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PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA ANIMAL

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**CONTRIBUIÇÕES AO CONHECIMENTO DE *Phyllocnistis* ZELLER, 1848
(LEPIDOPTERA: GRACILLARIIDAE), MINADORES DE FOLHAS
ASSOCIADOS À *Baccharis* L. (ASTERACEAE)**

PORTO ALEGRE
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Aprovada em ____ de _____ de ____.

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RESUMO

O gênero *Baccharis* L. (Asteraceae) é amplamente distribuído na região Neotropical com uma diversidade muito grande de espécies, sendo utilizado como planta hospedeira por várias famílias de Lepidoptera (Insecta), incluindo Gracillariidae, uma família de microlepidópteros cujas espécies são principalmente minadoras de folhas. Em viagens recentes de exploração em campo, minas de *Phyllocnistis* Zeller, 1848 (Gracillariidae) associadas a plantas de *Baccharis* foram encontradas nas encostas ocidentais dos Andes, no Peru. Considerando que só duas espécies deste gênero de minadores foliares foram reportadas previamente neste país, o objetivo geral deste estudo foi avaliar o estado taxonômico das populações descobertas. Para isso, o presente estudo foi dividido em duas partes, a primeira focada numa espécie associada a *B. alnifolia*, a segunda em duas espécies associadas a *B. latifolia*. Como resultado, dois artigos científicos foram produzidos. No primeiro se apresenta a descrição formal de *Phyllocnistis furcata* Vargas & Cerdeña, 2020 com base na morfologia do adulto e estágios imaturos (sap-feeding, spinning e pupa) coletados no departamento de Arequipa, além de caracterizar o padrão de dano foliar usando cortes histológicos e efetuar uma avaliação preliminar das relações da espécie com congêneras baseado em sequências de DNA mitocondrial. No segundo, duas espécies novas, dos departamentos de Lima e Tacna, são descritas com base na morfologia do adulto. Suas relações com congêneras são exploradas usando sequências de DNA mitocondrial, incluindo todas as espécies associadas a *Baccharis* até agora descritas. A localidade tipo de *Phyllocnistis baccharidis* Hering é confirmada mediante a coleta de estágios imaturos a 1300 m de altitude em Tucuman (Argentina) alimentando-se de *Baccharis tucumanensis*. Com a execução deste estudo, o número de espécies de *Phyllocnistis* nativas do Peru atinge cinco e novos caracteres morfológicos são relatados na genitália masculina e feminina, não observados previamente em nenhuma outra espécie Neotropical do gênero. Os resultados sugerem a importância de expandir as amostragens a outros ambientes neotropicais para melhorar o conhecimento da diversidade e evolução de *Phyllocnistis*.

Palavras-chave: Andes, barcoding, estágios imaturos, taxonomia, Phyllocnistinae

ABSTRACT

The genus *Baccharis* L. (Asteraceae) is widely distributed in the Neotropical region with a very large diversity of species, being used as a host plant by several families of Lepidoptera (Insecta), including Gracillariidae, a family of microlepidoptera whose species are mainly leaf-mining. On recent field exploration trips, mines of *Phyllocnistis* Zeller, 1848 (Gracillariidae) associated with *Baccharis* plants were found on the western slopes of the Andes in Peru. Considering that only two species of this genus of leaf miners were previously reported from this country, the general objective of this study was to assess the taxonomic status of the populations discovered. For this purpose, the present study was divided into two parts, the first focused on a species associated with *B. alnifolia*, the second on two species associated with *B. latifolia*. As a result, two scientific articles were produced. The first presents a formal description of *Phyllocnistis furcata* Vargas & Cerdeña, 2020 based on adult morphology and immature stages (sap-feeding, spinning and pupa) collected in the department of Arequipa, in addition to characterizing the pattern of leaf damage using histological sections and perform a preliminary assessment of species-congeneric relationships based on mitochondrial DNA sequences. In the second, two new species, from the departments of Lima and Tacna, are described based on adult morphology. Their congeneric relationships are explored using mitochondrial DNA sequences, including all *Baccharis*-associated species so far described. The type locality of *Phyllocnistis baccharidis* Hering is confirmed by collecting immature stages at 1,300 m elevation in Tucuman (Argentina) feeding on *Baccharis tucumanensis*. With the execution of this study, the number of *Phyllocnistis* species native to Peru reaches five and new morphological characters are reported in male and female genitalia, not previously observed in any other Neotropical species of the genus. The results suggest the importance of expanding the samplings to other neotropical environments to improve the knowledge of the diversity and evolution of *Phyllocnistis*.

Keywords: Andes, barcoding, immature stages, taxonomy, Phyllocnistinae

CAPÍTULO I

INTRODUÇÃO GERAL

Os insetos minadores de folhas consistem um grupo taxonomicamente diverso de insetos endófagos e as larvas se encontram, na maioria dos casos, dentro do tecido vegetal de seus hospedeiros durante todo o seu desenvolvimento, ou pelo menos em parte dele (Hering 1951; Kirichenko *et al.* 2018). Eles são representados por quatro ordens, Lepidoptera, Hymenoptera, Coleoptera e Diptera, contabilizando mais de 10.000 espécies em todo o mundo (Connor & Taverner 1997). Representam um grupo importante de insetos praga, causando altos impactos econômicos e ambientais (Argov & Rössler 1996; Kirichenko *et al.* 2018). A maioria dos insetos minadores de folhas pertence a Lepidoptera, sendo que pelo menos 40 famílias desta ordem exibem hábitos de mineração de folhas, com variação considerável entre as espécies (Connor & Taverner 1997; Powell *et al.* 1997; Steyn *et al.* 2020). A maioria pertencem a família Gracillariidae (Davis & Robinson 1998).

Gracillariidae se constitui num dos mais diversos grupos de lepidópteros, incluindo quase 2000 espécies descritas em mais de 100 gêneros (vanNieuwerkerken *et al.* 2011; De Prins & De Prins 2021) com muitos ainda a serem descritos (Lees *et al.*, 2013; Brito *et al.* 2016). Estudos filogenéticos recentes baseados em caracteres moleculares, suportaram fortemente a monofilia de Gracillariidae, dividindo a família em nove subfamílias: Acrocercopinae, Callicercopinae, Gracillariinae, Lithocolletinae, Phyllocnistinae, Marmarinae, Oecophyllembiinae, Ornixolinae, Parornichinae (Kawahara *et al.* 2017; De Prins *et al.* 2019; Li *et al.* 2021).

Larvas de Gracillariidae em sua maioria formam minas tipo serpentina nas folhas, mas algumas espécies minam frutos ou caules, enrolam folhas ou induzem galhas (Davis 1987; De Prins & De Prins 2021). Algumas espécies são mutualistas de polinização obrigatória, efetuada pelos adultos (Kawakita *et al.* 2004, 2010), enquanto outros podem atrasar a senescência das folhas abscizadas, das quais posteriormente se alimentam no solo (Giron *et al.* 2007; Kaiser *et al.* 2010; Gutzwiller *et al.* 2015). As larvas são únicas dentro dos lepidópteros porque passam por mudanças ontogenéticas e comportamentais atípicas quanto à morfologia e comportamento alimentar, sendo consideradas hipermetamórficas (Body

et al. 2005). Existe uma forma de alimentação sap-feeding (com cabeça e corpo achatados, mandíbulas modificadas, ausência de feira funcional e ausência de pernas) e uma forma de alimentação de tecido notavelmente diferente *tissue-feeding* (com um corpo cilíndrico, cabeça redonda, aparelhos bucais de mastigação, pernas e um feira funcional) que se assemelha a uma larva típica de lepidóptero (Wagner *et al.* 2000; Body *et al.* 2015). Também apresentam forma conhecida como *spinning* (=pré-pupa), caracterizada pela ausência de alimentação, onde todas as peças bucais são perdidas, sendo apenas o espinerete funcional, para que a larva possa tecer o casulo (Brito *et al.* 2017). 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 (Kawahara *et al.* 2017). Algumas larvas também têm um instar quiescente transicional que não alimenta (Wagner *et al.* 2000).

O gênero minerador de folhas *Phyllocnistis* com 113 espécies descritas é um dos mais diversos e amplamente distribuídos (De Prins & De Prins 2021). Os estágios imaturos estão associados a uma grande variedade de plantas hospedeiras, incluindo 26 famílias de plantas (De Prins & Kawahara 2009). Na região Neotropical, as plantas hospedeiras são conhecidas por cerca de 45% das espécies de *Phyllocnistis* (Brito *et al.* 2017), entre elas estão *P. ourea* Brito & Moreira, *P. baccharidis* Hering, *P. furcata* Vargas & Cerdeña, e uma ainda não descrita, que se alimentam todas de plantas do gênero *Baccharis* (Asteraceae) (Brito *et al.* 2017; Cerdeña *et al.* 2020).

O gênero *Baccharis* do Novo Mundo está amplamente distribuído na região Neotropical, adaptado a uma grande variedade de ecossistemas, com 440 espécies reconhecidas (Heiden *et al.* 2019). Famílias distintas de lepidópteros foram registradas alimentando-se de flores (e.g., Vargas 2010, 2020; Vargas & Duarte 2014), folhas (e.g., Stonis *et al.* 2016; Vargas 2019; Vargas & Moreira 2012) ou induzindo galhas (Vargas *et al.* 2015) em *Baccharis*.

Recentemente, em levantamento realizado nas encostas ocidentais da Cordilheira dos Andes do Peru, foram encontradas plantas de *Baccharis* apresentando danos foliares com padrão semelhante ao produzido por larvas de Gracillariidae. Detectou-se através de observações preliminares, que eram espécies desconhecidas da gênero *Phyllocnistis*. Nesse sentido, o presente estudo

teve como objetivo avaliar o status taxonômico dessas populações de *Phyllocnistis* associadas com *Baccharis* no Peru. Para isso, realizaram-se buscas e coletas ao longo da encosta oeste dos Andes peruanos, desde o Departamento de Lima em direção ao sul até o departamento de Tacna na fronteira com o Chile. O trabalho de campo foi realizado entre os anos de 2019 e 2021, em diferentes épocas do ano. 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 congêneros foram também conduzidas.

Os resultados obtidos neste trabalho são a descrição de três novas espécies de *Phyllocnistis* (Gracillariidae) associadas a duas espécies de *Baccharis* (Asteraceae), distribuídas nas encostas ocidentais dos Andes do Peru, entre 2000 e 3000 metros de altitude; e a confirmação da localidade tipo e planta hospedeira (*Baccharis tucumanensis*) de *Phyllocnistis baccharidis*, encontrado a 1300 m em Tucuman (Argentina).

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CAPÍTULO II

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Phyllocnistis furcata sp. nov.: a new species of leaf-miner associated with *Baccharis* (Asteraceae) from Southern Peru (Lepidoptera, Gracillariidae)

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Abstract

The southwestern Andes of Peru harbors a hidden taxonomic diversity of Lepidoptera. Here a new leaf-mining species of Gracillariidae (Lepidoptera) is described, *Phyllocnistis furcata* Vargas & Cerdeña, **sp. nov.**, from a dry Andean valley of southern Peru, at 2400 m above sea level. The morphological aspects of adults (male and female) and the immature stages associated with *Baccharis alnifolia* Meyen & Walp. (Asteraceae) are given, under optical microscopy and scanning electron microscopy. DNA barcodes show that its nearest neighbor is the Atlantic Forest species *Phyllocnistis ourea* Brito & Moreira, 2017 that feeds on *Baccharis anomala* DC. The importance of morphological characters from immature stages for diagnosis among congeneric species is also discussed. *Phyllocnistis furcata* represents the fourth species of *Phyllocnistis* Zeller for Peru, and first record from the south of Peru for the genus.

Keywords

Andes, Arequipa, barcoding, immature stages, Phyllocnistinae

Introduction

The Andes region of Peru contains hotspots of biodiversity for plants and animals (Myers et al. 2000). It includes global record highs of species richness and endemism rates for many taxa of Lepidoptera, particularly along the eastern slopes of the Andes (Lamas 2003; Pyrcz 2004; Hall 2005; Ignatov et al. 2011; Willmott et al. 2011; Pyrcz et al. 2014; Sublett et al. 2019). On the other hand, the level of knowledge of the lepidopteran fauna on the southwestern slopes of the Peruvian Andes is poor, based on a small number of studies of butterflies (Lamas 1977; Cerdeña et al. 2014; Farfán 2018; Farfán et al. 2020b), and with recent records of moths (Cerdeña et al. 2019; Farfán et al. 2020a), including the first record of a Gracillariidae species for this region (Davis et al. 2020).

Gracillariidae represents one of the most diverse families of micromoths with 1995 recognized species distributed in more than 100 genera (van Nieukerken et al. 2011; De Prins and De Prins 2020). Larvae are predominantly leaf-miners although some species mine stems or fruits (Guillén et al. 2001), and others bore into flowers (Vargas and Landry 2005), fruits (Hu et al. 2011) or stems (Davis et al. 1991), and may also be leaf-rollers or gall inducers (Hanson et al. 2014; Kawakita and Kato 2016; Vargas-Ortiz et al. 2019). In the Neotropics, more intense taxonomic work has been performed in the last decade, resulting in 28 newly described species (De Prins and De Prins 2020). Despite this effort of documenting the fauna, currently numbering 204 Neotropical species, there are gaps in information for gracillariids in several biodiversity hotspots, such as the Andes. Particularly in Peru, currently only 28 species of gracillariids are known (Kawakita et al. 2019; Davis et al. 2020; De Prins and De Prins 2020). From these, one is a non-native species introduced in the coastal area (Castillo and Cornejo 1996), two species were recently reported from southwestern Peru (Vargas 2010; Davis et al. 2020), five were described from northern Peru (Kawakita et al. 2019), and 19 species were described in the early last century by Edward Meyrick (Meyrick 1915, 1921) from material collected by Herbert Simpson Parish from the central Peruvian coast (Lima), central Andes (Matucana, Oroya, Huancayo, Jauja), and northeastern Peruvian Amazon (Iquitos, Yurimaguas) during two collecting trips to the tropics in 1914 and 1920 (Alexander 1916, 1921). These species in particular remain only known from the type specimens, some of which have deteriorated (Brito et al. 2017; De Prins et al. 2019).

Phyllocnistis Zeller, 1848 is a genus of Gracillariidae with 112 named species distributed in all biogeographic regions except Antarctica (Brito et al. 2016, 2017; Fochezato et al. 2018; Kirichenko et al. 2018; De Prins and De Prins 2020). A total of 28 species has been reported for the Neotropical region (De Prins et al. 2019), with 16 species recorded in the last ten years (Kawahara et al. 2009; Davis and Wagner 2011; Brito et al. 2012, 2017, 2019; Fochezato et al. 2018). However, only three species were registered from Peru: *Phyllocnistis sciophanta* Meyrick, 1915, *P. sexangula* Meyrick, 1915, and *P. citrella* Stainton, 1856; the first two species, collected from the center of Peru (Department of Lima) more than 100 years ago, remain with their host plant and immature stages unknown (Brito et al. 2017; De Prins et al. 2019). The third

species, with a worldwide distribution, known to be a pest in citrus fruits, is a native from Asia (Castillo and Cornejo 1996; De Prins et al. 2019). This lack of data is mainly due to two conditions that prevailed for a long time, not only in Peru but also in other countries of the Neotropical region (Brito et al. 2016): low collection intensity and scarce taxonomy activity on Gracillariidae and Microlepidoptera in general.

The hypermetamorphic development of the larvae of *Phyllocnistis* typically comprises two endophytic forms (e.g., Fochezato et al. 2018; Brito et al. 2019). The early, sap-feeding larva actively mines specific host tissues; later, the spinning larva does not feed and has most of the buccal apparatus atrophied, but has a functional spinneret that is used to expel silk to construct the pupal cocoon.

A variety of host plants are associated with *Phyllocnistis* in the Neotropical region, including 15 genera from 13 different plant families (Brito et al. 2017). Three species are known to be associated with the genus *Baccharis* (Asteraceae): *P. baccharidis* Hering, 1958, *P. ourea* Brito & Moreira, 2017, and an undescribed species associated with *Baccharis trimera* (Brito et al. 2017), the first from Argentina and other two from Brazil. This Neotropical plant genus is characterized by the tufted indumentum of leaves and stems, and by the unisexual florets generally in separate specimens (Müller 2006), currently comprising 440 species (Heiden et al. 2019).

Recently, as part of an ongoing study on the diversity of microlepidopterans in the Andes in southern Peru, we found a leaf-mining species of *Phyllocnistis* associated with *Baccharis*. Comparison at both morphological and molecular levels showed that it does not conform to any known *Phyllocnistis* species. The morphological description of adults (male and female) and immature stages of this new species is herein given. We also present a preliminary analysis of mitochondrial (COI) DNA sequences including congeneric Neotropical species.

Materials and methods

Larvae and pupae found in mines on leaves of *Baccharis alnifolia* Meyen & Walp. (Asteraceae) in the locality of Characato (16°27'S, 71°28'W), 2400 m, Characato Municipality, Arequipa Department, Peru, were collected and reared in plastic cups, at constant abiotic conditions (20 ± 2 °C, 13:11 h photoperiod) in the laboratory of Area de Entomología, Museo de Historia Natural, Universidad Nacional de San Agustín, Arequipa city, Peru, during September 2018, April 2019, and November 2019.

In total, 58 specimens have been studied: 23 adults, 17 larvae, 20 pupae. Adults that emerged from the mines were pinned and dried, and immature stages were fixed with Dietrich's fluid and preserved in 70% ethanol. Genitalia were cleared by heating in hot 10% KOH for ~ 15 minutes. They were subsequently stained with Chlorazol black and Eosin, and then slide-mounted with Euparal.

Morphological observations were performed with the aid of a Zeiss Stemi305, and structures selected to be illustrated were photographed with a Nikon SMZ25 stereomicroscope. Vectorized line drawings were then made with the software CorelDraw X4, using

the corresponding digitalized images as a guide. The terminology used for descriptions of adult wing pattern, genitalia and immature stages follows Brito et al. (2017, 2019).

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 JSM6060 scanning electron microscope at the Centro de Microscopia Eletrônica (CME) of Federal University of Rio Grande do Sul (UFRGS).

For plant anatomical descriptions, field-collected leaf portions (approx. 0.3 cm²) of *B. alnifolia* containing mines of *P. furcata* were fixed in FAA (37% formaldehyde, glacial acetic acid, and 50% ethanol, 1:1:18, v/v) for 24 h. They were then dehydrated in a series of ethanol (40%, 70%, 90%, 96%); embedded in paraffin and sectioned transversely (7 µm) on a rotary microtome. The sections were adhered to a microscope slide glass, then observed and photographed without staining, by using a Nikon SMZ25 stereomicroscope.

Total genomic DNA was extracted from larval tissue (last sap-feeding instar) of five specimens (H86–H90), using the CTAB method (Doyle and Doyle 1987), to support the hypothesis that the morphologically distinct specimens studied confirm a new *Phyllocnistis* species, and explore the phylogenetic placement among Neotropical congeners. We amplified part of the mitochondrial gene cytochrome oxidase I (COI – 639 bp) using primers and conditions described by Folmer et al. (1994). PCR products were purified using Exonuclease I (GE Healthcare Inc.) and Shrimp Alkaline Phosphatase (SAP), sequenced with forward and reverse primers using 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 new sequences obtained in this study are publicly available in GenBank and BOLD (DS-GRANEO) databases (Table 1). To explore the phylogenetic position of the new taxon and its specific classification we combined our COI data with a published dataset of ten species and 13 undescribed lineages of Neotropical *Phyllocnistis* (Davis and Wagner 2011; Brito et al. 2012, 2017, 2019; Lees et al. 2013) (Table 1). This includes *P. ourea* that feeds on *Baccharis anomala*, and *Phyllocnistis* sp. 12 (Bruto et al. 2017) associated to *Baccharis trimera* (Less) DC. *Angelabella tecomae* Vargas & Parra, 2005 (Oecophyllembiinae) and *Marmara arbutiella* Busck, [1904] (Marmarinae) were used as outgroups as they represent subfamilies closely related to Phyllocnistinae (Kawahara et al. 2017). A distance tree based on Neighbor-joining (NJ) method was generated from 31 nucleotide sequences using Kimura 2-parameters (K2P) model in MEGA X (Kumar et al. 2018). The evolutionary history was inferred by using the Maximum Parsimony (MP) analysis using the Tree-Bisection-Regrafting (TBR) algorithm with search level 1, with initial trees obtained by random addition of sequences (10 replicates) also in MEGA X. A Maximum Likelihood (ML) analysis was also performed, with the substitution model GTR+G+I according to the Akaike Information Criterion (AIC) estimated by JMODELTEST (Posada 2008), using PHYML 3.0 (Guindon et al. 2010). Initial trees for the heuristic search were obtained automati-

Table 1. Specimens used for molecular analyses of *Phyllocnistis furcata* sp. nov. Both the Sample ID and Process ID codes are unique identifiers linking the record in the BOLD database and the voucher specimen from which the sequence is derived. The asterisk(s) indicates those specimens associated with the *Baccharis* as host plant: **B. alnifolia*, ***B. anomala*, ****B. trimera*.

Species	Sample ID	Process ID	GenBank accession	Reference
<i>Phyllocnistis furcata</i> sp. nov.*	H86	MISA051-20	MT832361	This study
<i>Phyllocnistis furcata</i> sp. nov.*	H87	MISA052-20	MT832362	This study
<i>Phyllocnistis furcata</i> sp. nov.*	H88	MISA053-20	MT832363	This study
<i>Phyllocnistis furcata</i> sp. nov.*	H89	MISA054-20	MT832364	This study
<i>Phyllocnistis furcata</i> sp. nov.*	H90	MISA055-20	MT832365	This study
<i>Phyllocnistis hemera</i>	LMCI 292-25C	MISA019-17	MG264519	Fochezato et al. 2018
<i>Phyllocnistis kawakitai</i>	AK0105	GRANO105-11	KF460801	Lees et al. 2014
<i>Phyllocnistis norak</i>	CLV1381	LNOCUC318-10	JN276191	Lees et al. 2014
<i>Phyllocnistis obsbimai</i>	CLV1367	LNOCUC304-10	JN276189	Lees et al. 2014
<i>Phyllocnistis ourea</i> **	LMCI 297-15B	MISA013-16	KY006927	Brito et al. 2016
<i>Phyllocnistis petronellii</i>	IO0536	LEPPC2394-16	KY682706	Brito et al. 2017
<i>Phyllocnistis perseifolia</i>	DDAV-D555	RDOPO393-10	HM382096	Davis and Wagner 2011
<i>Phyllocnistis phoebus</i>	LMCI 263-9	MISA014-16	KY006929	Brito et al. 2016
<i>Phyllocnistis selene</i>	LMCI 263-22	MISA015-16	KY006928	Brito et al. 2016
<i>Phyllocnistis tethys</i>	LMCI 174-55-1	GBMIN15477-13	JX272049	Brito et al. 2012
<i>Phyllocnistis</i> sp. 2	AK0198	LNODU2290-12	KF460914	Lees et al. 2014
<i>Phyllocnistis</i> sp. 3	AK0210	LNODU2302-12	KF460586	Lees et al. 2014
<i>Phyllocnistis</i> sp. 4	AYK-FG10-135	LNOCUC1229-11	KF460667	Lees et al. 2014
<i>Phyllocnistis</i> sp. 5	CLV1284	LNODU1191-12	KF460613	Lees et al. 2014
<i>Phyllocnistis</i> sp. 7	CLV1368	LNOCUC305-10	JN276190	Lees et al. 2014
<i>Phyllocnistis</i> sp. 9	CLV2993	LNODU336-11	KF460927	Lees et al. 2014
<i>Phyllocnistis</i> sp. 10	CLV3313	LNODU489-11	KF460904	Lees et al. 2014
<i>Phyllocnistis</i> sp. 11	CLV4347	LNODU776-12	KF460865	Lees et al. 2014
<i>Phyllocnistis</i> sp. 12***	CLV5900 and CLV5901	GRPAL1220-13 and GRPAL1221-13	KY682713 and KF460659	Lees et al. 2014
<i>Phyllocnistis</i> sp. 13	CLV5902	GRPAL1222-13	KY682713	Lees et al. 2014
<i>Phyllocnistis</i> sp. 15	LEAFMINE2015-0006	LEPPC1378-15	KY682712	Brito et al. 2017
<i>Phyllocnistis</i> sp. 16	LEAFMINE2015-0008	LEPPC1380-15	KY682711	Brito et al. 2017
<i>Phyllocnistis</i> sp. 17	LEAFMINE2015-0010	LEPPC1382-15	KY682704	Brito et al. 2017

cally with BioNJ algorithm to a matrix of pairwise distances. Monophyly-confidence limits of all analysis were assessed with the bootstrap (BS) method after 1000 bootstrap iterations. Sequence divergences were quantified using K2P model for (i) the genus *Phyllocnistis* (using 35 named species deposited in BOLD; Table 1), (ii) the Neotropical *Phyllocnistis* (10 described + 13 undescribed species, Table 1), and (iii) the new species vs. *Baccharis*-feeding lineages.

Abbreviations for the museum collections and institutions from which specimens were examined are:

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

MUSA Museo de Historia Natural, Universidad Nacional de San Agustín de Arequipa, Arequipa, Perú.

MUSM Museo de Historia Natural, Universidad Nacional Mayor San Marcos, Lima, Peru.

Results

Phyllocnistis furcata Vargas & Cerdeña, sp. nov.

<http://zoobank.org/F54378EF-7ADF-425D-9FDD-B8F2223D1381>

Figs 1–7

Type locality. Peru, Arequipa, Characato [16°27'S, 71°28'W], 2400 m.

Specimens examined. Holotype: Peru • ♂; Arequipa, Characato; 16°27'S, 71°28'W; 2400 m a.s.l.; VIII–IX.2018; J. Cerdeña, H. Vargas & J. Farfan leg.; reared from pupae collected on *Baccharis alnifolia* (Asteraceae); MUSM. **Paratypes:** same data as for holotype • 1 ♂, 1 ♀; MUSM; • 1 ♂, 1 ♀; MUSA_ENT 015142, 015143; • 2 ♂, 2 ♀; LMCI.

Other material. Adults, pinned and dried, 5 ♂, 8 ♀, same data as for holotype, MUSA_ENT 015144, 015145, 015146, 015147, 015148, 015149, 015150, 015151, 015152, 015153, 015154, 015155, 015156. Genitalia preparations (MUSA_Gent_015142, 015143, 015146, 015147, 015148), mounted on slides, with the same collection data. Immature stages (11 sap-feeding larvae, 06 spinning larvae, 20 pupae) preserved in 70% ethanol, with the same collection data, but with dates VIII–IX.2018, IV.2019 or XI.2019, MUSA.

Diagnosis. Adults of *P. furcata* can be distinguished from all other known species of Neotropical *Phyllocnistis* in the forewing pattern by a combination of the following characters: ground color silver, four distinct transverse fasciae; transverse fasciae 1 reduced to the costal margin and mesally fused to longitudinal fascia, both not connected to transverse fascia 2; transverse fascia 3 almost reaching the middle portion of the wing. In the male abdomen, by the presence of two pairs of coremata on abdominal segment VIII, one pair consisting of wide rounded flat scales, a character not found in other Neotropical *Phyllocnistis*. In the female genitalia, by presenting a remarkable forked-shaped signum with four elongated spines on the distal margin. This species is similar to *P. nygodzinskyi* Hering, 1958 and *P. sexangula* Meyrick, 1915, in having similar patterns of fasciae. However, *P. nygodzinskyi* has a large black blotch at the inner border of the longitudinal fascia, and *P. sexangula* presents a small blotch close to the inner border of the longitudinal fascia, while *P. furcata* has no additional mark on the forewing.

Adult. (Figs 1, 2). **Description. Male:** Forewing length 3.10–3.33 mm ($N = 5$). Head: Vestiture silvery pale brown, completely covered with smooth, broad, scales slightly overlapping anterior margin of eyes (Fig. 1B). Antennae light brown dorsally becoming dark towards apex and silvery white ventrally, approximately equal to length of forewing (Fig. 1A, F). Labial palpus slender, ~ 0.4 mm in length, covered with light grey scales (Fig. 1F). Proboscis without scales, slightly longer than labial palpus (Fig. 1F). Thorax: Forewing ground color silvery white; with light orange longitudinal (lf) and transverse (tf) fasciae (Fig. 1A, E); if bordered by dark brown scales, extending 2/3 length of wing from base of costa, and connected with tf1 apically; tf1 not reach the inner margin, restricted between the costal margin and lf; tf2 separate from tf1,



Figure 1. *Phyllocnistis furcata* sp. nov. adult morphology **A, B** holotype of *P. furcata*, male, with head in detail, dorsal view (MUSM) **C, D** paratype of *P. furcata*, female, with head in detail, dorsal (MUSA_ENT 015143) **E** detail of right forewing with terminologies adopted, lf: longitudinal fascia; tf (1–4) transverse fascia(e) **F** lateral view of a male head with labial palpus (indicated by closed arrow head) and antenna (indicated by open arrow head). Scale bars: 2 mm (**A, C**), 1 mm (**E**), 0.4 mm (**F**).

lightly convex, crossing the wing entirely; tf3 separate from tf2, but not reach the inner margin; tf4 separate from tf3, crossing the wing entirely. Apex of forewing with a well-marked black spot. Costal strigulae, light orange, emerge from the base of transverse fasciae. Apical strigulae, dark brown, emerge from black spot. Inner marginal fringe varies from orange to dark brown. Hindwings light pale brown gray, with long light brown fringes. Legs light gray except dark brown over dorsal surface of femur, tibia, and tarsus of foreleg. Abdomen length ~ 2.0 mm, dark grey covered with silvery pale brown scales, two pairs of coremata present laterally on segment VIII (Fig. 2A), one pair consisting of a set of flat and long scales and the other pair consisting of wide rounded flat scales (Fig. 2D). Whether the wide rounded flat scales function as coremata by themselves or appendages of the long ones remains unknown.

Male genitalia: Uncus absent. Tegumen membranous, approximately equal to length of the valva, with spines arranged laterally from the base to the medial region. Saccus V-shaped, well developed, $\sim 0.8 \times$ the size of valva. Valvae digitiform and slightly convergent from the base to the apex, apex with small spine, setae randomly arranged along the valva getting shorter in the distal part (Fig. 2C). Phallus slender and with a slightly convex apex, weakly sclerotized, wrinkled cylinder, lightly longer than valva; cornuti absent (Fig. 2B).

Female: Forewing length 3.30–3.41 mm ($N = 5$). Color and pattern very similar to that of male, but head vestiture with light silvery scales (Fig. 1D). Hindwings light silvery gray with long silvery fringes and abdomen color light brown covered with silvery scales (Fig. 1C). VII abdominal sternum trapezoid, anterior margin thickened.

Female genitalia: Papillae anales slightly sclerotized, covered with hair-like setae. Posterior apophyses $\sim 2.4 \times$ length of anterior apophyses (Fig. 2F). Ostium bursae posterior to sternum 7. Ductus bursae completely membranous, slender, elongate, over $6.0 \times$ length of posterior apophyses (Fig. 2E). Corpus bursae slightly elongated, $\sim 0.3 \times$ length of ductus bursae, mainly membranous with three signa; a prominent fork-shaped signum on basis, resembling a garden fork, that occupies $\sim 0.5 \times$ length of corpus bursae with four elongated spines distally projected, and two small signa irregular in shape with minute dark spots on distal portion and also scattered sclerotized pellets on the bursa wall (Fig. 2G). Ductus seminalis membranous, narrow, inserted in base of corpus bursae.

Immature stages. The number of larval instars was not determined, with three sap-feeding instars suspected and one spinning instar.

Egg. (Fig. 7C). Flat, slightly ellipsoid; $\sim 0.4 \times 0.25$ mm; chorion translucent; aeropyles, micropyles, and external ornamentation not observed.

Sap-feeding larva. (Figs 3A, 4, 7E). Body flattened dorsoventrally, yellowish translucent (Fig. 7E). Length of largest larva examined ~ 4.5 mm. Head brown, prognathous, setae absent (Fig. 4A–C). Two pairs of small stemmata located in the lateral region (Fig. 4F). Antenna 3-segmented, with four sensilla, two stout ones located on the second segment and two on the distal segment, one spiniform and other stout (Fig. 4F). Labrum slightly bilobed with small epipharyngeal spines, which are of greater size in the lateral region (Fig. 4D). Labium slightly bilobed with small spines near distal margin (Fig. 4E). Spinneret present, in the form of a transverse slit. Maxillary

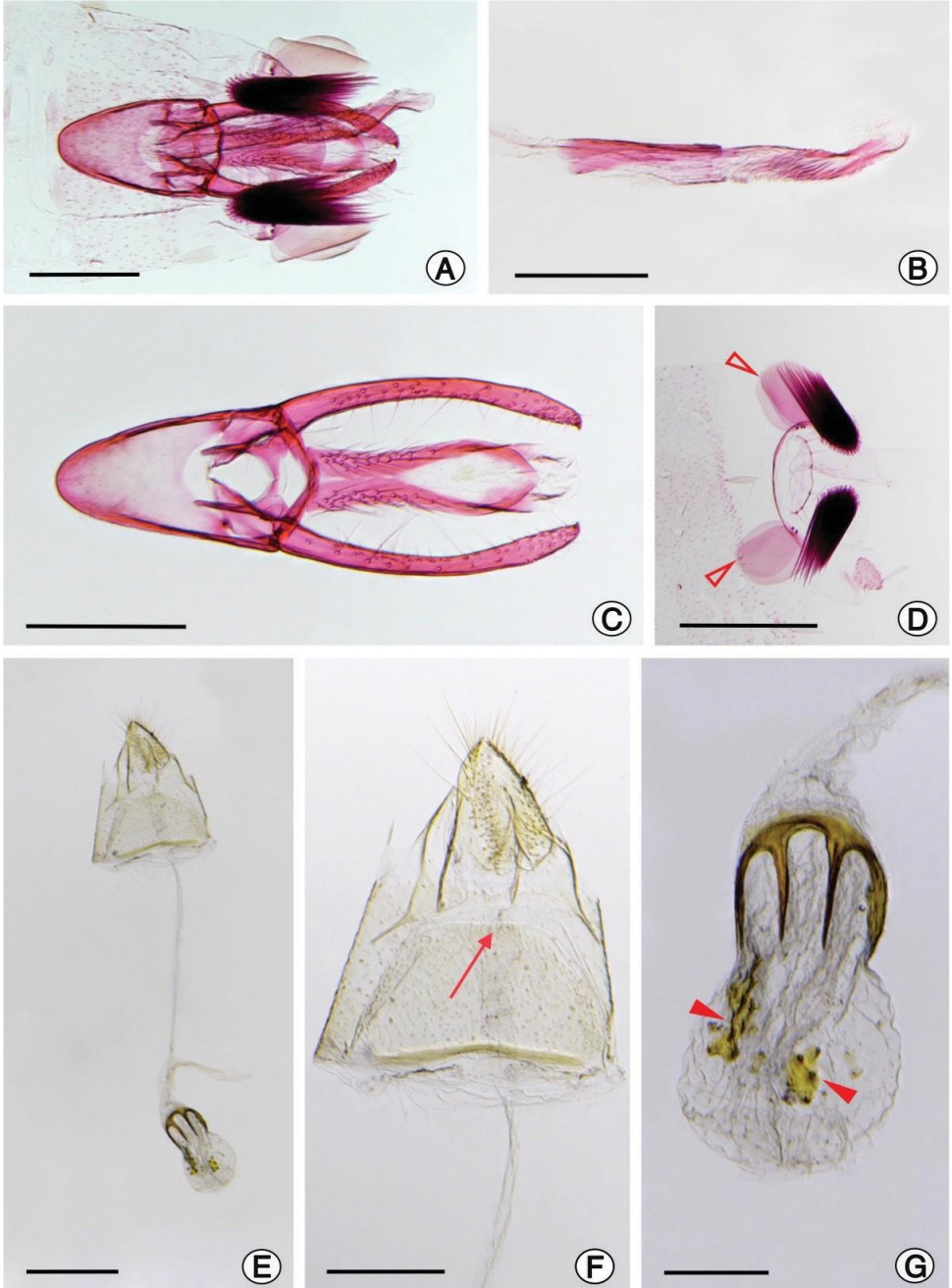


Figure 2. *Phyllocnistis furcata* sp. nov. genital morphology under light microscopy **A** male genital segments, ventral view **B** phallus, lateral **C** male genitalia with phallus removed, ventral **D** coremata (open arrows indicate wide rounded flat scales) **E** female genitalia, ventral **F** female last abdominal segments, in detail (seta points to ostium bursae) **G** corpus bursae in detail (closed arrows indicate two small signa with minute dark spots). Scale bars: 0.2 mm (**A-C, F, G**), 0.3 mm (**D**), 0.4 mm (**E**). Genitalia slides: MUSA_Gent_015142 (**A**), MUSA_Gent_015146 (**B-D**), MUSA_Gent_015148 (**E-G**).

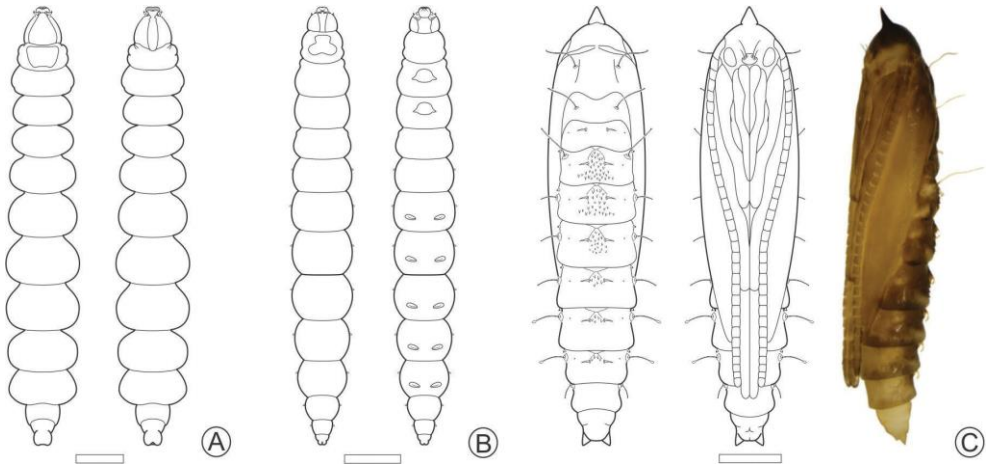


Figure 3. Larval and pupal morphology of *P. furcata* sp. nov. under light microscopy **A** sap-feeding larva, dorsal and ventral view **B** spinning larva, dorsal and ventral **C** pupa, dorsal, ventral, and lateral, respectively. Scale bars: 500 μ m.

and labial palpi absent. Legs and prolegs absent (Fig. 3A). Thorax with prothoracic light-brown dorsal shield in the form of a trapezoid (Figs 3A, 4G). Thoracic and abdominal segments without setae. Circular spiracle laterally on segments T1 and A1–A8 (Fig. 4H). Caudal abdominal segment slightly bilobed distally (Fig. 4I).

Spinning larva. (Figs 3B, 5, 7F). Body yellowish, cylindrical, wider along the thorax and first abdominal segments, narrowing towards the posterior region, covered with microtrichia (Figs 3B, 7F). Approx. 5.00 mm maximum length. Head capsule weakly sclerotized, with anteriorly pronounced trophic lobe (Figs 3B, 5A–C). Stemmata absent. Antenna short, three-segmented, with five sensilla (Fig. 5F). Clypeal region with three pairs of setae (Fig. 5D). Maxillary palpi, represented by a pair of short sensilla. Spinneret short (Fig. 5E). Thorax with slightly pronounced prothoracic dorsal shield (Fig. 3B). Legs and prolegs absent. A single ambulatory callus ventrally on center of meso- and metathorax (Figs 3B, 5G). One pair of smaller ambulatory calli ventrally on A3–A7 (Figs 3B, 5I, J). One pair of lateral campaniform sensilla on A2–A9 (Fig. 5K). Caudal abdominal segment slightly bilobed distally (Fig. 5L).

Pupa. (Figs 3C, 6, 7I). 1 Coloration changing from light yellowish during early stage of pupation to yellowish brown later in development (Fig. 7I). Approx. 5.00 mm maximum length. Cocoon-cutter triangular, concave dorsally (Fig. 6A–C) with serrated lateral edges (Fig. 6D). Frons with two pairs of large frontal setae close labrum (Fig. 6E). Labrum ellipsoidal (Fig. 6B). Antenna long and straight, extending to abdominal segment A7; forewing extending to A6 (Fig. 3C); prothoracic, mesothoracic and metathoracic legs reaching segments A3, A5 and A8, respectively (Fig. 3C). A pair of long setae, latero-dorsally on meso-, metathorax and A2 (Figs 3C, 6F). Lateral setae on abdominal segments A3–A7 (Fig. 3C); those of meso-, metathorax, A2–5 with dentate apex (Fig. 6I), those of A6–7 with clavate apex (Fig. 6H). A8 segment with a

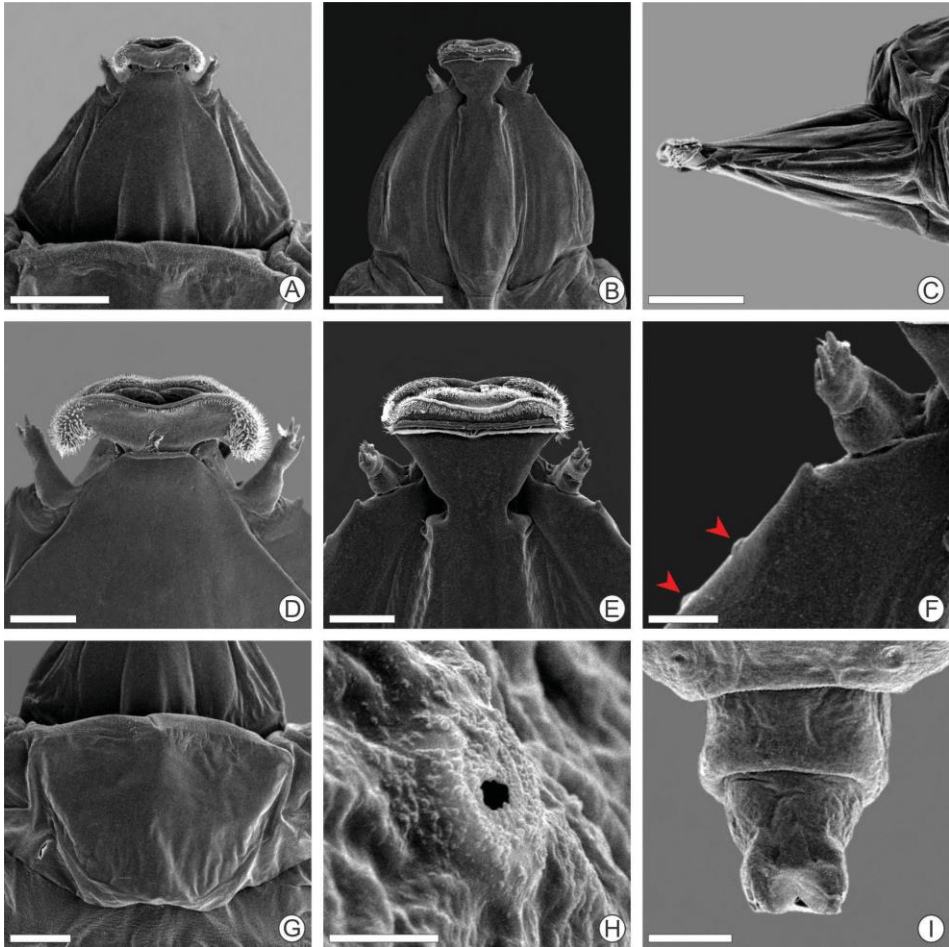


Figure 4. Scanning electron micrographs of *P. furcata* sp. nov. sap-feeding larva **A–C** head under dorsal, ventral, and lateral views **D** labrum, dorsal **E** labium, ventral **F** antenna and stemmata (indicated by arrow), ventral **G** prothoracic shield, dorsal **H** abdominal spiracle, lateral **I** last abdominal segment, ventral. Scale bars: 200 μm (**A**, **B**), 150 μm (**C**), 50 μm (**D**, **E**), 25 μm (**F**), 100 μm (**G**, **I**), 5 μm (**H**).

pair of acute setae latero-dorsally directed posteriorly (Fig 6K). One pair of conspicuous spiracles up to A2–A7 (Fig. 6J). Dorsum of A1–A7 with a pair of curved, large spines, projecting laterally, from A2 to A7 with a variable sized patch of smaller spines projecting posteriorly between them (Fig. 6G). One pair of small lateral spines on the pleural region from A1 to A7 (Fig 6H). Pleural region of body and last four abdominal segments covered by microtrichia (Fig. 6K, L). A pair of slightly divergent acute processes from caudal apex on last abdominal segment (Fig. 6K, L).

Etymology. The species name *furcata*, from the Latin adjective *furcatus*, *furca* meaning fork, alludes to the large and prominent form of the signum present in the female genitalia, resembling a garden fork.

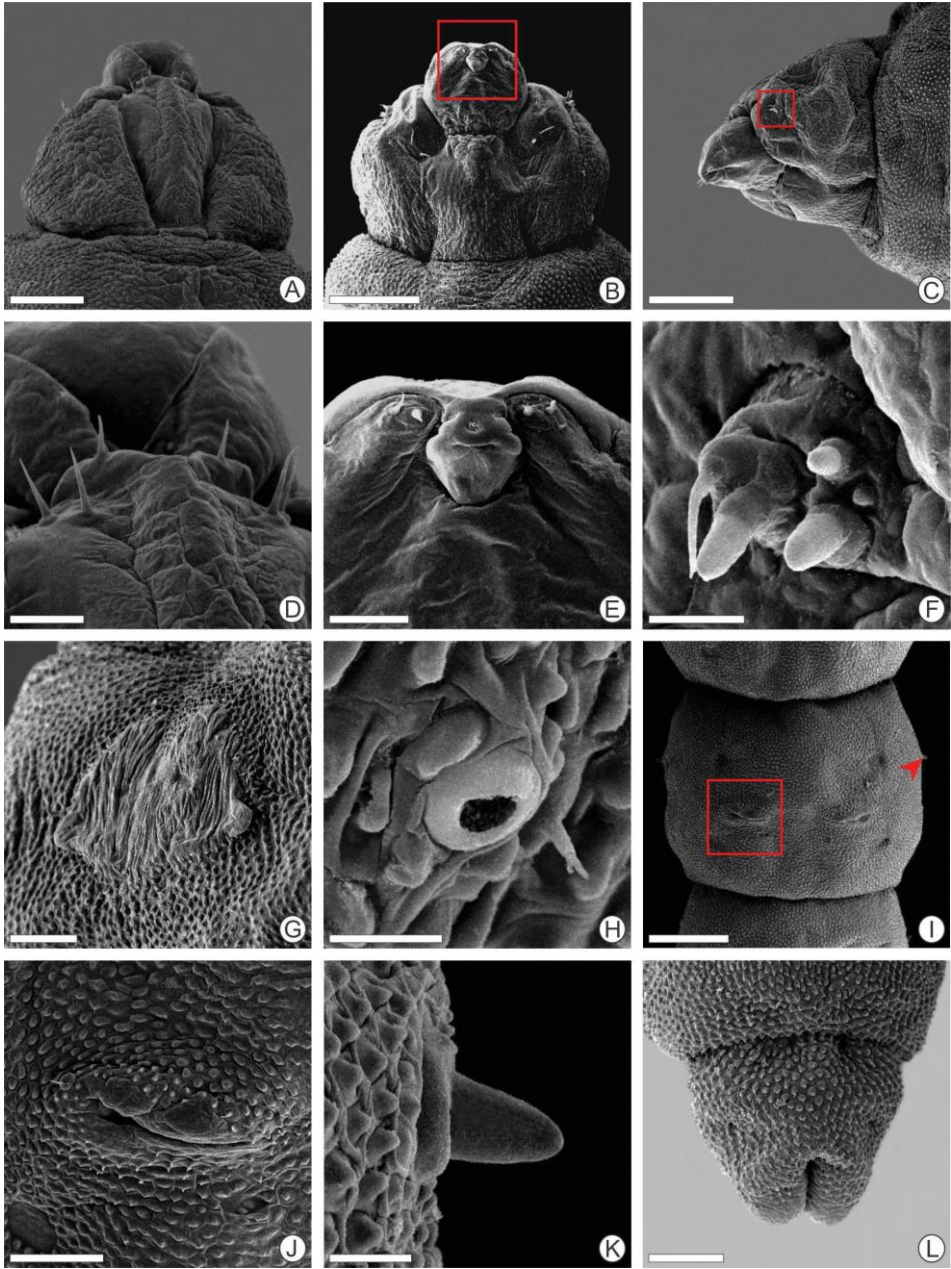


Figure 5. Scanning electron micrographs of *P. furcata* sp. nov. spinning larva **A–C** head under dorsal, ventral, and lateral views respectively **D** detail of trophic lobe and clypeal region, dorsal **E** spinneret, (indicated by square in **B**) **F** antenna, lateral (indicated by square in **C**) **G** mesothoracic ambulatory callus in detail, ventral **H** abdominal spiracle, lateral **I** abdominal segment A6, ventral (campaniform sensilla indicated by arrow) **J** abdominal ambulatory callus in detail, ventral (indicated by square in **I**) **K** campaniform sensilla in detail, ventral (indicated by arrow in **I**) **L** last abdominal segment, dorsal. Scale bars: 100 μm (**A**, **B**), 150 μm (**C**, **I**), 25 μm (**D**, **E**), 10 μm (**F**, **H**, **K**), 50 μm (**G**, **L**), 40 μm (**J**).

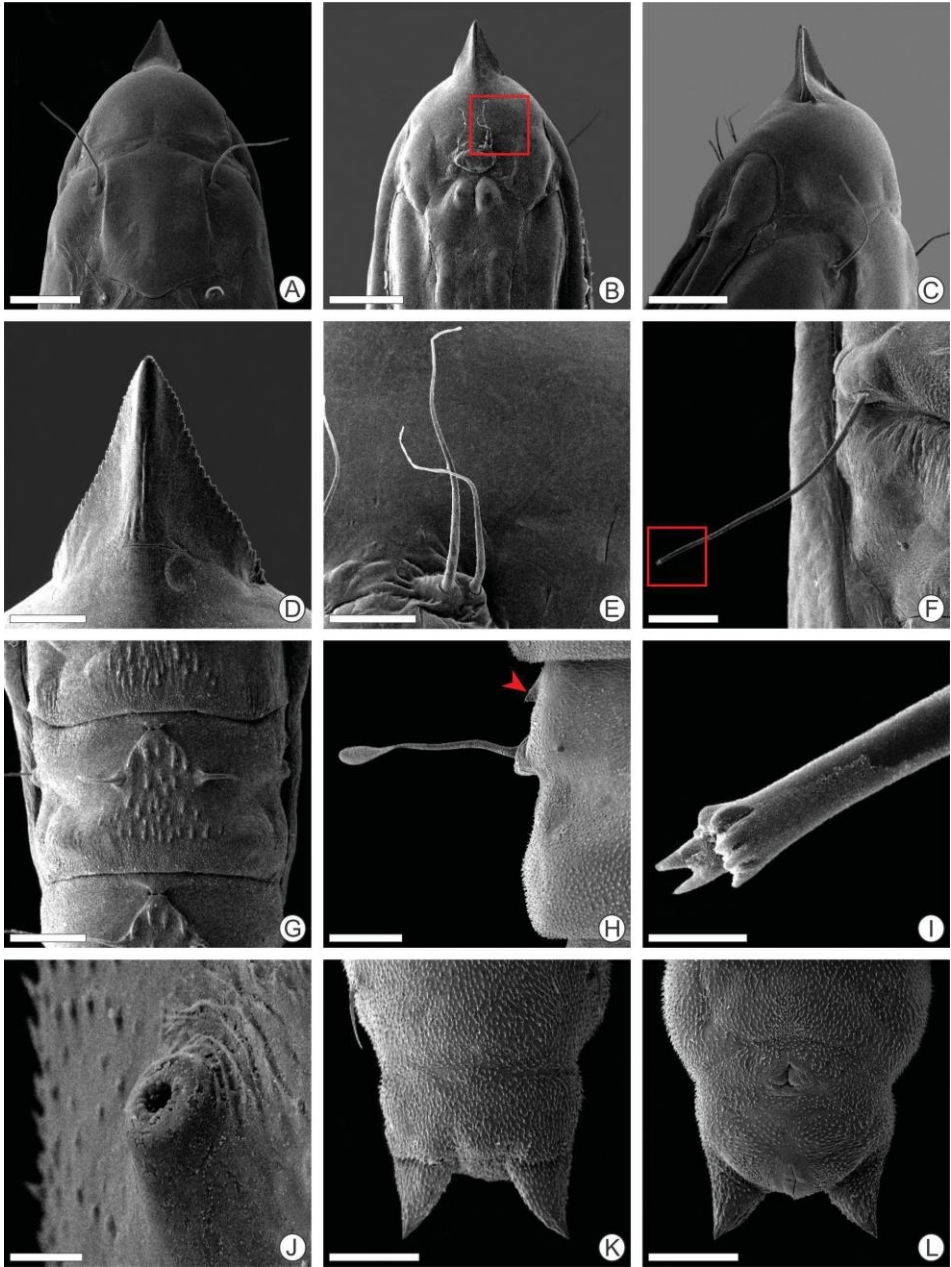


Figure 6. Scanning electron micrographs of *P. furcata* sp. nov. pupa **A–C** head under dorsal, ventral, and lateral views **D** cocoon-cutter, ventral **E** setae on clypeus, ventral (indicated by square in **B**) **F** lateral seta on abdominal segment A2, dorsal **G** detail of abdominal segment A3, dorsal **H** lateral seta with clavate apex, adjacent to spiracle (indicated by arrow) and close to small spine on abdominal segment A6, dorsal **I** detail of lateral seta distal portion, with dentate apex from abdominal segment A2, (indicated by square in **F**) **J** abdominal spiracle, lateral **K**, **L** last abdominal segments, dorsal and ventral, respectively. Scale bars: 200 μm (**A–C**, **G**), 50 μm (**D**), 40 μm (**E**), 100 μm (**F**, **H**, **K**, **L**), 10 μm (**I**, **J**).

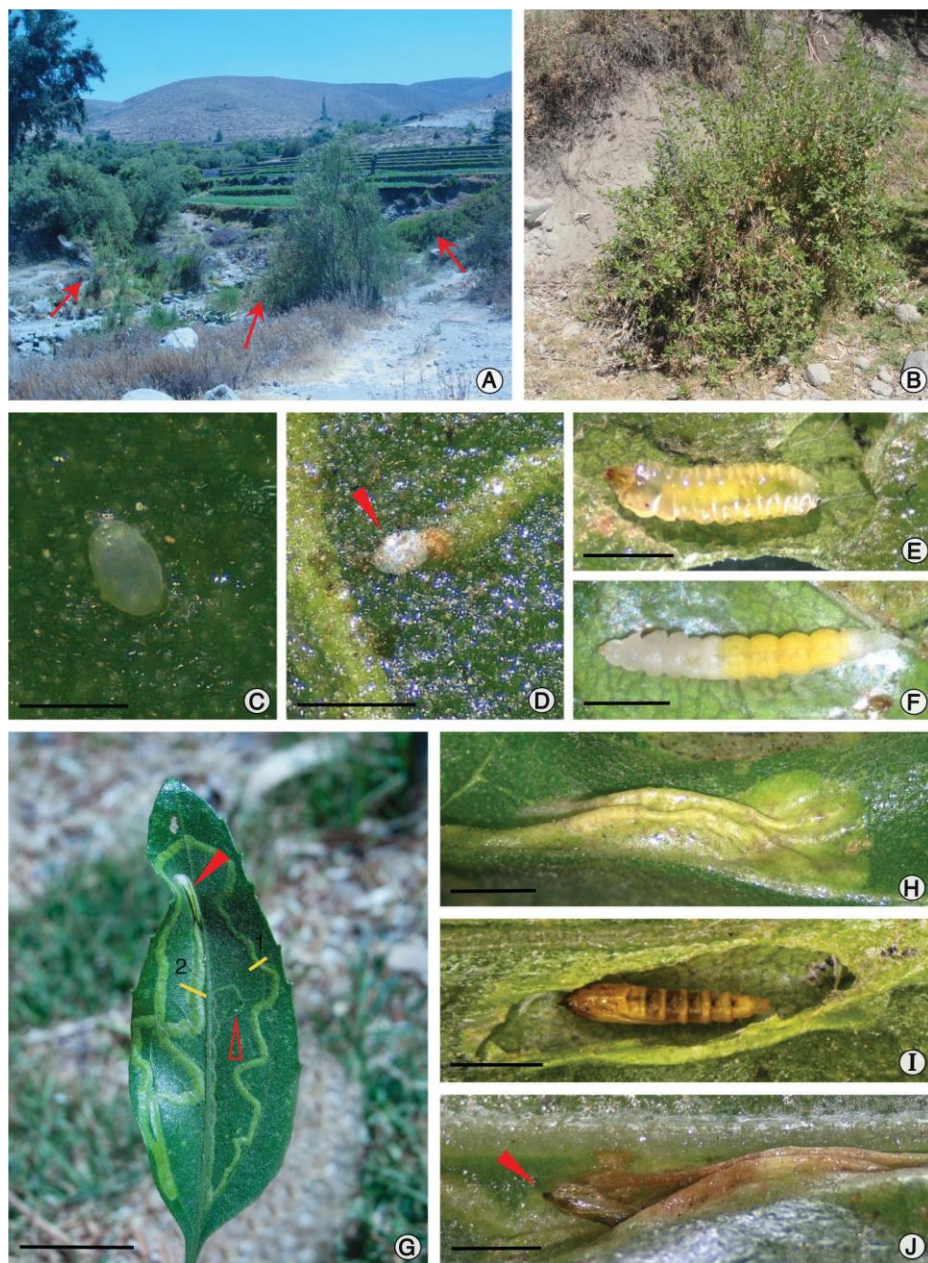


Figure 7. Natural history of *P. furcata* sp. nov. **A** type locality in Characato valley, Arequipa, southern Peru (setae point to *Baccharis* host-plants) **B** *B. alnifolia* plant, under close view **C** egg containing developing embryo **D** early mine with attached egg shell remains (pointed by arrow head) **E** sap-feeding larva, dorsal **F** spinning larva, dorsal view **G** leaf with a single *P. furcata* mine on adaxial surface (numbers indicate position of histological sections presented in Fig. 8; open and closed arrows indicated respectively the beginning and ending of the mine) **H** pupal cocoon, latero-dorsal **I** pupa, dorsal **J** pupal exuvium protruded from cocoon after adult emergence (close arrows points to pupal exuvium). Scale bars: 0.25 mm (**C**), 0.5 mm (**D**), 2 mm (**E, F**), 20 mm (**G**), 2.5 mm (**H-J**).

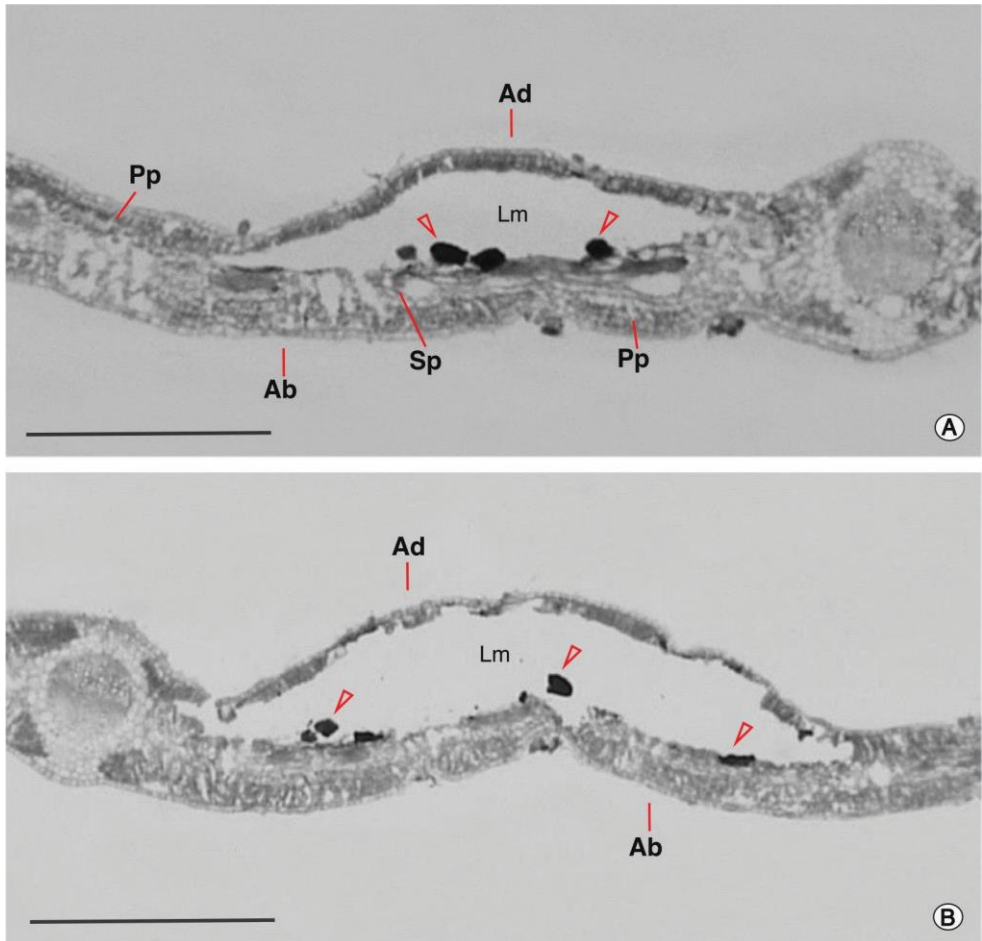


Figure 8. Transverse histological sections of *P. furcata* sp. nov. mine on *Baccharis alnifolia* Meyen & Walp. (Asteraceae) leaf **A** intermediate portion (location indicated by line 1 in Fig. 7G) **B** final portion (location indicated by line 2 in Fig. 7G). Feces are indicated by open arrows. Ab, epidermis of abaxial surface; Ad, epidermis of adaxial surface; Lm, leaf mine; Pp, palisade parenchyma; Sp, spongy parenchyma. Scale bars: 0.5 mm.

Host plant. (Fig. 7B). *Baccharis alnifolia* Meyen & Walp. (Asteraceae) is the only host plant known for the immature stages of *P. furcata*. This species is distributed from Peru to northern Chile, with an altitudinal range between 2400–3800 m (Beltran et al. 2006; Rodriguez et al. 2018). In Peru, *B. alnifolia* inhabits the western slopes of the Andes, distributed from the departments of La Libertad to Tacna (Beltran et al. 2006). It is commonly known as “chilca”, a shrub that reaches a height of 1.5 to 3 meters, and grows predominately on river banks (Brako and Zarucchi 1993).

Distribution. *Phyllocnistis furcata* is known only from the type locality, Characato, Arequipa, Peru (Fig. 7A).

Life history. (Figs 7C–J, 8). *Phyllocnistis furcata* mines are serpentine throughout their length, initially narrow, increasing in width to the end of the mine, covering most of the area of the leaf (Fig. 7G). Mines were found either on young leaves un-

der development or fully expanded ones, and almost all began near the midrib and extended along it. We found the majority of mines on the abaxial side of the leaf, and fewer on the adaxial side of the leaf. Most mines were found singly on a leaf; however, sometimes mined leaves carried two mines, either two on the abaxial side or one on each side. Mature mines are light green in color (Fig. 7G). Larvae are sap-feeders during the first instars (Fig. 7E) and are specialized in the palisade parenchyma, leaving the epidermis layers and generally the spongy parenchyma intact (Fig. 8). During the last spinning instar, it does not feed, but spins a cocoon within which pupation occurs (Fig. 7F). The cocoon is endophyllous, located on the final portion of the mine, during construction leading to a fold outside the leaf typical for *Phyllocnistis* (Fig. 7H). Before adult emergence, the anterior half of the pupa (head and thorax) protrudes out, while the posterior half remains in the pupal cocoon (Fig. 7J). In the examined mines, ~20% had a living and not parasitized larva or pupa. The remaining mines (~80%) were either empty or contained larvae or pupae which were either dead or parasitized by unidentified species of Hymenoptera; the affected stages varied from early sap-feeding larvae to pupal stage. Our field collection data indicate that the species may occur all year around in the area, with higher densities found in April and November.

Molecular data. (Fig. 9). The five DNA barcodes obtained for *P. furcata* (intraspecific distance = 0%) fall within the same clade, supporting the identification of the new species (Fig. 9). The nearest neighbor (BS = 57) is *P. ourea* (Fig. 9A), a *Baccharis*-feeding species. This pattern is consistent in MP and ML analysis (Fig. 9B, C, respectively), with node support (BS = 59). The mean distance between *P. furcata* and Neotropical *Phyllocnistis* (14.8%) is near the overall divergence within the genus (15.3%) and Neotropical groups (15.3%) (Table 2). The lowest divergence was observed between *P. furcata* and *P. ourea*. However, the *Baccharis*-feeding lineage *Phyllocnistis* sp. 12 showed high divergence distance (14.7%), similar to other species from the Neotropics.

Discussion

Phyllocnistis is one of the most species-rich genera of gracillariids, in which a number of taxa were recently described in the Neotropical region, predominantly from tropical and subtropical forests (Davis and Wagner 2011; Brito et al. 2012, 2016,

Table 2. Mean genetic distance (minimum – maximum) based on COI sequences using Kimura-2 parameters method for distinct phylogenetic arrangement of *Phyllocnistis* species, with special reference to *Phyllocnistis furcata* sp. nov. N, number of specimens used in the dataset.

Group	N	Mean (minimum – maximum)
All named species of <i>Phyllocnistis</i>	35	15.4% (3.3–22.1)
Neotropical <i>Phyllocnistis</i> (described + undescribed species)	29	15.3% (8.9–21.6)
<i>P. furcata</i> vs Neotropical <i>Phyllocnistis</i>	29	14.8% (11.9–18.5)
<i>P. furcata</i> vs. <i>P. ourea</i>	6	11.9%
<i>P. furcata</i> vs. <i>Phyllocnistis</i> sp. 12	7	14.7%

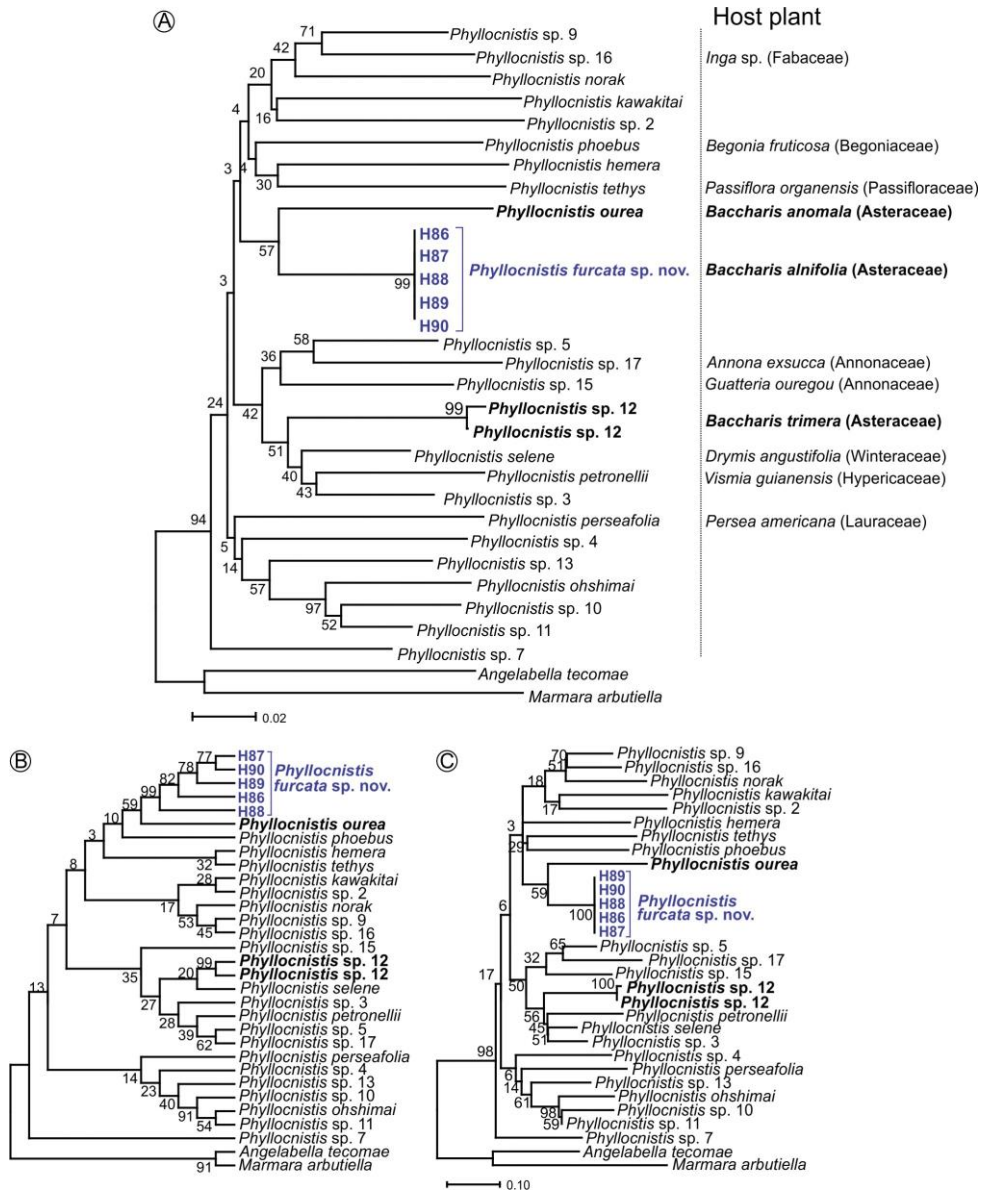


Figure 9. COI trees showing the specific classification of *Phyllocnistis furcata* sp. nov. (blue), and its position among 23 Neotropical *Phyllocnistis* lineages **A** phylogeny inferred using the Neighbor-Joining method with Kimura 2-parameter model. Host plants, when known, are indicated for each species [data were obtained from Brito et al. (2017) and BOLD database] **B** maximum parsimony consensus tree (length 1006, consistency index 0.3315, and retention index 0.4675) **C** maximum likelihood tree using the general time reversible model of sequence evolution. $-\ln$ likelihood = 4913.93 The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches in A, B, and C. *Angelabella tecomae* Vargas & Parra, 2005 (Oecophyllembiinae) and *Marmara arbutiella* Busck, [1904] (Marmarinae) were used as outgroups. Bold indicates species/lineages that use *Baccharis* species as host plants.

2017; Lees et al. 2013). Herein we describe an Andean new species, *P. furcata*, based on morphological and molecular characters that clearly separate it from congeneric species. The COI tree showed a monophyletic status for the new species, and different methods of reconstruction support the inference of its sister relationship with *P. ourea* among Neotropical lineages.

Overall, adults of *P. furcata* resemble the majority of Neotropical *Phyllocnistis* in general aspects of forewing pattern (Brito et al. 2017, 2019; Fochezato et al. 2018); nevertheless, comparing *P. furcata* with *P. ourea* and *P. baccharidis*, all associated with *Baccharis* as host plants, there are no characters in the wing pattern that group them together; however, they share the presence of a small stout spine at the apex of the valva in the male genitalia. However, a high genetic distance (11.4%) is found between *P. furcata* and *P. ourea*, suggesting either an ancient divergence of these sister species or incomplete sampling of Neotropical species, taking into account that much more *Phyllocnistis* species could be expected associated with the large genus *Baccharis* (440 sp), and what could also be expected the close relationships among these micromoths.

Phyllocnistis ourea clustered in all COI analysis as the closest related to *P. furcata*, sharing the same genus of host plant (*Baccharis anomala*), which could indicate a clade which is associated only with *Baccharis*. However, the undescribed *Phyllocnistis* sp. 12 (Brito et al. 2017), which feeds on *Baccharis trimera*, did not cluster in that group. Moreover, this lineage presents a high genetic divergence (ca. 15%) from *P. furcata*, near to the mean divergence found of all *Phyllocnistis* species, suggesting a convergent evolution of host plant use. Such pattern is distinct to described for a species group of *Phyllocnistis* that feed on Salicaceae, recovered as a single evolutionary clade in a COI tree (Kirichenko et al. 2018). Further inclusion of *P. baccharidis*, and eventually more species/lineages (currently unknown) associated with *Baccharis*, together with a multi-locus approach, will allow one to make more robust phylogenetic inferences and shed light on the diversification of Neotropical *Phyllocnistis* related to its host plants.

Interestingly, males of *P. furcata* possess two pairs of coremata on abdominal segment VIII. Only one pair, formed by long, slender, flattened scales is found generally in species of *Phyllocnistis* in which the coremata are described (e.g., Kawahara et al. 2009; Brito et al. 2012, 2019; Kirichenko et al. 2018). *Phyllocnistis furcata* presents one pair similar to those in other congeneric species, and a second pair formed by wide rounded flat scales, which may represent the first report of this type of structure for the genus. Furthermore, another interesting aspect is observed in females of *P. furcata*, where one of the three signa of the corpus bursae is prominent ($\sim 0.5 \times$ length of corpus bursae) with a particular fork-shaped appearance not observed in any other species of *Phyllocnistis*. This partially resembles the only signum of *P. tropaeolicola* Kawahara, Nishida & Davis, 2009, which has the form of a narrow band with two spines projecting inwards (Kawahara et al. 2009), but the spines are more prominent in *P. furcata*. *Phyllocnistis drimiphaga* Kawahara, Nishida & Davis, 2009 is another species with one of its two signa of large size, but five short spines arise from this signum. The remaining species of this genus mostly contain a small pair of fusiform signa or a single signum that occupies less than 2/3 of corpus bursae (Kirichenko et al. 2018; De Prins

et al. 2019). Also, there is no information about the variability of coloration pattern between females and males in other species of *Phyllocnistis*, notably that males are slightly darker than females in *P. furcata*. This has not been observed in other species of the genus, and should be reviewed in detail in the future.

The knowledge of immature stages of *Phyllocnistis* is also insufficient. In the majority of the species of the genus whose pupal morphology is described, pupae are characterized by a well-developed cocoon cutter and some abdominal terga (generally A2–A7) with a pair of prominent laterally curved spines and many small spines medially (Kobayashi and Hirowatari 2011; Brito et al. 2012, 2019; Fochezato et al. 2018; Kirichenko et al. 2018; Liu et al. 2018). However, a few species do not match this pattern perfectly. For instance, *P. subpersea* Davis & Wagner, 2011 has the cocoon cutter in the form of a pair of stout conical processes with a strongly recurved subapical spine (Davis and Wagner 2011). Curved spines of the abdominal terga are absent in two Neotropical species (Brito et al. 2012, 2019) and are present only on two segments of *P. citrella* (Kobayashi et al. 2013). The pupa of *P. furcata* matches well the general pattern of the genus. In addition, the prominent curved spines are present on A1–A7, a condition also found in *P. ourea*, whose larvae also feed on *Baccharis* (Brito et al. 2019). The apex of the lateral setae of A6 and A7 is clavate in the pupae of these two *Baccharis*-feeding *Phyllocnistis*. However, this condition is also found in other species whose larvae are associated with other plant families (Kawahara et al. 2009; Davis and Wagner 2011; Brito et al. 2019). In the spinning larva, a single ambulatory callus placed ventrally at the center of the meso- and metathorax is found in *P. ourea* (Brito et al. 2019), *P. furcata*, and also in the Thymelaeaceae-feeding *P. hemera* Brito & Fochezato, 2018, while lateral sensilla on abdominal segments are found in *P. hemera* (Fochezato et al. 2018) and *P. furcata*. In the sap-feeding larva, the presence of two stemmata, like in *P. furcata*, has been described for *P. ourea* and the Winteraceae-feeding *P. selene* Brito & Moreira, 2017 (Brito et al. 2019). Certainly, the external morphology of the immature stages should be explored in additional species of *Phyllocnistis* to have a more realistic perspective of the actual variation and its relationship with ecology and evolution.

The few studies in which the damage pattern caused by leaf miner larvae of Gracillariidae has been characterized using histological sections, suggest that its feeding activity can either be restricted to specific tissues throughout the leaf miner stage or the consumed tissues can change with larval ontogeny (Brito et al. 2012; Body et al. 2015; Moreira et al. 2018; Pereira et al. 2019; Vargas-Ortiz et al. 2019). In the case of *Phyllocnistis*, the larvae of *P. citrella* feed only in the epidermis of *Citrus* (Rutaceae) (Achor et al. 1997), and those of *P. tethys* Moreira & Vargas, 2012 on the spongy parenchyma of *Passiflora organensis* Gardn. (Passifloraceae) (Brito et al. 2012). Furthermore, larvae of *P. hemera* feed initially on the epidermis and later on the palisade parenchyma of *Daphnopsis fasciculata* (Meisn) Neveling (Thymelaeaceae) (Fochezato et al. 2018). The feeding behavior of larvae of *P. furcata* was found to be restricted to the palisade parenchyma of *B. alnifolia*.

The mines of *P. furcata* are found on adaxial and abaxial surfaces of the leaf. In contrast, those of *P. ourea*, another *Baccharis*-feeding *Phyllocnistis*, are restricted

to the adaxial surface of the leaf of its host *B. anomala* (Brito et al. 2019). Despite histological descriptions of leaves of *B. anomala* mined by *P. oura* not being available, it appears that the remarkable difference between the distribution pattern of the mines of the two species in the leaves of their hosts could be due, at least in part, to differences in leaf anatomy of the two hosts and the ability of the larvae to feed on palisade parenchyma. The organization of the mesophyll in *B. alnifolia* is isobilateral, with two or three layers of palisade parenchyma in each side and one or two layers of spongy parenchyma in the middle, a pattern reported for several species of *Baccharis* (Budel et al. 2018; Ornellas et al. 2019), while the organization of the mesophyll of *B. anomala* is dorsoventral, with two or three layers of palisade parenchyma and approximately three layers of spongy parenchyma (Budel and Duarte 2008). Given this diversity in leaf tissue structure, additional studies are needed to better understand the ecology and evolution of herbivory in *Phyllocnistis*. Certainly, histological descriptions of leaves mined by additional species of this genus will be helpful to propose hypotheses.

The known distribution of endemic species of *Phyllocnistis* in the western slopes of Peruvian Andes was previously restricted to the type localities of the two species described at the beginning of the last century by Edward Meyrick (1915, 1921) around the Department of Lima, in central Peru. Thus, the discovery of *P. furcata* in the Arequipa Department, as a result of recent evaluations of the Microlepidoptera fauna, provides the first record of this genus from southwestern Peru, at 2400 m, where the vegetation is dominated by xeric shrublands with abundant cacti (Montesinos et al. 2012, Heim 2014). However, as already mentioned, the taxonomic diversity of gracillariid species remain poorly studied in Peru, due to the absence of local specialists and collections of micromoths in general. As discussed in Brito et al. (2016), there exists a “taxonomic impediment” for the progress of studies on Neotropical gracillariids in general. Therefore, regional revisions of micromoth faunas would represent an important advance to the knowledge of this diverse group in Peru (e.g., Davis et al. 2020), particularly in areas that are subject to the highest rates of anthropic environmental degradation, like the environments of the southern Andes of Peru.

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CAPÍTULO III

ARTIGO FORMATADO CONFORME NORMAS EDITORIAIS DA REVISTA ZOOTAXA

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A contribution to the knowledge of leaf-mining *Phyllocnistis* Zeller, 1848 associated with *Baccharis* (Asteraceae), with description of two new species from Peru (Lepidoptera: Gracillariidae)

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Abstract

We provide new information on Neotropical *Phyllocnistis* Zeller (Gracillariidae) associated with *Baccharis* (Asteraceae) in the Andes region. Two new species are

described, *P. canta* Cerdeña & Farfán, **n. sp.** and *P. elongata* Cerdeña & Farfán, **n. sp.**, from the western slopes of the Andes of Peru between 2,000 and 3,000 m elevation, both associated with *Baccharis latifolia*. The type locality of *P. baccharidis* Hering is confirmed; immature stages were found at 1,300 m feeding on *Baccharis tucumanensis* in Quebrada las Higueras (Tucumán, Argentina). Four new locality records for *P. furcata* Vargas & Cerdeña are provided, extending its distribution by 450 km to the north of the type locality.

Key words: Andes, Lima, Tacna, Tucumán, Phyllocnistinae, taxonomy

Introduction

The leaf-mining genus *Phyllocnistis* Zeller, with 113 species described, is one of the most diverse and widely distributed genera in Gracillariidae (De Prins & De Prins 2021). The immature stages are associated with a great variety of host plants, including 26 recognized plant families (De Prins & Kawahara 2009). Host plants are known for about 45% of the *Phyllocnistis* species in the Neotropical region (Brito *et al.* 2017), among them *P. ourea* Brito & Moreira, *P. baccharidis* Hering, *P. furcata* Vargas & Cerdeña and one undescribed species, which feed on species of the genus *Baccharis* (Asteraceae) (Brito *et al.* 2017; Cerdeña *et al.* 2020).

The New World genus *Baccharis* is widely distributed in the Neotropical region, adapted to a great variety of ecosystems, with 440 recognized species (Heiden *et al.* 2019). Various families of Lepidoptera have been recorded feeding either on flowers (e.g. Vargas 2010, 2020; Vargas & Duarte 2014), or leaves (e.g. Stonis *et al.* 2016; Vargas 2019; Vargas & Moreira 2012) or inducing galls (Vargas *et al.* 2015) in *Baccharis*. Only *P. furcata* has been reported using this genus as a host plant in Peru (Cerdeña *et al.* 2020).

A survey carried out recently on the western slopes of the Andes of Peru found *Baccharis* plants showing damage of leaf miners with a pattern similar to that produced by Gracillariidae larvae. Adults of three species of *Phyllocnistis* were obtained by rearing the immature stages; *P. furcata* and two new species that are described herein based on adult morphology. Mitochondrial DNA sequences (COI) of those species and *P. baccharidis* were used to calculate the genetic distances and infer the nearest neighbors among Neotropical *Phyllocnistis* species.

Material and methods

Material examined. Pupae dissected from leaf mines on *Baccharis* spp. from the western slopes of the Andes of Peru between 2000 and 3000 m elevation from the Lima, Ica, Arequipa and Tacna Departments were reared in plastic cups during June, 2020, September, 2020, and July, 2021. An intensive search for pupae in mines on leaves of *Baccharis* spp. was also carried out in the type locality of *Phyllocnistis baccharidis* (Tucumán, Argentina) during March, 2020, with the purpose of confirming its type locality and obtaining further material for molecular analyses. The adults obtained were pinned and dried following standard procedures.

Morphological analysis. The abdomen of each specimen was removed and cleared by heating in hot 10% KOH for ~ 10 minutes; in localities where adults did not emerge, pupae in late stages of development were used to examine the genitalia, following the same procedure. Genitalia were stained with Chlorazol black and stored in microvials with glycerol, pinned together with specimens. Morphological observations were performed with the aid of a Zeiss Stemi305 stereomicroscope, and structures selected to be illustrated were photographed with a Nikon DS-Ri2 camera through a Nikon SMZ25 stereomicroscope. Images were assembled and edited in Nikon NIS-Elements and Photoshop CS5.1. Terminology used for descriptions of adult wing patterns and genitalia follows Brito *et al.* (2017, 2019).

Molecular analysis. Genomic DNA was extracted from six pupae (Table 1) following the procedures described in Huanca-Mamani *et al.* (2015). A fragment of 658 base pairs of the COI gene was amplified by polymerase chain reaction (PCR) with the primers LEP-F1 and LEP-R1 (Hebert *et al.* 2004). PCR reactions were performed in a final volume of 20 μ L. Each reaction contained 1 μ L DNA extract, 10 pmoles each primer, 2.5 mM each dNTP, 2 mM MgCl₂, 1X PCR buffer (KCl), 1 unit Taq DNA polymerase (Thermo Scientific) and sterile distilled water. The amplification program was 5 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 47 °C, 1 min at 72 °C, and a final elongation step of 10 min at 72 °C. Three μ L of each PCR product was visualized on 1.5% agarose gels stained with gel-red (Biotium). Reactions containing fragments of the expected size were purified and sequenced directly by a commercial facility (Macrogen, South Korea). The new sequences obtained in this study are publicly available in the BOLD (project DS-GRANEO) database. To explore the genetic distances and the nearest neighbors of the new species we combined our COI data with a data set obtained from the literature (Table

1). A distance tree based on the Neighbor-joining (NJ) method was generated from 34 sequences of COI and rooted with *Marmara arbutiella* Busck (Marmarinae) (*Marmara arbutiella*_MG4656949) using MEGA X (Kumar *et al.* 2018), with 1000 bootstrap replications. Sequence divergences were quantified using the Kimura 2-parameter model in MEGA.

Abbreviations for the institutions where specimens were deposited are:

MUSA Museo de Historia Natural, Universidad Nacional de San Agustín de Arequipa, Arequipa, Perú.

MUSM Museo de Historia Natural, Universidad Nacional Mayor San Marcos, Lima, Perú.

Results

Three species of *Phyllocnistis* were found in association with *Baccharis* plants between 2,000 and 3,000 m elevation on the western slope of the Andes of Peru: *P. furcata* and two new species that are described below. Four new record localities for *P. furcata* were obtained: Llauta (14°14'45"S / 74°55'14"W; 2,600 m; Ica Department), Chuquimaran (14°48'58"S / 74°37'52"W; 2,700 m; Ica Department), Cotahuasi (15°11'19"S / 72°52'46"W; 2,600 m; Arequipa Department) and Chuquibamba (15°50'57"S / 72°37'56"W; 2,500 m; Arequipa Department), extending its distribution by approximately 450 km to the north of its type locality. *P. baccharidis* was located in its type locality (Tucumán, Argentina) at 1,300 m elevation on *Baccharis tucumanensis*.

Phyllocnistis canta Cerdeña & Farfán, n. sp.

(Figs. 1, 2, 3)

Type locality. Peru, Lima, Canta [11°28'S; 76°38'W], 2450 m.

Type material: Holotype m#: Peru, Lima, Canta, 2450m, 11°28'48"S / 76°38'13"W, ix.2020, J. Cerdeña & J. Farfán [leg.], reared from pupae collected on *Baccharis latifolia* (Asteraceae); deposited in MUSM; Paratypes (3m#, 2f#): 1m# and 1f#: Peru, Lima, Matucana, 2300m, 11°51'11"S / 76°24'0.88"W, ix.2020, J. Cerdeña & J. Farfán [leg.], reared from pupae collected on *Baccharis latifolia* (Asteraceae), deposited in MUSA; 2m# and 1f#: Peru, Lima, Yauyos, 2900m, 12°20'09"S / 75°49'30"W, vi.2020, J. Cerdeña

& J. Farfán [leg.], reared from pupae collected on *Baccharis latifolia* (Asteraceae), deposited in MUSA.

Other material. Immature stages preserved in 70% ethanol, all deposited in MUSA: 12 pupae: Peru, Lima, Canta, 2450m, 11°28'48"S / 76°38'13"W, ix.2020, J. Cerdeña & J. Farfán [leg.]; 07 pupae: Peru, Lima, Matucana, 2300m, 11°51'11"S / 76°24'0.88"W, ix.2020, J. Cerdeña & J. Farfán [leg.]; 06 pupae: Peru, Lima, Yauyos, 2900m, 12°20'09"S / 75°49'30"W, vi.2020, J. Cerdeña & J. Farfán [leg.].

Diagnosis. Adults of *P. canta* **n. sp.** can be distinguished from all other known species of Neotropical *Phyllocnistis* in the forewing pattern by a combination of the following characters: ground color silver with light-brown color on inner margin below the longitudinal fascia; four distinct transverse fasciae; transverse fascia 1 reduced to the costal margin and fused to the longitudinal fascia, both connected to transverse fascia 2; transverse fascia 3 reaching the inner margin of the wing. This species can still be recognized with robust male genitalia by an invagination in the middle of arch of the tegumen, and in the female abdomen by a large expansion of VIII abdominal tergum. *P. canta* **n. sp.** is similar to *P. furcata* in wing size and fasciae patterns. However, in *P. furcata* the inner margin of the wing below the longitudinal fascia is silver, while in *P. canta* **n. sp.** it is light brown.

Description. MALE (Fig. 1. A, B): Forewing length 3.10 - 3.33 mm (N = 4). *Head*: Vestiture silvery light brown. Antennae brown, becoming dark towards apex; approximately equal to length of forewing. Labial palpus slender, covered with light grey scales. Proboscis without scales. *Thorax*: Forewing ground color silvery white with a light brown area close to the inner margin below light orange longitudinal fascia (lf). Also, three transverse (tf) fasciae; lf bordered by dark brown scales, extending 2/3 length of wing from base of costa and connected to tf1 apically; tf1 does not reach the inner margin, restricted between the costal margin and lf; tf2 connected to lf+tf1, slightly convex, crossing the wing entirely; tf3 separate from tf2, reaching the inner margin; tf4 separate from tf3, crossing the wing entirely. Apex of forewing with a well-marked black spot. Costal strigulae light orange, emerging from the base of transverse fasciae. Apical strigulae dark brown, emerging from black spot. Inner marginal fringe varying from orange to dark brown. Hind wings and legs light grey. *Abdomen*: generally dark grey, covered with silvery pale, brown scales. Two pairs of coremata present laterally on segment VIII; one pair consisting of a set of flat, long scales and the other formed by wide rounded flat ones. *Genitalia* (Fig. 2. A, B): Uncus absent. Tegumen membranous,

approximately equal to length of the valva, with spines arranged laterally from the base to the medial region, and with a conspicuous invagination in the middle of the arch. Saccus V-shaped, well developed, $\sim 0.5\times$ the size of valva. Valvae digitiform and slightly convergent from the base to the apex, which bears a small spine. Phallus wider in the proximal part and with a slightly convex apex, weakly sclerotized, slightly longer than valva; cornuti absent. FEMALE: Forewing length 3.30 - 3.41 mm (N = 2). Color and pattern similar to that of male. Tergum of VIII abdominal segment with large, medium distal expansion. *Genitalia* (Fig. 3. A, B, C): Papillae anales slightly sclerotized, covered with hair-like setae. Posterior apophyses $\sim 2\times$ length of anterior apophyses. Ostium bursae posterior to sternum VII. Ductus bursae completely membranous, slender, elongate, over $7.0\times$ length of papillae anales. Corpus bursae slightly elongated, $\sim 0.2\times$ length of ductus bursae, mainly membranous. A prominent signum on base of corpus bursae, with two short, acute to rounded, flattened externally projected spines. Ductus seminalis membranous, narrow, inserted in base of corpus bursae.

Etymology. The specific epithet is based on the type locality of Canta (Lima, Peru) where this new species was found. A feminine noun.

Distribution. *Phyllocnistis canta* n. sp. is known from the western slopes of the Andes in Department Lima, Peru. This species was recorded from three river basins: Chillón, Ríma, and Cañete, between 2,000 and 3,000 m elevation. (Fig. 4).

Host plant. *Baccharis latifolia* (Asteraceae), which is a shrub 1 to 2 m in height, widely distributed in South America; it is abundant in Peru, where it grows mainly on riverbanks between 1,000 and 4,000 m elevation (Beltrán 2016; Vilcapoma & Beltrán 2018).

***Phyllocnistis elongata* Cerdeña & Farfán, n. sp.**

(Figs. 1, 2, 3)

Type locality. Peru, Tacna, Tarata [17°29'S; 70°02'W], 2900 m.

nnnnnType material: Holotype m#: Peru, Tacna, Tarata, 2900m, 17°29'32"S / 70°02'42"W, vii.2021, J. Cerdeña & J. Farfán [leg.], reared from pupae collected on *Baccharis latifolia* (Asteraceae), deposited in MUSM; Paratypes (5 m#, 4 f#): 4m# and 4f#: same data as holotype, deposited in MUSA; 1m#: Peru, Tacna, Candarave, 2980m, 17°19'15"S / 70°14'52"W, vii.2021, J. Cerdeña & J. Farfán [leg.], reared from pupae collected on *Baccharis latifolia* (Asteraceae), deposited in MUSA.

Other material. Immature stages preserved in 70% ethanol, all deposited in MUSA: 10 pupae: same data as holotype; 03 pupae: Peru, Tacna, Candarave, 2980m, 17°19'15"S/70°14'52"W, ix.2020, J. Cerdeña & J. Farfán [leg.]; 05 pupae: Peru, Tacna, Candarave, 2960, 17°19'19"S/70°11'38"W, ix.2020, J. Cerdeña & J. Farfán [leg.].

Diagnosis. Adults of *P. elongata* **n. sp.** can be easily distinguished from all other known species of Neotropical *Phyllocnistis* in the forewing pattern by the light-brown color on the inner margin below the longitudinal fascia that bears two equidistant brown spots. The male genitalia of *P. elongata* **n. sp.** is remarkably similar to that of *P. furcata*. However, the two species can be accurately separated based on the female genitalia, as *P. elongata* **n. sp.** lacks the two small irregular signa that are present in *P. furcata*.

Description. MALE (Fig. 1. C, D): Forewing length 4.12–4.58 mm (N = 6). *Head*: Vestiture silvery brown. Antennae light brown, becoming dark towards apex, approximately equal to forewing in length. Labial palpus slender, covered with light grey scales. Proboscis without scales. *Thorax*: Forewing ground color silvery white with light brown area below longitudinal fascia bearing two equidistant, brown spots. One light orange longitudinal (lf) and three transverse (tf) fasciae; lf bordered by dark brown scales, extending 2/3 length of wing from base of costa and connected with tf1 apically; tf1 does not reach the inner margin, restricted between the costal margin and lf; tf2 connected to lf+tf1, lightly convex, crossing the wing entirely; tf3 separate from tf2, reaching the inner margin; tf4 separate from tf3, crossing the wing entirely. Apex of forewing with a well-marked black spot. Costal strigulae light orange, emerging from the base of transverse fasciae. Apical strigula dark brown, emerging from black spot. Inner marginal fringe varies from orange to light brown. Hindwings and legs light grey. *Abdomen*: dark grey covered with silvery scales. Two pairs of coremata present laterally on segment VIII; one pair consisting of a set of flat, long scales and the other formed by wide, rounded flat scales. *Genitalia* (Fig. 2. C, D): Uncus absent. Tegumen membranous, approximately equal to valve in length, with spines arranged laterally from the base to the medial region. Saccus V-shaped, well developed, ~ 0.6× the size of valva. Valvae digitiform and slightly convergent from the base to the apex, that bears a small spine. Phallus slender, with a slightly convex apex, weakly sclerotized, slightly longer than valva; cornuti absent. FEMALE: Forewing length 3.80 - 4.32 mm (N = 4). Color and pattern similar to that of male. *Genitalia* (Fig. 3. D, E, F): Papillae anales slightly sclerotized, covered with hair-like setae. Posterior apophyses ~ 2× length of anterior apophyses. Ostium bursae posterior to sternum VII. Ductus bursae completely membranous, slender, elongate, over 6.0×

length of papillae anales. Corpus bursae slightly elongated, $\sim 0.2\times$ length of ductus bursae, mainly membranous. A prominent signum on base of corpus bursae with two large flattened spines projecting from one side, varying from acute to rounded. Ductus seminalis membranous, narrow, inserted in base of corpus bursae.

Etymology. The specific epithet is the feminine of the Latin participle “elongatus”, meaning 'prolonged' or 'elongated', and is in reference to the elongate wings of this species, reaching up to 4.58 mm of forewing length, one of the longest wings among Neotropical *Phyllocnistis*.

Distribution. *Phyllocnistis elongata* **n. sp.** is known from the western slopes of the Andes in the Tacna Department, Peru. This species is recorded from two river basins: Locumba, and Sama, between 2,800 and 3,000 m elevation. (Figure 4).

Host plant. *Baccharis latifolia* (Asteraceae), as described for *P. canta* **n. sp.**

Discussion

As a result of our study, the diversity of *Phyllocnistis* in the Andes has increased to ten species: *P. abatiae* Hering, *P. aurilinea* Zeller, *P. baccharidis* Hering, *P. canta* **n. sp.**, *P. elongata* **n. sp.**, *P. furcata* Vargas & Cerdeña, *P. perseafolia* Davis & Wagner, *P. puyehuensis* Davis, *P. sexangula* Meyric, and *P. wygodzinskyi* Hering. However, this species richness is still underestimated, considering the variety of Andean ecosystems and habitats and taking into consideration that most of these species were described in the early twentieth century based on few specimens and low sampling effort (Brito *et al.* 2017). The present study also found two undescribed species associated with the same plant species (*Baccharis latifolia*) within an area that covers a small altitudinal band of 2,000 – 3,000 m elevation in a xerophytic zone, characterized by very low diversity compared to the eastern slopes of the Andes. Thus, it is highly probable that the number of Andean species of *Phyllocnistis* will increase greatly in the future, and the prediction made by Brito *et al.* (2016), that the number of gracillariid species in the Neotropical region is approximately 20 times greater than currently known could be correct.

The first species of *Phyllocnistis* reported with immature stages feeding on *Baccharis* was *P. baccharidis* from Argentina, collected in March, 1953 by Dr. Petr W. Wygodzinsky (also called Peter, Pedro, and affectionately “Wygo”) (Schuh & Hernan 1988), whose type locality in the original description is detailed as “*Quebrada de las Higueras, Choromoro, Tucumán (800 m)*” (Hering 1958). Our search for this species in the type locality was carried out near the town of Choromoro, from 700 to 1,500 m

elevation in the Quebrada de las Higueras; a large number of mines were located only on *Baccharis tucumanensis* at circa 1,300 m (26°24'31"S / 65°33'19"W), near the Gonzalo village. However, it should be noted that between 700 and 1,200 m elevation no mine damage was found on the leaves of any *Baccharis* species in the surroundings of Choromoro and Quebrada las Higueras. Therefore, the altitude recorded in the original description of *P. baccharidis* corresponds to the altitude where the town of Choromoro is located, which may not be exactly where the material of *P. baccharidis* was originally collected.

The COI tree indicates that both *P. elongata* **n. sp.** and *P. canta* **n. sp.** are monophyletic and highly divergent (7-20%) from other Neotropical *Phyllocnistis* (Fig. 5, Table 2). *Phyllocnistis furcata* is the nearest neighbor (bootstrap = 58%) of *P. elongata* **n. sp.**, whereas *P. canta* **n. sp.** is close (bootstrap = 25%) to the cluster ((*P. furcata* + *P. elongata*) (*P. ourea* + *P. baccharidis*)), which forms a group associated with *Baccharis* (Figure 5). A notable exception is *Phyllocnistis* sp12, an undescribed species from Brazil associated with *Baccharis trimera*, placed outside this ‘*Baccharis* clade’ (*P. canta* ((*P. furcata* + *P. elongata*) (*P. ourea* + *P. baccharidis*)). *Phyllocnistis canta* **n. sp.** and *P. elongata* **n. sp.** use the same host plant *Baccharis latifolia*, but are not sister species. Although COI provided enough variation to calculate genetic distance of the Neotropical species of *Phyllocnistis*, to achieve a robust assessment of the evolutionary relationships and infer a potential convergence in the use of *Baccharis* as host plant, a substantial increase in the number and type of loci investigated is necessary.

The adults of three Andean species reported here from Peru present consistent differences in wing patterns, mainly in their ground color (Figure 1), despite being very close to each other in the COI sequence analysis (7% genetic distance) (Figure 5; Table 2) and using the same host plant genus (*Baccharis*). The closest related species according to molecular data are *P. furcata* and *P. elongata* **n. sp.**, but they show a greater difference in their wing ground color in relation to other Neotropical congeners. This could indicate that the wing patterns of *Phyllocnistis* may be considered as homoplastic characters, a hypothesis to be confirmed in a future study where phylogenetic relationships should be based also on morphological characters.

The morphology of the male and female genitalia of the two new species described herein generally appears relatively uniform among the species of *Phyllocnistis*. The male genitalia of all the species associated with *Baccharis* revised here (*P. canta* **n. sp.**, *P. elongata* **n. sp.**, *P. furcata* and *P. baccharidis*) possess two pairs of coremata on

abdominal segment VIII described and illustrated by Cerdeña *et al.* (2020), whereas the two remaining species (*P. ourea* and *P. sp. 12*) should be reviewed in detail in order to confirm whether they share this character. However, *P. canta* **n. sp.** differs from the other species by the presence of an invagination of the tegumen arch not observed in any other Neotropical *Phyllocnistis* (Figure 2A). The female genitalia among the revised species shows the shape and the number of signa as a diagnostic character; *P. canta* **n. sp.** is the only species that exhibits an elongated, pointed abdominal tergum VIII (Fig. 3. A, B), also a new and unique feature for the genus.

The three Peruvian species of *Phyllocnistis* recorded on *Baccharis* inhabit xeric shrubland of the Andes, and are found exclusively between 2,000 and 3,000 m elevation (Figure 4A). This distribution pattern, added to the evidence provided by our preliminary phylogenetic analysis, reflects a probable allopatric speciation pattern, in particular of *P. furcata* and *P. elongata*, which form a monophyletic clade and share the same host-plant species. A similar pattern was recently described in the same geographic area for allopatric, possibly cryptic, species of *Angelabella* Vargas & Parra, 2005 (Gracillariidae) based on DNA barcode analysis (Vargas-Ortiz *et al.* 2020). The main probable geographical barriers to *Phyllocnistis* associated with *Baccharis* in Peru are the northern valleys in the Department of Ica and the river Tambo valley in the Department of Moquegua in the south. In these valleys leaf-miner damage on *Baccharis* plants attributable to *Phyllocnistis* was not found. In order to elucidate the mode in which these speciation events have occurred, it is necessary to carry out an exhaustive analysis with distribution models that take into account not only the evolutionary history of this group of species but also that of *Baccharis*.

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(Legends to the figures)

Figure 1. Adults of *Phyllocnistis canta* n. sp. (A-B), *Phyllocnistis elongata* n. sp. (C-D) and *Phyllocnistis furcata* Vargas & Cerdeña (E-F). (A, C, E) dorsal view, (B, D, F) detail of right forewing. Scale bars: 2 mm (A, C, D, E), 1 mm (B, F).

Figure 2. Male genitalia of *Phyllocnistis canta* n. sp. (A-B), *Phyllocnistis elongata* n. sp. (C-D) and *Phyllocnistis furcata* Vargas & Cerdeña (E-F), under light microscopy. (A, C, E) ventral view, (B, D, F) aedeagus, lateral view. Scale bars: 0.2 mm (A, C, E), 0.1 mm (B, D, F). Arrow indicates invagination in the middle of tegumen arch (A).

Figure 3. Female genitalia of *Phyllocnistis canta* n. sp. (A-C), *Phyllocnistis elongata* n. sp. (D-F) and *Phyllocnistis furcata* Vargas & Cerdeña (G-I) under light microscopy. (A, D, G) lateral view, (B, E, H) last abdominal tergum in dorsal view, (C, F, I) corpus bursae in detail. Scale bars: 0.5 mm (A, D, G), 0.2 mm (B, E, H), 0.1 mm (C, F, I). Arrows indicate expansion of VIII abdominal segment (A: also marked with asterisk in B), signum bearing two small spines (C) and additional sclerotized areas bearing microspines (I).

Figure 4. Distribution of *Phyllocnistis* (Gracillariidae) species associated with *Baccharis* (Asteraceae) in Peru. (A) Records are for *P. canta* n. sp. (green circles), *P. furcata* Vargas & Cerdeña (red circles) and *P. elongata* n. sp. (yellow circles). (B) and (C) Type localities of *P. canta* n. sp. (Canta, Lima) and *P. elongata* n. sp. (Tarata, Tacna), respectively. *Baccharis* host plants of *P. canta* n. sp. and *P. elongata* n. sp. are indicated in both cases by red arrows.

Figure 5. Distance tree (Neighbor-joining) based on sequences of a 658-bp fragment of the cytochrome c oxidase I (COI) gene, showing the monophyletic status of the new species of *Phyllocnistis* and their nearest neighbors among Neotropical *Phyllocnistis* lineages, and the position of *P. baccharidis* Hering. The use of species of *Baccharis* as host plant by members of Neotropical *Phyllocnistis* is indicated.

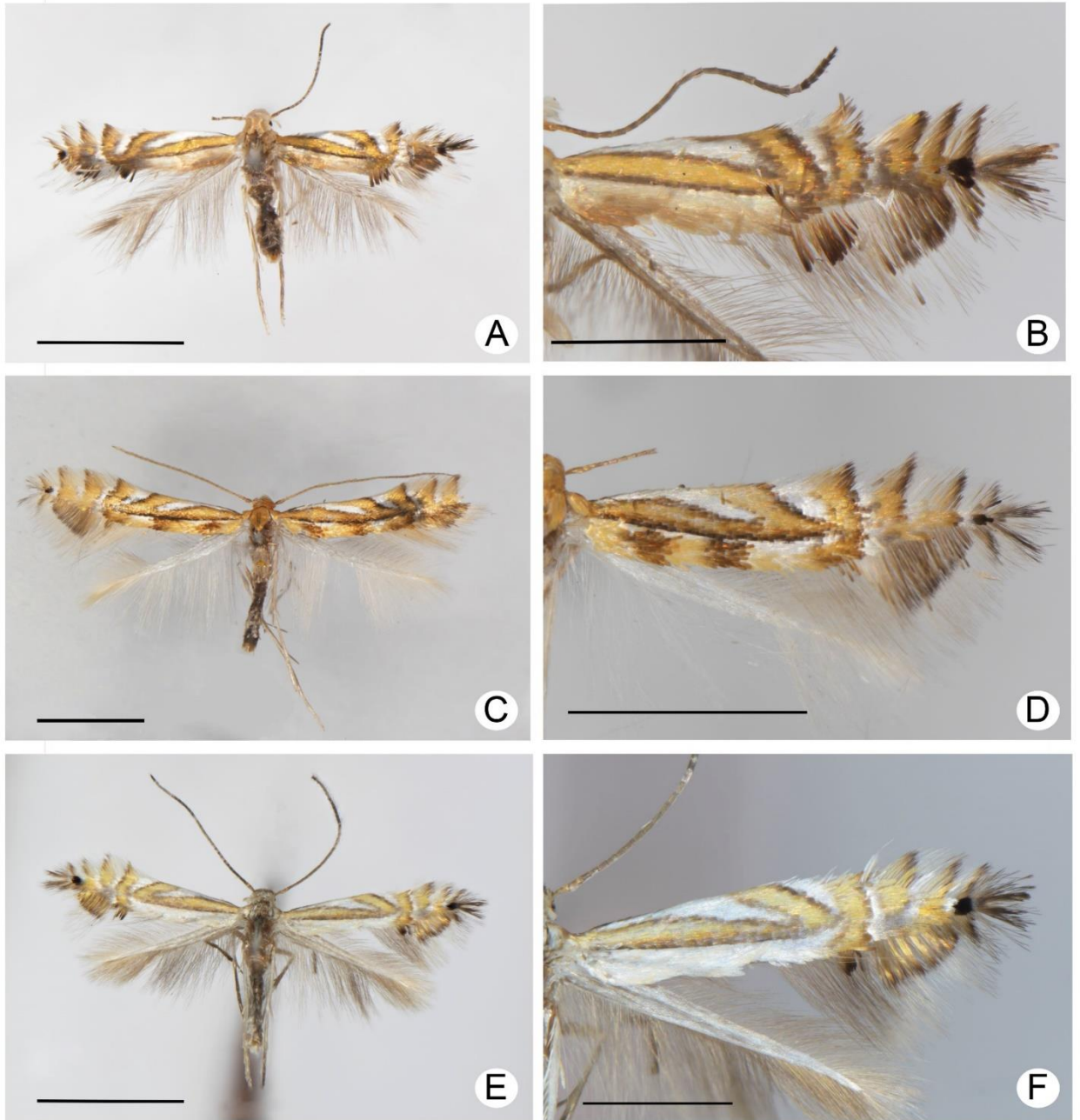
Table 1. Neotropical *Phyllocnistis* used for molecular analyses. Both the Sample ID and Process ID codes are unique identifiers linking the record in the BOLD database and the voucher specimen from which the sequence is derived. Asterisks indicate those specimens associated with *Baccharis* as host plant.

Species	Sample ID	Process ID	Reference
<i>Phyllocnistis baccharidis</i> *	J147	MISA059-21	This study
<i>Phyllocnistis canta n. sp.</i> *	J146	MISA060-21	This study
<i>Phyllocnistis elongata n. sp.</i> *	J122	MISA056-21	This study
<i>Phyllocnistis elongata n. sp.</i> *	J125	MISA057-21	This study
<i>Phyllocnistis elongata n. sp.</i> *	J145	MISA058-21	This study
<i>Phyllocnistis furcata</i> *	J148	MISA061-21	This study
<i>Phyllocnistis furcata</i> *	H86	MISA051-20	Cerdeña <i>et al.</i> 2020
<i>Phyllocnistis furcata</i> *	H87	MISA052-20	Cerdeña <i>et al.</i> 2020
<i>Phyllocnistis hemera</i>	LMCI 292-25C	MISA019-17	Fochezato <i>et al.</i> 2018
<i>Phyllocnistis kawakitai</i>	AK0105	GRANO105-11	Lees <i>et al.</i> 2013
<i>Phyllocnistis norak</i>	CLV1381	LNOUC318-10	Lees <i>et al.</i> 2013
<i>Phyllocnistis ohshimai</i>	CLV1367	LNOUC304-10	Lees <i>et al.</i> 2013
<i>Phyllocnistis ourea</i> *	LMCI 297-15B	MISA013-16	Brito <i>et al.</i> 2016
<i>Phyllocnistis petronellii</i>	IO0536	LEPPC2394-15	Brito <i>et al.</i> 2017
<i>Phyllocnistis petronellii</i>		LEPPC2394-16	Brito <i>et al.</i> 2017
<i>Phyllocnistis perseafolia</i>	DDAV-D555	RDOPO393-10	Davis & Wagner 2011
<i>Phyllocnistis perseafolia</i>	DDAV-D556	RDOPO393-10	Davis & Wagner 2011
<i>Phyllocnistis phoebus</i>	LMCI 263-9	MISA014-16	Brito <i>et al.</i> 2016
<i>Phyllocnistis selene</i>	LMCI 263-22	MISA015-16	Brito <i>et al.</i> 2016
<i>Phyllocnistis tethys</i>	LMCI 174-55-1	GBMIN15477-13	Brito <i>et al.</i> 2012
<i>Phyllocnistis</i> sp. 2	AK0198	LNOUD2290-12	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 3	AK0210	LNOUD2302-12	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 5	CLV1284	LNOUD1191-12	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 7	CLV1368	LNOUC305-10	Lees <i>et al.</i> 2013

Species	Sample ID	Process ID	Reference
<i>Phyllocnistis</i> sp. 9	CLV2993	LNOUD336-11	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 10	CLV3313	LNOUD489-11	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 11	CLV4347	LNOUD776-12	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 12*	CLV5900	GRPAL1220-13	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 12*	CLV5901	GRPAL1220-13	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 13	CLV5902	GRPAL1222-13	Lees <i>et al.</i> 2013
<i>Phyllocnistis</i> sp. 15	LEAFMINE2015-0006	LEPPC1378-15	Brito <i>et al.</i> 2017
<i>Phyllocnistis</i> sp. 16	LEAFMINE2015-0008	LEPPC1380-15	Brito <i>et al.</i> 2017
<i>Phyllocnistis</i> sp. 17	LEAFMINE2015-0010	LEPPC1382-15	Brito <i>et al.</i> 2017

Table 2. Pairwise genetic distances between Neotropical *Phyllocnistis* based on 658 base pair sequences of the cytochrome oxidase I (CoI) gene using the Kimura 2-parameter model. Divergence to the outgroup (*Marmara arbutiella*) is also presented. Bold indicates comparisons of the new species, *P. canta n. sp.* and *P. elongate n. sp.*

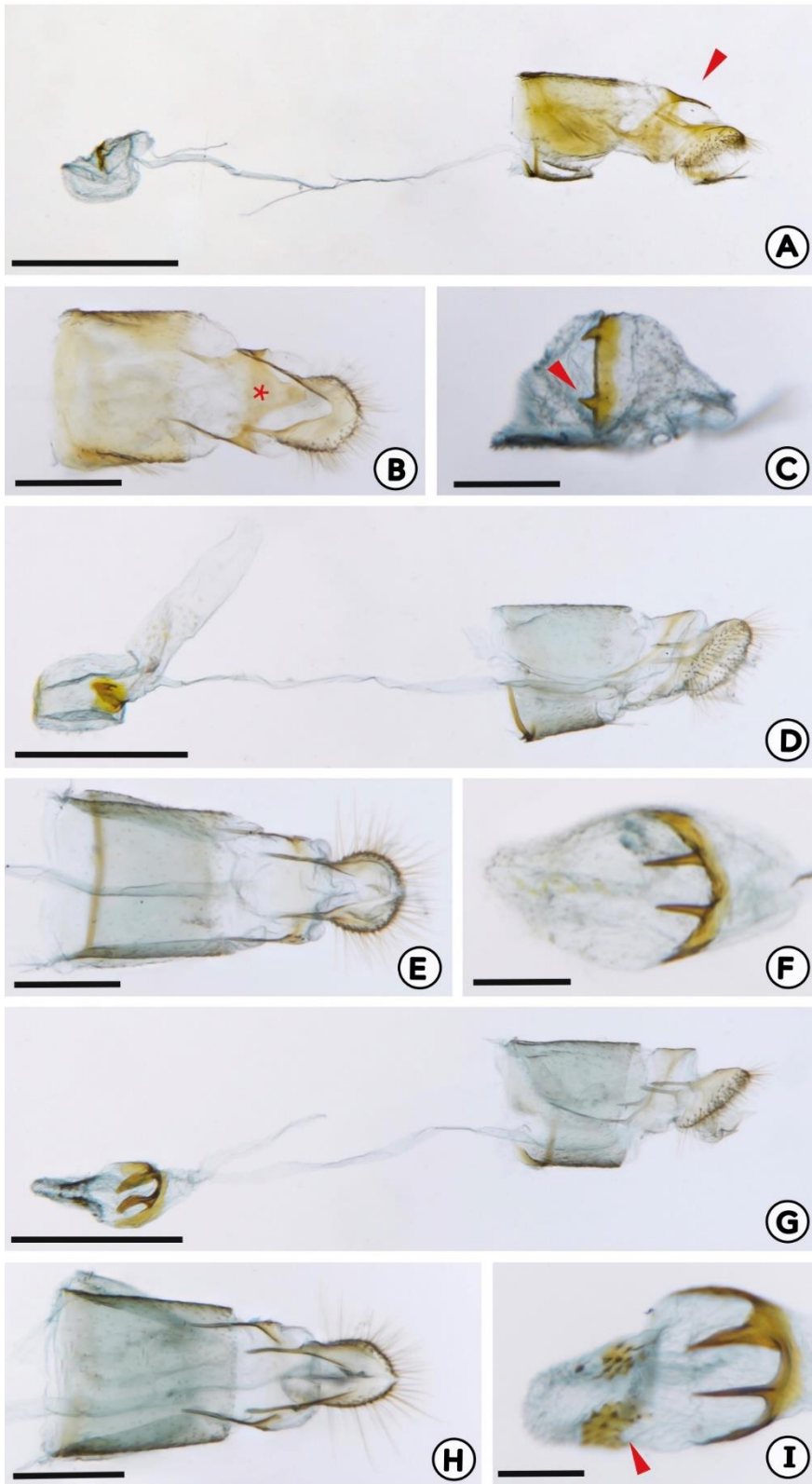
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.	16.	17.	18.	19.	20.	21.	22.	23.	24.	25.	26.
1. <i>P. baccharidis</i>																										
2. <i>P. canta n. sp.</i>	0.15																									
3. <i>P. elongata n. sp.</i>	0.12	0.12																								
4. <i>P. furcata</i>	0.08	0.11	0.07																							
5. <i>P. hemera</i>	0.17	0.15	0.14	0.14																						
6. <i>P. kawakitai</i>	0.18	0.18	0.20	0.17	0.20																					
7. <i>P. norak</i>	0.20	0.17	0.19	0.18	0.20	0.18																				
8. <i>P. ohshimai</i>	0.17	0.16	0.16	0.14	0.18	0.16	0.15																			
9. <i>P. ourea</i>	0.12	0.14	0.14	0.12	0.20	0.19	0.19	0.19																		
10. <i>P. perseafolia</i>	0.18	0.19	0.17	0.16	0.19	0.21	0.16	0.18	0.17																	
11. <i>P. phoebus</i>	0.16	0.16	0.17	0.17	0.20	0.19	0.19	0.22	0.15	0.21																
12. <i>P. petronellii</i>	0.17	0.17	0.15	0.14	0.19	0.22	0.20	0.17	0.18	0.20	0.21															
13. <i>P. selene</i>	0.15	0.15	0.13	0.13	0.15	0.19	0.16	0.15	0.15	0.19	0.16	0.12														
14. <i>P. tethys</i>	0.18	0.17	0.20	0.18	0.18	0.22	0.19	0.20	0.20	0.19	0.17	0.22	0.17													
15. sp2	0.20	0.20	0.19	0.16	0.19	0.19	0.16	0.22	0.20	0.19	0.21	0.21	0.17	0.20												
16. sp3	0.15	0.14	0.13	0.13	0.18	0.20	0.20	0.16	0.16	0.18	0.18	0.09	0.08	0.17	0.19											
17. sp5	0.16	0.17	0.12	0.12	0.17	0.19	0.19	0.15	0.18	0.18	0.18	0.12	0.12	0.20	0.19	0.11										
18. sp7	0.18	0.15	0.15	0.14	0.18	0.21	0.18	0.15	0.16	0.16	0.18	0.20	0.16	0.17	0.21	0.16	0.15									
19. sp9	0.16	0.15	0.14	0.14	0.15	0.16	0.11	0.14	0.17	0.16	0.16	0.16	0.14	0.17	0.18	0.16	0.14	0.17								
20. sp10	0.17	0.19	0.18	0.16	0.20	0.20	0.16	0.10	0.19	0.17	0.19	0.17	0.14	0.18	0.21	0.17	0.14	0.15	0.14							
21. sp11	0.14	0.17	0.14	0.13	0.15	0.19	0.15	0.07	0.16	0.13	0.18	0.17	0.13	0.18	0.20	0.16	0.15	0.14	0.10	0.07						
22. sp12	0.15	0.16	0.15	0.14	0.19	0.22	0.20	0.17	0.18	0.21	0.17	0.14	0.10	0.20	0.21	0.11	0.15	0.16	0.17	0.16	0.16					
23. sp13	0.15	0.17	0.14	0.13	0.17	0.21	0.16	0.13	0.15	0.15	0.16	0.18	0.15	0.19	0.17	0.18	0.13	0.17	0.14	0.12	0.09	0.17				
24. sp15	0.21	0.16	0.17	0.18	0.18	0.20	0.19	0.18	0.18	0.17	0.19	0.17	0.15	0.22	0.21	0.16	0.15	0.18	0.18	0.18	0.16	0.17	0.18			
25. sp16	0.17	0.17	0.16	0.16	0.18	0.18	0.14	0.17	0.18	0.16	0.17	0.19	0.16	0.18	0.17	0.16	0.16	0.18	0.10	0.16	0.14	0.18	0.13	0.18		
26. sp17	0.18	0.19	0.16	0.16	0.18	0.21	0.18	0.16	0.22	0.18	0.18	0.15	0.15	0.21	0.24	0.13	0.11	0.20	0.15	0.17	0.17	0.17	0.15	0.15	0.18	
Outgroup	0.27	0.26	0.25	0.25	0.26	0.26	0.25	0.23	0.26	0.25	0.25	0.26	0.25	0.27	0.29	0.23	0.23	0.23	0.24	0.23	0.21	0.26	0.23	0.25	0.25	0.25



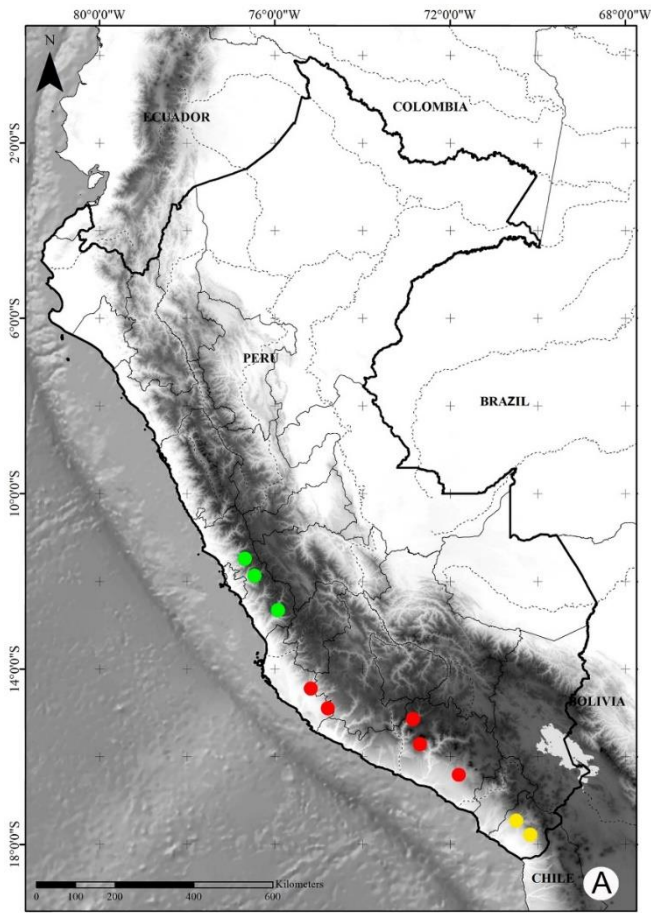
Cerdeña *et al.* – Figure 01



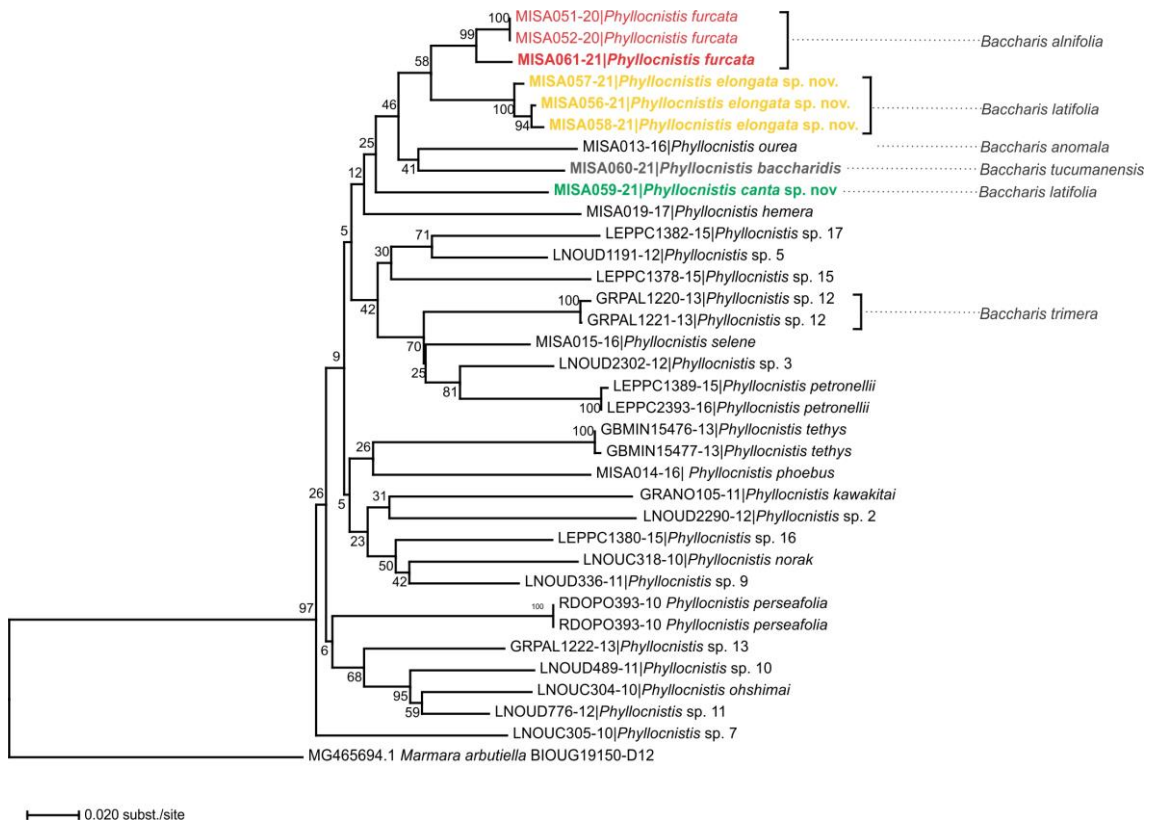
Cerdeña et al. – Figure 02



Cerdeña et al. – Figure 03



Cerdeña *et al.* – Figure 04



Cerdeña *et al.* – Figure 05

CAPÍTULO IV

CONCLUSÕES GERAIS

Com base nos resultados obtidos, conclui-se que as populações de *Phyllocnistis* (Gracillariidae) encontradas nas encostas ocidentais dos Andes, no Peru, correspondem a três novas espécies associadas a duas espécies de *Baccharis* (Asteraceae), distribuídas entre 2 e 3 mil m de altitude. Uma primeira espécie, *Phyllocnistis furcata* Vargas & Cerdeña, 2020 está associada à espécie *Baccharis alnifolia* no sul do Peru. As outras duas espécies foram encontradas em associação com *Baccharis latifolia*, porém, uma espécie distribuída no extremo sul do Peru, próximo à fronteira com o Chile, e a outra espécie distribuída no centro do país.

Phyllocnistis furcata representa a primeira espécie do gênero relatada no Peru em associação com o gênero *Baccharis*. Esta espécie apresenta padrão de asa semelhante a outras espécies neotropicais, porém caracteres únicos foram encontradas em suas estruturas genitais, como possuir dois pares de coremata em machos no último segmento abdominal, um par formado por largas escamas achatadas arredondadas, não mencionado para as demais espécies congênicas. Além disso, outro aspecto interessante é observado nas fêmeas, por possuírem três signa, onde um é proeminente com uma aparência em forma de garfo não observada em nenhuma outra espécie congênica. Em relação aos seus estágios imaturos, eram formados por quatro estágios: ovo, sap-feeding, spinning e pupa; o último apresentou caracteres que podem ser usados no diagnóstico para separar espécies. A arquitetura da mina foliar apresenta-se em forma de serpentina, característica das outras espécies do gênero, e o comportamento alimentar das larvas de *P. furcata* ficou restrito ao parênquima paliçádico. Na análise molecular esta espécie foi agrupada com *Phyllocnistis ourea*, uma espécie do sul do Brasil, associada a *Baccharis anomala*.

A descrição das duas novas espécies adicionais junto com a confirmação da localidade tipo de *P. Baccharidis* forneceu informações valiosas para as espécies associadas com *Baccharis*. Em primeiro lugar, permite inferir que todas as espécies associadas com *Baccharis* revisadas até o momento possuem dois pares de coremata no segmento abdominal VIII descritos e ilustrados apenas para *P. furcata*; segundo, através da análise molecular podemos evidenciar que todas as espécies conhecidas até hoje associadas com *Baccharis* formam um clado, exceto para uma espécie não descrita do Brasil associada com *B. trimera*, o que deve ser melhor investigado no futuro.