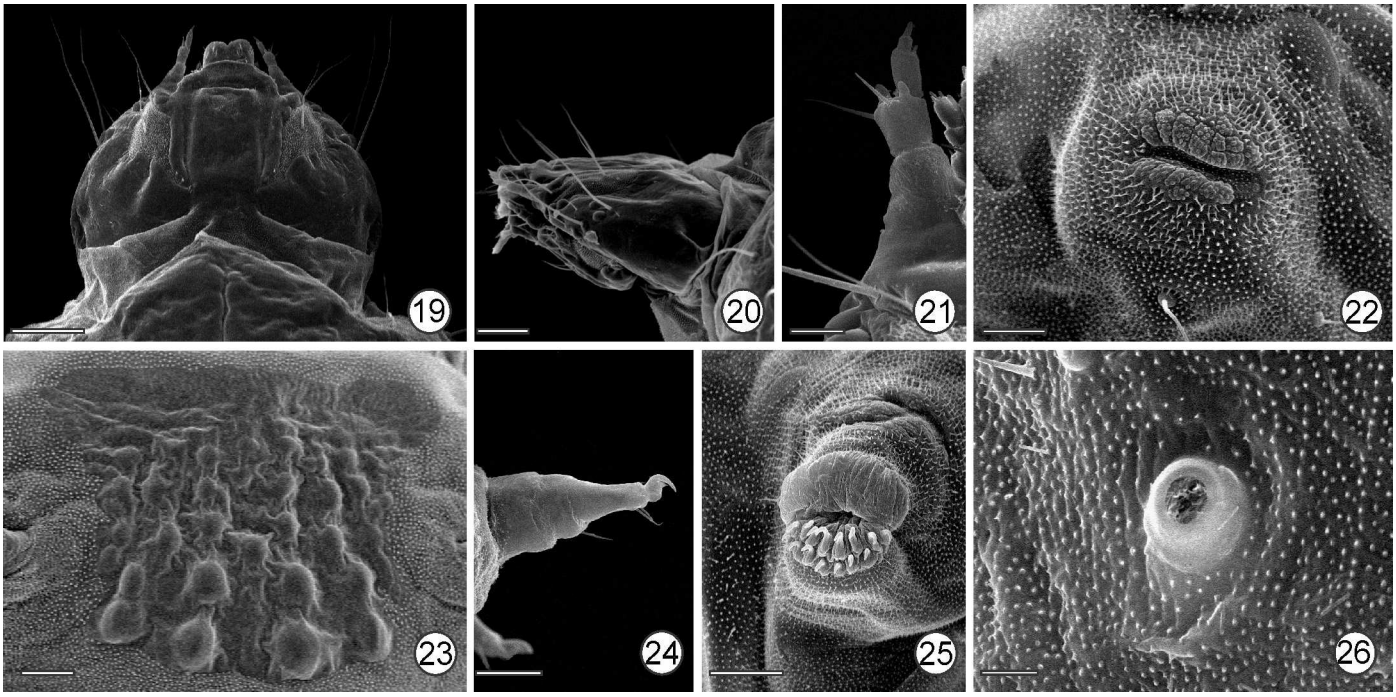
Larva (Figs. 15, 16, 19–26).

There are three morphotypes and five intars, which are similar in morphology to those described by Davis et al. (2011) for the type species, except for the last instar described below. The first instar is of a "sap-feeding" type, having mandibles modified for slicing the epidermis cells, differing from the remaining four instars that are tissue-feeders, which have mandibles used for chewing the leaf parenchyma. These can be identified by measuring their head capsule width, which do not overlap in size (Table 2). Corresponding exponential growth curve for the four tissue-feeding instars of *L. chilenis* reared on *L. molle* was: $y = 0.0494e^{0.4787x}$; $n = 50$; $r = 0.98$; $p < 0.0001$.



Figs. 15–18. Last instar (15, 16) and pupal (17–18) morphologies of *Leurocephala chilensis* under light microscopy, in dorsal and ventral views, respectively. Scale bars = 0.5 mm.

Last instar (Figs. 15, 16, 19–26). Maximum body length = 5.5 mm (n = 10). Head (Figs. 15, 16, 19, 20). Brown, semicircular in dorsal view, vertex partially covered by the prothorax, slightly depressed dorsoventrally, mostly smooth with a rhomboid-like area covered by short spine-like microtrichia between the frontoclypeous and the stemmata; epicranial notch U-shaped, broad and deep; frontoclypeous rectangle-like, about two times longer than wide; six circular stemmata, with stemmata 1 and 2 close to seta A3, stemmata 3–5 in a diagonal line ventral to seta A1, stemma 5 slightly displaced to the ventral surface of the head, stemma 6 isolated on the ventral surface of the head, almost equidistant to setae S2 and SS2. Antenna three-segmented; first segment annular, second segment cylindrical, about two times longer than the first segment, with sensillae distally, and third segment cylindrical, similar in



Figs. 19–26. Last larval instar of *Leurocephala chilensis* under scanning electron microscopy: (19–20) head, under dorsal and lateral views, respectively; (21) antenna, dorsal; (22) callum of first abdominal segment, ventral; (23) sternal prothoracic plate, ventral; (24) mesothoracic leg, lateral; (25) pseudopodium of fifth abdominal segment, ventral; (26) spiracle of eighth abdominal segment, lateral. Scale bars = 100, 50, 20, 50, 25, 50, 50 and 10 μm, respectively.

Table 2
Variation in size of head capsule width among instars of *Leurocephala chilensis* reared on *Schinus molle* ($n = 10$ per instar).

Instar	Head capsule width (mm)		
	Mean ± standard error	Range	Growth rate
I	0.127 ± 0.026	0.117–0.143	–
II	0.127 ± 0.003	0.117–0.143	–
III	0.211 ± 0.009	0.182–0.260	1.661
IV	0.340 ± 0.007	0.312–0.377	1.611
V	0.534 ± 0.005	0.507–0.559	1.571

length to second segment, about a half the diameter the second segment, with sensillae at apex. Mouthparts of the chewing type; labrum bilobed, four short hair-like setae on the external surface; epipharyngeal spines close to the distal margin of the labrum, one pair of plain epipharyngeal sclerites close to the each group of epipharingeal spines; mandible well-developed, with five distal cusps; maxilla with well-differentiated galea and palpus; labium with a well-developed cylindrical spinneret at apex and a pair of bi-segmented palpi laterally to the spinneret; hypopharynx provided with long hair-like projections. Chaetotaxy. AF group bisetose, AF1 and AF2 as microsetae close to the dorso-median apex of the patch of microtrichiae. A group bisetose, A1 close to antenna, A3 dorsal to stemma, about two times the length of A1. CD group of microsetae trisetose. C group unisetose, C1 as a microseta. F group of setae absent, Fa pore present. L group unisetose, L1 as a short hair-like seta posteroventral to A3. MG group of microsetae bisetose. P group bisetose, P1 at middle of the patch of microtrichiae, size similar to A1, P2 greatly reduced, slightly greatest CD setae. S group bisetose, S1 about halfway between stemmata 1 and 3, S2 about halfway between stemmata 1 and 6. SS group trisetose, SS1 ventromedial to stemma 5, SS2 about halfway between stemmata 4 and 6, SS3 posteromedial to stemma 6. Thorax and abdomen sculptured by short spine-like microtrichiae.

Thorax (Figs. 15, 16, 22–24). Prothorax. Dorsal shield grayish brown, smooth, in the form of two subtriangular plates separated medially by a narrow membranous stripe; each plate with the anterior margin slightly convex, medial margin straight, lateral margin widely concave close to the anterior margin and almost parallel to the medial margin on the distal 2/3, posterior vertex widely rounded. An ellipsoid shield postero-ventral to SV group. Ventral shield square-like, lateral sides slightly concave close to the anterior margin, posterior margin slightly convex; sculptured by granular projections variable in size, the largest restricted to the posterior third of the plate, clearly separated from each other by a distance similar to the diameter of the respective projection. A circular spiracle with slightly elevated peritrema laterally close to the posterior margin of the segment. A longitudinally oriented callus-like structure between the lateral margin of the dorsal shield and the SV group; another callus-like structure postero-lateral to the coxa. Chaetotaxy: D group bisetose, D1 greatly reduced, on the posterior half of the dorsal shield close to the lateral margin, D2 about three times the length of D1 between the dorsal shield and the callus-like structure. XD group bisetose, XD1 anterior to D2, XD2 ventral to XD1. SD group bisetose, lateroventral to the callus-like structure, SD1 similar in size to D2, SD2 about 4–5 times longer SD1. L group bisetose, similar to SD2 in size, clearly anterior to the spiracle. SV group bisetose, antero-dorsal to the ellipsoid shield. V group unisetose, V1 between the coxa and the ventral shield.

Meso and metathorax without dorsal, lateral or ventral shields. A transversally oriented callus-like structure anterior to D2; another callus-like structure postero-lateral to the coxa. Chaetotaxy: D group bisetose, D1 anterior and D2 posterior to the callus-like structure. SD group bisetose, ventro-lateral to the callus-like structure. L group bisetose. SV and V groups unisetose. Legs moderately well developed, bearing large tarsal claws.

Abdomen (Figs. 15, 16, 25, 26). All segments bearing dorsal and ventral smooth shields varying in shape. Dorsal shield of A1–6 in the form of a small irregular plate, those of A7 and A8 little developed, in the form of a small dot; dorsal shield of A9 ellipsoidal, well-developed, transversally arranged; dorsal shield of A10 in the form of two widely separated plates close to the posterior margin of the segment. Ventral shield of A1–2 and A8 circle-like, little evident on A3–5, between the respective prolegs; those of A6–7 similar in size and shape to those of A3–5; ellipsoidal on A9, transversally arranged, smaller than the dorsal shield of the same segment; absent on A10. Chaetotaxy: A1–2, 6–7: D group bisetose, D1 anterior and D2 posterior to the callus-like structure. SD group bisetose, SD1 latero-ventral to the callus-like structure, SD2 greatly reduced, dorsal to the spiracle. L group unisetose, L1 postero-ventral to the spiracle. SV bisetose, dorso-lateral to the callus like structure. V group unisetose, V1 between the callus-like structure and the ventral shield. A3–5: similar to the preceding segments; SV dorso-lateral to the proleg. A8 similar to preceeding segment, except that SV group unisetose. A9 similar to preceeding segment, except that SD group unisetose. A10 with D and SD groups bisetose; D1 anterior and D2 posterior to the dorsal shield, SD1, SD2 on the margin of the lateral part of the dorsal shield; L, SV and V groups unisetose. Spiracles round, with moderately elevated peritreme. Prolegs present on A3–5 and A10; crochets arranged in a staggered caudal varying from 10 to 16 hooks on A3–5 (Fig. 25); A10 with crochets reduced to 4 hooks.

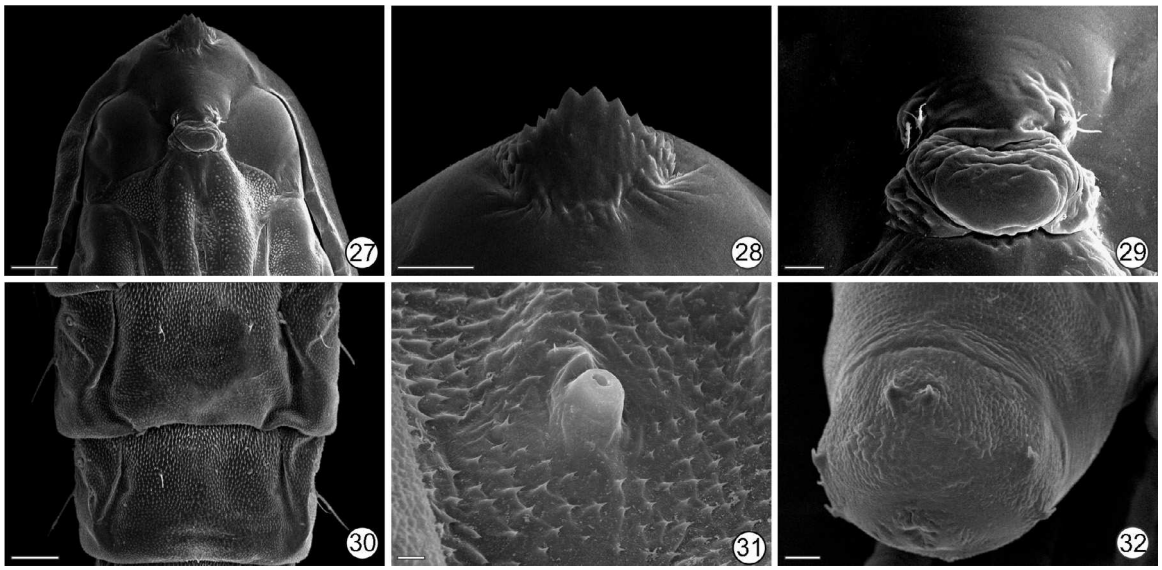
Pupa (Figs. 17, 18, 27–32).

Maximum body length: 4 mm ($n = 10$). Similar in color and general appearance to *L. schinusae*, as described by Davis et al. (2011) for *L. schinusae*. Minutely and densely spinose, particularly the dorsal abdomen. Cocoon cutter subtriangular, with outer ridge having numerous minute teeth, the central three teeth the largest. Antennae long, surpassing the abdomen in length. Labial palpi ca. 1/3 the length of proleg. Proboscis as long as the proleg. Forewings narrow, well separated, extending to abdominal segment A7. Hindlegs extending to abdominal segment 9 + 10. Setae D1, SD1, and L1 present on A1–7; only SD1 present on A8. Abdominal spiracles round, with elevated peritreme (Fig. 31). Cremaster formed by three pairs of slightly curved spines, two lateral and one dorsal (Fig. 32).

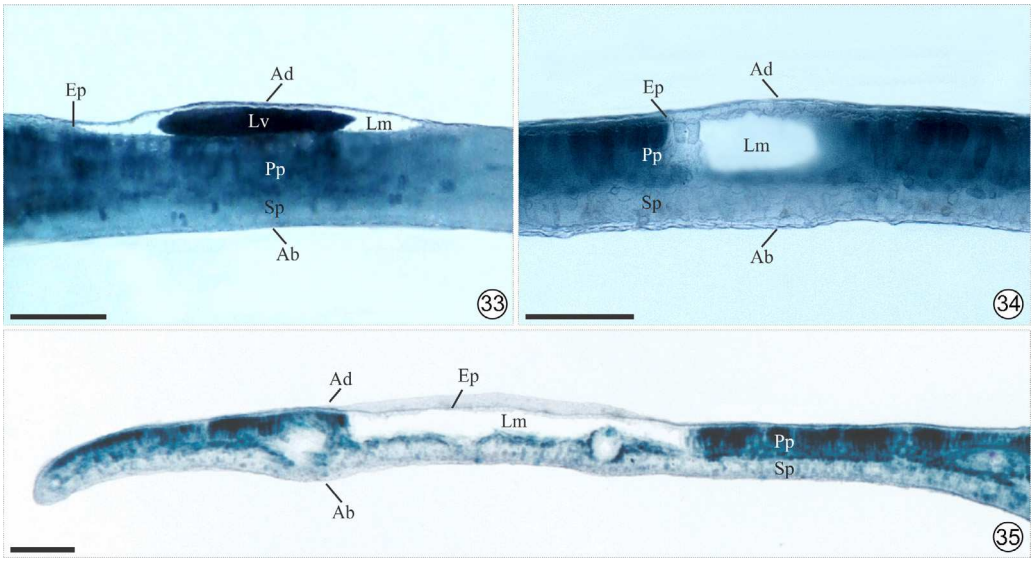
Etymology. The specific epithet is derived from the country of the type locality.

Distribution. *L. chilensis* is known only from Azapa (type locality) Valley in the Atacama Desert of northern Chile.

Life history (Figs. 2–7). Eggs are laid individually (Fig. 2) on the adaxial surface of the leaflet, mostly close to the main leaflet vein. After hatching, the first, sap-feeder instar introduces itself into the leaflet, constructing a small, superficial, blotch-like mine a short distance from the empty chorion (Fig. 3). The feces are deposited into the lumen of the chorion during the time that the anal apex of the first instar remains there. The second instar constructs a narrow serpentine mine (Fig. 4) on the adaxial surface of the leaflet. Subsequent instars construct a conspicuous blotched mine on the adaxial surface whose diameter increases with the sequence of the instars, generally covering more than 50% of the leaflet when fully



Figs. 27–32. Pupa of *Leurocephala chilensis* under scanning electron microscopy: (27) head, ventral view; (28) "cocoon-cutter" in detail, ventral; (29) labrum in detail, ventral; (30) sixth and seventh abdominal segments, dorsal, (31) spiracle of eighth abdominal segment, lateral; (32) last abdominal segments, dorso-posterior. Scale bars = 100, 25, 100, 100, 5 and 100 μm, respectively.



Figs. 33–35. Variation in transversal histological sections of *Leurocephala chilensis* on *Schinus molle* leaf, according to larval ontogeny; (33) first, *sapfeeding* instar (position indicated by letter "a" in Fig. 4); (34) early *tissue-feeding* instar (letter "b" in Fig. 4); (35) last *tissue-feeding* instar (letter "c" in Fig. 4). Ab, abaxial surface of epidermis; Ad, adaxial surface of epidermis; Lm, leaf mine; Ep, epidermis; Pp, palisade parenchyma; Sp, spongy parenchyma. Scale bars = 0.2 mm.

developed (Fig. 5). A large number of feces are glued on the internal side of the "epidermal" surface of the blotch mine. The fully developed fifth instar makes a short slit on the margin of the mine to exit from it to search for a site for pupation. Pupation mostly occurs on the abaxial surface of a leaflet of the same plant; previously the fifth instar constructs an ellipsoidal smooth cocoon, generally with one of the lateral margins touching the lateral margin of the leaflet, and externally deposits silk bubbles (Fig. 6) secreted by the anus. When metamorphosis is completed, the pupa makes a slit on the cocoon using the cephalic cocoon-cutter to enable the adult to emerge, after which the pupal exuvium typically appears protruded with the posterior body portion remaining in the cocoon (Fig. 7). The first, sap-feeding instar feeds only on the epidermis cells (Fig. 33), while the other four tissue-feeding instars feed on the palisade parenchyma, leaving the spongy parenchyma intact (Figs. 34, 35).

Molecular analyses. Two reciprocally monophyletic lineages were found within the genus *Leurocephala*: the currently recognized *L. schinusae* and the new species *L. chilensis* (Fig. 36). Genetic divergence estimated between these lineages was 12% (±1%) and of both species together versus the outgroups (*Spinivalva*, *Parectopa* and *Epicephala*), varied from 16 to 18% (±1%, for any comparison).

Discussion

Morphological resemblance with the type species enables us to include this new gracillariid species in the formerly monotypic Neotropical genus *Leurocephala*. No differences were found in body color or wing venation in the adult stage for the two species. In addition, we were unable to separate *L. chilensis* from *L. schinusae* based on the external morphology of the pupa; also, no differences were found between their last larval instar chaetotaxy. However,

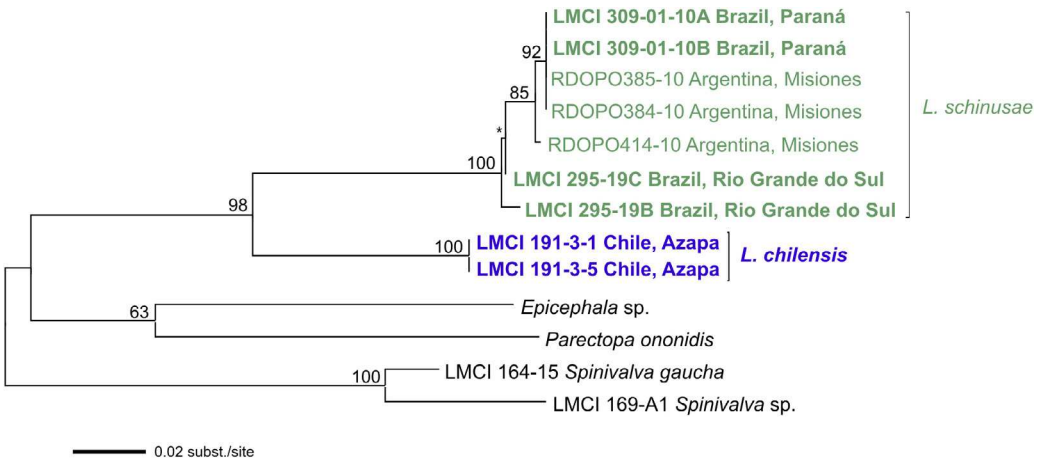


Fig. 36. Maximum likelihood phylogenetic tree of *Leurocephala* inferred based on 658 bp of DNA barcode sequences (cytochrome oxidase subunit I gene). Asterisk above branch indicate bootstrap support lower than 50%. Species of *Spinivalva, Parectopa* and *Epicephala* were used as outgroup; see Table 1 and text for further description.

as already mentioned, highly constant differences were found to separate the two species in the adult genitalia, and in the ventral prothoracic plate of larvae. In addition, subtle differences were found in the shape of the mine built by the second, tissue feeding instar. These morphological differences were reinforced by the molecular phylogenetic analysis, as the two species were grouped as closely related, but reciprocally monophyletic taxa that diverge from each other by ca. 12% (±1%) in DNA barcode sequences.

The known distribution of *Leurocephala* was previously restricted to the southern part of the Atlantic Forest, including portions of Argentina, Brazil and Paraguay (Davis et al., 2011). Thus the discovery of *L. chilensis* in the Atacama Desert provides the first record of *Leurocephala* from west of the Andes Range, expanding remarkably the geographic range of the genus. Additional surveys will be needed to characterize adequately the host(s) and geographic range of *L. chilensis*. It is very likely to find this species in other coastal valleys of the Atacama Desert, either in northern Chile or southern Peru, in the same way that other Gracillariidae were found in this hyperarid area (Maita-Maita et al., 2015), since the host plant *S. molle* is widespread in the region. The type species *L. schinusae* is able to breed on several species of *Schinus*, and also on species of two other genera in Anacardiaceae (Mc Kay et al., 2012). Adults of *Leurocephala* specimens were recently reared from leafmines collected on *S. polygamus* (Cav.) Cabrera, and mines typical of *Leurocephala* were also found on leaves of *Schinus latifolius* (Gill. ex Lindl.) Engler in two localities of Central Chile not included in the present study. These new findings suggest that all the species of *Schinus* distributed in south-central Chile (see Rodríguez et al., 1983) should be surveyed to know the effective range and diversity of *Leurocephala* west of the Andes. In our opinion, further taxonomic decisions on this regard should wait until comparative, fine scale analyses with an integrative taxonomic approach are performed, and after a broader survey for this micromoth genus been conducted throughout its range in South America. According to Davis et al. (2011), the type material used in the original description of *L. schinusae* was reared from mines collected on *S. terebinthifolius* Raddi, but similar mines were also found by them on other *Schinus* species, and also on an additional anarcadeacean, *Astronium balansae* Engl.

Additional monospecific genera of Neotropical Gracillariidae have been described during the last decades (e.g., Davis, 1994; Vargas and Landry, 2005; Vargas and Parra, 2005; Mundaca et al., 2013a,b), whose monotypic status might be associated with low sampling effort. The discovery of a second species of *Leurocephala* with morphology very close to the type species but with relative high level of genetic divergence, and with larvae feeding on a plant of the same family as the type species suggests that analogous studies should be carried out also for these other apparently monotypic genera. In other words, host-plant shifts between closely related plants and the existence of cryptic species in association should be further explored in such Neotropical gracillariid genera, as already suggested by Brito et al. (2013).

Finally, our results illustrate the existence of wide diversity of feeding habits at fine scale throughout ontogeny and among gracillariid lineages, which should be better explored. As far we are aware, feeding confined to single-celled epidermis, as demonstrated here for the first sap-feeding instar of *L. chilensis* has not been described for any species within the *Parectopa* group. This very specialized feeding behavior was demonstrated for the sap-feeding larvae of *Phyllocnistis citrella* Stainton (Achor et al., 1997) and *Marmara arbutiella* Busck (Wagner et al., 2000). Sap-feeding instars of other gracillariids are supposedly associated primarily with the outer layers of parenchyma (e.g. Brito et al., 2012). Feeding on palisade parenchyma is restricted to tissue-feeding instars in *L. chilensis*, which is also the case for all tissue-feeding instars of *Spinivalva gaucha* Moreira & Vargas, as described by Brito et al. (2013). Our data give further support in the sense that the three larval morphs (sap-feeder, apodal and legged) *Leurocephala* found (see Davis et al., 2011) might be associated with the three mining types described here (the epidermic, serpentine and blotch types, respectively).

Conflicts of interest

The authors declare no conflicts of interest.

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CAPÍTULO IV

Artigo aceito para publicação na revista Zootaxa, em março de 2019:

Fwd: a manuscript, accepted by me: Pereira et al.: Vallissiana universitaria: a new genus and species

Gilson Rudinei Pires Moreira Fri, Mar 8, 8:12 AM (23 hours ago)

to me, Helber, Paolo, Rosângela, Rosy, Gislene

I enclose to this message a manuscript, accepted by me to be published in *Zootaxa*.

Title: *Vallissiana universitaria* (Lepidoptera: Gracillariidae): a new genus and species of leaf-mining moth associated with *Erythroxylum* (Erythroxylaceae) in the Atlantic Forest of Brazil
Authors: CRISTIANO M. PEREIRA, HELBER A. ARÉVALO-MALDONADO, PAOLO TRIBERTI, ROSÂNGELA BRITO, ROSY M. S. ISAIAS, GISLENE L. GONÇALVES & GILSON R. P. MOREIRA
e-mail of corresponding author: *gilson.moreira@ufrgs.br*

Number of new genera: 1

Number of new species: 1

Number of image plates: 9

Number of references: 54
Running title: Pereira *et al.*: *Vallissiana universitaria*: a new genus and species
Subject: Lepidoptera: Gracillariidae

Please find the illustration plates following this link:
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Kind regards,

Jurate De Prins

Editor

Vallissiana universitaria (Lepidoptera, Gracillariidae): a new genus and species of leaf-minering moth associated with *Erythroxylum* (Erythroxylaceae) in the Atlantic Forest of Brazil

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Abstract

Vallissiana universitaria Pereira & Arévalo, a new genus and species of leaf-miner moth, (Gracillariidae: Gracillariinae) is described and illustrated with the aid of optical and scanning electron microscopy, including adults, larva, pupa and the mine. Its monophyletic status is confirmed within the subfamily based on a DNA barcode *CoI* tree. The immature stages are associated with *Erythroxylum argentinum* O. E. Schulz (Erythroxylaceae) and four larval instars are found, all forming a round blotch mine from the beginning of ontogeny. The first two instars are sap-feeders, using only the epidermal cells, whereas the last two are tissue-feeders, mining the parenchyma cells. Pupation occurs inside the leaf mine within a flimsy, silk-made cocoon. This is the third endemic genus of gracillariid moths described from the Atlantic Forest of Brazil and the first associated with *Erythroxylum* P. Browne. Characteristics found on the forewing and in the last abdominal segments of the adult were determinant for the proposition of the new genus. The *CoI* tree indicated that it is closely related to *Aspilapteryx*, while this genus was recovered as polyphyletic in the analyses. Morphological evidence supports this polyphyly. Consequently, *Sabulopteryx* Triberti, 1985, **stat. nov.** is considered a valid genus.

Key words: gracillariids, Gracillariinae, leaf-mining moths, microlepidoptera, Neotropical region.

Introduction

Leaf-mining is a particular habit frequently found in holometabolous insects, characterized by consuming leaf structures in an endophagous manner. This action is performed by the larva within the leaf, where food and protection are found during development (Hering 1951).

Lepidoptera include a wide range of families with a leaf mining habit. They show striking features, such as a high specificity to the host plant and the type of tissue mined, stereotyped behavior in the manner that mines are built, and small size in all developmental stages (Hering 1951; Connor & Taverner 1997).

Gracillariidae is the most diverse family of leaf-mining lepidopterans, with 107 genera and approximately two thousand recognized species (Davis 1987; De Prins & De Prins 2019). Of these, 28 genera and 199 species have been described from Neotropical region (De Prins *et al.* 2016; De Prins & De Prins 2019), among them ten genera and 39 species are found in Brazil and only twelve species are known from the Atlantic Forest (Brito & Duarte 2018). This number of gracillariid species is low, considering the extension and richness of this biome. Although strongly degraded by humans, the Atlantic Forest still hosts one of the most rich animal and plant diversity on the planet, with a high endemism degree (Myers *et al.* 2000).

Reduced numbers of gracillariid species known to occur in Brazil result from low sampling effort (Brito *et al.* 2016). New collections have generally led to the establishment of new genera, which are often either monotypic or with a small number of species described (e.g. *Cactivalva* Moreira & Vargas, *Leurocephala* Davis & McKay, *Parectopa* Clemens, *Spanioptila* Walsingham, *Spinivalva* Moreira & Vargas). Thus, morphological characteristics and genetic divergence of newly described species are expected to be highly distinct with regard to already existing taxa.

Larvae described in this study were found mining the leaves of the evergreen shrub *Erythroxylum argentinum* O. E. Schulz (Erythroxylaceae) in South Brazil. Morphologically and genetically, the new leaf miner species corresponds to Gracillariinae (Gracillariidae), but it does not conform to any of the 25 genera known in this subfamily (*sensu* Kawahara *et al.* 2017). We

describe and illustrate all life stages of this new genus and species, with the note of its life history and leaf mine histology. An analysis of mitochondrial DNA barcode sequences including members of related genera is also provided, with elevation of subgenus *Sabulopteryx* Triberti to the genus status.

Material and methods

Leaves of *E. argentinum* with leaf mines (*ca*. 150 units) were collected from 2015 to 2017 in the Campus do Vale, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre municipality, Rio Grande do Sul (RS), Brazil. They were kept in small plastic vials containing moistened cotton at room temperature in the Laboratório de Morfologia e Comportamento de Insetos (LMCI) at the Zoology Department of UFRGS. They were inspected daily for emergence of adults, which were pin-mounted and dried.

Immature stages were dissected from additional leaf mines sampled in the same location, fixed in Dietrich´s fluid and preserved in 75% ethanol. For morphological description, the specimens were prepared using the technique described in Kumata (1977) but with fixation on the slide with Euparal instead of Canada balsam. Morphological characters of immature and mature insects were examined under Leica® M125 stereomicroscope, and all measurements were made using an attached ocular micrometer (precision = 0.01 mm). To determined growth pattern of larvae, their head capsule widths were adjusted to an exponential equation. Morphological structures of the insects selected to be drawn were photographed with a Sony® Cyber-shot DSC-H10 digital camera attached to the stereomicroscope and Nikon AZ 100M stereomicroscope. Vectorized line drawings were then made with the software Corel Draw® X7, using the corresponding digitalized images as a guide.

The description of adults was done according to: Comstock (1918) for wing venation, Kumata (1982) and Triberti (1985) for abdominal and genitalia structures, Kumata (1982) for vestiture, and Kuznetzov (1989) for wing pattern. The format of the larval description generally followed Stehr (1987).

For scanning electron microscope (SEM) analyses, additional larvae and pupae (at least five specimens per stage) were dehydrated in a Bal-tec® CPD030 critical-point dryer, mounted with double-sided tape on metal stubs and coated with gold in a Bal-tec® SCD050 sputter coater. They were examined and photographed in a JEOL® JSM6060 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of UFRGS.

Museum collections

Abbreviations of the specimen depositories are the following:

DZUP Coll. Padre Jesus S. Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, Paraná, Brazil;

LMCI Laboratório de Morfologia e Comportamento de Insetos, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Plant anatomical analysis

For histological sections, leaf fragments (0.5 cm^2) with mines in three developmental stages (initial, middle and final; n = 6 per stage) were fixed in FAA (37% formaldehyde, acetic acid, 50% ethanol, 1:1:18, v/v) for 48 h, dehydrated in an n-butyl series, and embedded in Paraplast. After dewaxing and rehydration, transverse sections (12 µm) obtained in a rotary microtome

(Jung Biocut) were stained in safranin-astrablue (2:8, v/v) (Bukatsch 1972, modified to 0.5%), washed, dehydrated, and mounted in colorless varnish (Paiva *et al*. 2006). Photographs were taken under a Leica® DM 2500-LED light microscope with a Leica® DFC7000T camera.

DNA sequencing and analysis

DNA was extracted from two larvae of *V. universitaria* (LMCI 002-7A and LMCI 002-7B) using the PureLink genomic DNA extraction kit (Invitrogen) following the manufacturer's instructions. Polymerase chain reaction (PCR) was performed to amplify 658 base pairs (bp) of the mitochondrial cytochrome c oxidase subunit I (*CoI*) gene, i.e. the DNA barcode region (*sensu* Hebert *et al.* 2003), with universal primers and conditions proposed by Folmer *et al.* (1994). PCR products were treated with exonuclease I and FastAP thermosensitive alkaline phosphatase (Thermo Scientific), sequenced using BigDyeTerminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, USA) through standard conditions provided by the manufacturer and analyzed in an ABI3730XL (Applied Biosystems, USA). Sequences were edited in the program ChromasPro 2.1.5 (www.technelysium.com.au/ChromasPro.html) and automatically aligned using the algorithm Clustal X in MEGA v5 (Tamura *et al.* 2011) running in full mode.

To explore the phylogenetic position of the new species and its generic classification we used the *CoI* data of the new taxon together with a published dataset of 20 species of Gracillariinae and an outgroup (Table 1). These taxa were chosen based on morphological diagnosis in association with the topology proposed by Kawahara *et al*. (2017). The outgroup was composed of members of the Acrocercopinae and Ornixolinae, the subfamilies most closely related to Gracillariinae (Kumata et al. 1988; Kawahara *et al.* 2017), while the ingroup was composed of representative species of Gracillariinae, especially species proposed by Kumata

(1982) since morphological characters established of *V. universitaria* corresponded to this group of genera.

The two sequences of *V. universitaria* were deposited in both GenBank (MK058444 and MK058445) and BOLD Systems (GRABR011-18 and GRABR012-18) (Table 1). The *CoI* tree was constructed using Bayesian inference, performed in BEAST v1.8.4 (Drummond *et al.* 2012) using an uncorrelated lognormal clock and a Yule prior on branching rates with GTR+G as substitution model. Four independent runs of 10 million generations and a burn-in period of 10,000 (the first 1,000 trees were discarded) were implemented; the remaining trees were summarized in TreeAnnotator v1.6.2 (Drummond & Rambaut 2007) and used to infer a maximum *a posteriori* consensus tree. Bayesian posterior probabilities (BPP) were used as an estimate of branch support. Consensus trees were visualized and edited in FigTree 1.4.2 (http://tree.bio.ed.ac.uk/software/201/). Sequence divergences were quantified using the Kimura 2-parameter model in MEGA v5.

Results

Molecular data. *V. universitaria* was supported as a monophyletic lineage within Gracillariinae, confirming the identification of a new genus and species (Fig. 1). The closest lineages recovered in the *CoI* tree were *Aspilapterix inquinata* and *Aspilapterix limosella*. The pairwise genetic distance of these to the new species was 13% in average (Table 2). Similarly, *Aristae pavoniella*, *Caloptilia acericola* and *Gracillaria syringella* presented lowest distance to *V. universitaria* (i.e., 12% of divergence). All genera were monophyletic except *Aspilapterix*, which was recovered as polyphyletic with two distantly related groups: one formed by *A. inquinata* and *A. limosella* and close to *Gracillaria*, *Povolnya* Kuznetzov and *Caloptilia* Hübner, and a second group that

included *A. tringipennella* (Zeller) and *A. multipunctella,* related to *Euspilapteryx* and *Eucalybites* Kumata. The genetic distance estimated between *V. universitaria* and species of Gracillariinae ranged from 12% to 17%.(Table 2). Divergence of the new species to representative taxa of Acrocercopinae and Ornixolinae varied from 15 to 16%.

***Vallissiana* Pereira & Arévalo gen. nov.**

(Figs. 2–3).

Type species. ***Vallissiana universitaria* Pereira & Arévalo sp. nov.**

Diagnosis. *Vallissiana* belongs to the Gracillariinae based on the presence on males of the mostly membranous eighth abdominal segment with a reduced tergite and sternite (Kawahara *et al*. 2017). It conforms to the *Gracillaria* group proposed by Kumata (1982) based on the following characteristics: 1) legs with appressed scales ("smooth-scaled"), and with foretibiae, midfemur and tibiae bearing long, bristled scales ventrally, appearing as a thickening; 2) forewing Rs and R_1 faint from base to just beyond the region of R_1 branching; 3) forewing R_1 branching near base; 4) hindwing R_{2+3} parallel to short $Sc+R_1$.

The new genus shows similarity in the male genitalia with *Aspilapteryx* in the shape of the valva, with the ventroapical corner ending as a rounded lobe. Affinity exists also with *Sabulopteryx* due to the presence of a process on the mesal face on the basal third of the valva, even if some doubt remains on the homology with the finger-shaped process in *Sabulopteryx*. The female genitalia are similar to those of *Euspilapteryx* Stephens, *Calybites* Hübner, *Ectropina* Vári, *Gracillaria* Haworth and *Caloptilia theivora* (Walsingham) by the presence of one signum on the corpus bursae of the female and to *Aspilapteryx* by the wide and simple ostium bursae.

However, the new genus differs from the others in forewing venation by R_5 and M_1 stalked and the absence of coremata in the male abdomen.

Description of the adult. Small-sized moth, forewing 3.8 – 4.0 mm (n = 5) long. L/W index 6.4, male and female similar in size and coloration (Fig. 2).

Head (Figs. 2B–C).Vertex with long appressed scales ("smooth-scaled") directed anteriorly; occiput with long appressed scales directed towards vertex; frontoclypeus with long appressed scales directed ventrally; ocelli absent. Antenna filiform, ~1× forewing length; scape pectinate; flagellomeres covered by single, dense row of slender scales, each flagellomere 0.5× scape length, except for first one, ~2× longer than others. Labrum bilobed; pilifers developed, subtriangular; maxillary palpus short, 0.3× labial palp length; labial palpus upturned, three-segmented; second palpomere with porrect bristled, enlarged, roughly scaled ventrally; third palpomere 1.5× second palpomere in length; haustellum elongated, well developed, naked.

Thorax (Fig. 2A). Forewing narrow, lanceolate, acute apically, posterior piliform scales (=fringe) long, 1.1× longer than width of forewing; racket-shaped scales with apical margin dentate with three to five cusps at outer margin. Venation (Fig. 3A): 12 veins, discal cell almost rectangular, with distal margin almost straight. Veins R_1, R_2, R_3, R_4, R_5+ M_1, M_{2+3} +CuA_{1+2} branching from discal cell; R_1 to R_4 well separated at their bases; Rs and R_1 faint from base to just beyond the region where R_1 branches; R_1 branching near base; R_2 branching more basal on cell than CuA_{1+2} branching; R_5 and M_1 stalked; M_2 and M_3 fused; CuA_1 stalked with M_{2+3} ; CuP entirely faint; anal vein simple, almost straight, extending from base to 2/5 of wing length. Retinaculum: female, hooked piliform scales; male, curved triangular fold of subcostal vein. Hindwing narrow, lanceolate, sharply pointed; marginal scales long, ~3× longer than width of

hindwing. Venation (Fig. 3A): 9 veins, radial veins with two branches; R_{2+3} parallel with $Sc+R_1$, the latter short, extending to 0.4× hindwing length; discal cell open between M_2 and M_3; M_1 and M_2 stalked; CuA_1 and M_3 branching from CuA_2; M_3 branching from CuA_1; anal vein almost straight, short. Frenulum: female with two stout bristles, anterior ~2× thicker than posterior; male with one stout bristle. Legs: epiphysis present; tibial spur pattern 0-2-4, tibial length ratios ~0.4/0.6/1, covered with appressed short scales; foretibia, midfemur and midtibia with long, bristled scales ventrally appearing as a thickening. Tibial spurs of mid leg at subapex, mesal spur ~0.5× tibia length, lateral spur ~0.6× mesal spur length; tibial spurs of hind leg at 1/4 of tibia length and at subapex, meso-basal spur ~0.4× tibia length; latero-basal spur ~0.2× meso-basal spur length, meso-apical spur ~0.2× tibia length, latero-apical spur ~0.9× meso-apical spur; hind tarsus ~1.5× longer than tibia.

Abdomen. Male seventh and eighth segments membranous (Fig. 3B), retracted into sixth segment; seventh segment elongated, 1.2× longer than sixth segment; sternite and tergite of segment seventh reduced to thin transverse band; eighth segment short, 0.3× shorter than sixth segment. Female segments unmodified.

Male genitalia (Figs. 3C–G). Tegumen mostly membranous, slightly sclerotized. Anal tube nude, protruding posterior to tegumen; subscaphium very slender, from sub-base to posterior margin of anal tube. Valve symmetrical, sub-rectangular, slightly upturned, costal margin relatively straight and distally rounded, cucullus densely covered by long piliform setae, with distal portion membranous, sacculus with sparse setae and a membranous margin, with rounded cuticular projection on mesal surface at basal third, ventral margin with medial region curved, ending distally as a rounded lobe. Vinculum not differentiated; saccus broadly U-shaped;

juxta membranous. Phallus tubiform ~1× valva length, base slightly wider than apex, cornuti a group of spines in apical third, apex with two small projections.

Female genitalia (Figs. 3H–J). Papillae anales ~0.6× wider than long, connected dorsally, covered with setae and microtrichia; anterior apophysis with wide arms from base to middle, progressively shortening towards apex, reaching beyond distal margin of seventh segment; posterior apophysis curved, reaching sterigma. Lamella postvaginalis united with lamella antevaginalis; ostium bursae placed on intersegmental area between seventh and eighth abdominal segments; corpus bursae with one falciform signum; ductus seminalis arising from caudal region of ductus bursae.

Etymology. The genus name is derived from the Latin word *Vallis* (= valley) in reference to Campus do Vale – Universidade Federal do Rio Grande do Sul (UFRGS), the type locality. To be treated as feminine.

Vallissiana universitaria **Pereira & Arévalo sp. nov.**

(Figs. 2-–9)

Diagnosis. *V. universitaria* is similar to *Gracillaria albicapitata* Issikii and *G. syringella* (Fabricius) in forewing pattern, but easily distinguished from them by the venation and the male genitalia. Male genitalia show affinity with all species of *Aspilapteryx* in the shape of the valva, with a deep concavity in the ventral margin and a rounded ventroapical lobe. However, in *V. universitaria* the phallus is straight, not with more or less helical curves, with the exception of the South African *A. seriata* (Meyrick). Some similarity exists with *Sabulopteryx inquinata*

Triberti due to the presence of a finger-shaped process on the mesal surface at the basal third of the valva. However, the shape of the valva is completely different, with the ventral margin almost straight in *S. inquinata*. The female genitalia are very similar to those of *A. spectabilis* Huemer, *A. multipunctella* (Chretien) and *A. magna* Triberti by the simple sterigma and a wide ostium bursae located at posterior margin of eighth segment. However, in *V. universitaria* the ostium is placed more posteriorly, on the intersegmental membrane between the seventh and eighth segments, and there is only one signum on the corpus bursae.

Description of adult (Fig. 2, 3). Forewing 3.8 – 4.0 mm (n = 5) long. L/W index 6.4.

Head. Creamy white mottled with dark brown; inter-ocular index 0.83; antenna 1.1× forewing length; scape creamy white basally and brown apically, dilated, 4× pedicel length; pecten with 5 to 6 piliform scales (Fig. 2B); flagellomeres creamy white basally and dark brown apically. Maxillary palpus creamy white with dark brown subapical band; labial palpus 0.61mm, ~2.8× longer than eye diameter; second palpomere dark brown, bristled scales ~0.8× second palpomere length; third palpomere creamy white, with two dark brown bands at apex and subapex (Fig. 2C); haustellum ~2.0× labial palpus length.

Thorax. Dark brown laterally and ventrally, notum, patagium and tegula creamy white mottled with brown. Forewing ground color dark brown. Three white transverse fasciae at 1/3, 1/2 and 3/4 of wing length; three ochreous yellow transverse fasciae, the first two ochreous yellow fascia distal to the first two white fascia and the last ochreous yellow fascia basal to the last white fascia; one ochreous yellow posterior strigula at subapex; marginal scales white basally and dark brown apically (Fig. 2A). Legs: forelegs with coxa, trochanter, femur and tibia dark brown mottled with creamy white; tarsus white with dark brown spot at apex of each

tarsomere; midlegs with femur dark brown mottled with creamy white, two white spots dorsally at 1/2 and 4/5 of femur length, bristled scales racket-shaped, apical margin entire; tibia colored like femur, bristled scales racket-shaped, apical margin dentate with three cusps; spur dark brown with white spot at apex; lateral spur colored like mesal spur; tarsus colored like fore tarsus; hind legs with femur white bearing two dark brown bands laterally at 1/3 and 3/4 of femur length; tibia dark brown with three bands at base, 1/3 and apex; spurs white basally and dark brown apically; tarsus andfirst tarsomere dark brown with white band at 4/5 tarsomere length; second to fifth tarsomeres white with dark brown spot at apex of each tarsomere.

Abdomen (Figs. 2A, 3B). Tergum brown; sternum with a creamy white band anteriorly and dark brown posteriorly. Male: posterior region of seventh segment covered with four lamellar, lanceolate rows of scales (Fig. 3B). Female: posterior margin of seventh segment with slight notch mesoventrally.

Male genitalia (Figs. 3C–G). As described for genus.

Female genitalia (Figs. 3H-–J). As described for genus.

Type material. All from BRAZIL: Campus do Vale, Universidade Federal do Rio Grande do Sul (UFRGS), 30°4'23"S 51°7'33"W, 41 m, Porto Alegre municipality, Rio Grande do Sul state (RS). All adults were reared, preserved and pinned by the senior author, from leaf mines found on *Erythroxylum argentinum* (Erythroxylaceae). The material was part of the private collection of C. M. Pereira (CMP) and is deposited as follows: HOLOTYPE: ♂ (CMP002-22), 30.VIII.2015, deposited in LMCI (337–01). PARATYPES: 1♂ with genitalia on slide (CMP002-102 / HAA147), 30.VIII.2016, deposited in LMCI (337-02), 1♀ with genitalia on slide

(CMP002-23/HAA146), 30.VIII.2015, deposited in LMCI (337-02), 1♂ with genitalia on slide (CMP002-46), 19.X.2015, deposited in DZUP (DZ 33.241), 1♀ with genitalia on slide (CMP002-06), 30.VIII.2015, deposited in DZUP (DZ 33.251).

Other specimens examined. All deposited in LMCI. Wings mounted on slide: CMP002-90, 16.VIII.2017 (LMCI 337-10). Larvae and pupae inside their mines fixed in Dietrich's fluid and preserved in 70% ethanol solution: six sap-feeding larvae, CMP002-48, 30.VIII.2015 (LMCI 337–11); five last instar larvae, CMP002-03, 30.VIII.2015, (LMCI 337–12). Five pupae, CMP002-89, 30.VIII.2015 (LMCI 337-14). Last instar larva on slide, CMP002088, 05.V.2017 (LMCI 337-13).

Etymology. The specific name *universitaria* (= university student) comes from Latin and is thus an allusion to the students of Campus do Vale where this new species was first found. To be treated as feminine.

Immature stages

Larva (Figs. 4A–D, G; 5A-–I; 6A-–M). With hypermetamorphic development, two morphotypes and four instars, all leaf-miners. The first two instars are sap-feeders and the subsequent two tissue-feeders. They can be identified by measuring their head capsule width (Table 3). The exponential growth curve adjusted for the four instars of *V. universitaria* reared on *E. argentinum* was: $y = 0.861e^{0.391x}$; $n = 40$; $r = 0.985$; $p < 0.0001$.

Sap-feeding larva (second instar) (Figs. 4A, B; 5A–I; 8E). Average length ± standard deviation = 1.75 ± 0.55 mm; n = 8. Body flattened, head with brown coloration and body creamy white, setae reduced or absent.

Head: prognathous, extremely flattened (Figs. 5A–C). Antenna three-segmented with onesensillum at the apical segment, four stout sensilla with round apex on middle segment and three setiform setae on basal one (Fig. 5D). Stemmata absent. Slicing mouthparts (= sap-feeding). Mandibles flattened and rounded, with anterior surface smooth, mesal area serrated. Labrum reduced and slightly bilobed; labium reduced, hypopharynx with setae projected forward and reaching labrum; spinneret rudimentary with apical opening; labial palpus reduced, fused with labium (Fig. 5E).

*Thorax***:** Sclerotized plates disposed in parallel on pro- and mesothorax (Figs. 4A–B); anterior portion of these segments corrugated, posteriorly smooth in dorsal view (Figs. 4A, 5G); metathorax with parallel and perpendicular plates in dorsal view (Fig. 4A). Spiracle rounded, without elevated peritreme, located at lateral margin of the prothorax (Fig. 5F). Ventral calli present on all thoracic segments (Fig. 5I).

*Abdomen***:** Spiracles rounded, without elevated peritreme, located laterally on A1–A8. Abdominal segments partially covered with microtrichia. Ventral calli present on A3–A5 and A10.

Tissue-feeding larva (fourth instar) (Figs. 4C–D, G; 6A–M; 8F). Average length + standard deviation = 4.8 ± 0.67 mm; n = 9. Body cylindrical, covered with microtrichia, head dark brown, body creamy white (Fig. 8F).

Head. Hypognathous, frontoclypeus not reaching epicranial notch, elongated, ~0.7× head length; ecdysial line ending far from epicranial notch (Figs. 4C, 6A–C). Antenna three-segmented, with six sensilla on apical segment, one stout and bifurcate, two setiform, three rounded, and one minute (Fig. 6G). Stemmata absent. Mouthparts of chewing type (=tissue-feeding). Labrum bilobed with four pairs of microsetae near anterior edge and two centrally. Mandibles with four teeth, partially covered by labrum in dorsal view (Fig. 6D). Maxilla stout, maxillary palpus and galea differentiated, palpus with five setae (Fig. 6F). Labium wide, spinneret well developed with functioning apical opening (Fig. 6E); palpus bi-segmented, basal segment ca. 5x longer than the apical, both with one apical sensillum.

Thorax. Prothoracic shield sub-rectangular (Fig. 4C), weakly melanized and slightly apparent in scanning electron microscopy; prothoracic spiracle rounded with elevated peritreme, located laterally. Legs well developed, with five setae on coxa, two on tibia, five ontarsus, all with a hook-shaped apical claw (Figs. 6H, I).

Abdomen. Spiracles rounded, with elevated peritreme, located laterally on A1–A8 (Fig. 6K). Pseudopodia on A3–A5 and A10. Crochets reduced and hook-shaped, in uniordinal circle (Fig. 6J). Anal shield rounded (Fig. 6L), not melanized, apparent under scanning electron microscopy; last abdominal segment slightly divided into two lateral lobules (Fig. 6M).

Chaetotaxy of the last-instar larva (Fig. 4G).

Head. MD group trisetose; P group bisetose, P1 longer than P2 ; AF bisetose, AF1 closer to AF2 than P1 to P2; L unisetose; A group trisetose, A1 and A3 longer than A2; S group trisetose, S2 longer than S1 and S3; SS group trisetose; C bisetose; MG1 present, minute.

Thorax. **T1**: D and XD bisetose, both located on prothoracic shield; D2 longer than D1; XD1-2 similar; SD bisetose, both located at margin of prothoracic shield, SD2 lateral and shorter than SD1; L bisetose, L1 longer than L3, both anterior to spiracle; SV bisetose, SV1 longer than SV2; V unisetose. **T2-3**: D1-2 as onprothorax; SD bisetose; L and V1 as on prothorax; SV unisetose; MD1, MSD1, MSD2 and MV3 present.

Abdomen. **A1**: D and MD1 as on previous segments; SD bisetose, SD2 minute; L bisetose; SV and V unisetose; MV3 present; **A2**: as A1, SV bisetose. **A3-5**: SV trisetose. **A6-8**: SV2 and SV3 absent. **A9**: SD2 and L3 absent. **A10**: D1-2 and SD1 located on the anal shield; SD2 near shield margin; L trisetose, PP1 located dorsally to L; group SV composed of four setae; V group unisetose.

Pupa (Figs. 4E, F; 7A–I; 8G). Average length $\pm$ standard deviation = 4.2 $\pm$ 0.20 mm; n = 5. Light brown.

Head. Vertex without projections or setae (Figs.7A–C). Front wide, two pairs of microsetae close to labrum (Fig.7B). Labial palpi reaching middle of A1; proboscis reaching middle of A3; antennae reaching beyond distal margin of abdomen.

Thorax. Prothorax narrow with two comma-shaped depressions dorsally (Figs.7A, D). Meso and metathorax wide, each segment with a pair of dorsal setae. Forewings reaching A6; pro-, meso- and metathoracic legs reaching A4, A6 and A10, respectively.

Abdomen. A1 with three pairs of setae; a shorter dorsal pair, and two longer dorso-lateral. Spiracles rounded, with elevated peritreme. A group of small, posteriorly projected spines on anterior margin of terga A2–7; located between such spines a pair of dorsal setae of medium size; two pairs of dorso-lateral setae posteriorly to the spiracles (Figs. 7E–F). A8 with three pairs

of long setae, a dorsal pair and two dorso-lateral. Last abdominal segment bearing small tubercles, two dorsal and two lateral (Figs. 7G–I).

Biology (Figs. 8A–H; 9A–C). Blotch mines of *V. universitaria* are easily located on the adaxial surface of *E. argentinum* leaves (Figs. 8A, B). The oval-shaped, translucent egg is laid individually on the adaxial leaf surface, usually in between secondary veins (Fig. 8C). The first instar larva starts to mine immediately after hatching, moving into the epidermis, where it feeds also during the second instar (Figs. 8D; 9A). Both instars build mines centrifugally, which is shown by the distribution pattern of frass inside the leaf mine (Fig. 8E). The mine is a round blotch, with a space between the cuticle and the palisade parenchyma(Fig. 8D). Third instar larvae feed initially on palisade parenchyma (Fig. 9B), and the last on the spongy parenchyma (Figs. 8F; 9C). Mines are initiated by cutting the anticlinal cell walls of the adaxial epidermis (Fig. 9A). The larva goes deeper into leaf tissues and consumes all adaxial epidermal cell walls except the outer periclinal walls (Fig. 9B). Mines with mature larvae have almost all chlorophyllous cells consumed by the larvae, only remaining 2-4 layers of spongy parenchyma, the vascular bundles, which are protected by lignified sheath, and the abaxial epidermal cells (Fig. 9C). At the end, the leaf becomes twisted in the portion of the leaf mine. Before pupation, the larva spins a thin layer of silk that protects the pupal cocoon; pupation occurs inside the leaf mine (Fig. 8G). Prior adult emergence the pupa makes a slit on the leaf mine by pressuring the head to the wall, through which the adult emerges, leaving the pupal exuviae partially protracted (Fig. 8H). During the years of sampling (2015–2017), *V. universitaria* mines were found throughout the year in the field, but in greater numbers from September to December.

Host plant. *Erythroxylum argentinum* (Fig. 8A), a tree commonly called "cocão" that is native to southern Brazil (Atlantic Forest), Argentina and Uruguay. It is used mainly in the recovery of degraded areas and urban afforestation (Lorenzi 2002).

Distribution.*V. universitaria* is known only from the type locality, Porto Alegre Municipality, Rio Grande do Sul, Brazil.

Sabulopteryx Triberti, 1985, **stat. nov**.

Aspilapteryx (*Sabulopteryx*) Triberti, 1985: 4; Huemer, 1994.

http://zoobank.org/

Our analysis of mitochondrial DNA barcode sequences including members of related genera suggests that *V. universitaria* is mostly related to *Aspilapteryx* Spuler, and that this genus, including subgenera *Aspilapteryx* and *Sabulopteryx*, is polyphyletic. Thus *Sabulopteryx*, regarded until today as a subgenus of *Aspilapteryx* or simply as synonym (De Prins & De Prins 2019), belongs to a different clade and should be regarded as a valid genus: *Sabulopteryx* Triberti, 1985 **stat. nov**.

Type species: *Aspilapteryx (Sabulopteryx) limosella* (Duponchel, 1843) by original designation.

Diagnosis. According to the original description, as a subgenus (Triberti 1985), *Sabulopteryx* shows an affinity with *Aspilapteryx* in the wing pattern and forewing venation. However, the hindwing venation with M_3 always missing, the presence of coremata on the seventh and eighth

abdominal segments and the very different male genitalia, with the phallus straight and short, and the valva small and without ventroapical lobe separate it clearly. Also, a high divergence (from 14 to 16%) in DNA barcode sequences further supports this consideration. The two species originally included in subgenus *Sabulopteryx* are here confirmed to belong with *Sabulopteryx* as a genus: *S. limosella* and *S. inquinata* (Fig. 1).

Additions to original description. Head and face smooth; labial palpi long, upturned, smooth, terminal segment about as long as second; maxillary palpi smooth, from 1/3 to 1/4 as long as labial palpi; ocelli present. Antenna ~1x forewing length; scape with pecten of a few hairs. Forewing 12-veined, Rs and R_1 faint from base to just beyond the branch of R_1; M_2 and M_3 fused. Hindwing 8-veined, discal cell opened between M_2 and CuA_1.

Male genitalia. Anal tube naked; phallus about as long asvalva or shorter, truncated obliquely at apex.

Female genitalia. Posterior apophysis longer than anterior; ductus seminalis arising from posterior end of ductus bursae.

Immature stages. To be described in a separate paper.

Biology. Only the natural history of *S. limosella* is known. Its larvae mine leaves of *Teucrium chamaedrys* L. and *T. montanum* L. (Lamiaceae). Other hosts are also cited in the literature: *T. scordium* L., *Genista tinctoria* L. (Fabaceae) and *Jurinea cyanoides* (L.) Rchb (Asteraceae) (De Prins & De Prins 2019). However, these data are dubious, and no confirmation of their veracity

has been reported. The mine is on the lower-surface and tentiform. with the leaf curling up very similarly to a *Phyllonorycter* Hübner mine. From the the upper surface, mine is purplish brown. Frass is deposited in a corner. According to Klimesch (1951), on *T. montanum* larvae vacate mines and initiate new ones up to four times, depending on the size of the leaf. Pupation occurs usually in a cocoon in the mine the species hibernates as pupae. The larvae are present in May–July and August–September; thus, supposedly there are two generations per year.

Distribution. Central and Southern Europe (excluding Iberian Peninsula), South of European part of Russia, Western Asia.

Discussion

The decision of whether or not to propose a new genus, especially when monotypic, is difficult (Huemer *et al.* 2016). In this study, the morphological and *CoI* data provided the necessary basis and were determinant in the decision. Characteristics present in the genitalia are most of the time essential for distinguishing species of Gracillariinae. More comprehensive morphological characters, such as the presence or absence of coremata, wing venation and the wing color pattern are effective for the distinction of a given genus (Vári 1961; Kumata 1982; Triberti 1985). Substantial advances have been made in an attempt to clarify the morphological and phylogenetic patterns within Gracillariidae (De Prins & Kawahara 2012; Kawahara *et al.* 2017; De Prins & De Prins 2019). Despite of this, many groups still have a confusing taxonomic history that requires additional analyses.

This is the case for the genera belonging to the *Gracillaria* group which was proposed by Kumata (1982) based on morphological evidence, and that has not had its monophyly tested yet. However, morphological attributes are consistent in this group that includes *Vallissiana* gen. nov., and into which a recently described genus, *Mercantouria* Huemer, Lopez-Vaamonde &

Triberti, was also placed (Huemer *et al.* 2016). Wing venation characters, the presence and shape of coremata, and one or more membranous abdominal segments are diagnostic in the corresponding distinction from other Gracillariinae. In *Vallissiana*, the presence of the seventh and eighth membranous abdominal segments and the absence of coremata are important characteristics that separates it from the other genera within the *Gracillaria* group. These aspects associated with the unique feature found on the forewing venation also support the proposition of this new genus.

In addition to morphology, differences between *V. universitaria* and *Sabulopteryx* spp. are found in *CoI* sequences. They were placed as close related in the tree, diverging in ca. 12% . Some similarity was observed with species of *Aspilapteryx*, but there are considerable morphological differences as well as higher genetic distances (ca. 15%) with *V. universitaria*. Therefore, given the congruence between morphological and *CoI* sequences, *Sabulopteryx* and *Aspilapteryx* can be better understood as two distinct genera.

The hypermetamorphic development of Gracillariidae may involve different larval forms and various feeding behaviors, the presence of one or more sap-feeding instars being characteristic (Kumata 1978; Davis 1987). The last larval instar of *V. universitaria* does not follow the standard diagnostic proposed for Gracillariinae by Kawahara *et al.* (2017) by not exhibiting stemma, in contrast to the full complement of six stemmata cited by the authors. Also, the chaetotaxy of *V. universitaria* shows additional differences by the presence of the bisetose lateral group (L1 and L3) on the mesothorax and metathorax, in contrast to the trisetose condition in the other genera of the *Gracillaria* group (Kumata 1982) and also in Gracillariinae (Kawahara *et al.* 2017). Setae present on the last abdominal segment have been illustrated in the literature for closely related groups (e.g. Kumata 1982; Triberti 1985), although they have not

been named. We inferred the chaetotaxy in this case by comparing locations of setae in previous abdominal segments to Hinton´s system (Stehr 1987); their corresponding homologies should be explored in future in comparison to other related genera.

A remarkable characteristic in *V. universitaria* pupa is the absence of a cocoon cutter, for which we have no clear explanation. The thin upper layer of its mines and flimsy cocoon within which pupation of this species occurs may not need much efforts to be cut to facilitate adult emergence. However, this structure can be found in species with similar life habits, that is, in pupal stages developing in the interior of the leaf mines (e.g., Brito *et al*. 2012). Also in the pupa of *V. universitaria*, the two comma-shaped depressions dorsally on the pronotum are notable, a characteristic shared with other genera of the *Gracillaria* group such as *Caloptilia* (Patočka & Zach 1995; Patočka & Turčani 2005). The number and shape of this structure may provide key taxonomic features and should be further explored.

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Figure Legends:

FIGURE 1. Bayesian consensus tree for *Vallissiana universitaria* based on the analysis the mitochondrial cytochrome oxidase c subunit I gene ('DNA barcode' region). Colored branches indicate Bayesian posterior probability (BPP), as indicated in the legend.

FIGURE 2. Adult male of *V. universitaria*: (A) wing spread moth, dorsal; head (B) dorsal, and (C) lateral. Scale bars: (A) 1 mm, (B–C) 200 μm.

FIGURE 3. Wing venation, abdomen and genitalia morphology of *V. universitaria*: (A) forewing and hindwing venation; (B) male last, dorsal; (C) male genitalia, ventral (phallus omitted); (D) valva, region of cucullus (indicated by rectangular area marked in C), ventral; (E) valva, detail of process at basal third (indicated by area marked in C), ventral; (F) phallus, latero-ventral; (G) spines of cornuti in detail (indicated by rectangular area marked in F), latero-ventral; (H) female genitalia, ventral; (I) signum (indicated by square area marked in H), ventral; (J) sterigma in detail, (indicated by rectangular area marked in H) ventral. Scale bars: (A) 1mm; (B) 200, (C) 100, (D) 15; (E) 10; (F) 50; (G) 20; (H) 200; (I)10; (J) 50 μm.

FIGURE 4. Schematic representation of *V. universitaria* larva and pupa: sap-feeding larva of the second instar, (A) dorsal, (B) ventral; tissue-feeding larva of the fourth instar, (C) dorsal, (D)

ventral; pupa, (E) dorsal, (F) ventral; (G) chaetotaxy of last larval instar. Scale bars: (A–B) 100, (C–F) 500 µm, (G) 1 mm.

FIGURE 5. Morphology of *V. universitaria* sap-feeding larva (second instar): head, (A) dorsal (B) ventral, (C) lateral; (D) antenna in detail (indicated by rectangular area marked in A), dorsal; (E) mouthparts in detail (indicated by rectangular area marked in B), (asterisks indicate labial palpi and arrow points to spinneret), ventral; (F) spiracle on T1, lateral; prothoracic and mesothoracic segments, (G) dorsal, (H) ventral; (I) callus on T1 (indicated by square area marked in H), ventral. Scale bars: (A–C) 50, (D) 5, (E) 10, (F) 5, (G–H) 50 (I) 5 µm.

FIGURE 6. Morphology of *V. universitaria* tissue-feeding larva (fourth instar): head, (A) dorsal, (B) antero-ventral, (C) lateral; (D) labrum, dorsal; (E) spinneret, lateral; (F) maxilla, lateral; (G) antenna, lateral; (H) mesothoracic leg, lateral; (I) detail of tarsal claw (indicated by rectangular area marked in H), latero-posterior; (J) pseudopodium on A3, ventral; (K) spiracle on A4, lateral; (L) anal plate, postero-dorsal; (M) pseudopodia on A10, ventral. Scale bars: (A–C) 100, (D) 20, (E–G) 10, (H) 25, (I) 5, (J) 10, (K) 25, (L) 5, (M) 50 µm.

FIGURE 7. Pupal characters of *V. universitaria*. Head (A) dorsal, (B) ventral, (C) lateral; (D) left prothoracic depression in detail (indicated by rectangular area marked in A), dorsal; (E) spiracle on A3, latero-dorsal; (F) sixth and seventh abdominal segments, dorsal (right spiracles are indicated by arrows); last abdominal segment, (G) lateral, (H) dorsal; (I) lateral spine of last

abdominal segment in detail (indicated by square area marked in H). Scale bars: (A–C) 100, (D) 20, (E) 10, (F) 100, (G–H) 50, (I) 10 µm.

FIGURE 8. Life history of *V. universitaria*: (A) host plant *Erythroxylum argentinum* at the type locality; (B) leaf mines; (C) egg on the adaxial surface; (D) leaf mine bearing a sap-feeding larva (viewed through transparent epidermis asindicated by arrow); dissected leaf-mines showing (E) sap-feeding, (F) tissue-feeding larvae, and (G) pupa, dorsal; (H) pupal exuvium partially protruding from leaf-mine. Scale bars: (B) 10, (C) 0.1, (D) 5, (E) 0.5 (F–H) 1 mm.

FIGURE 9. Transverse histological sections of *V. universitaria* mine on *Erythroxylum argentinum* leaf, showing changes in damage throughout larval ontogeny. (A) sap-feeding instar uses the adaxial epidermis (closed arrows point to cut cell walls of adaxial epidermis); (B) first tissue-feeding instar starts using the upper cell layers of palisade parenchyma (open arrows point to cell fragments of parenchyma left attached to damaged epidermis); (C) last tissue-feeding instar causes general damage, consuming all parenchyma cells.. **Ad**, adaxial surface of epidermis; **Ab**, abaxial surface of epidermis; **Lm**, leaf mine; **Pp**, palisade parenchyma; **Sp**, spongy parenchyma. Scale bars = (A-C) 100µm.

Table 1. Specimens those DNA barcode sequences were used in this study to infer relationships of *V. universitaria* gen. et sp. n. within Gracillariidae.

Subfamily	Genus	Species	Sample ID	Accession Number		Reference
				GenBank	BOLD	
Gracillariinae	**Ingroup**					
	Aristaea	*pavoniella*	TLMF Lep 09206	KP253609	PHLAI644-13	Huemer & Hebert (unpub.)
	Aristaea	sp. n.	CLV16407	KU380288	GRACI054-07	Kawahara *et al.*(2017)
	Sabulopteryx	*inquinata*	TLMF Lep 10404	KP150259	LEATB227-13	Huemer (2014)
	Sabulopteryx	*limosella*	CLV2267	KX045193	GRSLO652-11	Mutanen *et al.* (2016)
	Aspilapteryx	*multipunctella*	TLMF Lep 03530	KX042619	GRSLO652-11	Mutanen *et al.* (2016)
	Aspilapteryx	*tringipennella*	STG34	KX042203	TIPSY413-12	Mutanen *et al.* (2016)
	Caloptilia	*acericola*	RN-12	LC127790	-	Nakadai & Kawakita (2016)
	Caloptilia	*kurokoi*	RN-10	LC127788	-	Nakadai & Kawakita (2016)
	Caloptilia	*kisoensis*	RN-09	LC127787	-	Nakadai & Kawakita (2016)
	Calybites	*trimaculata*	RN-88	LC127819	-	Nakadai & Kawakita (2016)
	Calybites	*phasianipennella*	G114AK	GU816418	-	Kawakita *et al.* (2010)
	Cupedia	*cupediella*	PHA1	KX049810	LNOUD383-11	Mutanen *et al.* (2016)
	Eucalybites	*aureola*	CLV21407	GU073228	GRACI102-07	De Prins *et al.* (2009)
	Euspilapteryx	*auroguttella*	NHMO-06060	KX049179	LON060-08	Mutanen *et al.* (2016)
	Gracillaria	*syringella*	CLV2540	KX045513	GRPAL477-11	Mutanen *et al.* (2016)
	Gracillaria	*ussuriella*	RN-20	LC127798	-	Nakadai & Kawakita (2016)
	Gracillaria	cf. *japonica*	RN-2016	LC127810	-	Nakadai & Kawakita (2016)
	Mercantouria	*neli*	TLMF Lep 16938	KU687411	LECRT028-15	Huemer *et al.* (2016)
	Vallissiana	*universitaria* gen. et sp. n.	CMP 002-7A	MK058444	GRABR011-18	This study
	Vallissiana	*universitaria* gen. et sp. n.	CMP 002-7B	MK058445	GRABR012-18	This study
	Povolnya	*obliquatella*	SWC-06-0265	KF523817	LTOLB088-08	Mitter *et al.* (unpub.)
	Povolnya	*leucapennella*	G04leuc	KU687409	GRACI444-09	Huemer *et al.* (2016)
	Outgroup					
Acrocercopinae	*Acrocercops*	*brongniardella*	CLV2514	KX045761	GRPAL451-11	Mutanen *et al.* (2016)
Ornixolinae	*Parectopa*	*robiniella*	BC ZSM Lep46386	KX040633	FBLMX070-11	Mutanen *et al.* (2016)

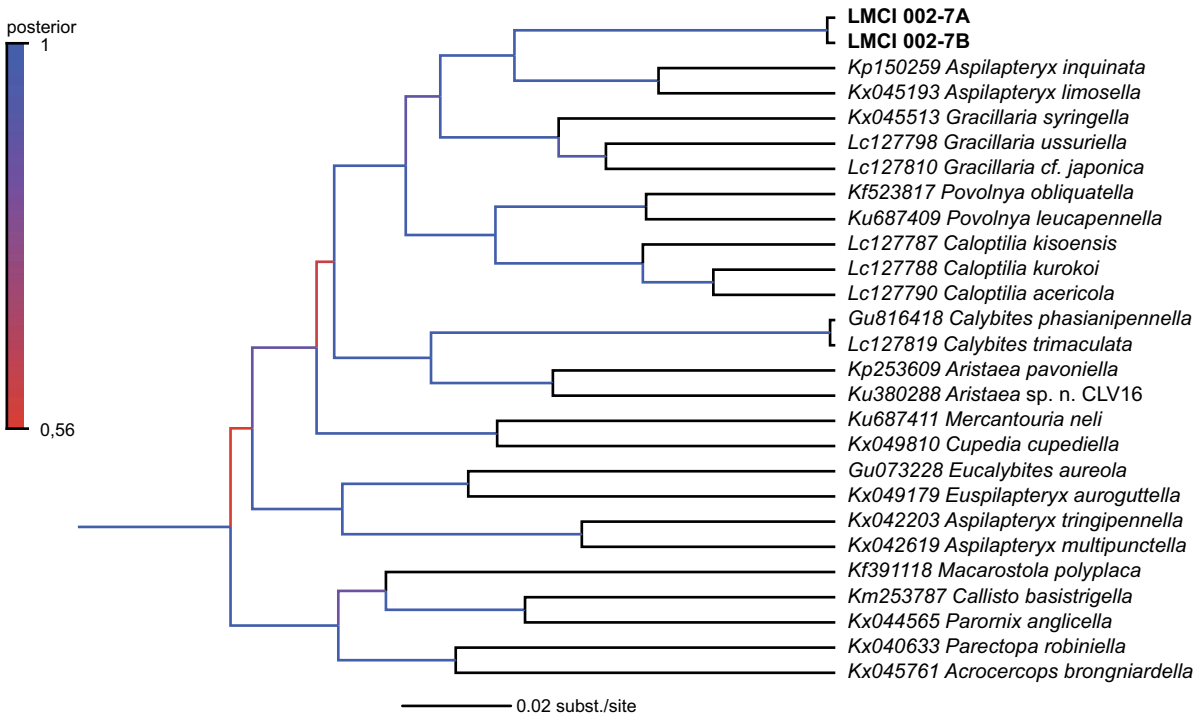
Table 2. Pairwise genetic distance between *V. universitaria* and representative species of the subfamily Gracillariinae, based on 658 base pair sequences of the cytochrome oxidase I (*CoI*) gene using the Kimura 2-parameter model. Divergence to species of the subfamilies Acrocercopinae and Ornixolinae, treated as outgroups, is also presented.

			1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.
Gracillariinae	1.	*Aristaea pavoniella*	-	-																					
	2.	*Aristaea* sp. n.	0.11	-																					
	3.	*Aspilapteryx multipunctella*	0.12	0.15	-																				
	4.	*Aspilapteryx tringipennella*	0.14	0.15	0.09	-																			
	5.	*Caloptilia acericola*	0.13	0.13	0.12	0.13	-																		
	6.	*Caloptilia kurokoi*	0.15	0.14	0.14	0.15	0.04	-																	
	7.	*Caloptilia kisoensis*	0.16	0.15	0.15	0.14	0.07	0.08	-																
	8.	*Calybites trimaculata*	0.15	0.16	0.15	0.16	0.15	0.16	0.15	-															
	9.	*Calybites phasianipennella*	0.15	0.18	0.17	0.18	0.16	0.18	0.17	0.00	-														
	10.	*Cupedia cupediella*	0.13	0.10	0.12	0.13	0.12	0.12	0.13	0.16	0.17	-													
	11.	*Eucalybites aureola*	0.15	0.16	0.15	0.15	0.15	0.15	0.17	0.19	0.21	0.17	-												
	12.	*Euspilapteryx auroguttella*	0.14	0.15	0.13	0.14	0.12	0.13	0.14	0.17	0.19	0.13	0.15	-											
	13.	*Gracillaria syringella*	0.15	0.15	0.14	0.16	0.12	0.12	0.13	0.18	0.20	0.15	0.15	0.13	-										
	14.	*Gracillaria ussuriella*	0.13	0.18	0.14	0.16	0.11	0.12	0.12	0.17	0.18	0.15	0.16	0.15	0.10	-									
	15.	*Gracillaria cf. japonica*	0.15	0.15	0.13	0.15	0.11	0.12	0.11	0.16	0.17	0.13	0.17	0.12	0.08	0.08	-								
	16.	*Mercantouria neli*	0.15	0.15	0.17	0.16	0.14	0.13	0.15	0.18	0.20	0.12	0.19	0.17	0.14	0.14	0.14	-							
	17.	***Vallissiana universitaria* gen. et sp. n.**	**0.12**	**0.13**	**0.14**	**0.14**	**0.12**	**0.13**	**0.13**	**0.16**	**0.17**	**0.12**	**0.14**	**0.14**	**0.12**	**0.13**	**0.15**	**0.14**	-						
	18.	*Povolnya obliquatella*	0.12	0.15	0.14	0.13	0.09	0.10	0.11	0.14	0.15	0.12	0.15	0.12	0.14	0.12	0.12	0.15	**0.13**	-					
	19.	*Povolnya leucapennella*	0.12	0.15	0.13	0.13	0.09	0.09	0.10	0.15	0.17	0.11	0.14	0.12	0.12	0.11	0.11	0.14	**0.13**	0.06	-				
	20.	*Sabulopteryx inquinata*	0.12	0.14	0.14	0.14	0.13	0.14	0.12	0.16	0.17	0.12	0.16	0.15	0.13	0.12	0.13	0.14	**0.12**	0.11	0.11	-			
	21.	*Sabulopteryx limosella*	0.13	0.15	0.16	0.16	0.15	0.15	0.14	0.16	0.17	0.13	0.17	0.15	0.15	0.14	0.14	0.15	**0.14**	0.14	0.13	0.07	-		
Acrocercopinae	22.	*Acrocercops brongniardella*	0.17	0.16	0.17	0.16	0.11	0.13	0.14	0.16	0.18	0.15	0.17	0.16	0.17	0.17	0.16	0.18	**0.15**	0.14	0.13	0.15	0.17	-	

Ornixolinae	23.	*Parectopa robiniella*	0.17	0.17	0.17	0.17	0.13	0.14	0.16	0.19	0.21	0.15	0.18	0.18	0.17	0.16	0.15	0.18	**0.16**	0.16	0.15	0.16	0.16	0.15	-

Table 3 Variation of head capsule width among instars of *V. universitaria* reared on *E. argentinum* (n = 10 per instar).

Head capsule width (mm)			
Instar	**Mean ± standard error**	**Range (min – max)**	**Growth rate**
I	0.19±0.002	0.182–0.195	-
II	0.26±0.003	0.260–0.286	1.40
III	0.35±0.003	0.338–0.364	1.31
IV	0.47±0.003	0.455–0.494	1.33



Pereira et al.
Fig 1.

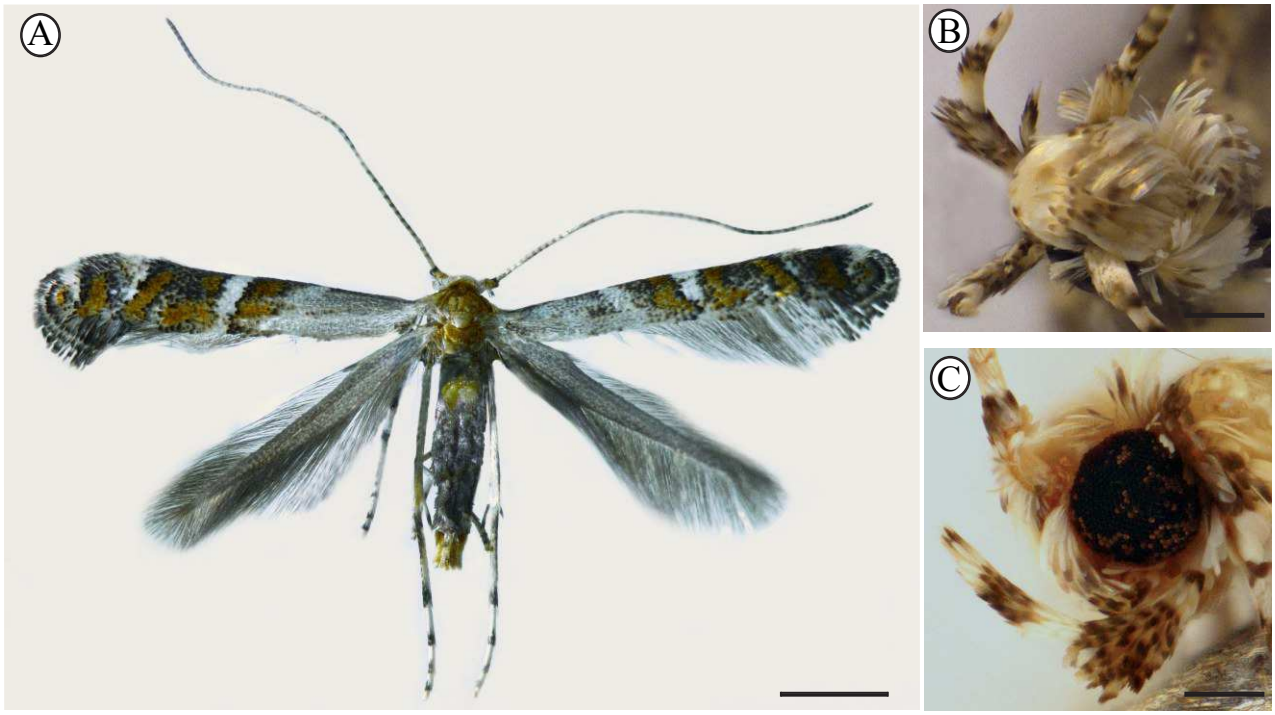


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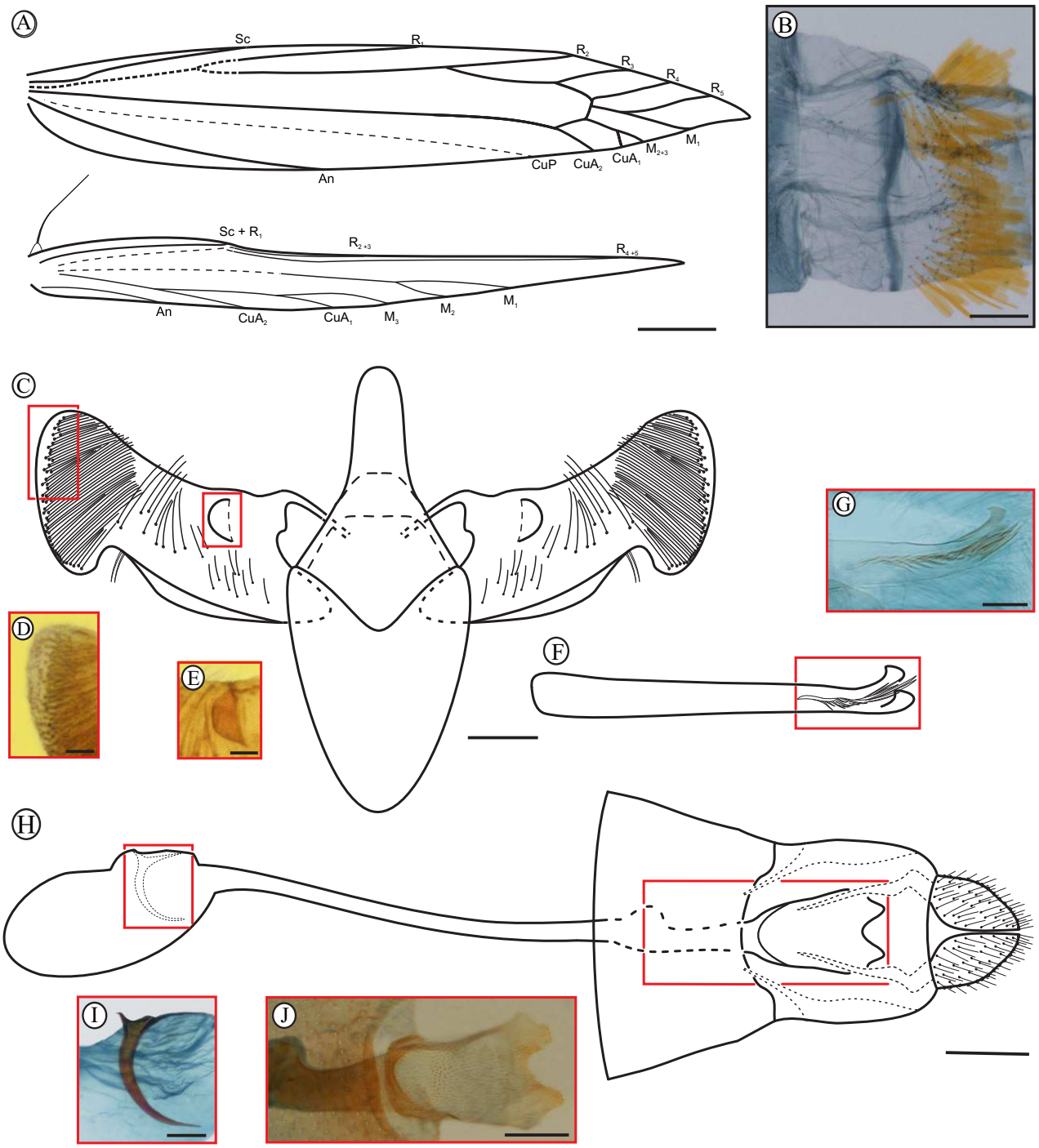
Fig 2.



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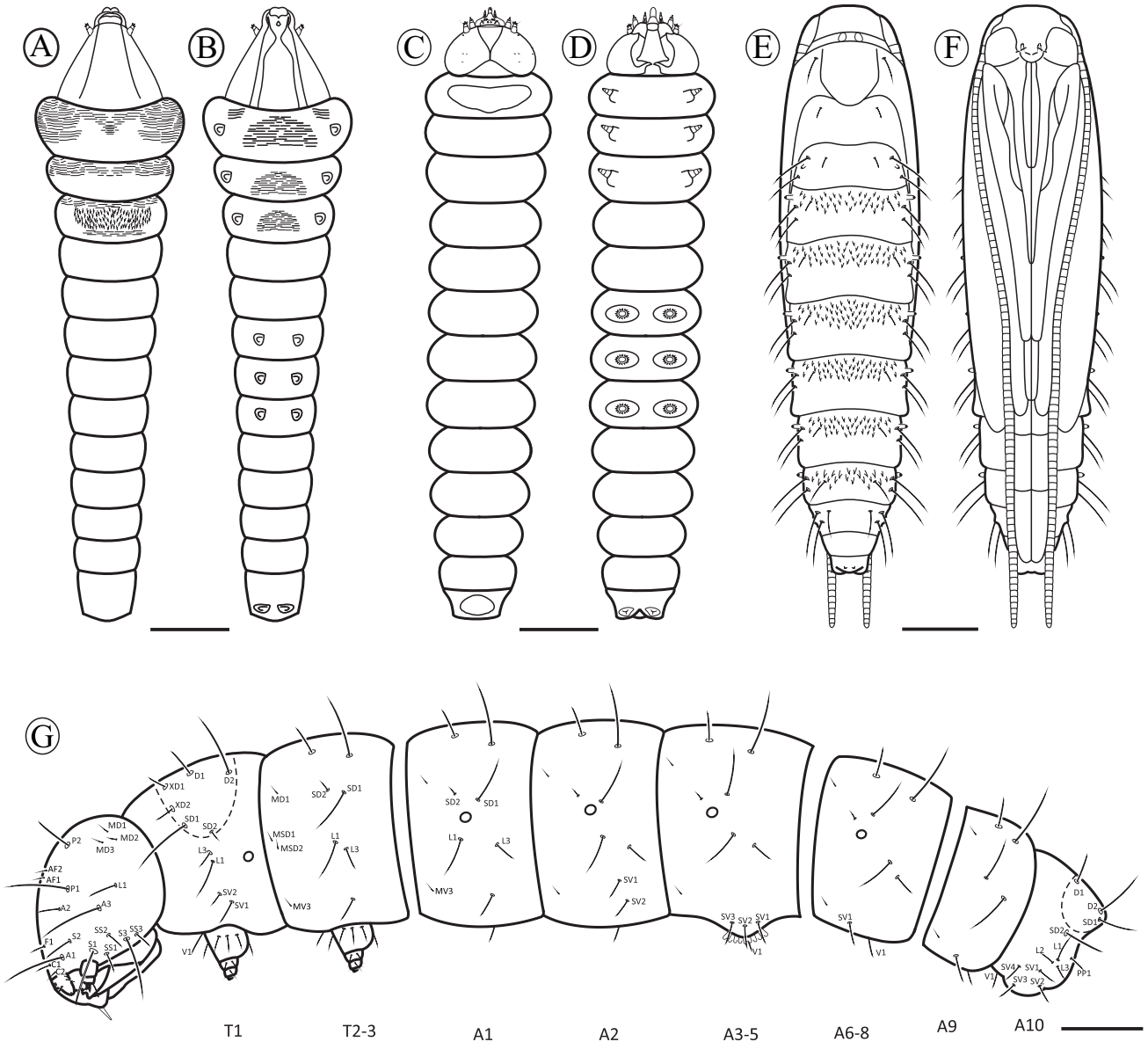

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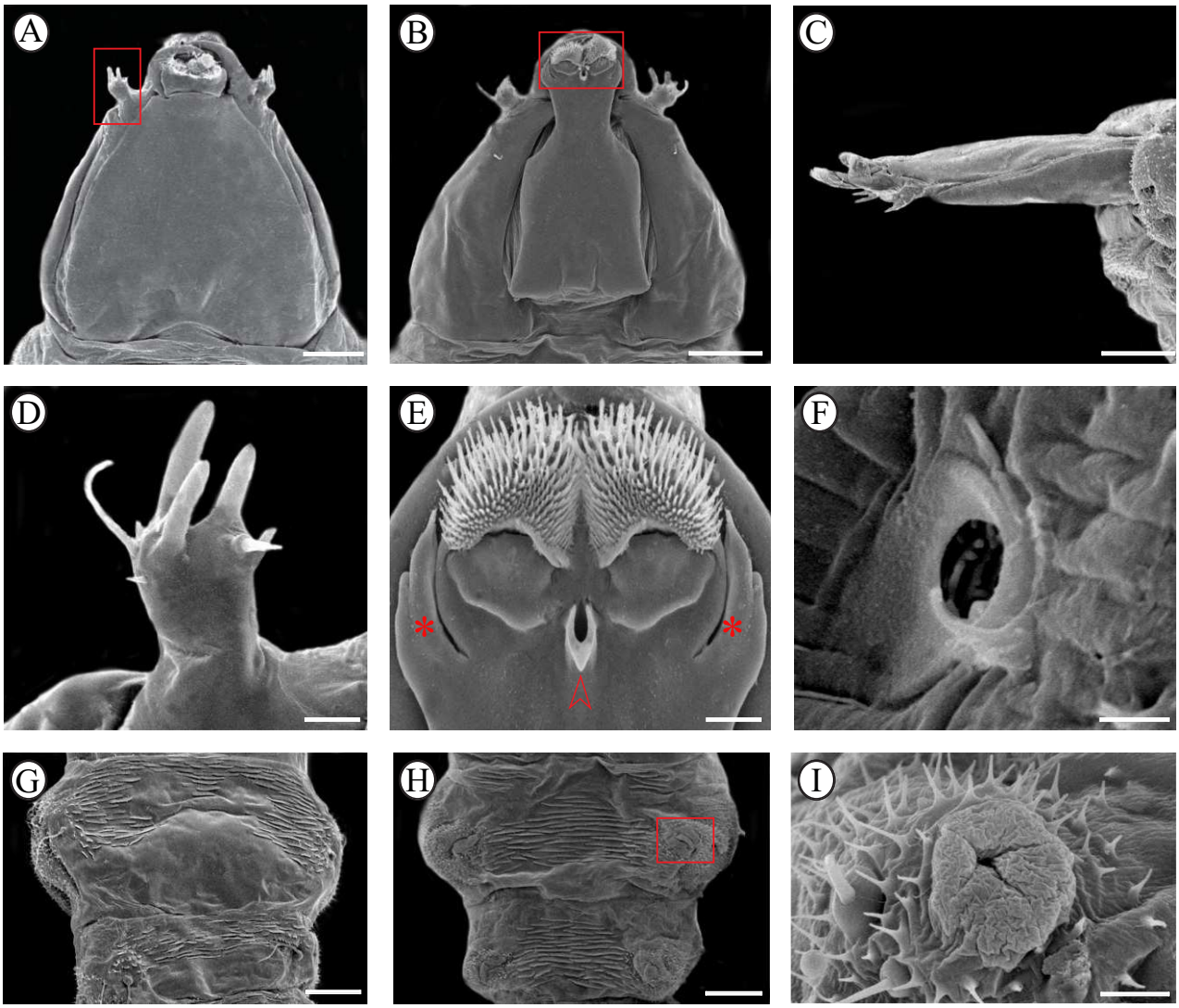
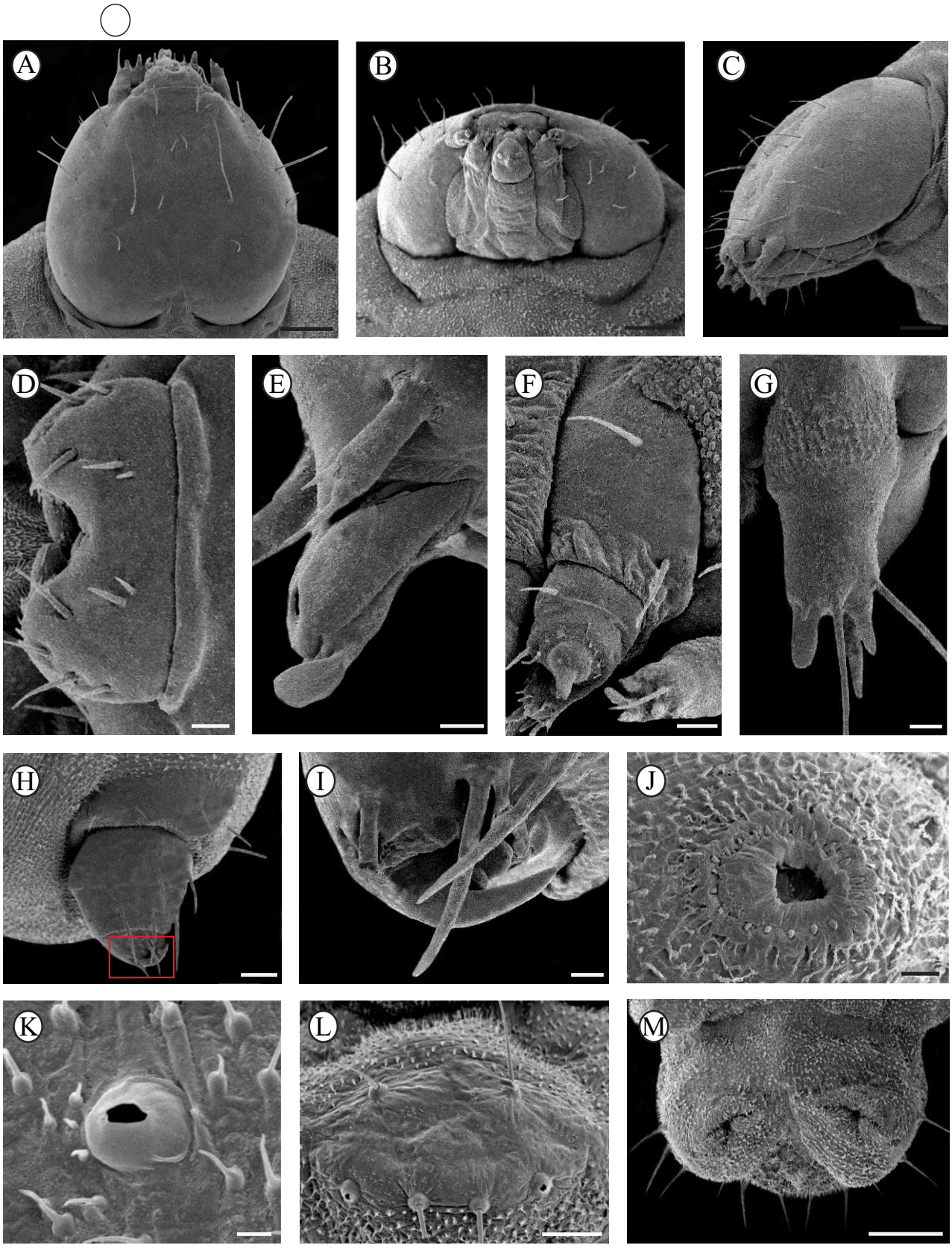
Fig 4.



Pereira et al.
Fig 5.



Pereira et al.
Fig 6.

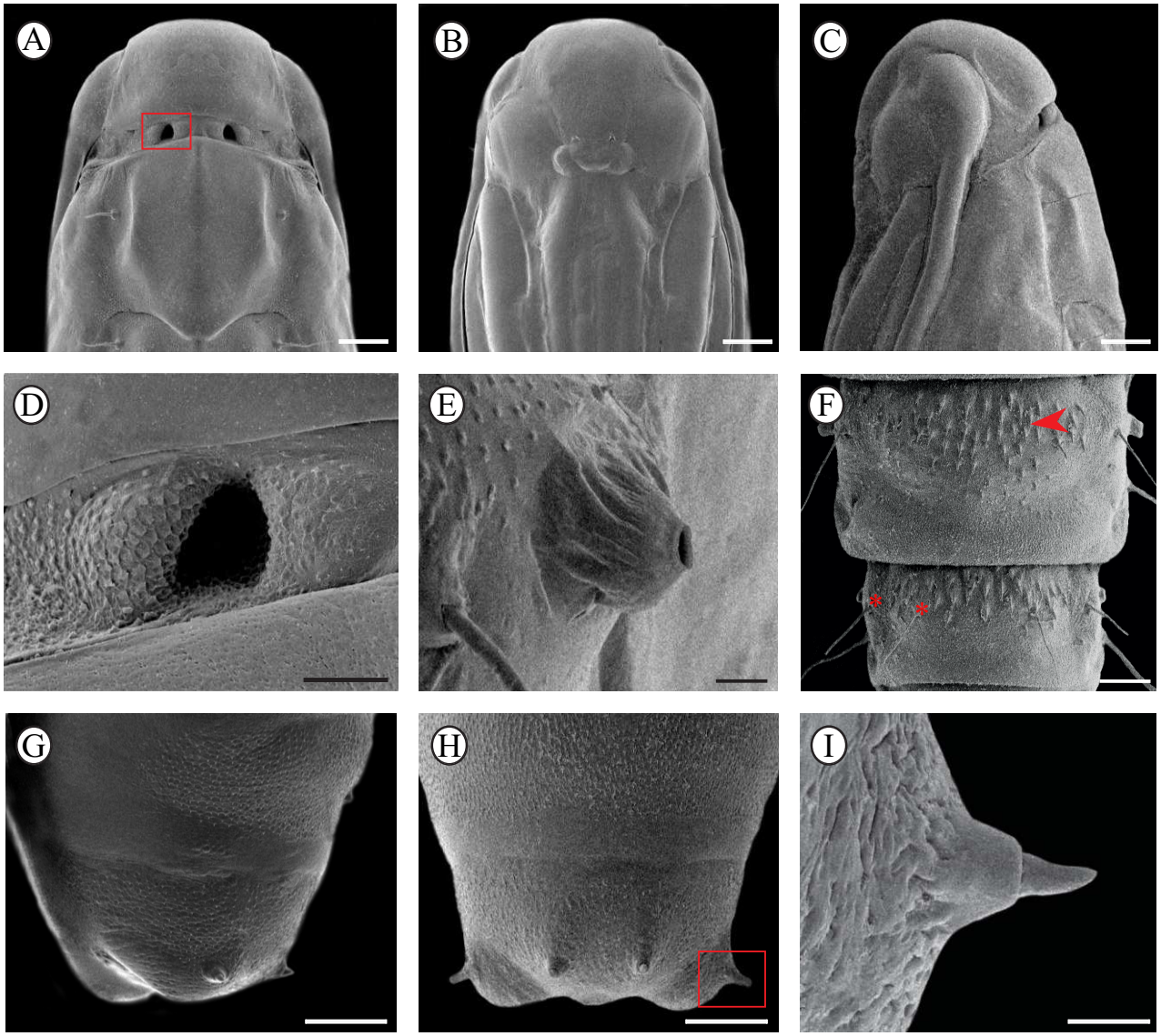


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Fig 7.

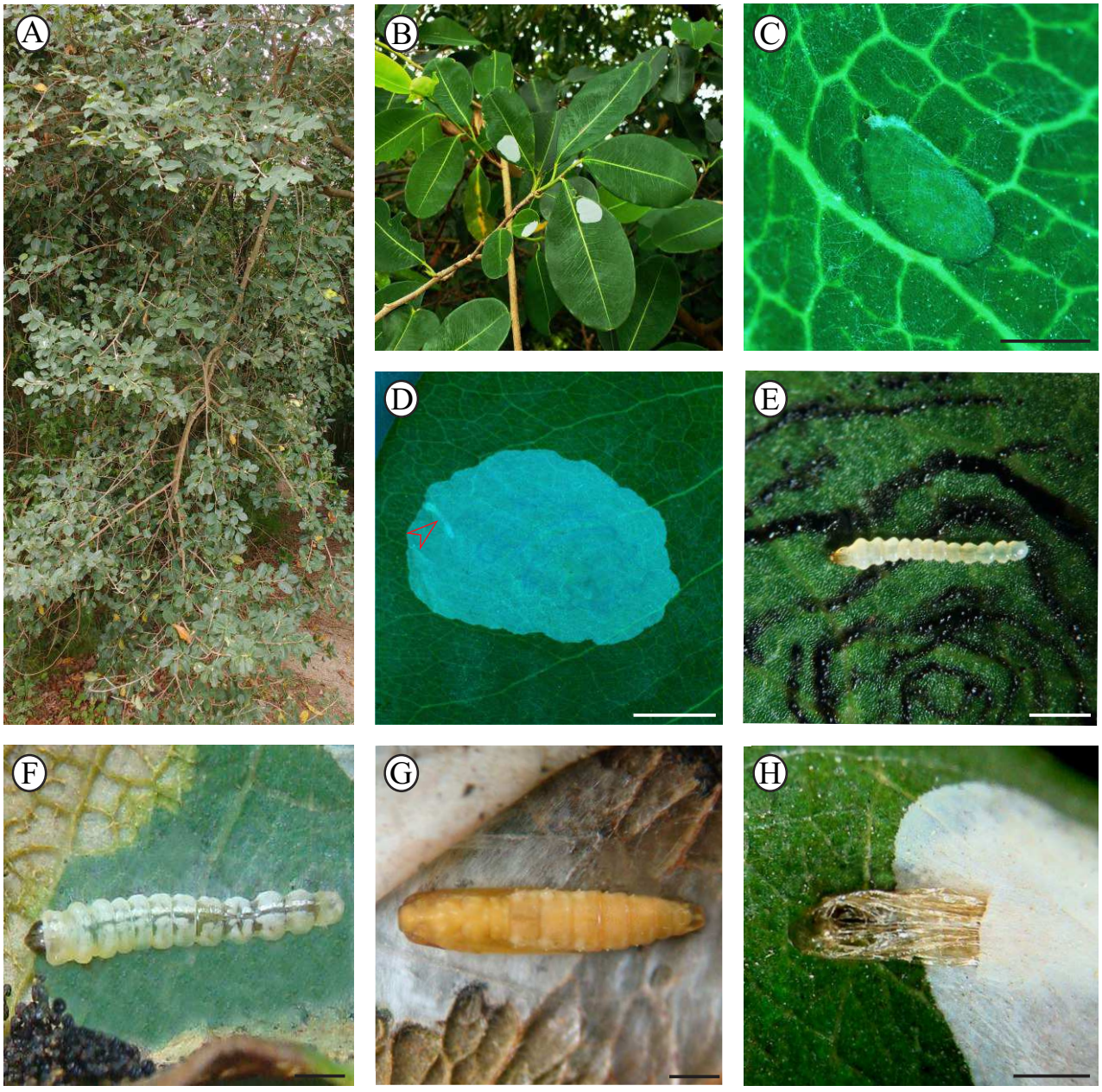

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Fig 8.

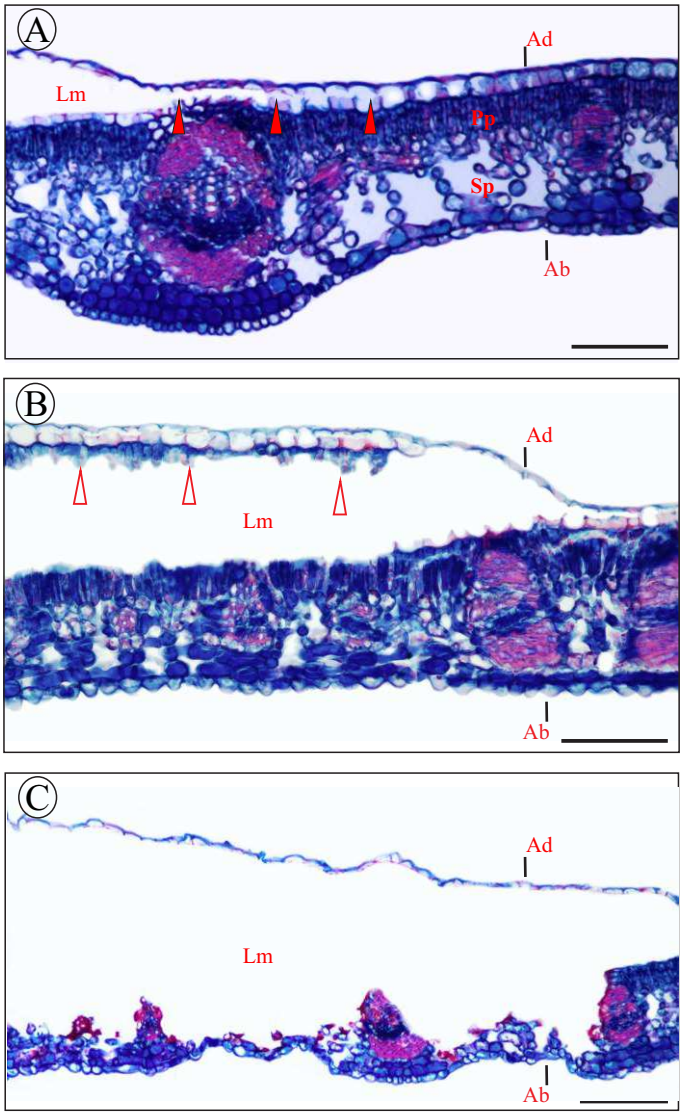

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Fig 9.

CAPÍTULO V

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Running title: A new many-plumed moth from the Brazilian Cerrado

A new cecidogenous species of many-plumed moth (Alucitidae) associated with *Cordiera* A. Rich. ex DC. (Rubiaceae) in the Brazilian Cerrado

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Abstract. Larvae of many-plumed moths (Alucitidae), especially in the world-wide genus *Alucita* Linnaeus, are known as feed-borers or gall-inducers on flowers, fruits and shoots of a few dicotyledonous families, including Bignoniaceae, Caprifoliaceae and Rubiaceae. However, there is

no study available on the biology of the monotypic, Neotropical genus *Prymnotomis* Meyrick, except for its original description that was based on a single male, the holotype of *P. crypsicroca* from Espirito Santo, Brazil. We describe here a second species for this genus, *Prymnotomis cecidicola* sp. n. whose larvae induce galls on *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae), a dioecious plant with dimorphic inflorescences found in the Brazilian Cerrado, Planaltina City, Federal District. Adults, larvae, pupae and galls are illustrated under light and scanning electron microscopy. Galls are green, spherical, unilocular and develop individually on *C. elliptica* flower buds. During development they look like fruits in shape and colour but are larger, do not have style scars when on female plants, and are induced also in male inflorescences. Pupation occurs outside the gall within a silk cocoon, presumably in the litter. A preliminary analysis of DNA barcode sequences including putative members of other alucitid lineages and Neotropical BINs (Barcode Index Number) supports *Prymnotomis cecidicola* sp. n. as an independent phylogenetic unit, with 12 to 18% divergence. Its nearest-neighbour was the BIN cluster 5 (BOLD:AAA0842) that includes specimens from Costa Rica.

Key words. Alucitid moths, Brazilian Savanna, insect galls, *Prymnotomis*, taxonomy

Introduction

Alucitidae is a small, worldwide family of apoditrysian moths with nine valid genera and *ca.* 216 species (Gielis 2003, Nieukerken et al. 2011). Several additional alucitid species have been described for the family recently, particularly in the tropics (e.g. Vargas 2011, Ustjuzhanin & Kovtunovich 2016, Ustjuzhanin et al. 2018), and many are supposed yet to be discovered in these regions. A total of 26 species of alucitids are found in the Neotropics, of which only six have been recorded in Brazil (Gielis 2003, Vargas 2011). These micromoths are well known by their

specialized fore- and hindwings, which are multiply divided into lobes that look like bird feathers. Larvae are usually feed-borers or gall-inducers, associated with flowers, fruits and shoots of a few plant families including the Bignoniaceae, Caprifoliaceae and Rubiaceae (Dugdale et al. 1998). In contrast to the adults, their immature stages are poorly known, especially in the Neotropical region where the host plants for a few species have been documented, particularly within *Alucita* Linnaeus, the most speciose, worldwide genus (e.g. Vargas 2011). Four other endemic genera have been described for the region (*Hexeretmis* Meyrick, *Prymnotomis* Meyrick, *Paelia* Walker, and *Alinguata* Fleming); to the best of our knowledge, none of the host plants or the immature stages of these genera have been identified so far (see also Lima 1945 and Pastrana 2004).

Prymnotomis was proposed by Meyrick (1931) to include *P. crypsicroca* Meyrick, that was described briefly based on the general morphology of only one adult male from Espírito Santo, Brazil. It has remained as a monotypic genus since the original description, and there was no information added on its biology afterwards. This study concerns a second, new species of *Prymnotomis* that induces conspicuous, spherical galls on inflorescences of *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae), a native shrub of the Brazilian Savanna (Cerrado Biome). A preliminary comparison of genitalia structures suggested that it is congeneric but does not conform to *P. crypsicroca*. Thus in this study we describe and illustrate the adult, larva, pupa and the gall under light and scanning electron microscopy, and provide information on the natural history of this new species. An analysis of DNA barcode sequences including putative members of other alucitid lineages, as well as Neotropical BINs (Barcode Index Number; Ratnasingham & Hebert 2013) is also provided to estimate the phylogenetic position and genetic distances of the new species.

Material and Methods

Adult specimens were reared by VO Becker from galls collected during October of 1981, 1982, and 1983 at the Centro Nacional de Pesquisa Agropecuária dos Cerrados (Embrapa Cerrados), Planaltina City, Federal District, Brazil (15°36'26.4"S, 47°42'52.4"W), and maintained in small plastic pots under room temperature in the Laboratório de Entomologia of the same Institution. They were checked daily for the emergence of adults, which were pin-mounted and dried. Immatures used for descriptions were dissected from additional galls that were collected by C.M. Pereira and A. Specht at same locality during September 2018, and brought to the Laboratório de Morfologia e Comportamento de Insetos (LMCI), Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre city, Rio Grande do Sul state, Brazil, where they were reared in a similar manner. They were fixed in Dietrich´s fluid and preserved in 75% ethanol. Additional larvae used for DNA extraction were preserved in100% ethanol at -20 ^{o}C.

For descriptions of adults, genitalia were dissected and cleared in a 10% potassium hydroxide (KOH) solution, stained with either eosin or Chlorazol black E and slide-mounted in Canada balsam, following Robinson (1976). Last instar larvae were prepared similarly for the study of 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 Photo-Paint® X7, using the corresponding digitalized images as a guide.

Additional specimens were used for scanning electron microscope analyses. They 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 examined and photographed in a JEOL® JSM6060 scanning electron microscope at the Centro de Microscopia e Microanálise (CMM) of UFRGS.

Terminology used in descriptions followed Heppner (1987), Patočka & Turčani (2005) and Landry & Landry (2004) for the larva, pupa and adults.

DNA sequencing and analysis

DNA was extracted from larvae of four specimens (CMP 008-01A, D) of *Prymnotomis* sp. n using the PureLink Genomic DNA extraction kit (Thermo Fisher Scientific, Carlsbad, California). Extracted DNA was resuspended in 80 mL of Tris: EDTA (10 mm Tris-HCl, 1 mm EDTA, pH 8.0). DNA barcoding PCR was conducted using primers LCO1490 and HCO2198 (Folmer et al. 1994), which amplify ca. 500 bp of the mitochondrial gene cytochrome oxidase (COI). PCR reactions were conducted using 2 µL of the extracted DNA. The thermal cycler profile consisted of 35 cycles of 94 ^{o}C for 45 s, 48 ^{o}C for 45 s and 72 ^{o}C for 45 s. Excess dNTP and primers were removed and the amplified DNA concentrated using exonuclease I and FastAP thermosensitive alkaline phosphatase (Thermo Fisher Scientific). Samples were sequenced in both directions using BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific) following standard procedure according to manufacturer's instructions, and analysed in an ABI3730XL (Thermo Fisher Scientific, Waltham, USA) automatic sequencer. The new data were deposited in BOLD Systems (http://www.boldsystems.org/) under the project MISA. The barcode sequences were aligned using CodonCode Aligner (CodonCode Corp, Massachusetts, USA).

To explore the phylogenetic position of the new species within the family we used the COI data generated for *Prymnotomis cecidicola* sp. n. with a published dataset of 40 Alucitidae (Table 1). Specifically, the genera *Alucita* Linnaeus and *Pterotopteryx* Hannemann were included in the analysis, as well as five BIN clusters (BOLD: AAH5751, AAG9907, AAJ6491, AAU0280 and AAA0842) that were identified in the Neotropics, publicly available in BOLD (Table 1). The tree was rooted with *Isonomeutis amauropa* Meyrick (Copromorphidae) according to the phylogeny

proposed by Mutanen et al. (2010). We used the maximum likelihood algorithm, which was performed in PHYML v3.0 (Guindon et al. 2010) using 1000 replicates of heuristic search, with random addition of sequences and TBR branch swapping. The Tamura-Nei substitution model was selected based on the Akaike information criterion run in MEGA v6 (Tamura et al. 2013). Monophyly confidence limits were assessed with the bootstrap method at a 50% cut-off after 1000 iterations. Pairwise genetic distances between the new species and other Alucitidae were quantified using the Kimura 2-parameter model in MEGA v6.

Museum collections

AMNH – American Museum of Natural History, New York, NY, USA;

DZUP -- Coll. Padre Jesus S. Moure, Departamento de Zoologia, Universidade Federal do Paraná, Curitiba, PR, Brazil;

LMCI -- Coll. Laboratório de Morfologia e Comportamento de Insetos, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil;

NM -- Natural History Museum, Vienna, Austria;

NHMUK -- Natural History Museum, London, United Kingdom;

VOB -- Coll. Vitor O. Becker, Reserva Serra Bonita, Camacan, BA, Brazil.

Results

Molecular data

Sequencing of COI resulted in an average amplicon size of 500 bp. The aligned data matrix had 683 characters, of which 227 (33%) were phylogenetically informative. Maximum likelihood analysis recovered an optimal ML ln likelihood tree = 5937 with nucleotide frequencies of A=31.9%, C= 15.2%, G=14.6% and T=38.3%. %. In the preliminary barcode tree all Neotropical

specimens clustered, including *Prymnotomis cecidicola* sp. n. and the five BIN taxa. Specimens of *Alucita* and *Pterotopteryx* grouped in a second clade, the Nearctic + Palearctic (Fig. 1). The nearest neighbour of *P. cecidicola* was the BIN cluster 5 BOLD:AAA0842 (ca. 12% genetic distance), which included specimens from Costa Rica (data from BOLD). Pairwise genetic distance of the new taxon to BIN clusters and lineages within Alucitidae ranged from 12% to 18%, with the highest divergence to *Pterotopteryx dodecadactyla* (Hübner) (Table 2).

Taxonomy

***Prymnotomis cecidicola* Moreira and Becker, new species** (Figs. 2-48, 51, 54)

Diagnosis

P. cecidicola shares with the closely related *Hexeretmis* Meyrick the porrect maxillary palpi, forewing cleft only to 1/5 from termen, and pattern of wing venation. However, it differs from this genus in general appearance in coloration pattern and by presenting shallower hindwing clefts (*ca.* 1/3 of wing length), as pointed out by Meyrick (1931). *P. cecidicola* can be separated from the congeneric *P. crypsicroca* Meyrick by the smaller size of the latter, whose forewing length of the type specimen measures *ca.* 6 mm, and by the less contrasting coloration of *P. crypsicroca*, especially in relation to the hindwing. The basal process of the valva of the male genitalia is upturned, finger-like, covered with sparse filiform setae in *P. crypsicroca*, while in *P. cecidicola* it is turned down, looking like a claw, bearing short, stout spines on the base.

Description of adults (Figs. 2-19)

Male. Forewing length (mean + standard error) = 9.34±0.45 mm; n = 6. Body with most scales greyish brown, interspersed by either isolated or small patches of either entirely light grey or

bicoulored scales, mostly with whitish beige at the base and pale greyish brown at the apex (Fig. 2). Palpi and thoracic legs a little lighter at distal portion of each segment (Figs. 7, 8). Abdominal segments also lighter ventrally. Head bearing paired tufts of scales on posterior vertex that project mesally, forming a collar (Fig. 6). Similar to description of *P. crypsicroca* provided by Meyrick (1931): maxillary palpus small; labial palpus well developed, porrected, with middle segment more than double the other two in length (Fig. 7); antennae filiform, reaching *ca.* 2/3 forewings in length, with flagellomeres ventrally ciliated; forewings mostly dark grey; a wavy whitish line on costal edge marking alternate dark fuscous spaces; a short and fine whitish, transversal bar on end of cell; three wavy whitish parallel lines crossing the terminal lobes (Fig. 2), proximal one weakly defined, restricted to basal intersections (Fig. 3); fringe concolours with adjacent scales; hind wings mostly dark grey distally, with basal half whitish, densely permeated on base with dark grey scales (Fig. 4); three fine white wavy lines on lobes and associated fringes (Fig. 5), similar to description of forewings.

Genitalia (Figs. 9-16)*:* Uncus narrow, downcurved near middle and spatulate (Figs. 9, 11, 13), with distal margin showing medially a slightly developed, pointed process. Median arm of gnathos strait and flattened, with a bow-shaped, distal margin bearing filiform setae (Figs. 9, 11, 14). Tegumen short, compact, rounded dorsally (Fig. 9). Juxta straight, narrow and flat, forked on both ends (Figs. 11, 16) with a pair of long, cylindrical arms bearing minute and sparse setae on widened distal ends (Figs. 11, 15). Valva membranous, with cucullus well developed, widened in distal half, bearing long filiform setae on distal, rounded margin; basal process well developed and sclerotized, claw-like, bearing a few stout spines on base (Figs. 9-11). Aedeagus similar in length to valva, tubiform, slightly curved, bearing a pair of indistinctly shaped, sclerotized plates apically. Vesica with elongated and narrow plates of indistinct shape. Coecum penis *ca.* two-thirds of total length of aedeagus (Figs. 9, 12).

197

198 *Female*. Virtually no differences from male regarding size and coloration. Genitalia (Figs. 17-19):
199 Papillae anales elongated, narrowly rounded apically, bearing sparse, long and short setae (Fig.
200 19). Eighth tergum broad with relatively long setae at distal on ventral margin. Posterior and
201 anterior apophyses thin, similar to each other in length. Ostium bursae broad, opening near
202 posterior margin of eighth sternum; antrum narrower, wide medially, slightly sclerotized on distal
203 margin; ductus bursae membranous, wide medially, similar to anterior apophyses in length. Ductus
204 seminalis inserted on distal third of ductus bursae. Corpus bursae membranous, ovoid, *ca.* 2/3
205 ductus bursae in length, wall covered with minute microtrichea (Fig. 18), without signum.

206

207 *Etymology*

208 The species name is derived from the Greek *kekis –idos* = gall + the Latin *co –col* = with; to be
209 treated as feminine.

210

211 *Material examined*:

212 All specimens examined came from galls associated with *Cordiera elliptica* (Cham.) Kuntze
213 (Rubiaceae) at Embrapa Cerrados, as already described. Adults were reared by VO Becker, from
214 galls collected during October 1982-83 (LMCI 313 series). Immatures were either dissected or
215 reared by CM Pereira and Alexandre Specht, from galls collected by CM Pereira (LMCI 349
216 series) on 19.x.2018. Additional galls were collected by GRP Moreira & J Fochezato (LMCI 346
217 series) on 1-4.xii.2018. Holotype ♂, BRAZIL: DF, Planaltina, 1100 m, 14.i.1985, ex *Cordiera*
218 *elliptica*(Cham.) Kuntze (Rubiaceae) (*Becker,* 57100) (VOB). Paratypes: 11 ♂♂, 4 ♀♀,
219 3.xii.1984-8.i.1985, same data as holotype; 1 ♀, same data as holotye, but 20.ii.1976, at light
220 (*Becker,* 19564); 2 ♂♂, 1 ♀, same data as holotype, but 15-30.xii.1982 (*Becker,* 40740); 1 ♂,

same data as holotype, but 15.x.1982, at light (*Becker,* 40613); 11 ♂♂, 1 ♀, same data as holotype, but 14.xi-23.xii.1983, at light (*Becker,* 41731); 1 ♂, same data as holotype, but 5.i.1984, at light (*Becker,* 56053). Additional paratypes, same data as holotype: 3♂♂ (VOB 57100, 3.xii.1984, deposited LMCI 313-869; VOB 41731, 26.xii.1983, deposited at LMCI 313-874; LMCI 313-879 / VOB 40740, donated to DZUP/DZ 33.402), and 3♀♀ (VOB 57100, 19.xii.1984, deposited LMCI 313-870 ; VOB 41731, 5.xii.1983, deposited at LMCI 313-876; VOB 41731, 14.xii.1983, donated to DZUP/DZ 33.412). Not paratypes: 1 ♀, GO, Ipameri, 10.X.1988 (*Becker,* 59682); MG, Nova Lima, 850m: 2 ♂♂, 25-27.xii.1982; 2 ♂♂, 1-10.i.1985; 1 ♀, 30.xii.1988; all at light (*Becker,* 50290, 55772, 60523) (VOB, USNM, NHMUK). Pinned-dried adults with genitalia preparations mounted in Canada balsam on slides – 2♂♂ (VOB 40740, 17.i.1983, deposited at LMCI 313-867; VOB 40740, 15.xii.1982, deposited at LMCI 313-880); 2♀♀ (VOB 57100, 6.xii.1984, deposited at LMCI 313-872; VOB 40740, 30.xii.1982, deposited at LMCI 313-878). Immatures fixed in Dietrich´s fluid and preserved in 70% ethanol - three last instar larvae (VOB 1519, 2.x.1984, deposited at LMCI 313-865A); five pupae (VOB 1519, 2.x.1984, deposited at LMCI 313-865B); twelve mature galls (CMP 008-01, donated LMCI 349-1); five empty, senescent galls (LMCI 346-01). Also, two last instar larvae, preserved in 100% ethanol at -20 ^{o}C, used for DNA extraction (CMP 008-04, donated to LMCI 349-2). Two last instar larvae preparations, also mounted in Canada balsam on a slide (VOB 1519, donated to LMCI 313-865C). ***Additional material examined*** (pinned-mounted adults): *Prymnotomis crypsicroca* Meyrick, Holotype ♂, BRAZIL: ES, [Baixo] Guandú, ES, 1920 (*Hoffmann*) (NM, Vienna) (g.s. NM 13322). *Hexeretmis pontopora* Meyrick, Holotype ♂, BRAZIL: PA, Taperinha, 11-20.vi.1927 (*Zerny*) (NM, Vienna) (g.s. NM 13321. *Alinguata neblina* Fleming, Alotype ♂, VENEZUELA: [Aragua], Rancho Grande, 3.vii.1946 (*Fleming*) (AMNH) (g.s. VOB).

Description of immature stages

Last instar larva (Figs. 20-34, 51): Head capsule width (mean ± standard error) = 1.88±0.01 mm; body length = 15.24±1.31 mm, n = 5. Body slightly cuneiform, proportionally wider in the middle abdominal segments (Fig. 20). Head tan-brown, with frontoclypeal area, labrum and mandibles darker (Figs. 21-23, 51). Thorax and abdomen light yellow (Fig. 51), turning into reddish prior to pupation. Prothoracic shield slightly melanized, with faint patches of light brown spots located laterally. Anal plate and thoracic legs not melanized.

Head: subretangular, with lateral margins convex, semiprognathus; a sculptured area latero-dorsally on posterior margin (Figs. 24, 27). Frontoclypeus subtriangular, with adfrontal sutures extending to apex of epicranial notch (Fig. 21). Five poorly developed, laterally located stemmata (Figs. 22, 24). Labrum (Fig. 25) slightly bilobed, with three pairs of setae on distal margin, two pairs laterally on proximal margin and another pair mesally. Antenna (Fig. 26) 2-segmented; basal segment with five sensilla on distal margin, two short and stout, one minute, and two long with more than 3x the length of the others; distal segment much thinner and shorter, bearing one short sensilla on distal margin. Mandible well developed with four cusps along distal margin and two setae basally on external surface. Maxilla (Figs. 28) with palpus and galea well developed, stipes bearing well-developed flap-like, distally forked protrusions that project mesally (Fig. 29). Spinneret short, conical (Figs. 28, 29). Labial palpus (Fig. 29) bi-segmented; distal segment thinner and much shorter, both with well-developed apical seta. Chaetotaxy (Fig. 20): MD group trisetose; F unisetose; C group bisetose; A group trisetose, with A1 longer than A2 and A3; AF group bisetose; S group trisetose, with S2 and S3 longer than S1; L unisetose, SS group trisetose. Thorax (T) and abdomen (A): Integument covered with microtrichia (Figs. 30, 33, 34). Thoracic legs well developed (Fig. 30), with stout tarsal claw bearing a tooth on ventral basis (Fig 31). Circular spiracles (Fig. 32) with slightly elevated peritreme, laterally on T1, and A1–8. Abdominal

pseudopodia short (Figs. 33, 34), on A3-6 and A10, with crochets arranged on uniserial and uniordinal, as a penellipse. Chaetotaxy (Fig 20): T1 with D, XD and SD group bisetose, all on the dorsal shield; D2 longer than D1; XD similar to each other in length; SD2 shorter than SD1; L group bisetose, both on the same pinacula, with L1 shorter and latero-dorsal to L2; SV bisetose, with SV1 shorter than SV2. T2-3 with D group bisetose, similar to prothorax; SD bisetose; L1 trisetose, with L1-2 anterior to L3, and posterior to SD; SV and V unisetose. Abdominal segments (A) with only short setae that are more or less aligned on the middle region of each segment, which are herein tentatively named. A1 with D and L groups bisetose; SD and SV unisetose; V present. A2 with D and L groups bisetose; SV trisetose; SD unisetose; V present. A3-6 with D and L groups bisetose; SD unisetose; SV trisetose, with SV2 and SV3 on proleg; MV3 and V present. A7-9 with D, SV and L groups bisetose; SD unisetose; MV3 and V present. A10 with D and SD groups bisetose, located within the anal plate; L bisetose; SV trisetose; V present.

Pupa (Figs. 35-48). Body cylindrical, yellowish brown, mean length ($\pm$ standard error) = 9.6 ± 1.74 mm; maximum width = 4.08 ± 0.07 mm; n = 5. Head with vertex deprived of setae and without a differentiated gall-cutter (Figs. 38-41). Frons wide, posteriorly expanded mesally to the eyes. Clypeus subtrapezoidal, also without setae. Antennae filiform, reaching the distal end of the middle legs. Mandibles small, rounded, latero-posterior to the clypeus. Maxillary palpi small, rounded, latero-posterior to the eyes. Proboscis well developed, reaching A2. Prothorax fairly developed, deprived of setae, bearing postero-laterally a wide open spiracle (Fig. 43). Hindwings concealed by forewings; the latter with six well marked longitudinal lobes on external surface (Fig. 44), extending to sixth abdominal segment. Protho-, meso- and methatoracic legs reaching the third, fifth, and seventh abdominal segments, respectively. Thoracic and abdominal setae extremely reduced in size (Fig 45); one pair dorsally on mesothorax and A1; two pairs of such

setae on metathorax and A2-7, one dorsal and the other lateral, dorsally to spiracles. Abdominal spiracles rounded, with slightly elevated peritreme (Fig. 46), laterally on A2–7; spiracle on A8 partially closed (Fig 47). Distal margin of the last abdominal segment with six pairs of stout, distally hooked setae (Fig 48).

Distribution

P. cecidicola is known from the Brazilian Savanna, within the biome called "Cerradão" (= Cerrado *stricto sensu*; for a description, see Parron et al. 1998) where their host-plant and associated galls were found. The adults of this species do not come readily to light, as shown by the few specimens studied. The third author collected all over the Cerrado region of Brazil for over 30 years. At the type locality (Planaltina) he collected this species regularly in places very close to the host plant where the galls were common.

Host plant

Galls of *P. cecidicola* have been found only in association with *Cordiera elliptica* (Cham.) Kuntze (Rubiaceae) (Fig. 49), a plant native to the Brazilian Savanna (Cerrado Biome), with distribution ranging from northeast Bahia to southeast São Paulo state. It is a dioecious shrub (1.5 to 3.0 m high), with thin, cylindrical, glabrous branches, having dimorphic inflorescences; these are fasciculate on male plants, but only solitary flowers appear on female ones (Matsuoka 2018). Also according to this author, flowers appear during the dry season (from July to September), fruits maturing later on, during the peak of the rainy season (from November to December), which was confirmed by our field observations. At the type locality, *C. elliptica* is commonly known as "marmelinho" and "marmelada-de-pinto", being found scattered on vegetation, particularly along trails. The fruits are consumed by native people either fresh or as a home-made jelly.

317

318 *Natural history*

319 Mature galls of *P. cecidicola* (Figs. 50-53) measure on average (± standard error) 1.80 ± 0.34 cm
320 (n = 5) in diameter. They are green when active, spherical, unilocular and develop individually on
321 *C. elliptica* inflorescences of both male and female plants. Thus we infer they are induced early on
322 flower buds, since male flowers are dehiscent. *C. elliptica* fruits are also green and spherical
323 during development, but a little smaller (maximum diameter = 1.49 ± 0.14 cm; n = 5) and turn
324 brown when mature (Fig. 55). Contrary to *P. cecidicola* galls that have a smooth surface, *C.*
325 *elliptica* fruits show conspicuous style scars distally (Fig. 50). Empty galls of *P. cecidicola* dry up,
326 turning black, remaining attached to the plant (Fig. 56).

327 Larvae of *P. cecidicola* assume an arched position within their galls by placing the body around
328 the fecal pellets left inside the gall. These are packaged and positioned centrally, as a sphere,
329 firmly attached to the gall wall (Fig. 51), except when leaving the gall, when fecal pellets are left
330 aside (Fig. 53). This sphere also contains the larval exuviae packaged within the feces. It is
331 apparently increased in size and modelled periodically, being covered each time by a fine,
332 blackish, silk net.

333 The body of full-grown *P. cecidicola* larvae progressively changes to red before pupation,
334 when they leave the gall through a circular orifice made laterally on the gall wall (Fig. 52).
335 Invariably when offered sandy soil and dried-broken leaves at the bottom of the rearing plastic
336 pots in the laboratory, they built a flat, semi-rectangular, tied, silk-woven cocoon with debris
337 attached (Fig. 54). Exit orifices were present on the surface of empty galls found in the field. Since
338 we did not encounter cocoons attached to the host plant bearing empty galls under these
339 conditions, we presume pupation occurs in the litter, which should be explored further.

C. elliptica plants bearing galls of *P. cecidicola* were found scattered in the field, density varying from one to four galls per plant. Field collections suggested that at the type locality *P. cecidicola* is a univoltine species. Galls and associated larvae were noticeable during the end of the dry season (August) up to beginning of the rainy season (October), thus coinciding in phenology with the reproductive phase of the host plant, described above. Full-grown larvae and pupae were obtained in late October. Emergence of adults under laboratory conditions was recorded from November to January.

Discussion

This study sheds light on the biology of *Prymnotomis*, a poorly known genus of Neotropical many-plumed moth. Lack of morphological and molecular data on the other three alucitid genera endemic to the region makes a broad discussion about the descriptions presented here difficult. However, field collections in Costa Rica, Puerto Rico and French Guiana that resulted in several barcoded specimens (clustered in five BINs) allowed us to compare *Prymnotomis* with other Neotropical material from a genetic perspective. As expected, our sequence clustered with these BINs and was most closely related to cluster #5, presenting ca. 12% genetic distance. Thus a revision is needed in Alucitidae, also to reduce the gap of lineage coverage in the analysis, which likely influences the large genetic divergence between taxa. The most distant affinity was found between *Prymnotomis* and *Pterotopteryx*, a Paleartic genus, not represented in the Neotropics, and that contrary to *Prymnotomis* shows deeply divided wings. *Prymnotomis* is expected to be more closely related morphologically to the genera *Hexeretmis* and *Paelia*, according to Meyrick (1931). This should be further explored, also taking into account morphological characteristics of immature stages, their host plants and larval feeding habitats not only for these genera but also

363 including *Alinguata*, also endemic to the Neotropics. Their original descriptions were based mostly
364 on coloration and relative depth of wing lobes.

365 The unusual flap-like protrusion on stipes described here for the larva of *P. cecidicola* has been
366 found in other alucitds, and also in the closely related copromorphid and carposinid lineages
367 (Hepner 1987). We confirmed the suggestion of this author that these structures are functionally
368 associated with the spinneret. As described by him, the tip of the spinneret is nested within these
369 flaps in *P. cecidicola*. Additional observation demonstrated that they are used to retain partially a
370 brownish dark, liquid substance over the spinneret´s tip, which is used to soak the silk strands
371 continuously when they emerge during the weaving process. This substance then solidifies, sealing
372 the silk threads of the net that is used, for example, to cover the faecal pellet described here, and
373 that thus remains isolated from the larva within the gall. We also noted under laboratory conditions
374 that an orifice artificially made on the gall wall is immediately covered by the larva in this manner.
375 Whether this substance is regurgitated and/or produced by an exocrine gland associated with its
376 buccal apparatus should be further explored.

377 The absence of a differentiated cocoon-cutter and abdominal spines on the pupa of *P.*
378 *cecidicola* and the presence of curved-pointed hooks on the terminal portion of the abdomen
379 suggest that adult emergence in this species occurs inside the cocoon, which should be further
380 examined. The emergence of the adult on the pupation site apparently appeared earlier in
381 Lepidoptera evolution, within the Gellechioidea (e.g. Powell 1973, Becker 1982, Luz et al. 2014).
382 Also interesting are the six raised lobes that appear externally on the *P. cecidicola* pupa forewing,
383 particularly under scanning electron microscopy. Further studies should explore whether this
384 characteristic is unique to and how variable it is within the Alucitidae, in order to rank its value as
385 a diagnostic character for the family in this stage. From an ontogenetic perspective, such lobes
386 supposedly correspond to early divisions in the wing that are present on the adults (for a

387 description of the corresponding position related to wing veins in adult alucitids, see Dugdale et al.
388 1998).
389
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401
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464

465 **Figure Legends:**

466 Figure 1. Maximum likelihood tree based on COI sequences for 41 Alucitidae species and lineages –
467 ln likelihood = 5937. The phylogenetic position of *Prymnotomis cecidicola* sp.n. (CMP 008) is
468 indicated in orange. Bootstrap values are indicated for nodes with more than 50% support (1000
469 replications).
470

471 **Figures 2-8.** Pinned-dried *adult of Prymnotomis cecidicola* sp.n. (2), dorsal view, and
472 corresponding morphology (3-7) in detail: (3) right forewing apical angle (indicated by rectangle
473 in Fig. 2); (4) left hind wing discal cell area (marked by asterisk in Fig. 2); (5) right hind wing
474 outer margin (delimited by brackets in Fig. 2); (6,7) head in dorsal and lateral views, respectively;
475 (8) left hind leg, anterior view. Scale bars = 2.5, 1, 1, 1, 0.5, 0.5, 2 mm, respectively.
476

477 **Figures 9-16.** Male genitalia morphology of *Prymnotomis cecidicola* sp.n. under light microscopy:
478 (9, 11) general, lateral and ventral views, respectively (aedeagus omitted in Fig. 11); (10) valva,
479 lateral (area marked with rectangle in Fig. 9; asterisk indicates basal process); (12) aedeagus,

480 lateral; (13) apex of uncus, ventral (indicated by asterisk in Fig. 11); (14) distal portion of gnathos
481 (pointed by open arrow in Fig. 11); (15) arms of juxta, lateral (indicated by closed arrow in Fig.
482 11); (16) base of juxta (pointed by seta in Fig. 11). Scale bars = 200, 100, 200, 200, 100, 100, 70,
483 100 µm, respectively.
484
485 **Figures 17-19.** Female genitalia morphology of *Prymnotomis cecidicola* sp.n. under light
486 microscopy: (17) general view, ventral; (18, 19) detail of papillae anales and corpus bursae,
487 respectively (areas marked with rectangles in Fig. 17). Scale bars = 200, 30, 50 µm, respectively.
488
489 **Figures 20-23.** Last larval instar of *Prymnotomis cecidicola* sp.n. under light microscopy: (20)
490 chaetotaxy, lateral view; (21-23) head under dorsal, ventral and lateral views, respectively. Scale
491 bars = 1 mm; 100, 100, 100 µm, respectively.
492
493 **Figure 24-34.** Morphology of *Prymnotomis cecidicola* sp.n. last larval instar under scanning
494 electron microscopy: (24) head, lateral view; (25) labrum, dorsal; (26) antenna, lateral; (27) latero-
495 dorsal area of head in detail (pointed by closed arrow in Fig. 24); (28) maxillae and labium,
496 antero-ventral; (29) labium in detail (area marked by rectangle in Fig. 28; asterisk indicates
497 associated flap-like protrusions of yy stipes); (30) mesothoracic leg, postero-lateral; (31) tarsal
498 claw in detail, posterior (open arrow indicates basal spine); (32) prothoracic spiracle, lateral; (33)
499 proleg of fifth abdominal segment, lateral; (34) last two abdominal segments, lateral. Scale bars =
500 200, 50, 50, 50, 100, 25, 100, 25, 50, 50, 200 µm; 0.5, 0.5, 0.5 mm, respectively.
501
502 **Figures 35-37.** *Prymnotomis cecidicola* sp.n. pupa under light microscopy, in dorsal (35), ventral
503 (36) and lateral (37) views. Scale bar = 0.5 mm.

Figure 38-48. Morphology of *Prymnotomis cecidicola* sp.n. pupa under scanning electron microscopy: (38-40) head, under dorsal, ventral and lateral views, respectively; (41) vertex of head, anterior; (42) buccal appendages, ventral; (43) prothoracic spiracle, dorsal; (44) left forewing, lateral; (45) mesothoracic seta, dorsal; (46, 46) prothoracic and eight abdominal spiracles, respectively, lateral; (48) dorsal hooks of last abdominal segment, lateral. Scale bars = 500, 100, 200, 100, 500, 25, 50, 50, 50 µm, respectively.

Figure 49-56. Natural history of *Prymnotomis cecidicola* sp.n. on *C. elliptica*: (49) host plant at the type locality; (50) young fruit and gall on female plant (gall is marked by asterisk; closed arrow indicates style scar of the fruit); (51) dissected gall showing last instar larva (seta indicates sphere made of faeces and exuviae attached to the gall wall); (51) external aspect of empty gall, showing larval exit orifice (pointed by open arrow); (52) dissected empty gall, showing sphere of packaged faeces and exuviae with fecal pellets left aside (pointed by closed arrow) by the larva before leaving for pupation; (54) fresh cocoon made by last larval instar in association with sand grains and dead-broken leaves under laboratory conditions; (55) mature fruit; (56) senescent empty gall. Scale bars = 3, 3.5, 4, 1, 3.5, 3.5 mm, respectively.

528 **Table 1.** Sample information for specimens used in this study.

		Acession number		
Species	**Sample ID**	**Genbank**	**BOLD COI-5P**	**BIN clusters**
INGROUP				
Alucita adriendenisi	CGWC-3887	-	LOWCE127-06	
Alucita cancellata	TLMF Lep 03910	JN307745	PHLSA260-11	
Alucita debilella	TLMF Lep 03912	JN307747	PHLSA262-11	
Alucita desmodactyla	TLMF Lep 09154	KP253214	PHLAI592-13	
Alucita grammodactyla	JBA-05-0004	-	LTOL071-06	
Alucita hexadactyla	TLMF Lep 03909	JN307744	PHLSA259-11	
Alucita lalannei	jflandry2557	-	MECC537-06	
Alucita montana	BIOUG21945-E01	-	SMTPL7338-15	
***Prymnotomis cecidicola* sp.n.**	**CMP 008-04A**	**xx**	**xx**	
***Prymnotomis cecidicola* sp.n.**	**CMP 008-04D**	**xx**	**xx**	
Pterotopteryx dodecadactyla	TLMF Lep 08735	KM573355	PHLAH931-12	
alucitBioLep01	BioLep698	HQ936333	BLPDT350-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HM411224	BLPDM2386-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HQ555946	BLPDQ816-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HM402693	BLPDN1595-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HM402110	BLPDN1038-10	BOLD:AAA0842
alucitBioLep01	BioLep698	HQ555644	BLPDQ346-10	BOLD:AAA0842
Alucita	BioLep698		LTOL788-07|	BOLD:AAA0842
Lepidoptera	BioLep698		BLPDW575-11	BOLD:AAA0842
alucitBioLep01	BioLep698	HM403333	BLPDN2246-10	BOLD:AAA0842
alucitBioLep01	BioLep696	JN296976	BLPEA475-11	BOLD:AAU0280
alucitBioLep01	BioLep696	JN295546	BLPDX574-11	BOLD:AAU0280
Lepidoptera			NOUD2153-12	BOLD:AAG9907
Lepidoptera			BLPEF6714-14	BOLD:AAG9907
Lepidoptera			BLPEF6357-14	BOLD:AAG9907
alucitBioLep01	BioLep693	HQ934518	BLPDR306-10	BOLD:AAG9907
Lepidoptera			MHMYS2880-13	BOLD:AAG9907
alucitBioLep01	BioLep693	HM375200	BLPDF869-09	BOLD:AAG9907
Alucitidae			LMEMB642-09	BOLD:AAG9907
Lepidoptera			BLPEE4426-14	BOLD:AAH5751
Lepidoptera			BLPEE3748-14	BOLD:AAH5751
Lepidoptera			BLPEE3457-14	BOLD:AAH5751
Lepidoptera			BLPEE4540-14	BOLD:AAH5751
alucitBioLep01	BioLep694		BLPDY391-11	BOLD:AAH5751
alucitBioLep01	BioLep694		BLPDL1914-10	BOLD:AAH5751
alucitBioLep01	BioLep694		BLPDY535-11	BOLD:AAH5751
Lepidoptera			BLPEE3749-14	BOLD:AAH5751
Lepidoptera			BLPEE4316-14	BOLD:AAH5751
alucitBioLep01	BioLep694	HQ934494	BLPDR282-10	BOLD:AAH5751

Lepidoptera			BLPEE3667-14	BOLD:AAH5751
Lepidoptera			BLPEE3751-14	BOLD:AAH5751
Lepidoptera			BLPEE3747-14	BOLD:AAH5751
OUTGROUP				
Isonomeutis amauropa	MM11203	GU828850	GBGL9477-12	

Table 2. Genetic distance between *Prymnotomis cecidicola* sp. n. and members of Alucitidae based on 683 base pairs of the DNA barcode sequences using the Kimura 2-parameter model. BIN clusters are identified in Figure 1.

	1.	2.	3.	4.	5.	6.	7.	8.
1. *Prymnotomis cecidicola* sp. n	-							
2. *Alucita* spp.	0.16	-						
3. BIN cluster1 (BOLD:AAH5751)	0.16	0.17	-					
4. BIN cluster2 (BOLD:AAG9907)	0.14	0.15	0.08	-				
5. BIN cluster3 (BOLD:AAJ6491)	0.14	0.14	0.09	0.03	-			
6. BIN cluster4 (BOLD:AAU0280)	0.17	0.16	0.15	0.12	0.12	-		
7. BIN cluster5 (BOLD:AAA0842)	0.12	0.15	0.17	0.13	0.14	0.14	-	
8. *Pterotopteryx dodecadactyla*	0.18	0.14	0.17	0.15	0.15	0.17	0.13	-

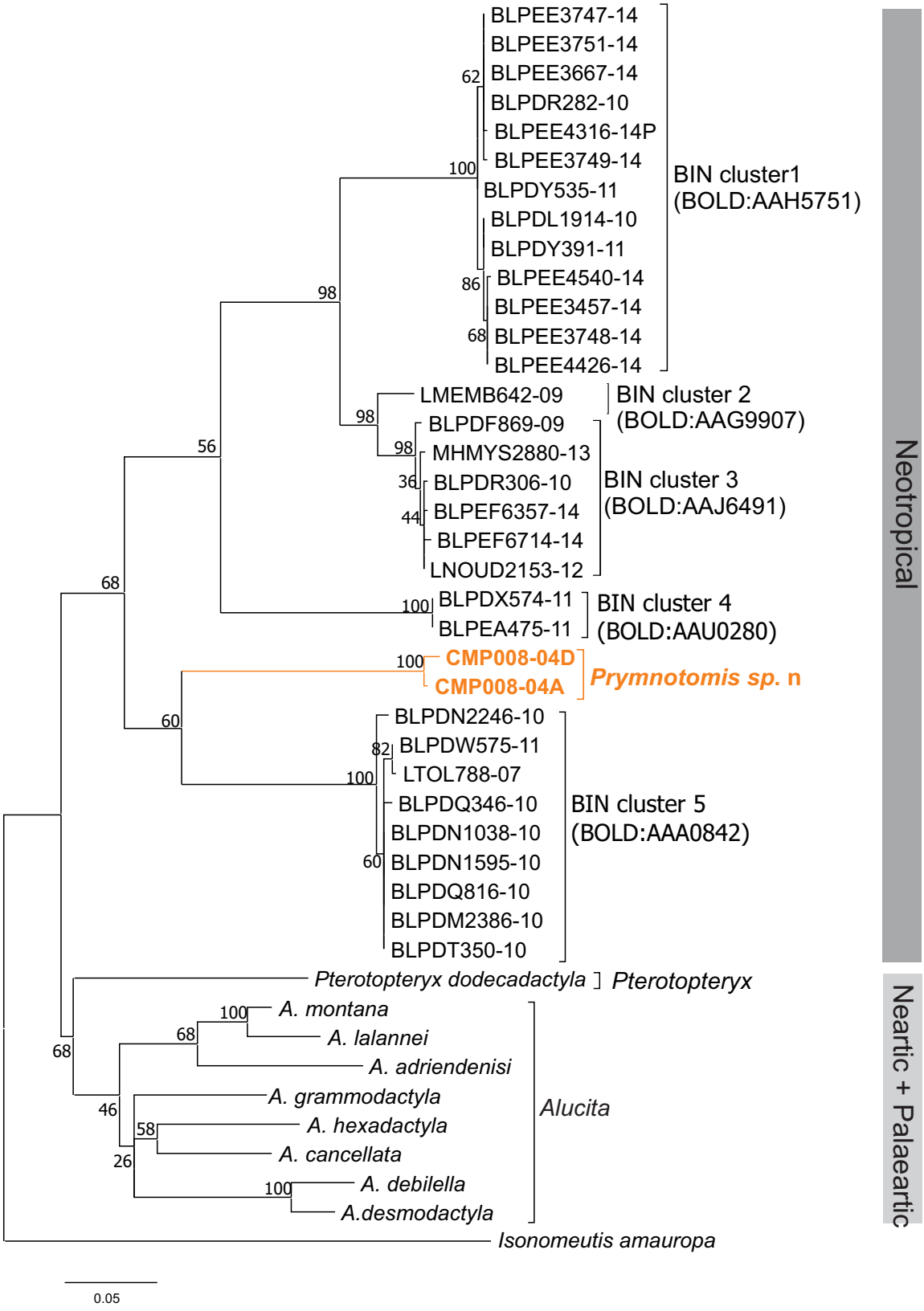

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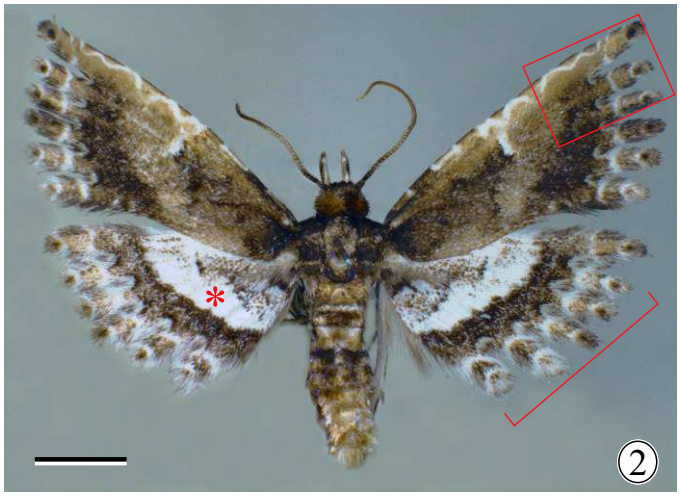
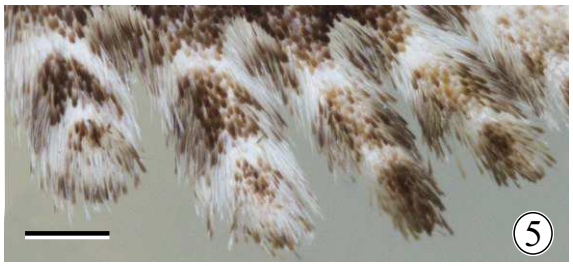
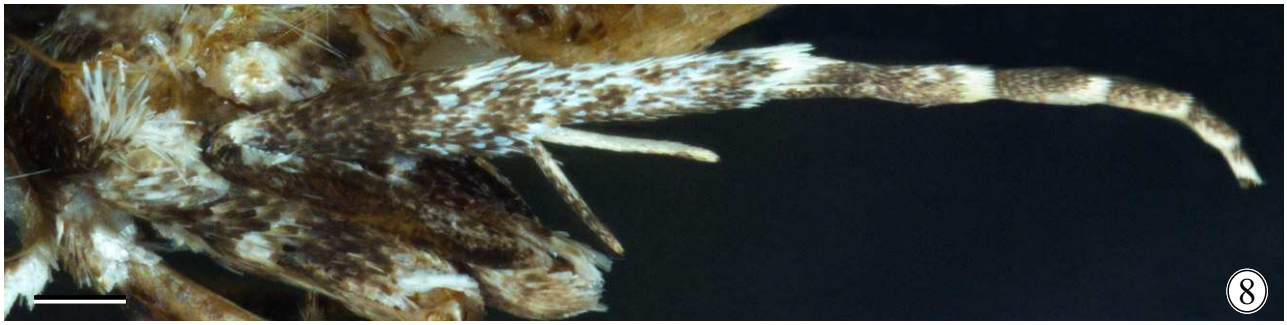
Fig. 1







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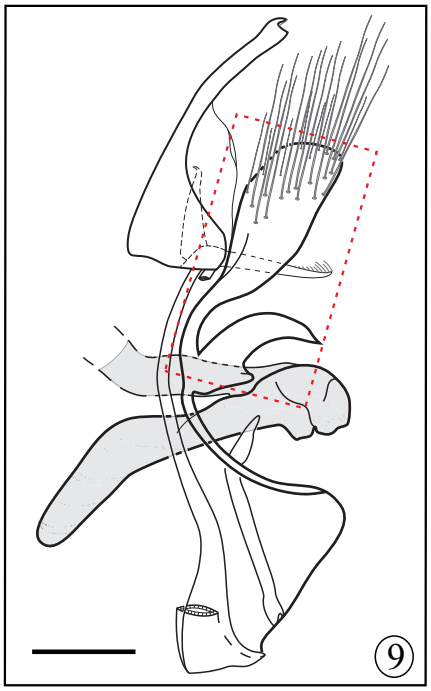
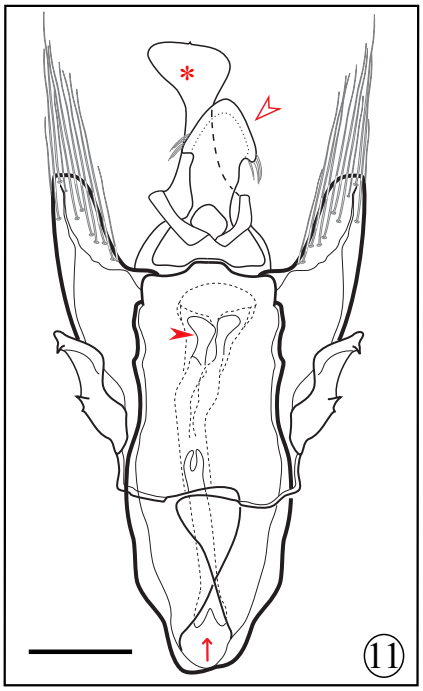
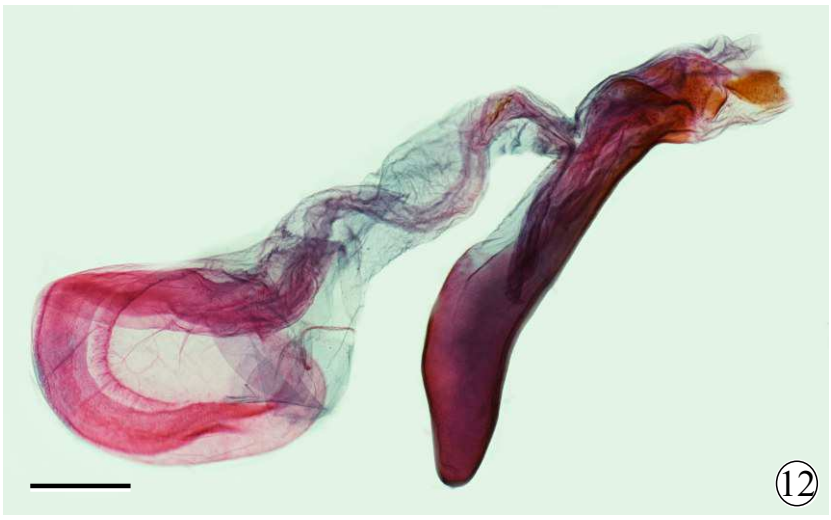
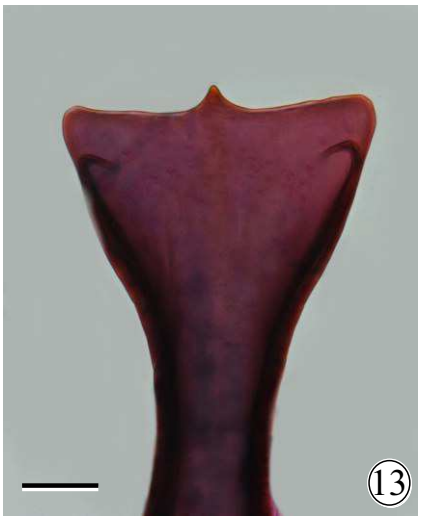
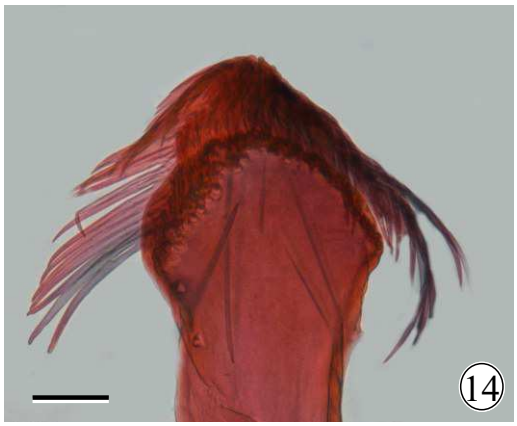
Figs. 2-8

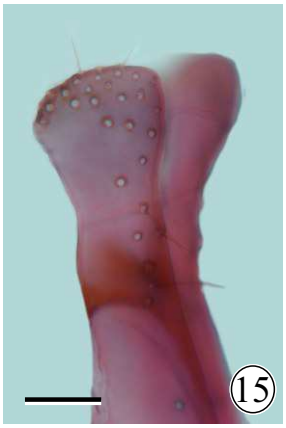


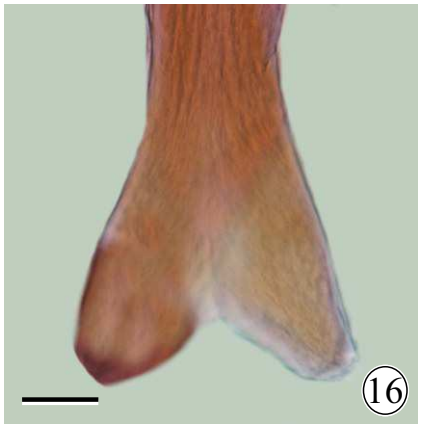












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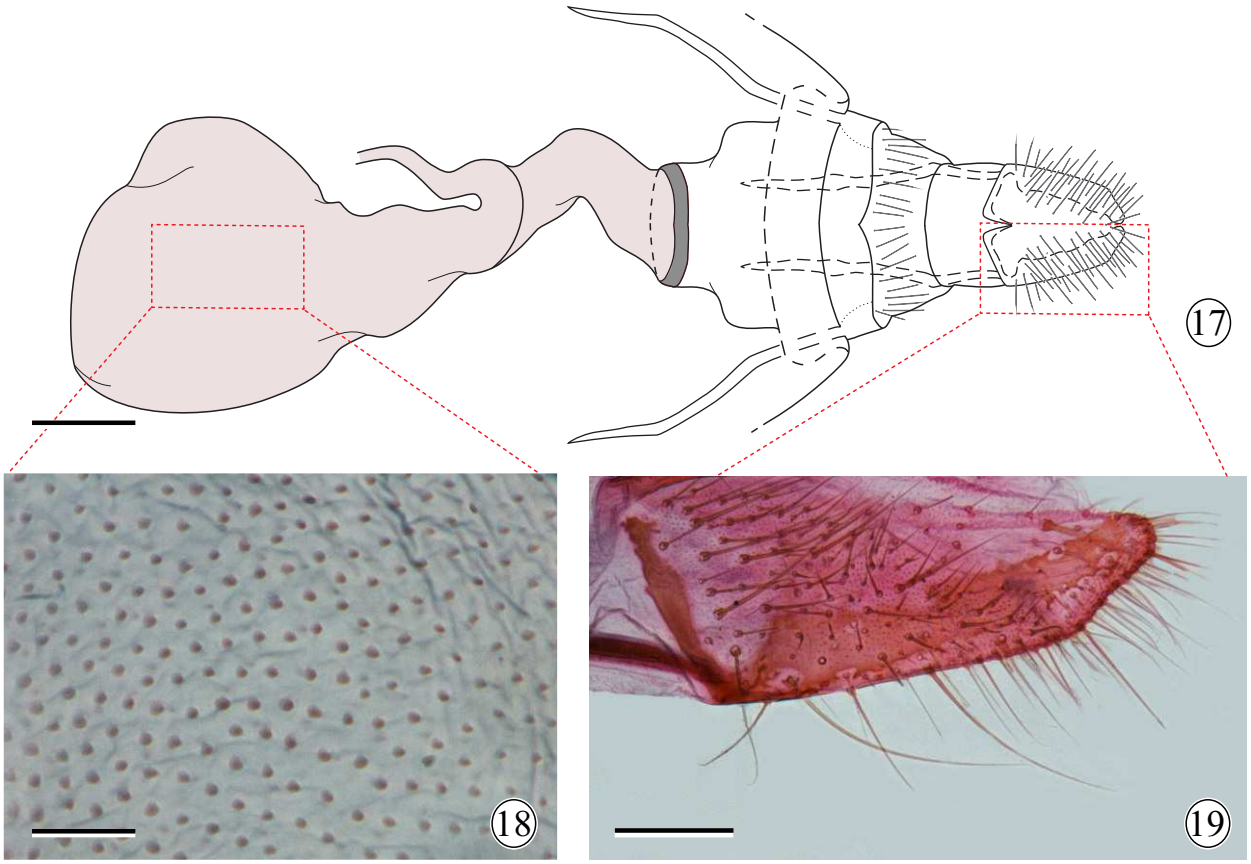
Figs. 9-16



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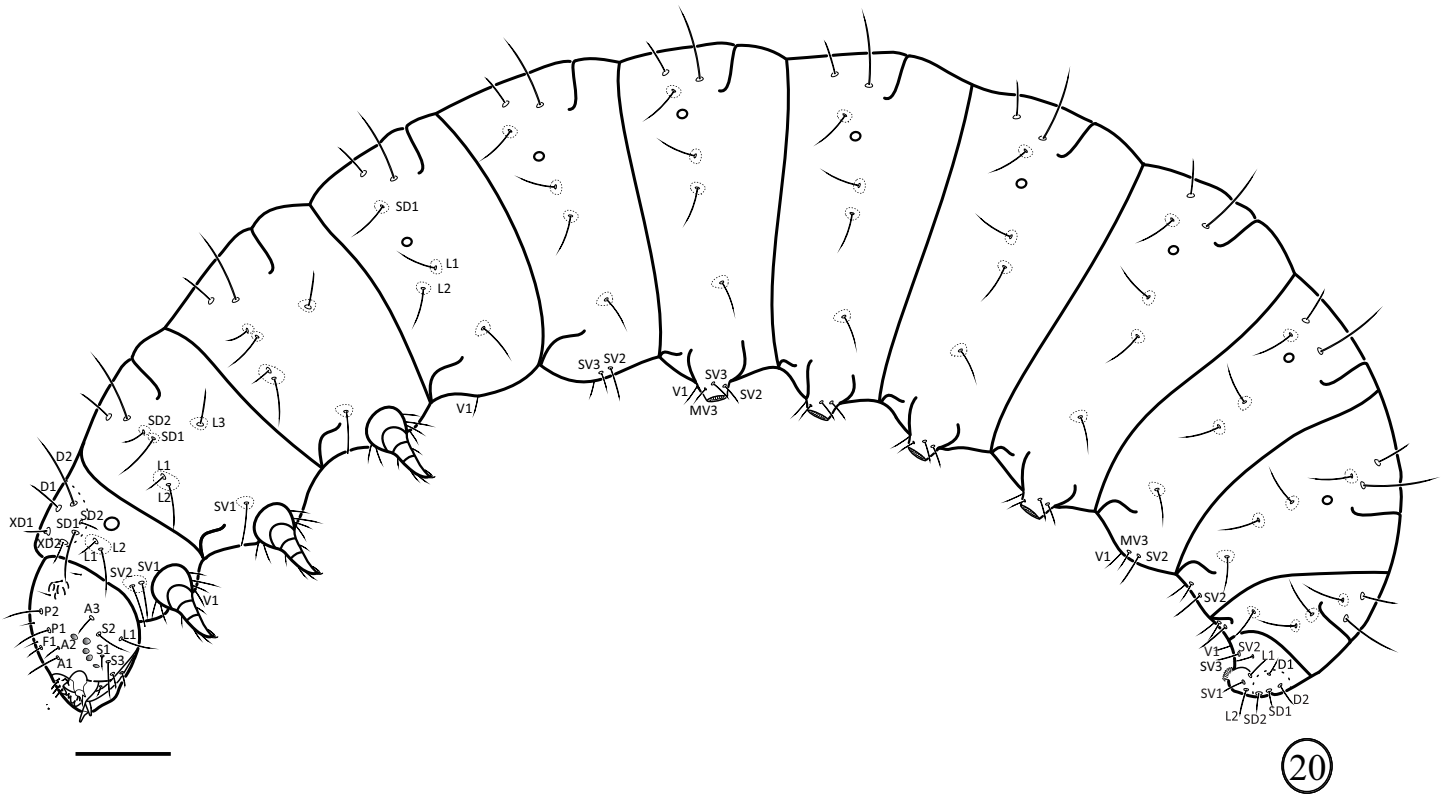
Figs. 17-19



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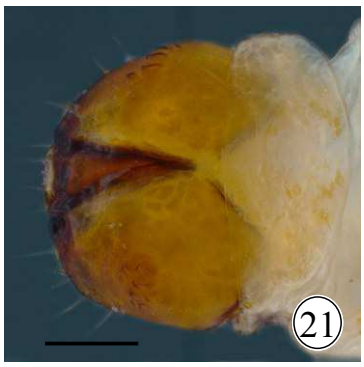

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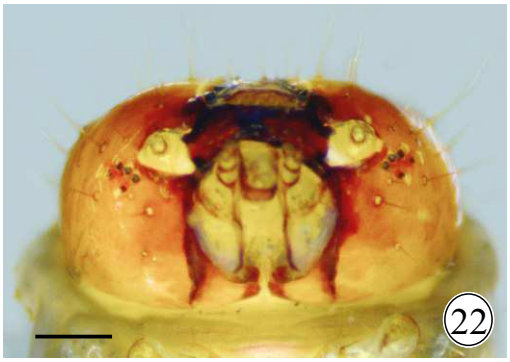

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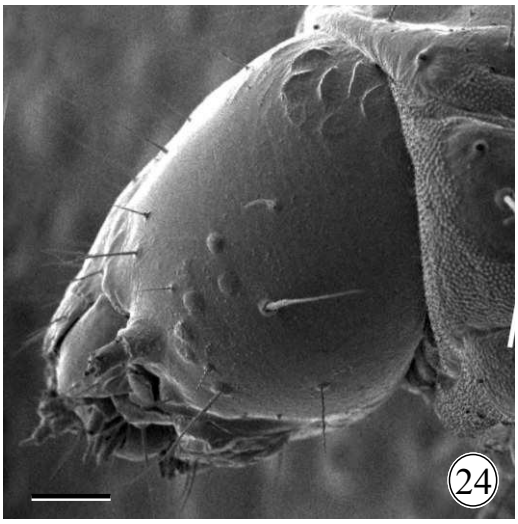
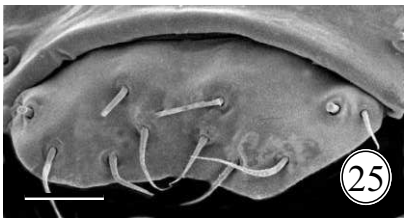
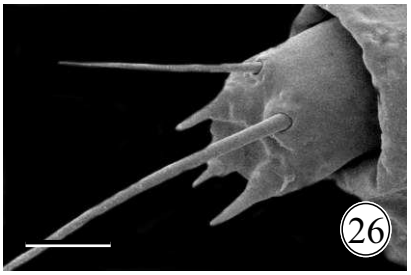
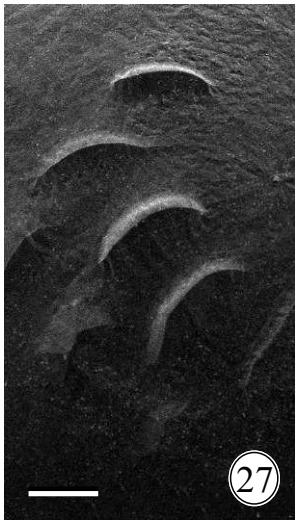
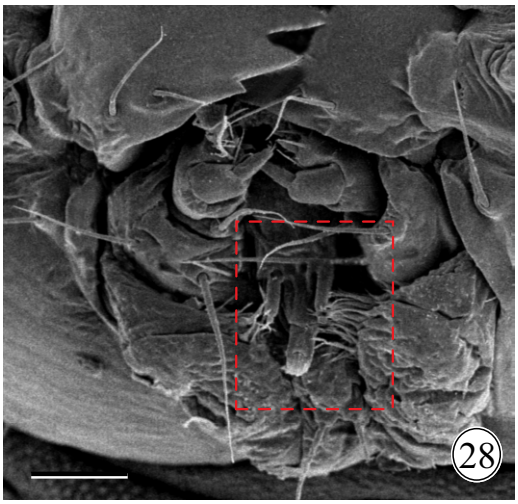
Figs. 20-23

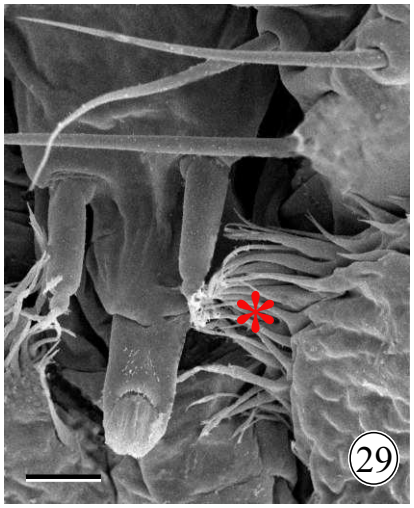


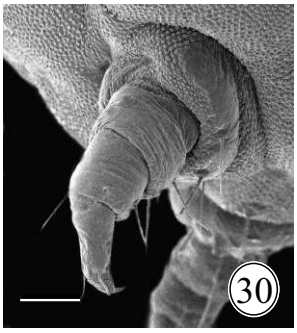


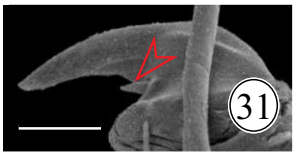


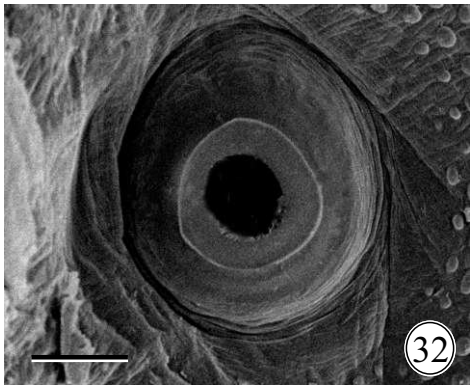


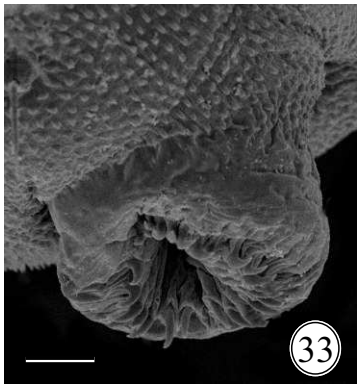


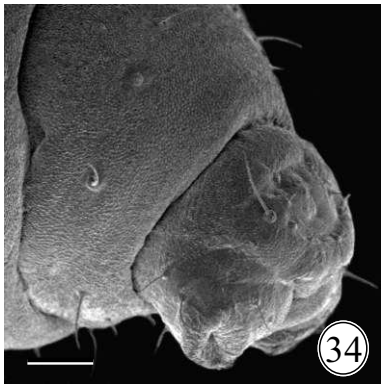












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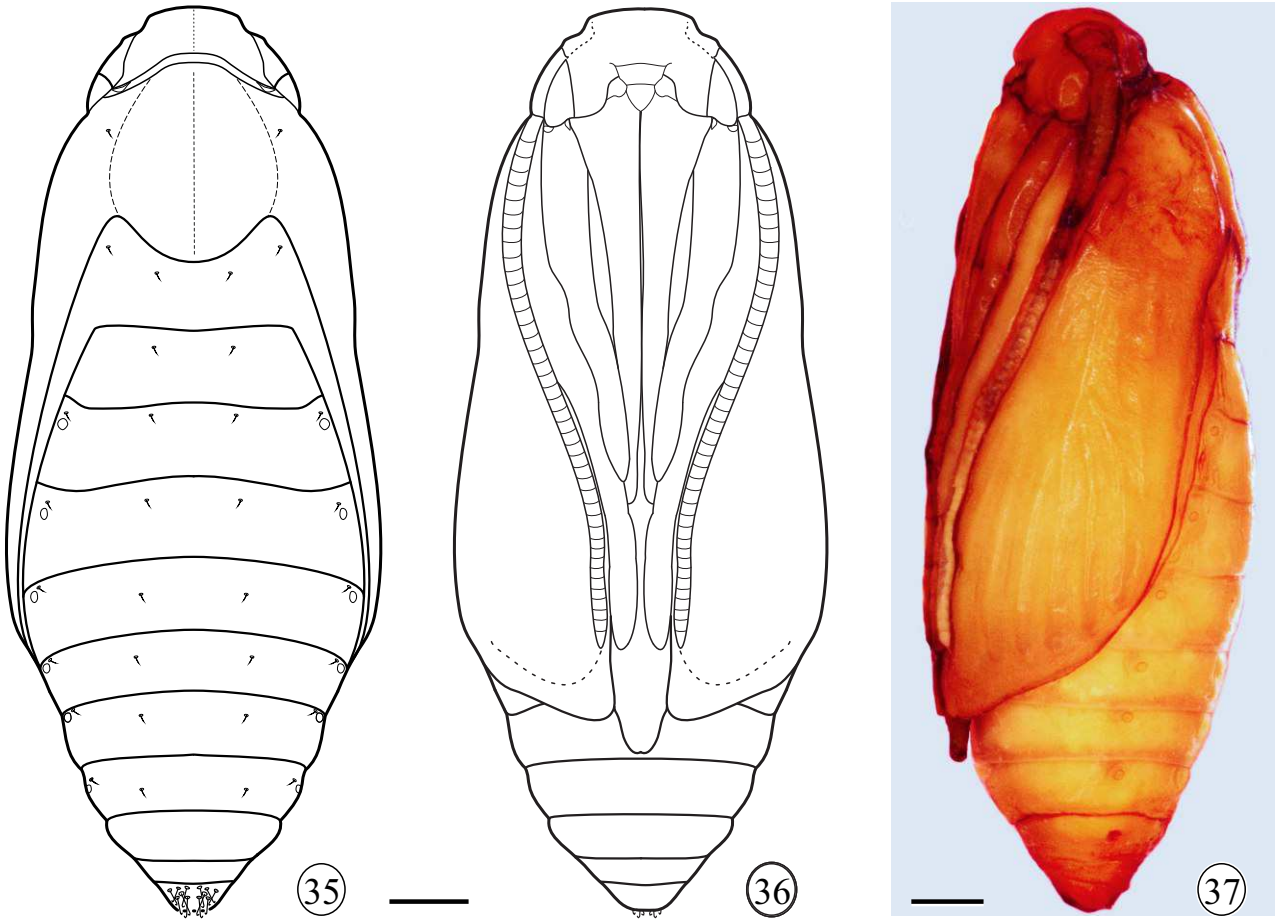
Figs. 24-34



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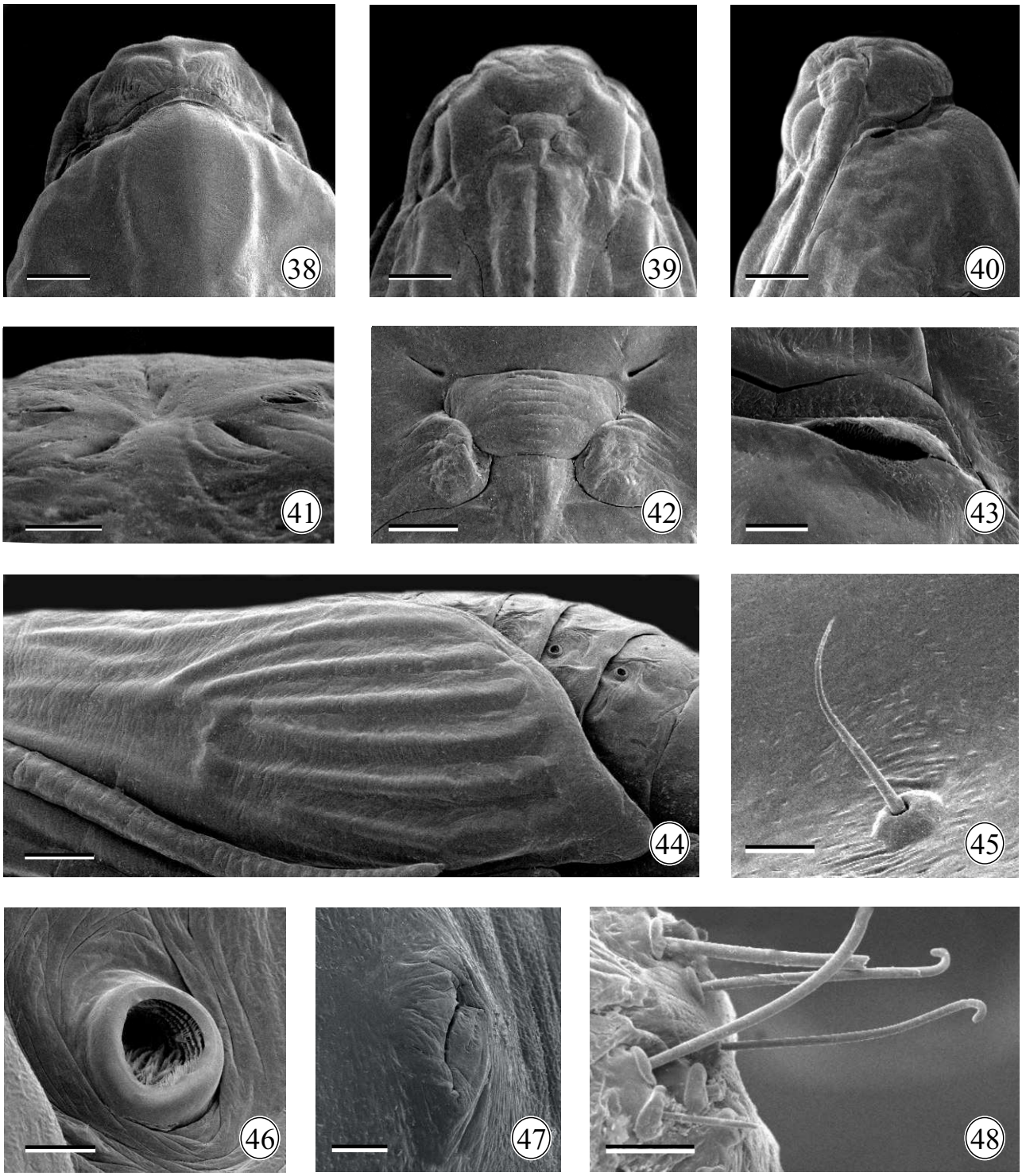
Figs. 35-37



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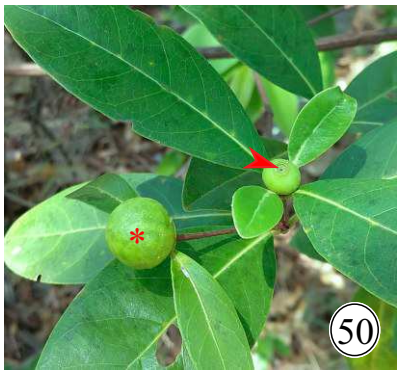
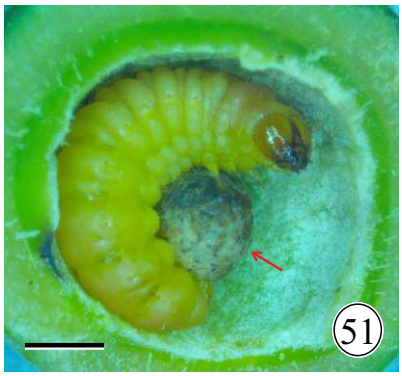
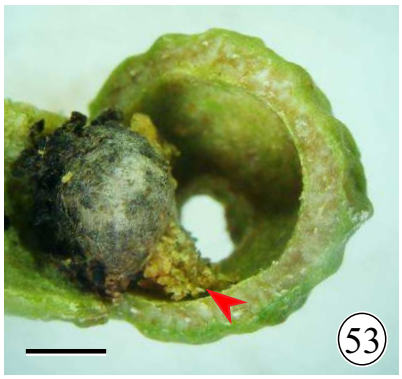
Figs. 38-48







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Figs. 49-56

CAPÍTULO 6

Considerações Finais

Neste trabalho de tese, foram descritas três novas espécies e um novo gênero de microlepidópteros, minadores e galhadores, envolvendo as famílias Nepticulidae, Gracillariidae e Alucitidae. De forma inédita, os estágios imaturos, danos histológicos causados e aspectos da história de vida de todas as novas espécies aqui citadas foram descritos e ilustrados.

No capitulo 2 foram descritos em detalhes a morfologia dos estágios imaturos e a história de vida de *Stigmella schinivora* van Nieukerken, 2016 (Nepticulidae), uma das linhagens mais ancestrais junto a guilda dos lepidópteros minadores foliares. Apesar do gênero possuir mais 400 espécies, muito pouco era conhecido a respeito da morfologia dos estágios imaturos, principalmente com um enfoque em análise por microscopia eletrônica, tal como o aqui utilizado. A planta hospedeira é a aroeira-vermelha *Schinus terebinthifolius*, (Anacardiaceae) e este foi o primeiro registro deste gênero de minador foliar no Brasil.

O capítulo 3 tratou da descrição de um nova espécie de minador foliar encontrada em Arica, Deserto do Atacama, extremo norte do Chile. A planta hospedeira é a aroeira-salsa, *Schinus molle* (Anacardiaceae). A espécie foi alocada na família Gracillariidae, e nomeada como *Leurocephala chilensis* Vargas & Moreira, 2016. Esta foi a segunda espécie descrita para o gênero, e além de diferenças morfológicas encontradas na genitália masculina, a análise molecular e aspectos da morfologia larval e da história de vida, foram determinantes para a descrição.

No capitulo 4 foram descritos um novo gênero e uma nova espécie de minador foliar, também alocados na família Gracillariidae. O gênero *Valissiana* foi criado baseado em evidências morfológicas presentes no adulto, tal como venação diferencial e morfologia da genitália e segmentos abdominais do macho. Estas diferenças foram reforçadas pela análise molecular. *Valissiana universitaria* ocorre na região sul do Brasil, minando folhas da espécie nativa *Erythroxylum argentinum* (Erythroxylaceae) conhecida como cocão. Neste mesmo capítulo, o subgênero *Sabulopteryx* Triberti foi elevado a categoria de gênero. Esta decisão também foi pautada em diferenças substanciais na morfologia dos adultos, e reforçada pela divergência apontada em análise molecular.

O capítulo 5 tratou da descrição de uma nova espécie de galhador do gênero *Prymnotomis*. Esta é a segunda espécie descrita para o gênero, o qual foi criado há cerca de 88 anos. A espécie induz galhas que afetam os frutos da planta marmelada-de-pinto *Cordiera elliptica* (Rubiaceae), uma espécie nativa, e ocorrente na região central do Brasil, principalmente no Bioma Cerrado. Características presentes na genitália masculina foram determinantes para a descrição da espécie, e tanto a morfologia dos estágios imaturos, quanto aspectos da história de vida foram descritos pela primeira vez para este gênero.

As espécies aqui descritas e as informações morfológicas, ecológicas e moleculares levantadas nesta tese, tiveram como objetivo, aumentar o conhecimento a respeito dos microlepidópteros minadores e galhadores ocorrentes na região Neotropical. Esta foi sem dúvida uma tarefa árdua e desafiadora, e continuará sendo, para todos aqueles que tiverem o mesmo objetivo. Por outro lado, é difícil descrever o quão interessante e motivador foi vencer cada obstáculo apresentado nesta jornada, e como em um gigantesco jogo de quebra cabeça, conseguir peça a peça, ir construindo o cenário e a história de cada nova espécie descoberta. Espécies estas, que assim como nós, habitam esta tão rica e diversa região geográfica, e que precisam ter o seu valor e a sua importância reconhecidas.