ROSÂNGELA BRITO

BIOLOGIA DE MICROLEPIDÓPTEROS (LEPIDOPTERA: GRACILLARIIDAE) ASSOCIADOS À PASSIFLORACEAE NO RIO GRANDE DO SUL

Dissertação apresentada ao Programa de Pós-Graduação em Biologia Animal do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, como parte dos requisitos para obtenção do título de Mestre em Biologia Animal.

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Biologia de microlepidópteros (Lepidoptera: Gracillariidae) associados à Passifloraceae no Rio Grande do Sul

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“Sem sonhos, as perdas se tornam insuportáveis,

as pedras do caminho se tornam montanhas,

os fracassos se transformam em golpes fatais.

Mas, se você tiver grandes sonhos...

seus erros produzirão crescimento,

seus desafios produzirão oportunidades,

seus medos produzirão coragem.”

(Augusto Cury)

Dedico este trabalho aos meus pais Alcemiro (*in memoriam*) e Magda pela compreensão e carinho despendidos durante minha trajetória acadêmica.

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E de forma especial a Deus e a vida.

SUMÁRIO

RESUMO

Os maracujás (*Passiflora* L.; Passifloraceae) encontram-se amplamente distribuídos na região sul do Brasil e são geralmente associados como hospedeiras de algumas linhagens de Lepidoptera. Dentre elas, destaca-se, de forma inédita neste estudo, uma família de microlepidópteros conhecidas como Gracillariidae, a qual apresenta o hábito de construir minas na epiderme das folhas. Objetivou-se descrever duas novas espécies de gracilarídeos associados a quatro espécies de passifloráceas, ambas ocorrentes no estado do Rio Grande do Sul (RS), abordando aspectos referentes à morfologia e história de vida. Para isso, realizaram-se coletas nos municípios de Porto Alegre e São Francisco de Paula, RS. Os espécimes foram descritos e ilustrados usando microscopia óptica e microscopia eletrônica de varredura, tanto os adultos quanto estágios imaturos. A anatomia das minas foliares correspondentes é também descrita, com base em cortes histológicos. Análises de DNA mitocondrial (COI) incluindo membros congenéricos foram também conduzidas. *Phyllocnistis tethys* Moreira & Vargas, 2012 associada com *Passiflora organensis*, foi registrada no município de São Francisco de Paula e a larva caracteriza-se por apresentar quatro instares endofíticos, sendo os três primeiros *sap-feeding*, com aparelho bucal adaptado a dilaceração da folha e sucção da seiva, associados com o parênquima esponjoso da folha e, um instar *spinning*, cuja larva não se alimenta, sendo responsável pela construção do casulo junto à porção final da mina. *Spinivalva gaucha* Moreira & Vargas gen. nov., sp. nov, foi encontrada sob ramos de *Passiflora actinia*, *P. misera* e *P. suberosa*, nos municípios de São Francisco de Paula e Porto Alegre. Todos os estágios imaturos são endofíticos apresentando cinco instares larvais, todos *tissue feeding* associados com o parênquima paliçádico da planta hospedeira. De forma inédita, neste último estudo foi descrito a primeira espécie não *sap-feeding* pertencente à Gracillariidae.

CAPÍTULO I

INTRODUÇÃO GERAL

Insetos minadores são conhecidos como os herbívoros especializados em se alimentar de tecidos internos da planta, criando túneis ou galerias principalmente nas folhas. Entre esses, se encontram os representantes de Gracillariidae que constituem a maior família de microlepidópteros minadores de folhas (Davis & Robinson 1998). Fósseis de folhas minadas por larvas de mariposas pertencentes à família são conhecidos do início da idade Cemoniana (Cretáceo Superior), indicando que o surgimento desse grupo de organismos esteja possivelmente relacionado à irradiação das angiospermas (Davis 1994).

Gracillariidae apresenta aproximadamente 1880 espécies descritas para o mundo, exceto na região da Antártica, sendo registradas mais de 180 espécies para a região Neotropical e em torno de 29 espécies para o Brasil (De Prins & De Prins 2013). Grande parte dos autores dividem os representantes dessa família em três subfamílias: Gracillariinae, Lithocolletinae e Phyllocnistinae. No entanto, alguns autores propõem a adição de outras subfamílias baseados em caracteres morfológicos, como Oecophyllembiinae (Davis & Miller 1984; De Prins & Kawahara 2009). Estudos filogenéticos recentes, baseados em caracteres moleculares, suportaram fortemente a monofilia para Gracillariidae, no entanto, a relação entre a maioria das espécies pertencentes à família permanece incerta (Kawahara *et al*. 2011).

As principais sinapomorfias relacionadas à Gracillariidae abrangem a construção de minas ao longo da ontogênese e hipermetamorfose com presença de instares iniciais

do tipo *sap-feeding* (Davis 1994; Davis & Robinson 1998). Os representantes dessa família apresentam uma ampla diversidade quanto à história de vida. Algumas espécies podem utilizar tanto os tecidos das folhas como frutos e pecíolos para alimentação, podendo alterar sua forma de alimentação durante seu próprio desenvolvimento. Os danos causados associados às diferentes formas de alimentação podem ter importância econômica para diversas culturas vegetais, em diversas partes do mundo (Davis 1987).

Os ovos geralmente são depositados individualmente sobre a superfície da folha, sendo geralmente de forma achatada e elípticos. Após a eclosão, a larva penetra nas internas camadas foliares dando início a alimentação e a construção da mina (Davis 1987). As larvas de Gracillariidae podem ser divididas em quatro formas distintas ao longo da sua ontogênese, variando de acordo com a espécie. A primeira forma, a que ocorre após a eclosão do ovo, é conhecida como *sap-feeding*, a qual é caracterizada pelo achatamento do corpo e da cabeça. As peças bucais nessa fase são prognatas, o espinerete é atrofiado, e o corpo, na maioria das vezes, desprovido de cerdas. O aparelho bucal, principalmente as mandíbulas, é modificado, não sendo utilizado para morder, mas sim dilacerar o tecido vegetal e sugar a seiva liberada pela destruição correspondente. As pernas e larvópodos estão ausentes e as larvas podem apresentar algumas vezes tubérculos (= *calli*) utilizados para a locomoção (Kumata 1978). A segunda forma chamada *tissue feeding* é também conhecida como forma cilíndrica por alguns autores, sendo similar a larva dos macrolepidópteros. A cabeça é redonda e parcialmente prognata, apresenta seis estemas e um espinerete funcional. O aparelho bucal apresenta-se adaptado à mastigação. As pernas estão geralmente presentes assim como os larvópodos, do terceiro ao quinto e no décimo segmento abdominal. Essa forma ocorre usualmente nos últimos instares larvais, precedida geralmente de uma forma *sap-feeding* (Kumata 1978; Davis 1987). A forma conhecida como *spinning* (=

pré-pupa) é caracterizada pela ausência de alimentação durante essa fase. Todas as peças bucais são perdidas, sendo apenas o espinerete funcional, para que a larva possa tecer o casulo. Essa forma é característica do gênero *Phyllocnistis* Zeller, 1848, podendo ser encontrada em algumas espécies do gênero *Marmara* Clemens, 1863, *Cameraria* Chapman, 1902, *Metriochroa* Busck, 1900 e *Chrysaster* Kumata, 1961 (Kumata 1978). A forma quiescente é observada em poucas espécies, constitui geralmente de um período curto e representa a forma imóvel ou independente da larva. São conhecidas somente em algumas espécies de *Marmara*, *Dendrorycter* Kumata, 1978 e *Chrysaster* (Kumata 1978).

A localização do casulo pode variar entre as espécies, podendo ser construída uma câmara ao final da mina foliar internamente para representantes de Lithocolletinae e Phyllocnistinae. O casulo pode ser construído externamente na mesma ou em outras folhas da planta hospedeira como alguns representantes de Gracillariinae. São constituídos de seda e podem ou não apresentar ornamentos. A pupa é distinguida principalmente pelo processo frontal conhecido como *cocoon-cutter*, sendo utilizado para rasgar a seda durante a emergência do adulto (Davis 1987; Davis & Robinson 1998).

A mina foliar pode apresentar variações, podendo ser utilizada como um caracter diagnóstico entre as espécies (Davis 1987). Para a maioria dos gêneros, a mina inicia estreita, com formato serpentino, podendo formar manchas próximo ao estágio de empupamento. No entanto, para algumas espécies as minas podem apresentar formato de grandes manchas cobrindo parcialmente ou totalmente a superfície foliar. As fezes liberadas pela larva também podem auxiliar na identificação de algumas espécies; isto é, podem ser secas em forma de *pellets*, ou escuras sem estruturação, formando um rastro fino que geralmente acompanha a trajetória da larva na mina (Davis 1987).

A ampla habilidade para explorar diversos grupos de plantas hospedeiras está associada ao sucesso na diversificação da família; no entanto, a quantidade de espécies descritas atualmente não ultrapassa 20% do total que se espera encontrar no hemisfério sul (Grimaldi & Engel 2005; Davis & Wagner 2011). Detectou-se através de observações preliminares, que o gênero *Passiflora* L., conhecido popularmente pelas plantas do maracujá e amplamente distribuído no estado do Rio Grande do Sul, abrigava espécies desconhecidas da família Gracillariidae.

Assim, objetivou-se descrever duas novas espécies de gracilarídeos associados a quatro espécies de passifloráceas, ambas ocorrentes ao estado do Rio Grande do Sul (RS), abordando aspectos referentes à morfologia e história de vida. Para isso, realizaram-se coletas nos municípios de Porto Alegre e São Francisco de Paula, RS. Os espécimes foram descritos e ilustrados usando microscopia óptica e microscopia eletrônica de varredura, tanto os adultos quanto estágios imaturos. A anatomia das minas foliares correspondentes é também descrita, com base em cortes histológicos. Análises de DNA mitocondrial (COI) incluindo membros congenéricos foram também conduzidas. Os resultados correspondentes integram dois artigos, um já publicado e outro submetido para publicação.

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CAPÍTULO II

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A new species of *Phyllocnistis* Zeller (Lepidoptera: Gracillariidae) from southern Brazil, with life-history description and genetic comparison to congeneric species

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Abstract

Male, female and immature stages of *Phyllocnistis tethys* Moreira & Vargas sp. nov. (Lepidoptera; Gracillariidae) from the Atlantic Rain Forest, coastal mountains of southern Brazil, are described and illustrated, using both optical and scanning electron microscopy. A preliminary analysis of mitochondrial (COI) DNA sequences including putative members of congeneric species is also provided. The immature stages are associated with the passion vine *Passiflora organensis* (Passifloraceae). The hypermetamorphic, endophyllous larva has four instars; the first, second and third instars are sap-feeders, associated primarily with the spongy parenchyma, and construct a blotch mine in the lower surface of the lamina; the fourth, non-feeding (spinning) instar constructs a flimsy endophyllous cocoon at the end of the mine, where pupation occurs. This is the first species of *Phyllocnistis* Zeller described from Brazil, and the first leaf-mining gracillariid associated with Passifloraceae.

Key words: leaf-mining moths, gracillariids, Neotropical region, hypermetamorphosis, passion vines

Introduction

Gracillariidae is one of the largest groups of leaf-mining Lepidoptera, with 1,885 species recognized worldwide, of which 181 are recorded in the Neotropical region (De Prins & De Prins 2012). *Phyllocnistis* Zeller, 1848 is a poorly studied genus of minute moths (wingspans generally not exceeding 5 mm) that has been assigned to different families of Gracillarioidea and only lately has been included in the Gracillariidae, within the Phyllocnistinae (Davis & Miller 1984; Kawahara *et al*. 2011; Nieukerken *et al*. 2011). The taxonomic history of the genus was reviewed recently by De Prins & Kawahara (2009), and information on the general biology was provided by Davis & Wagner (2011). The existence of subepidermal, sap-feeding instars early in the larval stage and a specialized, non-feeding last instar that spins an endophyllous cocoon prior to pupation are shared characteristics among all known species of *Phyllocnistis*. Adults show consistent differences in wing patterns (*e. g*., conspicuously colored fasciae and strigulae) at the species level, but they vary little in the structure of their genitalia compared to other gracillariids and lepidopterans in general. They are, however, relatively diverse in some pupal structures, which may provide valuable species-level differences, such as in the frontal process of the head (= cocoon cutter) and in the shape and arrangement of tergal spines present on the abdomen (Davis & Wagner 2011).

A total of 126 species have been recognized for the genus *Phyllocnistis* worldwide (De Prins & De Prins 2012). Ten species were listed for the Neotropical region by Davis & Miller (1984), with type localities in

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Argentina, Colombia, Guyana, Costa Rica, Ecuador and Peru. Subsequent to this list, a new species was described from southern Chile (*P. puyehuensis*) by Davis (1994), three from Costa Rica (*P. drimiphaga*, *P. maxberryi*, and *P. tropaeolicola*) by Kawahara *et al*. (2009), and recently also from Costa Rica, *P. perseafolia* by Davis & Wagner (2011). Thus, only 16 species have so far been recorded for the genus in the Neotropics, although none for Brazil except *P. citrella* Stainton, a cosmopolitan, citrus pest species of Asiatic origin (De Prins & Kawahara 2009; De Prins & De Prins 2012). This number is supposedly greatly underestimated, probably the result of low collecting effort, in particular regarding the central and southern areas of the Neotropics where microlepidopterans in general have been historically less collected. Preliminary results from a survey of *Phyllocnistis* species for a localized area in Costa Rica conducted by Davis & Wagner (2011), for example, led to the conclusion that the tropical diversity for the genus may comprise hundreds of species.

Members of *Phyllocnistis* exploit a wide range of host plants worldwide, and are known to feed on plants from at least 20 families (De Prins & Kawahara 2009). A large proportion of species found in the Nearctic region are specialized to feed on ancient angiosperms whose origins date to the Cretaceous (*e.g.*, Lauraceae, Magnoliaceae and Hamamelidaceae) (Davis & Wagner 2011). At least one Neotropical species (*P. drimiphagha*) was recently associated with the archaic Winteraceae (Kawahara *et al*. 2009). In fact, phyllocnistine leaf mines are among the oldest known fossils of Ditrysia Lepidoptera, dating to the Late Cretaceous (Labandeira *et al*. 1994; Davis & Wagner 2011; Sohn *et al*. 2012). However, it is still uncertain whether these associations constitute evidence that *Phyllocnistis* species are ancient or that they have only recently colonized these host plants. This question will remain unanswered until more information on host-plant diversity is attained, and a consistent phylogeny is established for the genus (Davis & Wagner 2011).

Recently, as part of an ongoing study on the diversity of microlepidopterans in the Atlantic Rain Forest in southern Brazil, we found for the first time a leaf-mining gracillariid, belonging to *Phyllocnistis* and associated with Passifloraceae. Herein, we describe and illustrate all the life stages of this new species, and provide a preliminary characterization of its life history, including histological aspects of the leaf mine. We also present a preliminary analysis of mitochondrial (COI) DNA sequences including putative members of congeneric species.

Material and methods

Specimens used in the study were reared in small plastic vials under controlled abiotic conditions (14 h light / 10 h dark; 20 ± 2°C) in the Laboratório de Morfologia e Comportamento de Insetos, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre city, Rio Grande do Sul State (RS), Brazil, from eggs, larvae and pupae collected on *Passiflora organensis* Gardner (Passifloraceae), during May 2011 and March 2012, in the Centro de Pesquisas e Conservação da Natureza (CPCN Pró-Mata / PUCRS; 29 °28’36’’S, 50 °10’01’’W), 900 m, São Francisco de Paula Municipality, RS.

Immature stages were fixed with Dietrich´s fluid and preserved in 75% ethanol. At least five specimens were used for the descriptions of each life stage or instar. For observations of 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, where structures selected to be illustrated were photographed with an attached Sony® DSC-H10 digital camera. Vectorized line drawings were then made with the software CorelDraw® X4, using the corresponding digitalized images as a guide. Adult wing-pattern nomenclature follows Kawahara *et al*. (2009), and for the general description of larvae and pupae, Davis (1987) and Davis & Wagner (2011).

For scanning electron microscope analyses, 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 then examined and photographed in a JEOL® JSM5800 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of UFRGS.

For plant anatomical descriptions, field-collected leaf portions of *P. organensis* containing mines of *P. tethys* were fixed in FAA (37% formaldehyde, glacial acetic acid, and 50% ethanol, 1:1:18, v/v), and preserved in 70% ethanol. Under the stereomicroscope in the laboratory, leaf portions containing the last sap-feeding larval instars were later selected (n = 15). They were then progressively hydrated, immersed in 10% potassium hydroxide for 20 min, stained for 12 h with rose bengal (aqueous solution: 200 mg/liter), and then mounted whole in glycerin 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.

Molecular analysis. Total genomic DNA was extracted from last sap-feeding larval instar specimens using the CTAB method (Doyle & Doyle 1987), in order to evaluate the phylogenetic status of *Phyllocnistis* sp. nov. using molecular characters. We surveyed four specimens to amplify part of the mitochondrial gene cytochrome oxidase I (COI—639 bp) using primers and conditions described by Folmer *et al*. (1994) (Table 1). PCR products were purified using Exonuclease I (GE Healthcare Inc.) and Shrimp Alkaline Phosphatase (SAP), sequenced with a BigDye kit and analyzed on an ABI3730XL (Applied Biosystems Inc.). Chromatograms obtained from the automatic sequencer were read and sequences were assembled using the software CodonCode Aligner (CodonCode Corporation). The COI sequences obtained in this study were deposited in GenBank (Table 1). Phylogenetic trees were constructed using maximum likelihood (ML) in the software PHYML 3.0 (Guindon *et al*. 2010). The program JMODELTEST (Posada 2008) was used to estimate the substitution model GTR + G [General Time-Reversible model (Rodríguez *et al*. 1990), with gamma distribution (G)] for ML according to the Akaike Information Criterion (AIC). Monophyly-confidence limits were assessed with the bootstrap method (Felsenstein 1985) at 60% cutoff after 1000 bootstrap iterations.

TABLE 1. Specimens used in this study to reconstruct the phylogenetic status of *Phyllocnistis tethys* based on cytochrome oxidase subunit I sequences.

Family	Species	Voucher number	GenBank accession number
Bucculatricidae	*Bucculatrix canadensisella*	UBC-2007-0541	FJ412220
Gracillariidae	*Acrocercops astericola*	08-JDWBC-1948	HQ682752
Gracillariidae	*Caloptilia stigmatella*	BIOUG(CAN): 04HBL007525	GU438783
Gracillariidae	*Phyllocnistis labyrinthella*	MM00041	GU828587
Gracillariidae	*P. citrella*	SK-013	AB614513
Gracillariidae	*P. hyperpersea*	DDAV-D557	HQ971045
Gracillariidae	*P. perseafolia*	DDAV-D555	HM382097
Gracillariidae	*P. populiella*	08-JDWBC-2658	HQ683340
Gracillariidae	*P. saligna*	SK-011	AB614511
Gracillariidae	*P. gracilistylella*	SK-010	AB614510
Gracillariidae	*P.* sp. 1 (BOLD AAF6349)	DDAV-D565	HM382102
Gracillariidae	*P.* sp. 2 (BOLD AAF6349)	DDAV-D566	HM382102
Gracillariidae	*P.* sp. *'longipalpus'*	DDAV-D562	HM382099
Gracillariidae	*P. tethys* sp. nov.	LMCI 174-55-1	JX272049
Gracillariidae	*P. tethys* sp. nov.	LMCI 174-55-3	JX272050
Gracillariidae	*P. tethys* sp. nov.	LMCI 174-55-5	JX272051
Gracillariidae	*P. tethys* sp. nov.	LMCI 174-55-9	JX272052
Gracillariidae	*P. vitegenella*	2VE	JQ412575

Museum collections. Abbreviations of the institutions from which specimens were examined are:

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

MCNZ Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

MCTP Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

Results

***Phyllocnistis tethys* Moreira & Vargas, sp. nov.**
Figs. (1–8)

Type material. BRAZIL: Centro de Pesquisas e Conservação da Natureza Pró-Mata (CPCN Pró-Mata; 29º28'36'S, 50º10'01'W; 900 m), São Francisco de Paula Municipality, Rio Grande do Sul State, Brazil. All adults were preserved dried and pinned, and reared by the senior author from larvae and pupae collected on 05-11.V.2011 by G.R.P. Moreira, R. Brito & K. Barão, on *Passiflora organensis* Gardner (Passifloraceae). HOLOTYPE: ♂ (LMCI 155-58), deposited in DZUP (22.623). PARATYPES: 2 ♀♀ (LMCI 155-41 and 155-43), deposited in DZUP (22.633 and 22.643); 1 ♂, 1 ♀ (LMCI 155-31 and 155-26), deposited in MCNZ (81901 and 81902); 1 ♂, 1 ♀ (LMCI 155-35 and 155-30), deposited in MCTP (28635 and 28636**)**.

Other specimens examined. Adults, dried and pinned, 4 ♂♂, with the same collection data, deposited in LMCI (155-25, 27, 32, 33); 2 ♀♀, fixed in Dietrich's fluid and preserved in 70% ethanol, with the same collection data, deposited in LMCI (155-20). Genitalia preparations, mounted in Canada balsam on slides, with the same collection data, deposited in LMCI under the following accession numbers: 5 ♂♂ (GRPM 50-10, 13, 14, 15 and 16); 4 ♀♀ (GRPM 50-8, 17, 18, and 19). Immature stages, fixed in Dietrich's fluid and preserved in 70% ethanol, with the same collection data, deposited in LMCI under the following accession numbers: 3 eggs (LMCI 155-14), 2 first-instar (sap-feeding) larvae (LMCI 155-3 and 4), 5 third-instar (sap-feeding) larvae (LCMI 155-12 and 13), 4 fourth-instar (spinning) larvae (LMCI 155-16), and 8 pupae (LMCI 155-18 and 19). Mature leaf mines (n = 24) containing exuvia of all instars, mounted in glycerin on slides and stained with rose bengal, with the same collection data, 26.III.2012, deposited in LMCI, under accession numbers LMCI 174-1 to 24.

Diagnosis. Adults of *P. tethys* can be readily distinguished from all other known species of Neotropical *Phyllocnistis* in the forewing pattern, primarily by the absence of longitudinal and costal fasciae. Of the five species of *Phyllocnistis* known from neighboring Argentina and Chile (Davis & Miller 1984, Davis 1994), only two (*P. abatiae* Hering and *P. puyehuensis* Davis) lack the basal longitudinal fascia. However, *P. abatiae* possesses a pair of small, isolated costal fasciae; and *P. puyehuenis* has a single, broad, isolated pale-gold costal fascia that crosses the wing. In addition, in these species the presence of yellowish-orange scales on the subapical part of the forewing is restricted to a small circular area adjacent to the black spot. Also, in contrast to *P. tethys*, in these species the tornal fringes are uniform in color.

Adult (Figs. 1, 2). Male and female similar in size and color (Fig. 1). Forewing length 2.41–2.72 mm (n = 5). *Head*: Vestiture moderately smooth, with a pair of latero-dorsal light-gray scale tufts that curve forward to the frons. Eyes medium in size (interocular index ranging from 0.51 to 0.72; n = 4). Antenna mostly dark gray, ~ equal to length of forewing, covered with lanceolate scales; a single row of scales encircling each flagellomere. Labial palpus slender, ~ 0.3 mm in length, covered with dark-gray scales. Proboscis without scales, slightly longer than labial palpus. *Thorax*: Forewing light gray; longitudinal and costal fasciae absent; transverse fascia C-shaped, with faint dark border filled in with sparse light-gray scales; apical to subapical area bright yellowish orange, medially interspersed on costal strigulae and transverse fascia, and with large black spot; three slender, dark costal strigulae, three slender dark apical strigulae, and one dark tornal strigula arising from the apical black spot; fringe along tornal margin light gray with a wide dark basal band of scales; ventral surface dark gray. Hindwing dark gray. Legs light gray; foretibia and tarsomeres mostly dark gray. *Abdomen:* Length ~ 1.7 mm, covered with dark-gray scales. *Male genitalia:* Tergum VIII small, semicircular; sternum VIII reduced to a narrow transverse band. A pair of coremata present meso-laterally on segment VIII, consisting of inflatable tubular extensions bearing a terminal cluster of long, wide and flat scales (Fig. 2D). Tegumen formed by a basal, narrow transverse band that continues caudally up to approximately the length of the valvae, as an elongate, mostly membranous, basally spinose cylinder that encloses the anal tube (Fig. 2A); saccus well developed, ~ 0.3 length of valve, U- shaped with rounded anterior end and sinuous posterior margin having pronounced concavity medially; valvae digitiform, slightly curved medially and long, ~2.0 length of saccus, with moderately broad base formed by two wide dorsal and ventral

projections that converge, reaching each other medially; setae of medium size are scattered found on median surface of valve, and short setae distally. Aedeagus (Fig. 2B) subcylindrical, weakly sclerotized, ~ equal to length of valva, having basal 2/3 portion slightly dilated and with subapical, dorsally located concave aperture. Vesica with several short spiniform cornuti (Figs. 2B, E). *Female genitalia*: Sternum VII subrectangular, with concave anterior margin more heavily sclerotized, and posterior margin slightly concave (Fig. 2C); tergum VIII reduced to narrow transverse band, with large subtriangular, latero-ventral projections; anterior apophysis similar in length to subtriangular projections of sternum VIII; anal papillae connected dorsally, covered with long piliform setae and microtrichia (Figs. 2C, F); posterior apophyses similar in length to anterior ones; ostium bursae broad , located on posterior margin of sternum VII; ductus bursae membranous, broader at base and narrow distally; corpus bursae membranous, pear-shaped, ~ twice length of ductus bursae, with a conspicuous, proximal, diagonally oriented, and hook-shaped signum that is directed posteriorly into the lumen (Figs. 2C, G); ductus seminalis membranous, narrow, inserted in apex of corpus bursae.

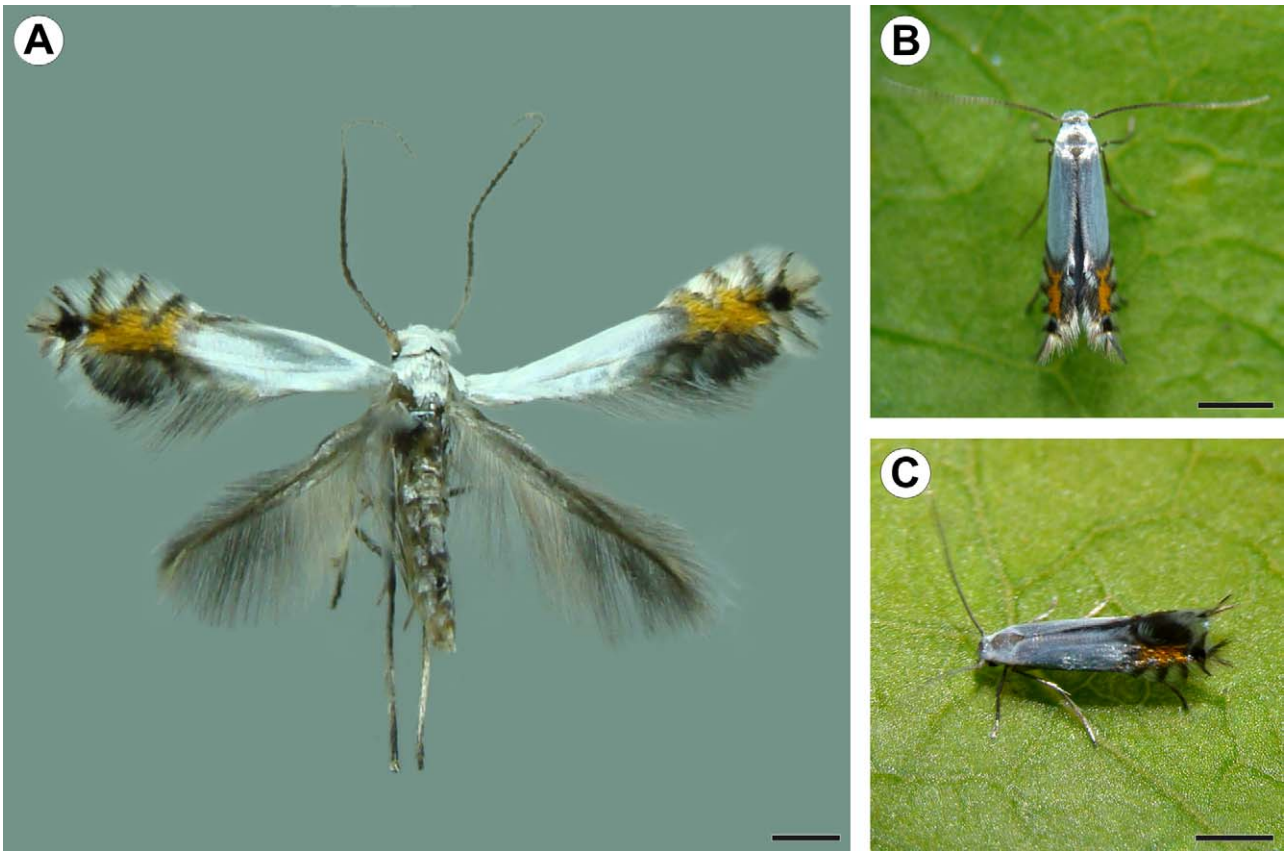

FIGURE 1. *Phyllocnistis tethys* adult: wings spread, pinned, dorsal view (A); wings folded, on *Passiflora organensis* leaf, in dorsal (B) and lateral (C) views. Scale bars = 0.5, 1.0 mm, respectively.

Immature stages. Egg (Fig. 4A; 7C). Flat, slightly ellipsoid; chorion translucent, without external ornamentation, and white at deposition; larva can be seen by transparency before emergence; aeropyles and micropylar area were not observed.

Larva (Figs. 3A–C; 4B–I; 5; 7B, E, G). Leaf-miner, with hypermetamorphic development and four instars, all endophyllous. The first three instars are sap feeders, prognathous and apodous, with highly modified buccal apparatus and depressed body; maximum length of larvae examined 4.79 mm. The prothorax and mesothorax of first-instar larvae are somewhat longer than the metathorax, which is not the case in the following instars. However, we found no stable differences either in shape or coloration among the sap-feeding instars of *P. tethys*. Instars can be correctly identified through measurements of the head capsule, since there is no overlap between the head-capsule size of succeeding instars (Table 2). For the three sap-feeding instars, the following exponential growth equation was adjusted for the head-capsule width: $y = 0.073e^{0.504x}$; $n = 45$; $r = 0.98$; $p < 0.0001$. The fourth instar (= non-feeding, “spinning”) is also prognathous and apodous, but has the mouth parts either reduced

or absent, except for the functional spinneret; maximum length of larvae examined 4.17 mm. Body color uniformly white in all instars.

TABLE. 2. Variation in size among head capsules of sap-feeding instars of *Phyllocnistis tethys* (n = 15 per instar).

Instar	Head capsule width (mm)		
	Mean ± standard error	Range	Growth rate
I	0.121 ± 0.003	0.116–0.158	-
II	0.197 ± 0.005	0.179–0.242	1.63
III	0.333 ± 0.004	0.305–0.368	1.69

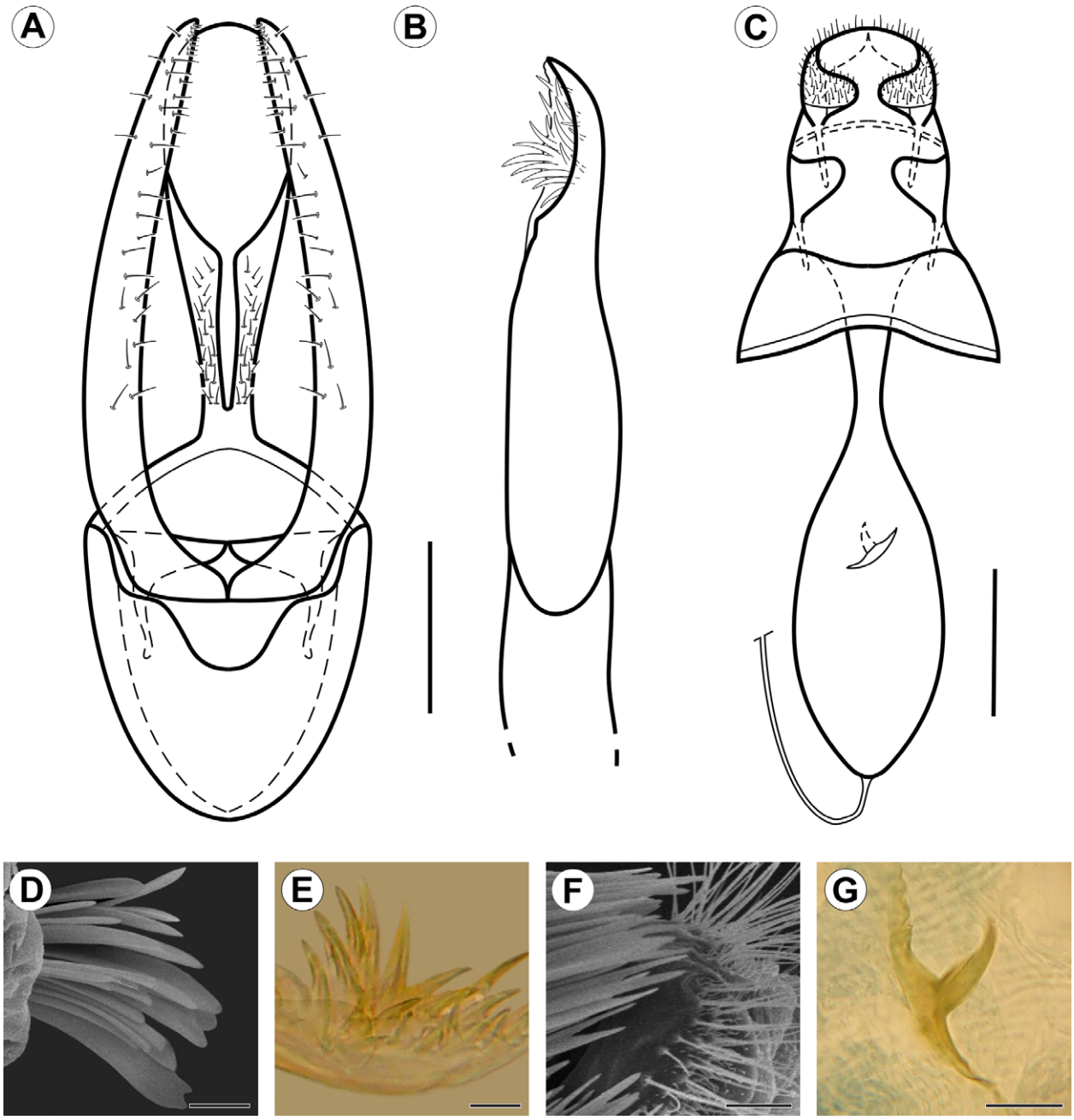

FIGURE 2. Genital morphology of *Phyllocnistis tethys* under light and scanning electron microscopy: (A) male genitalia, ventral view (aedeagus omitted); (B) aedeagus, lateral view; (C) female genitalia, ventral view ; (D) male coremata, lateral view; (E) male cornuti in detail, lateral view; (F) female papilla annalis in detail, latero-dorsal view; (G) female signum in detail, lateral view. Scale bars = 100, 200, 25, 50, 25, 25 µm, respectively.

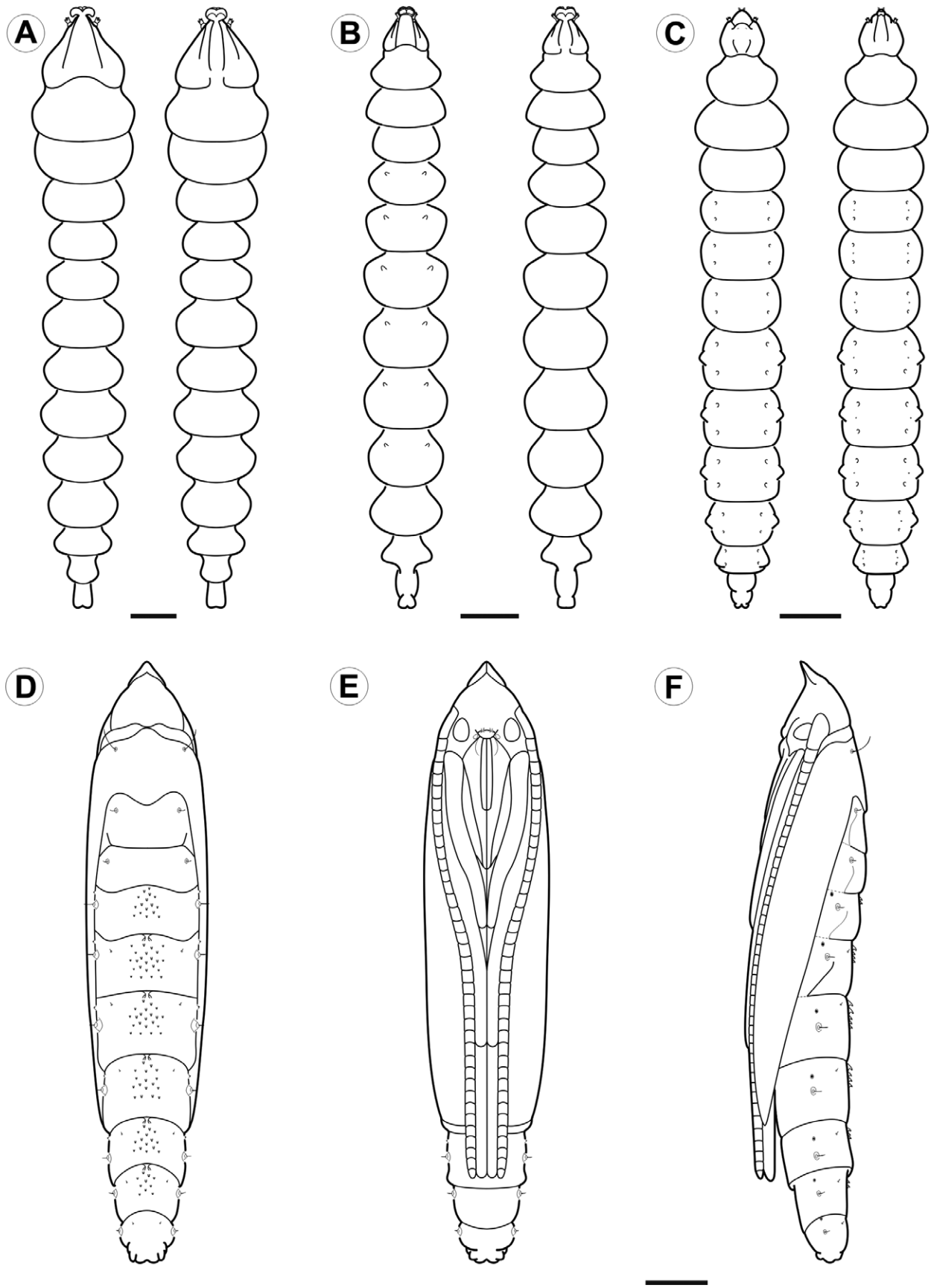

FIGURE 3. Larval and pupal morphology of *Phyllocnistis tethys* under light microscopy: (A) first larval ("sap-feeding") instar, dorsal and ventral views; (B) third larval ("sap-feeding") instar, dorsal and ventral views; (C) fourth larval ("cocoon-spinning") instar, dorsal and ventral views; (D–F) pupa, dorsal, ventral and lateral views. Scale bars = 100, 400, 400, 300 µm, respectively.

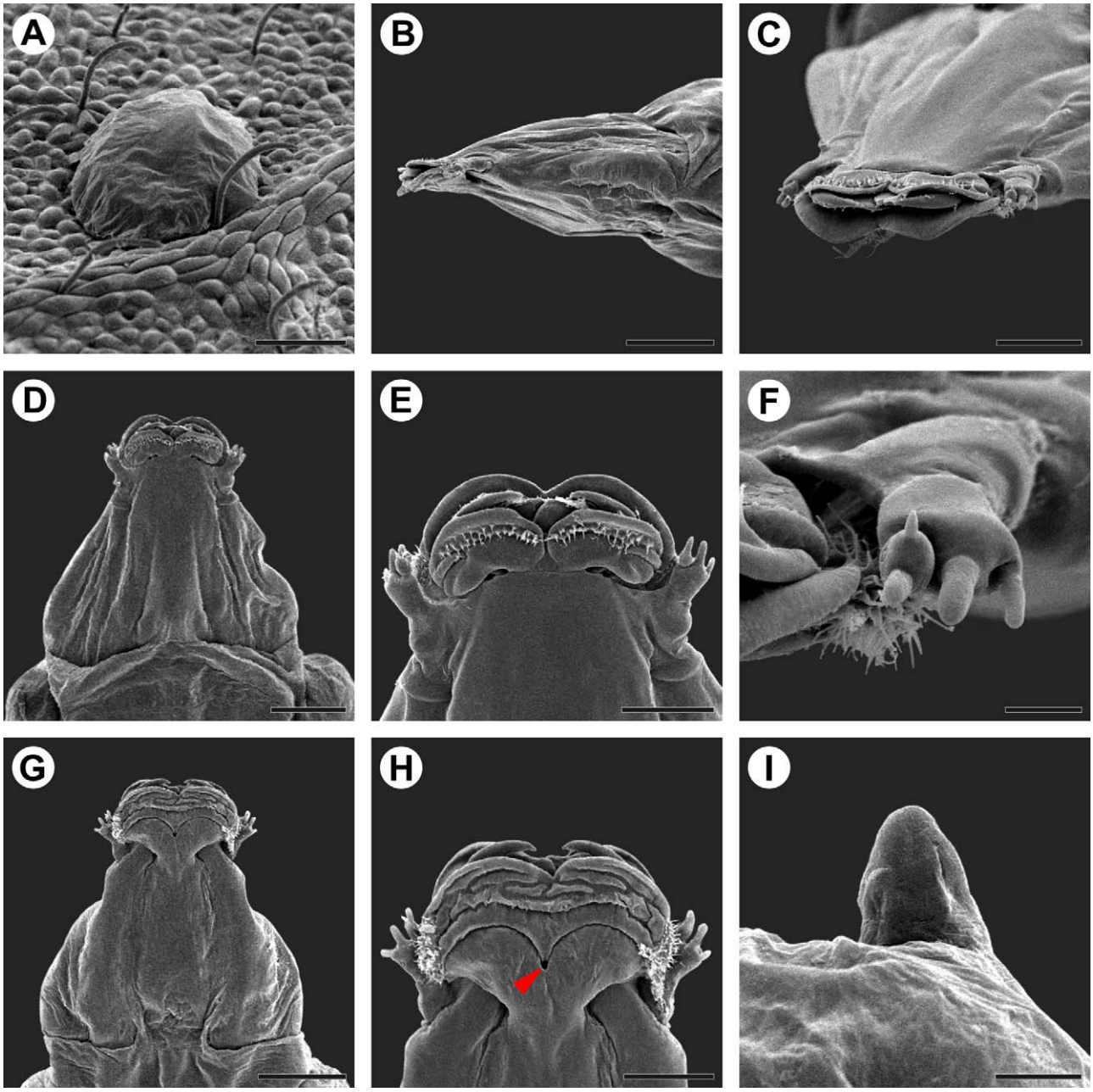

FIGURE 4. Scanning electron micrographs of *Phyllocnistis tethys* egg and third larval "sap-feeding" instar: (A) egg, on abaxial surface of a *Passiflora organensis* leaf; (B–D) head, lateral, anterior and dorsal views; (E) labrum and mandibles, dorsal view; (F) antenna, anterior view; (G) head, ventral view; (H) labium, ventral view (arrow indicates the spinneret); (I) abdominal lobe, dorsal view. Scale bars = 100, 100, 50, 100, 50, 10, 100, 50, 20 µm, respectively.

Sap-feeding instars (Figs 3A, B; 4B–I; 7B, E). Head prognathous, greatly depressed (Figs. 4B–D, G); primary setae either lost or reduced; stemmata absent. Antenna 3-segmented (Fig. 4F); second segment more slender than first, with 2 moderately stout sensilla; third segment less than 1/3 the length of second, with 2 apical sensilla. Labrum (Figs. 4D, E) with well-developed lateral lobes; antero-lateral margins rounded; anterior submargin densely spinose; posterior margins slightly concave. Mandibles large, rounded, flattened plates; anterior surface smooth, lateral area with single tooth, and mesal area with minute serrations. Labium with well-developed lateral lobes, conspicuous rugose cuticular band extending across anterior margin, and cluster of short hypopharyngeal spines laterally. Spinneret rudimentary (Fig. 4H), without extension of cuticle covering aperture. Maxillary and labial palpi absent. Thorax and abdomen without setae. Legs and prolegs absent; one latero-dorsal pair of rounded lobes on each of terga A1–6 (Fig. 3B, 4I).

Spinning instar (Figs. 3C; 5; 7G). Body cylindrical, with all appendages and setae greatly reduced. Head capsule weakly sclerotized, with anteriorly pronounced trophic lobe (Figs. 5A–D); integument finely corrugated. Stemmata absent. Antenna short (Fig. 5F), one-segmented, nearly flush with head capsule, with 4 short sensilla. Maxilla rudimentary (Fig. 5E), flush with head capsule, represented by one moderately long and a pair of short sensilla chaetica. Spinneret short, with simple terminal opening (Fig. 5E). Legs and prolegs absent. Two pairs of weakly differentiated, ventral and dorsal callosities (Fig. 5G) on A1–8; pair of microsetae laterally between the ventral callosities; pair of ventral and dorsal lobes laterally on A4–8. Pleural region of body and last two abdominal segments partly covered by microtrichia (Figs. 5H, I).

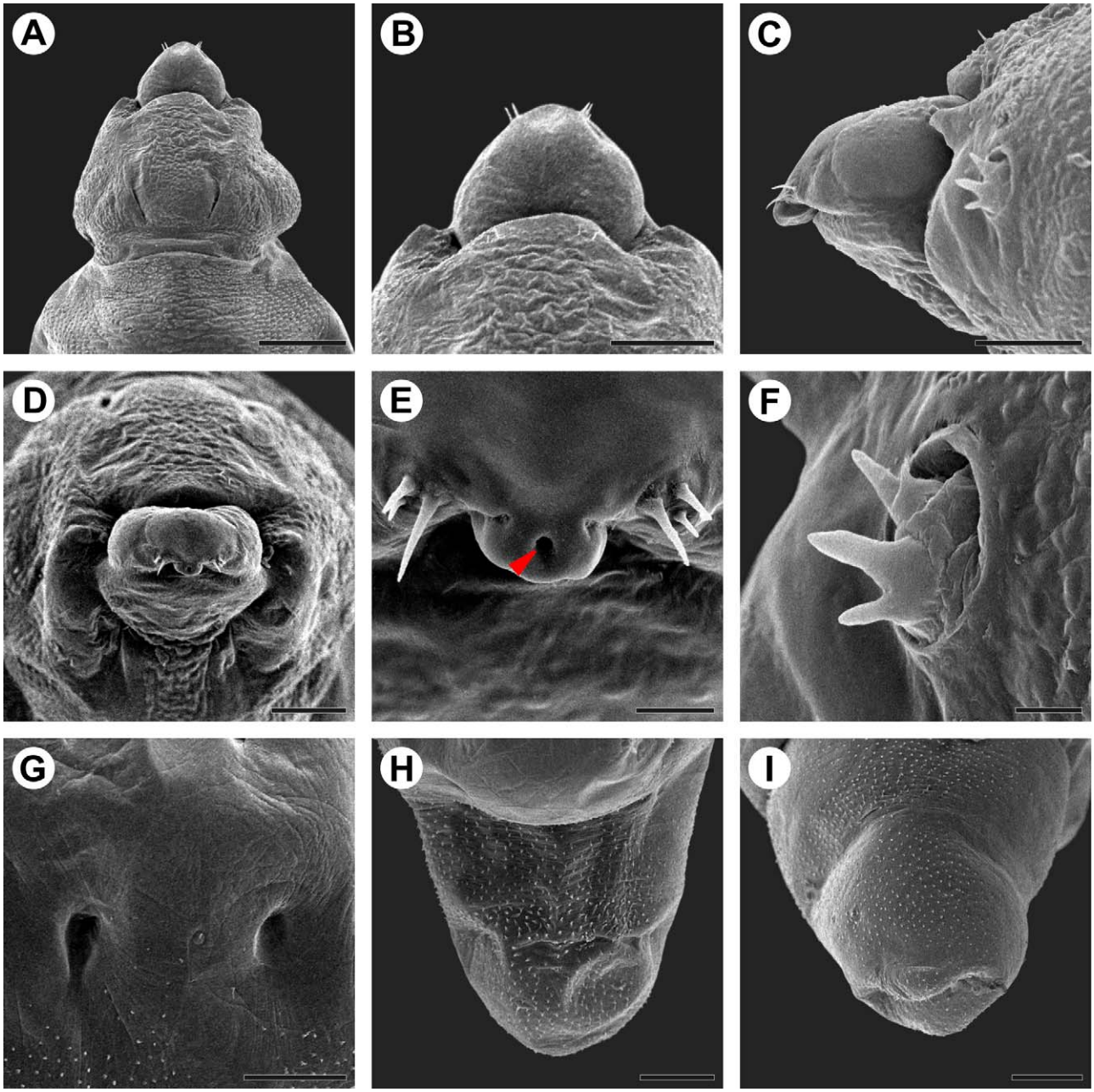

FIGURE 5. Scanning electron micrographs of *Phyllocnistis tethys* fourth larval (cocoon-spinning) instar: (A) head, general, dorsal view; (B–C) detail of head, dorsal and lateral views; (D) head, general, anterior view; (E) spinneret (indicated by arrow), anterior view; (F) antenna, lateral view; (G) invaginations of integument on abdominal sterna, ventral view; (H) caudal end of abdominal , ventral view; (I) dorsal view of fig. H (last segment retracted). Scale bars = 100, 50, 50, 50, 10, 10, 50, 50, 50 µm, respectively.

Pupa (Figs. 3D–F; 6B–O; 7H–K). Maximum length of specimens examined ranging from 2.59 to 3.20 mm. Coloration changing from light yellowish during early stage of pupation to yellowish brown (Fig. 7J) later in development. Vertex with large, subtriangular acute process (= cocoon cutter; Figs. 6B–E) with serrated anterior edge. Frons with 2 pairs of short frontal setae (Fig. 6F). Antenna long and straight, extending almost to abdominal segment A7; forewing extending almost to A6 (Figs. 3E, F). A pair of relatively long setae, latero-dorsally on meso-, metathorax and A1–8, those of A2–8 on chalaza (Fig. 6J); a second pair of micro-setae, meso-dorsally on anterior margin of A3–8; spiracles (Figs. 6K, L) on prothorax and from A1–8, anterior to latero-dorsal setae (Fig. 6J). Six mid-dorsal spine clusters, arranged in V-shaped pattern (Figs. 6G–I) on anterior margin of A2–7; each cluster with row of similar, low, posteriorly curved spines. Tenth abdominal segment with two pairs of relatively short, stout, digitate caudal projections located latero-dorsally and latero-ventrally (Figs. 6M–O). Pleural region of body and last two abdominal segments partly covered by microtrichia (Figs. 6J, M–O).

Pupal cocoon (Figs. 6A; 7I). Endophyllous, constructed at the end of the mine; spherical, covered by sparse silk threads (Fig. 6A), and without external ornamentation (Fig. 7I). Spun by the non-feeding (spinning) fourth-instar larva prior to molting.

Etymology. *Phyllocnistis tethys* is named after Tethys, a Titan goddess in the Greek mythology; the wife of Oceanus, and the mother of rivers, springs, streams, fountains and clouds. Thus, the name also alludes to the cloudy and humid nature of the area of the Brazilian Atlantic Rain Forest where the new species was first found. Proposed as a noun in apposition.

Host plant (Fig. 7A). The only host plant known for the immature stages of *P. tethys* is the passion vine *Passiflora organensis* Gardner (Passifloraceae) (Fig. 7A). This passion vine is found mainly on forest edges in the coastal mountains of southern Brazil, where it is endemic, ranging in distribution from the states of Minas Gerais to Rio Grande do Sul (details of the biology and distribution of *P. organensis* were given by Mondin *et al*. 2011 and Moreira *et al*. 2011, respectively).

Distribution. *Phyllocnistis tethys* is known only from the type locality, the Dense Umbrophilous Forest (= Brazilian Atlantic Rain Forest *sensu stricto*) portions of the CPCN Pró-Mata, São Francisco de Paula Municipality, Rio Grande do Sul, Brazil.

Life history. *Phyllocnistis tethys* eggs (Figs. 4A, 7C) are deposited mostly on the abaxial leaf surface, adhered by a cement substance, usually on the secondary veins. Eclosion occurs through the surface of the egg adhered to the leaf; the first-instar larva enters progressively into the leaf, loading frass to the outside, empty space covered by the chorion (Fig. 7D), since initially the posterior part of the body remains within the chorion. Larvae are sap-feeding leaf miners during the first three instars. By feeding in circles, they form a blotch mine that widens as the larvae develop (Figs. 7B, D). The feeding paths of a larva can be traced by following the dark-green, non-granular frass lines left and head capsule exuvia shed in the mine (Figs. 7F, 8A, B). The three sap-feeding instars are specialized in the abaxial spongy parenchyma, leaving the two epidermis layers and generally the palisade parenchyma intact (Figs. 8C–E). In conditions of low larval density, the adaxial palisade parenchyma may be partly used by later instars (Fig. 7F), and in this case the feeding damage appears as white scars visible through the transparent upper leaf surface (Fig. 7A). However, if a leaf is intensively attacked, at the end of development the palisade parenchyma can be almost completely consumed; leaves then appear mostly deprived of green color (Fig. 7H, I). We could not find a distinct weaving pattern for the flimsy endophyllous cocoon constructed at the end of the mines by the last larval (spinning) instar (Fig. 7I). During adult emergence, the pupal cocoon is ruptured by the frontal process of the pupa (cocoon cutter). Generally after the adult emerges, the anterior half of the pupal exuvium (head and thorax) protrudes outside, while the posterior half remains in the pupal cocoon (Fig. 7K).

At the type locality, *P. tethys* mines are common in *P. organensis* plants. One to several mines may be present per leaf (up to 13 young mines have been found in a single leaf) and may cover almost the entire lamina later in development (Figs. 7A, H). Our field collection data indicate that the species may have more than one generation per year, with adults emerging primarily in summer and autumn.

Molecular phylogeny. A total of 639 nucleotide sites were analyzed, in which 231 were variable and 173 parsimony-informative. ML and MP analyses showed identical topology and similar bootstrap supports, and we therefore show only the former (Fig. 9). According to our phylogenetic hypothesis, *P. tethys* was strongly supported as a monophyletic clade, showing high branch length in relation to the other 11 species surveyed. Additionally, it was placed as the most basal lineage within *Phyllocnistis*.

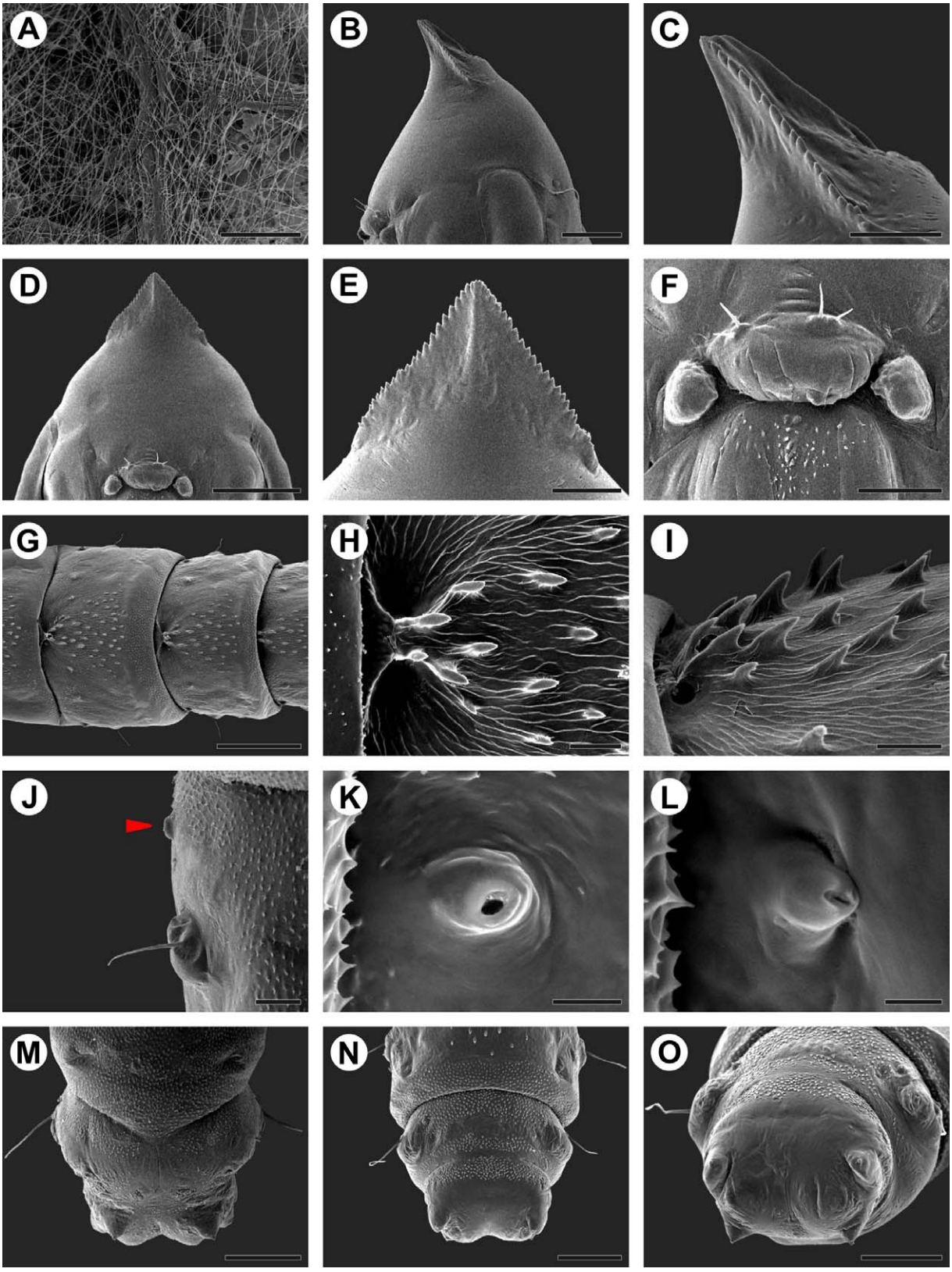

FIGURE 6. Scanning electron micrographs of *Phyllocnistis tethys* pupal cocoon and pupa: (A) weaving pattern of lower surface of pupal cocoon; (B) head, lateral view; (C) cocoon-cutter, lateral view; (D) head, ventral view; (E) cocoon-cutter, ventral view; (F) frons, ventral view; (G) abdominal segments 4 and 5, dorsal view; (H) spines on tergum 5, dorsal view; (I) spines on tergum 4, lateral view; (J) spiracle (arrow) and lateral seta on A5, dorsal view; (K) spiracle A3, lateral view; (L) spiracle A8, lateral view; (M–O) last abdominal segments in ventral, dorsal and posterior views, respectively Scale bars = 100, 100, 50, 150, 50, 50, 200, 25, 25, 25, 10, 5, 100, 100, 100 µm, respectively.

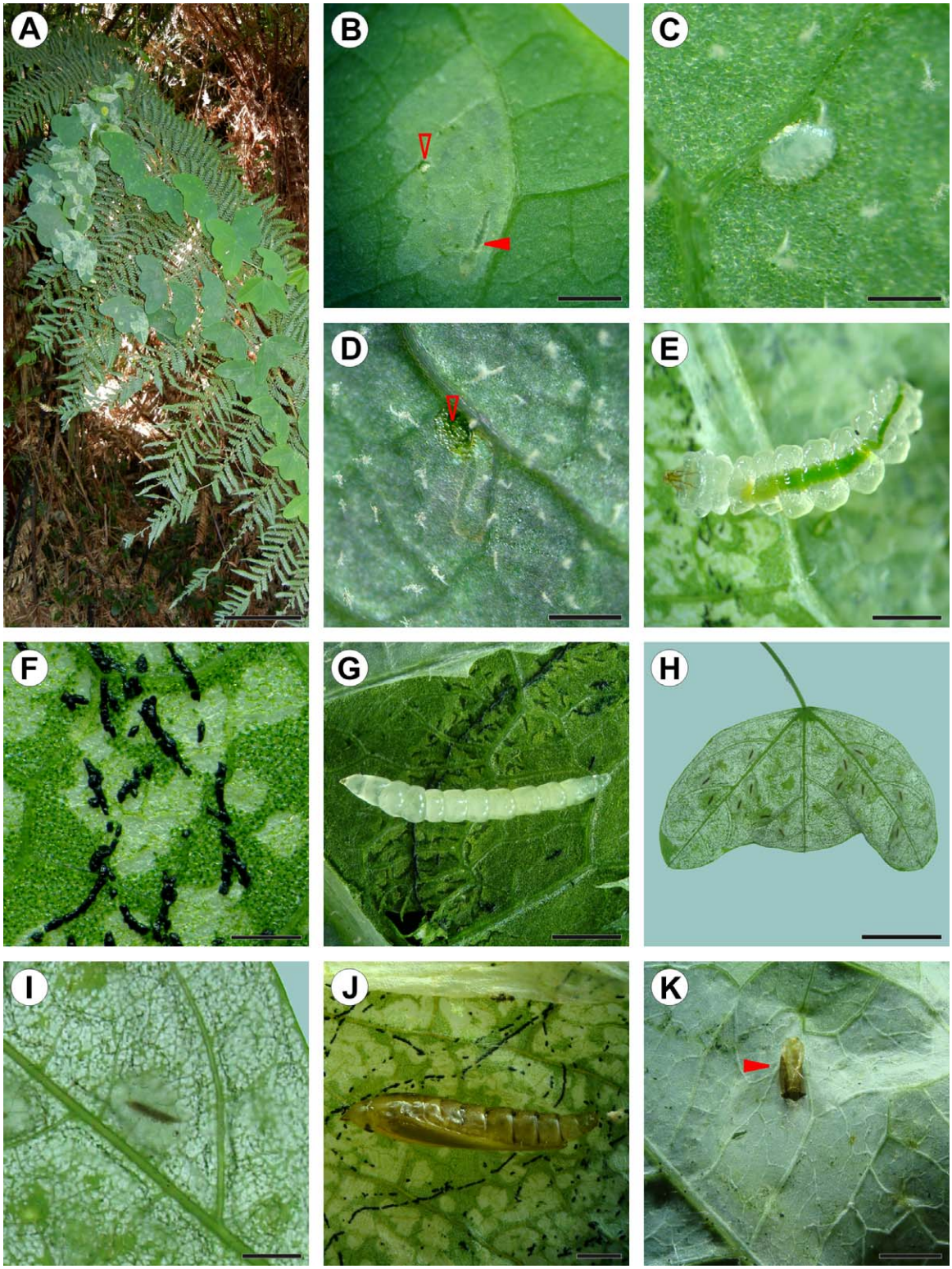

FIGURE 7. Life history of *Phyllocnistis tethys*: (A) *Passiflora organensis* shoot twining around on a fern at the type locality, showing several leaves with leaf mines at different development stages; (B) leaf mine on abaxial leaf surface (open and closed arrows, respectively, indicate empty chorion on leaf surface, and sap-feeding larva seen through transparent mine); (C) egg containing developing embryo; (D) freshly hatched larva (indicated by closed arrow; open arrow indicates green frass lines left within the egg chorion; (E) third-instar (sap-feeding) larva; (F) detail of frass lines and damage on leaf parenchyma, left by the larva within the mine; (G) fourth-instar (spinning) larva; (H) *Passiflora organensis* containing several pupae, seen by transparency (indicated by arrows); (I) a pupal chamber in detail, showing a pupa by transparency; (J) pupa, lateral view; (K) pupal exuvium protruded (arrow) from mine exit hole, just after the adult emergence. Scale bars = 100, 1, 0.2, 0.3, 1, 1, 1, 20, 5, 0.5, 2 mm, respectively.

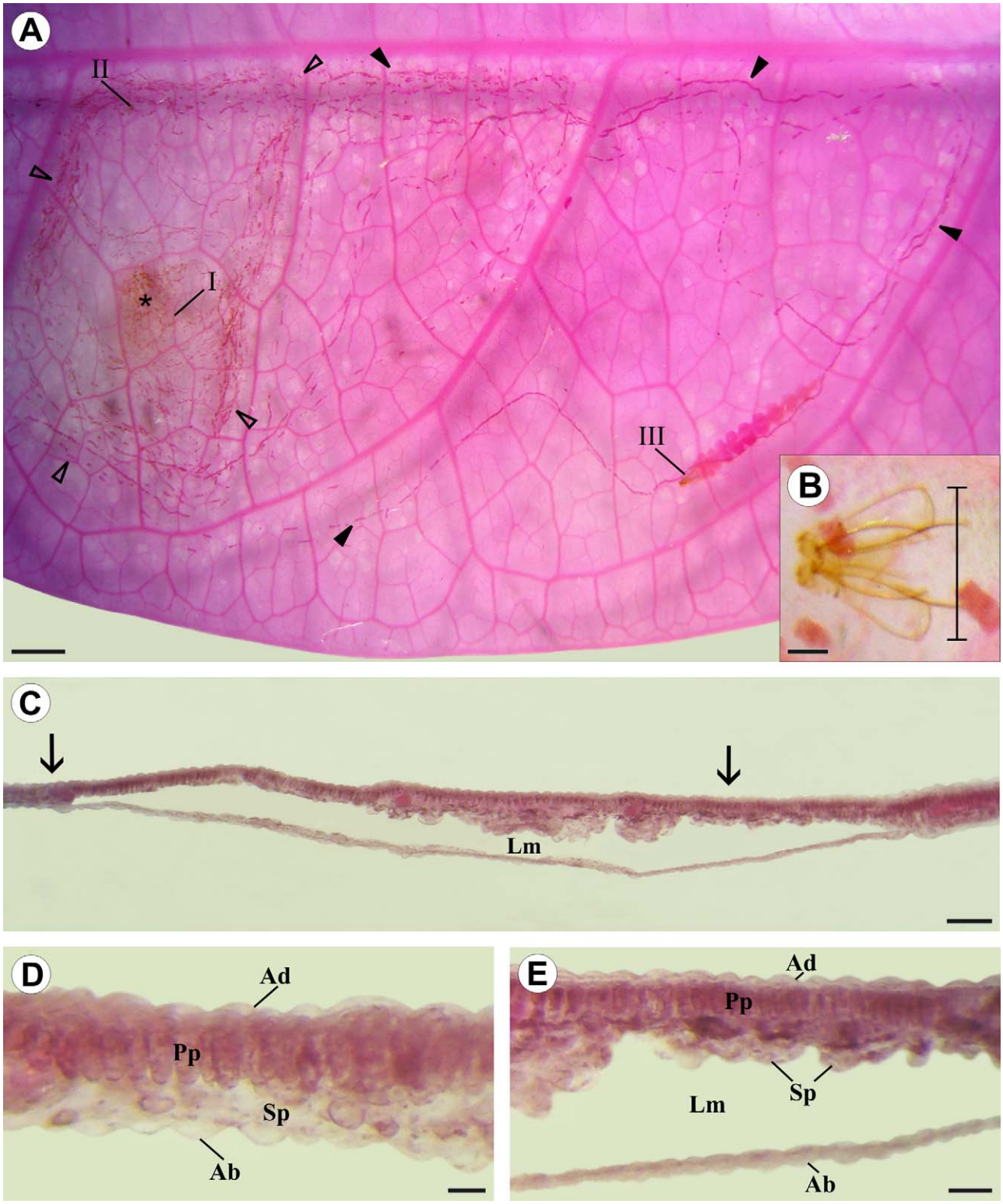

FIGURE 8. Diaphanized portion and histological sections of a *Passiflora organensis* leaf, showing by transparency the organization levels of a *Phyllocnistis tethys* mine in relation to larval ontogeny: (A) general aspect of the mine, containing a third-instar "sap-feeding" larva; asterisk indicates feeding area of the first instar; Roman numerals indicate larval instar numbers and corresponding positions of head capsules in the mine; open and closed arrows indicate the limit areas of non-granular frass lines left by second- and third-instar larvae, respectively; (B) detail of head capsule shed by the second-instar larva (bar indicates position for measurement of head-capsule width); C) transverse section of a mine; (D) transverse section of intact portion of leaf lamina (indicated by left arrow in C); (E) transverse section of mined portion of leaf lamina (indicated by right arrow in C). **Ab**, abaxial surface of epidermis; **Ad**, adaxial surface of epidermis; **Lm**, leaf mine; **Pp**, palisade parenchyma; **Sp**, spongy parenchyma. Scale bars = 1 mm, 50, 100, 25, 50 µm, respectively.

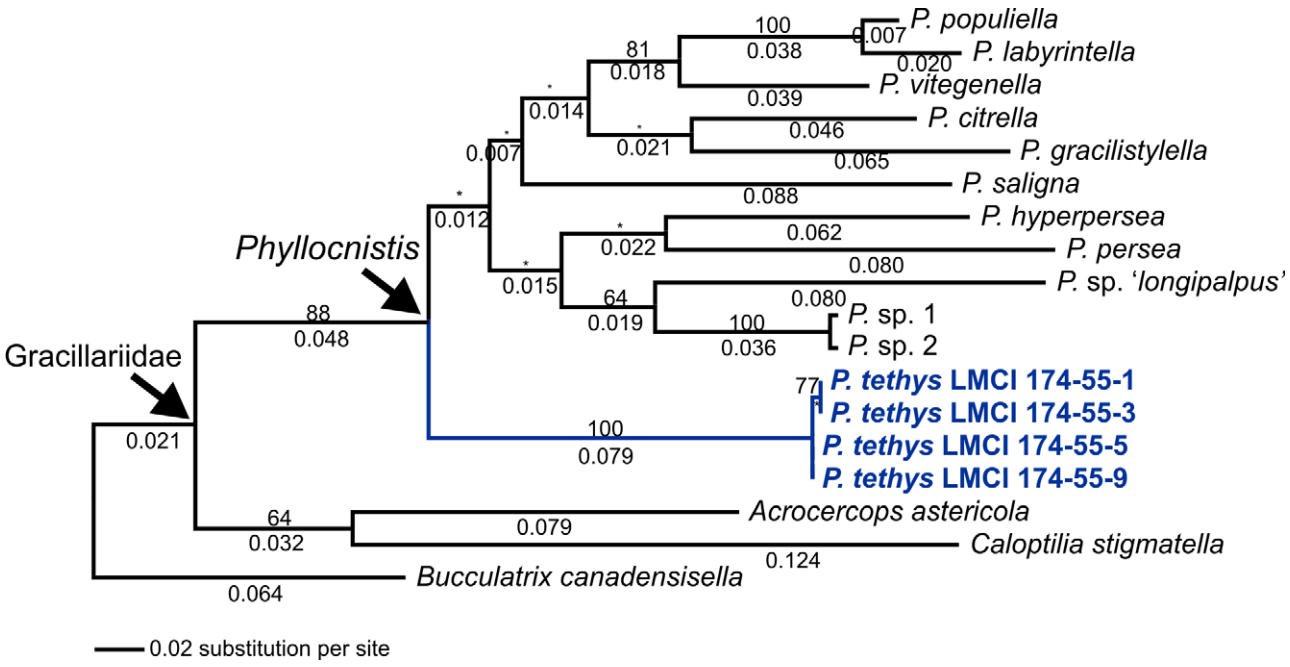

FIGURE 9. Maximum-likelihood tree of *Pyllocnistis* species based on 639 bp of the mitochondrial gene cytochrome oxidase subunit I (COI). Numbers above branches indicate bootstrap support higher than 60%; branch lengths are indicated below. Asterisks indicate bootstrap values < 60% and branch lengths < 0.001. Species of Bucculatricidae (*Bucculatrix canadensisella*) and Gracillariidae (*Acrocercops* and *Caloptilia*) were used to root the tree, according to the phylogeny proposed by Kawahara *et al*. (2011); see Table 1 and text for further description.

Discussion

Adults of *P. tethys* can be readily distinguished from all other known species of Neotropical *Phyllocnistis* by the forewing pattern, and also by differences in the genitalia and in the morphology and life history of the immature stages. For example, the cornuti of *P. tethys* are conspicuous, whereas their existence is not mentioned for other congeneric Neotropical species (*e.g*., Hering 1958; Davis 1994; Kawahara *et al*. 2009; Davis & Wagner 2011). In most female *Phyllocnistis*, the ostium bursae is a slender duct and the corpus bursae contains a pair of fusiform signa, bearing a short median projection (Davis & Wagner 2011); in the case of *P. tethys*, the ostium bursae is broad and there is only one fusiform signum in the corpus bursae. All Neotropical *Phyllocnistis* where the pupal stage has been described in detail have a pair of large recurved spines on the abdominal terga, and between these spines is a concentration of smaller spines arranged in a V-shaped pattern (Kawahara *et al*. 2009; Davis & Wagner 2011). Corresponding pairs of large spines are absent on all abdominal segments of *P. tethys*. Furthermore, all known phyllocnistine larvae construct long, serpentine leaf mines (Davis 1994; De Prins & Kawahara 2009). This is not the case for *P. tethys*, whose mines are clearly of the blotch type for all larval instars. Thus, most life stages of *P. tethys* show conspicuous differences in biology compared to other species of *Phyllocnistis* known for the Neotropical region. Also, phylogenetic reconstruction based on CO-I sequences indicated that this species is the basal lineage within *Phyllocnistis*, with higher differentiation (i.e., branch length) in relation to all other taxa in the genus for which sequences are known. However, we retain here the traditional nomenclatural status of the genus, until further evidence becomes available. This is the first species of *Phyllocnistis* described from Brazil. Future fieldwork in neighbouring Neotropical areas may reveal other undescribed congeneric species that are more closely related to *P. tethys*, and in that case the current taxonomic status should be re-evaluated.

According to our knowledge, this is the first gracillariid that has been found in association with a member of Passifloraceae. Our preliminary observations suggest this association is not occasional, because at least two additional undescribed leaf-mining gracillariid species are found on these plants in southern Brazil. Our discovery raises several questions regarding such a peculiar insect-plant association. Passion vines are toxic to most herbivorous insects, which is the reason that they have been successfully used as a food resource by only a few

ectophagous lepidopteran lineages, for example the Heliconiini (Nymphalidae) (for reviews, see Benson *et al*. 1975; Brown 1981; Gilbert 1991). Cyanogenic glycosides present in their leaves, for example, can negatively affect the feeding of some species, but on the other hand indirectly benefit other herbivorous insects. The heliconians in particular may either sequester or modify, or alternatively synthesize *de novo*, and use these substances for their own defense against vertebrate predators (Nahrstedt & Davis 1983; Spencer 1988; Engler-Chaouat & Gilbert 2007). The mechanisms by which these gracillariid larvae deal with the chemical compounds existing in the leaf parenchyma of their *Passiflora* host plants, where they are confined and feed throughout the larval stage, are of primary interest to be explored in the near future. The corresponding consequences, if any, for the adult stage of these gracillariids should also be investigated.

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CAPÍTULO III

ARTIGO SUBMETIDO À REVISTA ZOOKEYS

EM 26/02/2013.

BRITO R, GONÇALVES GL, VARGAS HA, MOREIRA GRP (2013). Description of the Brazilian gracillariid moth *Spinivalva gaucha*, gen. nov., sp. nov. with notes on life-history and DNA analysis of related genera. Zookeys (submetido em 26/02/2013).

Running title: NEW GRACILLARIID FROM BRAZIL

Description of the Brazilian gracillariid moth *Spinivalva gaucha*, gen. nov., sp. nov. with notes on life-history and DNA analysis of related genera

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Abstract. Male, female, pupa, larva and egg of a new genus and species of Gracillariidae, *Spinivalva gaucha* Moreira and Vargas from southern Brazil are described and illustrated with the aid of optical and scanning electron microscopy. A preliminary analysis of mitochondrial DNA sequences including members of related lineages is also provided. The immature stages are associated with *Passiflora actinia*, *P. misera* and *P. suberosa* (Passifloraceae), and build mines on the adaxial leaf surface. Initially the mines are serpentine in shape, but later in larval ontogeny become a blotch type. Unlike other gracillariids, there is no sap-feeding instar in *S. gaucha*; the larva feeds on the palisade parenchyma, thus producing granular frass during all instars. Pupation occurs outside the mine; prior to pupating, the larva excretes numerous bubbles that are placed in rows on the lateral margins of the cocoon external surface. This is the second genus of gracillariid moth described for the Atlantic Rain Forest, and the second gracillariid species known to be associated with Passifloraceae.

Keywords. Atlantic Rain Forest, gracillariids, leaf-mining moths, Neotropical region, passion vines.

Introduction

Gracillariidae is a diverse and speciose lineage of leaf-mining Lepidoptera, with a total of 102 recognized genera (1,880 species), distributed worldwide except for Antarctica; 24 of the genera (181 species) have been recorded in the Neotropical region (De Prins and De Prins 2013). Only four genera are recognized as endemic to South America; one occurs in the Atlantic Rain Forest of Brazil (*Leurocephala* Davis and McKay) and three in Chile: one in the southern Valdivian forests (*Prophyllocnistis* Davis) and two in the northern coastal valleys of the Atacama Desert (*Angelabella* Vargas and Parra, and

Chileoptilia Vargas and Landry) (Davis 1994, Vargas and Landry 2005, Vargas and Parra 2005, Davis et al. 2011). Only 29 gracillariid species have been recorded up to now for the Amazon and Atlantic rain forests of Brazil. This small number likely results from low collecting effort, since microlepidopterans in general have been undercollected in these biomes. Recent surveys conducted in a relatively small area of Central America suggested that a single gracillariid genus (*Phyllocnistis* Zeller) may include hundreds of species (Davis and Wagner 2011).

Almost all of what is known about the diversity of Brazilian gracillariids is concerned with the adult stage, in general associated with the original species descriptions, which were provided primarily by the pioneer work of Meyrick (1920, 1921, 1924, 1928, 1932). Several recent studies have suggested that the most informative characters for distinguishing species of leaf-mining moths might be found in the pupal morphology (*e.g.*, Patočka 1989, Fujihara et al. 2001, Kawahara et al. 2009, Kobayashi et al. 2011). However, studies that include the description of immatures are still in their infancy for microlepidopterans in general, in both the Amazon and Atlantic regions of Brazil (*e.g.*, Brown et al. 2004, Becker and Adamski 2008, Brito et al. 2012, Moreira et al. 2012), and thus should be taken as a priority in research on this group.

The Atlantic Rain Forest, where only six species of gracillariids have been recorded up to now (Davis and Miller 1984, Davis et al. 2011, Brito et al. 2012), originally extended for more than 3,300 km along the eastern Brazilian coast and covered more than 1.1 million km^2 (for a general description, see Morellato and Haddad 2000, Oliveira-Filho and Fontes 2000). Although now restricted to less than 8% of its earlier range, this biome is still among the areas with the greatest diversity of plants and animals on earth, and has long been recognized as extremely rich in endemics (Myers et al. 2000, Carnaval et al. 2009), including Lepidoptera (Freitas et al. 2011). For example,

Stehmann et al. (2009) listed 14,552 species of vascular plants for the entire Atlantic Rain Forest, of which 6,933 (49%) are endemic. Considering the wide range of host plants used and the high level of host specificity usually found for the leaf-mining gracillariids in general (Davis 1987), it seems reasonable to predict that hundreds of gracillariid species await description in this understudied, species-rich biome, to which probably most of them are also endemic.

In the course of an ongoing survey on the diversity of microlepidopterans in the Atlantic Rain Forest in southern Brazil, we recently found a leaf-miner gracillariid associated with Passifloraceae. A literature comparison indicated that this taxon is distinct from other described genera of Gracillariidae, and therefore a new genus is proposed herein. We describe and illustrate all the life stages of this new species, and provide a preliminary characterization of its life history, including histological aspects of the leaf mine. We also present a preliminary analysis of mitochondrial DNA sequences, including members of related genera.

Materials and Methods

Specimens used in the study were reared in small plastic vials under controlled abiotic conditions (14 h light / 10 h dark; 25 ± 2 ºC) in the Laboratório de Morfologia e Comportamento de Insetos, Departamento de Zoologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre city, Rio Grande do Sul State (RS), Brazil, from May 2011 through December 2012. They came from field-collected leaves bearing eggs, mines with feeding larvae inside, and pupae on shoots of *Passiflora actinia* Hook. (São Francisco de Paula municipality, RS), *P. misera* Kunth and *P. suberosa* L. (Porto Alegre municipality, RS) plants.

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. Structures selected to be drawn were previously photographed with a Sony® Cyber-shot DSC-H10 digital camera mounted on the stereomicroscope. Vectorized line drawings were then made with the software Corel Photo-Paint® X3, 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® JSM5800 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of UFRGS.

Descriptions of plant anatomy were based on diaphanized, field-collected leaf-mines (n =5) from *P. actinia* shoots that were fixed in FAA (37% formaldehyde, glacial acetic acid, and 50% ethanol, 1:1:18, v/v), stained with rose bengal (aqueous solution: 200 mg/1) and mounted either whole or in freehand section in glycerin on slides, following a procedure described in detail by Brito et al. (2012).

Molecular analysis. High-quality DNA was purified from larval tissue using the organic method of Cetyl Trimethyl Ammonium Bromide (CTAB) to investigate (i) levels of genetic variation within *Spinivalva* specimens collected in different localities and from different host plants (*Passiflora misera, P. suberosa* and *P. actinia*) and (ii) reconstruct phylogenetic relationships of this new genus among and within the *Parectopa* group of gracillariids. A total of nine field-collected specimens from three

populations: 1) Porto Alegre, RS, from *P. suberosa* and *P. misera* (Pop. 1); 2) São Francisco de Paula, RS, from *P. actinia* (Pop. 2) and 3) Curitiba, PR, also from *P. actinia* (Pop. 3). They were used to amplify 1.5 kb of mitochondrial genes cytochrome *c* oxidase subunit I (CO-I), transfer RNA (tRNA-Leu), and cytochrome *c* oxidase subunit II (CO-II). For the PCR amplification we used the primer pairs Jerry + Pat II for the first segment (700 bp), and Patrick + Eva for the second (800 bp), following the procedure described by Caterino and Sperling (1999). Additionally, we amplified genetic material from three specimens of *Spinivalva,* using the universal barcode primers LCO1490 (5'-ggtcaacaaatcataaagatattgg-3') and HCO2198 (5'-taaacttcagggtgaccaaaaaatca-3'), following the procedure of Folmer et al. (1994). We obtained variants that exactly matched the region previously sequenced in 6 representative taxa of the *Parectopa* group of gracillariids, downloaded from GenBank and incorporated into our analysis (Table 1). The remaining PCR products were treated with exonuclease I and shrimp alkaline phosphatase (ExoSAP) (Fermentas Inc.), sequenced using the BigDye sequencing kit and analyzed in an ABI 3730XL DNA Analyzer (Applied Biosystems Inc.). Sequences were aligned and visually inspected using the algorithm Clustal X in MEGA 5 (Tamura et al. 2011) running in full mode with no manual adjustment. The dataset of 1.5 kb generated for specimens of *Spinivalva* from three different localities was deposited in GenBank under the accession numbers KC512114- 512123. The phylogenetic tree was reconstructed based on Bayesian inference and implemented in BEAST 2.0 (Drummond et al. 2012) to recover (i) the evolutionary distance within *Spinivalva* taxa from different localities and host plants, and (ii) relationships of *Spinivalva* among the lineages of gracillariids surveyed in this study. In both trees, the HKY85 model of sequence evolution (Hasegawa et al. 1985) was used with empirical base frequencies and 4 gamma categories. A relaxed uncorrelated log-normal clock was

used, with no fixed mean substitution rate and a Yule prior on branching rates. We used four independent runs of 10 million generations, with the first 500,000 of each run discarded as burn-in. Posterior probabilities were used as an estimate of branch support. The species-level tree was unrooted, while the genus-level was rooted with a species of Bucculatricidae (*Bucculatrix ulmella*).

Museum collections. Abbreviations of the institutions from which specimens were examined are:

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.

MCNZ Museu de Ciências Naturais, Fundação Zoobotânica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

MCTP Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Results

Spinivalva Moreira and Vargas, new genus

(Figs. 1-11)

Type species. *Spinivalva gaucha* Moreira and Vargas, sp. nov. new species

Diagnosis. *Spinivalva* males show several abdominal and genital features that in conjunction differentiate this taxon from all known gracillariid genera: 1) saccular extension of valva abruptly narrowing distally, forming a single, medially bent process bearing a stout sensillum at the apex; 2) aedeagus tubiform, slender, straight and long,

ending as a sharply pointed spine; 3) saccus with anterior process long and tubiform; 4) two pairs of coremata, each with two unit types that are formed by an external hair pencil and a tubular, membranous, corrugated pouch. In the female genitalia, the circular ostium bursae is located near the anterior margin of sternum VII, having a membranous corpus bursae associated with an accessory bursa, with no signum. The larvae construct mines on the adaxial surface of passion-vine leaves; initially the mines are serpentine in shape but later in ontogeny become a blotch type. Unlike all known stages of other leaf-miner gracillariids, *S. gaucha* has no larval sap-feeding instars; all instars of its larvae have a conspicuous spinneret and mandibles of the chewing type, and feed on the palisade parenchyma after hatching. Pupation occurs outside the mine; the larva excretes numerous bubbles that are aligned on the lateral margins of the cocoon surface prior to pupation.

Description

Adult (Figs. 1-4). Male and female similar in size and color. Small moth, forewing length 2.78–3.61 mm (n = 5). *Head* (Fig. 2A): Vestiture moderately smooth, with a large, light-gray dorsal scale tuft that curves forward to the frons; scales slender, with apices slightly rounded. Eye relatively large, rounded, with dorsal margin slightly concave; vertical diameter ~ double minimum interocular distance across frons (n = 6). Antenna filiform, long, exceeding length of forewing; scape slightly elongate, ~ 2.4x length of pedicel; flagellomeres completely encircled by single, dense row of slender scales. Labrum trilobed, pilifers well developed, triangular. Mandible absent. Haustellum naked, elongate, ~ 2.0x length of labial palpus. Maxillary palpus short, smoothly scaled, 4-segmented; ratios of segments from base: ~1.0 : 2.2 : 3.6 : 3.5. Labial palpus smoothly scaled, moderately long, bent anteriorly and upward; ratio of

segments from base: ~1.0 : 4.6 : 0.3. *Thorax*: Forewing (Fig. 2B) lanceolate, with 12 veins, all arising separately from the cell and reaching the margin; L/W index ~ 7.3; retinaculum consisting of few subcostal, narrow, flat, longer, loosely coiled scales (Fig. 2C); discal cell ~ 0.8x length of forewing (n = 4) ending near distal fifth of wing margin; R5-branched; R1 ending near proximal third of wing margin; M3-branched, CuA not branched, and faded basally; CuP weak proximally and not stalked, with 1A+2A that is well developed, extending past midlength of posterior margin. Hindwing (Fig. 2B) extremely lanceolate, L/W index ~ 9.6, ~ 1/8 forewing in length; male frenulum (Fig. 2D) a single stout bristle; female with frenulum divided at base, then fused for nearly its entire length and appearing as a single stout bristle; pseudofrenulum consisting of ~8 modified scales arising in two to three irregular rows near $Sc+R_1$ ending at circa 1/5 anterior margin; Rs faded proximally, ending at circa 1/3 anterior margin; M and CuA unbranched, both faded proximally and weak distally, ending at circa 1/3 and 2/3 of posterior margin. Legs with tibial spur pattern 0-2-4; epiphysis present. Tibial length ratios (anterior / middle / posterior legs) ~ 0.55/0.85/1.0. *Abdomen*: Male with segments VII-VIII complex and reduced, except for enlarged tergum VIII; segment VII reduced to narrow, almost completely sclerotized ring; tergum VIII elongate, hoodlike, partly covering tegumen; sternum VII bearing two pairs of coremata, arising from distal apex of rodlike sclerites that protrude from intersegmentary membrane VII-VIII; each coremata (Fig. 3D) bearing two types of units – an external hair pencil (~ valva in length) and a tubular, membranous, corrugated pouch; pouches of anterior pair ~ ½ hair pencil in length; those of caudal pair double in size (near to hair pencil in length). Female postabdominal segments unmodified.

Male genitalia (Figs. 3A-C, 4A, B, D, E). Uncus absent. Tegumen broad, hood-shaped, mostly membranous, with shallow apical notch. Pair of long, distally tapering affilated, membranous lobes arising ventrally beneath tegumen. Vinculum long, broadly V-shaped, extending laterally along base of valva. Saccus well developed, U-shaped; anterior process long and tubiform, ~1/2 length of valva, apex slightly capitate. Transtilla an arched, sclerotized plate joining bases of valvae. Juxta small, a dorsally concave, membranous plate, attached to middle of aedeagus. Aedeagus (Figs. 3B, 4E) tubiform, slender, straight and long (~2x valve length), slightly dilated caudally, with subapical, dorsally located concave aperture and ending as sharply pointed spine; entry of ductus ejaculatorius located at anterior end; vesica without cornuti. Valva (Figs. 3C, 4A, B, D) broad at base, and deeply divided; costal margin relatively straight and distally rounded; cucullus densely covered by long piliform setae; sacculus with broad lobe abruptly narrowing distally, ending as a medially bent process with apex bearing a stout, blunt sensillum.

Female genitalia (Figs. 3E, 4C, F). Sternum VII subtriangular; anterior margin linear; posterior margin with narrow notch. Tergum VIII subtriangular. Anterior apophysis with arms slightly curved, similar in length to posterior apophyses. Anal papillae connected dorsally, covered with long piliform setae and microtrichia. Ostium bursae moderately wide, located on anterior margin of sternum VII. Ductus bursa membranous, wider in middle, forming an accessory bursa ~ 1/3 length of corpus bursae. Corpus bursae membranous, gradually broadening posteriorly, ~ twice length of ductus bursae. Ductus seminalis membranous, narrow, inserted on distal portion of accessory bursa. Signum absent.

Etymology. The genus name is derived from the Latin *spina* (spine) and *valva* (valve), in reference to the conspicuous spine-like process present on the male valvae.

Spinivalva gaucha Moreira and Vargas, new species

(Figs. 1-11)

Diagnosis. As discussed for the genus.

Description. Adult (Fig. 1). *Head*. Frons light gray; vertex covered mostly by white scaled tuft that curves forward to the frons. Antennae mostly dark gray; scape white ventrally with pecten of light-gray hairlike scales; pedicel and flagellum ventrally whitish gray. Maxillary and labial palpi mostly white, with scattered dark-gray scales laterally. *Thorax*. Forewing mostly covered by dark-gray scales. Narrow stripe of white scales along posterior margin; a zigzag edge, formed by short, oblique white fascia, separates this stripe from the remaining, mostly dark-gray area; distal portion of apical fascia bearing brownish scales. Apical portion with transverse bar of light-gray scales that separates distally two well-defined dots, one dark gray (toward anterior margin) and one white (toward posterior margin). Fringe with scales of two sizes, mostly white at base and dark gray apically. Hindwing completely covered by dark-gray scales and with concolorous fringe. Forelegs mostly dark gray, with some white scales basally and apically on each podite, particularly on coxa. Midlegs mostly white with scattered light-brown scales, and transverse dark-gray stripes on femur, tibia, tibial spurs and tarsomeres. Hindlegs similar to midlegs, but with hair-like scales on tibia. *Abdomen*. Mostly dark gray, with transverse, V-shaped white stripes on ventral surface of segments III-VI.

Male genitalia (Figs. 3A-D; 4A, B, D, E). As described for genus.

Female genitalia (Figs. 3E; 4C, F). As described for genus.

Type material. BRAZIL: Condomínio Alpes de São Francisco, 29º27'9.2''S, 50º37'6.6''W, São Francisco de Paula Municipality, Rio Grande do Sul State (RS), Brazil. All preserved dried and pinned, reared by the senior author from larvae and pupae collected on *Passiflora actinia* Hook. (Passifloraceae): LMCI 186, 26.V.2012, by G.R.P. Moreira, H.O. Vargas and S. Bordignon; LMCI 199, 19.XII.2012 by G.R.P. Moreira, R. Brito and F.A. Luz. HOLOTYPE: ♂ (LMCI 199-01), donated to DZUP (24.976). PARATYPES: 1♀ (LMCI 199-02), donated to DZUP (24.986); 1♂, 1♀ (LMCI 199-03 and 186-12), donated to MCNZ (81900 and 81903, respectively); 1♂, 1♀ (LMCI 199-04 and 186-15), donated to MCTP (28637 and 28639, respectively).

Other specimens examined. LMCI 156: Floresta Nacional de São Francisco de Paula, 29º25'21.4''S, 50º23'26.6''W, São Francisco de Paula Municipality, RS, Brazil, collected by K.R. Barão, 13-15.V.2011, on *P. actinia*. LMCI 157: Condomínio Alpes de São Francisco, 29º27'9.2''S, 50º37'6.6''W, São Francisco de Paula Municipality, RS, Brazil, collected by G.R.P. Moreira, R. Brito and G.L. Gonçalves, 28.V.2011, on *P. actinia*. LMCI 164: Campus da Vale, Universidade Federal do Rio Grande do Sul (UFRGS), 30º04'12.9''S, 51º07'11.5''W, Porto Alegre Municipality, RS, Brazil, collected by R. Brito, on *P. misera* Kunth and *P. suberosa* L. (Passifloraceae). LMCI 169: Centro Politécnico da Universidade Federal do Paraná, 25º26'44.1''S, 49º13'56.8''W, Curitiba Municipality, Paraná State, Brazil; 5 larvae dissected from mines collected by G.R.P. Moreira, on *P. actinia*; used for DNA extraction only. Adults, dried and pinned, with the same collection data, deposited in LMCI: 4♂♂ (LMCI 156-9, 164-6, 7, 10); 1♀, (LMCI 164-9). Genitalia preparations, mounted in Canada balsam on slides, with the same collection data, deposited in LMCI: 4♂♂ (GRPM 50-11, 13, 21, 22); 4♀♀ (GRPM 50-12, 23, 32, 34). Immature stages, fixed in Dietrich's fluid and preserved in 70% ethanol, with the same collection data series,

deposited in LMCI: 2 eggs (LMCI 157-2), 4 first-instar larvae (LMCI 157-8), 5 third-instar larvae (LCMI 157-4), 6 fifth-instar larvae (LMCI 157-10), and 9 pupae (LMCI 157-5, 6). Mature leaf mines (n = 5) containing larval exuvia, mounted in glycerin on slides and stained with rose bengal, with the same collection data, deposited in LMCI, under accession numbers LMCI 186-3, 7 and LMCI 199-5, 6, 7.

Etymology. The specific name is derived from the Portuguese "Gaúcho", a term commonly used for natives of Rio Grande do Sul, the southernmost state of Brazil, where this new species was first found.

Immature stages

Egg (Fig. 10C). Flat, ellipsoid, laid on the abaxial surface, usually close to the leaf veins; chorion translucent, larva visible through transparent area of leaf before eclosion; chorionic ultrastructure, aeropyles and micropylar area not observed.

Larva (Figs. 5A, B, 6, 7, 8, 10F, H). Head brown, thorax and abdomen yellowish. Leaf-miner, with hypermetamorphic development and five instars, all endophyllous, prognathous and tissue feeders; that is, there is no sap-feeder instar, and all larvae have a typical spinneret and functional mandibles of the chewing type. Instars change gradually in external morphology during ontogeny, and can be identified through measurements of the head capsule, since there is no overlap between the head-capsule size of succeeding instars (Table 2). The following exponential growth equation was adjusted for the head-capsule width from larvae reared on *Passiflora actinia*: $y = 0.078e^{0.420x}$; $n = 25$; $r = 0.99$; $p < 0.0001$. Preliminary observations suggested that the number of instars may vary from four to five as a function of the host plant, which should be further explored.

First instar (Figs. 5A, 6A-F). Body depressed, without setae, legs or pseudopodia. Antennae (Fig. 6C) reduced, one-segmented, nearly flush with head capsule, with four short sensilla. Stemmata absent. Labrum (Fig. 5A) moderately bilobed, with slight central notch. Mandibles (Fig. 6B) of chewing type, with three blunt teeth. Maxilla (Fig. 6E) rudimentary, uni-segmented, with two finger-like, terminal lobes. Labium relatively broad, with well-developed tubular spinneret having flared terminal opening (Fig. 6D). Hypopharynx bearing few papilliform projections basally (Fig. 6D). Labial palpi (Fig. 6F) vestigial, reduced to pair of closely appressed, slender setae.

Third instar (Figs. 7A-F). Similar to first instar, but with body partly depressed and setae greatly reduced. Prothoracic shield slightly differentiated (Fig. 7F), nearly colorless; legs and pseudopodia absent. Antennae (Fig. 7E) bi-segmented, first segment stouter than second segment, each bearing three sensilla. Stemmata absent. Labrum (Fig. 7B) bilobed, with pronounced median notch, and several ventral, posteriorly bent papilliform projections. Mandibles of chewing type, with three teeth. Maxilla well developed, as shown in Fig. 7C. Labium broad, with spinneret similar to that of first instar, but shorter and stouter (Fig. 7C). Labial palpi (Fig. 7C) short, bi-segmented, each bearing apical sensillum. Hypopharynx with several papilliform projections basally (Fig. 7C).

Fifth instar (Figs. 5B, 8A-L, 10F, H). Body subcylindrical, covered by microtrichia and with setae well developed; thoracic legs reduced; pseudopodia present on A3-5, A10. Head and prothoracic shield brown (Fig. 10F); remaining parts of body yellowish (Fig. 10F), changing to red before pupation (Fig. 10H). Maximum length of larvae examined 5.52 mm. Antennae (Fig. 8B) three-segmented, second segment longer than third, each bearing four sensilla. Stemmata six in number, five of them arranged

close to lateral margin of head, and one inconspicuous stemma located ventrally (Fig. 8C). Labrum (Fig. 8A) bilobed, with deep median notch, similar to previous instars, but with ventral papilliform projections curved anteriorly. Mandibles similar to those of previous instars. Maxilla well developed, as shown in Fig. 8F. Labium broad, with stout, tubular spinneret having subapical opening (Figs. 8G). Labial palpi (Fig. 8E) bi-segmented, basal segment longer than distal one, each bearing apical sensillum, that of distal segment longer. Hypopharynx with two sets of dense papilliform projections (Fig. 8E). Chaetotaxy: A group trisetose; L group unisetose; S group trisetose; SS group bisetose.

Thorax with prothoracic dark-brown dorsal shield well developed; one pair of legs on each thoracic segment; each leg with one pair of tarsal setae and one curved hook-like tarsal claw; one circular spiracle on each side of prothorax, near posterior margin and slightly displaced dorsally (Figs. 8H-J). Protothorax chaetotaxy: D group bisetose, both located on dorsal shield; XD group bisetose, XD1 on dorsal shield and similar in length to D1 and D2; XD2 lateral to dorsal shield, about three times XD1 in length; L group bisetose, L1 dorsal to L2, slightly longer than XD2 and about three times L2 in length; SV group bisetose, posteroventral to L2, both similar to L1 in length; V group absent. Meso- and metathorax chaetotaxy: D group bisetose, length of both setae similar to prothoracic D2; D2 posterolateral to D1; L group unisetose, L1 similar to that on prothorax in length; SV group bisetose, similar to prothoracic SV group in size and position; V group absent.

Abdomen with paired, circular spiracle laterally on A1-8; pseudopodia on A3-5 and A10, bearing uniordinal crochets in lateral penellipse (Figs. 8K-L). Chaetotaxy of A1-2, 6-7: D group bisetose, both setae very small, D1 anterolateral to D2 and posterior to spiracle; SD group unisetose, SD2 anteroventral to spiracle, length of SD2 about half

of D1; L group unisetose, L1 length similar to anterior segment; SV group unisetose, length of SV1 about half of L1; V group unisetose, length of V1 similar to L1. A3-5: Similar to anterior segment, but V1 located on proleg and extremely reduced. A8: Similar to anterior segment, but SV group absent. A9: All setae lost except L group, which is similar to anterior segment. A10: D group unisetose, D2 on posterior margin; SD group unisetose, SD1 lateral to D2; L group unisetose, L1 about 1/3 length of corresponding seta on A9; SV group on proleg, bisetose; V group unisetose.

Pupa (Figs. 5C-E, 9D-L, 10J). Maximum length of specimens examined ranged from 3.69 to 5.10 mm. General coloration yellowish, with head, thorax, and corresponding appendices darkening later in development (Fig. 10J). Vertex bearing subtriangular acute process (= cocoon cutter; Figs. 9D-F) with serrated anterior edge, formed by several pointed projections that are fewer and larger at apex. Frons with 2 pairs of short frontal setae (Fig. 9D). Antennae long and slender, extending longer than pupal length; forewing reaching anterior margin of A6; proboscis extending to A2; prothoracic, mesothoracic and metathoracic legs reaching A3, A5 and A9, respectively (Figs. 5C-E). Abdominal integument dorsally covered with michotrichia (Figs. 9G-I). Intermediate abdominal segments with lateral margin of terga corrugated (Figs. 9G-H). From A1 to A7, two micro-setae, located medially on anterior margin of terga; additional micro-setae are found laterally, located posteriorly to spiracles (Figs. 9H-I). Last abdominal segment with two pairs of spines dorsally and one pair laterally, on posterior margin of tergum (Figs. 9J-L).

Host Plants. Passifloraceae: *Passiflora actinia* Hook, *P. misera* Kunth and *P. suberosa* L. The former, where *S. gaucha* was most frequently collected, is found primarily in forest edges in the coastal mountains of southern Brazil, where it is endemic, distributed from the Brazilian states of Espírito Santo to Rio Grande do Sul. *Passiflora suberosa*

and *P. misera* have broader distributions, extending to Central America, and also occur in relatively open areas occupied by shrubs and herbaceous vegetation. Details about the biology and distribution of these passion-vine species in southern Brazil were provided by Mondin et al. (2011) and Moreira et al. (2011), respectively.

Distribution. *Spinivalva gaucha* is known from the type locality (Condomínio Alpes de São Francisco) and the Floresta Nacional de São Francisco de Paula, both located in São Francisco de Paula Municipality, where *P. actinia* plants are used as larval host plants. A few scattered specimens were also collected from an additional population located in Porto Alegre Municipality. Both municipalities are located in Rio Grande do Sul (RS), Brazil. In the Porto Alegre population, *P. misera* and *P. suberosa* are used as hosts. We could not find conspicuous morphological differences among the specimens collected in RS, as also corroborated by the DNA analyses. Additional *Spinivalva* specimens were collected farther north in Curitiba Municipality, Paraná, also mining *P. actinia* leaves. However, as discussed below, analyses of the molecular data suggested that this population may correspond to a new species, a possibility that should be further explored. All these populations are located within the Atlantic Rain Forest domain *sensu lato* (Morellato and Haddad 2000).

Life history (Figs. 9A-C, 10A-E, G-I, K, 11). Eggs of *S. gaucha* are deposited on the abaxial leaf surface, adhered by a cement substance, close to the midrib or secondary veins (Fig. 10C). Hatching occurs through the surface of the egg adhered to the leaf; the first-instar larva moves directly into the leaf lamina, easily reaching the adaxial side of the leaf (Fig. 10D). Initially, the mine is narrow, slightly serpentine in shape, increasing in width progressively during ontogeny and becoming a blotch during the last larval instar. The feeding pattern is not altered during ontogeny; that is, the larva feeds on the palisade parenchyma from the beginning to the end of the mine (Figs. 10B, 11). Dark-

green granular frasses of increasing sizes are found in the larva's feeding path (Figs. 10D, E), as are the head-capsule exuviae**.**

After the fifth-instar larva leaves the mine through a slit made in the blotch section (Fig. 10G) and prior to pupal molting, it spins the pupal cocoon, usually on the adaxial leaf surface of adjacent leaves. The pupal cocoon is exophyllous, elliptical in general outline, transparent, from 7.76 to 8.74 mm long in the specimens examined. Silk filaments are woven in a tight pattern, forming a compact, flat wall that covers the pupa (Figs. 9A, 10I). The cocoon periphery is adorned with several irregularly spaced, minute, light-yellow bubbles (Fig. 10I). These are not compartmentalized, showing a finely granular structured surface (Fig. 9C). They are discharged from the anus by the mature larvae to the outside through a slit made with the mandibles in the cocoon wall, which is closed soon after deposition (Fig. 9B). Throughout this process, the bubbles are manipulated by the spinneret. During adult emergence, one end of the pupal cocoon is split by the frontal process of the pupa (cocoon cutter). Generally after the adult emerges, the anterior half of the pupal exuvium (head and thorax) protrudes outside, while the posterior half remains in the pupal cocoon (Fig. 10K).

At the type locality, *S. gaucha* mines occur at low numbers on *P. actinia* plants (Fig. 10A). In most cases, only one mine was found per leaf, but up to three were collected in a single leaf, and several leaves may be used per plant. There was no indication that this behavior differs from that of the other passion-vine species and populations studied. We could not find a clear pattern for the number of generations per year and the flight period.

Molecular phylogeny (Fig. 12). A total of 1583 nucleotide sites were analyzed for *Spinivalva* from different localities and host plants; 110 (7%) were variable. An unrooted Bayesian tree recovered two major groups (Fig. 12A). The first included

specimens from Porto Alegre (Pop. 1), hosted on either *P. suberosa* or *P. misera*, together with those from São Francisco de Paula / Condomínio Alpes de São Francisco (Pop. 2) hosted on *P. actinia*. The second group included individuals from Curitiba (Pop. 3) sampled on *P. actinia*. The genetic divergence between these major groups was 7% (Fig. 12A). The intraspecific difference between localities in the first group was 1%. In addition, the barcode fragment analyzed recovered 610 nucleotides, including 236 (38%) variable sites. According to our phylogenetic hypothesis, *Spinivalva* was strongly supported as a monophyletic lineage within the *Parectopa* group of gracillariids (Fig. 12B). Despite the strong statistical support within this lineage of gracillariids, the internal relationships for the genera included in the *Parectopa* group were poorly resolved. *Leurocephala schinusae* and *Liocrobyla desmodiella* were placed as closest to *Spinivalva* (showing lower genetic divergence), but with weak posterior probability of node support.

Discussion

The following characteristics suggest that *Spinivalva* gen. nov. belongs to the subfamily Gracillariinae (*sensu* Davis et al. 2011): 1) flat, scaled head; 2) maxillary palpi with four segments; 3) male abdomen bearing two coremata; 4) pupation occurring outside the mine; 5) adults resting with the anterior portion of the body inclined circa 45º. Our molecular analysis placed it within the *Parectopa* group (*sensu* Kawahara et al. 2011) in the Gracillariinae, near the genera *Leurocephala* Davis and McKay and *Liocrobyla* Meyr. From a morphological perspective, the forewing of adults of *Spinivalva* resembles those in the *Parectopa* group in general coloration, fascia arrangement, presence of apical dot, and venation pattern (Vári 1961). When compared to

Leurocephala, a recently described genus also found in the Atlantic Rain Forest of Brazil (Davis et al. 2011), additional similarities are found in the males, in particular regarding the reduced segment VII that bears two pairs of coremata, the elongated tergum VIII, and the presence of paired gnathal lobes. However, as noted above, males of *Spinivalva* differ markedly from those of *Leurocephala* and the remaining genera of the *Parectopa* group in relation to the valva. Unlike them, it has a saccular extension with a conspicuous process bearing a stout sensillum, in association with an aedeagus that is tubiform, long and slender, and a saccus with the anterior process long and tubiform. These differences extend to additional lineages related to the *Parectopa* group that were not included in our molecular analysis, for example *Micrurapteryx* Spul., *Neurobratha* Ely (Kawahara et al. 2011), and *Chileoptilia* Vargas and Landry (C. Lopez-Vamoonde, unpubl. data), and other genera described by Vári (1961). As far as we are aware, the existence of a saccular tubiform portion associated with the hair pencils in the coremata of *Spinivalva* has not been reported within Gracillariidae. However, detailed descriptions for coremata structures are rarely provided in the gracillariid literature, and thus one should use caution regarding the validity of this apomorphy. Bubbles adorning the pupal cocoon similar to those described here for *S. gaucha* have been found not only in other phylogenetically related genera such as *Conopomorpha*, *Epicephala* and *Leurocephala*, but also in other lineages that are not closely related to the *Parectopa* group (*e.g.*, Wagner et al. 2000, Davis et al. 2011, Hu et al. 2011).

The existence of at least one "sap feeder" instar early in larval ontogeny has been considered a characteristic shared by all Gracillariidae (Kumata 1978, Davis 1987, Davis et al. 2011). However, our data showed clearly that this is not the case for *S. gaucha*, where all instars are of the "tissue feeder" type. That is, early-instar larvae also

have mandibles of a chewing type combined with a well-developed spinneret, and with the remaining mouth parts differentiated; and after they hatch, these larvae feed on the palisade parenchyma. Palisade cells typically have well-developed, compact walls compared to those in the spongy parenchyma. The morphological characteristics in *S. gaucha* are associated with feeding on tough tissues after hatching, contrary to other gracillariid species that have sap-feeder early instars that feed by dilacerating either the leaf epidermic layers or the spongy parenchyma (*e.g.*, Kumata 1978, Wagner et al. 2000, Brito et al. 2012). The absence of a sap-feeder instar was suggested for the life cycle of *Chileoptilia yaroella* by Vargas and Landry (2005), although the first instar was not described by the authors at that time. Additional studies using scanning electron microscopy recently conducted by two of us (Vargas, H.A. and Moreira, G.R.P., unpublished data) confirmed this prediction; in this case, however, the first instar is not a leaf miner, but feeds on the tiny gynoecia within the calyx of flowers of *Acacia macracantha* Willd. (Mimosaceae) in northern Chile. These discoveries will certainly have important implications for future studies concerning the evolution of the wide diversity in feeding habits known to exist within Gracillariidae.

We found no conspicuous morphological differences at any life stage among populations of *Spinivalva* occurring in Rio Grande do Sul, independently of the host plant. These observations were corroborated by the molecular data, which showed a low divergence rate among the different populations. Consequently, we consider all these specimens to be co-specific; that is, a set of variations exists within *S. gaucha* species boundaries and among the host plants used. However, comparison of these specimens from Rio Grande do Sul with those collected from Curitiba revealed a greater divergence in mitochondrial DNA sequences. We did not study genitalia morphologies of the latter, or their immature stages, and so a decision about their taxonomic status

awaits further investigation. Thus, specific diversity within the genus *Spinivalva* might be higher than described here. As discussed for the flora in general, many passion-vine species occur in the Atlantic Rain Forest, and several of them are endemic to this biome (Stehmann et al. 2009). In the future, they should be searched for the presence of this and other lineages of gracillariids. Another gracillariid species, *Phyllocnistis tethys* Moreira and Vargas, has been associated with a different passion-vine species in southern Brazil (Brito et al. 2012). However, *Phyllocnistis* larvae use a wide range of plant families as hosts (Kawahara et al. 2009). Therefore, *Spinivalva* is the first genus that is known to be particularly associated with the Passifloraceae. Passion vines are toxic to most lepidopterans, and the biological implications, if any, for such a peculiar association in herbivory also remain unknown (Brito et al. 2012).

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Figure legends

Figure 1. *Spinivalva gaucha* adult, dorsal view: **A** wings spread, pinned, dorsal view; **B** wings folded, on *Passiflora actinia* leaf. Scale bars = 1.0 mm.

Figure 2. *Spinivalva gaucha* adult morphology: **A** head, anterior view; **B** fore- and hind-wing venation; **C** detail of retinaculum; **D** detail of basal frenulum (open arrow) and more distal pseudofrenulum (closed arrow). Scale bars = 0.2, 0.5 mm; 50, 100 µm, respectively.

Figure 3. Genital morphology of *Spinivalva gaucha* under light microscopy: **A** male genitalia, ventral view (aedeagus omitted; open arrow indicates gnathal lobe); **B** aedeagus, lateral view; **C** male right valve, median view; **D** units of the coremata anterior pair, ventral view (open and closed arrows indicate tubular pouch and hair pencil, respectively); **E** female genitalia, lateral view. Scale bar = 0.2 mm.

Figure 4. Genital morphology of *Spinivalva gaucha* under scanning electron microscopy: **A** male valvae (scales partly removed), showing saccular processes in crossed position and aedeagus (indicated by arrow), ventral view; **B** setae of costa valvular in detail (open arrow indicates distal portion of saccular process), median view; **C** female papilla annalis in detail, latero-dorsal view; **D** saccular processes in detail (squared area in A; asterisk indicates distal sensillum of the right process); **E** caudal portion of aedeagus, showing terminal spine (indicated by closed arrow) and vesica (indicated by asterisk), lateral view; **F** female ostium bursae, ventral view. Scale bars = 50, 25, 20, 10, 25, 50 µm, respectively.

Figure 5. Larval and pupal morphology of *Spinivalva gaucha* under light microscopy: **A** first larval instar, dorsal and ventral views; **B** fifth larval instar, dorsal and ventral

views; **C-E** pupa, dorsal, ventral and lateral views, respectively. Scale bars = 50 µm; 0.5, 0.5 mm, respectively.

Figure 6. Scanning electron micrographs of *Spinivalva gaucha* first larval instar: **A** head, general, dorso-lateral view; **B** mandible, dorsal view; **C** antenna, lateral view; **D** spinneret, lateral view; seta indicates hypopharyngeal papillae; **E** maxilla (asterisk), lateral view; **F** labial palpi. Scale bars = 20, 5, 5, 5, 3, 1 µm, respectively.

Figure 7. Scanning electron micrographs of *Spinivalva gaucha* third larval instar: **A** head, general, ventral view; **B** labrum, ventral view; **C** labium, ventral view (asterisk indicates the spinneret); **D** head, general, dorso-lateral view; **E** antenna, antero-ventral view; **F** left side of prothoracic shield, dorsal view. Scale bars = 75, 15, 15, 50, 10, 75 µm, respectively.

Figure 8. Scanning electron micrographs of *Spinivalva gaucha* fifth larval instar: **A** head and prothorax, general, dorsal view; **B** antenna, dorsal view; **C** stemmata (open arrow indicates sixth stemma), lateral view; **D** head and prothorax, general, ventral view; **E** labium, ventral view; **F** maxilla, lateral view; **G** spinneret, lateral view; **H** left side of prothoracic shield, dorsal view; **I** prothoracic spiracle, lateral view; **J** prothoracic leg, postero-lateral view; **K** pseudopodium on A4, antero-lateral view; **L** crochets of pseudopodium A4 in detail. Scale bars = 200, 25, 25, 200, 20, 20, 20, 75, 10, 25, 75, 10 µm, respectively.

Figure 9. Scanning electron micrographs of *Spinivalva gaucha* pupal cocoon and pupa: **A** weaving pattern of the pupal cocoon upper surface; **B** ornamental bubble (asterisk indicates a covered slit, used by the larva to attach the bubble on outside of the cocoon surface); **C** bubble surface in detail; **D** head, ventral view; **E-F** cocoon-cutter, ventral and lateral views, respectively; **G** abdominal segments, dorsal view; **H** abdominal

segment A3 (detail of area marked with a rectangle in G; open arrow indicates spiracle); **I** setae and microtrichia occurring on central portion of A1-A7; **J-K** last abdominal segments, dorsal and ventro-posterior views, respectively; **L** spine of last abdominal segment (detail of squared area marked in K). Scale bars = 20, 75, 5, 150, 50, 50, 200, 50, 10, 75, 75, 10 µm, respectively.

Figure 10. Life history of *Spinivalva gaucha*: **A** *Passiflora actnia* shoot at the type locality; **B** *Spinivalva gaucha* mine on upper leaf surface (leaf marked with an asterisk in A; open and closed arrows indicate respectively the beginning of a mine and a last-instar larva visible through transparent blotch section of the mine); **C** chorion of empty egg on lower surface; **D** first-instar larva (indicated by closed arrow) visible through transparent serpentine section of a young mine (open arrow indicates the beginning of the mine on the upper leaf surface); **E** initial portion of blotch section in detail, showing frass and damage to leaf parenchyma left by last-instar larva within the mine; **F** fourth-instar larva in the mine; **G** exit hole (arrow) used by a last-instar larva to leave the mine; **H** last-instar larva after changing color, building the cocoon outside the mine on the leaf surface; **I** cocoon, with pupa visible through transparency; **J** pupa in detail, after removing the cocoon; **K** pupal exuvium protruding from the cocoon exit hole onto plastic substrate, just after the adult emergence. Scale bars = 20, 10, 0.2, 1.5, 1, 0.5, 0.5, 2, 2, 1, 2 mm, respectively.

Figure 11. Diaphanized portion and histological sections of a *Passiflora actinia* leaf, showing through transparency the organization levels of a *Spinivalva gaucha* mine in relation to larval ontogeny: **A** general aspect of the mine, containing a last-instar larva; Roman numerals indicate larval instar numbers and corresponding positions of head capsules in the mine; closed arrow indicates the beginning of the mine; **B** detail of head capsule shed by the fourth-instar larva (bar indicates position used for measurement of

head-capsule width); **C** transverse section of serpentine portion of the mine (location indicated by the horizontal dashed line in A); **D** transverse section of blotch portion of the mine (location indicated by the vertical dashed line in A); **E** transverse section of intact portion of leaf lamina (indicated by left arrow in D); **F** transverse section of mined portion of leaf lamina (indicated by right arrow in D). **Ab**, abaxial surface of epidermis; **Ad**, adaxial surface of epidermis; **Lm**, leaf mine; **Pp**, palisade parenchyma; **Sp**, spongy parenchyma. Scale bars =1.5, 0.15, 2.0, 3.0 mm; 500, 600 µm, respectively.

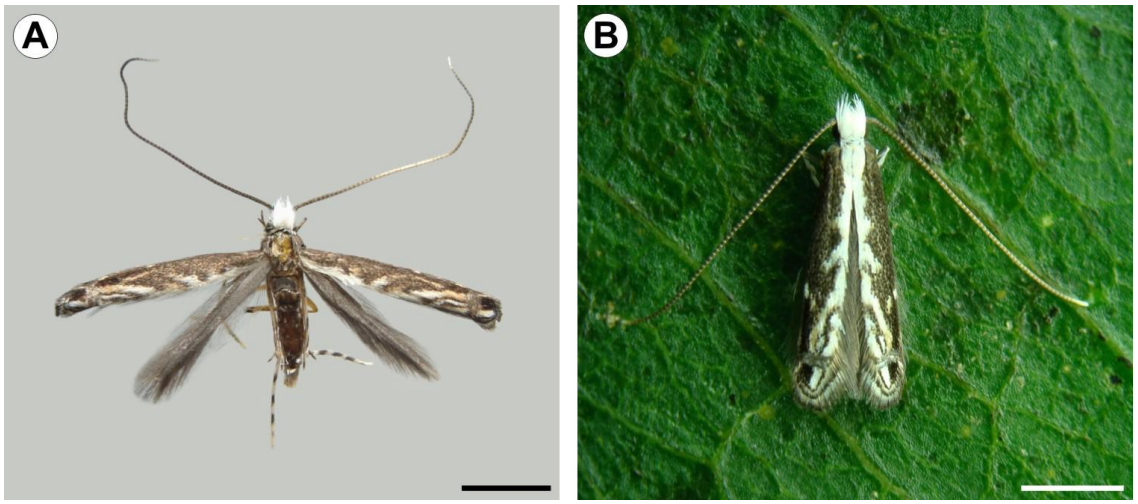
Figure 12. Bayesian consensus phylogeny of *Spinivalva.* **A** unrooted tree based on 1.5 Kb bp of the mitochondrial genes cytochrome oxidase *c* subunit I, tRNA-Leu and cytochrome oxidase *c* subunit II. Specimens from three different localities (termed Populations 1, 2 and 3; see Material and Methods for details), field-collected from different host plants (*P. suberosa* [ ], *P. misera* [ ] and *P. actinia* [ ]) were analyzed. Numbers indicate raw branch lengths. **B** phylogenetic relationships of *Spinivalva* within the *Parectopa* group of gracillariids (sensu Kawahara et al. 2011), based on 610 bp of the barcoding region (cytochrome oxidase *c* subunit I gene). Numbers above branch indicate node support (posterior probability); those located below represent the raw branch length. A species of Bucculatricidae (*Bucculatrix ulmella*) was used to root the tree.

Table1. Specimens used to investigate phylogenetic relationships of *Spinivalva* within the *Parectopa* group of gracillariids (following Kawahara et al. 2011). See Material and Methods for detailed description of *Spinivalva* specimens.

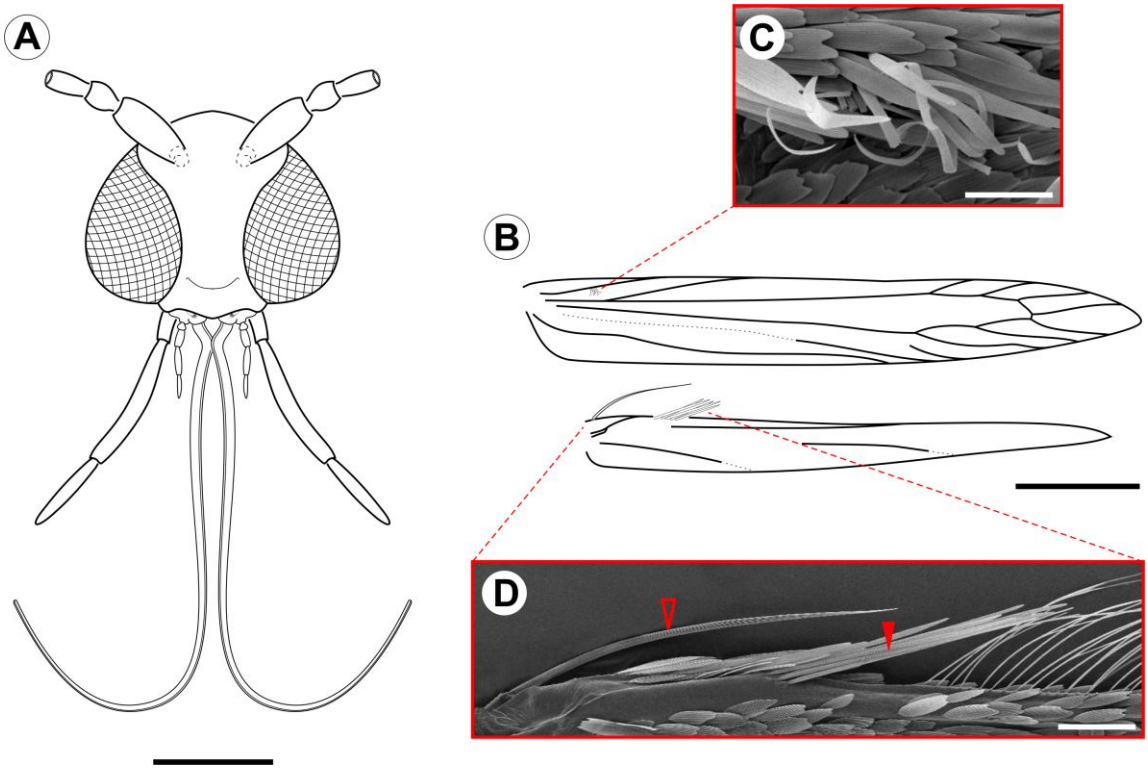
Taxa	Voucher	Genbank Acession Number
Ingroup		
GRACILLARIIDAE		
Conopomorpha sinensis	-	HQ824810
Epicephala mirivalvata	-	JX231168
Leurocephala schinusae	RDOPO385-10	HM382093
Liocrobyla desmodiella	G95AK	GU816416
Parectopa sp.	10-JDWBC-0213	HM863870
Spinalva gaucha sp.n.	LMCI 186-12	KC512112
	LMCI 164-15	KC512113
Spinivalva sp.1	LMCI 169-A1	KC512114
Stomphastis sp. n	USNM:ENT 00455002	JF415895
Outgroup		
BUCCULATRICIDAE		
Bucculatrix ulmella	RMNH.INS.18466	JX215365

Table. 2. Variation in size among head capsules of *Spinivalva gaucha* larvae reared on *Passiflora actinia* Hook (n = 5 per instar).

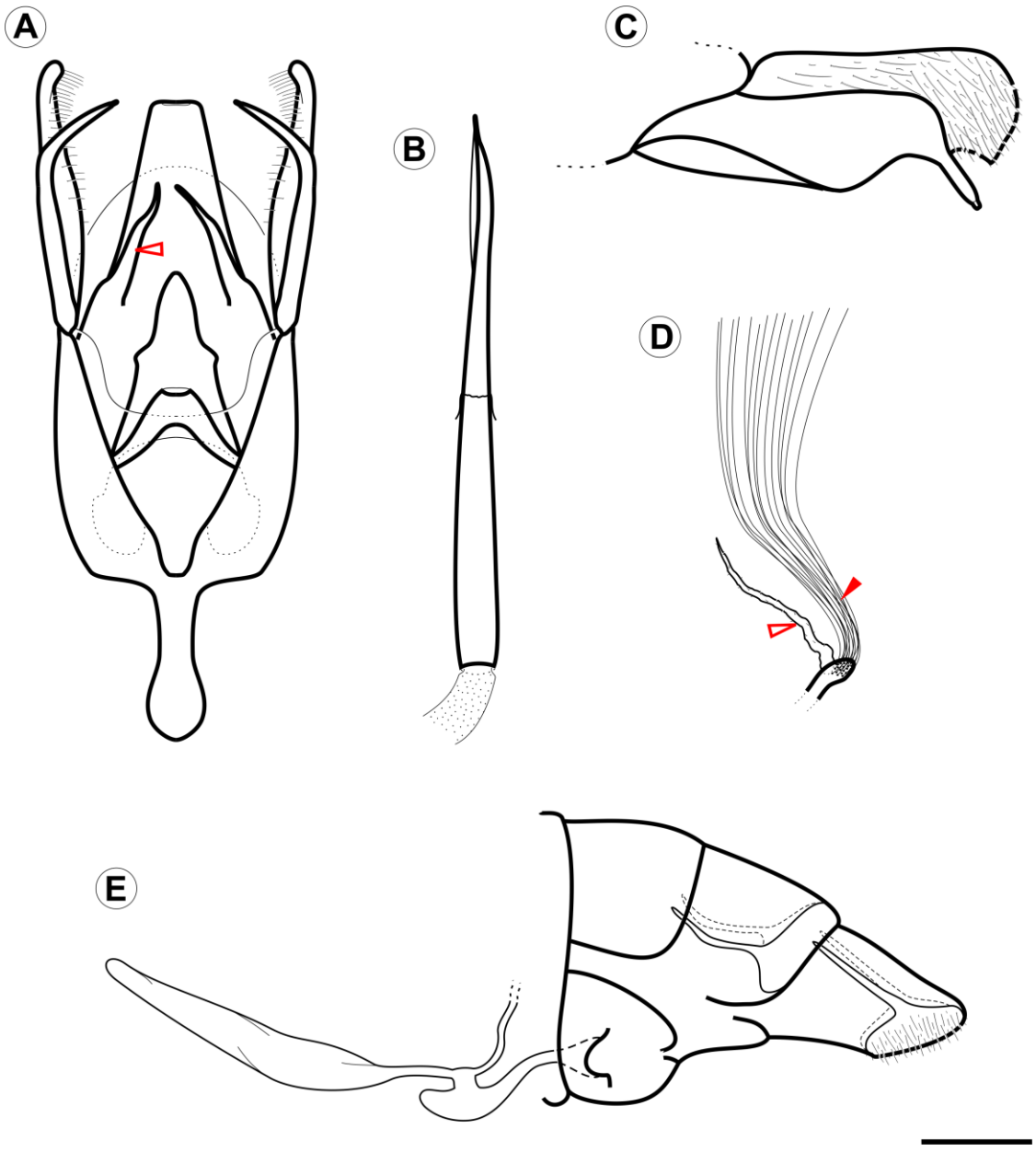
Instar	**Head capsule width (mm)**		
	Mean ± standard error	Range	Growth rate
I	0.113 ± 0.002	0.105 – 0.116	-
II	0.187 ± 0.004	0.179 – 0.200	1.65
III	0.284 ± 0.009	0.263 – 0.315	1.52
IV	0.431 ± 0.012	0.389 – 0.462	1.52
V	0.572 ± 0.140	0.567 – 0.578	1.33



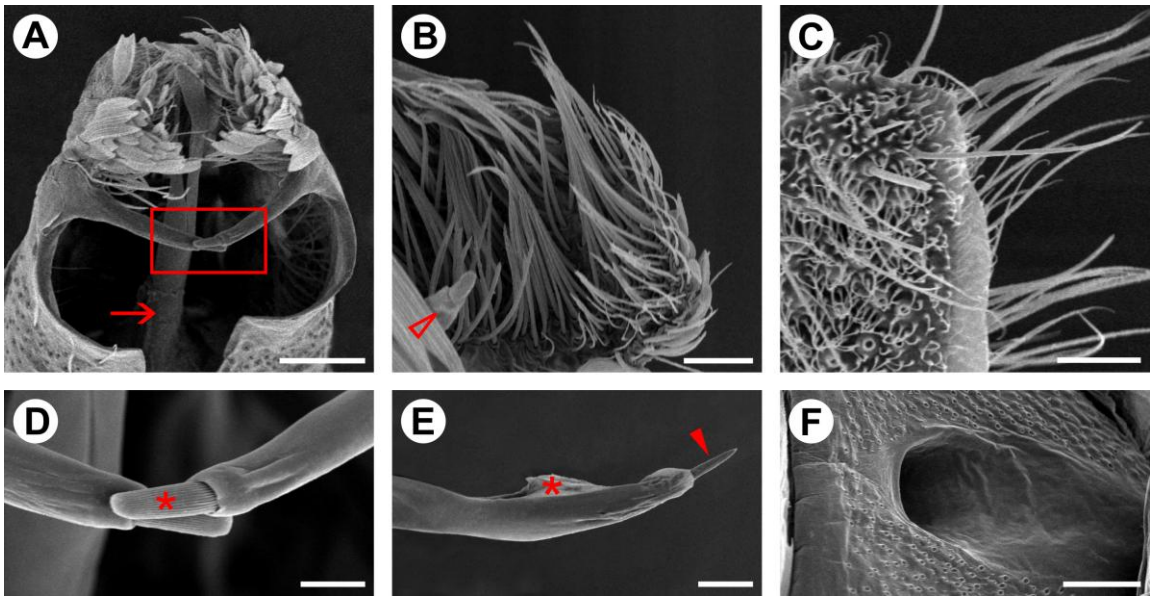
Brito *et al.* – Figure 01.



Brito et al. – Figure 02.



Brito et al. – Figure 03.



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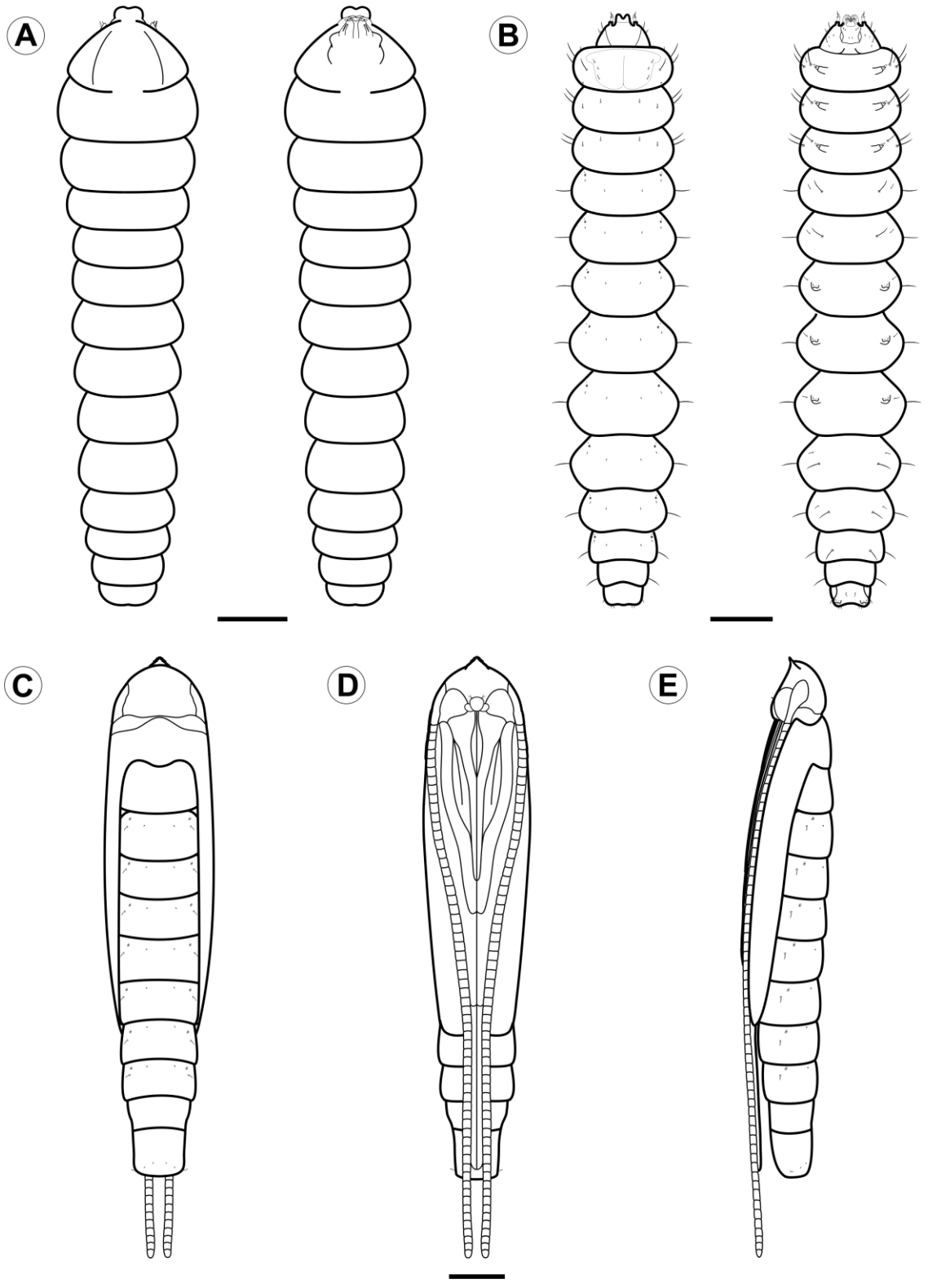

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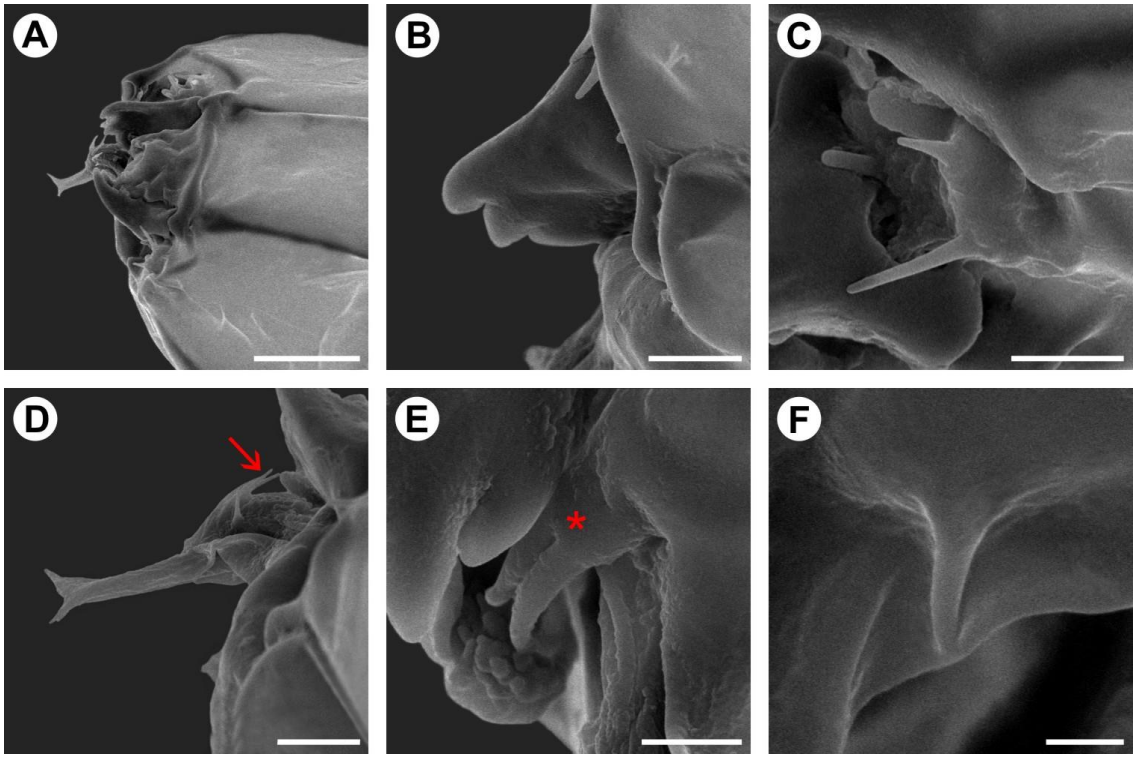

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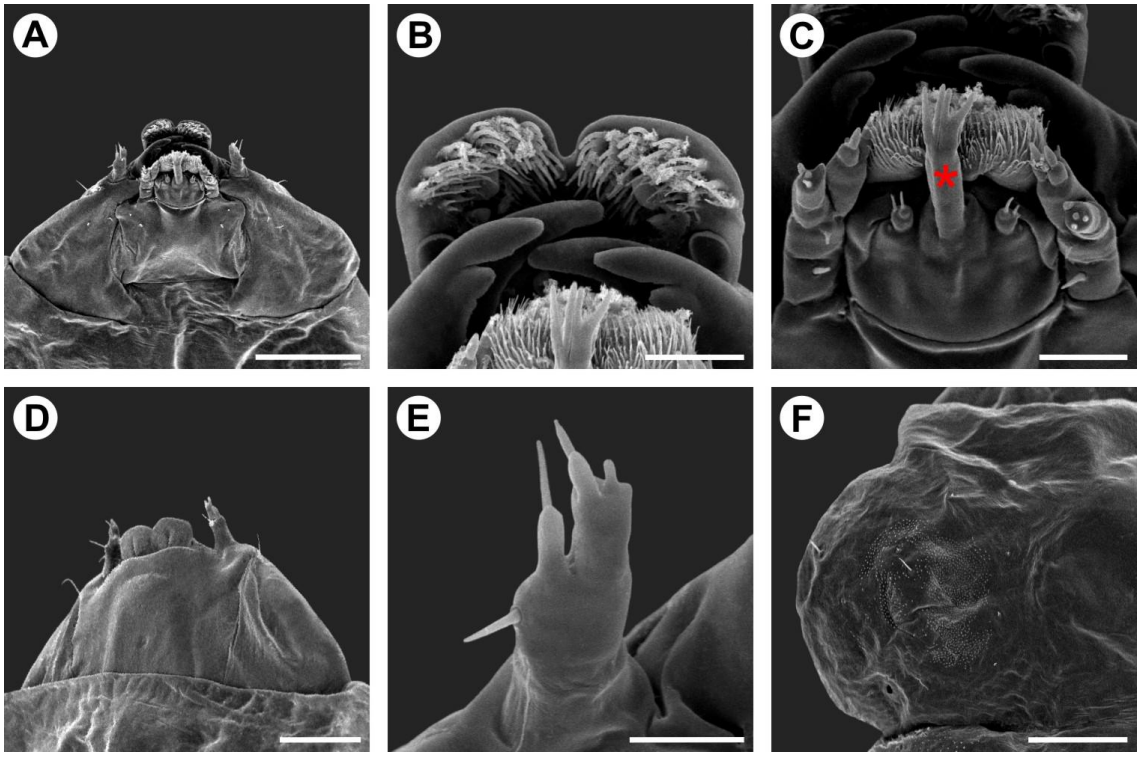
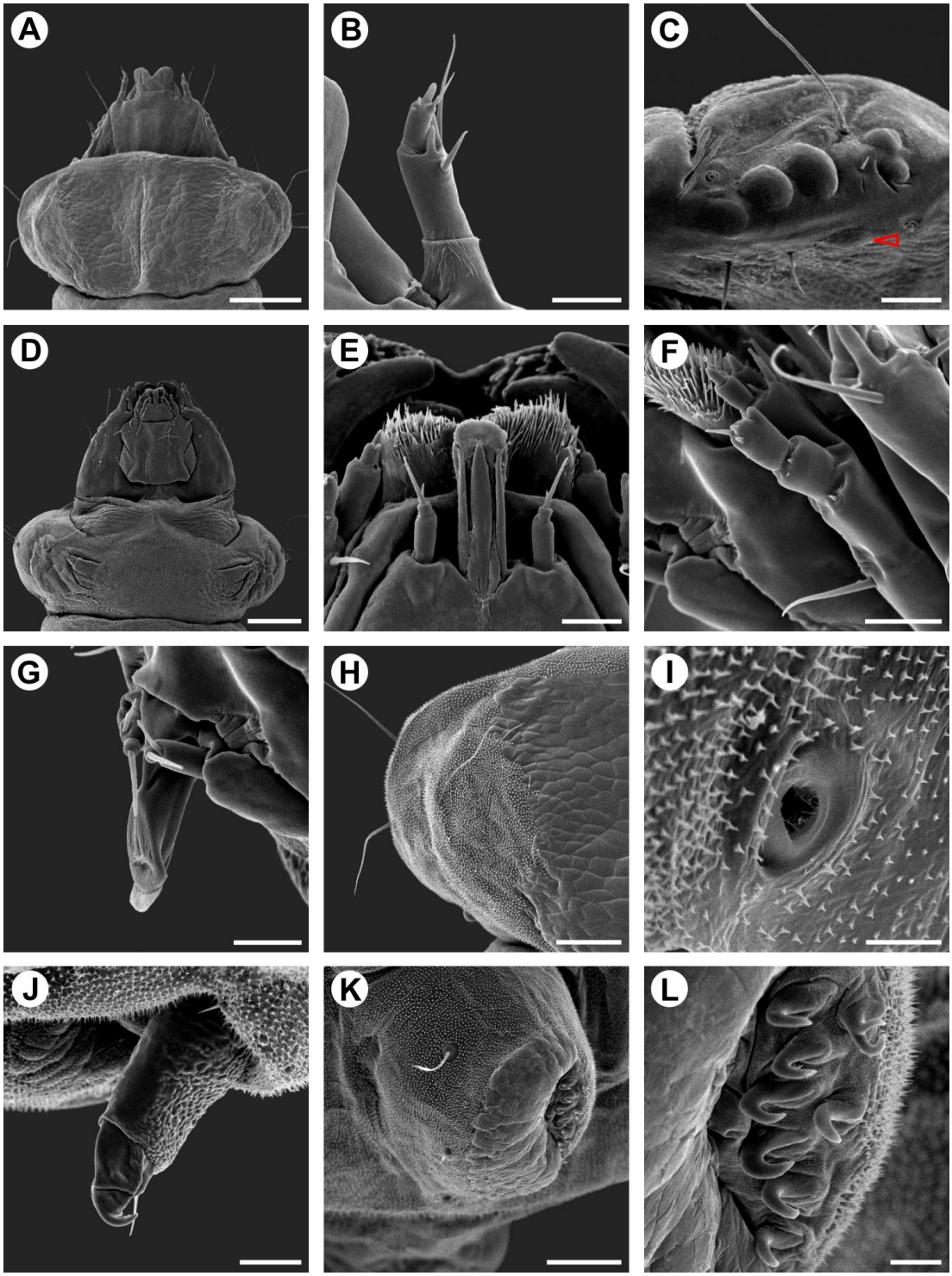


Brito et al. – Figure 07.



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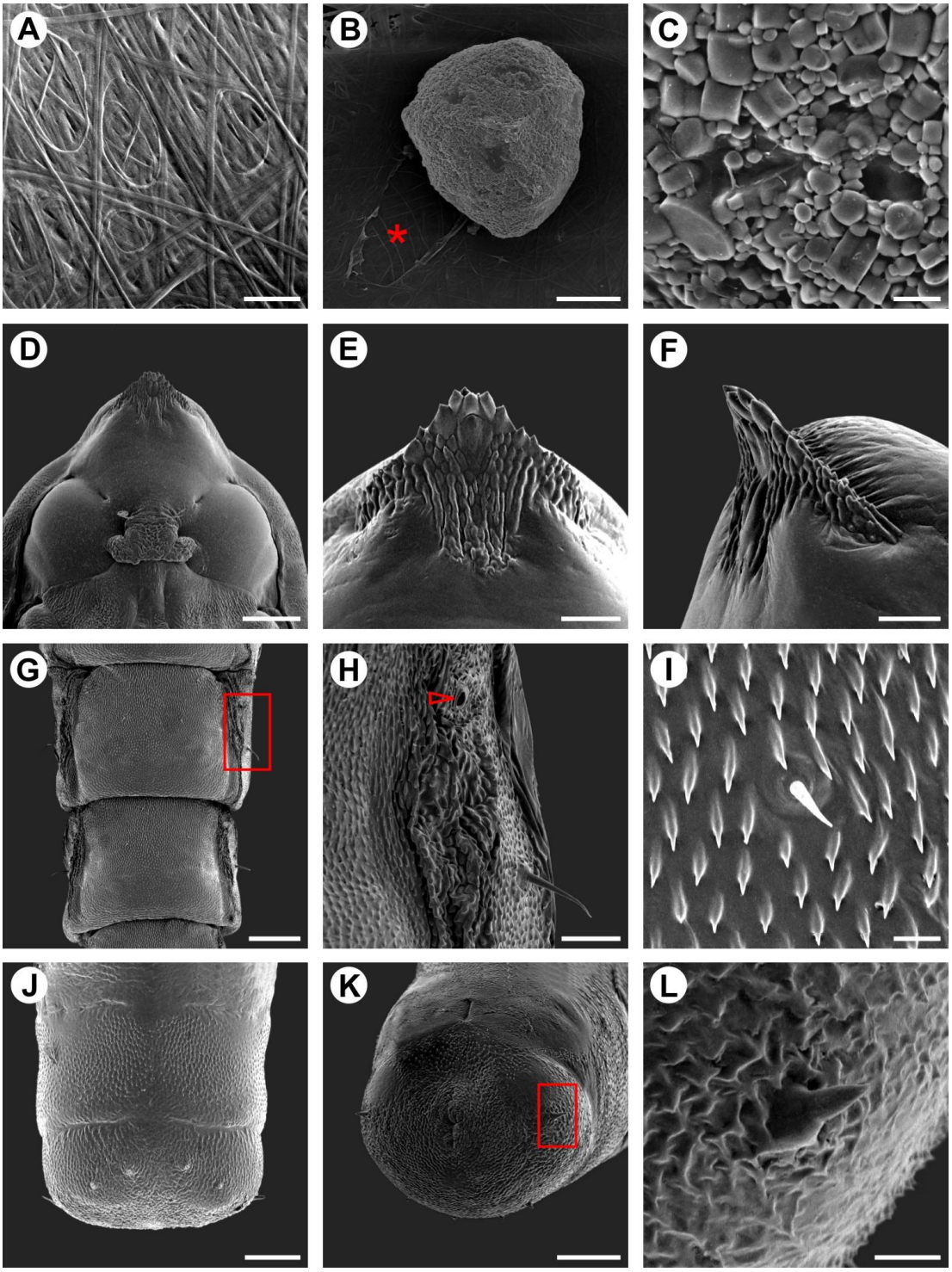

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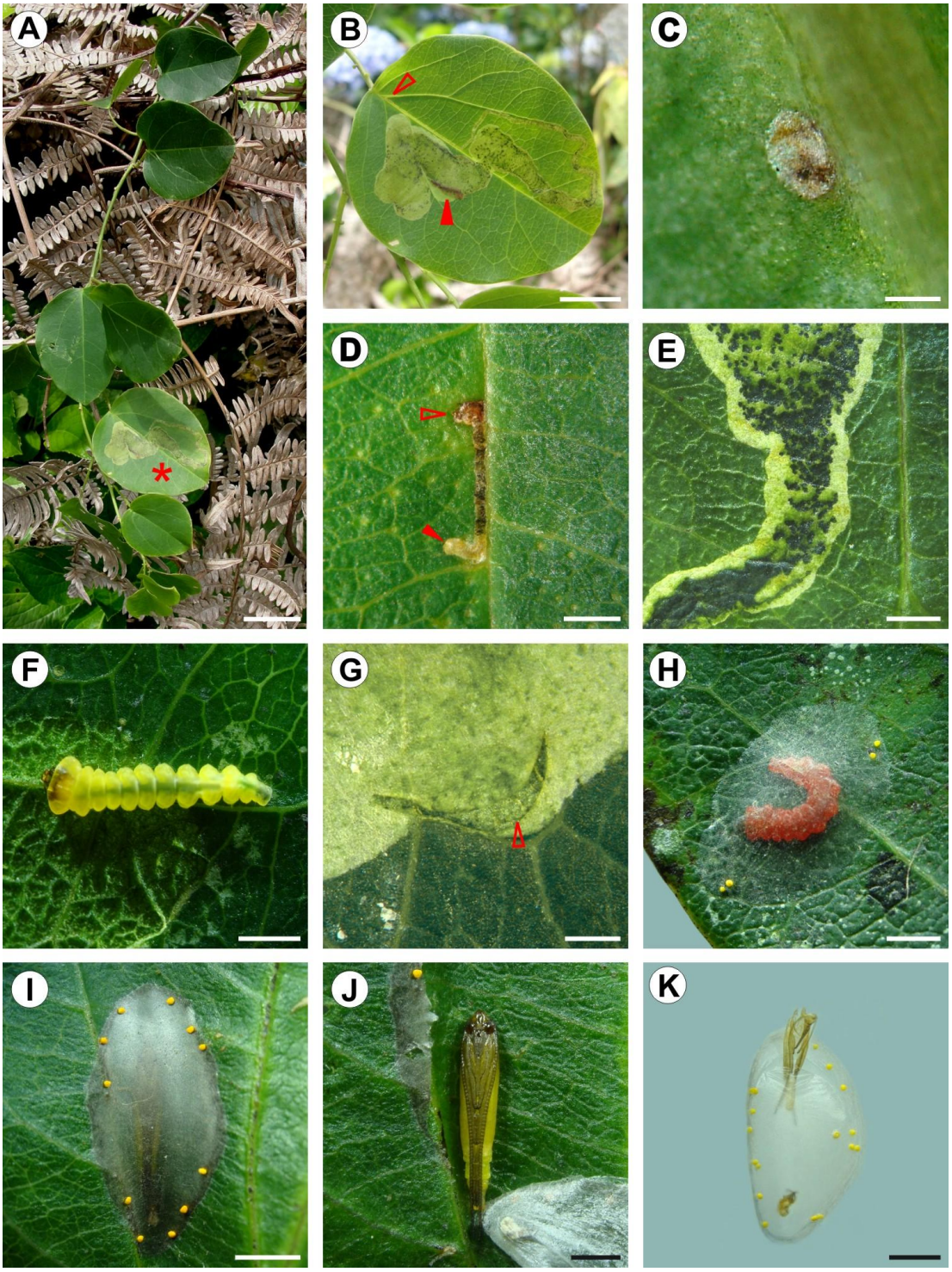
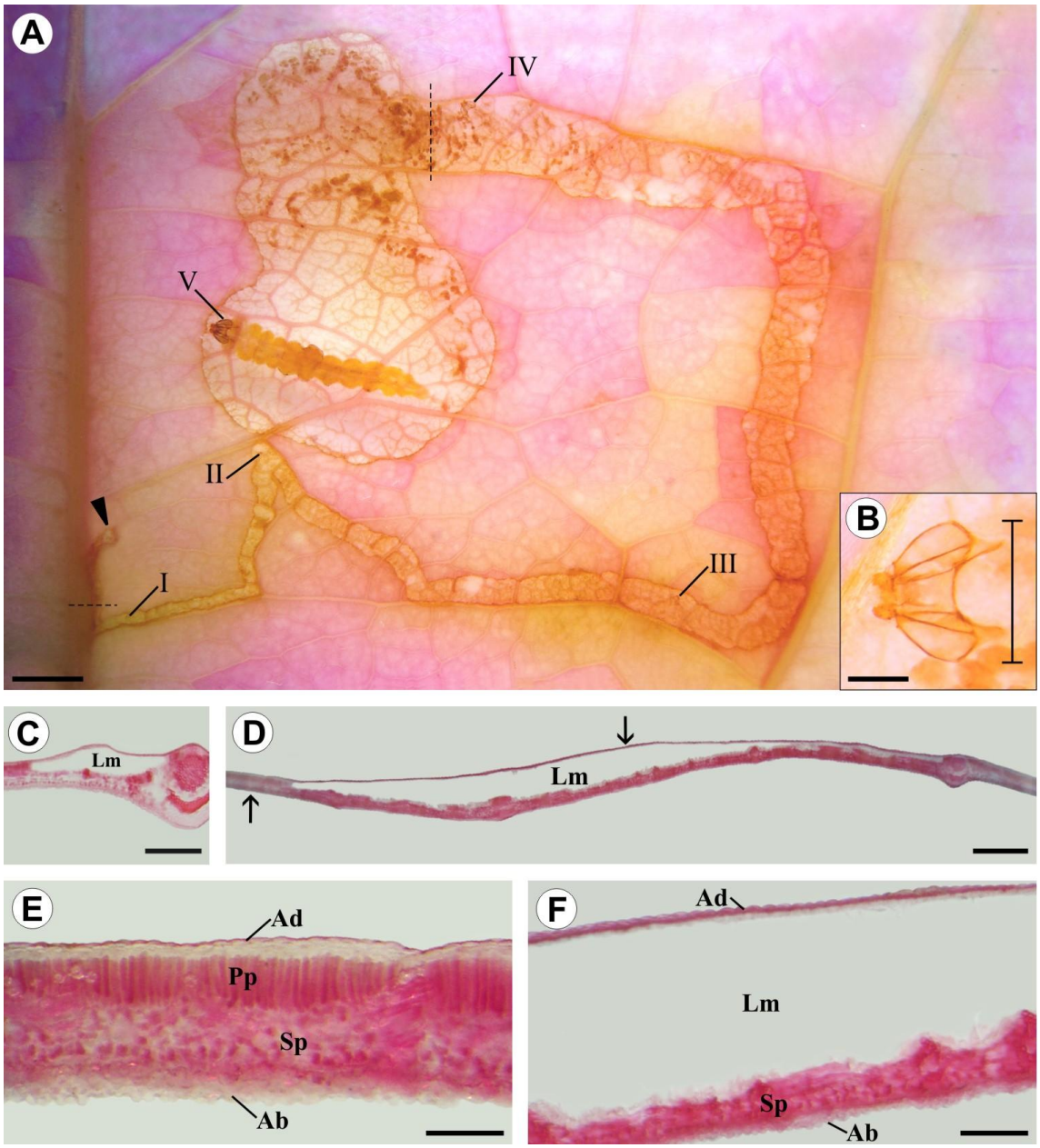


Brito et al. – Figure 10.



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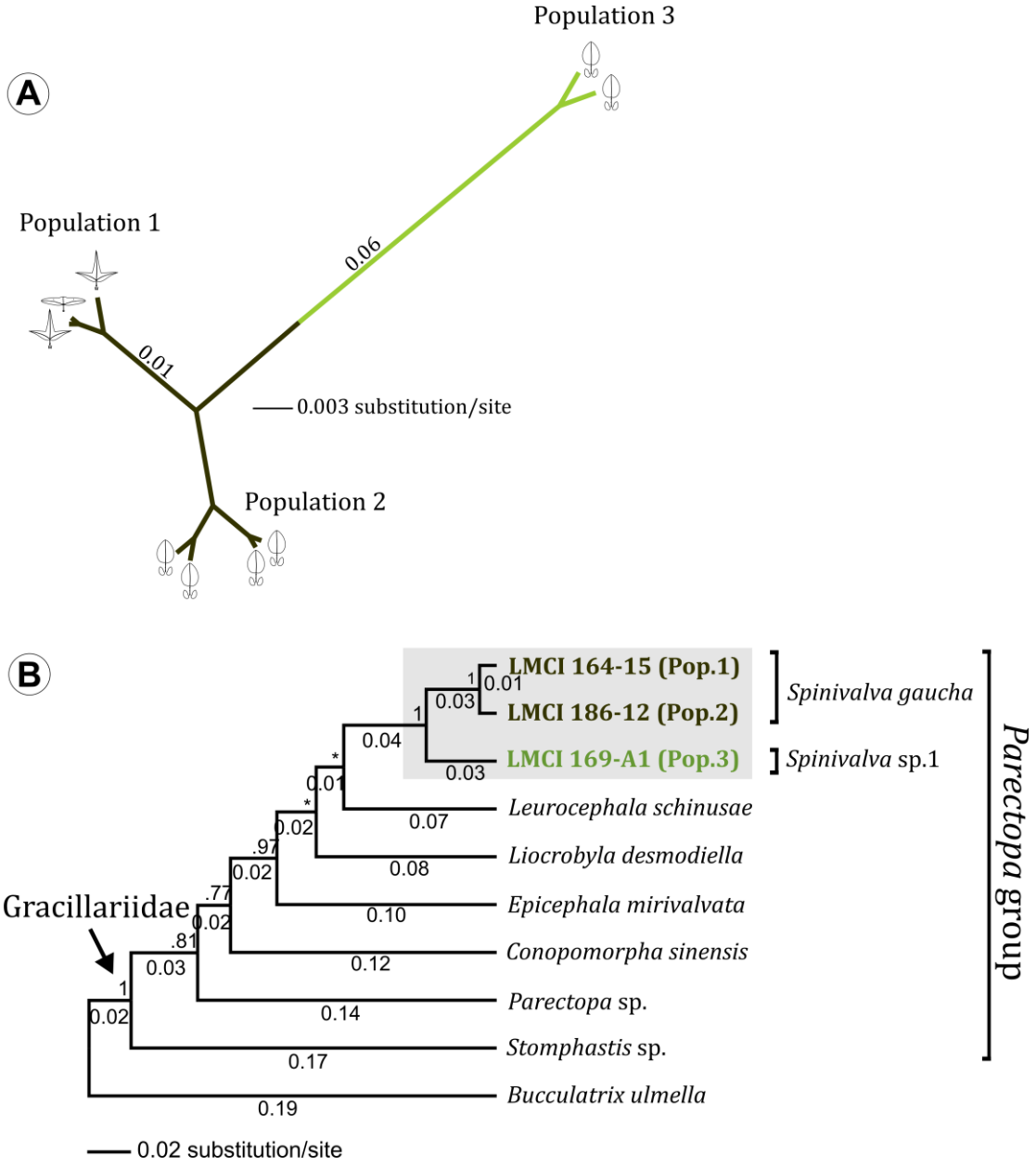

Brito et al. – Figure 12.

CAPÍTULO IV

CONSIDERAÇÕES FINAIS

Neste estudo descrevem-se, de forma inédita, duas novas espécies de Gracillariidae encontradas no sul do Brasil, as quais utilizam como planta hospedeira representantes da família Passifloraceae. A primeira espécie descrita pertence ao gênero *Phyllocnistis* e foi encontrada sobre ramos de *Passiflora organensis*; e a segunda, representando um novo gênero, foi descoberto sobre ramos de *Passiflora actinia*, *P. misera* e *P. suberosa* no estado do Rio Grande do Sul.

Phyllocnistis tethys é a primeira espécie desse gênero descrita no Brasil. Compartilham com as demais espécies do gênero o padrão de coloração das asas, como escamas laranjadas junto a asa anterior, três instares larvais *sap-feeding*, seguidos de um instar *spinning*, o qual não se alimenta. Como caracteres diagnósticos, além do padrão das asas, ressaltam-se algumas características referentes à genitália, como a presença de *cornuti* conspícuos nos machos, não mencionado para as demais espécies congenéricas e presença de um único *signum* em forma de gancho junto à genitália feminina. Em relação à arquitetura da mina foliar foi possível verificar que a mesma apresenta-se em forma de mancha, característica não visualizada para as demais espécies do gênero. Através da análise molecular podemos evidenciar que *P. tethys* possivelmente esteja associada a uma linhagem mais basal se comparada com algumas espécies da região Neártica e Neotropical utilizadas no trabalho, o que deve ser melhor investigado.

O terceiro capítulo contemplou a descrição de *Spinivalva gaucha* gên. nov., sp. nov. considerado o primeiro gênero de Gracillariidae que não apresenta nos instares

iniciais uma forma *sap-feeding*. Em todos os instares, as mandíbulas estão adaptadas à mastigação, utilizando apenas o tecido paliçádico durante todo desenvolvimento larval. *S. gaucha* é considerada um representante da subfamília Gracillariinae devido o compartilhamento de diversas características, tais como, a presença de duas corematas junto ao abdômen masculino, pupação desenvolvida junto à área externa da folha e a posição dos adultos quando em repouso, os quais elevam a parte anterior do corpo em aproximadamente 45^{o} apoiando-se sobre as pernas anteriores que ficam distendidas e largamente afastadas. Não foram detectadas diferenças morfológicas notáveis entre populações alimentadas por plantas hospedeiras diferentes no RS (*P. actinia*, *P. misera* e *P. suberosa*), o que foi corroborado pela baixa divergência genética correspondente visualizada junto às análises moleculares.

As espécies novas referentes ao estado do Rio Grande do Sul descritas neste trabalho permitem indicar a existência de ampla diversidade de plantas hospedeiras para Gracillariidae na região Neotropical. Como perspectivas futuras, visamos buscar novas informações referentes à biologia e filogenia das espécies descritas neste trabalho, bem como à taxonomia e sistemática de espécies adicionais. Assim, dando continuidade à descrição de novas espécies, visando explorar à ampla diversidade supostamente existente a respeito, mas desconhecida para a região Neotropical.

CAPÍTULO V

ANEXOS

1) NORMAS PARA PUBLICAÇÃO NA ZOOTAXA

Aim and scope

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

Based on length, two categories of papers are considered.

1) **Research article**

Research articles are significant papers of four or more printed pages reporting original research. Papers between 4 and 59 printed pages are published in multi-paper issues of

60, 64 or 68 pages. Monographs (60 or more pages) are individually issued and bound, with ISBNs.

Zootaxa encourages large comprehensive taxonomic works. There is no upper limit on the length of manuscripts, although authors are advised to break monographs of over 1000 pages into a multi-volume contribution simply because books over 1000 pages are difficult to bind and too heavy to hold.

Very short manuscripts with isolated descriptions of a single species are generally discouraged, especially for taxa with large number of undescribed species. These short manuscripts may be returned to authors without consideration. Short papers on species of economic, environmental or phylogenetic importance may be accepted at the discretion of editors, who will generally encourage and advise authors to add value to the paper by providing more information (e.g. checklist of or key to species of the genus, biological information...). Short papers of 4 or 5 pages accepted for publication may be shortened for publication in the Correspondence section.

2) **Correspondence**

High quality and important short manuscripts of normally 1 to 4 pages are considered to fill blank pages in multi-paper issues. *Zootaxa* publishes the following six types of correspondence:

- opinions and views on current issues of interests to systematic zoologists (e.g. *Zootaxa* 1577: 1-2);
- commentary on or additions/corrections to papers previously published in *Zootaxa* (e.g. *Zootaxa* 1494: 67-68);
- obituary in memory of deceased systematic zoologists (e.g. *Zootaxa* 545: 67-68)
- taxonomic/nomenclatural notes of importance;

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

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

Commentaries on published papers are intended for scholarly exchange of different views or interpretations of published data and should not contain personal attack; authors of concerned papers may be invited to reply to comments on their papers.

Special issues

Special issues with collected papers such as a Festschrift (see *Zootaxa* 1325 and *Zootaxa* 1599) within the scope of the journal are occasionally published. Guest editors should send the proposal to the chief editor for approval and instructions. Although guest editors for special issues are responsible for organizing the peer review of papers collected within these issues, they must follow Zootaxa's style, standard and peer review procedures. If any papers by the guest editors are to be included in the

special issue, then these papers must be handled by editors/colleagues other than the editor(s) involved. Special issues must be 60 or more pages. Normally funding is required to offset part of the production cost. Author payment for open access is strongly encouraged. Reprints can be ordered for the entire issue or for individual papers.

Preparation of manuscripts

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

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

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

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

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

A) **Journal paper**:

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

B) **Book chapter**:

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

C) **Book**:

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

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

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

Please note that (1) **journal titles must be written in full (not abbreviated)**; (2) journal titles and volume numbers are followed by a ","; (3) page ranges are connected by "n dash", not hyphen "-", which is used to connect two words. For websites, it is important to include the last date when you see that site, as it can be moved or deleted from that address in the future.

On the use of dashes: (1) Hyphens are used to link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use). (2) En-dash or en-rule (the length of an ‘n’) is used to link spans. In the context of our journal that means numerals mainly, most frequently sizes, dates and page numbers (e.g. 1977–1981; figs 5–7) and also geographic or name associations (Murray–Darling River; a Federal–State agreement). (3) Em-dash or em-rule (the length of an ‘m’) are used far more infrequently, and are used for breaks in the text or subject, often used much as we used parentheses. In contrast to parentheses an em-dash can be used alone; e.g. What could these results mean—that Niel had discovered the meaning of life? En-dashes and em-dashes should not be spaced.

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

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

8) **Keys** are not easy to typeset. In a typical dichotomous key, each lead of a couplet should be typed simply as a paragraph as below:

1 Seven setae present on tarsus I; four setae present on tibia I; leg I longer than the body; legs black in color ... Genus A

- Six setae present on tarsus I; three setae present on tibia I; leg I shorter than the body; legs brown in color ... 2

2 Leg II longer than leg I ... Genus B

- Leg II shorter than leg I ... Genus C

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

Deposition of specimens

Whenever possible, authors are advised to deposit type specimens in national or international public museums or collections. Authors are also advised to request registration numbers of deposited material in advance of the acceptance of papers to avoid unnecessary delay of publication. Some countries (e.g. Australia) require that

primary type specimens be deposited in collections of the country of origin; authors are advised to take this into consideration.

2) NORMAS PARA PUBLICAÇÃO NA ZOOKEYS

Focus and Scope

ZooKeys is a peer-reviewed, open-access, rapidly disseminated journal launched to accelerate research and free information exchange in taxonomy, phylogeny, biogeography and evolution of animals. *ZooKeys* aims to apply the latest trends and methodologies in publishing and preservation of digital materials to meet the highest possible standards of the cybertaxonomy era. *ZooKeys* will publish papers in systematic zoology containing taxonomic/faunistic data on any taxon of any geological age from any part of the world with no limit to manuscript size. *ZooKeys* will consider for publishing works on the following topics:

- new descriptions of taxa, if they are accomplished with proper diagnoses, keys and/or revision of at least at species group level;
- taxonomic revisions of extant (or "recent") and fossil animal groups;
- checklists and catalogues;
- phylogenetic and evolutionary analyses;
- papers in descriptive and/or historical biogeography;
- methodology papers;
- data mining and literature surveys;
- monographs, conspecti, atlases;
- collections of papers, Festschrift volumes, conference proceedings.

Extensive faunistic overviews on a group in a country or larger region are welcome. Short faunistic contributions may be considered if they are based on significant or unexpected discovery. Regular faunistic contributions may eventually be published in

special issues devoted to a region/country. Papers containing identification keys will be considered for publishing with priority. Extensive manuscripts consisting mostly of keys will be considered for publishing as well. The minimum requirements for publishing a description of a single species in *ZooKeys* is to provide: (1) a statement on type material and type locality, according to the ICZN requirements, (2) thorough description with good quality images, (3) a differential diagnosis, (4) identification key to at least the closest relatives of the new species, e.g. species group, (5) etymology, and (6) as much additional information as possible on the natural history, biology, distribution, and conservation status.

Descriptions of single species are encouraged if they form part of a work of broader importance (e.g. key or revision of the species in a wide region; revision of the particular species group; separation of widespread cryptic species), or are of particular scientific importance (e.g. disease vector, representative of a new genus, sister-group of a large clade), or are exceptional in some respect (e.g. species in danger of extinction, large extension of the geographical range of a higher taxon).

The following categories of papers will be considered:

- original research articles;
- reviews - longer articles, offering a comprehensive overview, historical analysis or/and future perspectives of a topic;
- monographs and collections of papers with no limit in size, published as 'special issues';
- short communications;
- letters and discussion papers;
- book reviews.

ISBN numbers will be assigned to large monographic papers (i.e., major revisions of taxa), monographs, collections of papers, Festschrift volumes, atlases, checklists, conspecti.

The journal will be published both as online and printed version. Both versions will be published on the same date in compliance with current ICZN requirements.

Author Guidelines

Main Text

Title: The title should be in a sentence case (only scientific, geographic or person names should be with a first capital letter, i.e. *Elater ferrugineus* L., Germany, etc.), and should include an accurate, clear and concise description of the reported work, avoiding abbreviations. The higher taxa within the title should be separated with commas and not with a semicolon, e.g.: (Coleoptera, Elateridae, Elaterini).

Authors and Affiliations: Provide the complete names of all authors, and their addresses for correspondence, including e.g., institutional affiliation (e.g. university, institute), location (street, boulevard), city, state/province (if applicable), and country. One of the authors should be designated as the corresponding author. It is the corresponding author's responsibility to ensure that the author list and the individual contributions to the study are accurate and complete. If the article has been submitted on behalf of a consortium, all consortium members and their affiliations should be listed after the Acknowledgements section.

Abstract and Keywords: Please have your abstract and keywords ready for input into the submission module.

Body Text: All papers should be in grammatically correct English. Non-native English speaking authors are required to have their manuscripts checked by a native English speaker prior to submission. Use either British/Commonwealth or American English provided that the language is consistent within the paper. A manuscript must be written with precision, clarity, and economy, whenever appropriate in active voice and first person. Avoid the use of parenthetical comments and italics or bold for emphasis. This journal discourages the use of quotation marks except for direct quotations, words defined by the author, and words used in unusual contexts. Short quotations should be embedded in the text and enclosed in double quotation marks ("). Long quotations should be on a separate line, italicized, but without quotation marks. Single quotation marks are to be used only for a quotation that occurs within another quotation.

Spacing, Fonts, and Page Numbering: Single-space all material (text, quotations, figure legends, tables, references, etc.). Separate paragraphs with a blank line. Use a 12-point font (preferably Times New Roman or Arial).

Capitals: First capital letters should be used only in the beginning of a sentence, in proper names and in headings and subheadings, as well as to indicate tables, graphs and figure/s within the text. Software programmers should be written with capital letters (e.g., ANOVA, MANOVA, PAUP).

Italicization/Underlining: Scientific names of species and genera, long direct quotations and symbols for variables and constants (except for Greek letters), such as p, F, U, T, N, r, but not for SD (standard deviation), SE (standard error), DF (degrees of freedom) and NS (non significant) should be italicized. These symbols in illustrations and equations should be in italics to match the text. Italics should not be used for

emphasis, and not in abbreviations such as e.g., i.e., et al., etc., cf. Underlining of any text is not acceptable.

Abbreviations: Abbreviations should be followed by '.' (full stop or period; for instance: i.e., e.g., cf., etc.). Note that you shouldn't add a full stop at the end of abbreviated words if the last letter of the abbreviation is the same as the last letter of the full word. For example, you should abbreviate „Eds", „Dr", and „Mr" without full stop at the end. All measures, for instance mm, cm, m, s, L, should be written without full stop.

On the use of dashes: (1) Hyphens are used to link words such as personal names, some prefixes and compound adjectives (the last of which vary depending on the style manual in use) (2) En-dash or en-rule (the length of an 'n') is used to link spans. In the context of our journal en-dash should be used to link numerals, sizes, dates and page numbers (e.g., 1977–1981; figs 5–7; pp. 237–258); geographic or name associations (Murray–Darling River; a Federal–State agreement); and character states combinations such as long–pubescent or red–purple. (3) Em-dash or em-rule (the length of an 'm') should be used rarely, only for introducing a subordinate clause in the text that is often used much as we use parentheses. In contrast to parentheses an em-dash can be used alone. En-dashes and em-dashes should not be spaced.

Footnotes: Avoid footnotes in the body text of the manuscript. It is always possible to incorporate the footnote into the main text by rewording the sentences, which greatly facilitates reading. Additionally, footnotes are not always handled well by the journal software, and their usage may cause a failure of submission. Footnotes are acceptable only below tables; instead of numbers, please use (in order): †, ‡, §, |, ¶, #, ††, ‡‡, §§, ||, ¶, ##.

Geographical coordinates: It is strongly recommended to list geographical coordinates as taken from GPS or online gazetteer, or georeferencer (http://wwold.gbif.org/prog/digit/Georeferencing). Geographical coordinates must be listed in one of the following formats:

Definition: The locality consists of a point represented by coordinate information in the form of latitude and longitude. Information may be in the form of:

- Degrees, Minutes and Seconds (DMS),
- Degrees and Decimal Minutes (DDM), or
- Decimal Degrees (DD).

Records should also contain a hemisphere (E or W and N or S) or, with Decimal Degrees, minus (–) signs to indicate western and/or southern hemispheres. Examples:

- Example 1: 36° 31' 21" N; 114° 09' 50" W (DMS)
- Example 2: 36° 31.46’N; 114° 09.84’W (DDM)
- Example 3: 36.5243° S; 114.1641° W (DD)
- Example 4: −36.5243; −114.1641 (DD using minus signs to indicate southern and western hemispheres).

Note on accuracy: Because GPS units are very commonly used today to record latitude/longitude, many authors simply give the GPS readings for their localities. However, these readings are much too accurate. For example, a GPS unit might give the latitude in decimal seconds as 28°16'55.87"N. Since one second of latitude is about 30 m on the ground, the second figure after the decimal in 55.87 represents 30 cm, yet a typical handheld GPS unit is only accurate at best to a few metres. We therefore recommend two ways to report GPS-based locations. If you give the GPS reading

without rounding off, make sure you include an uncertainty figure as a context for the over-accurate GPS reading. We recommend the Darwin Core definition of uncertainty (http://rs.tdwg.org/dwc/terms/index.htm#coordinateUncertaintyInMeters):

"The horizontal distance (in meters) from the given decimal Latitude and decimal Longitude describing the smallest circle containing the whole of the Location."

If you only give the GPS reading, please round it off to an implied precision appropriate to the error in the measurement, or to the extent of the area sampled. We suggest rounding off:

- to the nearest second in degree-minute-second format (28°16'56"N), which implies roughly ± 25-30 m at middle latitudes;
- to four decimal places in decimal degree format (28.2822°N), which implies roughly ± 10-15 m at middle latitudes;
- to two decimal places in decimal minute format (28°16.93'N), which implies roughly 15-20 m at middle latitudes.

Altitude: Many GPS users simply record the elevation given by their GPS unit. However, GPS elevation is NOT the same as elevation above sea level. GPS units record the elevation above a mathematical model of the earth's surface. The difference between this elevation and elevation above sea level can be tens of metres. In any case, the accuracy of a GPS elevation is often the same as the usual accuracy in horizontal position, so a GPS elevation such as '753 m' is much too accurate and should be rounded off to 'ca 750 m'.

We strongly recommend the use of Example 2 (the DDM format). The other three are also possible but will be recalculated to DDM during the process of online mapping from the HTML version of the paper.

The only restriction on format is in creating a KML (Keyhole Markup Language) file. KML latitudes and longitudes must be in the DD format shown above in Example 4.

Please also consider submitting a **table of localities** with your manuscript, either as a spreadsheet or in CSV text format. By doing so you will make your specimen localities much more easily available for use in biodiversity databases and geospatial investigations. The geospatial table will be put online as supplementary material for your paper. A minimum table will have three fields: species (or subspecies) name, latitude and longitude. A full table will have the same data for each specimen lot as appears in the text of your paper. Please check latitude/longitude carefully for each entry.

Units: Use the International System of Units (SI) for measurements. Consult Standard Practice for Use of the International System of Units (ASTM Standard E−380−93) for guidance on unit conversions, style, and usage.

Statistics: Use leading zeroes with all numbers, including probability values (e.g., $P < 0.001$). For every significant F−statistic reported, provide two values (numerator and denominator). Whenever possible, indicate the year and version of the statistical software used.

Web (HTML) links: Authors are encouraged to include links to other Internet resources in their article. This is especially encouraged in the reference section. When inserting a reference to a web-page, please include the http:// portion of the web address.

Supplementary files: Larger datasets can be uploaded separately as Supplementary Files. Tabular data provided as supplementary files can be uploaded as an Excel

spreadsheet (.xls), as an OpenOffice spreadsheets (.ods) or comma separated values file (.csv). As with all uploaded files, please use the standard file extensions.

Headings and subheadings: Main headings: The body text should be subdivided into different sections with appropriate headings. Where possible, the following standard headings should be used: **Introduction, Methods, Results, Discussion, Conclusions, Acknowledgements, and References**. These headings need to be in bold font on a separate line and start with a first capital letter. Please do not number headings or subheadings.

- **Introduction** − The motivation or purpose of your research should appear in the Introduction, where you state the questions you sought to answer, and then provide some of the historical basis for those questions.
- **Methods** − Provide sufficient information to allow someone to repeat your work. A clear description of your experimental design, sampling procedures, and statistical procedures is especially important in papers describing field studies, simulations, or experiments. If you list a product (e.g., animal food, analytical device), supply the name and location of the manufacturer. Give the model number for equipment used. Supply complete citations, including author (or editor), title, year, publisher, and version number, for computer software mentioned in your article.
- **Results** − Results should be stated concisely and without interpretation.
- **Discussion** − Focus on the rigorously supported aspects of your study. Carefully differentiate the results of your study from data obtained from other sources. Interpret your results, relate them to the results of previous research, and discuss the implications of your results or interpretations. Point out results that do not

support speculations or the findings of previous research, or that are counter-intuitive. You may choose to include a Speculation subsection in which you pursue new ideas suggested by your research, compare and contrast your research with findings from other systems or other disciplines, pose new questions that are suggested by the results of your study, and suggest ways of answering these new questions.

- **Conclusion** −This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.
- **References** − The list of References should be included after the final section of the main article body. A blank line should be inserted between single-spaced entries in the list. Authors are requested to include links to online sources of articles, whenever possible!

Where possible, the standard headings should be used in the order given above. Additional headings and modifications are permissible.

Subordinate headings: Subordinate headings (e.g. Field study and Simulation model or Counts, Measurements and Molecular analysis), should be left-justified, italicized, and in a regular sentence case. All subordinate headings should be on a separate line.

Citations and References

Citations within the text: Before submitting the manuscript, please check each citation in the text against the References and vice-versa to ensure that they match exactly. Citations in the text should be formatted as follows: Smith (1990) or (Smith 1990),

Smith et al. (1998) or (Smith et al. 1998) and (Smith et al. 1998, 2000, Brock and Gunderson 2001, Felt 2006).

References: It is important to format the references properly, because all references will be linked electronically as completely as possible to the papers cited. It is desirable to add a DOI (digital object identifier) number for either the full-text or title and abstract of the article as an addition to traditional volume and page numbers. If a DOI is lacking, it is recommended to add a link to any online source of an article. Please use the following style for the reference list:

A) **Published Papers**:

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

B) **Accepted Papers**:

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

C) **Electronic Journal Articles**:

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

D) **Paper within conference proceedings**:

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

E) **Book chapters**:

‘Mayr E (2000) The biological species concept. In: Wheeler QD, Meier R (Eds) Species Concepts and Phylogenetic Theory: A Debate. Columbia University Press, New York, 17-29.

F) **Books**:

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

G) **Book with institutional author**:

International Commission on Zoological Nomenclature (1999) International code of zoological nomenclature. Fourth Edition. London: The International Trust for Zoological Nomenclature.

H) **PhD thesis**:

Dalebout ML (2002) Species identity, genetic diversity and molecular systematic relationships among the Ziphiidae (beaked whales). PhD thesis, Auckland, New Zealand: University of Auckland.

I) **Link/URL**:

BBC News: Island leopard deemed new species http://news.bbc.co.uk/

Citations of Public Resource Databases: It is highly recommended all appropriate datasets, images, and information to be deposited in public resources. Please provide the relevant accession numbers (and version numbers, if appropriate). Accession numbers should be provided in parentheses after the entity on first use. Examples of such databases include, but are not limited to:

- ZooBank (www.zoobank.org)
- Morphbank (www.morphbank.net)
- Genbank (www.ncbi.nlm.nih.gov/Genbank)

- BOLD (www.barcodinglife.org)

Providing accession numbers to data records stored in global data aggregators allows us to link your article to established databases, thus integrating it with a broader collection of scientific information. Please hyperlink all accession numbers through the text or list them directly after the References in the online submission manuscript. All journal titles should be spelled out completely and should not be italicized.

Provide the publisher's name and location when you cite symposia or conference proceedings; distinguish between the conference date and the publication date if both are given. Do not list abstracts or unpublished material in the References. They should be quoted in the text as personal observations, personal communications, or unpublished data, specifying the exact source, with date if possible. When possible, include URLs for articles available online through library subscription or individual journal subscription, or through large international archives, indexes and aggregators, e.g., PubMedCentral, Scopus, CAB Abstracts, etc. URLs for pdf articles that are posted on personal websites only should be avoided.

Illustrations, Figures and Tables

Figures and illustrations are accepted in the following image file formats:

- EPS (preferred format for diagrams);
- TIFF (at least 300dpi resolution, with LZW compression);
- PNG (preferred format for photos or images);
- JPEG (preferred format for photos or images);
- GIF;
- BMP;

- SVG.

Should you have any problems in providing the figures in one of the above formats, or in reducing the file below 20 MB, please contact the Editorial Office at zookeys@pensoft.net.

Figure legends: All figures should be referenced consecutively in the manuscript; legends should be listed consecutively immediately after the References. For each figure, the following information should be provided: Figure number (in sequence, using Arabic numerals − i.e. Figure 1, 2, 3 etc.); short title of figure (maximum 15 words); detailed legend, up to 300 words. Please note that it is the responsibility of the author(s) to obtain permission from the copyright holder to reproduce figures or tables that have previously been published elsewhere.

Tables: Each table should be numbered in sequence using Arabic numerals (i.e. Table 1, 2, 3 etc.). Tables should also have a title that summarizes the whole table, maximum 15 words. Detailed legends may then follow, but should be concise.

Small tables can be embedded within the text, in portrait format (note that tables on a landscape page must be reformatted onto a portrait page or submitted as additional files). These will be typeset and displayed in the final published form of the article. Such tables should be formatted using the 'Table object' in a word processing program to ensure that columns of data are kept aligned when the file is sent electronically for review. Do not use tabs to format tables or separate text. All columns and rows should be visible, please make sure that borders of each cell display as black lines. Colour and shading should not be used; neither should commas be used to indicate decimal values. Please use a full stop to denote decimal values (i.e., 0.007 cm, 0.7 mm). Larger datasets can be uploaded separately as Supplementary Files. Tabular data provided as

supplementary files can be uploaded as an Excel spreadsheet (.xls), as an OpenOffice spreadsheets (.ods) or comma separated values file (.csv). As with all uploaded files, please use the standard file extensions.

Supplementary Files

Online publishing allows an author to provide data sets, tables, video files, or other information as supplementary information, greatly increasing the impact of the submission. Uploading of such files is possible in Step 4 of the submission process. The maximum file size for each Supplementary File is 20 MB. The Supplementary Files will not be displayed in the printed version of the article, but will exist as linkable supplementary downloadable files in the online version.

While submitting a supplementary file the following information should be completed:

- File format (including name and a URL of an appropriate viewer if format is unusual);
- Title of data;
- Description of data.

All supplementary files should be referenced explicitly by file name within the body of the article, e.g. 'See supplementary file 1: Movie 1" for the original data used to perform this analysis.

Ideally, the supplementary files should not be platform-specific, and should be viewable using free or widely available tools. Suitable file formats are:

For supplementary documentation:

- PDF (Adobe Acrobat)

For animations:

- SWF (Shockwave Flash)

For movies:

- MOV (QuickTime)
- MPG (MPEG)

For datasets:

- XLS (Excel spreadsheet)
- CSV (Comma separated values)
- ODS (OpenOffice spreadsheets)

As for images, file names should be given in the standard file extensions. This is especially important for Macintosh users, since the Mac OS does not enforce the use of standard file extensions. Please also make sure that each additional file is a single table, figure or movie (please do not upload linked worksheets or PDF files larger than one sheet).

Taxonomic Treatments

International Code for Zoological Nomenclature: *ZooKeys* will publish papers that strictly adhere the rules of the last edition of the International Code of Zoological Nomenclature. To assure this, authors are advised to follow the recommendations below.

General: Each first mentioning of an animal species name within the text must be provided with author(s)' name(s). Year of publication of an animal species should be

given in taxonomic revisions with quotation of the work providing the original species' description in the list of references.

New names: When new taxonomic alterations are proposed the taxonomic act should be indicated by adding its abbreviation, i.e., sp. n., comb. n., stat. n. after the taxon name. Same refer to high taxonomic ranks such as subfamily, family, suborder, etc. Authors names should be specified throughout the text if different from the authors of publication. Examples:

- Genus X-us Smith, new genus (author(s) of the publication and authority (-ies) of the taxon is/are identical);
- X-us albus Jones & Peters, new species (the publication is authored by persons different in composition or combination from the authority (-ies) of the taxon itself, e.g. Smith, Jones & Peters or Peters & Jones).

New family-group names: Although all family group names are derived/based on their type genus, the type genus is to be compulsorily designated in any description of a family-group name published after 31st December 1999 (Article 16.2). It is not sufficient that the type genus is mentioned as belonging to the new family-group name; it must be stated that this is the type genus. We recommend a single type line as: Type-genus: Musca Linnaeus, 1758.

New genus-group names: The origin ("etymology", or "derivatio nominum") of name and its gender should be indicated. The type-species and the character of the proposed taxonomic act should be specified for new genus-group names. The type species name should be given in its original combination with an author and year. If the type species is now considered a junior synonym there need to be a clear mention of that. The

fixation type should derive from the International Code of Zoological Nomenclature (see Articles 68 & 69; original designation, monotypy, absolute tautonymy, Linnaean tautonymy, subsequent monotypy, subsequent designation). Example:

- *Sympycnus* Loew

Type-species: *Porphyrops annulipes* Meigen, 1824 by subsequent designation of Coquillett (1910: 610) =pulicarius Fallen, 1823

New species-group names: According to the ICZN Art. 11.9, but also Art. 11.3 the origin "etymology", or "derivatio nominum") new species-group names should be supplemented by information on whether the epithet is an 1) adjective or participle in the nominative singular; 2) noun in the nominative singular; 3) a noun in the genitive case; 4) an adjective used a substative in the genitive case; or 5) an arbitrary combination of letters (ICZN Art. 11.3). For **species-group names**, there are two separate statements of type information that are needed:

- the statement of species' type locality – that is the exact place whence the primary type origins, including exact collecting dataplace with geographical coordinates, geographical or political unit (Area/ District/ State) and country; also, if possible, supplementary locality information should be included – habitat type, method of collecting, date, collector's names, host name (for parasites), etc.
- there should be a separate statement about the type specimen, exact quotation of its original label, condition of specimen (dry pinned, in alcohol, slide, fossil, etc.) and repository (organization's name and city).

Examples:

For a **new species:**

- Type-locality: USA, Viriginia: Fairfax County, Kingstowne, 38°46'N, 77°07'W, broad-leaf forest, under bark, 10 July 2000, J. Smith leg.
- Type-specimen: Holotype male, pinned, with genitalia in a separate microvial. Original label: "USA, VA, Fairfax, Kingstowne, 38°46'N, 77°07'W, 12 Oct 2003, BJ & FC Thompson" "USNM ENT00033805" [Code 49 barcode], "HOLOTYPE / Xylota / x-us / Thompson [red handwritten label].

For a **previously described species**:

Lectotype male, pinned … [details] here designated to fix the concept of X-us albus Jones and to ensure the universal and consistent interpretation of the same. Or… [details then] by designation of Smith (1976: 999).

Previously published names: For a **previously published name**, please provide the year of description. Also use the parentheses convention for subsequent new combinations. **Arranging sections within species treatments** (sections in square brackets are requested for new descriptions only!):

[Name]

[Material]

- [Type material]
- Other material

[Diagnosis]

[Description]

[Etymology]

Distribution

Ecology (including phenology)

Conservation status (optional, but very desirable)

Discussion (optional, but very desirable).