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**INSTITUTO DE BIOCÊNCIAS**  
**PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL**

MARCELO FELIPE VARGAS ORTIZ

**Taxonomia e variação genética de um microlepidóptero  
(Gracillariidae), minador foliar de *Morella pavonis* (Myricaceae)  
no deserto de Atacama, Chile.**

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

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Orientador: Prof. Dr. Gilson Rudinei Pires Moreira.

Co-orientadores: Dra. Gislene Lopes Gonçalves (UFRGS)

e Dr. Wilson Huanca Mamani (UTA)

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## INTRODUÇÃO GERAL

Lepidoptera é um dos grupos de organismos mais estudados e com maior diversidade, estando presente em quase todas as regiões e em uma grande variedade de habitats (Kristensen *et al.*, 2007; Friedlander *et al.*, 2000). Estima-se que existam mais de meio milhão de espécies no mundo, no entanto, apenas cerca de 160.000 espécies foram descritas. Na última década, em média, mais de mil novas espécies foram descritas por ano (Kristensen *et al.*, 2007). Existem mais de 14.000 gêneros descritos para a ordem, dos quais perto de 66% são considerados macrolepidopteros, e o restante pertencentes a microlepidoptera (van Nieukerken *et al.*, 2011).

Entre os microlepidópteros, a família Gracillariidae é considerada uma das mais diversas, com mais de 2.000 espécies descritas (De Prins & De Prins, 2017). A maioria são minadores de folhas, mas também há minadores de flores, frutos, brotos e caules (Davis, 1987). A morfogênese larval é caracterizada por apresentar uma notável hipermetamorfose (Kumata, 1978; Davis, 1987) que geralmente inclui, pelo menos, duas formas larvais e com diferentes hábitos alimentares. Conta da única família que apresenta larvas denominadas "*sap-feeding*", as quais tem uma morfologia altamente especializada e ocorrem normalmente nos primeiros ínstares do desenvolvimento (Kumata, 1978; Davis, 1987). Estas são ápodas com corpo e cápsula cefálica achatada dorsoventralmente e tem mandíbulas muito achatadas dorso-ventralmente, tendo um número de dentes apical reduzido, permitindo-a cortar/dilacerar o tecido da planta e absorver seiva a partir de células rompidas (Kumata 1978; Davis, 1987; Davis e Robinson, 1998). Existem, no entanto, espécies que não apresentam este morfotipo (e.g. Brito *et al.*, 2013). A outra forma larval mais comum de Gracillariidae é denominada "*tissue feeding*", que do ponto de visto ontogenético sucede o tipo "*sap-feeding*" no

desenvolvimento larval. Este morfotipo tem uma morfologia mais "comum" para Lepidoptera, com cabeça e corpo cilíndrico ou subcilíndrico, presença de pernas torácicas, e pseudopódios geralmente bem diferenciados nos segmentos abdominais A3-A5 e A10. As peças bucais são mais desenvolvidas, permitindo a alimentação de tecidos mais complexos, como células do parênquima das folhas. Outra forma larval encontrada em Gracillariidae é denominada "*spinning*", ou prepupa, que difere da forma anterior principalmente por não se alimentar (Kumata, 1978). Geralmente, a atividade desta forma larval destina-se exclusivamente para a formação do casulo pupal; apresenta alterações nas peças bucais, tais como um espinerete mais desenvolvido (Kumata, 1978), que é a peça bucal com o qual constrói o casulo. Outras peças bucais como maxilas, labro e mandíbulas, além das pernas torácicas e pseudopódios, podem também ser reduzidos, ou mesmo estarem ausentes, em algumas espécies (Kumata, 1978; Wagner *et al.*, 2000).

Em geral, as espécies de Gracillariidae tem uma estreita gama de hospedeiros, com muitos casos de monofagia ou oligofagia (De Prins & De Prins, 2014). No entanto, têm sido descritas algumas espécies polípagas, tais como *Marmara gulosa* Guillén & Davis, 2001, cujas larvas são minadoras de pelo menos cinco famílias vegetais (Guillén *et al.*, 2001).

Com relação à diversidade, cerca de 186 espécies de Gracillariidae são descritas para a região Neotropical (De Prins & De Prins, 2017). Porém, estudos recentes revelaram que existe uma grande quantidade de espécies desconhecidas nesta região, principalmente por ser ainda insuficientemente estudada (Lees *et al.*, 2014 ; Brito *et al.*, 2016). No Chile, apenas oito espécies de Gracillariidae foram descritas, das quais seis têm relatos da planta hospedeira (De Prins & De Prins, 2017). No norte em particular, foram relatadas quatro espécies nativas de Gracillariidae, com informações sobre suas

plantas hospedeiras, distribuídas nos vales transversais do deserto do Atacama; *Angelabella tecomae* Vargas & Parra, 2005, *Acrocercops serrigera serrigera* Meyrick, 1915, *Chileoptilia yaroella* Vargas & Landry, 2005, e *Leurocephala chilensis* Vargas & Moreira, 2017. A espécie descrita neste trabalho que é nativa do Deserto do Atacama, foi recentemente descoberta. Pertencente ao gênero *Caloptilia* Hübner, 1825, seu estágio imaturo é monofago e utiliza como planta hospedeira a árvore nativa "Guacano", *Morella pavonis* (Myricaceae).

O gênero *Caloptilia* Hübner, 1825, apresenta 20 espécies relatadas para a região Neotropical e a associação de Gracillariidae com plantas da família Myricaceae como planta hospedeira tem sido relatada para treze espécies de Gracillariidae no mundo, das quais seis pertencem a *Caloptilia*, mas nenhuma delas foi relatada para a região Neotropical (De Prins & De Prins, 2017). Dessa forma, *Caloptilia* sp1.Vargas-Ortiz & Vargas sp. nov., descrita neste trabalho, corresponde à primeira espécie deste gênero registrada para o Chile e também em associação com Myricaceae como planta hospedeira para a região Neotropical. A distribuição geográfica desta espécie está restrita a cinco vales transversais localizados no Deserto do Atacama, norte do Chile: Lluta (18° 24' S, 70° 07' O), Livilcar (18° 30' S, 69° 43 ' O), Codpa (18° 49' S, 69° 40' O), Camiña (19° 18' S; 69° 25' O) e Mamiña (20° 4' S; 69° 13' O).

O Deserto do Atacama é a região mais seca e uma das mais antigas do mundo neste contexto (Clarke, 2006). Sua biota nativa vive em condições extremamente frágeis e isoladas (Pinto *et al.*, 2006; Vargas & Moreira, 2012; Carevic *et al.*, 2013.). Em alguns casos, esta área de deserto representa uma importante barreira geográfica para o fluxo genico entre populações, gerando altos níveis de divergência intra-específica em vários organismos (Baranzelli *et al.*, 2014; Larridon *et al.*, 2015).



Os vales transversais do norte do Chile representam uma importante fonte de biodiversidade dentro do deserto do Atacama. Porém, são fortemente afetados pela atividade humana, principalmente associada com uma agricultura intensiva, o que levou a uma redução significativa da biota nativa (Luebert & Pliscoff, 2006; Estades *et al.*, 2007; Vargas & Parra, 2009; Mendez-Abarca *et al.*, 2012). Um desafio fundamental para a conservação da biodiversidade é identificar com precisão as unidades bióticas, diagnosticando aquelas que merecem planos de conservação (Forister *et al.*, 2007). Estudos de populações naturais, à nível geográfico e ecológico, permitem compreender os processos que determinam os padrões de diversidade observados na natureza. Deste ponto de vista, os estudos de genética de populações são muito importantes para compreender a história e o potencial evolutivo de espécies e populações (Burgman *et al.*, 1993).

Do ponto de vista ecológico, *Caloptilia* sp1 tem pelo menos duas características importantes: 1) é um microlepidóptero, com uma envergadura da asa anterior de 4 mm aproximadamente, o que pode significar uma baixa capacidade de dispersão; 2) é uma espécie monófaga, o que sugere um elevado grau de especialização aos habitats nos quais se encontra sua única planta hospedeira. Estes dois fatores podem levar a uma alta estruturação genética nas populações desta espécie, associada à sua distribuição geográfica. Ou seja, as grandes áreas de deserto que separam os vales transversais, aonde as plantas hospedeiras se encontram, poderiam estar atuando como barreiras geográficas, limitando a dispersão de insetos com baixa capacidade de vôo.

Cheng *et al.* (2016) indicam que o isolamento geográfico separa as populações, impede o fluxo de genes e leva à diferenciação genética, o que pode resultar na evolução de novas espécies. Broquet *et al.* (2010) indicam que a conectividade entre habitats é importante para a manutenção da variação genética em populações naturais, e

restrições e fragmentações destes podem levar a uma redução nas áreas de dispersão disponíveis e o tamanho da população da espécie, especialmente em pequenas populações que tem menor variabilidade genética.

Em Lepidoptera, há alguns estudos nos quais foi analisada a estruturação genética populacional de espécies para determinar a possível falta de conectividade entre populações. Nesse contexto, há casos nos quais se apresenta diferenciação genética atribuída a distribuição geográfica e outros em que as diferentes populações mantêm o fluxo gênico entre elas (Crawford *et al.*, 2011; Keyghobadi *et al.*, 2006; Habel *et al.*, 2013; Snall *et al.*, 2004; Kirichenko *et al.*, 2017), devido principalmente a que os adultos tem uma alta capacidade de dispersão.

Um método amplamente usado para a avaliação da diversidade genética de populações animais é a análise de variações no DNA mitocondrial (mtDNA) (Harper *et al.*, 2008; Gonçalves *et al.* 2009; Morales *et al.*, 2011; Silva-Brandão *et al.*, 2011; Siti-Balkhis *et al.*, 2011) devido ao fato que as mutações recentes (substituição, deleção e inserção de bases) ocorrem geralmente entre 5-10 vezes mais rápido do que no DNA nuclear (Brown *et al.*, 1979). Além disso, diferentes regiões do genoma mitocondrial evoluem a taxas diferentes, permitindo opções específicas para cada estudo. Visto que o mtDNA é herdado via materna (Avisé, 2009), a análise de sua variabilidade para estudos populacionais é uma ferramenta muito informativa, já que no contexto da dinâmica populacional a dispersão das fêmeas é um fator decisivo, pois são elas quem depositam os ovos (Snall *et al.*, 2004).

As espécies de microlepidoptera do norte do Chile têm sido pouco estudadas de uma maneira geral, embora alguns estudos a respeito tenham sido realizados usando ferramentas filogenéticas e populacionais (Vargas *et al.*, 2015 a; Maita-Maita *et al.*, 2015; Vargas *et al.*, 2015 b; Escobar-Suárez *et al.*, 2017). O objetivo principal deste

estudo foi fazer a descrição taxonômica desta nova espécie de *Caloptilia* do Deserto do Atacama, com base na caracterização morfológica dos estágios de desenvolvimento (ovo, larva, pupa, adulto) e da sua história natural com ênfase na interação com a planta hospedeira. Também foi feito um estudo de caracterização e análise da variação genética correspondente, usando sequências de DNA mitocondrial (região barcode do citocromo oxidase subunidade 1 - COI), com o objetivo de determinar as relações filogenéticas com espécies congênicas, e determinar padrões de estruturação genética populacional no Deserto do Atacama.

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***Caloptilia* sp1. Vargas-Ortiz & Vargas sp. nov. (Lepidoptera: Gracillariidae): a new leafminer feeding on *Morella pavonis* (Myricaceae) in the Atacama Desert of northern Chile, with notes on natural history and population genetic structure**

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Running title: a new leafminer *Caloptilia* from the Atacama Desert

## Abstract

The highly diverse and widely distributed micro-moth genus *Caloptilia* Hübner [1825] (Lepidoptera: Gracillariidae: Gracillariinae) is reported for the first time in Chile. Adults, immature stages and natural history, including the mine of *Caloptilia* sp1. Vargas-Ortiz & Vargas sp. nov. are described and illustrated with the aid of optical and scanning electron microscopy. Larva is a leafminer of the native tree "guacano" *Morella pavonis* (Myricaceae) in the transverse valleys of the Atacama Desert. DNA barcode sequences are provided and used to assess preliminarily the relationships with congeneric species and to investigate population genetic structure of this new species in northern Chile.

**Key words:** Micromoths; Gracillariids; Myricacean; Guacano tree; Neotropical region

## INTRODUCTION

The taxonomy and natural history of the Neotropical fauna of the highly diverse micro moth family Gracillariidae remain insufficiently studied. First, the number of species actually present in this biogeographic region is underestimated for several reasons, one of which is the poor sampling in many habitats (Lees *et al.*, 2014; Brito *et al.*, 2016). Second, a large number of the Neotropical species already described are known only from the type material, with original descriptions many times restricted to color patterns of the adult stage. Data regarding genitalia morphology, geographic ranges, host plants and immature stages remain unknown for many of them (Brito *et al.*, 2016; De Prins *et al.*, 2016). Surveys for larvae of Gracillariidae on native Neotropical plants are useful to either discover new species and help to improve knowledge about the morphology, geographic range and natural history of previously described species (e.g.: Mundaca *et al.*, 2013; Vargas *et al.*, 2013; Arévalo-Maldonado, 2014; Moreira *et al.*, 2017; Pereira *et al.*, 2017; Brito *et al.*, 2017).

Despite the fragility by being arid natural environments, the transverse valleys of northern Chile are recognized as among the most important in terms of containing biodiversity of the Atacama Desert (Luebert & Pliscoff, 2006; Estades *et al.*, 2007). However, its native micro moths remain partially collected and studied (Bobadilla & Vargas, 2015). Only four native species of Gracillariidae have been recorded previously

from these valleys: 1) *Acrocercops serrigera serrigera* Meyrick, 1915, whose leaf-miner larvae are associated with two Malvaceae (Vargas *et al.* 2013); 2) *Angelabella tecomae* Vargas & Parra, 2005, with leaf-miner larvae on the native shrub “chuve” *Tecoma fulva fulva* D. Don (Bignoniaceae) (Vargas & Parra, 2005); 3) *Chileoptilia yaroella* Vargas & Landry, 2005, whose larvae feed on inflorescences of “yaro” *Acacia macracantha* Wild. (Fabaceae) (Vargas & Landry, 2005); and, 4) *Leurocephala chilensis* Vargas & Moreira, 2017, with leaf miner larvae on *Schinus molle* (Anacardiaceae) (Pereira *et al.*, 2017).

*Caloptilia* Hübner [1825] (Gracillariidae: Gracillariinae) is a highly diverse genus with more than 320 species described worldwide, only 20 of which are represented in the Neotropical Region, with records from Argentina, Brazil, Colombia, Cuba, Ecuador, Guyana, Peru, Puerto Rico, United States, Saint Vincent and the Grenadines, and the U.S. Virgin Islands (De Prins & De Prins, 2017). The genus has been not recorded in Chile, although species are known from two (Argentina and Peru) of the three neighbouring countries (De Prins & De Prins, 2017). As part of a study of the Lepidoptera associated with the native plants of the arid environments of the Atacama Desert, some adults of an undescribed species of *Caloptilia* were recently reared by us from larvae collected on “guacano”, *Morella pavonis* (C.DC) (Myricaceae) in the Lluta Valley, Arica Province of northern Chile. Accordingly, one of the purposes of this contribution is to describe and illustrate the adults, immature stages and natural history of this new species. In addition, sequences of DNA barcodes are provided and used to assess preliminarily the relationships with congeneric species and to investigate intraspecific variation and population structure of this new species in the Atacama Desert of northern Chile.

The Atacama Desert is a remarkable biome to address population genetic studies; a continuously arid region with environments isolated in patches defined by valleys (Pinto *et al.*, 2006, Vargas and Moreira, 2012, Carevic *et al.*, 2013). Transverse valleys are separated by extensive areas of desert, which imposes a significant barrier to dispersal; this, reduces gene flow among populations, promoting high levels of intraspecific divergence (Baranzelli *et al.*, 2014). The impact of habitat fragmentation on genetic diversity, population differentiation, inbreeding and extinction risk depends on gene flow among populations, which is allowed by the dispersal of individuals between populations. Dispersal reduces the impact of habitat fragmentation, because with enough interchange of individuals among populations, gene flow can be sufficient

to maintain genetic diversity at levels similar to a contiguous population. Thus gene flow can prevent genetic differentiation by replacing the alleles lost through genetic drift, mitigating inbreeding depression (Frankham *et al.*, 2002; Freeland, 2005). The new species of *Caloptilia* is endemic to this region and occurs specifically in valleys which are located far from each other (ca.  $\geq 30$  Km) and highly anthropic, particularly due to agriculture. As in other micro moths, we suppose that this species is not able to disperse long distances. Thus we hypothesized that females of *Caloptilia* sp1. Vargas-Ortiz & Vargas sp. nov. are not able to disperse among these valleys, and the extensive desert areas might act as a geographic barrier, reducing the gene flow among populations. As mitochondrial DNA has been used successfully to infer polymorphism in the absence of codominant markers in other insects (Snäll *et al.*, 2004; Seraphim *et al.*, 2016; Piwczyński *et al.*, 2016; Peterson *et al.*, 2016; Kramp *et al.*, 2016; Velasco-Cuervo *et al.*, 2016; Sarswat *et al.*, 2016; Frantine-Silva *et al.*, 2016; Rosa *et al.*, 2016), we decided to use these markers to make our inferences and test this prediction.

## **MATERIAL AND METHODS**

### **Sampling and rearing**

Leaves of *M. pavonis* containing larvae of *Caloptilia* were collected between April 2010 and December 2016 in the Lluta, Livilcar, Codpa, Camiña and Mamiña Valleys, and brought to the Laboratorio de Entomología of the Facultad de Ciencias Agronómicas, Universidad de Tarapacá, Arica, Chile. Larvae representative of each instar were preserved in 75% ethanol for morphological description. Additional larvae were preserved in 100% ethanol at -20°C for DNA extraction. The remaining larvae were reared in the laboratory to obtain pupae. Some pupae were also preserved in 75% ethanol, while the remainder were kept at room temperature to obtain adults, which were pin-mounted and dried following standard procedures.

**Museum collections.** Abbreviations of the institutions where specimens examined were deposited are as follows:

MNNC: Museo Nacional de Historia Natural de Santiago, Santiago, Chile;

IDEA: Colección Entomológica de la Universidad de Tarapacá, Arica, Chile;

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

## **Morphological analysis**

For study the adult genitalia, the abdomen of adults was removed and cleaned with 10% KOH solution and subsequently slide-mounted with Euparal. A similar procedure was followed to dissect the larval integument to study the chaetotaxy. Morphological observations and measurements were performed on at least five individuals of each instar and/or stage with the aid of a Leica® M125 stereomicroscope and an Olympus BX51 optical microscope. Figures were constructed in the CorelDraw® X7 software based on photographs taken with a Sony CyberShot DSC-HX200V digital camera. For scanning electron microscopy 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 examined and photographed in a JEOL® JSM5800 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil.

In order to characterize the leaf damage of the different instars, fresh leaves of *M. pavonis* containing larvae at different ages were collected and cut freehand with a razor blade. The cross sections were photographed immersed in tap water following procedures similar to those described above for performing photography of the leaf miner morphology.

## **DNA extraction and sequencing**

Total genomic DNA was extracted from larval tissue stored at -20°C, following the method proposed by Huanca-Mamani *et al.* (2015). The barcode region (658 base pairs) was amplified through the polymerase chain reaction (PCR) using the universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') developed by Folmer *et al.* (1994). The PCR conditions used were: initial denaturation for 5 minutes at 95°C, continued by 35 cycles of 30 sec at 95°C, 30 sec at 55°C and 30 sec at 72°C, with a final extension for 2 minutes at 72°C. PCR products were purified and sequenced in both strands with the same forward and reverse primers using the Sanger method. The sequences generated were aligned using default parameters in Clustal W implemented in MEGA 6 (Tamura *et al.* 2013). Variable sites were checked using original chromatograms. The sequences generated were deposited in Genbank database (Table S1).

## **Molecular phylogeny**

To provide a preliminary assessment of the relationships of the new species, nine barcode haplotypes of *Caloptilia* sp1. sp. nov. were analyzed using the Maximum Likelihood (ML) method (Felsenstein, 1981). We took into account the available sequences of South American *Caloptilia* provided by Lees *et al.* (2014) and Nakadai & Kawakita (2016), all the available sequences of congeneric species that feed on Myricaceae (De Prins & De Prins, 2017) and additional congeneric species taken from GenBank and BOLD using the respective BLAST. One species of *Callisto* Stephens, 1834 was used to root the tree, as this genus belongs to Parornichinae, the sister subfamily of Gracillariinae (Kawahara *et al.* 2017) (Table S1). The GTR+G+I (General Time-Reversible model; Rodríguez *et al.* 1990) with gamma distribution (G) and invariable sites (I) was selected as the best model for the ML. The statistical support for each node was calculated by the bootstrap method (Felsenstein, 1985) using 1000 replicates. The pairwise distance among sequences was assessed using the Kimura 2-parameter model (Kimura, 1980). All the molecular analyses were performed in the software MEGA6 following the procedures described by Hall (2013).

## **Population analysis**

A total of 120 individuals of *Caloptilia* sp1. sp. nov. were analyzed from five geographically separated valleys of the Atacama Desert (Table S2; Fig. 2B): Lluta (18°23'58''S; 70°1'14''W), Livilcar (18°34'41''S; 69°47'16''W), Codpa (18°49'30''S; 69°41'10''W), Camiña (19°18'40''S; 69°25'81''W) and Mamiña (20°4'32''S; 69°13'5''W). Sequence distances were calculated using the Kimura 2-parameter method with MEGA 6. Intraspecific genealogy was constructed using the median-joining method (Bandelt *et al.*, 1999) with the software Network 5.0 ([www.fluxus-engineering.com](http://www.fluxus-engineering.com)). The molecular diversity indices number of polymorphic sites (S), haplotype diversity (Hd) and nucleotide diversity ( $\pi$ ) were calculated in Arlequin 3.0 (Excoffier *et al.*, 2006). Genetic differentiation between pairs of populations among valleys were measured using F-statistics. We inferred pairwise  $F_{ST}$  with Arlequin using haplotype frequency. We performed an AMOVA (Excoffier *et al.*, 1992) to infer whether geographic distance might have influenced the genetic variation in this species. To perform this analysis, we separated the samples into two major groups: i) northern (Lluta+Livilcar+Codpa) and ii) southern (Camiña +Mamiña). Although populations of the Camiña and Mamiña valleys are highly distant from each other, both are categorized

in a southern part of the desert. We also tested for positive Pearson correlation between genetic ( $F_{ST}$ ) and linear geographic distance (in kilometers) with the Mantel test using the software Arlequin 3.0. Population size changes during the evolutionary history of *Caloptilia* sp1 were investigated through neutrality tests (Tajima's D; Fu and Li's D and F; Fu's FS) calculated in the program DnaSP 5.0. Finally, we performed a mismatch distribution analysis (under the population growth model) to investigate patterns of population expansion. The shape of a mismatch distribution has been shown to be influenced by demographic events like past expansions or population bottlenecks (Rogers and Harpending, 1992).

## RESULTS

### Molecular phylogeny

The ML tree showed that *Caloptilia* sp1 is monophyletic, with strong node support (bootstrap = 80) (Fig. 1). The closest related taxon was an undetermined Peruvian species, *Caloptilia* sp., with genetic distance of 4.9-5.1% (K2P), and *C. agrifoliella* Opler (1971) with 4.13-4.28% divergence. The species from Peru, for which there is no detailed morphological information, uses a host plant of the genus *Morella* (Myricaceae) (Nakadai & Kawakita, 2016). Other species of *Caloptilia* which feed on Myricaceae were included, but did not form a clade with *Caloptilia* sp1., suggesting that a restricted evolutionary relationship between diversification of *Caloptilia* species and this host plant family might be ruled out. Additional species from the Neotropical (French Guyana) region were also included, but we do not have information on their host plant, and they formed a group apart from the Neotropical species of Chile and Peru.

### Population analysis

Ten variable sites were found in *Caloptilia* sp1., which resulted in nine COI haplotypes; haplotype diversity ( $H_d$ ) and nucleotide diversity ( $\pi$ ) were 0.722 and 0.003, respectively. No insertions or deletions were observed in any of the sequences. The nucleotide frequencies observed were A = 38.92, T = 32.45, C = 14.36, G = 14.27. Only one population (Codpa) presented a transversion (=1). Molecular diversity indices are given in Table 1. Genetic distances (K2P) were between 0.2 and 1.1%, with greater values between haplotypes 4 and 6 (Table 2). The median Joining network (Fig. 2D)

indicates that two main haplotypes (H\_2 and H\_3) are common to all populations, present in 39 and 47 individuals, respectively. The populations of Codpa, Lluta and Livilcar had one unique haplotype each (H\_4, H\_6 and H\_7, respectively) represented by only one individual. The Mamiña population presents one specific haplotype (H\_1) represented by 16 individuals. The Camiña one had two specific haplotypes, each represented by 9 (H\_8) and 1 (H\_9) individuals. The Livilcar and Codpa populations share one haplotype (H\_5) represented by 5 individuals. The pairwise  $F_{ST}$  was not significant for almost all comparisons between populations, except between Camiña and others that showed significant ( $p < 0.05$ ) differences (Table 3). The AMOVA analysis indicated that most of the genetic variation (85.38%) occurred within populations. Thus the F-statistic indicates low genetic differentiation over the geographical scale studied, although the distance between Mamiña and the other populations is fairly large ( $> 87$  Km; Table S2). Genetic variation among groups and among populations within groups were low (4.71 and 9.91, respectively) (Table 4). Isolation by distance assessed by the Mantel test did not show significant correlation (correlation coefficient = 0.19;  $P = 0.256$ ) between genetic and geographical distances among pairs of populations, i.e. populations are not isolated by distance (Fig. 2E). Neutrality tests indicated that the Mamiña population might be under a population expansion, since significant values for Tajima's D (2.72,  $p < 0.01$ ) and Fu and Li's F (1.791,  $p < 0.05$ ) were observed in this case (Table 1). However, none of the other populations showed evidence of historic demographic changes. By contrast, mismatch distribution analysis showed similar unimodal patterns for all populations, indicating a putative population expansion (Fig. 3).

## **TAXONOMY**

*Caloptilia* sp1. **Vargas-Ortiz & Vargas sp. nov.**

### **Material examined**

#### ***Holotype***

♂, CHILE: Lluta valley (18°23'58''S; 70°1'14''W), Arica, Chile, May 2010, H.A. Vargas coll., ex larva on *Morella pavonis* (MNNC).

#### ***Paratypes***

1 ♂, 2 ♀, same data and locality as holotype (MNNC); 3 ♂, 1 ♀, same data and locality



as holotype (IDEA); 1 ♂ same locality as holotype, September 2010, H.A. Vargas coll., ex larva on *Morella pavonis* (IDEA); 1 ♂, 1 ♀ Livilcar valley (18°34'41''S; 69°47'16''W), Arica, Chile, November 2016, M. Vargas-Ortiz coll. ex larva on *Morella pavonis* (IDEA); 1 ♀ Codpa valley (18°49'30''S; 69°41'10''W), Arica, Chile, January 2011, H.A. Vargas coll., ex larva on *Morella pavonis* (IDEA).

### ***Additional material***

Immature stages, all fixed in Dietrich fluid and preserved in 70% ethanol, collected at the type locality by H. A. Vargas and Gilson R.P. Moreira, on 1-15 August 2012, and deposited in LMCI were as follows: 2 eggs (LMCI 191-60), 10 first instar larvae (LMCI 191-61), 11 second instar larvae (LMCI 191-62), 15 third instar larvae (LMCI 191-63), 12 fourth instar larvae (LMCI 191-64), 8 fifth instar larvae (LMCI 191-65), and 6 pupae (LMCI 191-65).

### **Diagnosis**

The external appearance of *Caloptilia* sp1. resembles *C. immuricata* (Meyrick, 1915) described from Lima, Peru, which is also the geographically nearest *Caloptilia*. However, *C. immuricata* is characterized by the presence of a row of small, well-defined black spots on the costal margin of the forewings, which are not found in *Caloptilia* sp1. In addition, the male genitalia of the two species is clearly different, as the median finger-like expansion on the sacculus of *Caloptilia* sp1 is absent in *C. immuricata*, which has a similar projection on the middle of the ventral margin of the valva; the tip of the aedeagus is bifid in *C. immuricata*, while this is narrow in *Caloptilia* sp1. As the female genitalia of *C. immuricata* remain unknown it is not possible to compare with that of *Caloptilia* sp1.

Among the other Neotropical representatives of *Caloptilia*, the male genitalia of *Caloptilia* sp1 resembles that of *C. schinusifolia* Davis & Wheeler, 2011, described from coastal Brazil (Davis *et al.* 2011). However, the suddenly anteriorly narrowed saccus, the median finger-like expansion on the sacculus and the elongated cornutus on the vesica distinguish *Caloptilia* sp1., because in *C. schinusifolia* the anterior portion of the saccus is not suddenly narrowed, an expansion is not present on the sacculus and the vesica has a row of 9-11 spine-like cornuti. At level of the female genitalia, the narrow ostium bursae of *Caloptilia* sp1. is about 1/3 the length of the anterior apophyses; while the wide ostium bursae is about 2/3 the length of the anterior apophyses in *C.*

*schinusifolia*.

## **Description**

### **Male** (Fig. 4)

#### *Head*

Vertex and frons mostly yellowish white, a pair of yellowish-brown spots on the frons close to the compound eyes. Filiform antennae slightly longer than forewing, uniformly yellowish white. Maxillary palpi almost straight, length similar to the diameter of the compound eye, mostly yellowish brown, with some scattered yellowish-white scales. Labial palpi upward curved, about three times the length of the maxillary palpi, similar to maxillary palpi in color.

#### *Thorax*

Mostly yellowish-brown. Foreleg: coxa, femur and tibia mostly grayish-brown with a few scattered, yellowish-white scales; tarsi mostly yellowish-white with a few scattered grayish-brown scales. Middle leg similar to foreleg in color; a pair of apical spurs on the tibia. Hind leg mostly yellowish-white with a few scattered, grayish-brown scales; two pairs of tibial spurs. Forewing (5.0-5.5 mm length) mostly grayish-brown with yellowish-white and grayish-violet scales scattered; a yellowish-white area close to the costal margin at about the middle third; fringe with long, hair-like scales on the distal third of the posterior margin and short, smooth scales at apex. Hindwing completely covered by yellowish-white scales; fringe with color, long, hair-like scales.

#### *Abdomen*

Yellowish-brown. Segments VII and VIII mostly membranous. Tergum VII as a narrow transverse stripe (Fig. 5I), anterior margin slightly concave at middle, a narrow posterior projection at middle of the posterior margin; sternum VII as a narrow transverse stripe (Fig. 5J), a wide semicircular expansion at middle of the anterior margin. Tergum VIII as a narrow longitudinal stripe (Fig. 5K), anterior apex T-shaped, posterior apex widened as a semicircle; sternum VIII (Fig. 5L) as a narrow transverse stripe, anterior margin with a rectangular expansion at middle and a pair of triangle-like expansions laterally; a pair of coremata immediately posterior to the sternum VIII, each composed of two groups of different-sized scales (Fig. 5M).

### *Male genitalia* (Fig. 5A)

Tegumen narrow, slightly sclerotized. Subscaphium a narrow longitudinal stripe about half the length of the tegumen. Saccus triangle-like, about 1.3 times the length of the tegumen, posterior third wide, posterior margin widely concave, anterior 2/3 suddenly narrowed, apex round. Valvae mostly rectangle-like throughout the proximal half, dorsally expanded sub-apically; long hair-like setae mostly concentrated on the medial surface of the distal part of the valvae (Fig. 5C); costal margin with a spine-like ventral expansion, ventrally projected sub-basally; ventral margin mostly straight; distal margin widely convex; sacculus with a distinctly sclerotized finger-like median expansion dorsally projected (Fig. 5D). Aedeagus sub-cylindrical, length similar to saccus, progressively narrowing distally; vesica with several short cornuti clustered in a longitudinal area about 1/5 the length of the aedeagus (Fig. 5E).

### **Female**

Mostly similar to male.

### *Female genitalia* (Fig. 5F)

Papillae anales narrow, lobe-like, slightly sclerotized, with long hair-like setae. Anterior and posterior apophyses similar in length to the papillae anales; anterior apophyses dorsally continuous with the antero-lateral portion of the narrow tergum VIII, ventrally continuous with the narrow and slightly widened at middle posterior margin of sternum VIII. Ostium bursae on the membranous area between sterna VII and VIII. Ductus bursae elongated, narrow, mostly membranous, coiled distally; antrum short, mostly slightly sclerotized, a little membranous basally; ductus seminalis narrow, arising dorsally on the membranous part of the ductus bursae immediately distal to the antrum; corpus bursae membranous, pear-like, with two great claw-like signa having medial, saw-like margins (Fig. 5G).

### **Immature stages**

#### *Egg* (Fig. 11B)

Flat, ellipsoid, length from 0.23 to 0.25 mm, width from 0.11 to 0.12 (n = 2). Light yellow when recently deposited; chorion translucent, larva visible by transparency before eclosion.

## *Larva*

Hypermetamorphic with five instars; the first two sap-feeding; the last three tissue-feeding. No morphological differences were found between the two sap-feeding instars. Similarly, no major morphological differences were found among the three tissue-feeding instars, except that the third instar has head and prothoracic dorsal shield dark brown, which are light brown in the fourth and fifth instars. Instars can be identified by measurements of the head capsule (Table 5).

*First instar* (Figs 6, 8A). Prognathous, apodal; head, thorax and abdomen depressed, setae absent.

*Head.* Light brown, without stemmata. Antennae 1-segmented with five sensilla (Fig. 6F). Mouthparts typical of the sap-feeding type; labrum 2-lobed with a sharp cleft at the middle of distal margin; each lobe with pectinate distal margin and a few spine-like dorsal projections. Mandibles depressed; medial margin round, serrated; distal margin with two hooks mesally projected, the most distal with a small hook ventrally. Maxillae absent. Labium depressed; hypopharynx covered by short spine-like ornamentations distally and elongated granular projections ventrally (Fig. 6D); the first, located distally, with ca. 16 uniformly aligned sub-rectangular units, followed by another with about 24 irregular shaped, randomly arranged units bearing the spinneret in the central region; labial palpi as two reduced projections (Fig. 6E), the most medial short, spine-like, at the apex.

*Thorax.* Yellowish-white. Prothoracic dorsal shield well differentiated. Integument mostly smooth with transverse patches of granular microtrichia on the dorsal surface of T2-3 (Fig. 6G). Two callus-like projections ventrally on T1-3 (Fig. 6I).

*Abdomen.* Yellowish-white. Integument mostly smooth with transverse patches of granular microtrichia on the dorsal surface of A1-9. Two callus-like projections ventrally on A3-5. Spiracles circular (Fig. 6H), laterally on T1 and A1-8.

*Fifth instar* (Figs 7, 8B-C). Eruciform; hypognathous; maximum length 5 mm; legs on T1-3, prolegs on A3-5 and A10.

*Head.* Light brown. Frontoclypeous triangle-like, with lateral margins slightly sinuous. Six circular stemmata, laterally in semicircle-like distribution (Figs 7C, 8B). Antennae 2-segmented (Fig. 7H); first segment cylindrical with one sensillum laterally

and four sensilla at apex; second segment cylindrical, slightly shorter and about a third the diameter of the first segment, with three sensilla at the apex; antocoria with several granular projections. Mouthparts of the chewing type. Labrum 2-lobed (Fig. 7E); medial cleft on the distal margin extending up to near the middle of the labrum, with six pairs of hair-like setae on the external surface. Mandible with five teeth and two hair-like setae on the external surface. Maxillae with galea and palpus well differentiated (Fig. 7G), both with sensilla. Hypopharynx 3-lobed, covered with short spine-like projections. Labium with a cylindrical spinneret (Fig. 7F) at the apex with circular orifice sub-apically on the dorsal surface; a pair of 2-lobed, narrow, cylindrical palpi about two thirds the length of the spinneret, each bearing a sensillum at apex.

*Thorax.* Yellowish-white; covered with short spine-like microtrichia. Prothoracic dorsal shield light brown, elliptical, smooth (Fig. 7I). Circular spiracle with slightly elevated peritreme laterally on prothorax (Fig. 7K). Legs with a narrow and slightly curved claw at the apex (Fig. 7J).

*Abdomen.* Yellowish white; covered by short spine-like microtrichia. Anal shield semicircular (Figs 7M, 8C), smooth, light brown. Circular spiracle with slightly elevated peritreme laterally on A1-8. Crochets of A3-6 in biordinal circles (Fig. 7L); crochets of A10 in uniordinal mesoserries (Fig. 7N).

*Chaetotaxy of the fifth instar* (Figs 8B-C). All setae hair-like, with variable length.

*Head.* A group 3-setose; A1 anterior to stemma 3; A2 dorsal to A1; A3 posterolateral to A2. AF group 2-setose; AF1 and AF2, close to dorsal vertex of the frontoclypeus. C group 2-setose; C1 and C2 close to the ventral margin of the frontoclypeus. CD group 3-setose, almost in a straight line. F group 1-setose; F1 close to the lateral margin of the frontoclypeus. L group 1-setose; L1 posterolateral to A3. MG group 1-setose. P group 2-setose; P1 lateral to AF group; P2 dorsolateral P1. S group 3-setose; S1 between stemmata 2 and 3; S2 between stemmata 1 and 6; S3 between stemmata 5 and 6. SS group 3-setose; SS2 ventral to S3; SS1 anterior to SS2; SS3 posterior to SS2.

*Thorax:* **T1.** D, XD and SD groups 2-setose, with all setae on the prothoracic dorsal shield. L group 2-setose; L1 and L2 anterodorsal to spiracle. SV group 2-setose; SV1 and SV2 dorsal to coxa. **T2-3.** D and SD groups 2-setose; D1, D2, SD1 and SD2 almost in an aligned line with D1 dorsal and SD2 ventral. L group 3-setose with the three setae nearly in a straight line, with L2 anterior, L3 posterior and L1 in the middle. SV groups 1-setose; SV1 dorsal to coxa.

*Abdomen*: **A1**. D and SD groups 2-setose; D1 anterodorsal to D2; SD1 posterodorsal to spiracle; SD2 anterodorsal to spiracle. L group 2-setose; L1 posteroventral to spiracle, almost in a straight line with D1 and SD1. SV group 1-setose. V group 1-setose, close to SV1. **A2, 6-7**. Similar to A1, but with SV group 2-setose. **A3-5**. Similar to segment A2, but SV group 3-setose with all the setae on the lateral surface of the proleg. **A8**. Similar to segment A7, but SV 1-setose. **A9**. D, SD, L, SV and V groups 1-setose. **A10**. D and SD groups 2-setose with all setae close to margin of anal shield. L, PP, SV and V groups on the proleg. L group 3-setose; SV group 4-setose; V group 1-setose.

*Pupa* (Figs 9-10).

Light brown, maximum length = 5.5 mm.

*Head*. Cocoon cutter dorso-anteriorly projected (Figs 10C-D). Frons broad with two pairs of setae close to the labrum (Fig. 10E). Apex of the antennae exceeds the apex of the metathoracic legs. Maxillari palpi bean-like, located between the eye, antenna and prothoracic leg. Labial palpi about half the length of the proboscis. Apex of the proboscis about half the length of the metathoracic legs (Fig. 9).

*Thorax*. Prothorax as a smooth straight transversal stripe in dorsal view, provided with two comma-like depressions close to the middle (Fig. 10G). Mesothorax as a smooth, wide, transverse wide stripe in dorsal view, with anteriorly and posteriorly projected central vertices. Metathorax as a narrower square-like plate in dorsal view with a pair of hair-like setae laterally, close to the anterior margin. Apex of the prothoracic legs about half the length of the apex of the antennae. Apex of the mesothoracic legs slightly exceeds the apex of the prothoracic legs (Fig. 9).

*Abdomen*. Mostly covered by granular microtrichiae; one pair of hair-like setae dorsally on A1, three pairs (dorsal, dorso-lateral and lateral) on A2-7 and one pair laterally on A8. Dorsal surface of segments A1-8 covered by posteriorly curved spine-like projections (Figs 10H-I). Circular spiracles with slightly elevated peritreme laterally on A2-7 (Fig. 10J). Segment A10 with ten anteriorly curved, circularly arranged stout spine-like projections; two pairs dorsal, two pairs lateral and one pair ventral, the last pair with projections close to each other (Figs. 10K-L).

### **Etymology**

The species name is derived from the common name of the host plant ("guacano") and the Latin *voro* (= to eat, to devour).

## **Host plant**

*Morella pavonis* (Fig. 11A) is the only host plant currently known for *Caloptilia* sp1. This native tree has a relatively narrow distribution range, from central Peru to northernmost Chile (Parra-O, 2002). It is the only representative of the family Myricaceae in Chile (Muñoz-Pizarro, 1966). The Chilean populations of *M. pavonis* are in low abundance, restricted to valleys and ravines of the Atacama Desert with either permanent or semi-permanent water courses (Rodríguez *et al.*, 1983), and have been recently classified either as near threatened or vulnerable (Gatica-Castro *et al.*, 2015).

## **Natural history**

Eggs are deposited mostly on the abaxial surface of *M. pavonis* leaf (Fig. 11B). The first instar (sap-feeding) penetrates the epidermal cells and constructs a narrow serpentine mine by feeding upon the lower layer of spongy parenchyma adjacent to the abaxial epidermis (Figs 11C-12D). The second instar (sap-feeding) remains restricted to this layer, but its mine looks like a blotch, wider than the mine of the preceding instar (Figs 11D-E, 12E). The third instar (tissue-feeding) remains in the same leaf as the first two instars, the leaf lamina appearing coiled in transverse view (Figs 11F, 12F). The fourth instar (tissue-feeding) is not endophytic, exiting the mine and searching for another leaf which it folds like a cone with the aid of silk deposited on one of the lateral margins of the leaf (Fig. 11G) prior to feeding. Within this cone, the fourth instar feeds on abaxial epidermis, palisade and spongy parenchyma (Figs 11H, 12G); it is protected in this case by the external surface of the cone, mostly formed by the adaxial epidermis. The fifth instar (tissue-feeding) feeds inside the cone constructed by the fourth instar. After completion of development, it drills and exits the cone searching for another leaf whose abaxial surface, which is partially covered with silk, where the cocoon is constructed (Fig. 11I). It results in a longitudinal fold of the leaf in which the pupa remains protected. Adult emergence occurs through an orifice made with the aid of the cocoon cutter of the pupa on one of the tips of the pupal cell.

Eggs and active larvae of different instars have been found in all months, suggesting that this species is able to develop throughout the year in the study site.

## DISCUSSION

*Caloptilia* sp1. is the first species of *Caloptilia* described from Chile. This finding considerably expands the southern limit of the genus on the Pacific coast of South America, which was previously recorded for Lima, Peru (Meyrick, 1915) about 1,000 km north of the Lluta Valley.

### Host plant and geographic range

*Morella pavonis* is the only host plant known for *Caloptilia* sp1. until now. The range of this tree is restricted to central and southern Peru and northern Chile (Parra-O, 2002). In the case of Chile, *M. pavonis* has been recorded from a few additional transverse valleys of the Atacama Desert south the Lluta Valley (Rodríguez *et al.*, 1983; Luebert, 2004). In total, five valleys were surveyed to characterize the geographic distribution of *Caloptilia* sp1. The local populations of *M. pavonis* are currently considered either as near threatened or vulnerable in the different localities of its Chilean range (Gatica-Castro *et al.*, 2015). The nearby Peruvian populations of *M. pavonis* were not included in the present study, and thus should be surveyed for *Caloptilia* in future studies. Species of *Caloptilia* are able to feed on a wide range of host plants, with at least six species recorded as Myricaceae-feeding from Afrotropical, Nearctic, Palearctic and Oriental regions (De Prins & De Prins, 2017). The host plants previously known for other Neotropical *Caloptilia* include species of Anacardiaceae, Ericaceae, Fabaceae, Fagaceae, Lauraceae, Myricaceae and Rhamnaceae (Busck, 1920; Bourquin 1962; Landry, 2006; Davis *et al.*, 2011; Arévalo-Maldonado, 2014; Nakadai & Kawakita, 2016). Curiously, the only previous Neotropical record on Myricaceae is that of the Peruvian *Caloptilia* sp. on *Morella pubescens* (Nakadai & Kawakita 2016), the same species with nearest match to *Caloptilia* sp1. in GenBank. In fact, the DNA barcode divergence (4.9-5.1% K2P) suggests that *Caloptilia* sp. is effectively a different species from *Caloptilia* sp1. Accordingly, further sampling of *Caloptilia* feeding on *M. pubescens* is need to verify if the two species share the same host plant, and to assess whether they are closely related to each other morphologically and evolutionarily.

### Pre-genital segments and genitalia

The morphology of the VII and VIII abdominal segments of the male and female



genitalia is known only for a few of the described species of Neotropical *Caloptilia*: *C. camaronae* (Zeller, 1877); *C. cruzorum* Landry, 2006; *C. dondavisii* Landry, 2006; *C. galacotra* Landry, 2006 and *C. schinusifolia* Davis & Wheeler, 2011 (Landry, 2006; Davis *et al.*, 2011; Arévalo-Maldonado, 2014). Comparisons with corresponding descriptions and illustrations suggest that the presence of just one pair of coremata on the male abdomen could be a distinctive characteristic of *Caloptilia* sp1. That is, on the other five species there is only one pair of coremata on VII and another on the VIII abdominal segment, which appears to be a common pattern for many species of *Caloptilia* worldwide (e.g. Kumata, 1981, 1982; Davis *et al.*, 2013). However, an arrangement of coremata similar to that here reported for *Caloptilia* sp1. was described by Opler (1971) for the Nearctic Fagaceae-feeding *C. agrifoliella*. Interestingly, this species was found to be the nearest neighbor of *Caloptilia* sp1. (4.13-4.28% K2P) in the BOLD database. For the male genitalia, the sacculus with a distinctly sclerotized finger-like median expansion dorsally projected separates *Caloptilia* sp1. from the other five Neotropical *Caloptilia* species of which the genitalia is known. For the female genitalia, considering the same other five Neotropical species, a well-differentiated sternum VIII has been described for the three species of the Galapagos Islands (*C. dondavisii*, *C. cruzorum* and *C. galacotra*), all of which are different compared to *Caloptilia* sp1.

### **Immature stages and natural history**

The hypermetamorphic development of Gracillariidae can follow varied pathways, each involving different larval morphs and feeding behaviors (Kumata 1978; Davis *et al.* 1987; Wagner *et al.*, 2000; Brito *et al.*, 2013). The hypermetamorphosis of *Caloptilia* sp1. mostly fits the general morphological pattern described for the Gracillariinae, with early sap-feeding and later tissue-feeding larva (De Prins *et al.*, 2015). The two larval forms are involved in the mine construction in *Caloptilia* sp1., as the leaf mine is initially constructed by the two sap-feeding instars and continued by the third (first tissue-feeding) instar, while instars IV and V are external feeders concealed within a leaf cone. A similar pattern was described for *C. octopunctata* (Turner, 1894) from India (Kumata, 1981). Accordingly, the instar-related feeding behavior found here deserves further exploration at a broader scale in *Caloptilia*.

Most Gracillariidae larvae are plant miners on leaves, although other plant organs can be also mined; others bore into flowers, fruits or seeds, and others are either leaf rollers or gall inducers (Davis, 1987; Vargas & Landry, 2005; Hu *et al.*, 2011;

Kawakita & Kato, 2016). In the case of *Caloptilia*, leaf cone construction is the most common pattern for the later instars (Kumata, 1982), although a few species remain as leaf miners throughout the larval development, emerging from the mine only for pupation, while a Nearctic species is a gall inducer (De Prins *et al.*, 2015). Accordingly, the feeding behavior of the two later instars of *Caloptilia* sp1. fit the more widespread pattern described for the genus.

Kawahara *et al.* (2017) characterized the chaetotaxy of Gracillariinae by the presence of six labral setae, three lateral setae on the mesothorax and methatorax and two lateral setae on each abdominal segment. The chaetotaxy of *Caloptilia* sp1. mostly fit this pattern, except for the presence of only one lateral seta on A9 and three lateral setae on A10.

Based on the analysis of central European representatives, Patočka & Zach (1995) suggested that pupal morphology provides adequate characters for identification of *Caloptilia*. Pupal morphology in *Caloptilia* sp1. closely matches the general pattern described by Patočka & Zach (1995), fitting into what they called group three, whose species lack large frontal bristles.

### **Molecular phylogeny**

The usefulness of DNA barcodes to explore the biodiversity of microlepidoptera has been widely recognized, with several examples in Gracillariidae (e. g. Brito *et al.*, 2012, 2013, 2017; Lees *et al.*, 2014; Kirichenko *et al.*, 2015; Pereira *et al.*, 2017).

Interestingly, the two nearest neighbors of *Caloptilia* sp1. found in the GenBank and BOLD databases share some attributes with this species, as the host plant family (*Caloptilia* sp. from Peru) and the arrangement of coremata (*C. agrifoliella*), suggesting that further comparative studies involving the three species are required. Strong branch support of the ML tree was obtained for *Caloptilia* sp1. from the nearest species (*Caloptilia* sp. from Peru).

### **Population analysis**

All populations of *Caloptilia* sp1. included in this study present low haplotype diversity levels (Hd) and nucleotide diversity ( $\pi$ ) compared to other studies in Lepidoptera (Snall *et al.*, 2004; Wang *et al.*, 2016; Peterson *et al.*, 2016; Mori *et al.*, 2016). However, similar values were observed in areas of natural dispersion for another gracillariid,

*Cameraria orhidella* (Valade *et al.*, 2009). The maximum intraspecific genetic distance (K2P) observed reaches the 2% COI distance suggested for Lepidoptera by Mutanen *et al.* (2012) below the species level. Studies on genetic variation at the population level are very useful, for example to describe phylogeographic patterns and investigate the origin of an invasive species, but only a few have been carried out in micro-moths. Mori *et al.* (2016) investigated intraspecific variability and patterns of genetic structure in the red clover casebearer *Coleophora deauratella* (Coleophoridae) by analyzing the barcode region, i.e. a fragment of the mitochondrial cytochrome oxidase I subunit (COI) gene (sensu Hebert *et al.* 2003), and microsatellites. Escobar-Suárez *et al.* (2017) studied the genetic diversity using COI sequences in the tortricid leaf miner *Eugnosta azapensis* (Tortricidae) in the Atacama Desert of northern Chile and found four haplotypes potentially geographically structured in two valleys. Maita-Maita *et al.* (2015) addressed the connectivity among populations of the leaf-miner *Angelabella tecomae* (Gracillariidae) in the same region in the Atacama Desert using COI haplotypes and suggested some gene flow among valleys in the desert. Similarly, our results indicate a weak population structure among populations of *Caloptilia* sp1., except for the Camiña population that presents a moderate level of differentiation ( $F_{ST}$ ) compared to all other populations. The Mantel test suggested that this level of differentiation is not influenced by geographic distance. This suggests that females of *Caloptilia* sp1. from northern populations (Lluta, Livilcar and Codpa) might present gene flow and therefore could be able to disperse between transversal valleys through extensive desert areas. On the other hand, females from Camiña may have a lower migration rate than the other populations, which would decrease gene flow and favor genetic differentiation. Despite being the most distant population, Mamiña showed a non-significant  $F_{ST}$  with the other populations of the north. Except for this population, a low differentiation is suggested between the northern and southern populations. Therefore, it is necessary to clarify the absence of genetic differentiation among populations from the north and the southernmost (Mamiña), since they have the greatest geographical distance (over 147 km) among the sites analyzed. This may indicate recent geographic isolation, or it could reflect an ancestral polymorphism present in mtDNA lineages, regardless of extant connectivity. Futures studies on the genetic variation of populations of *Caloptilia* sp1. ideally should include analyses of additional molecular markers (e. g. Valade *et al.*, 2009; Shapiro *et al.*, 2008) with higher mutation rate and biparental inheritance in order to understand the ecology and evolution of the

geographically isolated populations of this micro-moth. It would also provide important information for conservation programs for these highly human-modified and extremely arid environments of the Atacama Desert.

Finally, mismatch distribution analysis indicated a unimodal pattern for all populations with differences in the smoothness strongly affected by genetic structure (Harpending, 1994) of peaks, which suggests that populations might be expanding throughout the area. Mismatch distributions are unimodal in populations having increased in the past as a consequence of a recent demographic expansion (Rogers and Harpending, 1992; Slatkin and Hudson, 1991) or through a range expansion with high levels of migration between neighboring demes (Ray *et al.*, 2003; Excoffier, 2004). However, it is difficult to untangle demographic expansions, which generally result from spatial expansions that follow a colonization event by relatively few founder individuals. Thus, further analysis of the expansion models to evaluate these events is very important to clarify the population dynamics of *Caloptilia* in the conspicuous environment of the northern Atacama Desert.

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## Figure Legends

**Figure 1.** Maximum-likelihood tree of *Caloptilia* species based on 654 bp of the mitochondrial gene cytochrome oxidase subunit I (COI). Numbers on branches indicate bootstrap support. Green bars indicate clades with Myricaceae hostplants. Black bars indicate clades with other hostplant families. Gray bar indicates clade without host plant information.

**Figure 2.** Sampling of *Caloptilia* sp1. **A.** Occurrence area in the Atacama desert, northern Chile. **B.** Corresponding enlarged geographic area for the five populations sampled (Red: Lluta; Blue: Livilcar; Yellow: Codpa; Purple: Camiña; Green: Mamiña). **C.** Photograph of a typical valley in such cases (Lluta). **D.** Median-joining network based on COI sequence data describing the relationship between haplotypes (colors indicate populations in the map). Nucleotide substitutions are shown on the branches as small transverse bars. Circle size is proportional to haplotype frequency. **E.** Isolation by distance plots of pairwise values for geographic distance and genetic distance across collected sites. Statistical significance was assessed using the Mantel test ( $p > 0.05$ ).

**Figure 3.** Mismatch distribution for each population of *Caloptilia* sp1. (see material and methods for details). Dashed lines (blue) represent the observed distributions and solid lines (red) the expected ones (under the population growth model).

**Figure 4.** Adult male of *Caloptilia* sp1., dorsal view. Scale bar: 1 mm.

**Figure 5.** Genitalia and postabdomen morphology of *Caloptilia* sp1. under light microscopy. **A.** Male genitalia, ventral view (aedeagus omitted). **B.** Aedeagus, lateral. **C.** apex of valve in detail, ventral. **D.** base of valve in detail, ventral. **E.** cornutus, lateral. **F.** Female genitalia, ventral. **G.** Signum, lateral. **H.** Male postabdominal segments VII and VIII with coremata, lateral. **I.** Tergum VII, dorsal. **J.** Sternum VII, ventral. **K.** Tergum VIII, dorsal. **L.** Sternum VIII, ventral. **M.** Left coremata, lateral. Scale bars: 200, 100, 50, 25, 25, 200, 25, 200, 40, 30, 40, 40, 100  $\mu\text{m}$ , respectively.

**Figure 6.** Scanning electron micrographs of *Caloptilia* sp1. first instar larva. **A-C.** Head, under lateral, dorsal and ventral views, respectively. **D.** Labium-hypopharynx complex, ventral (detail of squared area marked in C). **E.** Labial palp, ventral (detail of square area marked in D), open arrow indicates the spinneret. **F.** Antenna, antero-dorsal. **G.** Detail of mesothorax surface, dorsal. **H.** Spiracle A1, lateral. **I.** Mesothoracic sternal callus, latero-ventral. Scale bars: 50, 50, 50, 10, 5, 5, 5, 4, 10  $\mu\text{m}$ , respectively.

**Figure 7.** Scanning electron micrographs of *Caloptilia* sp1. fifth instar larva. **A, B.** Head, under lateral and dorsal views, respectively. **C.** Stemmata, lateral. **D.** Head, frontal. **E.** Labrum, frontal. **F.** Spinneret, frontal. **G.** Labial palp, antero-lateral. **H.** Antennae, antero-lateral. **I.** Prothoracic shield, dorsal. **J.** Prothoracic leg, lateral. **K.** Prothoracic spiracle, lateral. **L.** Pseudopodium on A3, lateral. **M.** Last abdominal (A10) segment, lateral. **N.** Crochets of pseudopodium A10 in detail, ventral. Scale bars: 200, 200, 50, 100, 500, 20, 40, 25, 200, 50, 10, 50, 100, 100  $\mu\text{m}$ , respectively.

**Figure 8.** Chaetotaxy of *Caloptilia* sp1. under light microscopy. **A.** First instar larva, lateral view. **B.** Head of fifth instar larval, frontal (left) and lateral (right). **C.** Fifth instar larva, lateral. Scale bars: 100, 200, 250  $\mu\text{m}$ , respectively.

**Figure 9.** Pupal morphology of *Caloptilia* sp1. under light microscopy, in ventral (**A**), lateral (**B**) and dorsal (**C**) views. Scale bar: 1 mm.

**Figure 10.** Scanning electron micrographs of *Caloptilia* sp1. pupa. **A.** Head, ventral view. **B.** Head, lateral. **C.** Cocoon cutter, lateral. **D.** Cocoon cutter, frontal. **E.** Labrum, frontal. **F.** Maxillary palp in detail, lateral. **G.** Middle prothoracic depressions in detail, dorsal. **H.** Third abdominal (A3) segment, lateral. **I.** Surface of A3 in detail, latero-

dorsal. **J.** Spiracle on A4, lateral. **K.** Last abdominal (A10) segment, postero-lateral. **L.** Spine of A10 segment in detail (square area marked in K). Scale bars: 200, 200, 20, 50, 100, 80, 50, 200, 20, 20, 100, 10  $\mu\text{m}$ , respectively.

**Figure 11.** Natural history of *Caloptilia* sp1. **A.** “Guacano” trees, *Morella pavonis* (Myricaceae) in a transverse valley of the Atacama Desert. **B.** Egg on abaxial surface of hostplant leaf, showing cephalic capsule of first instar larva by transparency. **C.** Mine produced by first instar larva. Open arrow indicates empty chorion near of beginning of the mine. Discontinuous line indicates histological section of fig. 9D. **D.** Second instar larva. **E.** Second instar mine. Discontinuous line indicates histological section of fig. 9E. **F.** Folded leaf produced by third instar larva “tissue feeder”. Discontinuous line indicates histological section of fig. 9F. **G.** Leaf cone produced by fourth instar larva. Fourth and fifth instar larvae live within the leaf cone. **H.** Internal damage of leaf cone in detail. Discontinuous line indicates histological section of fig. 9G. **I.** Cocoon, generally built on the abaxial surface within a transversally folded leaf. Scale bars: 0.2, 4, 0.2, 6, 6, 8, 4, 8 mm, respectively.

**Figure 12.** Histological sections of a *Morella pavonis* leaf, showing the organization levels of a *Caloptilia* sp1. mine in relation to larval ontogeny. **A.** General view of mine, containing first and second instar cephalic capsules, and third instar larva. **B.** Detail of second instar cephalic capsule. **C.** Transverse section of intact portion of leaf. **D.** Transverse section of mined portion produced by first instar larva (see figure 8C). **E.** Transverse section of mined portion produced by second instar larva (see figure 8E). **F.** Transverse section of folded portion produced by third instar larva (see figure 8F); open arrow indicates excreta of third instar larva; asterisk indicates damage produced by third instar larva. **G.** Transverse section of rolled portion (leaf cone) produced by fourth and fifth instar larva (see figures 8G-H). Endophytic feeding of the three first larval instars may be observed, while the fourth and fifth instars larvae have exophytic feeding (indicated by asterisks in G), although the last two instars larvae maintain protection within the leaf cone (see figure 8G). **Ad:** Adaxial surface; **Ab:** Abaxial surface; **Ep:** Epidermis; **Pp:** Palisade parenchyma; **Sp:** Spongy parenchyma; **Lm:** Leaf mine. Closed arrow indicates central vascular bundle leaf. Scale bars: 3, 0.1, 2, 2, 2, 2.5, 1.5 mm, respectively.

## Tables

**Table 1.** Molecular diversity indices and neutrality tests for *Caloptilia* sp1. populations from Atacama Desert. N, number of samples; S, variables sites; H, number of haplotypes; Hd, haplotypes diversity;  $\pi$ , nucleotide diversity.

Locality	N	S	H	Hd	$\pi$	Neutrality tests			
						Tajima D	Fu and Li's D	F	Fu' FS
Lluta	20	5	3	0.56±0.06	0.002±0.001	0.76 <sup>NS</sup>	-0.413 <sup>NS</sup>	-0.095 <sup>NS</sup>	2.787 <sup>NS</sup>
Livilcar	21	4	4	0.64±0.06	0.002±0.001	1.37 <sup>NS</sup>	0.157 <sup>NS</sup>	0.572 <sup>NS</sup>	1.301 <sup>NS</sup>
Codpa	23	4	4	0.66±0.06	0.002±0.001	1.39 <sup>NS</sup>	0.127 <sup>NS</sup>	0.560 <sup>NS</sup>	1.394 <sup>NS</sup>
Camiña	21	5	3	0.65±0.06	0.002±0.001	-0,02 <sup>NS</sup>	0.371 <sup>NS</sup>	0.302 <sup>NS</sup>	0.829 <sup>NS</sup>
Mamiña	35	4	3	0.59±0.04	0.003±0.001	2.72**	1.041 <sup>NS</sup>	1.791*	4.306 <sup>NS</sup>

<sup>NS</sup> = Not significant; \*\* = p<0.01; \* = p<0.05.

**Table 2.** K2P distances values among haplotypes.

	H1	H2	H3	H4	H5	H6	H7	H8
<b>H2</b>	0.002							
<b>H3</b>	0.006	0.005						
<b>H4</b>	0.009	0.008	0.003					
<b>H5</b>	0.006	0.005	0.003	0.005				
<b>H6</b>	0.005	0.003	0.008	<b>0.011</b>	0.008			
<b>H7</b>	0.005	0.003	0.008	0.009	0.006	0.006		
<b>H8</b>	0.005	0.003	0.008	0.009	0.006	0.006	0.003	
<b>H9</b>	0.008	0.006	0.002	0.005	0.005	0.009	0.009	0.009

**Table 3.** Pairwise  $F_{ST}$  values among localities.

	Lluta	Livilcar	Codpa	Camiña
<b>Livilcar</b>	-0.042			
<b>Codpa</b>	-0.027	-0.036		
<b>Camiña</b>	0.280*	0.281*	0.236*	
<b>Mamiña</b>	0.077	0.080	0.076	0.257*

\* = p<0.05.

**Table 4.** AMOVA analysis of mtDNA COI sequences in two population groups of *Caloptilia* sp1.

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	6.323	0.04779 Va	4.71
Among populations within groups	3	9.527	0.10061 Vb	9.91
Within populations	115	99.692	0.86688 Vc	85.38
Total	119	115.542	1.01528	

**Table 5.** Variation in size among head capsules of *Caloptilia* sp1. larvae reared on *Morella pavonis*.

Head capsule width (mm)				
Instar	N	Mean $\pm$ standard error	Range	Growth rate
I	10	0.1806 $\pm$ 0.0014	0.17 – 0.18	–
II	10	0.2741 $\pm$ 0.0025	0.26 – 0.28	1.52
III	10	0.3381 $\pm$ 0,0021	0.32 – 0.34	–
IV	10	0.4799 $\pm$ 0.0057	0.45 – 0.50	1.42
V	8	0.7114 $\pm$ 0.0131	0.64 – 0.74	1.48



## Supporting information

**Table S1.** Information of species included in phylogenetic analysis.

Genus	Species	Accession number		Biogeographic Region	Host plant
		GenBank	BOLD Systems		
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp1			Neotropic (Chile)	<i>Morella pavonis</i> (Myricaceae)
<i>Caloptilia</i>	sp2.	LC127817.1		Neotropic (Peru)	<i>Morella pubescens</i> (Myricaceae)
<i>Caloptilia</i>	<i>agrifoliella</i>			Nearctic	Fagaceae
<i>Caloptilia</i>	<i>alnivorella</i>	GU095593.1	MEC225-04	Nearctic, Palearctic	Betulaceae, Fagaceae, Sapindaceae
<i>Caloptilia</i>	sp3	KF460851.1	GRANO078-11	Neotropic (French Guiana)	
<i>Caloptilia</i>	sp4	HQ571713.1	LNOUA910-10	Neotropic (French Guiana)	
<i>Caloptilia</i>	sp5	KF460920.1	LNOUC373-10	Neotropic (French Guiana)	
<i>Caloptilia</i>	sp6	KF460847.1	GRANO077-11	Neotropic (French Guiana)	
<i>Caloptilia</i>	<i>populetorum</i>	KX045635.1	GRPAL485-11	Paleartic	Betulaceae
<i>Caloptilia</i>	<i>roscipennella</i>	KF808540.1	LNOUC1115-11	Paleartic	Juglandaceae
<i>Caloptilia</i>	<i>aurantiaca</i>	HQ957019	GRPAL078-10	Paleartic (Portugal/Spain)	<i>Morella faya</i> (Myricaceae)
<i>Caloptilia</i>	<i>coruscans</i>	KX044547	GRPAL788-12	Nearctic, Palearctic	<i>Morella faya</i> (Myricaceae)
<i>Caloptilia</i>	<i>stigmatella</i>		CGUKB687-09	Nearctic/Paleartic	<i>Myrica</i> sp. (Myricaceae)
<i>Caloptilia</i>	<i>flavella</i>	KR454374	CNMIC015-14	Nearctic	<i>Myrica gale</i> , <i>Myrica caroliniensis</i> , <i>Morella cerifera</i> (Myricaceae)
<i>Callisto</i>	<i>denticulella</i>	JN271899	FBLMW173-10	Nearctic, Palearctic	Rosaceae

**Table S2.** Geographic distances (km) among localities.

	<b>Lluta</b>	<b>Livilcar</b>	<b>Codpa</b>	<b>Camiña</b>
<b>Livilcar</b>	31,00			
<b>Codpa</b>	58,60	29,45		
<b>Camiña</b>	118,83	90,06	60,56	
<b>Mamiña</b>	204,06	176,54	147,10	87,32

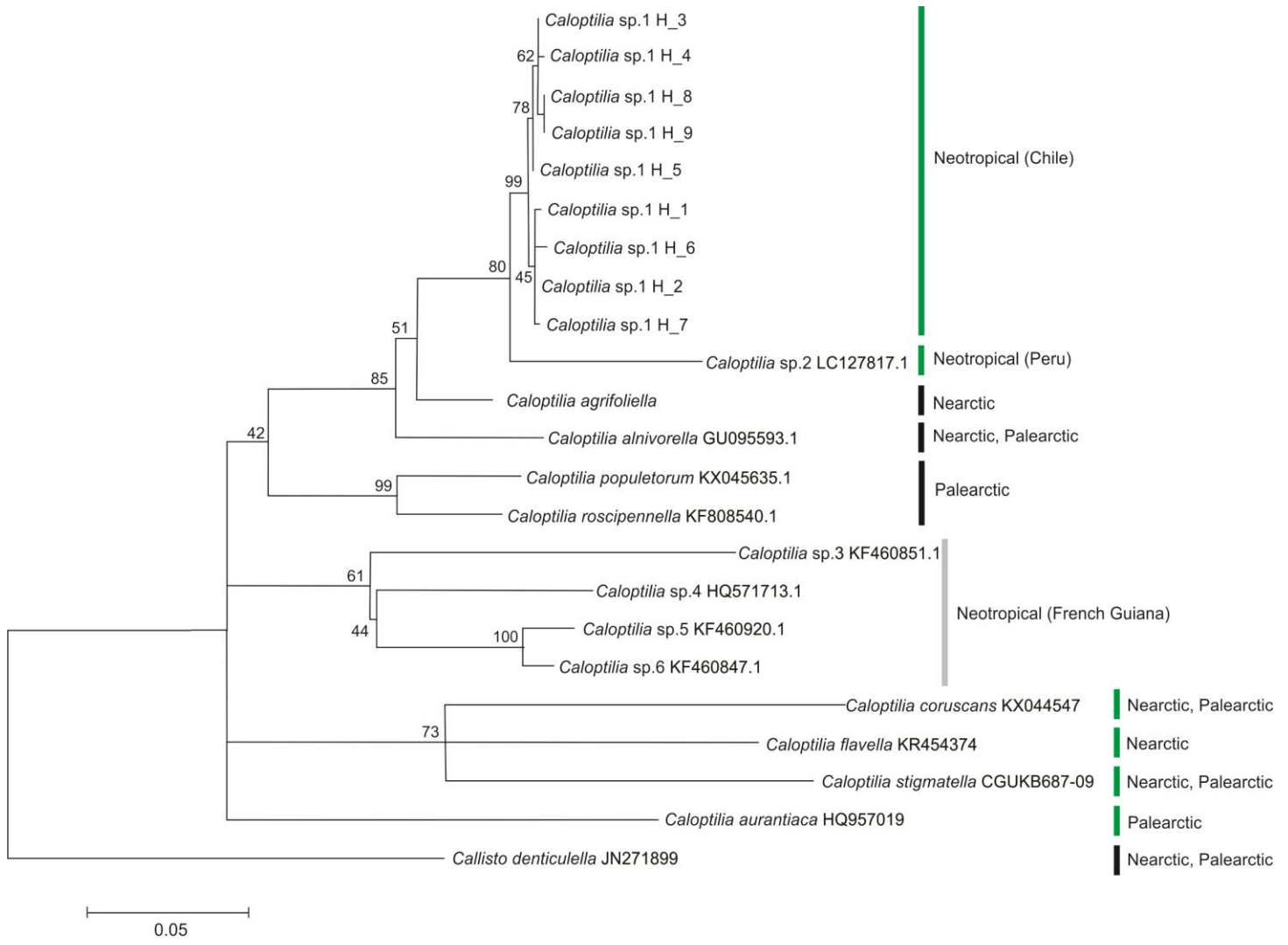


Figure 01 - Vargas-Ortiz *et al.*

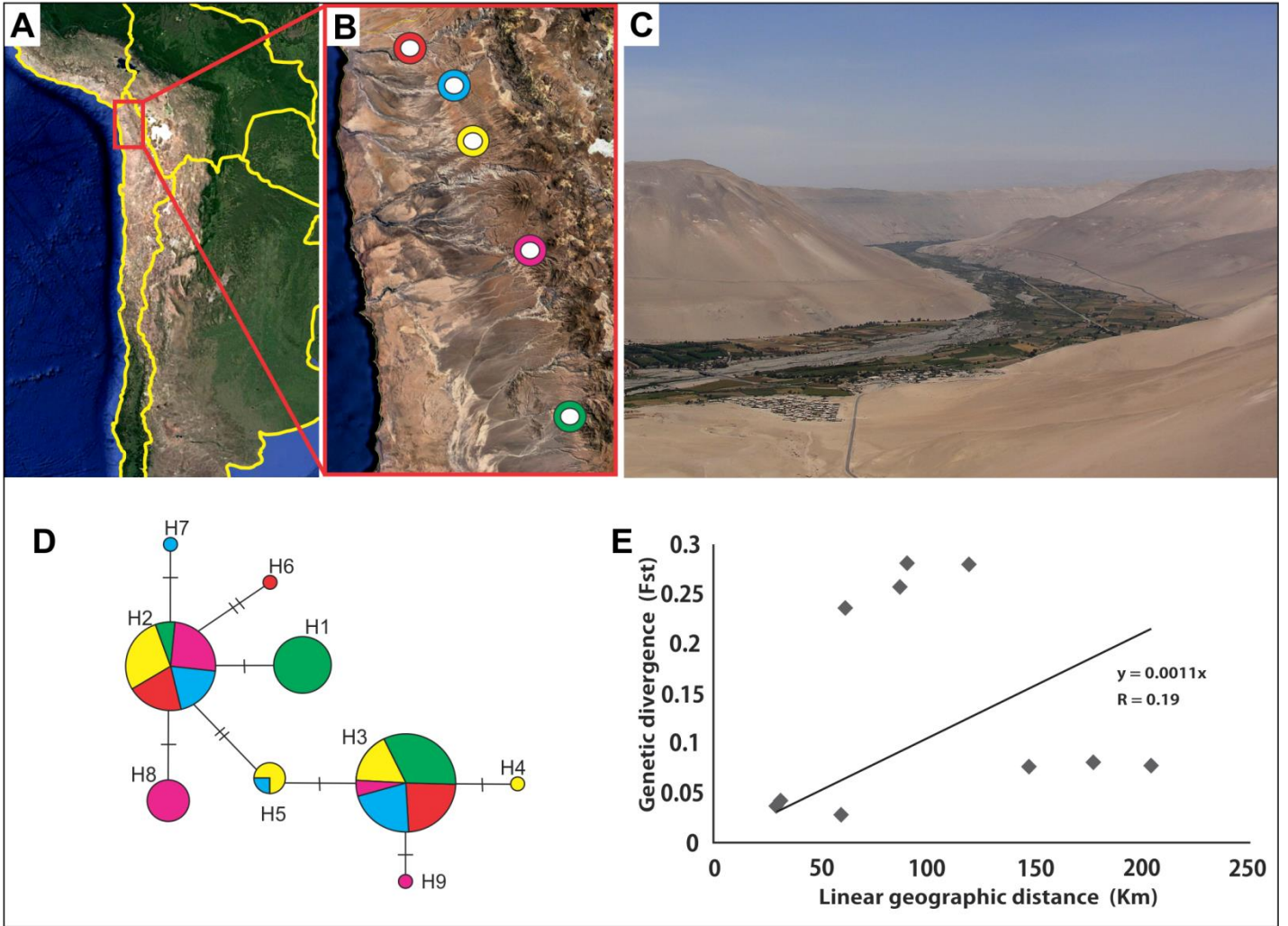


Figure 02 - Vargas-Ortiz *et al.*

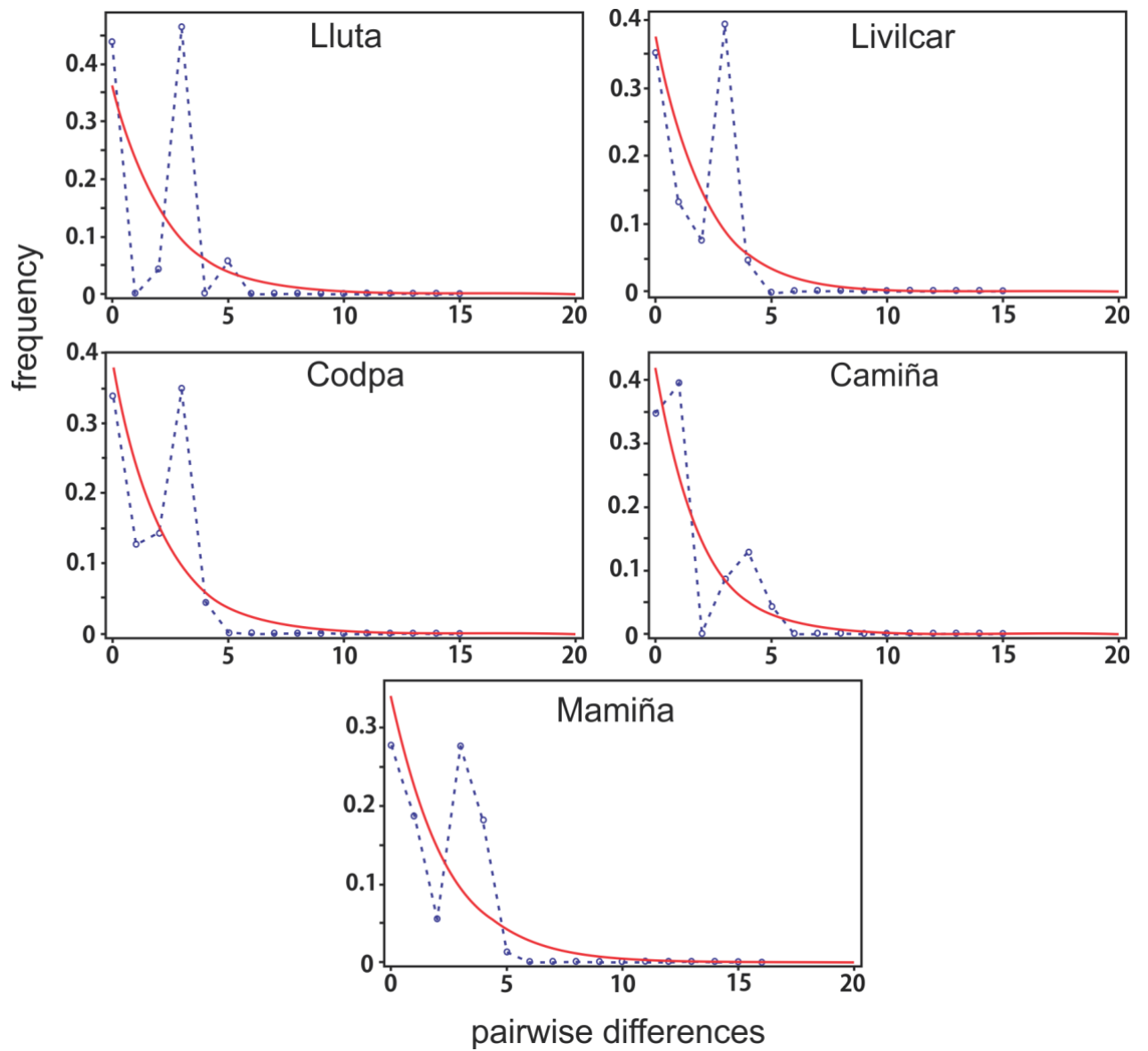


Figure 03 - Vargas-Ortiz *et al.*



Figure 04 - Vargas-Ortiz *et al.*

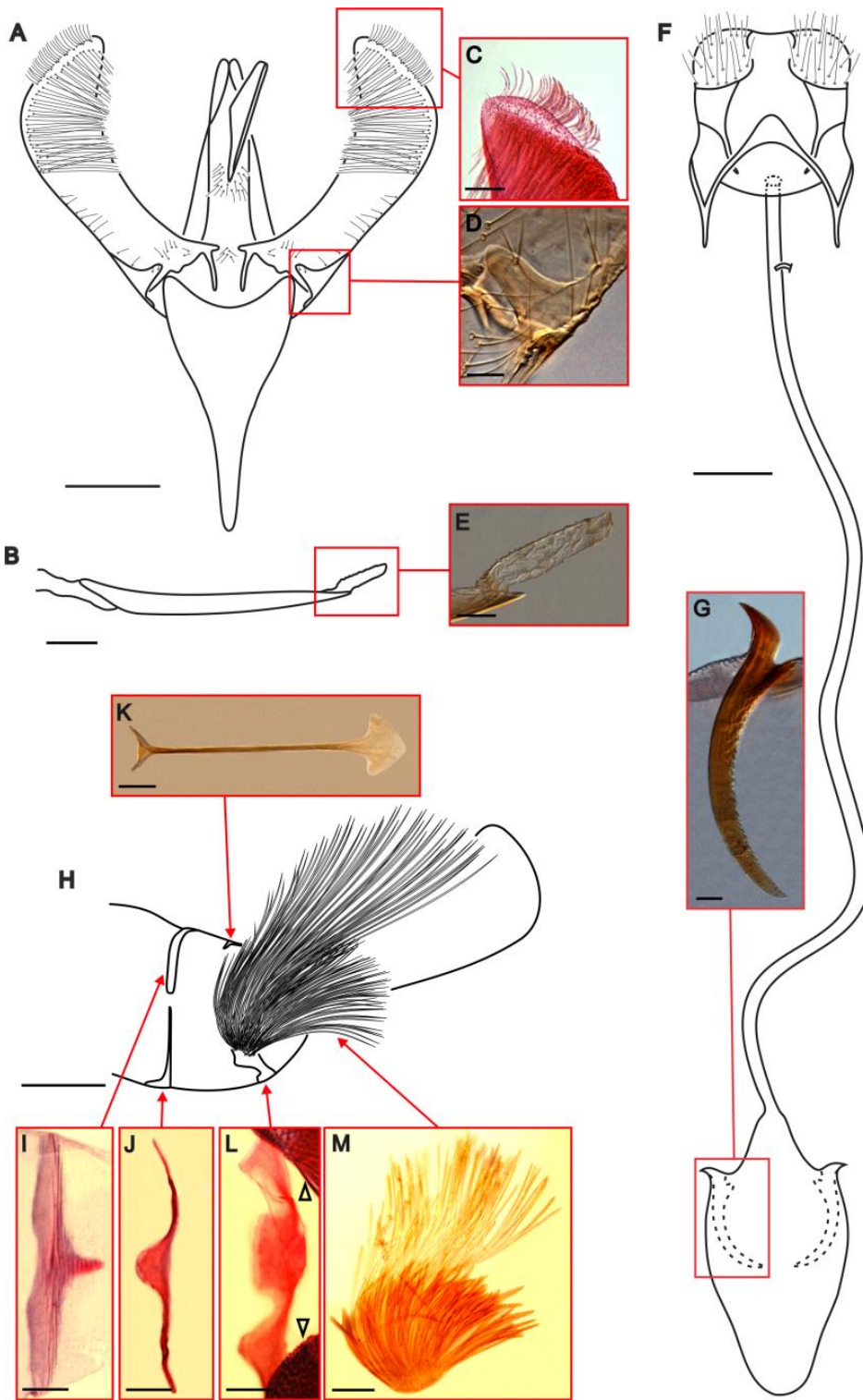


Figure 05 - Vargas-Ortiz *et al.*

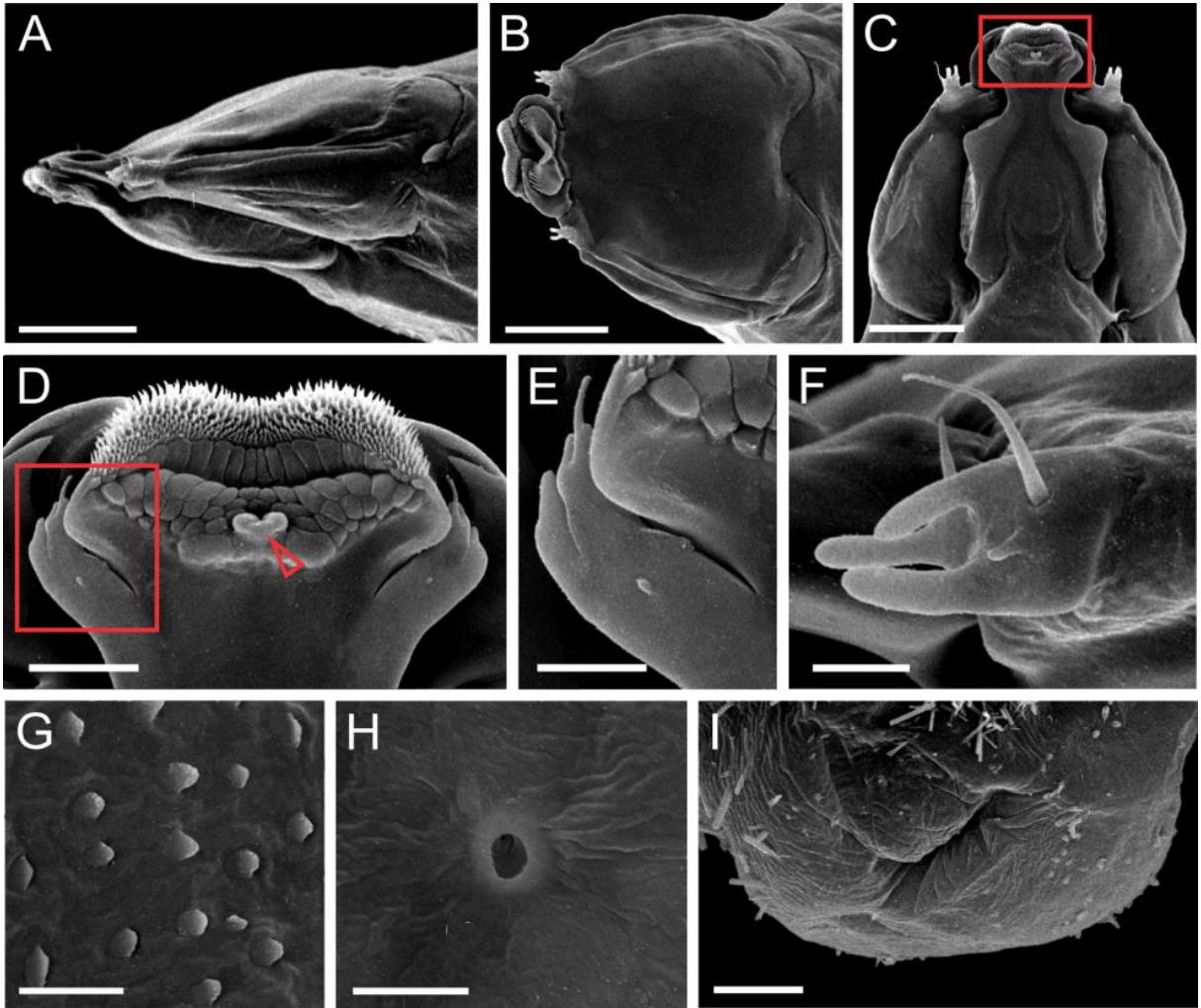


Figure 06 - Vargas-Ortiz *et al.*



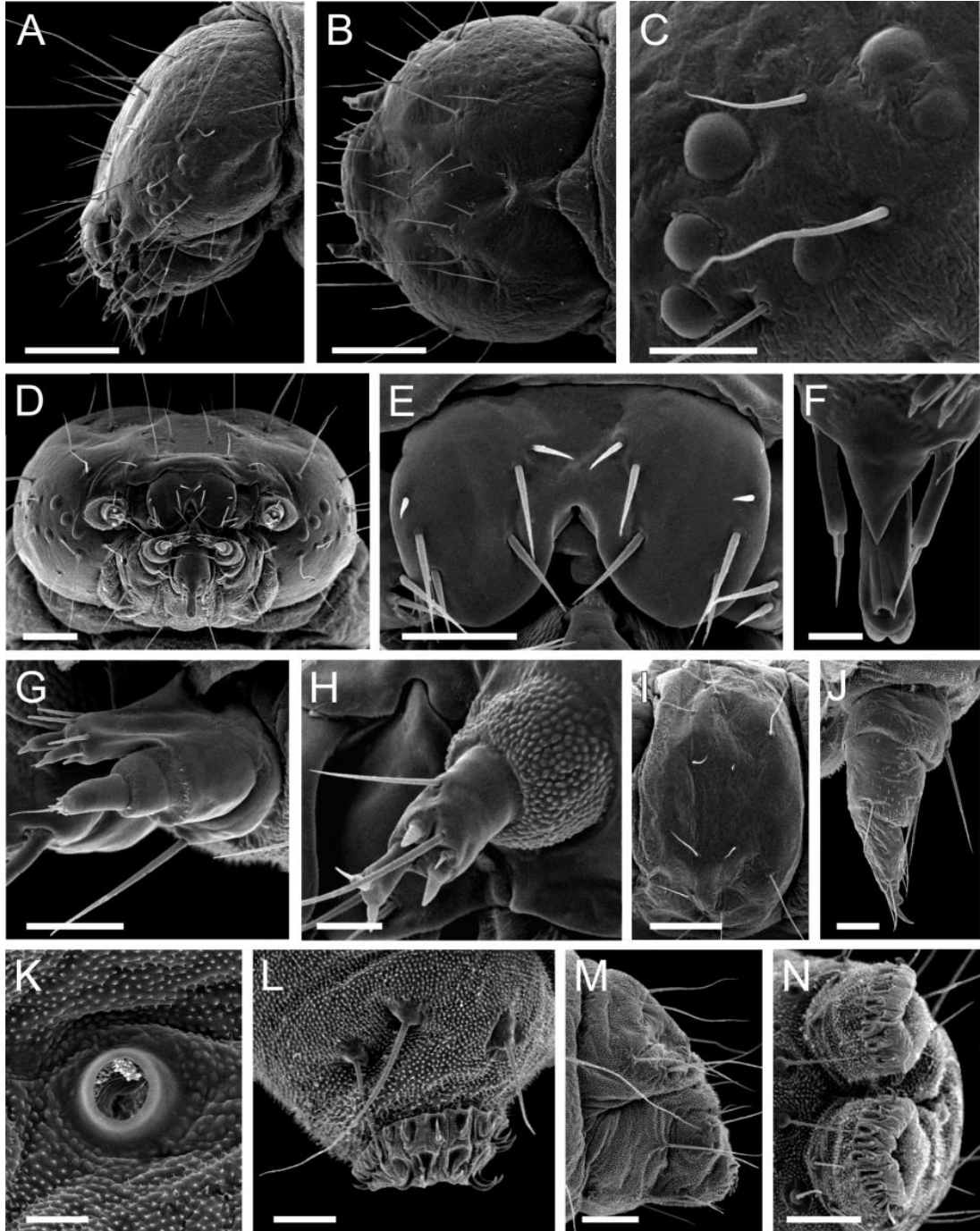


Figure 07 - Vargas-Ortiz *et al.*

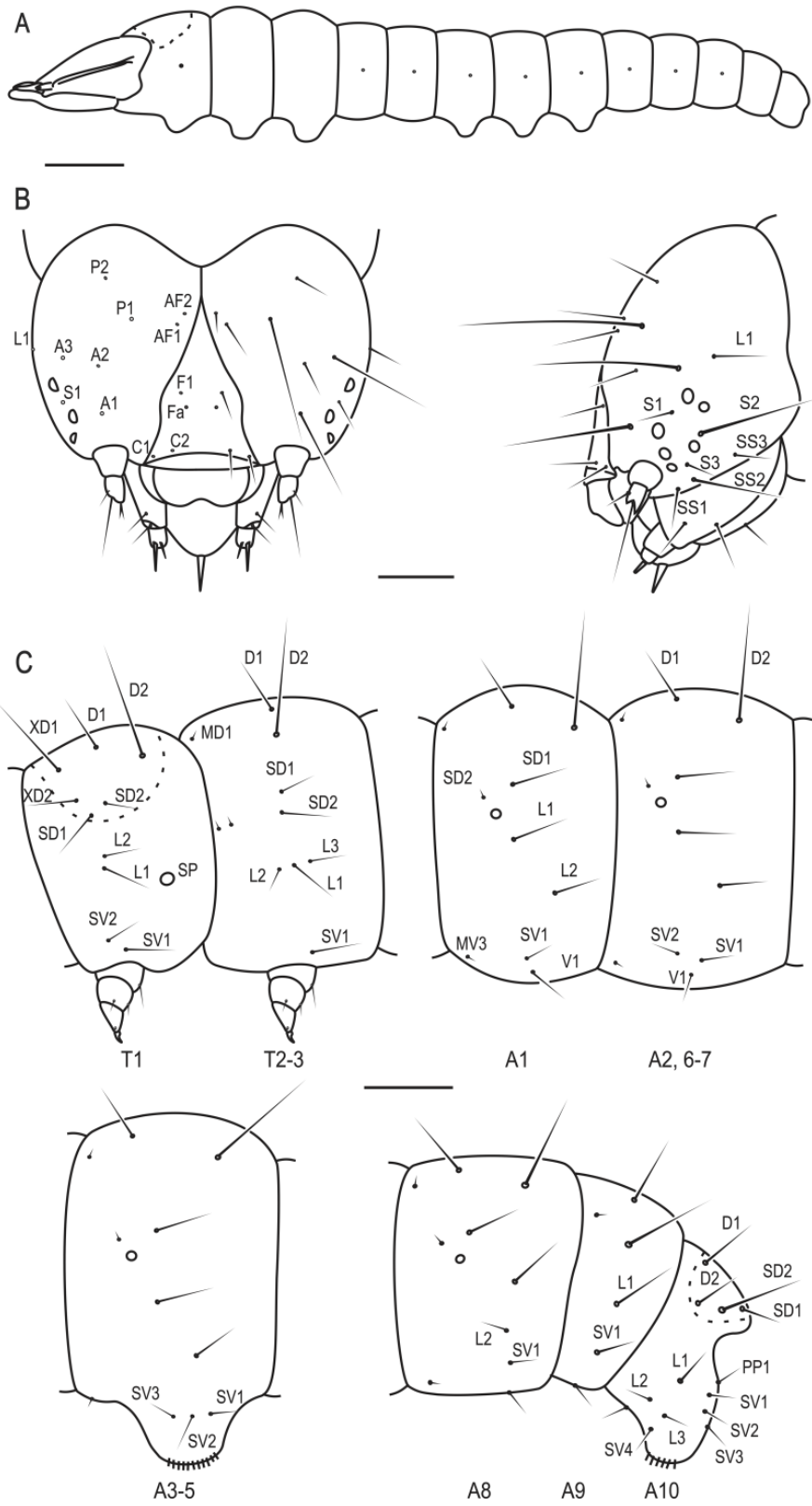


Figure 08 - Vargas-Ortiz *et al.*

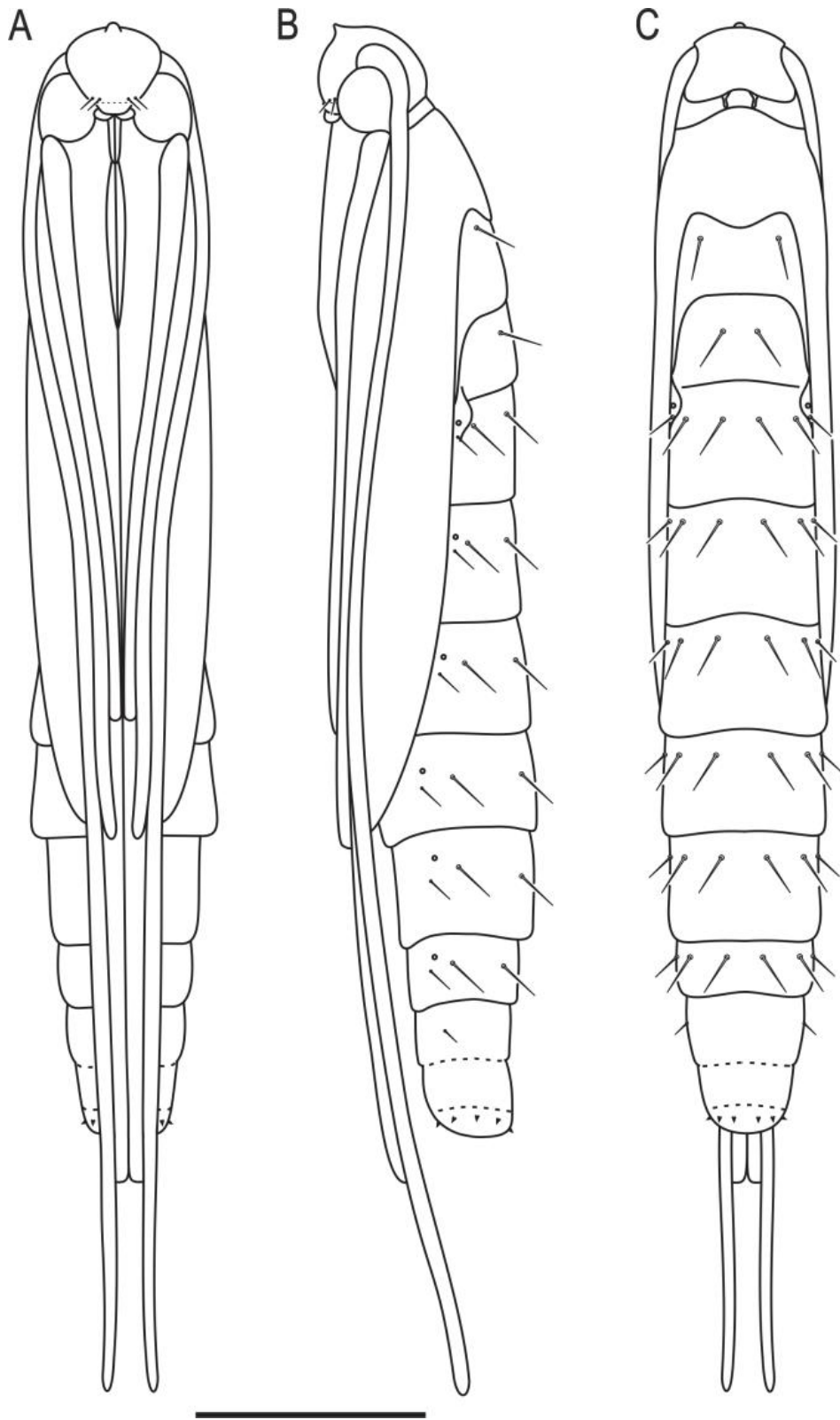


Figure 09 - Vargas-Ortiz *et al.*

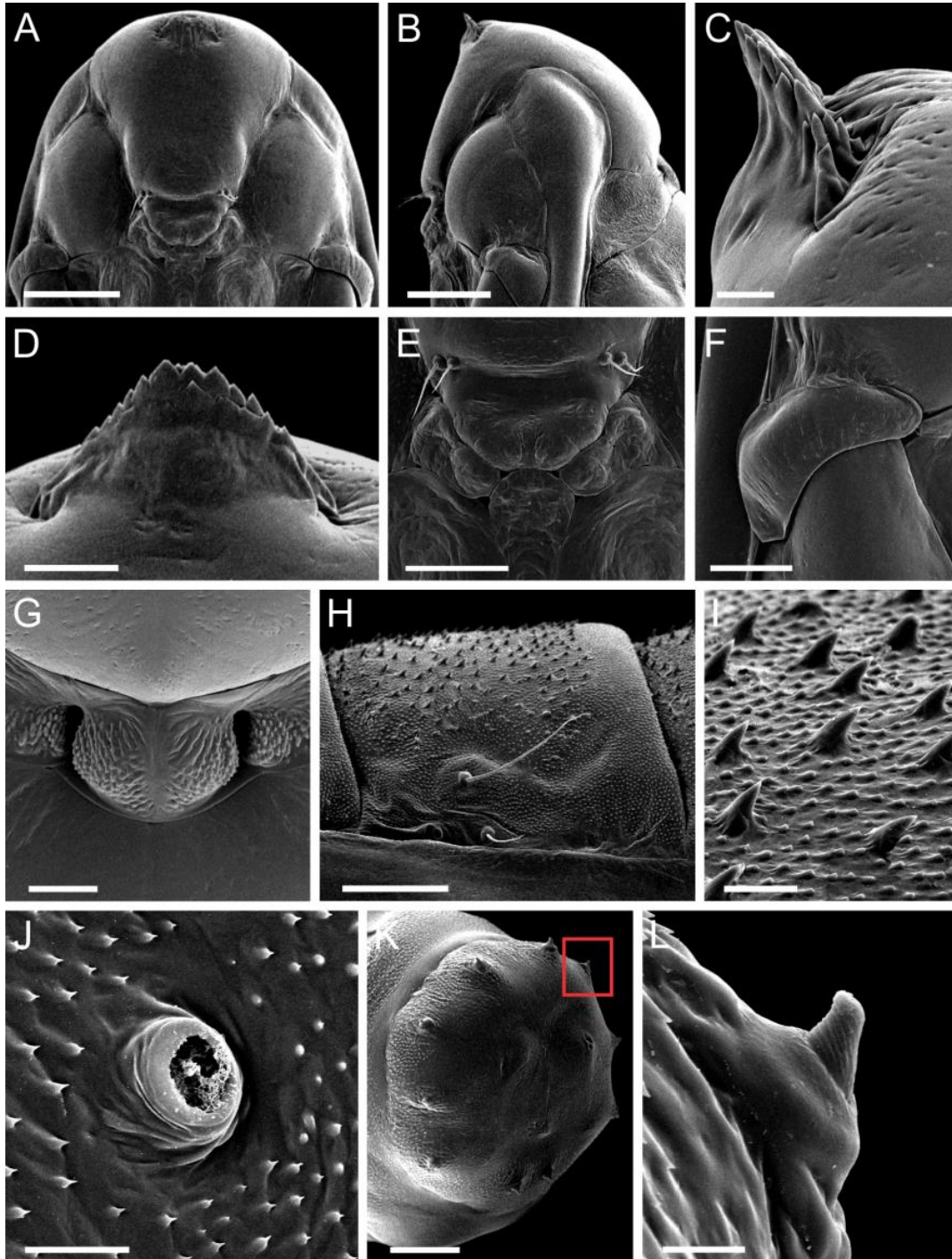


Figure 10 - Vargas-Ortiz *et al.*

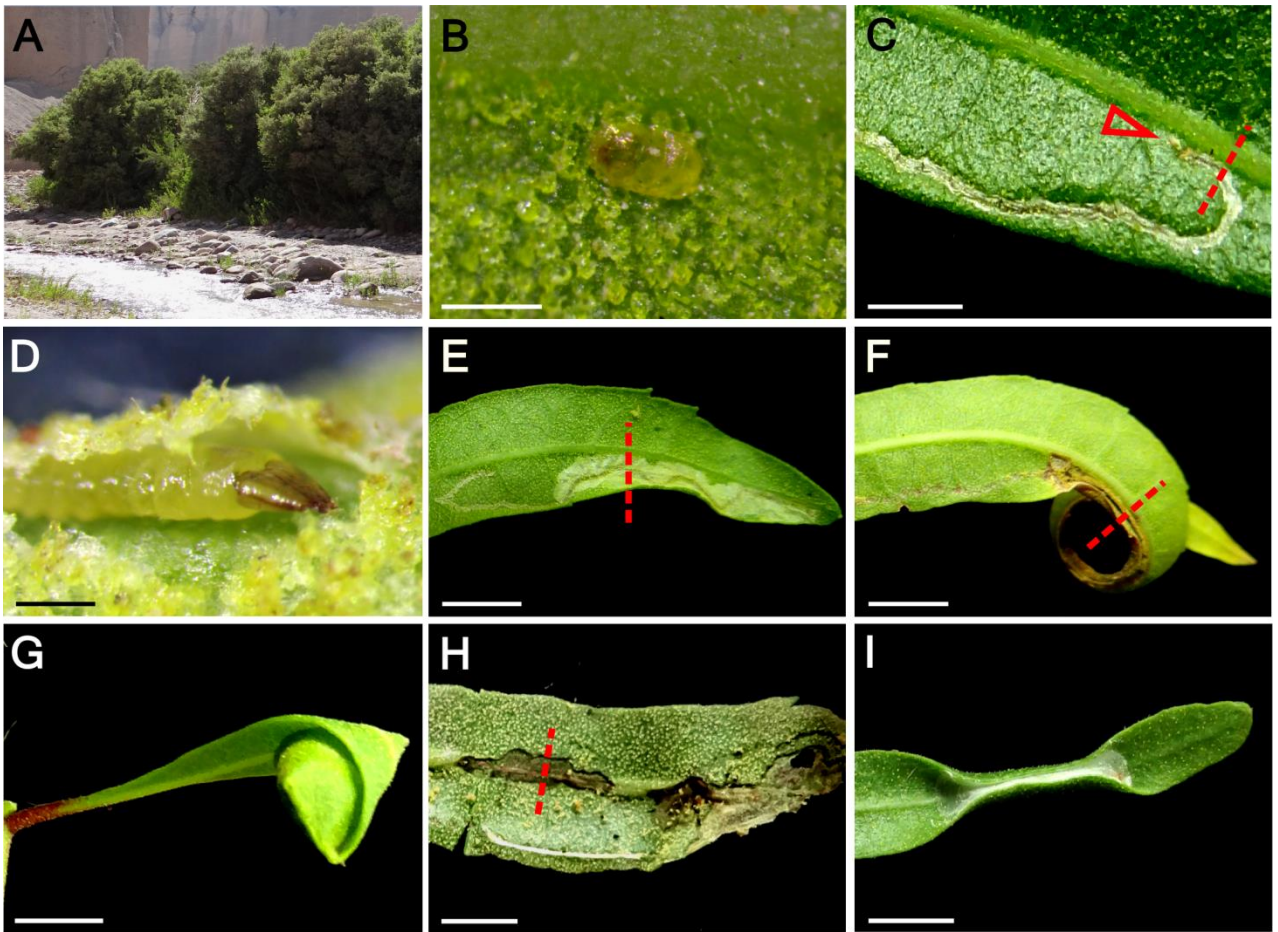


Figure 11 - Vargas-Ortiz *et al.*

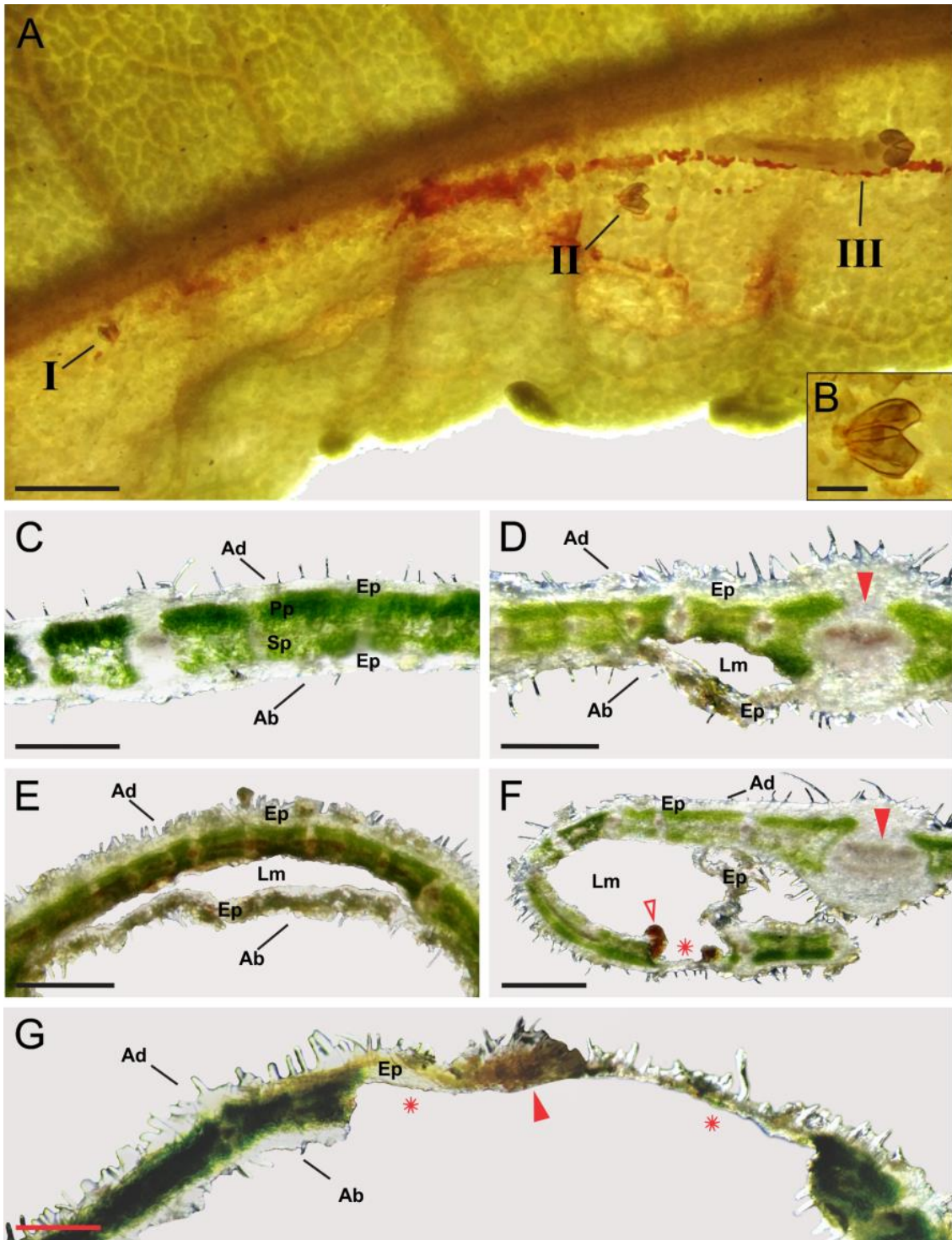


Figure 12 - Vargas-Ortiz *et al.*

## CONSIDERAÇÕES FINAIS

Neste estudo, foi descrita uma nova espécie de Gracillariidae, *Caloptilia* sp1. Vargas-Ortiz & Vargas sp. nov., presente nos vales transversais do Deserto de Atacama, norte do Chile. Essa espécie utiliza como planta hospedeira *Morella pavonis* (Myricaceae). Como caracteres diagnósticos para a identificação, foi detalhada a morfologia das genitálias do macho e fêmea, os estágios imaturos, história de vida e padrões de alimentação desta espécie com a planta hospedeira ao nível dos tecidos foliares. Um detalhe morfológico característico do adulto é a presença de só um par de coremata no abdômen, especificamente no segmento oitavo, em comparação a outras cinco espécies neotropicais de *Caloptilia* que tem dois pares de coremata (um par no segmento sétimo e outro no segmento oitavo).

As relações filogenéticas analisadas neste trabalho permitiram definir a *Caloptilia* sp1. como uma espécie monofilética. A espécie congênica mais próxima foi coletada em Peru, ainda sem informação a nível específico do ponto de visto morfológico. A distância genética entre elas, acima de 4% (COI), sugere fortemente que corresponde a uma espécie diferente. Um dado interessante é que ambas espécies utilizam como planta hospedeira membros do gênero *Morella* (Myricaceae). Outras espécies de *Caloptilia* relacionadas com Myricaceae quanto à planta hospedeira foram incluídas na análise filogenética, mas não formaram um grupo próximo a *Caloptilia* sp1., o que sugere de maneira preliminar uma evolução independente da relação *Caloptilia* – Myricaceae.

A análise da estruturação genética populacional sugere que as fêmeas de *Caloptilia* sp1. tem uma alta taxa de dispersão entre os vales transversais do Deserto de Atacama, devido a uma débil estruturação observada dos haplótipos em relação à

distancia geográfica. Ou seja, provavelmente as extensas áreas de deserto entre os vales não constitui uma barreira geográfica para a dispersão das fêmeas desta nova espécie.

Futuros estudos são necessários para explorar de forma mais abrangente os padrões filogeográficos desta espécie. Para isso, é necessário determinar a total área de distribuição, que provavelmente é a mesma que a planta hospedeira, o que significa que serão necessárias coletas também no centro-sul do Peru. Além disso, tais estudos devem preferentemente incluir análises com marcadores moleculares que tenham uma maior taxa mutacional e de descendência biparental (ex. marcadores nucleares), para podermos compreender de melhor maneira o papel do espaço em relação à evolução desta espécie no Deserto de Atacama.



## ANEXO

### NORMAS PARA PUBLICAÇÃO NA REVISTA AUSTRAL ENTOMOLOGY.

#### 1. AIMS AND SCOPE

*Austral Entomology* is a scientific journal of entomology for the Southern Hemisphere. It publishes Original Articles that are peer-reviewed research papers from the study of the behaviour, biology, biosystematics, conservation biology, ecology, evolution, forensic and medical entomology, molecular biology, public health, urban entomology, physiology and the use and control of insects, arachnids and myriapods. The journal also publishes Reviews on research and theory or commentaries on current areas of research, innovation or rapid development likely to be of broad interest – these may be submitted or invited. Book Reviews will also be considered provided the works are of global significance. Manuscripts from authors in the Northern Hemisphere are encouraged provided that the research has relevance to or broad readership within the Southern Hemisphere. All submissions are peer-reviewed by at least two referees expert in the field of the submitted paper. Special issues are encouraged; please contact the Chief Editor for further information.

*Austral Entomology* is the official publication of the [Australian Entomological Society](http://www.austentsoc.org.au/), an incorporated non-profit Australian company limited by guarantee. Membership of the Society is open to any person interested in entomology in its broadest sense. Application forms are available from the Australian Entomological Society website (<http://www.austentsoc.org.au/>).

#### 2. MANUSCRIPT CATEGORIES AND REQUIREMENTS

*Austral Entomology* publishes the following article types:

- Original Articles
- Reviews
- Book reviews
- Editorials

##### **Original Articles**

Please click on this link for further details on how Original Articles should be formatted: [Template for Original Articles](#).

## **Original Articles (Taxonomic Papers)**

For taxonomic papers, authors must follow the International Code of Zoological Nomenclature (ICZN). Revisionary papers and small monographs will be accepted provided they occupy no more than 25 printed journal pages due to competition for space. The Introduction of all taxonomic papers must provide a clear statement outlining the context and purpose of the paper - why the work was done and what it aims to achieve. Further detail about requirements for taxonomic papers are available here: [Template for Taxonomic Papers](#).

## **3. PREPARING YOUR MANUSCRIPT**

### **Style and Formatting**

For submission, the manuscript should preferably be submitted as a single file, with the figures embedded as low resolution files. Tables and figures should be inserted at the end of the manuscript. Name the manuscript file as: **authorname.doc**.

- Submissions should be typed in 12 pt Times New Roman and have 1.5 line spacing.
- All margins should be set to 2.5 cm.
- The first paragraph under each heading is not indented; indent following paragraphs, with no blank line between paragraphs.
- Ensure that all mark-up ('Track Changes') done during manuscript preparation is removed ('Accept All Changes' on Reviewing Toolbar) so that reviewers have a clean copy on which to insert suggested changes and comments.

### **Abbreviations and Units**

SI units (metre, kilogram etc.), as outlined in the latest edition of *Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors* (Royal Society of Medicine Press, London), should be used wherever possible. Give statistics and measurements in figures; that is, 10 mm, except where the number begins the sentence. When the number does not refer to a unit measurement, it is spelt out, except where the number is greater than nine. Use only standard abbreviations. Shorten the word 'Figure' to Fig. unless starting a sentence.

The journal uses Australian spelling and authors should therefore set the Language in MS Word to English (Australia) (accessible under the Tools menu in MS Word) and follow the latest edition of the Macquarie Dictionary. Manuscripts that do not conform

to this requirement and the following format will be returned to the author prior to review for correction.

## **Parts of the Manuscript**

### **Title page**

The title page should contain:

- (i) an informative title that contains the major key words. The title should contain the scientific name of the insect, with the order and family placed in parentheses;
- (ii) the full names of the authors;
- (iii) the author's institutional affiliations at which the work was carried out;
- (iv) a short running title of less than 50 characters including spaces.
- (iv) the email address of the author to whom correspondence about the manuscript should be sent.

### **Abstract**

All manuscripts must include a brief but informative abstract intelligible without reference to the main text. It should not exceed 350 words and should describe the scope of the work and the main findings. Both common and scientific names of the insect should be included. Authorities to species names are not required except for taxonomic papers. References to the literature should not be included. Use the passive voice in the Abstract. DO NOT use the uninformative phrase 'Results are discussed.'

### **Key Words**

Up to 10 additional key words should be provided below the Abstract.

### **Main Text Sections**

- *Introduction*: This section should include sufficient background information to set the work in context. The aims and goals of the manuscript should be clearly stated. The introduction should not contain findings or conclusions.
- *Materials and Methods*: This should be concise but provide sufficient detail to allow the work to be repeated by others.
- *Results*: This should be presented in a logical sequence in the text, tables and figures; repetitive presentation of the same data in different forms is not permissible. The results should not contain material appropriate to the Discussion.

- *Discussion*: This should consider the results in relation to any hypotheses advanced in the Introduction and place the study in the context of other work.

Details of sections required in taxonomic papers are set out here: [Template for Taxonomic Papers](#).

### **Acknowledgements**

The source of financial grants and other funding must be acknowledged, including a frank declaration of the author's industrial links and affiliations. Financial and technical assistance may be acknowledged here. If tables or figures have been reproduced from another source, or copyright is not held by any of the authors, then written permission from the copyright holder must be mentioned in the Acknowledgements. Do not acknowledge anonymous reviewers.

### **References**

The Harvard (author, date) system of referencing is used.

- In the text give the author's name followed by the year in parentheses: Sago (2000).
- When reference is made to a work by three or more authors, the first name followed by *et al.* should be used: Powles *et al.* (1998).
- Within parentheses, groups of references should be cited in chronological order.
- Personal communication, unpublished data and publications from informal meetings are not to be listed in the reference list but should be listed in full in the text (e.g. Smith A, 2000, unpublished data).
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## **References should be listed in the following form:**

### **a) Journal articles:**

North RC & Shelton AM. 1996. Ecology of Thysanoptera within cabbage fields. *Environmental Entomology* **15**, 520–526.

### **b) Books:**

Eberhard WG. 1985. *Sexual Selection and Animal Genitalia*. Harvard University Press, Harvard.

### **c) Chapters in books:**

Bray RA. 1994. The leucaena psyllid. In: *Forage Tree Legumes in Tropical Agriculture* (eds RC Gutteridge & HM Shelton) pp. 283–291. CAB International, Oxford.

### **d) Website:**

Bureau of Meteorology. 2014. Southern Oscillation Index Archives – 1876 to present. Available from URL: <http://www.bom.gov.au/climate/current/soihtm1.shtml> [Accessed 5 March 2014]

## **Tables**

Tables must be constructed using the ‘Table’ function of your word processor and must not have the Enter key used in any cell. Tables should be self-contained and complement, but not duplicate, information contained in the text. Tables should be numbered consecutively in Arabic numerals. Each table should be presented on a separate page at the end of the text with a comprehensive but concise legend above the table. Tables should be double-spaced and vertical lines should not be used to separate columns. Column headings should be brief, with units of measurement in parentheses; all abbreviations should be defined in footnotes. Use superscript letters (not numbers) for footnotes and keep footnotes to a minimum. \*, \*\*, \*\*\* should be reserved for *P*-values. The table and its legend/footnotes should be understandable without reference to the text.

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Legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement.

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Manuscripts must not present work that has been published, accepted for publication or is currently under consideration for publication elsewhere. Submission of a manuscript must be approved by all authors.

All manuscripts are reviewed by two specialist referees and a Subject Editor of the Editorial Board before acceptance. Manuscripts considered unsuitable for publication will be returned to authors who may resubmit after amendment. If a reviewed

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The Editors and the Publisher reserve the right to modify manuscripts to eliminate ambiguity and repetition, and to improve communication between author and reader. To encourage the valuable ongoing services of highly qualified reviewers and to avoid unnecessary delays, authors are strongly advised to have their manuscripts thoroughly reviewed by impartial colleagues prior to submission, especially if English is not their first language.

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The complete scientific name (genus and species), and cultivar or strain where appropriate, should be given for all animals when first mentioned; authorities are only needed for taxonomic papers. The generic name may be abbreviated to an initial in subsequent references except at the start of sentences and where intervening references to other genera would cause confusion. Common names of organisms, if used, should conform to the list on <http://www.ces.csiro.au/aicn/intro.htm>. All names must conform to the Articles and Recommendations of the fourth edition of the International Code of Zoological Nomenclature. Common names of pesticides listed in *Pesticides – Synonyms and Chemical Names* (Australian Dept of Health, Canberra) must be used.

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The zoobank registration numbers are to be added by the author after the manuscript has been accepted.

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All DNA sequences must be lodged with GenBank and should not be repeated in the paper unless highly relevant. The GenBank registration number must be provided for each sequence (e.g. in Supporting Information).

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