University of Nevada, Reno

It's the shape that matters! The diverse world of genitalia: A taxonomic and evolutionary exploration of the neotropical genus *Eois* Hübner (Lepidoptera: Geometridae: Larentiinae)

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Ecology, Evolution, and Conservation Biology

by

Lydia Mai Doan

Dr. Lee A. Dyer/Dissertation Advisor

Dr. Matthew L. Forister/Dissertation Co-advisor

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We recommend that the dissertation
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LYDIA M. DOAN

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DOCTOR OF PHILOSOPHY

Lee A. Dyer, Ph.D.
Advisor

Matthew L. Forister, Ph.D.
Co-advisor

John W. Brown, Ph.D.
Committee Member

Chris R. Feldman, Ph.D.
Committee Member

Marjorie D. Matocq, Ph.D.
Graduate School Representative

Markus Kemmelmeier, Ph.D., Dean
Graduate School

August, 2023

Abstract

Eois Hübner (Geometridae: Larentiinae) is a hyperdiverse genus of moths containing 267 valid species, but with estimates of 1,000 or more Neotropical species yet to be described. The paucity of contemporary descriptive and distributional data for these moths not only limits monitoring and conservation efforts of potentially vulnerable populations, but also hinders investigations into ecological and evolutionary factors underlying the diversity of *Eois.* To begin to remedy shortcomings in our knowledge of this moth group, I conducted research on three different aspects of *Eois* systematics and evolution.

1) I conducted morphometric analysis of cryptic species at a site in the Ecuadoran Andes, evaluating relationships between genetic and genitalic variation of morphologically similar (i.e., cryptic) species in the *Eois olivacea* clade, and investigating the extent that elevation and host plant associations influence evolutionary patterns across Ecuadorian populations. Based on 170 individuals sampled from different elevations and host plants at a single site within the Ecuadorian Andes, population genetic analyses revealed that samples can be assigned to four distinct taxa, with genetic divergence among taxa associated with different host plants. Morphometric analysis indicated that the adult samples belong to three distinct taxa, and molecular dating analysis implied that these taxa form a monophyletic clade that began diverging approximately five million years ago.

2) I circumscribed and described 16 new species of the *Eois olivacea* clade based on traditional morphological techniques using specimens from various institutional collections (UNR, AMNH, BMNH, USNM and McGuire Center), employing a data

matrix of 107 morphological characters. I defined the clade based on wing pattern and other morphological features, and then provided detailed diagnoses and descriptions of each new species, as well as re-examining the four previously described species in the clade.

3) I analyzed male vs. female features of the genitalia to further our understanding of sexual traits and evolution of genitalia in *Eois*. I considered different mechanisms of diversification of genitalia and differences among the sexes, including genetic drift, pleiotropy, female choice, cryptic female choice, male to male competition, sexual conflict, and the lock-and-key hypothesis. I also explored variation of female versus male traits. Using the morphological data matrix mentioned above, I developed a phylogeny for a sample of 99 species (94 *Eois* and five outgroup taxa), using maximum parsimony and maximum likelihood methods, and separate dendrograms based on male-only and female-only characters. An examination among trees revealed discordance between dendrograms based on male-only and female-only traits, suggesting at least partially independent evolution of traits between the sexes.

The Neotropical moth genus *Eois* is a remarkable group, and includes dazzling species with complex ecologies and fascinating evolutionary patterns. Nevertheless, understanding that diversity has been challenging, and if estimates of the species richness are correct, the genus is among the most species-rich in all of Lepidoptera. The results presented here provide a starting point in undertaking the challenging endeavor of describing the hundreds of new species of *Eois*. The results also represent a foundation for investigating sexual trait evolution in the genus, largely because knowledge of functional morphology of *Eois* genitalia is limited, as is information on mating

interactions, mating costs and benefits, the physical interaction of male and female genital structures, and rates of evolutionary divergence of these animals.

Dedication

To my one and only love Michael.

You have been my rock against all the waves and storms throughout this journey.

Your unwavering support and love have made this possible.

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I would like to thank my doctoral committee members, Dr. Lee Dyer, Dr. Matt Forister, Dr. John Brown, Dr. Marjorie Matocq, and Dr. Chris Feldman, for their patience, for providing exceptional mentorship, and for their constructive and thoughtful feedback that has been instrumental to this work.

I want to especially express my deepest gratitude to my advisors, Lee and Matt, for giving me multitudes of opportunities to learn and grow as a scientist. Thank you for seeing the potential in me, for always listening and answering every little question I had.

I am also very grateful to John. Your positive and upbeat nature made even the most technical, mundane task exciting. I appreciate your insightful critiques, and giving me the opportunity to develop valuable skills in taxonomy and systematics.

Additionally, I am very grateful for the support and feedback I received from the members of Robinson/Dyer lab, Forister lab, Plant-Insect Group and the wider EECB community. In particular, I would like to thank Aramee Diethelm, Saw Sudta, Kelli McKeegan, Ericka Kay, Cas Carroll, Christian Connors, Anna Tatarko, Tara Christensen, and Dr. Paul Hurtado for their immensely helpful comments for my defense presentation and talk!

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And last, but not least, I want to express my heartfelt gratitude to Jim Miller. My taxonomic journey started with you, and I truly could not have done it without your guidance. You taught me the tools of the trade as you patiently and encouragingly taught me how to dissect and diagnose characters. And you were always willing to lend an ear, and I will always remember your words of wisdom and think fondly of the time we spent together. Thank you for your mentorship and your friendship, I will always miss you whenever I look at an *Eois*.

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Introduction

Insects constitute a significant proportion of Earth's biodiversity, with over half of the estimated 1.5 million described species of animals belonging the class Insecta (Mayhew 2007; Stork et al., 2015; Stork 2018). As pollinators, predators, parasitoids, herbivores, decomposers, and food for innumerable other invertebrates and vertebrates, their diversity plays a critical role in maintaining ecosystem function, stability, and sustainability. While the actual number of insect species on the planet remains a mystery, estimates range from 2 million (Hodkinson and Casson 1991) to 80 million species (Stork 1993; Erwin 2004).

The documentation of insect biodiversity has always been a daunting challenge, and it has become even more so with the changing face of our planet, with species extinction rates increasing, driven largely by climate change and other anthropogenic activities (Sánchez-Bayo & Wyckhuys 2019; Engel et al., 2021; Isbell et al., 2023). Many species of invasive insects are translocated around the planet through globalization of commercial trade and international travel, while others are disappearing, particularly in tropical regions, as rainforest habitat is converted to agriculture. The greatest concentration of insect species lies in the neotropical regions of the world; according to Erwin (2004) one hectare of Amazonian forest may contain more than 100,000 arthropod species. Therefore, events such as deforestation can cause destruction of habitats for millions of arthropods. Based on satellite imagery, Brazil's National Institute for Space Research (INPE) found that from August 2015 to July 2016, the area of Amazon rainforest was reduced by 7,989 square kilometers, an increase of 29% compared to the previous year in which 6,207 square kilometers was lost (INPE (2019)). So, with such

devastating habitat losses, many species will become extinct before they are even discovered, and true estimates of biodiversity may never be uncovered.

Further hindering the advancement of the biodiversity sciences, there simply are not enough taxonomists being trained to deal with the overwhelming chore of documenting global biodiversity, and their declining numbers are manifested in the reduced output of taxonomic research (Coleman & Radulovici, 2020). Overall, taxonomists are not being trained fast enough to describe species before they go extinct, and contemporary methods of taxonomic study, such as species descriptions based on DNA sequence data, have caveats and shortcomings of their own (Coleman & Radulovici, 2020). For Lepidoptera, morphological features of the male and female genitalia have been long seen as standard for species circumscription and identification [CITE]. More recently, modern techniques such as DNA sequencing or barcoding have provided an additional potentially more quantitative tool for species identification (Coleman & Radulovici, 2020). Many contemporary evolutionary biologists consider genitalia dissections too time consuming, and many argue that the costs of training skilled morphology-based taxonomists are exorbitant in comparison to the lowering costs of DNA sequencing (Ebach & Holdrege, 2005; Engel et al., 2021). However, because in many insect groups, genital characters differ greatly from species to species, dissections are still a highly useful tool for species identification, especially in cases where DNA cannot be sequenced (e.g., older specimens). And though many contemporary systematists, ecologists, and agricultural entomologists opt for DNA barcoding for species identifications, this method depends on reference sequences from correctly identified specimens. However, there is no “universal” gene that exhibits enough

sequence variation for species discrimination in all taxa (Stoeckle 2003), and older holotype specimens may not provide enough DNA for barcoding, as DNA degrades over time (i.e., fragment length decreases after years of preservation, and is also degraded by some methods of collection, preparation, and storage) (Strutzenberger et al., 2012). Thus, in many cases, genitalia dissections may be a better tool for species identification, as they provide an array of physical structures that can be compared among specimens, and contain interesting morphological features that may form the basis of subsequent ecological and evolutionary research.

Of the 29 orders of the class Insecta, the holometabolous orders Coleoptera, Diptera, Hymenoptera, and Lepidoptera account for 81% of all described insect species (Foottit & Adler 2009). The order Lepidoptera includes moths and butterflies, and with about 180,000 described species, it is likely second or third in species richness [CITE]. It comprises 126 families arranged in 46 superfamilies (Capinera 2008; Mallet 2007). Lepidoptera not only play an important role as pollinators, herbivores, and food for many vertebrate and invertebrate predators, they can be useful indicators for monitoring climate change, as their populations, range dynamics, and even their physiology are sensitive to environmental changes (Kocsis & Hufnagel 2011; Parmesan et al., 1999; Wagner et al., 2021). The moth family Geometridae, commonly known as inch worms, loopers, or geometers, is one of the three largest of clades of Lepidoptera, consisting of more than 21,000 described species (Brehm et al., 2003; Enkhtur et al., 2020). Its members also have been shown to be reliable indicators of environmental change (Sánchez-Bayo & Wyckhuys 2019).

The geometrid genus *Eois* Hübner (1818) is a member of the subfamily Larentiinae, the second largest subfamily in the family Geometridae. The subfamily comprises more than 6,200 described species distributed predominantly in temperate regions of the globe (Õunap et al., 2016). An exception, however, is the genus *Eois*, which reaches its greatest species richness in the tropics, with 83% of its species in the Neotropics alone (Õunap et al., 2016, Brehm et al., 2011). Species of *Eois* are known to specialize on plants of the genus *Piper* (Piperaceae), and they have radiated on this diverse plant family in the Neotropics (Dyer et al., 2004; Rodríguez-Castañeda et al., 2010; Wilson et al., 2012).

Eois is comprised of 267 valid species, with estimates of 1,000 or more Neotropical species yet to be described (Brehm et al., 2011; Strutzenberger et al., 2017; Moraes et al., 2021). While the genus has attracted considerable attention from ecologists and evolutionary biologists over the past few decades (Brehm et al., 2008; Brehm et al., 2011), limited progress has been made on its alpha taxonomy. The lack of accurate taxonomic descriptions and the paucity of distribution data for these moths not only limits monitoring and conservation efforts of possibly vulnerable populations, but also hinders investigations into ecological and evolutionary factors underlying biodiversity of *Eois.*

Eois undoubtedly harbors many undescribed cryptic species leading to an underestimation of species richness (Strutzenberger et al., 2011; Moraes et al., 2021). One such complex of undescribed species is the *Eois olivacea* clade, a group of commonly collected species. Owing to their abundance in reared collections, they are a highly valuable candidate for exploring patterns and mechanisms of low-level

diversification. The olivacea clade, initially recognized by Strutzenberger et al. (2010), includes species related to *E. olivacea*, *E. auruda*, *E. goodmanii*, and *E. muscosa*. According to previous phylogenetic analyses (Strutzenberger 2010; Brehm et al., 2011; Moraes et al., 2021), there appears to be at least two species complexes within the olivacea clade: *E. olivacea* and *E. goodmanii*. My dissertation investigated diversity in *Eois*, starting with this clade, as the research collection of Lepidoptera from the University of Nevada, Reno Museum of Natural History (UNR) had numerous specimens matching the morphological descriptions of these moths. The careful curation of these animals has uncovered misidentifications and new species hidden within these collections. And as I embarked on describing new species, complex genitalic variation also caught my attention. Therefore, with the motivation of uncovering evolutionary patterns in *Eois* genitalia, I also investigated sexual traits, their function, and the possible mechanisms driving these patterns.

My dissertation is divided into three sections: 1) In collaboration with Dr. Kathryn Uckele and Dr. Thomas Parchman, I assisted in morphological morphometric analyses to evaluate the relationships between genetic and genitalic variation with species morphologically similar to *Eois olivacea*, as well as investigating the extent to which elevation and host plant association influence evolutionary patterns across Ecuadorian sympatric populations of the olivacea species complex. We conducted phylogenetic analyses to contextualize the pattern and timing of population divergence within the broader context of *Eois* diversification. Section 2) For the second part of my dissertation, I collaborated with the late Dr. James Miller and Dr. John Brown to further investigate the olivacea complex and diversity of *Eois*, we described 16 new species based on

morphology and/or DNA sequence data. Section 3) With the effort of Dr. James Fordyce, who provided expertise in phylogenetic tree reconstruction, we sought to understand morphological variation in sexual traits to increase our understanding of evolution of the genitalia in *Eois*. We considered different mechanisms responsible for trait evolution in male and female genitalia, including genetic drift, pleiotropy, female choice, cryptic female choice, male to male competition, sexual conflict, and the lock-and-key hypothesis. The results from the dissertation research reported herein represent a preliminary framework into investigating the many undescribed neotropical *Eois* species, exploring morphological taxonomic methods to understand cryptic speciation and diversity within the genus, a first step in assigning much needed descriptions to unknown taxa, as well as representing a first step in revealing patterns of male to female trait evolution in Lepidoptera and their possible functions.

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Cryptic divergence in the hyperdiverse geometrid genus *Eois* (Lepidoptera: Geometridae: Larentiinae)

Kathryn A. Uckele[1], Lydia M. Doan[2], Thomas L. Parchman[2], Joshua P. Jahner[3], Matthew L. Forister[2], Lee A. Dyer[2]

[1] Department of Ecology & Evolutionary Biology, University of California, Santa Cruz, CA, 95064, USA

[2] Department of Biology, Ecology, Evolution and Conservation of Biology, University of Nevada, Reno, NV 89557, USA

[3] Department of Botany, University of Wyoming, Laramie, WY 82071, USA

Running head: Cryptic divergence in *Eois*

Abstract

The majority of earth's biodiversity resides in the tropics. Understanding the processes that generate and maintain this diversity are key to conservation and a major goal of evolutionary biology. In hyperdiverse tropical systems, species interactions are posited to play a greater role than abiotic factors in the diversification of lineages and the coexistence of closely-related species; however, additional empirical studies are needed to examine this hypothesis. Herbivorous insects and their host plants comprise an outsized proportion of tropical diversity, and the strength of plant-insect interactions increases with decreasing latitude. Here, we investigate how patterns of evolutionary divergence in a lepidopteran lineage of extreme diet specialists (*Eois*) are associated with patterns of host plant association at a single site in the Ecuadorian Andes. We used population genetic analyses based on high-throughput sequencing data to resolve patterns of genetic differentiation and diversity across 137 putative *Eois olivacea* larvae sampled from different host plant species. A subset of larvae were raised to adulthood for microscopic morphological analyses (genitalia and wing) to assess the extent to which genetic divergence is associated with morphology. Our genetic analyses suggest that the samples belonged to four distinct taxa, with divergence associated with the use of different host plants. A small number of wing and genitalic characters were able to distinguish three of these four taxa. These findings shed light on ecological drivers of divergence in this young cohort of closely-related taxa and provide new insight into the evolutionary drivers of tropical insect diversity.

Key words: Tropical diversity, plant-insect interactions, Lepidoptera, genitalia, geometrid, moth, *Eois*, *Piper*

Introduction

Tropical forests are hotspots of diversity and endemism (Fischer 1960) and are important both for conservation and understanding the drivers of diversification and coexistence. Species interactions, which are generally more specific and exert stronger selective pressures in tropical than in temperate forests (Coley & Barone 1996; Dyer et al., 2007; Schemske et al., 2009; Forister et al., 2015), are posited to have an outsized role in the origin and maintenance of diversity in the tropics (Wallace 1878; Dobzhansky 1950; Ehrlich & Raven 1964; Janzen 1970; Connell 1971; Marquis 2005). Specifically, theory suggests that stronger biotic interactions at lower latitudes favor coevolutionary processes that drive geographic variation in adaptations, the formation of distinct lineages, and enhanced sympatric coexistence among close relatives (Horvitz & Schemske 2002; Schemske 2009; Schemske et al., 2009). More empirical work examining the interplay of biotic interactions and microevolutionary processes is needed, but these studies are often challenging due to rampant cryptic diversity and taxonomic uncertainty in hyperdiverse tropical ecosystems.

Phytophagous insects are excellent models for understanding the role of biotic and abiotic processes in generating patterns of diversity (Medeiros & Farrell 2020; Du et al., 2020). Multiple attributes of host plants affect insect fitness and are associated with life history parameters, including mating behavior, physiology, and immune function (Landolt & Phillips 1997; Thompson 1988; Näsvall et al., 2021; Dambroski & Feder 2007; Carper et al., 2019). As a result, the evolution of insects and their plant hosts is often linked, resulting in phenotypic matching of plant defensive and insect counter-defensive traits (Ehrlich & Raven 1964; Marquis et al., 2016; War et al., 2012).

Increasing host plant specialization with decreasing latitude may allow finer partitioning of niche space, and ostensibly higher rates of coexistence, evolution, and diversification (Forister et al., 2015; Büchi & Vuilleumier 2014; Hardy & Otto 2014). While macro-evolutionary patterns of plant-insect interactions have been investigated to a moderate degree, there has been relatively less research into the micro-evolutionary processes that underpin plant host use and taxonomic diversity within tropical insect lineages. Population genetic analyses, which may elucidate the ecological and geographic factors that underlie gene flow and selection, should bridge the gap in our understanding of the evolutionary role of biotic interactions (Ortego & Knowles 2020; Graciá 2020). Additionally, abiotic (climate) factors influence patterns of diversity but have received relatively less attention in the tropics. Abiotic processes may be especially important in tropical montane regions, which exhibit pronounced spatial turnover in climate and species composition (Rahbek et al., 2019). Furthermore, compared to temperate species, tropical insects exhibit narrower physiological tolerances, making them more susceptible to environmental gradients (Chen et al., 2009). Fine-scale spatial variation in temperature and microclimate is inherent to many mountain regions (Rahbek et al., 2019), which may partially explain why plant and insect species richness tends to peak locally on mountain slopes more often than in the lowlands (Rahbek, 1995; Beck et al., 2017).

Despite their importance for elucidating the biotic and abiotic drivers of population- and species-level differentiation, population genetic analyses are underrepresented in tropical ecosystems (Beheregaray et al., 2015). Furthermore, many of the past analyses that have been conducted in the tropics have relied on small numbers of traditional genetic markers (e.g., single locus mtDNA sequencing, fragment length

polymorphisms) to characterize taxonomic diversity and population differentiation (Dyer et al., 2014). However, modern high-throughput sequencing approaches have made genome-wide sampling affordable and efficient, even for organisms lacking genomic resources (Narum et al., 2013). These approaches routinely identify thousands to millions of polymorphisms, which can be instrumental in detecting patterns of genetic divergence across small geographic and ecological scales, and allow for improved resolution of evolutionary diversification across shallow evolutionary scales (Irissari et al., 2018; Cerca et al., 2023). Applying these approaches to tropical systems holds promise for uncovering complex patterns of differentiation that inform our understanding of tropical diversity and the processes that shape it.

Eois (Larentiinae: Geometridae) is a hyperdiverse genus of tropical lepidopterans that has experienced a recent evolutionary radiation in the Central and South American tropics (Strutzenberger et al. 2017; Jahner et al., 2017). *Eois* diversity is highest within the high-elevation wet tropical forests of the eastern Andes (Brehm et al., 2011), where *Eois* species can account for up to 8% of all adult geometrid moth species captured at an individual light trap (Brehm et al., 2005). *Eois* specialize on plants in the genus *Piper* (Piperaceae) (Dyer et al., 2004), which is likewise an extraordinarily diverse group that includes over 1,300 species (Martínez et al., 2015). Although most *Eois* species are specialists on *Piper*, they are not necessarily monophagous, with diet breadth ranging from 1-5 species (Dyer and Palmer 2004, LAD unpublished data). Due in part to its astounding diversity and high degree of host specificity, *Eois* has become a model group for understanding the ecological and geographic drivers of lineage diversification (Rodríguez-Castañeda et al., 2010; Wilson et al., 2012; Jahner et al., 2017). It is likely

that a combination of cryptic diversity, small, localized populations, limited migration, and low incidence at light traps have contributed to limitations in *Eois* collections, leading to an underestimation of species richness in the genus (Strutzenberger et al., 2011; Moraes et al., 2021 PeerJ). There are 256 described species of *Eois*, but based on undescribed species in collections, the niche breadth of the genus, and the geographic range of the host plants, over a thousand *Eois* species are predicted to occur across the range of the genus (Parsons et al., 1999; Rodríguez-Castañeda et al., 2010; Moraes et al., 2021 PeerJ). *Eois* species descriptions are largely based on wing and genitalia morphology, which vary considerably in the genus (Brehm et al., 2011). In particular, variation in the valvae and eversible vesica of the aedeagus, two genitalic structures that potentially contribute to lock-and-key prezygotic isolating mechanisms in lepidopterans, has been taxonomically valuable for delimiting *Eois* species that cannot be distinguished with macroscopic characters alone (Brehm et al., 2011; Strutzenberger et al., 2011 Insect Science).

Previous work in this group has identified many cases of nominal species that apparently contain elevated levels of genetic diversity or are represented by phylogenetically distinct accessions (Wilson et al., 2012; Jahner et al., 2017). Here, we target one of those taxa, *Eois olivacea*, as a candidate for exploring patterns and mechanisms of cryptic diversity and ecological divergence. Specifically, we evaluate the extent to which host plant association influences evolutionary patterns across sympatric populations (within 1000 ha area) of the *E. olivacea* species complex at a single site within the Ecuadorian Andes. We used high-throughput sequencing of reduced-representation libraries to generate genome-wide SNP data for 137 individuals sampled

from different *Piper* host plants. We used population genetic analyses to resolve patterns of genetic differentiation and genetic diversity among samples and leveraged complementary microscopic morphological analyses to evaluate the relationship between genetic and phenotypic variation. Finally, we conducted phylogenetic analyses on the samples and a number of additional *Eois* species to contextualize the pattern and timing of population divergence within the broader context of *Eois* diversification.

Materials and methods

Sample collection

We collected 137 *E. olivacea* caterpillars from three *Piper* host species: 60 individuals were collected from *P. baezanum*, 8 from *P. crassinervium*, 65 from *P. lancifolium*, 4 from *P. perareolatum*. All specimens were collected at the Yanayacu Biological Station located in the eastern Ecuadorian Andes (00’ 36’’ S, 77’ 53’’ W) at elevations ranging from 1,794 - 2,250 m. A subset of the caterpillars (15 from *P. baezanum*, 8 from *P. crassinervium*, and 10 from *P. lancifolium*) were raised to adulthood on the plant host they were collected from at the Yanayacu Biological Station and transported to the University of Nevada, Reno, Museum of Natural History (UNR). Adults were subsequently photographed and dissected for morphometric analyses.

DNA extraction and ddRADseq library preparation

We extracted DNA from whole caterpillars using Qiagen DNeasy Blood and Tissue Kits (Qiagen Inc., Valencia, CA, USA). To increase DNA yield for the adult samples, we extracted DNA from asmall portion of the anterior end of the abdomen and

hind legs with the Qiagen QIAamp DNA Micro Kit (Qiagen Inc., Valencia, CA, USA) which can provide above average yields of purified genomic DNA from small sample sizes (< 10 mg tissue). We quantified and assessed the quality of extracted DNA with a QIAxpert microfluidic analyzer (Qiagen Inc., Valencia, CA, USA). Sequencing libraries were prepared according to a ddRADseq protocol (Parchman et al., 2012; Peterson et al., 2012). First, genomic DNA from all individuals was digested with two restriction digest enzymes, *Eco*RI and *Mse*I. Second, Illumina base adaptors customized with unique nucleotide barcodes (8, 9, or 10 bases in length) were ligated to *Eco*RI restriction sites, and unmodified adaptors were ligated to *Mse*I restriction sites. Third, the ligated fragments were PCR amplified with a high-fidelity proofreading polymerase (Iproof polymerase, BioRad Inc., Hercules, CA, USA) and pooled for sequencing. The resulting PCR amplified samples were pooled into a single library, which was size-selected for fragments between 350 and 450 bp in length using a Pippin Prep System (Sage Sciences, Beverly, MA) at the University of Texas Genome Sequencing and Analysis Facility and sequenced on two distinct sequencing platforms: two lanes of single-end 100-base sequencing were executed with an Illumina HiSeq 2500 at the University of Wisconsin Madison Biotechnology Center, and one lane of single-end 100-base sequencing was executed with an Illumina NovaSeq 6000 at the University of Texas Genome Sequencing and Analysis Facility.

Assembly and variant calling

The raw sequence data obtained from the HiSeq and NovaSeq platforms were filtered in parallel for DNA contaminants *E. coli*, *Phi*X, Illumina adaptors or primers, and low quality reads using bowtie2_db (Langmead & Salzberg 2012) and a pipeline of bash

and Perl scripts (https://github.com/ncgr/tapioca). After this initial filtering step, the reads generated with HiSeq and NovaSeq were concatenated into a single file prior to demultiplexing and variant calling. A custom Perl script was used to trim barcode- and restriction site-associated bases and approximate string matching of sequence barcodes to assign each read to an individual. We used the mem algorithm in bwa (v. 0.7.17; Li & Durbin 2009) to align reads from each individual to a newly generated *E. olivacea* reference genome (Uckele et al. unpublished data). 60 individuals were in the lower 30th percentile of assembled reads (< 314,007 reads) and were removed from the analysis prior to variant calling. The remaining 137 SAM files were converted to BAM format and used to identify variant positions, call single nucleotide polymorphisms (SNPs), and estimate genotype likelihoods with samtools and bcftools (v. 1.8; Li & Durbin 2009). Briefly, we removed loci with site quality (QUAL) and genotype quality scores (GQ) lower than 20 or coverage depth (--max-depth) higher than 200, and we retained only bi-allelic SNPs. With vcftools (v. 0.1.16; Danecek et al., 2011), we randomly sampled one SNP per 100 base pairs to reduce the effects of linkage disequilibrium (LD), set the minimum minor allele frequency (MAF) to 5%, and retained only SNPs which were present in more than 70% of samples.

Population genetic analyses

To account for uncertainty caused by variation in sequencing depth across loci and individuals, we ran ENTROPY (Gompert et al. 2014; Shastry et al. 2021) to estimate genotype probabilities based on the genotype likelihoods. Similar to the correlated allele frequency model of STRUCTURE (Pritchard et al., 2000; Falush et al., 2003), the ENTROPY model uses estimates of individual ancestry coefficients and allele

frequencies to facilitate information sharing across loci and individuals to probabilistically estimate genotypes for loci with low-depth or missing data (Shastry et al., 2021). We used ENTROPY to estimate individual ancestry coefficients (q), or the proportion of an individual's genome that is derived from one of *K* source populations, and to estimate genotype probabilities for each locus in each individuals. We ran ENTROPY for values of *K* = 2-8 and used deviance information criteria (DIC) to compare model fit for alternative values of *K*. To aid MCMC convergence, we seeded each chain with cluster membership probabilities generated from a *k*-means clustering analysis based on a linear discriminant analysis of the principal components estimated from the genotype likelihoods using the MASS package in R v.4.0.3 (R Core Team 2020; Venables & Ripley 2002). We ran ENTROPY for four independent chains for each value of *K* = 2-8. Chains were run for 60,000 steps, after a 30,000 step burn-in, and thinned every tenth step. To assess adequate mixing and convergence, we plotted and visualized posterior chains for each value of *K* using the coda package in R v.4.1.2 (Plummer 2020).

To visualize the major axes of genetic variation across individuals, we performed principal components analyses (PCA) on the genotype probabilities estimated with ENTROPY. We quantified the magnitude of genetic differentiation among groups of individuals assigned to distinct clusters with ENTROPY using Nei's D (Nei 1972) and Hudson's FST (Hudson et al., 1992). To calculate the genetic diversity within the genetically differentiated groups determined by PCA and structure analysis, we used the *-doSaf* option and the *realSFS* program in angsd (v. 0.923; Korneliussen et al., 2014) to calculate π (Tajima, 1983) and Watterson's θ (Watterson, 1975) for each variant site.

These values were averaged across sites to produce genome-wide values of π and θ for each group.

Phylogenetic analysis

To place patterns of divergence in the context of genus-wide diversification, we conducted a phylogenetic analysis that included additional taxon sampling across the *Eois* genus. We obtained sequencing data for 133 additional *Eois* samples from Jahner et al. (2017), which utilized an identical ddRAD sequencing approach to this study. We included five representative samples from this study, representing four genetically differentiated lineages that were identified with PCA and structure analyses, resulting in a total of 138 samples for phylogenetic analysis. To generate multiple alignments for phylogenetic analyses, we utilized the assembly workflow of ipyrad (Eaton 2014). Briefly, after removing reads which contained more than five low quality bases, the remaining reads were mapped to the *E. olivacea* reference genome with bwa (v. 0.7.17; Li & Durbin 2009) and bedtools (v. 2.29.2; Quinlan & Hall 2010) according to a 90% similarity threshold (*clust_threshold*). Statistical base calls were made for all loci with a depth of at least 6, and consensus sequences with more than 5% ambiguous bases (*max_Ns_consens*) or heterozygous sites (*max_Hs_consens*) were discarded to remove poorly aligned regions. The remaining consensus sequences were clustered again, this time across individuals, and the resulting stacks were discarded if they contained more than 8 indels (*max_Indels_locus*), 20% variable sites (*max_SNPs_locus*), or one heterozygous site shared across more than 50% of the samples (*max_shared_Hs_locus*), all of which may indicate poor alignment or paralogy. Multiple species alignments produced with reduced representation sequencing data are often characterized by high

proportions of missing data, particularly when they span deep divergences. To improve our ability to recover deeper splits in the *Eois* phylogeny, we first retained all loci that were present in a minimum of four samples (*min_samples_locus*), which we analyzed with the multi-species coalescent (MSC) method SVDquartets (Chifman & Kubatko 2014) implemented with tetrad v.0.9.13 (Eaton et al. 2017). The analysis randomly sampled 1,083,779 quartets out of a total of 16,701,685 (6% of total), and support was estimated using 100 bootstrap replicates. Nine clades (see Supplementary Information) that were resolved with greater than 97% bootstrap support were used to constrain the tree topology in the Bayesian molecular dating analysis.

To calibrate the *Eois* phylogeny, we used Bayesian inference to estimate the species topology and node ages. We used RevBayes v.1.0.12 (Höhna et al. 2017) to specify the model and conduct MCMC sampling. For this analysis, we set the ipyrad *min_samples_locus* filtering parameter to 110, retaining all loci that were present in at least 110 of 137 samples. Though this level of filtering was necessary to ensure reasonable computational time and resources, it likely introduced bias by excluding loci with the highest mutation rates, which could bias divergence time estimates to be more recent (Huang & Knowles 2014). We compared the relative fits of the JC, HKY, GTR, GTR+Gamma, and GTR+Gamma+I substitution models using Bayes factors. The topology was modeled as a constant rate birth death process where the prior on diversification rate was specified with an exponential distribution ($\lambda = 10$) and the prior on rate of turnover was specified with a beta distribution ($\alpha = 2$, $\beta = 2$). Highly-supported (> 85% bootstrap support) clades resolved with the SVDquartets analysis were used to constrain the Bayesian topology, such that clade membership could not change, but the

relationships within and between clades could vary. To calculate the sampling proportion ρ, we divided the number of sampled species (137) by the estimated number of *Eois* species (1,710; Brehm et al. 2011). We relaxed the assumption of a global molecular clock by allowing each branch-rate variable to be drawn from a lognormal distribution where the mean and variance are drawn from exponential hyperpriors. Due to the paucity of *Eois* fossils, we used the estimated age of *Eois* from Strutzenberger et al. (2017), which was informed by the minimum age of the fossil *Geometridites larentiiformis*. We calibrated the root of the tree with a uniform prior (min = 23.3, max = 32.3), based on the reported 95% highest posterior density (HPD) (Strutzenberger et al., 2017). Six independent MCMC chains were run for 400,000 generations with a burn-in of 10,000 generations and sampled every 10 generations. Chains were visually assessed for convergence with Tracer v.1.7.1 (Rambaut et al., 2018) and quantitatively assessed with effective sample sizes and the Gelman-Rubin convergence diagnostic (Gelman & Rubin, 1992) using the *gelman.diag* function in R (CODA package; Plummer at al., 2006).

Morphometric measurements and analyses

Dissections were conducted following Robinson (1976), with the exception that genitalic structures were slide mounted using euparal. Images of adults and genitalia were taken using a Canon EOS 40D digital SLR (Canon U.S.A., Lake Success, NY) mounted on a Visionary Digital BK Lab System (Visionary Digital, Palmyra, VA). Taxonomic classification follows Holloway (1997) and Viidalepp (2011); to delineate each taxon in this study, adult specimens were identified using a series of type photos, and use of a morphological data matrix (Appendix A). Figures were constructed in Adobe Photoshop

(v24.6) and Illustrator (v27.6. 1) both part of the Adobe Creative Cloud 2023 (v5.11.0.522.1) (Adobe Inc. 2023).

After careful diagnosis and from prior unpublished work, we compiled a morphological data matrix of 107 characters (37 binary and 70 multi-state), including 16 external characters (characters 1–16), 58 male characters (characters 17–75), and 33 female characters (characters 76–107); Appendix A. We chose a select few features shared by these closely related taxa of the olivacea species complex, taking into consideration presence or absence of pronounced features unique to each species, either unique to the complex, or used to distinguish one specimen from another shown in Tables 1 and 2 (reported in detail in the supplementary materials). Genitalic structures such as the ventral margin of the saccus and the cornuti of the vesica in male genitalia, and ductus bursae, multispined signum, and papillae anales in female genitalia (Fig. 1 & 2) (Moraes et al., 2021; Brehm et al., 2011). These genitalic structures have complex functionality in Lepidotera and have been suggested to be involved in sexual selection. For example, the sclerotized spikes or nub-like cornuti, which are attached to the apex of the eversible membranous vesica of the male phallus, is hypothesized to cause trauma to the female corpus bursa, the copulatory chamber that houses the sperm envelope (spermatophore) disposited during copulation (Cordero & Baixeras 2015). Another example is the sclerotized, horn-shaped signum located in the corpus bursae in females which might serve as a complementary locking structure to the male phallus. This structure may stimulate ejaculation (either by breaking a physical barrier or stimulating a sensory structure in the male aedeagus), protect the corpus bursae from damage inflicted

by phallus structures during copulation, or even break open the spermatophore to release sperm, among other posited functions (Galicia et al., 2008; Meslin et al., 2015).

Throughout the text we refer to the subset of the caterpillars (15 from *P. baezanum*, 8 from *P. crassinervium*, and 10 from *P. lancifolium*) raised to adulthood on the plant host they were collected as the following: depending on host, taxa will be referred as "nr. *E. auruda* 1", "nr. *E. auruda* 2" (and etc.), or "nr. *E. olivacea* 1", "nr. *E. olivacea* 2" (and so on). This is based off of these specimens' morphological similarity to *E. auruda* and *E. olivacea* male specimen type photographs from USNM (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA) and NHML (Natural History Museum, London, U.K.) respectively. Photos of the male genitalia of the co-type of *E. auruda* confirmed that our Yanayacu populations do not contain *E. auruda* taxa. We could not, however, confirm the identification of *E. olivacea* within our populations as the type specimen is missing its abdomen, rendering identification impossible.

Results

Assembly and variant calling

After demultiplexing and removing potential contaminants, 357,018,284 reads were retained, of which 294,678,158 (~83%) aligned to a newly assembled *E. olivacea* reference genome. We removed 42 individuals with less than 500,000 aligned reads, leaving 156 individuals for variant calling. After filtering for sequencing quality, minor allele frequency, sampling coverage, and linkage we retained 5,076 SNPs for

population genetic analyses with an average depth of coverage of 16x per individual and average marker density of approximately 6 SNPs every megabase.

Evolutionary divergence in the context of host plant association

Population genetic analyses consistently resolved a continuum of evolutionary divergence among four main *Eois* groups. Comparison of ENTROPY DIC values supported a model of four ancestry groups ($K = 4$). The first two principal component axes also resolved four major groups that corresponded to those inferred with ENTROPY. Between-population measures of F_{ST} demonstrated a continuum of divergence among groups, ranging from 0.28 to 0.56, that was consistent with the range of divergences inferred from PCA and the phylogenetic analyses.

The majority of individuals contained ancestry from a single ancestry group, and host plant association was useful for classifying individuals to ancestry groups. The group with the largest number of sampled individuals (N = 63), was collected from *Piper lanceifolium*. The group with the smallest number of sampled individuals (N = 8), was associated primarily with *P. crassinervium*, but was also collected once from *P. baezanum*. The two remaining groups, which included 17 and 48 individuals respectively, were both associated with *P. baezanum*, however, individuals from the latter of the two groups (hereafter referred to as the *P. baezanum*-associated "white" group) were also collected once from *P. lanceifolium* and four times from *P. perarolatum*. The former of the two groups (hereafter referred to as the *P. baezanum*-associated "dark blue" group) were exclusively collected from *P. baezanum*.

Genetic diversity within groups was variable, ranging from 0.0007 to 0.0035. A number of admixed individuals (N = 13) were detected with PCA and structure analyses (Fig. 3). Nine of the 13 admixed individuals contained ancestry from the *P. lanceifolium*-associated group and the *P. crassinervium*-associated group. These individuals were collected from three different host plant species: *P. baezanum* (N = 2), *P. lanceifolium* (N = 2), and *P. pubinervulum* (N = 5). They possessed a slightly greater proportion of ancestry from the *P. crassinervium*-associated group, and variation in the proportion of *P. crassinervium*-associated ancestry was low across individuals. Additionally, there were four individuals that possessed ancestry from both the *P. lanceifolium*-associated group and the *P. baezanum*-associated "white" group. These ancestry proportions were more variable across the four admixed individuals. Two of the four admixed individuals possessed predominantly *P. lanceifolium*-associated ancestry, and were collected from *P. baezanum* and *P. lanceifolium*, respectively. The other two individuals possessed predominantly *P. baezanum*-associated "white" ancestry, and were also collected from *P. baezanum* and *P. lanceifolium*. There were no discernible hybrids created via admixture with the *P. baezanum*-associated "dark blue" group. Additionally, no admixture was detected between the *P. crassinervium*-associated group and the *P. baezanum*-associated "white" group.

Phylogenetic relationships and evolutionary patterns

The two multi-species sequence alignments that were used as input for SVDquartets and Bayesian inference differed in their proportions of missing data. To maximize the information available for deep splits, the SVDquartets alignment contained all loci that occurred in at least 4 of 138 samples. This filtering regime generated 341,754

unlinked SNPs for analysis, however, 96% of the data across variable sites was missing, compared to only 37% for the Bayesian alignment. To reduce computational load, the Bayesian alignment contained all loci that occurred in at least 110 of 138 samples, which produced 57 concatenated loci and 1,368 SNPs for analysis.

Both phylogenetic inference methods provided better resolution of recent splits than deeper relationships along the backbone of the tree. The accessions that were collected for this study formed a highly supported monophyletic clade (Fig. 4). The phylogenetic relationships among the samples from this study were consistent with estimates of divergence based on PCA and F_{ST}. The *P. lanceifolium*-associated and *P. baezanum*-associated "dark blue" accessions formed a monophyletic group that was sister to a clade of two unidentified accessions from Ecuador. The *P. baezanum*-associated "white" accession formed a strongly supported monophyletic clade with another accession from Ecuador that was also associated with *P. baezanum*. The *P. crassinervium*-associated accession formed a monophyletic group with four other accessions that were associated with *P. crassinervium.* The *P. baezanum*-associated "white" and *P. crassinervium*-associated clades were sister to one another and formed a highly supported clade that was sister to the highly supported monophyletic clade containing the *P. lanceifolium*-associated and *P. baezanum*-associated "dark blue" accessions (Fig. 4).

The most recent common ancestor of the four lineages was dated to 5.52 million years before present (95% HPD: 2.63 – 8.44). According to the analyses, the *P. lanceifolium*-associated and *P. baezanum*-associated "dark blue" lineages diverged approximately 3.34 million years ago (95% HPD: 1.1 – 5.81), and the *P. baezanum*-

associated “white” lineage diverged from the *P. crassinervium*-associated lineage approximately 4.06 million years ago (95% HPD: 1.76 – 6.49).

The morphometric analyses resolved four distinct taxa that were consistent with the ancestry assignments inferred with the genetic analyses. For the adult males, variation in the shapes of the ventral margin of the saccus and configurations of the cornuti arising from vesica separated three of the four lineages: 1) *P. baezanum*-associated “white” lineage, 2) the *P. crassinervium*-associated lineage, and 3) the clade containing the *P. baezanum*-associated “dark blue” lineage and the *P. lanceifolium*-associated lineage. For the adult females, variation in the width of the ductus bursae, the location of the signum, and the shape of the papillae anales separated two of the four lineages: 1) the *P. crassinervium*-associated lineage from 2) the clade containing the *P. baezanum*-associated “dark blue” lineage and the *P. lanceifolium*-associated lineage. None of the trait variation differentiated the *P. baezanum*-associated “dark blue” lineage from its sister lineage, the *P. lanceifolium*-associated lineage. The remaining 102 morphological traits from the analysis did not vary across individuals. Comparisons of the adult samples to the type specimens of nr. *Eois olivacea* and related taxa enabled species-level determinations as follows (Fig. 1, 2, 5-7): the *P. crassinervium*-associated adults were identified as nr. *Eois auruda*, the *P. baezanum*-associated “dark blue” adults and the *P. lanceifolium*-associated adults were both identified as nr. *Eois olivacea* (1 & 2 respectively), and the *P. baezanum*-associated “white” adults were determined to be *Eois parumsimii*.

Wing pattern and coloration were distinct for the three taxa described above: nr. *E. olivacea*, nr. *E. auruda*, and *Eois parumsimii.* Superficially, the wing patterns are

as follows: forewing and hindwing dorsum ground color of nr. *E. olivacea* are a light moss green with varying ivory colored antemedial, medial, and postmedial lines, with dark red-brown discal spot, banded cream and dark red-brown costal margin, dark red-brown submarginal band, and light-yellow fringe (Fig. 3). Forewing and hindwing dorsum ground color of nr. *E. auruda* are a brownish green with varying light yellow colored antemedial, medial, and postmedial lines, with dark orange-brown discal spot, costal margin is light yellow with distal portion of wing apex with variable markings of dark orange-brown, submarginal band is dark orange-brown, and fringe is two- toned, mostly light yellow with a portion between M2 and M3 being red-brown (Fig. 5). And lastly, forewing dorsum ground color of *Eois parumsimii* is an olive green with varying light yellow colored antemedial, medial, and postmedial lines, with clay brown discal spot, fringe is two- toned, mostly light yellow with a portion between M2 and M3 being red-brown, while hindwing dorsum ground color is clay brown with varying light yellow colored antemedial, medial, and postmedial lines (Fig. 5).

Discussion

Cryptic diversity, characterized by the presence of multiple morphologically similar species, has been extensively documented in tropical lepidoptera (Herbert et al., 2004) and has caused significant underestimations of species richness (Bickford et al., 2007; Li & Wiens 2023). Various insect species share similar morphological characteristics, and this is particularly true for the immature stages of holometabolous insects (Truman & Riddiford 2019). The lack of accurate taxonomic descriptions and distribution data for tropical lepidopterans not only limits monitoring and conservation efforts for these

vulnerable populations but also hinders investigations into the ecological and evolutionary factors underlying biodiversity (Doré et al., 2022; Isbell et al., 2023). In this study, we employed population genetic and morphometric analyses to clarify the evolutionary relationships of cryptic, sympatric *Eois* populations at a single site in the Ecuadorian Andes. Our results indicated that the samples belonged to four distinct taxa, and genetic divergence among taxa was associated with the utilization of different host plants. Morphological assessment of adult samples representing these four groups revealed consistent differences in trait variation that provide further support for the inference of genetic differentiation and suggest cryptic differentiation of *Eois* spp. in this region of the Andes. These findings shed light on ecological drivers of speciation in this cohort of cryptic taxa and provide a starting point for future research into the evolutionary history of this cryptic species complex.

Patterns of cryptic diversity are associated with host plants

Our population genetic analyses provide evidence that the samples in this study represent four closely related, cryptic taxa that span a gradient of evolutionary divergence. Less divergence (F_{ST} = 0.33) was observed between the sister lineages of *E. olivacea* in which one lineage feeds on *Piper baezanum* and the other is found on *P. lancifolium*. A similar level of evolutionary divergence (F_{ST} = 0.28) was found between the two remaining taxa, *E. auruda* and an undescribed *Eois* species, that were associated with *P. crassinervium* and *P. baezanum*, respectively. The deepest divergence was observed between these sister species and *E. olivacea sensu lato* (F_{ST} = 0.39 – 0.56). According to the molecular dating analysis, the focal taxa form a monophyletic clade that

began diverging approximately five million years ago. The four taxa are associated with distinct *Piper* host plant species apart from the undescribed *Eois* spp. and one of the *E. olivacea* lineages, which both utilize *P. baezanum*. Concordance between patterns of genetic divergence and host plant utilization suggests a potential role of species interactions in maintaining species boundaries in this cryptic species complex.

The *Eois* genus is characterized by extreme diet specialization, most commonly with plants in the *Piper* genus. It is well understood that diet specialization can promote the emergence or maintenance of host-associated lineages of insect herbivores as evidenced in examples of contemporary evolution in host-associated races such as of those of the apple maggot fly, *Rhagoletis pomonella* (McPheron et al., 1988) and the soapberry bug, *Jadera haematoloma* (Carroll & Boyd, 1992). Host-associated lineages of herbivorous insects are common as host plant affiliations and are a major driver of radiations among herbivorous insects. These lineages can arise when the effects of host chemistry, biogeography, and population-level variation influence host selection, speciation, or specialization in insects (Percy et al., 2004). Host specialization can promote the formation and maintenance of host-associated lineages, as *Eois* species are particularly susceptible to processes that facilitate the formation of host-associated lineages (Strutzenberger et al. 2017; Dyer et al., 2004). For example, the spatial distributions of *Eois* and *Piper* are tightly coupled, such that variation in the distribution of *Piper* across the landscape can influence patterns of genetic structure across *Eois* populations (Strutzenberger et al. 2011; Strutzenberger et al. 2017; Jahner et al., 2017). *Eois* fitness is likewise highly influenced by interactions with *Piper*, and the utilization of

a novel *Piper* species dramatically changes the topology of the fitness landscape (Brehm et al., 2011; Glassmire et al., 2016; Jahner et al., 2017).

As has been well-documented in many insects, novel host plant utilization is associated with many physiological and behavioral adaptations that can limit heterospecific encounters or decrease the fitness of heterospecific mating (Geiselhardt et al., 2012; Hue et al., 2014; Zhang et al., 2015; Vuts et al., 2018). Our results support a continuum of evolutionary divergence that is associated with patterns of host plant utilization. Our analyses resolved four distinct lineages that were associated with different *Piper* host plant species; these lineages are substantially diverged and nearly isolated from one another. Diet specialization can contribute to the formation or maintenance of host-associated differentiation, as well as contribute to evolutionary divergence in many lepidopteran systems where host shifts have involved divergent selection on morphological, behavioral, and physiological traits (Cohen et al., 1992; Dambroski & Feder 2007). Diversification of specialists and their life histories can be tied to their host associations, in particular diapause life history (Dambroski & Feder 2007) or female oviposition preference (Thompson 1988).

Morphology partly reflects genetic differentiation and host plant association.

Host plant affiliations are recognized as a major driver of radiations among herbivorous insects, with divergence resulting from interactions with their host plants. However, evolutionary divergence can also be associated with morphology, in particular, genitalic traits. Genitalia of male and female Lepidoptera are complex organs, composed of several structures that exhibit great diversity of shapes, sizes, and positions, suggesting

that they have evolved in a relatively rapid and divergent way (Cordero & Baixeras 2015; Sánchez et al., 2018). There are several proposed mechanisms for the evolution of genitalia, specifically the possible effects of sexual selection on male genital morphology (Cordero & Baixeras 2015) and these range from selective pressures that include genetic drift, pleiotropic effects, female choice, cryptic female choice, to sperm competition, male-female conflicts of interest, sexual conflict or coevolution, and the lock-and-key isolating mechanism (Mayr 1963; Arnqvist 1997; Simmons 2014; Sloan & Simmons 2019). Any of these mechanisms, or combinations of mechanisms could be responsible for the patterns seen in our data. For example, in female choice, females may exert preference for particular male genital morphologies, or mechanical or sensory components of male genitalia. It has been shown that female genitalia can diverge between populations with different male genital preferences, resulting in a pattern of correlated evolution of male and female traits (Simmons 2014; Eberhard & Lehmann 2015). Another example would be the lock-and-key hypothesis in which mechanical differences between two species provides a reproductive isolating barrier, where male genitalia act as “keys” to female genitalia “locks” leading to species-specific structures to evolve to prevent copulation with heterospecific individuals via lack of morphological fit or stimulation (Masly 2011). Therefore, a general pattern of evolution we may expect to observe would be a stepwise evolutionary pattern in which small changes in female genitalia would be closely tracked by changes in male genitalia (Simmons 2014).

However, these mechanisms are difficult to observe or evaluate when solely assessing morphological structures in pinned specimens in research collections, and not observing mating behavior of males and females, the interactions of their genitalia, and

observing their gametes (Eberhard 1996, Eberhard & Lehmann 2015). Furthermore, the literature on functional morphology of Lepidopteran genitalia is incomplete, as there are missing methods to test the function of interacting male and female genitalia (Cordero & Baixeras 2015): methods such as freezing specimens during different stages of copulation and dissection of females for evidence of anatomic damages that arise from traumatic copulation, as observed in other insect species such as Coleoptera (Dougherty et al., 2017).

The morphological results reported here overlapped with genomic analysis with the exception of the two cryptic species identified as nr. *Eois olivacea* for which genetic analysis revealed distinct taxa based across the host plants, *P. baezanum* and *P. lanceifolium.* There may be morphological differences between the two genetically distinct taxa within nr. *Eois olivacea*, but these could be subtle and easily overlooked or misinterpreted. The discrepancy between the species numbers identified by morphological traits (3) versus genetic data (4) underscores some limitations of traditional taxonomy in detecting cryptic species and highlights the valuable role of genomics in revealing the true richness and complexity of tropical Geometridae. This subtle difference in number of taxa serves as an important contribution to future research on the evolutionary history and ecological dynamics of *Eois*. Importantly, the three taxa that were revealed were clearly differentiated based on genetics and associated with different sympatric host plants.

Conclusion

Tropical lepidopteran diversity has been greatly underestimated for a variety of reasons (Bickford et al. 2007; Herbert et al., 2004), including unrecognized cryptic diversity. Many insect species share similar morphological characteristics, and this is especially true for the immature stages of holometabolous insects. The paucity of accurate taxonomic descriptions, host affiliations, distribution data, and descriptions of tropical insects undermines research on ecological and evolutionary aspects of biodiversity as well as conservation efforts (Doré et al., 2022; Isbell et al., 2023). This study on cryptic *Eois* individuals from a single site in the Ecuadorian Andes revealed four distinct taxa using both traditional morphological and modern population genomics methods. The genetic divergence observed among these taxa was linked to the utilization of different host plants, which provides valuable insights into ecological factors potentially influencing speciation in this group of cryptic species.

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Figures

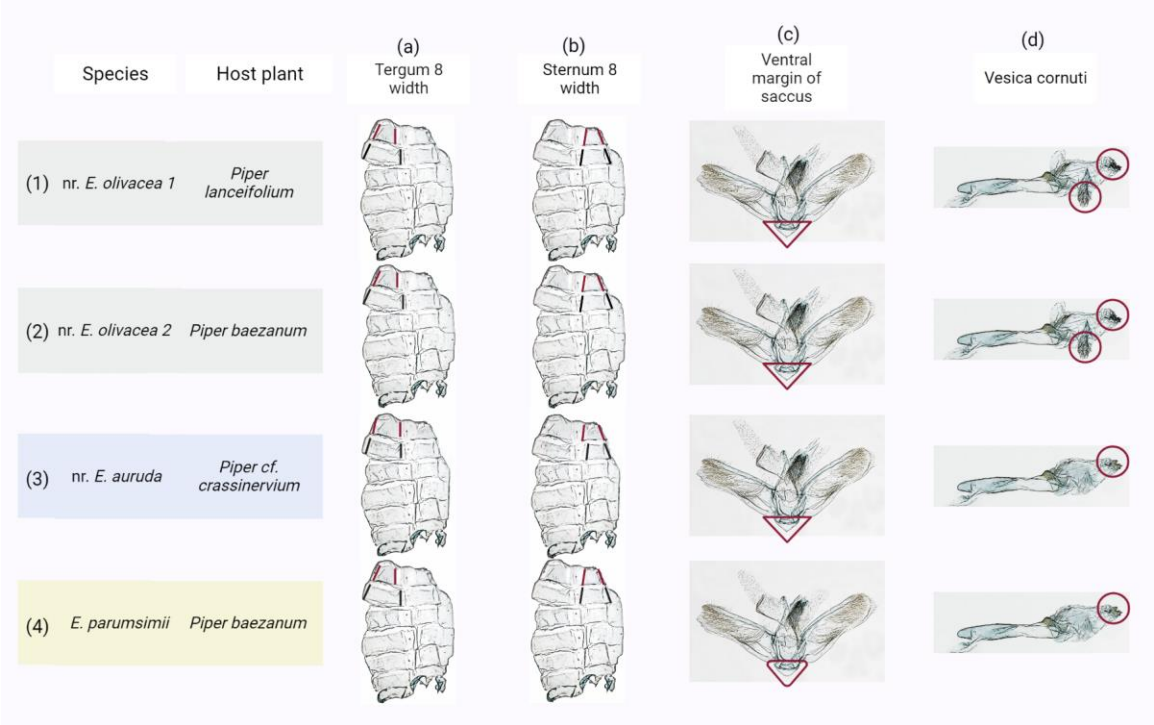

Figure 1. Simple illustrations* of male genitalia characters among the 4 cryptic species of (1) "nr. *E. olivacea* 1" reared *P. lanceifolium* at ~2100m elevation, (2) "nr. *E. olivacea* 2" reared on *P. baezanum* at ~2100m, (3) "nr. *E. auruda* " reared on *P. crassinervium* at ~1800m and (4) "*Eois parumsimii* "reared on *P. baezanum* at ~1800m. Differences in genitalia structures are as follows: (a) Tergum 8 widths of both nr. *E. olivacea* 1 & 2, and *E. parumsimii* are slightly narrower than tergum 7, while for nr. *E. auruda*, tergum 8 width is roughly equal in width to tergum 7; (b) Sternum 8 widths of nr. *E.* olivacea 1 & 2, *E. parumsimii* are narrower than sternum 7, however for nr. *E. auruda*, sternum 8 width is roughly equal in width to sternum 7; (c) Ventral margin of saccus forms a small, transverse-ovoid pocket in nr. *E.* olivacea 1 & 2, and nr. *E. auruda* 1, whereas the ventral margin of saccus forms a blunt conical pocket in nr. *E. auruda* 2; (d) Vesica bears two distal group of spine-like cornuti in nr. *E.* olivacea 1 & 2 while for vesica in nr. *E. auruda* & *E. parumsimii* bears a single distal group of cornuti.
**Illustrations based off of male genitalia slides, not accurate depictions of each cryptic species.*

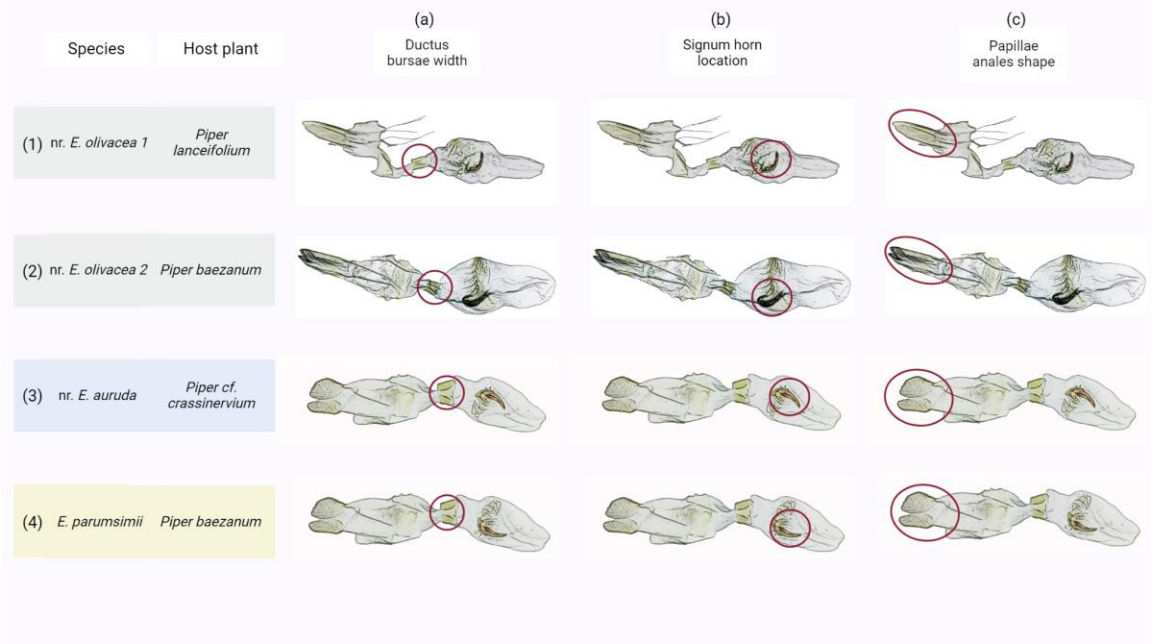

Figure 2. Simple illustrations* of female genitalia characters among the 4 cryptic species of (1) "nr. *E. olivacea* 1" reared *P. lanceifolium* at ~2100m elevation, (2) "nr. *E. olivacea* 2" reared on *P. baezanum* at ~2100m, (3) "nr. *E. auruda*" reared on *P. crassinervium* at ~1800m and (4) new species "*Eois parumsimii*" reared on *P. baezanum* at ~1800m. Difference in genitalia structures are as follows: (a) Ductus bursae widths of nr. *E.* olivacea 1 & 2 are narrow, while for nr. *E. auruda* and *E. parumsimii*, ductus bursae widths are wide; (b) Signum horns of nr. *E.* olivacea 1 & 2, and nr. *E. auruda* slightly protrudes beyond outer wall on left side of corpus bursae, whereas the signum horn of *E. parumsimii* partially protrudes beyond outer wall on ventral side of corpus bursae; (c) Papillae anales are narrow in nr. *E. olivacea* 1 & 2, and papillae anales are in nr. *E. auruda* and *E. parumsimii* are trapezoidal shape.
**Illustrations based off of female genitalia slides, not accurate depictions of each cryptic species.*

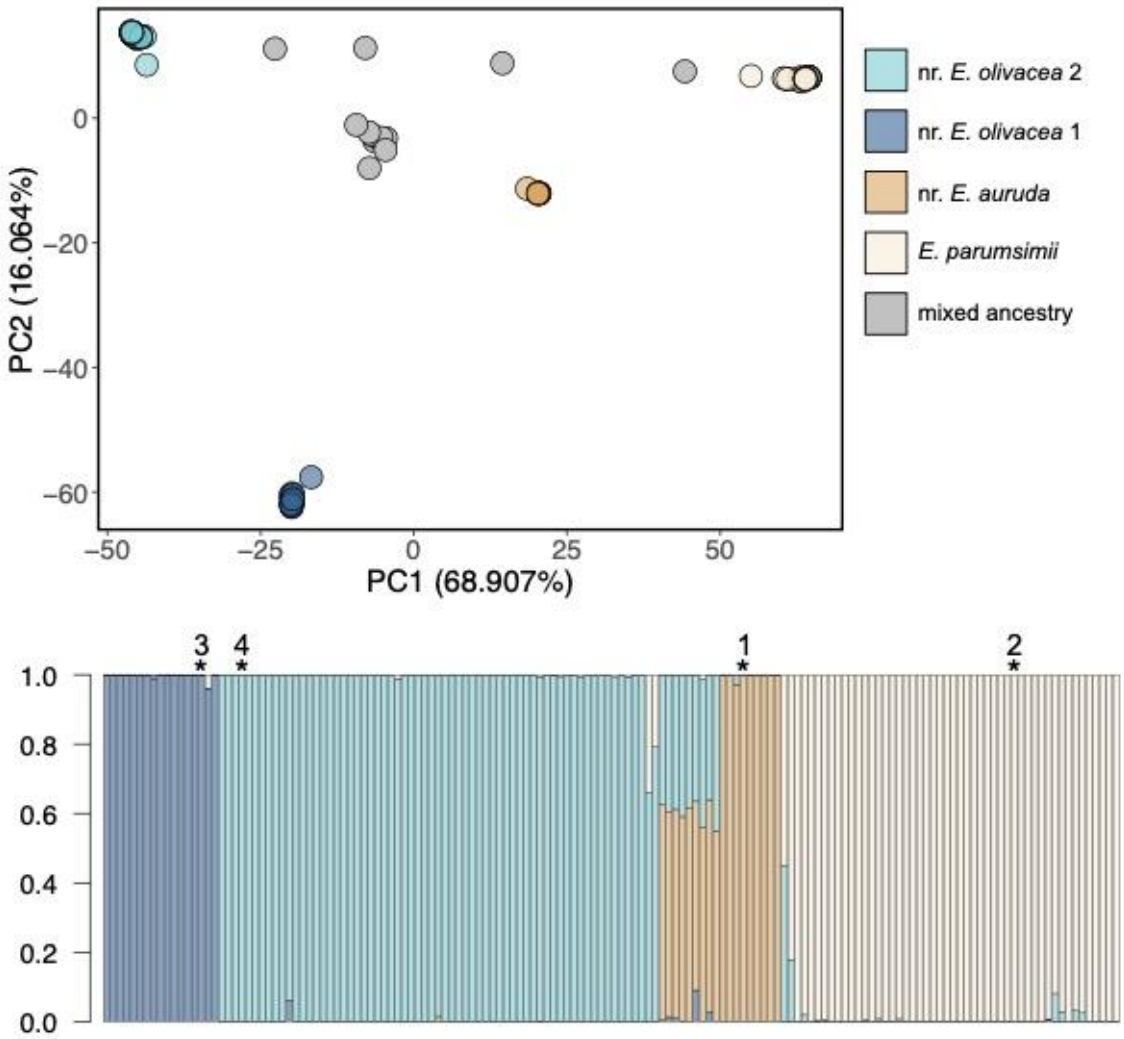

Figure 3. Genetic variation across *Eois* individuals is associated with *Piper* host plant species rather than elevation or locality. Four distinct source populations were resolved with (a) principal components analysis and (b) Bayesian clustering analysis, corresponding to distinct taxa based on phylogenetic analyses (Fig 2). Dark blue and white individuals were associated with *P. baezanum*, light blue individuals with *P. lanceifolium*, and orange individuals with *P. crassinervium*. Asterisks and numbers indicate the individuals that were included in the phylogenetic analysis (Figure 4).

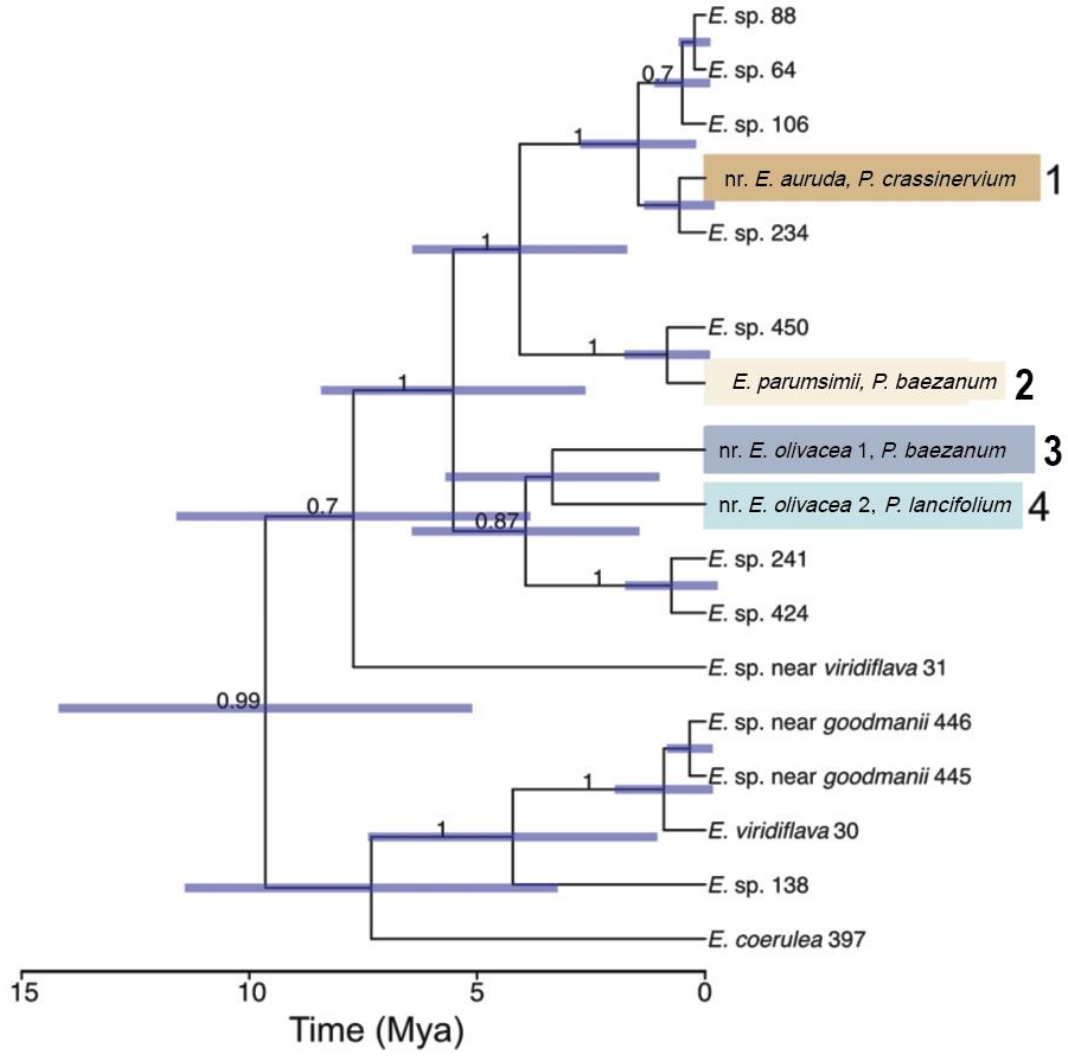

Figure 4. A pruned time-calibrated phylogeny of the focal taxa and their close relatives from a genus-wide species tree of 137 *Eois* species. Posterior node support > 0.7 is displayed, and the 95% highest posterior density intervals for node ages are represented by blue bars. Focal taxa are highlighted with the same colors utilized in Fig. 3 to designate cluster analysis assignments. Numbers to the right of focal species correspond to the same individuals indicated in the bar plot of Fig. 3.

Figure 5. Images of male and female adult representatives from groups sampled. First row (male left, female right): "nr. *E. olivacea*" reared on *P. lanceifolium* at ~2100m; Second row (male left, female right): *"*nr. *E. olivacea*" reared on *P. baezanum* at ~2100m elevation; Third row (male left, female right): *"*nr. *E. auruda"* reared on *P. crassinervium* at ~1800m; Fourth row (male left, female right): "*Eois parumsimii*" reared on *P. baezanum* at ~1800— 2100m elevation.

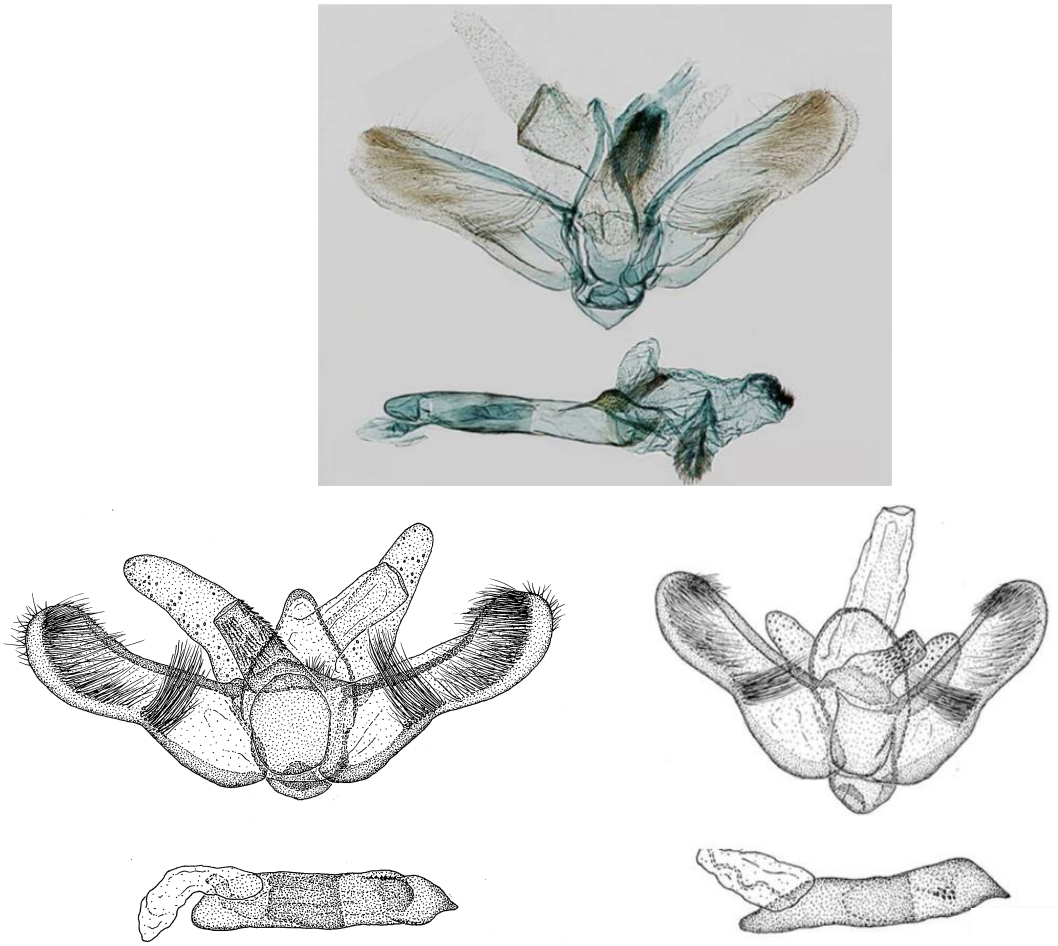

Figure 6. Images and illustrations of male genitalia of adult representatives from groups sampled. First row: Representative male genitalia of "nr. *E. olivacea*" 1 and 2 reared on *P. lanceifolium* and *P. baezanum* at ~2100m respectively. Second row left: Male genitalia of *"*nr. *E. auruda"* reared on *P. crassinervium* at ~1800m; second row right: Male genitalia of "*Eois parumsimii*" reared on *P. baezanum* at ~1800— 2100m elevation.

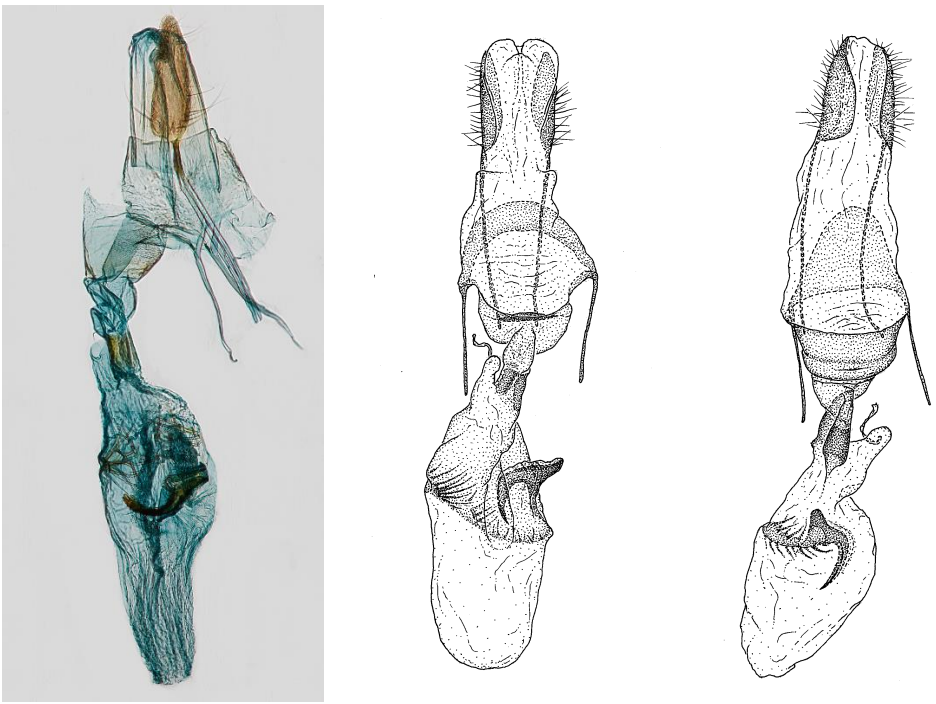

Figure 7. Images and illustrations of female genitalia of adult representatives from groups sampled. Left to right: Representative female genitalia of "nr. *E. olivacea*" 1 and 2 reared on *P. lanceifolium* and *P. baezanum* at ~2100m; "nr. *E. auruda*" reared on *P. crassinervium* at ~1800m; "*Eois parumsimii*" reared on *P. baezanum* at ~1800— 2100m.

	Orange	White	Dark blue	Light blue
Orange		0.28	0.39	0.40
White	0.28		0.53	0.56
Dark blue	0.39	0.53		0.33
Light blue	0.40	0.56	0.33	

Table 1. Genome-wide estimates of F_{ST} among the groups that were inferred with PCA and Bayesian clustering analysis. The names of the groups correspond to the colors in the PCA, bar plot, and phylogenetic tree in Figs. 3 and 4.

Supplementary Materials

Table S1. Sampling information for all samples included in the genetic analysis. Individuals which were raised to adults and harvested for morphological analysis are indicated in the most right-hand column.

Piper host species	Yanayacu locality	Taxonomic group	Elevation	Number of samples	Number of adults
P. baezanum	Plot 568	white	1883	3	3
P. baezanum	San Isidro	white	2100	1	0
P. baezanum	Rock Trail	white	2110	1	0
P. baezanum	Steam Trail	white	2127	36	0
P. baezanum	Plot 613	white	2210	1	0
P. perareolatum	Plot 613	white	2210	4	0
P. baezanum	Macuculoma Trail	dark blue	2100	3	3
P. baezanum	San Isidro	dark blue	2100	1	0
P. baezanum	Rock Trail	dark blue	2110	2	0
P. baezanum	Steam Trail	dark blue	2127	7	7
P. baezanum	Sierra Azul	dark blue	2250	1	0
P. lancifolium	Entrada a Caucheras	light blue	2093	1	1
P. lancifolium	YY Road	light blue	2100	40	7
P. baezanum	Rock Trail	light blue	2110	1	0
P. lancifolium	San Benjamin	light blue	2124	1	1
P. lancifolium	Sierra Azul	light blue	2250	21	0
P. baezanum	Baeza Granja Integral	mustard	1794	1	1
P. crassinervium	Plot 434	mustard	1869	7	7
P. lancifolium	YY Road	mustard	2100	1	0
P. baezanum	Steam Trail	mustard	2127	2	0

Table S2. Diversity estimates for each group inferred with structure analyses and PCA.

	Pi	W
P. crassinervium-associated	0.00202	0.00233
P. baezanum-associated "white"	0.00074	0.00075
P. baezanum-associated	0.00274	0.00329
P. lanceifolium-associated	0.00311	0.00349

original:

Lineage	Pi	Pi_low	Pi_high	W	W_low	W_high
grp1_drkblue	**0.00368015**	**0.00367607**	**0.00368423**	**0.0056467**	**0.00564272**	**0.00565068**
grp4_white	**0.00488115**	**0.00487622**	**0.00488608**	**0.00577686**	**0.0057728**	**0.00578092**
grp3_ltblue	**0.00520844**	**0.00520399**	**0.00521289**	**0.00663696**	**0.0066339**	**0.00664001**
grp2_mustard	**0.00389922**	**0.00389206**	**0.00390638**	**0.00430013**	**0.00429368**	**0.00430658**

Table S3. Nei's D estimates used to quantify the magnitude of genetic differentiation among groups of individuals assigned to distinct clusters with ENTROPY.

	P. baezanum	*P. baezanum* (mid)	*P. crassinervium*	*P. lancifolium*
P. baezanum	0	0.290	0.187	0.298
P. baezanum (mid)		0	0.360	0.250
P. crassinervium			0	0.341
P. lancifolium				0

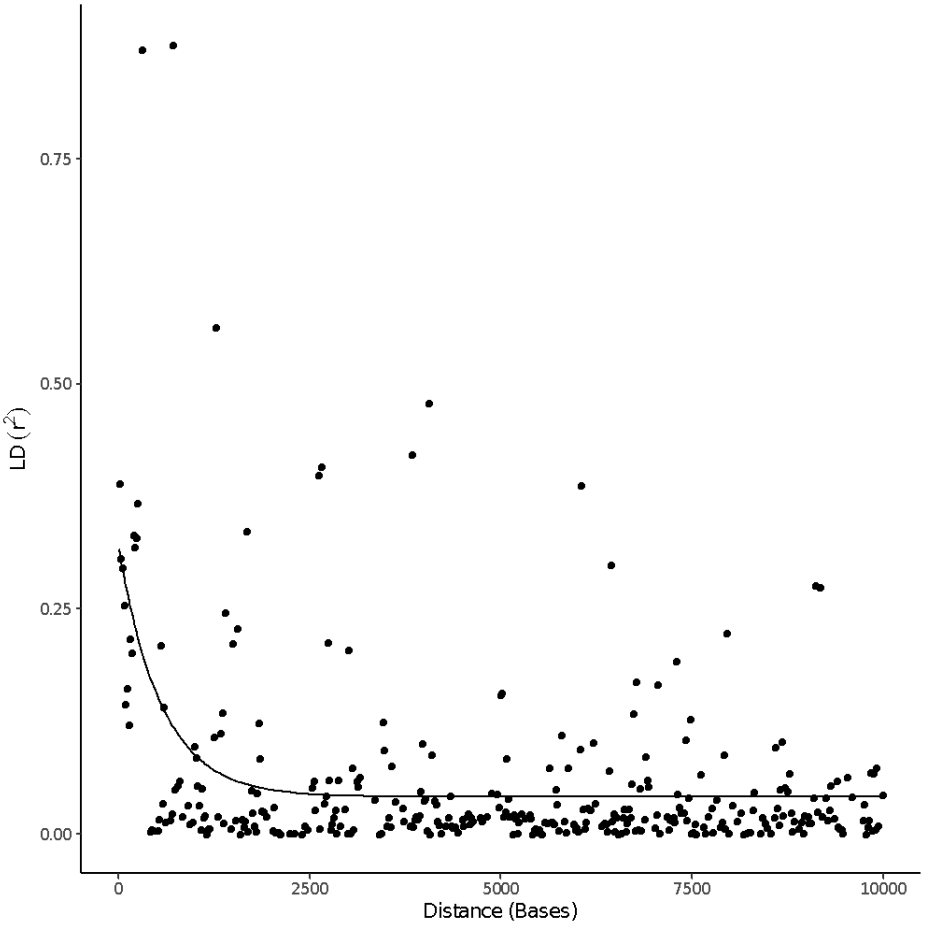

Figure S1: Linkage disequilibrium decay. Calculated mean of binned r^2 values for every 200 bp of physical distance and plotted means against the midpoints of the distance bins.

Adult Morphological Characters in *Eois olivacea* species complex

Below we present a morphological guide to distinguish species within this complex, with brief descriptions of each morphological character, followed by a number/score, or character state, in brackets (e.g. **[1]**, **[2]**, **[3]**, etc.) which denotes variation of that character. Abbreviations for morphological structures in the guide are as follows: Tg7 = tergum 7; Tg8 = tergum 8; St7 = sternum 7; St8 = sternum 8; CB = corpus bursae.

Male-only characters

1) Tergum 8 roughly equal in width to Tg7 **[0]**; Tg8 much narrower than Tg7 **[1].**

2) Sternum 8 equal in width to St7 **[0]**; Sternum 8 much narrower than St7 **[1].**

3) Ventral margin of saccus broadly triangular **[0]**; ventral margin of saccus forming a small, transverse-ovoid pocket **[1]**; ventral margin of saccus forming a blunt conical pocket **[2].**

4) A uniform field of spines surrounding phallus base **[0]**; spines surrounding phallus base arranged in two longitudinal rows **[1]**.

5) Apex of phallus simple **[0]**; apex of phallus forming a large, blade-like ventral process **[1]**.

6) Vesica lacking spine-like cornuti **[0]**; vesica bearing a single distal group of one or more coarse, spine-like cornuti **[1]**; vesica with two distal groups of spine-like cornuti **[2].**

Female-only Characters

7) Ductus bursae relatively narrow **[0]**; ductus bursae wide **[1]**.

8) Lateral margins of ductus bursae simple **[0]**; lateral margins of ductus bursae rolled upward, ductus U-shaped in cross section **[1].**

9) Signum comprising an ovoid patch of short spines **[0]**; signum horn-like, its base partially protruding from CB **[1].**

10) Signum located ventrally **[0]**; signum located laterally on left side of CB **[1].**

11) Internal part of horn-like signum narrow, curved, dentate along lateral margin **[0]**; internal part of horn-like signum wing-shaped, lateral margins serrate **[1].**

12) Corpus bursae without a sclerite arising from signum **[0]**; a spinose sclerite arising from signum, sclerite narrow, strap-like, wrapping around CB **[1].**

13) Papillae anales extremely narrow **[0]**; papillae anales short, trapezoidal **[1].**

Sixteen new species of the hyperdiverse geometrid genus *Eois* (Lepidoptera: Geometridae: Larentiinae) from the New World tropics

Lydia M. Doan[1], James S. Miller[2], John W. Brown[3], Matthew L. Forister[1], & Lee A. Dyer[1*]

[1] Department of Biology, Ecology, Evolution and Conservation of Biology, University of Nevada, Reno, NV 89557, USA (Doan: https://orcid.org/0000-0002-7039-8814; Forister: 0000-0003-2765-4779; Dyer: 0000-0002-0867-8874)

[2] Entomology Department, American Museum of Natural History, New York, NY, 10024, USA (deceased)

[3] Entomology Department, National Museum of Natural History, Smithsonian Institution, Washington, DC, 20560, USA; https://orcid.org/0000-0001-5610-9855

* Correspondence: ldyer@unr.edu

Running head: New species of *Eois*

Abstract

The hyperdiverse geometrid genus *Eois* Hübner, estimated to encompass over 1,000 species, is among the most species-rich genera in all of Lepidoptera. While the genus has attracted considerable attention from ecologists and evolutionary biologists in recent decades, limited progress has been made on its alpha taxonomy. This contribution focuses on the Olivacea Clade whose monophyly has been recognized previously through molecular analyses. We define the clade from a morphological perspective and then recognize the following species based on morphology and/or DNA sequence data: *E. olivacea* (Felder and Rogenhofer); *E. tochensis* Doan, **new species**; *E. pseudolivacea* Doan, **new species**; *E. auruda* (Dognin), **revised status**; *E. beebei* (Fletcher), **revised status**; *E. espadera* Doan, **new species**; *E. braziliana* Doan, **new species**; *E. ocherata* Doan, **new species**; *E. boliviensis* (Dognin); *E. pseudoboliviensis* Doan, **new species**; *E. cochabamba* Doan, **new species**; *E. fallera* Doan, **new species**; *E. multilineata* Doan, **new species**; *E. sclerobursana* Doan, **new species**; *E. nubesilva* Doan, **new species**; *E. pijao* Doan, **new species**; *E. dognini* Doan, **new species**; *E. altoparana* Doan, **new species**; *E. parumsimii* Doan, **new species**; and *E. heppneri* Doan, **new species**. We provide descriptions and illustrations of the egg, larva, and pupa a species reared from *Piper* (Piperaceae) in Ecuador.

Key words: genitalia, morphology, Neotropics, *olivacea* species group, *Piper*, taxonomy

Introduction

Larentiinae are the second largest subfamily of the highly diverse and worldwide family Geometridae, commonly known as geometers, loopers, or inch worms owing to the unusual gait of the caterpillars. Larentiines are primarily denizens of temperate regions, with more than 6,200 described species. However, the larentiine genus *Eois* Hübner is strictly tropical, and 83% of the described species are restricted to the Neotropics (Brehm et al. 2005, Brehm et al. 2011, Strutzenberger et al. 2010, 2011, 2017, Õunap et al. 2016). The genus is comprised of 267 formally described species – 220 in the Neotropics (Moraes et al. 2021a, b), 30 in Southeast Asia, and 17 in Africa (Herbulot 2000, De Prins and De Prins 2023). Remarkably, it is estimated that an additional 1,000 or more Neotropical species of *Eois* remain to be described (Brehm et al. 2011, Strutzenberger et al. 2017, Moraes et al. 2021a, b). If these estimates are correct, the genus is among the most species-rich in all of Lepidoptera. Based on Lepidoptera inventories in Central and South America, *Eois* appears to reach its greatest diversity in high elevation (above 2,000 m) habitats of the eastern Andes (Brehm et al. 2005, Brehm et al. 2011, Õunap et al. 2016), where the larvae specialize on shrubs and vines of the genus *Piper* (Piperaceae).

Species of *Eois* are generally small, with wingspans of 12–20 mm. Wing shape and pattern are diverse, featuring ground colors of yellows, greens, or browns, some with finely reticulated networks of lines, some with spots or bands, and others nearly uniform in color (Moraes et al. 2021a). Larvae are mostly green, with brown, red, or black spots or bands; others are completely dark (Brehm et al. 2011: fig. 6; also see http://caterpillars.org). In some clades, larvae are elongate and transparent greenish, whereas in others they are stout and brightly colored; one species even appears to mimic

bird-droppings (Brehm et al. 2011). The larvae exhibit a typical geometrid ground plan, with slender bodies, the absence of prolegs on abdominal segments 3–5, and slight modifications to the basic setal pattern found in other larentiines (McGuffin 1958).

The majority of *Eois* species was described between 1891 and 1920; but the first was named by Hübner in 1818 and the most recent by Moraes in 2021 (Parsons 1999; Moraes et al. 2021). While progress on the taxonomy of the genus has been slow since the 1950s, over the past two decades *Eois* has experienced a resurgence in attention from ecologists and evolutionary biologists focused on the interactions of *Eois* immatures with their host plants and associated parasitoids (e.g., Dyer and Palmer 2004, Connahs et al. 2009, Brehm et al. 2011). *Eois* larvae are specialized feeders on shrubs and vines of *Piper*, and their diversification is similar to the substantial diversification of their larval hosts (Rodriguez-Castaneda et al. 2010, Jahner et al. 2017).

A preliminary molecular phylogeny of the genus based on the mitochondrial gene cytochrome oxidase subunit 1 (COI; 1220 bp) and the nuclear gene elongation factor 1 alpha (Ef-1a; 1066 bp), evaluating 142 taxa, was presented by Strutzenberger et al. (2010), and this was followed by a checklist of the Neotropical species compiled by Brehm et al. (2011). A second analysis by Strutzenberger et al. (2017) using the same genes and a total of 221 *Eois* species, confirmed and reinforced their previous findings. The most recent molecular study of the genus by Moraes et al. (2021b) suggests that *Eois* potentially harbors an unparalleled array of cryptic diversity. Taken together, these molecular studies provide a preliminary phylogenetic framework for taxonomic progress at the species level, through the identification of many well-defined clades that can now be investigated based on their monophyly.

In each of the molecular studies, a well-defined “Olivacea Clade” was recognized that is rich in undescribed species from South America, primarily Ecuador (Strutzenberger et al. 2010; Strutzenberger et al. 2017; Moraes et al. 2021b), and this is supported by the large number of BINs represented in BOLD (Barcode of Life Database). Given the paucity of recent descriptive taxonomic work on the genus, coupled with its enormous undescribed diversity, we embarked on a study to describe and illustrate new species of *Eois*, and we begin this study focused on the Olivacea Clade based primarily on morphological data.

Materials and Methods

Specimens examined. The bulk of the material used in this treatment is from two sources: the collection of the National Museum of Natural History, Washington, D.C. (USNM), and an inventory of the caterpillars at Yanayacu Biological Station, Napo Province, Ecuador (Miller and Dyer 2009, Sudta et al. 2022). The Yanayacu site is located at 2200 m elevation in the Quijos Valley, Napo Province, in the Andes Mountains of northeastern Ecuador. The station lies just south of the equator (00°35.9’S, 77°53.4’W) in one of the world’s last remaining unexplored regions of high-elevation cloud forest. The survey has run continuously from 2001 to present.

At Yanayacu, caterpillars were discovered in the field primarily using visual searches. Larvae were taken to the laboratory where they were placed in plastic bags that were coded, imaged, tagged, and hung on clothes lines. Periodically, observational notes were taken on the larvae, and additional host material was added as needed. When an adult moth or butterfly, or sometimes its parasitoid, emerged, it was preserved and

labeled. Each specimen received a unique voucher number in the form of a serial number (e.g., 15328), with time of year, elevation, latitude and longitude, host plant, and other data. In the text, we use “r.f.” (= reared from) to denote larval host plants. Some specimens were preserved in alcohol and examined for setal patterns and other important larval characters such that a general description of shared characters among *Eois* larvae from this location is possible.

Dissections and Photography. Dissection methods followed those presented by Robinson (1976), except all parts were slide mounted using Euparal rather than Canada balsam. Initially, we attempted to evert the vesica, teasing it out with a 000 pin, but owing to the small size of the phallus, the process frequently inflicted more damage than the value of viewing features of the everted vesica. Also in the male genitalia, a membranous region surrounding the phallus typically supports a dense field of small spines. Although we attempted to leave the membrane in situ, more often it was detached with the phallus. For mated females, any spermatophore and associated material were removed.

Images of adults and genitalia were captured using a 65 mm lens attached to a Canon EOS 40D digital SLR (Canon U.S.A., Lake Success, NY) mounted on a Visionary Digital BK Lab System (Visionary Digital, Palmyra, VA). Multiple images were stacked using Helicon Focus software and subsequently enhanced using Adobe PhotoShop and GIMP 2.10 software. Plates were constructed in Adobe Photoshop CC 2020 (v21. 0).

Terminology. Descriptions of morphology and wing maculation are based on the examination of specimens using a Zeiss Stemi 2000-C stereomicroscope with SCHOTT EasyLED ring-light illuminator. Colors in the descriptions refer to the RAL color

standard (https://www.ral-farben.de/en/home/). Forewing length was measured to the nearest 0.5 mm using an optical micrometer. Terms for genital structures and forewing pattern elements follow Holloway (1997) and Viidalepp (2011). However, an unusual structure in the male genitalia of members of the several clades of *Eois* appears to lack a term. It is a membranous, flat, lateral flap of variable size and length attached to the sides of the tegumen and/or transtilla, which supports long, fine male scent scales. We suggest the term "lacina" for these structures and use that term throughout the manuscript. Viidalepp (2011) indicates that "The paired, heavily sclerotized, long and tapered projections from the posterior side of tegumen, which are the peculiarity of two species of the genus *Solitanea* Djakonov (the tribe Solitaneini Leraut) can be identified neither with socii nor with gnathos." Although we are uncertain of the homology of lacina with the structures in *Solitanea*, they appear to occupy the same position in the male genitalia.

Abbreviations for morphological structures in the text are as follows: Tg7 = tergum 7; Tg8 = tergum 8; St7 = sternum 7; St8 = sternum 8; PVP = postvaginal plate; DB = ductus bursae; DS = ductus seminalis; CB = corpus bursae.

Depositories. We examined specimens deposited in the following institutions:

CNC = Canadian National Collection of Insects, Ottawa, Canada;

INABIO = Instituto Nacional de Biodiversidad, Quito, Ecuador;

MGCL = McGuire Center for Lepidoptera and Biodiversity, Gainesville, Florida, USA;

MNCR-A = Museo National de Costa Rica, Arthropoda, Santo Domingo de Heredia, Costa Rica;

NHML = The Natural History Museum, London, England;

UNR = University of Nevada, Reno, USA (specimens on loan from INABIO);

USNM = National Museum of Natural History, Washington, D.C., USA (all USNM specimens are labelled with a unique USNM ENT barcode label, the numbers of which are given in the text).

Results

The Olivacea Clade

The Olivacea Clade was first recognized by Strutzenberger (2010) based on an analysis of 142 morphospecies of *Eois*, employing sequence data from two genes: COI (1220 bp) and Ef-1a (1060 bp). Sixteen morphospecies linked together to form the clade, all of which were assumed to be undescribed. The monophyly of the clade was subsequently confirmed by Strutzenberger et al. (2017) based on considerably broader taxon sampling (n = 221 morphospecies) but with the same genes, expanding the number of morphospecies in the clade to 23. A recent molecular study by Moraes et al. (2021) found support for an *olivacea* species group, but not for the clade; however, their study was based entirely on the mitochondrial gene COI.

Brehm et al. (2011: 1093) noted that all species belonging to the clade "have a green ground color and yellowish fringes…" They also commented that the caterpillars "show particularly contrasting patterns, including bright and dark spots dorsally…and pink spots laterally…in some species, whereas others exhibit merely some pale patches…" (Brehm et al. 2011: 1094–1095). They concluded that all species of the clade feed on species of *Piper* (Piperaceae). They further recognized that conspicuous morphological differences could be found even among closely related species within the group. For example, the male genitalia of some taxa possess numerous stout cornuti in

the vesica, whereas others lack them altogether. Within the Olivacea Clade as defined by Strutzenberger et al. (2010, 2017), there appears to be three species complexes: an *E. olivacea* complex, an *E. goodmanii* complex, and an *E. muscosa* complex (although the last is represented by a single morphospecies) (Figs. 1–3). Although the first two species complexes were recognized as monophyletic by Moraes et al. (2021), they were included as part of a larger clade, and *muscosa* was not included in their study. We focus our current efforts on the *E. olivacea* species complex. However, because diagnostic morphological characters to separate the *E. olivacea* species complex from the *E. goodmanii* species complex are yet to be discovered, one or more of the species treated herein may belong to the latter.

Eois olivacea species complex

As currently defined, the *E. olivacea* species complex includes *E. olivacea* (Felder and Rogenhofer 1875) (TL: Bogotá, Colombia; type in NHML), *E. beebei* (Fletcher, 1952) (TL: Rancho Grande near Maracay, Venezuela; type in NHML), *E. auruda* (Dognin, 1900) (TL: Loja, Ecuador; type in USNM), and *E. boliviensis* (Dognin, 1900) (TL: Bolivia, type in USNM), along with nearly a dozen undescribed morphospecies treated by Strutzenberger et al. (2017). BOLD (Barcode of Life Database) includes 27 BINs, mostly from Ecuador, with many fewer from Peru and Colombia, that likely represent species in the *olivacea* species complex. Herein, we describe 16 new species based nearly entirely on morphology of the adults. We also provide superficial descriptions and illustrations of the egg, larva, and pupa of *Eois*.

To minimize redundancy in the species descriptions, we first provide a general diagnosis and description of the *E. olivacea* species complex, which includes features shared by all the included taxa. In the individual species descriptions that follow, we include diagnoses and descriptions that include details of the features treated broadly in the general description of the complex. Because the *olivacea* species complex was recognized solely on the basis of molecular data, our morphological definition of the clade may be viewed as preliminary.

Diagnosis. Ovum (Fig. 50). The eggs are uniformly cream colored, oval, and flattened without sculpturing, and are deposited horizontally (i.e., not upright). They are laid singly on a leaf, but infrequently multiple eggs, as many as 12, can be found on a single leaf.

Larva (Figs. 51, 52). The caterpillars are typical of those of Larentiinae. The head is rounded with a standard arrangement of stemmata, a spinneret that is long and pointed but not extending beyond the labium, and a retinaculum on each mandible. The thoracic prolegs each have 6–8 setae. Abdominal segments 1–5 are approximately twice as long as those posterior to A5. There are no ridges, bumps, swollen segments, scoli, or filaments. Abdominal segments exhibit typical larentiine chaetotaxy with a few exceptions: A2–A7 lack the extra L seta found in temperate larentiines, and A1 has an arrangement of setae that includes two D setae, three L setae, two SV setae, and one V seta. All spiracles are round with a single seta immediately dorsal. The abdomen bears prolegs only on A6 and A10; each A6 proleg has 5 setae, and A10 prolegs have 6 setae each. Crochets are biordinal and arranged in two groups that surround a large pad in a hemi-ellipse. There

are paired paraprocts on A10, and the anal shield is rounded. Larvae scrape the bottoms of leaves, leaving characteristic windows of upper epidermal tissue on the host leaf.

Pupa (Fig. 53). The pupae of *Eois* are similar to those of other geometrids; they are 45–55 mm in length and dark brown. They are attached to the undersides of leaves with by hooked spines of the cremaster.

Imago. Adults of the *E. olivacea* species complex all share extremely similar wing color and pattern, with a pale yellowish to pale gray-green ground color, usually with one to three faint, jagged or wavy, narrow, whitish fasciae (i.e., antemedial, medial, and postmedial lines) extending from the costa to the hind margin of the forewing, and continuing across the hindwing. The postmedial fascia is usually well defined, whereas the submedian and median fasciae are often reduced or lacking altogether (especially in worn specimens). The outer margin of the wings (termen) bears an extremely narrow line of red-brown to maroon scales, and the fringe is bright yellow throughout, in contrast to the wing ground color and the terminal line. There usually is a small brown dot near the apex of the discal cell in both the forewing and hindwing, but the dot is occasionally weakly expressed or absent.

On the head, the chaetosemata are represented by small rounded patches posterior to the bases of the antennae, connected by a narrow, continuous row of setae located in a naked region near the posterior margin of the head (Fig. 4), typical of members of the tribe Asthenini (Viidalepp 2011). The male genitalia are characterized by the absence of an uncus, with the semi-sclerotized scaphium occupying this position; the dorsal part of the tegumen narrow; the presence of lacina; and a patch of spines in the membrane surrounding the phallus, often arranged in two longitudinal rows. The vesica in all

species examined bears a small, variable, semicircular plate with a saw-toothed margin (or field) around the curved side. Clusters of large, elongate cornuti are present in the vesica of a few species but absent in most. All of these morphological features appear to be shared with members of the *E. goodmanii* and *E. muscosa* species complexes.

Members of the *E. goodmani* species complex are superficially similar to those of the *olivacea* complex, but the wing ground color is usually a darker green or darker gray-green; the postmedial line is ill-defined and either dark rather than pale, extremely faint, or absent altogether; and the fringe is interrupted by brown patches rather than entirely yellow. Members of the *muscosa* species complex are superficially dissimilar to the other two groups, with the pale green forewing lacking medial and antemedial lines; instead with the inner two-thirds of the wing featuring a large, ill-defined patch of pale brown scales, and the fringe not contrasting with the forewing ground color (Figs. 1–3).

Description. Male. *Head*: Scales of frons smooth appressed, fawn brown; scales of vertex slightly paler; vertex between bases of antennae with narrow, transverse band of snow-white scales, separating fawn brown scales of frons from paler scales of vertex. Ocellus absent. Chaetosema a rounded patch posterior to base of antenna, with narrow continuous row across vertex in naked region near posterior margin of head. Compound eye large, comprising greater than 0.66 of head. Antenna cylindrical, bipectinate in males, with long, slender rami biciliate to tip, rami absent in distal 0.25 of antenna; dorsum of flagellomeres with white scales. Labial palpus with segment 2 approximately 0.5 length of segment 1; segment 3 short, approximately 0.25 as long as segment 1; length of all segments combined 0.5–0.7 times diameter of compound eye.

Thorax: Concolorous with forewing dorsum ground color. Legs long, slender, densely covered in scales, usually concolorous with thorax; tibia of mid- and hindlegs with conspicuous tibial spurs, midtibia with one, hindtibia with two, approximately 0.25 length of tarsomere 1; sclerotized tips of tibial spurs simple, elongated. Forewing broadly triangular, length 1.2 times width at termen, outer margin evenly convex, with discal cell less than 0.5 wing length, accessory cell long, originating from distal costal margin of discal cell. Ground color variable from pale yellow to pale greenish gray; antemedial line usually faint, wavy, ill defined, ivory; medial line either extremely faint or lacking altogether; postmedial line usually well defined, wavy, ivory, angled perpendicularly toward costa beyond M_3; region from postmedial line to termen sometimes with a faint, narrow, zigzag, ivory line; discal spot usually well defined, somewhat oblong-round, red-brown; costal region usually faintly tinged pale pinkish brown, irregularly marked with small cream blotches; termen with narrow, dark red-brown line, concolorous with discal spot, variable from nearly straight to conspicuously scalloped. Fringe pale yellow. Forewing underside usually ivory, suffused with faint reddish brown, darkest in costal portion, with or without trace of dorsal pattern; discal spot round, orange-brown, faint to absent. Fringe pale yellow. Hindwing concolorous with forewing; antemedial line ill defined, ivory; postmedial line well defined, wavy, ivory; termen and fringe as in forewing. Hindwing underside ivory to yellowish gray, with ill-defined pattern similar to upperside; discal spot faint. Hindwing rounded, outer margin evenly convex, with discal cell approximately 0.33 as long as wing, M_3 and CuA_1 stalked; frenulum with one thick spine in male, 6–8 weaker spines in female.

Abdomen: Concolorous with thorax, usually with narrow row of white to cream scales at posterior end of each segment. Slender, extending beyond anal angle of hindwing. Tg8 somewhat narrower posteriorly; St8 slightly tapered posteriorly; St8 equal in width to St7; Tg8 roughly equal in width to Tg7; St8 equal in width to Tg7; posterior margin of St8 with shallow, U-shaped mesal excavation. Genitalia with uncus absent; tegumen narrow, dorsal part band-like, with arms forming rounded dorsal arch; lacina of variable size and shape from lateral margins of tegumen or transtilla; junction of tegumen and vinculum forming a shallow angle. Anal tube (scaphium) long, with ventral surface bearing a long, relatively wide, sclerotized band. Saccus shallow with transverse dorsal margin at base of valva; ventral margin forming small, transverse-ovoid pocket. Transtilla weakly sclerotized, distinctly bilobed. Membrane surrounding phallus with one or two variable fields of short spines. Juxta gradually narrowing dorsally with wide dorsal margin and U-shaped mesal excavation. Area between phallus and juxta simple. Valva elongate-subrectangular to rounded, with dorsal and ventral margins roughly parallel, but ventral margin variably constricted near middle, with patch of long, fine setae at constriction; costa long, narrow, band-like, extending to apex. Apex of valva with fine, hair-like setae, similar to those covering remainder of valva; sacculus narrow, lightly sclerotized, 0.3–0.5 as long as valva, ventral margin contiguous with ventral margin of valva, without secondary group of robust setae near apex of sacculus; inner margin of sacculus lacking row of setae. Phallus usually ca. as long as valva, wide, with broad distal opening, narrowed basally, with rounded base; apex developed into blade-like ventral process. Vesica with at least one semicircular plate with a saw-toothed margin; cornuti variable: two clusters of fine short cornuti, a single group of large, spine-like cornuti, or

cornuti absent altogether; base of vesica minutely scobinate with a pair of narrow, curved scobinate sclerites.

Female. *Head*: Essentially as described for male, but antenna filiform, lacking rami.

Thorax: Essentially as described for male.

Abdomen: Essentially as described for male. Genitalia with papillae anales narrow to roughly triangular, distal portion rounded. Tg8 either narrow, quadrate, or U-shape, with transverse posterior margin, bearing transverse striations. Dorsal membrane between Tg8 and papillae anales simple, with a small membranous invagination. Posterior apophyses longer than anterior apophyses. Ostium forming a large, dorso-ventrally compressed, vase-like structure. Region between ostium and ductus bursae broadly membranous, bearing a ventral appendix. Ductus bursae narrow, lightly sclerotized with lateral margins rolled upward, ductus U-shaped in cross-section. Ductus bursae arising from a small, narrow appendix at base of corpus bursae ventrally, curving to right. Corpus bursae oblong with variable lateral band of long spines; rounded anteriorly, membranous, continuous with remainder of corpus. Signum horn-like with base partially protruding beyond outer wall on left side of corpus bursae. Internal part of signum comprised of long, curved spines. Narrow, strap-like, spinose sclerite arising from signum, wrapping around corpus bursae; remainder of corpus bursae beyond signum smooth and simple. Corpus bursae often composed of two parts, smooth distal portion broadly attached to remainder of corpus.

Few members of the species complex can be distinguished reliably based on facies alone. However, structures of the male and female genitalia provide morphological

characters for separating these similar-looking adults. We divide the species complex into two subgroups based on the arrangement and size of the cornuti in the vesica of the male phallus: Group I species have either one or two small patches of small cornuti (usually less than 0.15 the length of the phallus) or lack them altogether; and Group II species have one or two patches of large, elongate, robust cornuti that are >0.25 the length of the phallus.

GROUP I

Eois olivacea (Felder and Rogenhofer, 1875)

Figs. 1, 5, 25

Jodis olivacea Felder and Rogenhofer 1875: pl. 128, fig 13.

Eois olivacea: Parsons et al. 1999: 279; Brehm et al. 2011: 1106.

Type. Holotype ♂, Colombia, Bogota [ca. 2630 m] (NHML).

Additional specimens examined. Colombia: Fasaogasuga, [1770 m], [no date] (1♂), Dognin Collection, USNM slide 154,479, USNM ENT 01906870 (USNM).

Remarks and diagnosis. The holotype male of this species (Fig. 1) lacks an abdomen; hence, comparisons of the genitalia to congeners is impossible. However, among several candidates from Colombia (USNM) that are potential conspecifics of *E. olivacea*, a male from Fasaogasuga possesses the distinctive jagged line midway between the postmedial line and the termen of the forewing that is characteristic of the type of *E. olivacea* (Fig. 5). Hence, we provisionally assign that specimen to *E. olivacea*. The

question may be resolved through molecular analyses that are beyond the scope of this contribution. In the redescription below, details of external features are based on the holotype, and those of the genitalia are based on the putative conspecific. Although the genitalia of the specimen from Fasaogasuga is damaged, the important characters are intact.

If our association is correct, *E. olivacea* has the simplest vesica of any member of the complex, with the possible exception of *E. beebei*, with a single semicircular plate bearing teeth along the curved margin. The lacina are longer than those of *E. tochensis* and shorter than those of *E. pseudolivacea*, two species that share a very similar shape of the valvae with *E. olivacea*.

Redescription. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.0 mm (n = 1); forewing ground color pale gray-green; antemedial and medial lines faint, postmedial line well defined, ivory; distinctive, ivory, zigzag line midway between postmedial line and termen; discal spot small; costal region lightly tinged pale pinkish brown, irregularly and faintly marked with small cream blotches; termen with narrow, dark red-brown line, concolorous with discal spot, consisting of uninterrupted series of inward-directed scallops. Forewing underside as described for species complex. Hindwing concolorous with forewing, with antemedial line ill defined, postmedial line well defined; termen and fringe as in forewing. *Abdomen*: Genitalia (Fig. 25) with tegumen slender, lacina broad basally, upcurved, narrower and somewhat parallel-sided in distal 0.5, rounded apically; valva subrectangular, slightly constricted near middle with patch of long fine setae at constriction, sacculus well defined, terminating at constriction of valva, outer margin

weakly angled subbasally; phallus ca. as long as valva; vesica with semicircular, saw-toothed plate, lacking large cornuti; membrane surrounding phallus with a pair of dorsal fields of short spines.

Female. Unknown.

Distribution and biology. This species is known from the holotype from near Bogotá and a second specimen from Fasaogasuga, Colombia. The early stages remain unknown.

Eois tochensis Doan, new species

Figs. 6, 26

Type. Holotype ♂, Colombia, Río Toche, 2400 m, Dognin Collection, USNM slide 154,450, USNM ENT 01906871 (USNM).

Remarks and diagnosis. This species is described from a single male collected before the turn of the century that was identified as *E. olivacea* in the USNM collection. The holotype is similar in ground color to *E. olivacea*, but lacks the distinctive, narrow, jagged line midway between the postmedial line and the termen that is present in the latter. The male genitalia are most similar to those of *E. pseudolivacea*. The two share a subrectangular valva that is weakly constricted near the middle; a sacculus that is weakly angled subbasally; a large field of spines in the membranous region surrounding phallus; and two small patches of short cornuti in the vesica. The two species are separated by (1) the shape of the lacina: broad basally, arched dorsally, and 0.5 the length of the valvae in *E. tochensis* vs. mostly parallel-sided and nearly as long as the valvae in *E.*

pseudolivacea; (2) the length if the tegumen: shorter in *E. tochensis*; and (3) the shape of the transtillar lobes: more triangular in *E. tochensis* vs. more rounded in *E. pseudolivacea*.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.0 mm (n = 1); forewing ground color pale gray-green; antemedial and postmedial lines faint, medial line well defined; discal spot well defined; costal region slightly tinged pale pinkish brown, irregularly marked with small cream blotches; termen with narrow, maroon line consisting of uninterrupted series of inward-directed scallops concolorous with discal spot. Forewing underside as described for species complex, with vague trace of upperside markings. Hindwing concolorous with forewing, with antemedial line ill defined, postmedial line well defined, contiguous with postmedial line of forewing; termen and fringe as in forewing. *Abdomen*: Essentially as described for species complex. Genitalia (Fig. 26) with lacina broad in basal 0.33, arched dorsally (as in *espadera*); valva comparatively long, narrow, ventral margin weakly angled subbasally, slightly constricted at termination of sacculus near mid-valva; membrane surrounding phallus with large patch of spines, ca. 0.3 length of phallus; phallus ca. as long as valva with pointed triangular process apically, vesica with semicircular, toothed plate and two patches of small cornuti, one with minute slender spines, other with short thicker spines.

Female. Unknown.

Distribution and biology. This species is known only from the holotype from Río Toche, Colombia. The early stages remain unknown.

Etymology. The species name refers to the type locality of Río Toche, Colombia.

Eois pseudolivacea Doan, new species

Figs. 7, 27, 42

Types. Holotype ♂, Ecuador, Napo, Yanayacu Biological Station, 2163 m, 00°35’0.9”S, 077°53’0.4”W, Mar 2010, r.f. 46067, Earthwatch, slide 69575 (UNR).

Paratypes (5♂, 6♀). ECUADOR: Napo: Yanayacu Biological Station, 2066.8 m, 00°34’0.001”S, 77°52’.001”W, Jun 2013, r.f. 75714, 75718 (2♂), July 2013, r.f. 78585, 78550, 78563 (1♂, 2♀), Sept 2014, r.f. 86500 (1♀), Aug 2015, r.f. 88081 (1♂), Earthwatch (UNR). ECUADOR: Napo: Yanayacu Biological Station, 2096.6 m, 00°35’7.02”S, 77°52’31.379”W,” Oct 2013, r.f. 80578, 80648, 80696 (3♀), Earthwatch (UNR). [no further locality data] r.f. B1409 (1♂), Earthwatch (UNR).

Remarks and diagnosis. *Eois pseudolivacea* is described from Ecuador, where it occurs in sympatry with several very similar congeners. Superficially, *E. pseudolivacea* is nearly indistinguishable from other members of the species complex. However, the male genitalia are easily recognized by the length of the lacina, which is nearly as long as the valva. Other features of the male genitalia, including the valvae, sacculus, and phallus, are most similar to those of *E. tochensis.*

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for the species complex, except forewing length 9.5 mm (n = 12); forewing ground color light moss; antemedial line faint, ivory, uniform in width throughout; medial line faint, wavy, ivory; postmedial line prominent, well defined, wavy, ivory, perpendicularly angled toward costa beyond M_3; region from postmedial

line to termen with two narrow, wavy, ivory lines; discal spot well defined, round, red-brown; costal margin banded cream and dark red-brown; termen with narrow, dark red-brown line, concolorous with discal spot. Fringe light yellow. Forewing underside ivory, suffused with faint reddish brown, with faint trace of dorsal pattern; discal spot round, orange-brown, faint to absent. Fringe light yellow. Hindwing concolorous with forewing; antemedial line ivory; postmedial line wavy, cream, perpendicularly angled toward costa beyond CuA. Hindwing underside with pattern similar to upperside, with faint red-brown antemedial and postmedial lines, region from postmedial line to termen with two, wavy, red-brown lines; discal spot faint. *Abdomen*: Genitalia (Fig. 27) with lacina nearly as long as valva, somewhat parallel-sided; membrane surrounding phallobase bearing large dorsal field of short spines arranged in two longitudinal rows; vesica bilobed, each lobe with distal group of small spine-like cornuti.

Female. *Head* and *Thorax*: Essentially as described for male, but antenna slightly narrower, lacking rami. *Abdomen*: Genitalia (Fig. 42) with papillae anales narrow; ductus bursae narrow; corpus bursae oblong, with large, curved, spinelike signum located laterally on left side.

Distribution and biology. This species is known only from Napo Province, Ecuador. It was reared from larvae discovered on *Piper lanceifolium* (n = 456) and *P. baezanum* (n = 49).

The eggs, larvae, and pupae of *A. pseudolivacea* have all the general characteristics described above for *Eois* with no modifications (Figs. 50–53). For larvae, the first two instars are similarly colored, with a clear beige head capsule, yellowish green thorax, abdomen, pinacula, and setae. The thoracic legs and prolegs are clear. Instars 3-5 have

this same color pattern but also have paired broad cream patches subdorsally, stretching across all segments from T2 to A8, A9-10 are usually light cream colored, pinacula are chalky white with brown setae, and mandibles are dark brown. A thin white lateral stripe connects all spiracles. Pupae are usually pressed along major leaf veins on the underside of the leaf.

Etymology. The species name refers to the superficial similar of this species to *E. olivacea*.

Eois auruda (Dognin, 1900)

Figs. 8, 28

Amaurinia auruda Dognin 1900: 443.

Eois auruda: Parsons et al. 1999: 279; Brehm et al. 2011: 1106.

Types. Holotype ♂, Ecuador, [Loja Province], environs de Loja, 1889, Dognin Collection, USNM type 32227, USNM ENT 01906872 (USMN).

Paratype (1♂). Ecuador, Loja, valley of Zamora [S 00°35.9” W 77°53, 2163 m], May 1886, USNM slide 154,179, USNM ENT 01906873 (USMN).

Additional material examined. ECUADOR: [Loja Province], Environs de Loja, 1891 (1♂), USNM slide 154,650, USNM ENT 01906874 (USNM).

Remarks and diagnosis. *Eois auruda* was described by Dognin based on two males from “Loja et vallée de Zamora, Equateur.” One specimen is clearly labelled “type” and the other “co-type.” Superficially, *E. auruda* is paler yellowish orange than the pale green

of most members of the species complex, somewhat similar to the color of *E. braziliana* and *E. ocherata*; and the maroon scallops of the termen are weakly interrupted by yellowish brown. The male genitalia are most like those of *E. tochensis*, with similar shaped valvae and lacina. However, the phallus of *E. auruda* lacks the two patches of small cornuti present in the vesica of *E. tochensis*; the membranous region surrounding the phallus has a considerably smaller patch of spines; and the lacina are slightly narrower in the distal half than in *E. tochensis*.

Description. Male. *Head*: Essentially as described for complex. *Thorax*: Essentially as described for complex, except forewing length 9.0–10.0 mm (n = 3). Forewing ground color pale yellowish green; postmedial line prominent throughout; antemedial line faint; discal spot, small, round, faint, reddish brown; costal region slightly tinged with pale brown; termen with dark maroon scalloped line, concolorous with discal spot; fringe two-toned, mostly pale yellow with small incursions of red and/or brown between veins M_2 and M_3. Forewing underside pale yellow, suffused with pale reddish brown, with faint indication of upper surface markings; antemedial line absent. Discal spot round, faint to absent, orange-brown; termen with dark orange-brown line. Hindwing ground color brownish pale yellowish green; antemedial line absent or very faint; postmedial line well defined; region between postmedial line and termen with several extremely faint, interrupted wavy, pale-yellow lines; discal spot well defined, red-brown. Fringe two-toned, mostly yellow, interrupted by pale red veins M_2 and M_3. Hindwing underside with pattern and coloration similar to forewing underside. *Abdomen:* Essentially as described for genus. Genitalia (Fig. 28) with tegumen slender, lacina broad basally, upcurved, somewhat parallel-sided in distal 0.5; valva subrectangular, slightly constricted near

middle with patch of long fine setae, sacculus well defined, rounded subbasally, terminating at constriction of valva; phallus long, broad; vesica with semicircular, saw-toothed plate, lacking large cornuti; membrane surrounding phallus with very small dorsal field of short spines.

Female. Unknown.

Distribution and biology. This species is known from three specimens from Loja Province, Ecuador, collected before the turn of the twentieth century.

Eois beebei Fletcher, 1952, revised status

Figs. 9, 29

Racheospila beebei Fletcher 1952: 101.

Eois beebei: Parsons et al. 1999: 279; Brehm et al. 2011: 1106.

Type. Holotype ♂, Venezuela, Rancho Grande near Maracay, W. Beebe, No. 481604 (NHML).

Remarks and diagnosis. Fletcher (1952) described this species from a single male collected by William Beebe at Rancho Grande (now known as Henri Pittier National Park), in the Venezuelan Costal Range, an historically well-known collecting locality. Fletcher's description is somewhat outdated, as is his rather crude drawing of the male genitalia. The species was treated as a synonym of *E. olivacea* by Parsons et al. (1999), without the benefit of a comparison of the genitalia with those of the latter.

As in many members of Group I, the phallus of *E. beebei* has a large, conspicuous, scobinate plate with a saw-toothed edge situated near the distal end of the vesica, but lacks long cornuti. The species can be distinguished from all other members of the species complex by the well-defined sacculus in the basal half of the valva.

Redescription. Male. *Head*: Frons and vertex pale pinkish buff with distinct white bar between bases of antennae; labial palpus pale pinkish buff, length ca. 0.5 diameter of compound eye; pectinations of antenna ca. four times as long as the diameter of the shaft. *Thorax*: Pale olive; forewing ground color pale olive, anterior 0.5 irrorate with pale grayish brown, costa lightly irrorated with cream-brown, postmedial fascia white, discal spot fuscous. Fringe chalcedony yellow. Forewing undersurface white, glossy; discal spot minute. *Abdomen*: Pale olive, each segment edged posteriorly with white. Male genitalia (Fig. 29) with top of tegumen broadly rounded; lacina supporting long androconial scales; valva subrectangular with distinct sacculus along venter of basal 0.5. Phallus with weakly sclerotized patch near apex; vesica with two scobinate plates in apical 0.5, lacking elongate cornuti. [Need to get an image from David Lees.]

Distribution and biology. Known only from the type locality.

Eois espadera Doan, new species

Figs. 10, 30

Type. Holotype ♂, Colombia, Antioquia, Queb. [de] Espadera, 7 km E Medellin, 6 Mar 1984, C. M. & O. S. Flint, USNM slide 154,651, USNM ENT 01906875 (USNM).

Remarks and diagnosis. This species is described from a fairly recently collected male (1984). The forewing ground color is similar to that of *E. boliviensis,* but the postmedian line is slightly brighter. The male genitalia are most similar to those of *E. tochensis*. The two share a comparatively long, narrow valva that is weakly constricted in the middle; a sacculus that is weakly angled subbasally; and a large field of spines in the membranous region surrounding phallus. However, *E. espadera* can be distinguished by the absence of cornuti in the vesica, the long distal tip of the phallus, and smaller, more rounded lobe of the vinculum.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.0 mm (n = 1); forewing ground color pale gray-green; antemedial and medial lines extremely faint or absent, medial line well defined, whitish; discal spot well defined, reddish brown; costal region slightly tinged pale pinkish brown, irregularly marked with small, faint, cream dashes; termen with narrow, red-brown line, concolorous with discal spot, consisting of uninterrupted series of inward-directed scallops; region between postmedial line and termen with or without faint whitish striae. Forewing underside pale whitish green, suffused with pale red-brown, darkest along costa. Hindwing concolorous with forewing, with postmedial line well defined, contiguous with postmedial line of forewing; termen and fringe as in forewing. *Abdomen*: Essentially as described for species. Genitalia (Fig. 30) with lacina broad in basal 0.33, arched dorsally (as in *tochensis*); valva comparatively long, narrow, ventral margin weakly angled subbasally, slightly constricted at termination of sacculus near mid-valva; membrane surrounding phallus with large patch of spines, ca. 0.3 length of phallus; phallus ca. as long as valva with elongate, slender, pointed process

apically, vesica with semicircular, toothed plate; additional patches of small cornuti absent.

Female. Unknown.

Distribution and biology. This species is known only from Colombia. The early stages are unknown.

Etymology. The specific epithet is the Spanish word for waterfall, and refers to the type locality of this species - Quebrada de Espadera.

Eois braziliana Doan, new species

Figs. 11, 31, 43

Type. Holotype ♂, Brazil, Paraná, Castro, [no date], Wm. Schaus Collection, USNM slide 154,458, USNM ENT 01906876 (USNM).

Paratypes (3♂, 1♀). Brazil: Paraná, Castro, [no date] (1♂, 1♀), Wm. Schaus Collection, USNM slide 154,171, USNM ENT 01906877, 01908878 (USNM). [no further locality data] (1♂), F. Johnson Collection, USNM ENT 01906879 (USNM). São Paulo: "S.E. Brazil," [no date] (1♂), Wm. Schaus Collection, USNM ENT 01906880 (USNM).

Remarks and diagnosis. This species is described from four males and a female from Paraná (n = 4) and São Paulo (n = 1), Brazil, collected over a century ago. Although the specimen from São Paulo is superficially identical to the others, it lacks an abdomen; hence, its genitalia cannot be examined to confirm its conspecificity with the other

specimens. Nonetheless, it is included as a paratype based on its superficial similarity to the other specimens.

***Eois braziliana* is externally most similar to *E. auruda* and *E. ocherata*, with a slightly more yellowish orange** forewing ground color than most members of the complex, although not nearly as dark as that of *E. ocherata*. In the forewing of ***E. braziliana* there is a faint row of small cream markings in the region between the postmedial line and the termen that is much fainter in *E. auruda*. The male genitalia of the *E. braziliana* and *E. auruda* are also similar, but those of *E. braziliana* can be distinguished by the two parallel fields of spines on the membrane surrounding the phallus (in *E. auruda* there is a single broader patch of spines) and by the slenderer saw-toothed plate in the vesica.**

Description. Male. *Head*: Essentially as described for complex. *Thorax*: Essentially as described for complex, except forewing length 9.0–9.5 mm (n = 3). Forewing ground color pale yellowish green; postmedial line prominent throughout, antemedial more-or-less complete; discal spot round, reddish brown; costal region very slightly tinged with pale brown, with a few small, irregular, cream dashes; termen with reddish brown scalloped line, concolorous with discal spot; fringe pale yellow throughout. Forewing underside pale yellow, suffused with pale reddish brown in costal region, with faint indication of upper surface markings; antemedial line absent. Discal spot round, faint to absent, orange-brown; termen dark reddish brown line. Hindwing ground color pale yellowish green; antemedial line absent or very faint; postmedial line well defined; region between postmedial line and termen with several extremely faint, interrupted wavy, pale-yellow lines; discal spot well defined, red-brown. Fringe pale yellow. Hindwing

underside with pattern and coloration similar to forewing underside. *Abdomen:* Essentially as described for genus. Genitalia (Fig. 31) with tegumen slender, lacina broad basally, upcurved, somewhat parallel-sided in distal 0.5; valva subrectangular, slightly constricted near middle with patch of long fine setae, sacculus well defined, rounded subbasally, terminating at constriction of valva; phallus long, broad; vesica with semicircular, saw-toothed plate, lacking large cornuti; membrane surrounding phallus with two parallel fields of short spines.

Female. *Head* and *Thorax*: Essentially as described for species complex. *Abdomen*: Genitalia (Fig. 43) with papillae anales broad, somewhat ovoid; sterigma a weakly sclerotized lateral band; antrum membranous, broadest posteriorly, confluent with ductus bursae; ductus bursae short, broad, with wide, band-shaped colliculum at junction with corpus bursae; corpus bursae oblong, with large hook-shaped signum, expanded at base, extending slightly beyond outer wall of corpus bursae; an irregularly linear patch of 12–14 long, slender, curved spines from left side of corpus.

Distribution and biology. **This species is known from a handful of specimens collected in Paraná and São Paulo, Brazil**. The early stages are unknown.

Etymology. The species name is derived the country of the type locality, Brazil.

Eois ocherata Doan, new species

Figs. 12, 44

Type. Holotype ♀, Colombia, Carmen [El Carmen de Viboral?], 21 Aug 1908, Fassel, Dognin Collection, USNM slide 154,453, USNM ENT 01906881 (USNM).

Remarks and diagnosis. ***Eois ocherata*** is described from a single female that has considerably more pale ocherous reddish-brown overtones on the forewing than in all other species of the group (Fig, 12). It is somewhat similar to *E. braziliana* and *E. auruda*, but with darker pinkish hues, and the underside is conspicuously darker throughout. The female genitalia are distinguished by triangular rather linear papillae anales, a broader colliculum, and a comparatively small patch of spines on the left side of the corpus bursae originating from a small, warty diverticulum. The corpus bursae also bears a large sickle-shaped signum, serrate along one margin, typical of other females of the species complex.

Description. Male. Unknown.

Female. *Head*: Essentially as described for complex. *Thorax*: Essentially as described for complex, except forewing length 9.5 mm (n = 1); forewing ground color pale ocherous reddish-brown; wavy, cream, postmedial line prominent from costa to hind margin; antemedial and medial lines absent; spot of discal cell rather faint, reddish brown; costal region slightly tinged darker pale pinkish brown in distal 0.5, with a few small, irregular, cream dashes; termen with uninterrupted, reddish brown scalloped line, concolorous with discal spot; fringe yellow throughout. Forewing underside pale ocherous reddish-brown, faintly suffused with pale reddish brown throughout, darkest in costal region; ill-defined trace of pale postmedial line; discal spot round, orange-brown; termen with fine, scalloped, dark reddish brown line. Hindwing ground color concolorous with forewing; antemedial and medial lines absent; postmedial line present; discal spot well defined, red-brown; fringe pale yellow. Hindwing underside with pattern and coloration similar to forewing underside. *Abdomen:* Essentially as described for species

complex. Genitalia (Fig. 44) with papillae anales somewhat triangular; sterigma a weakly sclerotized lateral band; ductus bursae nearly as broad as sterigma, with wide colliculum immediately before junction with corpus bursae; corpus bursae oblong, with large sickle-shaped signum, weakly serrate along outer (long) margin; a small, invaginated, sclerotized, warty, diverticulum on left side, with 6–8 long, slender, curved, spines, some branched.

Distribution and biology. **This species is known from a single specimen from Colombia**. The early stages are unknown.

Etymology. The specific epithet refers to the rather pale ocher color of the wings.

Eois boliviensis (Dognin, 1900)

Figs. 13, 32

Thalassodes boliviensis Dognin 1900: 215.

Eois boliviensis: Parsons et al. 1999: 275; Brehm et al. 2011: 1105.

Type. Holotype ♂, Bolivia, USNM slide 154,454, USNM ENT 01906882 (USNM).

Remarks and diagnosis. Dognin (1900) described this species from a single male from Bolivia, without a specific locality. There are two additional specimens in the USNM from the Dognin collection identified by him as *boliviensis*, but neither is conspecific with the holotype. This species was transferred to *Amurinia*, now considered

a synonym of *Eois*, and later treated as *Eois* by Parsons et al. (1999) and Brehm et al. (2011).

Eois boliviensis is superficially similar to other members of the species group, but the ground color is a distinctive darker gray-green, most similar to that of *E. pseudoboliviensis*. The male genitalia are also typical of other species, with a coarsely toothed, scobinate plate near the distal end of the vesica, and the absence of long cornuti. The species can be distinguished from all other members of the species complex by the small, free, triangular lobe at the distal end of the sacculus ca. 0.33 the distance from the base to the apex of the valva (Fig. 32), the relatively smaller toothed plate in the vesica, and the reduced patch of spines in the membranous region surrounding the phallus in the male genitalia.

Redescription. Male. *Head*: Frons and vertex pale green, with white bar between bases of antennae; labial palpus pale grayish green. *Thorax*: Essentially as described for species complex, except forewing length 10.0 mm (n = 1); forewing ground color gray-green, with straw colored, wavy, postmedial line; small spot in cell, red-brown; terminal line more wavy than scalloped, reddish brown; fringe straw. Underside pale gray-green, entirely suffused with pale reddish brown. Hindwing ground color and postmedial line concolorous with those of forewing. Underside pale grayish green. *Abdomen*: Pale green. Male genitalia (Fig. 332) with tegumen arms joined dorsally forming a weakly bilobed process; lacina broad in basal 0.4, narrower and weakly attenuate in distal 0.6 with rounded outer margin, bearing long, hair-like androconial scales; valva elongate-subrectangular, ca. 3 times longer than wide, parallel-sided, with rounded outer margin; sacculus angled subbasally, with small triangular process at termination, ca. 0.33 distance

from base to apex of valva; phallus ca. as long as valva, attenuate basally, somewhat truncate apically; membrane surrounding phallus with broad, weakly developed field of spines; vesica with small, coarsely toothed plate in apical 0.5, lacking elongate cornuti.

Female. Unknown.

Distribution and biology. *Eois boliviensis* is known from holotype collected in Bolivia without additional locality data. Nothing is known of the biology.

Eois pseudoboliviensis Doan, new species

Figs. 14, 33

Type. Holotype ♂, Bolivia, Chaco, USNM slide 154,666, USNM ENT 01906883 (USNM).

Remarks and diagnosis. This species is described from a single male from Chaco, Bolivia identified by Dognin as *Thalassodes boliviensis*. Superficially, it is nearly identical to *E. boliviensis*, with a slightly greater forewing length and darker gray-green forewing ground color than most members of the *olivacea* species complex, and the fringe paler yellow than that of other species. It can be distinguished from all other species by the conspicuously rounded ventral margin of the sacculus; it lacks the triangular lobe of the sacculus that is diagnostic for *E. boliviensis*.

Description. Male. *Head*: Frons and vertex pale green, with white bar between bases of antennae; labial palpus pale grayish green. *Thorax*: Essentially as described for species complex, except forewing length 10.0 mm (n = 1); forewing ground color gray-green; postmedial line, straw colored, wavy; antemedian and medial lines absent; brown

spot in discal cell; terminal line nearly straight, pale reddish brown; fringe straw colored. *Abdomen*: Pale green. Male genitalia (Fig. 33) with tegumen arms joined dorsally forming a broadly rounded hood; lacina short, broad in basal 0.4, narrower and parallel-sided in distal 0.6 with round distal margin; valva broad in basal 0.5, narrower in distal 0.5, with rounded outer margin; sacculus comparatively broad, with conspicuously rounded ventral margin; phallus ca. as long as valva, pointed apically; membrane surrounding phallus with small field of tiny spines; vesica with small, coarsely toothed plate in apical 0.5, lacking elongate cornuti.

Female. Unknown.

Distribution and biology. *Eois pseudoboliviensis* is known from the male holotype collected in Chaco, Bolivia. Nothing is known of the biology.

Eois cochabamba Doan, new species

Figs. 15, 34

Type. Holotype ♂, Bolivia, Incachaca, Cochabamba, [no date], J. Steinbach, Wm. Schaus Collection, USNM slide 154,491, USNM ENT 01906884 (USNM).

Paratypes (4♂). Bolivia, Incachaca, Cochabamba, [no date], J. Steinbach, Wm. Schaus Collection, USNM slide 154,179, USNM ENT 01906885, USNM ENT 01906886, USNM ENT 01906887, USNM ENT 01906888 (USNM).

Remarks and diagnosis. This species is described from a series of five male specimens, all with identical collection data – Cochabamba, Incachaca, Bolivia. Superficially, it is nearly indistinguishable from most other members of the species

complex, with a pale greenish forewing ground color, and a well-defined postmedial line, with the antemedial and medial lines weak or absent, and a conspicuously scalloped terminal line. The male genitalia are much like those of *E. pijao*, with a slightly deeper constriction in the middle of the valva (at the termination of the sacculus), with the venter of the valva slightly rounded, both the sacculus and in the distal half of the valva beyond the sacculus. ***Eois cochabamba*** can be distinguished from of *E. pijao* by the absence of a prominent patch of large cornuti in the vesica.

Description. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 8.0-9.0 mm (n = 5); forewing ground color pale gray-green; antemedial weak in lower 0.5 of wing, medial line absent, postmedial line well defined, slender, pale ivory; discal spot well defined, reddish brown; costal region from wing base to apex faintly tinged pale pinkish brown, irregularly marked with small cream dashes; region between postmedial line and termen with indistinct pale markings; terminal line narrow, red-brown, concolorous with discal spot, consisting of uninterrupted series of inward-directed scallops. Forewing underside pale whitish green, suffused with pale reddish brown, darkest along costa. Hindwing concolorous with forewing, with postmedial line well defined, contiguous with postmedial line of forewing; termen and fringe as in forewing. *Abdomen*: Male genitalia (Fig. 34) with tegumen slender; lacina broad in basal 0.33, extending latero-dorsally, mostly parallel-sided; transtilla with a pair of large, membranous, triangular lobes directed dorsally; valva comparatively long, constricted at termination of sacculus near mid-valva, with sacculus and margin beyond sacculus both weakly rounded; membrane surrounding phallus with large patch of spines, ca. 0.3 length of phallus; phallus ca. as

long as valva with elongate, pointed process apically; vesica with semicircular, toothed plate, stout cornuti absent; membrane surrounding phallus with two, large, dense, longitudinal fields of fine spines.

Female. Unknown.

Distribution and biology. ***Eois cochabamba*** is known from a series of males collected at Cochabamba, Bolivia, at an elevation of ca. 2570 m. The early stages are unknown.

Etymology. The species name refers to the type locality of the species – Cochabamba.

Eois fallera Doan, new species

Figs. 16, 35

Type. Holotype ♂, Bolivia, Incachaca, Cochabamba, [no date], J. Steinbach, Wm. Schaus Collection, USNM slide 154,170, USNM ENT 01906889 (USNM).

Remarks and diagnosis. This species is described from a single male with collection data exactly the same as that for *E. cochabamba*. Superficially, it is nearly indistinguishable from *E. cochabamba*, with a pale greenish forewing ground color, and a well-defined postmedial line, with the antemedial and medial lines weak or absent, and a conspicuously scalloped terminal line. However, the male genitalia are easily distinguished by the conspicuously rounded ventral margin of the valva beyond the sacculus, resulting in a valva that is broadest subapically, reminiscent of the valva of *E. braziliana*, but broader still.

Description. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.5 mm (n = 1); forewing ground color pale gray-green; postmedial line well defined, slender, wavy, pale ivory; discal spot well defined, reddish brown; costal region from wing base to apex faintly tinged pale pinkish brown, irregularly marked with small cream dashes; terminal line narrow, red-brown, concolorous with discal spot, consisting of uninterrupted series of inward-directed scallops. Forewing underside pale whitish green, suffused with pale reddish brown, darkest along costa. Hindwing concolorous with forewing, with postmedial line well defined, contiguous with postmedial line of forewing; termen and fringe as in forewing. *Abdomen*: Male genitalia (Fig. 35) with tegumen slender, joined dorso-posteriorly forming rounded arch; [lacina damaged]; valva constricted immediately basad of termination of sacculus near mid-valva, with sacculus and margin beyond sacculus both conspicuously rounded; membrane surrounding phallus with large patch of spines, ca. 0.3 length of phallus; phallus ca. as long as valva with elongate, pointed process apically; vesica with semicircular, toothed plate, stout cornuti absent; membrane surrounding phallus with two, linear rows of large spines.

Female. Unknown.

Distribution and biology. ***Eois fallera*** is known from a single male collected at Cochabamba, Bolivia, at ca. 2570 m elevation. The early stages are unknown.

Etymology. The species name is from the Latin *fallere* – to deceive or mislead.

Eois multilineata Doan, new species

Figs. 17, 36, 45

Types. Holotype ♂, Venezuela, Mérida, La Carbonerra Forest, 25 km SE La Azulita, 7100’ [2168 m], old *Podocarpus* forest, 20 Feb 1978 (1♂), J. B. Heppner, USNM ENT 01906890 (USNM).

Paratypes (4♂, 3♀). Venezuela: Mérida: Murcuy Fish Hatchery, 7 km E Tabay, 6600 [2015 m]’, 10–13 Feb 1978 (3♂), blacklight, J. B. Heppner, USNM slide 154,477, USNM ENT 01906891, USNM ENT 01906892, USNM ENT 01906893 (USNM). La Carbonerra Forest, 25 km SE La Azulita, 7100’ [2168 m], old *Podocarpus* forest, 20 Feb 1978 (1♂), J. B. Heppner, USNM ENT 01906894 (USNM). Lara: Yacambú National Park, 13 km SE Sanare, 4800’ [1463 m], 4–7 Mar 1978 (3♀), cloud forest, J. Heppner, USNM ENT 01906895, USNM ENT 01906896, USNM ENT 01906897 (USNM)

Remarks and diagnosis. *Eois multilineata* is described from a series of five males from Mérida and three females from Lara, Venezuela. The sexes were associated by similarity in forewing maculation; hence, it is not beyond question. It is one of few species in the complex that can be distinguished by facies alone: the forewing has a pale gray-green ground color with numerous slender, pale cream, wavy lines of variable width extending from the costa to the hind margin, and the discal spot is absent. The male genitalia are likewise easily distinguished from those of all other species, with a robust, sclerotized triangular lobe at the distal termination of the sacculus bearing a dense patch of long, fine setae. The vesica has two small patches of slender, spine-like cornuti.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 8.0–9.0 mm (n = 5); forewing ground color pale gray green, with numerous, pale cream, wavy lines of

variable width, extending from costa to hind margin, creating a pattern of cream lines alternating with pale gray-green lines; discal spot absent; costal margin strongly suffused with reddish brown hue, with small, subsquare, yellowish blotches along length of costa; termen with faint, narrow, maroon-brown marks alternating with pale yellow. Fringe pale yellow. Forewing underside pale grayish green, suffused with pale grayish brown; terminal line represented by small brown dots at end of each vein. Fringe pale yellow. Hindwing concolorous with forewing, with numerous wavy lines, discal spot absent; terminal line represented by small, brown dots at termination of veins. Fringe pale yellow. Hindwing underside pale gray green without suffusion. *Abdomen*: Genitalia (Fig. 36) with lacina broad basally, upturned, relatively short, ca. 0.5 length of costa of valva, rounded apically; valva comparatively short, broad, rounded, conspicuously bilobed by indentation at termination of sacculus; sacculus ca. 0.5 length of valva, weakly angled near middle of ventral margin, with large, triangular, lobe-like distal termination bearing dense patch of long, fine setae; ventral margin of valva beyond sacculus broadly rounded; membrane surrounding phallus with two dense fields of short spines; vesica with semicircular, saw-toothed plate small; two clusters of relatively small, slender cornuti, ca. 0.25 times length of phallus; apex of phallus with long, pointed process.

Female. *Head* and *Thorax*: Essentially as described for species complex; forewing length 8.5–9.0 mm (n = 3). *Abdomen*: Genitalia (Fig. 45) with papillae anales slender, pointed apically; sterigma a broad, lightly sclerotized band; antrum cup-shaped, as broad as sterigma, membranous; ductus bursae extremely short, slightly less than 0.5 length of antrum, with conspicuous split colliculum; corpus bursae large, ovoid, length ca. 3.5 times that of papillae anales, with large, somewhat blade-shaped signum, ca. 0.3 of which

projects beyond outer wall of corpus, inner portion slightly broadened basally, weakly divided along basal margin; an irregular, narrow row of long spines around inner circumference of corpus near middle, 1–3 spines wide.

Distribution and biology. This species is known from males collected in Mérida and three males collected in Lara, Venezuela, between 1475 to 2185 m elevation. Although all specimens were collected in February and March, this likely represents collecting bias rather than flight period. Nothing is known of the biology.

Etymology. The specific epithet refers to the numerous wavy lines of the forewing.

Eois sclerobursana Doan, new species

Figs. 18, 46

Types. Holotype ♀, Venezuela, Aragua, Rancho Grande, 1100 m, 22–23 Jan 1978, cloud forest, J. B. Heppner, USNM slide 154,649, USNM ENT 01906898 (USNM).

Paratype (1♀). Venezuela: Lara: Yacambú National Park, 13 km SE Sanare, 1560 m, 1–5 Aug 1981, cloud forest, J. Heppner, USNM slide 154,490, USNM ENT 01906899 (USNM).

Remarks and diagnosis. This species is described from two females, one from Aragua and the other form Lara, Venezuela, that have identical genitalia. The specimen from Aragua (holotype) has conspicuous forewing markings that are absent in the specimen from Lara, the latter of which appears to be rather worn. The gray-green ground color of the forewing of *E. sclerobursana* is more similar to that of members of the *E. goodmanii* species complex, but the narrow, wavy, pale, median and postmedian lines are

like those of other species in the *E. olivacea* complex. The maroon terminal line is nearly straight compared to the more scalloped line of most other species. The female genitalia are easily distinguished from those of all other species in the complex by the large region of sclerotization near the middle of the corpus bursae, extending posteriorly, bearing numerous stout thorns. Because males are unknown, the assignment of this species to our Group I is somewhat arbitrary.

Description. Male. Unknown.

Female. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 7.5–8.0 mm (n = 2); forewing gray green, with well-defined, uninterrupted, white, wavy postmedial line, and slightly less defined wavy antemedial line; discal spot conspicuous, reddish brown; costal margin suffused with faint brown-pinkish hue, with several faint, irregular, cream dots and/or dashes along length of costa; termen with narrow, nearly straight, maroon line. Fringe pale yellow. Forewing underside cream, with faint, pale reddish brown suffusion in costal region. Fringe pale yellow. Hindwing concolorous with forewing; antemedial and postmedial lines as in forewing; discal spot present; termen with narrow, maroon line, similar to forewing termen. Fringe pale yellow. Hindwing underside cream, without reddish suffusion. *Abdomen*: Genitalia (Fig. 46) with papillae anales comparatively short, broad mesally; sterigma a broad, unsclerotized band, contiguous with short, membranous, cup-shaped antrum; ductus bursae extremely short, with conspicuous, sclerotized colliculum; corpus bursae large, ovoid-oblong, with large patch of sclerotization near the middle, extending posteriorly, bearing numerous stout thorns; signum large, hook-

shaped, trifurcate apically, with small lateral spines; base of hook projecting slightly beyond outer wall of corpus bursae.

Distribution and biology. This species is known from two females collected in Aragua and Lara, Venezuela, between 1100 and 1560 m elevation, one in January, one in August. The larval foodplants and early stages are unknown.

Etymology. The specific epithet refers to the patch of sclerotization in the corpus bursae of the female genitalia.

Eois nubesilva Doan, new species

Figs. 19, 47

Types. Holotype ♀, Venezuela, Lara, Yacambú National Park, 13 km SE Sanare, 1560 m, 28–31 Jul 1981, cloud forest, J. Heppner, USNM slide 154,494, USNM ENT 01906900 (USNM).

Paratypes (2♀). Venezuela: Lara: Yacambú National Park, 13 km SE Sanare, 1560 m, 28–31 Jul 1981 (1♀), cloud forest, J. Heppner, USNM slide 154,503, USNM ENT 01906901 (USNM); 4800’ [1463 m], 4–7 Mar 1978 (1♀), cloud forest, J. Heppner, USNM slide 154,653, USNM ENT 01906902 (USNM).

Remarks and diagnosis. This species is described from three females from Yacambú National Park, Venezuela. The forewing has a pale greenish ground color with numerous faint, whitish wavy lines, slightly reminiscent of *E. multilineata*, but with the lines less defined, and with the reddish brown terminal line scalloped rather than straight (Fig. 19). The female genitalia (Fig. 47) are distinguished by a large hook-like, apically

toothed signum, accompanied by a small patch of long, slender spines on the opposite wall of the corpus bursae. In other species of the complex, the patch of long spines is more extensive, usually continuing in a lateral band around most of the inner perimeter of the corpus bursae. Like *E. nubesilva*, the assignment of this species to our Group I is provisional until a conspecific male is recognized.

Description. Male. Unknown.

Female. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 7.5 mm (n = 3); forewing pale green, with numerous faint, white, wavy lines; discal spot small, reddish brown; costal margin faintly suffused with pinkish brown hue; termen with narrow, scalloped, reddish brown line. Fringe pale yellow. Forewing underside cream, with faint, pale reddish brown suffusion in costal region. Fringe pale yellow. Hindwing concolorous with forewing; antemedial and postmedial lines as in forewing; discal spot present; termen with narrow, scalloped, maroon line, similar to forewing termen. Fringe pale yellow. Hindwing underside cream, without reddish suffusion. *Abdomen*: Genitalia (Fig. 47) with papillae anales narrow, pointed posteriorly; sterigma a broad, mostly membranous band, contiguous with short, membranous, cup-shaped antrum; ductus bursae extremely short, with split colliculum; corpus bursae large, ovoid-oblong, with weakly sclerotized patch opposite of signum bearing a field of long, slender spines; signum large, hook-shaped, toothed apically, with small knob at base projecting beyond outer wall of corpus bursae.

Distribution and biology. This species is known from three females collected in Yacambú National Park in Venezuela, at 1463 to 1560 m elevation. The biology is unknown.

Etymology. The specific epithet comes from the Latin “nubes,” for cloud, and “silva” for forest.

Eois parumsimii Doan, new species

Figs. 20, 37, 48

Types. Holotype ♂, Ecuador, Napo, Yanayacu Biological Station, 2113.9 m, 00°35’48.998”S, 77°53’17.998”W, Nov 2012, r.f. 71361, Earthwatch (UNR).

Paratypes (5♂, 2♀). ECUADOR: Napo: Yanayacu Biological Station, 2066.8 m, 00°34’0.001”S, 77°52’0.001”W, Feb 2005, r.f. 1673, 1674 (2♂), Sept 2010, r.f. 51792 (1♂), Earthwatch (UNR); 2188.4m, 00°35’54”S, 77°53’44.34”W, Nov 2005, r.f. 9442 (1♂), Earthwatch (UNR); 1240.7 m, 00°43’38.798”S, 77°46’22”W, Jun 2014, r.f. 84256 (1♀), Earthwatch (UNR); 1871.9 m, 00°31’31.2”S, 77°52’35.399”W, Aug 2014, r.f. 85960, 85963 (1♂, 1♀), Earthwatch (UNR).

Diagnosis. This species is described from specimens reared from larvae collected at Yanayacu Biological Station in Ecuador. Externally, *E. parumsimii* is distinct from all of species in the genus, with a much broader, yellow, postmedial line on a pale pinkish gray ground color (Fig. 20). The male genitalia of *E. parumsimii* can be distinguished from those of other members of the complex by the following combination of character states: the ventral margin of the saccus forming a blunt conical pocket (vs. a small, transverse-ovoid pocket in most other species); and the membrane surrounding the phallus with a large dorsal field of short spines arranged in a series of longitudinal rows (vs. arranged in two longitudinal rows most other in species). The female genitalia of *E. parumsimii* have

short, trapezoidal papillae anales, whereas the papillae anales are slenderer in most other species; and the signum is located laterally on the left side of the corpus bursae, whereas it located ventrally in many other species of the complex.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 8.0–9.0 mm (n = 8); ground color manzanilla olive, anterior portion of antemedial line very faint, posterior portion prominent, pale-yellow; postmedial line well defined, wavy, pale yellow; discal spot well defined, red brown; basal 0.66 of costal margin concolorous with ground color, distal portion with clay brown markings; termen with slender clay brown line; fringe two-toned, mostly pale yellow, with red brown incursion between veins M_2 and M_3. Forewing underside ground color pale yellow, suffused with red brown, inverse to dorsal pattern; antemedial line absent; postmedial line prominent, width variable, but broader near costa and posterior margin; discal spot round, faint, clay brown; termen clay brown. Fringe pale yellow. Hindwing upperside ground color clay brown; antemedial line faint; postmedial line wavy, pale yellow; discal spot round, well-defined, orange-brown; termen clay brown. Fringe pale yellow. Hindwing with pattern similar to forewing, with prominent pale yellow antemedial and postmedial lines and faint discal spot. *Abdomen*: Genitalia (Fig. 37) with tegumen arms forming rounded triangular dorsal arch, curving slightly posterad; ventral margin of saccus forming a blunt conical pocket; transtilla slender, V-shaped; juxta wide basally, abruptly narrowed in distal 0.33 with acute dorsal part; dorsal margin of juxta narrow, truncate, with a small down-curved lip; valva subrectangular, weakly constricted on ventral margin near distal end of sacculus; a brush of bristle-like setae near apex, contrasting with remaining setae; setae at apex of sacculus

longer than width of valva; ventral margin of sacculus bowed outward; base of phallus narrow, horn-shaped, membrane surrounding phallus with large dorsal field of short spines arranged in longitudinal rows; phallus ca. as long as valva; vesica bifurcated distally with two appendices, one with distal group of spine-like cornuti, the other at base of vesica with a single, large scobinate plate.

Female. *Head* and *Thorax*: Essentially as described for male except lacking rami on antenna. A*bdomen*: Genitalia (Fig. 48) with papillae anales short, trapezoidal. Dorsal membrane between Tg8 and papillae anales bearing a large dorsal sac. Ductus bursae wide; ductus seminalis arising from elongate, triangular appendix at base of corpus bursae; Corpus bursae oblong, without mesal constriction, distal appendix absent; signum wing-shaped with serrate lateral margins, located laterally on left side of corpus bursae, internal part horn-like.

Biology and distribution. This species is known only from Napo Province, Ecuador. Adults were reared from larvae (n = 25) discovered in the field on *Piper baezanum*, which is a threatened species, endemic to Ecuador (Santiana and Pitman 2004).

The eggs, larvae, and pupae of *E. parumsimii* have all the same general characteristics described above for *Eois* with no modifications (Figs. 50–53), and are difficult to distinguish from those of *E. pseudolivacea*. All larval instars (until the prepupal stage) are similarly colored, with a translucent beige head capsule, light green thorax and abdomen, translucent pinacula, and paired subdorsal yellow spots on all segments form T2-A6. Abdominal segments are separated by slight constrictions. The prothoracic legs and abdominal prolegs are tan. The prepupa is clear.

Etymology. The species epithet *parumsimii* is a patronym for Michael Lumibao, who is the partner and long-time supporter of the first author; the name is derived from his Chinese zodiac animal.

GROUP II

Eois pijao Doan, new species

Figs. 21, 38

Type. Holotype ♂, Colombia, Quindui [misspelling of Quindío?; now Departamento de Tolima], Río Toche, 2400 m, Dognin Collection, USNM slide 154,176, JWB-DNA-22-151, USNM ENT 01906903 (USNM).

Remarks and diagnosis. *Eois pijao* is described from a single male from Colombia, collected before the turn of the century, that was previously misidentified as *E. olivacea* (USNM). Superficially, it is similar to other members of the species complex, but the forewing is slightly darker green, and the scallops of the maroon line of the termen are interrupted by small whitish dots, especially conspicuous on the hindwing (Fig. 21), creating a more zigzag pattern than in other species. The male genitalia (Fig. 38) are distinguished by the shorter valvae with a broadly rounded outer margin of the sacculus, and slender, strap-like lacina. As in other Group II species, the vesica has a prominent patch of large, stout cornuti.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.0 mm (n = 1); forewing ground color pale green; antemedial line represented by a yellow dash at costa,

medial line absent; postmedial line prominent, well defined, wavy, ivory; region between postmedial line and termen without conspicuous markings; discal spot well defined, rounded, reddish brown; costal margin suffused with faint pinkish hue, with several small, irregular, cream dots and/or dashes along length of costa; termen with narrow, reddish, scalloped line, concolorous with discal spot, with tiny yellowish spot separating each scallop. Fringe pale yellow. Forewing underside pale grayish, suffused with pale reddish brown; postmedial line well defined; subterminal line weakly defined; discal spot faint. Fringe pale yellow. Hindwing concolorous with forewing; antemedial and postmedial lines absent; medial line well defined, wavy, ivory; discal spot well defined, concolorous with discal spot of forewing; termen with narrow, reddish brown, scalloped line, concolorous with discal spot, with tiny yellow spot separating each scallop. Fringe pale yellow. Hindwing underside pale gray green without reddish suffusion. *Abdomen*: Genitalia (Fig. 38) with lacina short, subrectangular, parallel-sided, rounded apically, from near middle of tegumen; valvae comparatively short, sacculus with broadly rounded ventral margin; membrane surrounding phallus with small dorsal field of short spines; vesica with semicircular, saw-toothed plate and cluster of large cornuti, ca. 0.33 times length of phallus.

Female. Unknown.

Distribution and biology. This species is known from a single male collected at Río Toche, Department of Quindío, in the west-central portion of Colombia, at an elevation of 2400 m. Nothing is known of the life history,

Etymology. The specific epithet “pijao” refers to the indigenous tribes of the region prior to the Spanish conquest of Colombia.

Eois dognini Doan, new species

Figs. 22, 39

Type. Holotype ♂, Colombia, Fassl, Dognin Collection, USNM slide 154,647, USNM ENT 01906904 (USNM).

Remarks and diagnosis. *Eois dognini* is described from a single male from Colombia, lacking further locality data. Unlike other members of the complex, the forewing is totally devoid of lines, except for the very weakly developed scalloped terminal line (Fig. 22). The male genitalia are similar to those of *E. pijao*, with comparatively short valvae, a broadly rounded outer margin of the sacculus, and a slender, elongate lacina. It can be distinguished by the more rounded ventral margin of the sacculus and ventral margin of the valva beyond the sacculus; by the slightly longer lacina; and by the long, pointed apical process of the phallus. As in other Group II species, the vesica has a distinct patch of large cornuti.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.0 mm (n = 1); forewing ground color pale green, faintly mottled with slightly darker gray-green; antemedial, medial, and postmedial lines all lacking; discal spot faint, reddish brown; costal margin suffused with faint yellow-pinkish hue, with several faint, irregular, cream dots and/or dashes along length of costa; termen with narrow, reddish line, only weakly scalloped. Fringe pale yellow. Forewing underside pale grayish, suffused with pale reddish brown. Fringe pale yellow. Hindwing concolorous with forewing; antemedial and

postmedial lines absent, medial line vaguely represented by slightly darker gray green scales; discal spot absent; termen with narrow, reddish brown line, similar to forewing termen. Fringe pale yellow. Hindwing underside pale gray green without reddish suffusion. *Abdomen*: Genitalia (Fig. 39) with lacina elongate, parallel-sided, rounded apically, ca. 0.5 length of costa of valva, originating from near middle of tegumen; valva comparatively short, sacculus with broadly rounded ventral margin; ventral margin of valva beyond sacculus broadly rounded; membrane surrounding phallus with dense field of short spines; phallus ca. as long as valva, apex with long, pointed process, ca. 0.4 length of remainder of phallus, minutely serrate in basal 0.64, vesica with semicircular, saw-toothed plate and cluster of large cornuti, ca. 0.33 times length of phallus.

Female. Unknown.

Distribution and biology. This species is known from a single male collected in Colombia, without additional locality information. Nothing is known of the life history.

Etymology. The specific epithet is a patronym for the French entomologist Paul Dognin (1837–1941), whose collection resides primarily in the USNM and includes several previously undescribed species of *Eois* from Colombia and Bolivia.

Eois altoparana Doan, new species

Figs. 23, 40, 47

Types. Holotype ♂, Paraguay, Alto Paraná, Centro [de Formaciòn de Tecnico Superior] For.[estal] Alto Paraná, [190 m], 25°10’S, 54°44’W, 14–16 April 1984, M. Pogue & M. Solis, USNM slide 154,185, USNM ENT 01906905 (USNM).

Paratypes (4♂). Same data as holotype, USNM ENT 01906906, USNM ENT 01906907, USNM ENT 01906908, USNM ENT 01906909 (USNM).

Additional material examined (4♂, 1♀). Paraguay, Paraguarí Dept., 25 km SE Ybycui, Ybycui National Park, [70 m], 12–24 Apr 1980, P. Spangler et al. USNM slides 154,502, 154,495, 154,496, USNM ENT 01906910, USNM ENT 01906911, USNM ENT 01906912, USNM ENT 01906913 USNM ENT 01906914 (USNM).

Remarks and diagnosis. *Eois altoparana* is described from a series of five males from Alto Paraná, Paraguay. The gray-green forewing ground color is similar to that of *E. boliviensis*, *E. pseudoboliviensis*, and *E. dognini*, and like those species, the terminal line is only weakly scalloped (Fig. 23). All four of these species bear a superficial resemblance to members of the *E. goodmanii* complex, to which they may belong. The male genitalia of *E. altoparana* are similar to those of *E. boliviensis* with a somewhat parallel-sided valva, but they lack the triangular lobe of the sacculus that is diagnostic for the latter, and the vesica possesses a patch of exceedingly large cornuti, lacking in the three aforementioned species. The male genitalia share with *E. pijao* the patch of large cornuti in the vesica, but the venter of the valvae, including the sacculus, is less rounded than in *E. pijao*, and the cornuti are much larger and longer than in any other member of the species complex.

Five additional specimens (four males and a female) from Ybycui National Park are virtually identical to specimens from Alto Paraná; however, the lacina in the male genitalia of these specimens is conspicuously shorter. Hence, they are not included as paratypes; however, the female is illustrated and described below.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 9.0–9.5 mm (n = 5); forewing ground color pale gray-green, with slender whitish postmedial line and trace of median line; discal spot faint, reddish brown; costal margin suffused with faint pale greenish-pink hue, with several faint, irregular, cream dots and/or dashes along costa; termen with narrow, reddish line, only weakly scalloped. Fringe pale yellow. Forewing underside pale greenish, suffused with pale reddish brown. Fringe pale yellow. Hindwing concolorous with forewing; antemedial and postmedial lines absent, medial line slender, wavy, ivory; discal spot faint; termen with narrow, reddish brown line, similar to forewing termen. Fringe pale yellow. Hindwing underside pale gray-green without reddish suffusion. *Abdomen*: Genitalia (Fig. 40) with lacina elongate, parallel-sided, rounded apically, ca. 0.6 length of costa of valva; valva slightly broader at base, remainder more-or-less parallel-sided, sacculus with rounded ventral margin, ventral margin of valva beyond sacculus relatively straight; membrane surrounding phallus with field of stout spines; vesica with small, semicircular, saw-toothed plate and cluster of large cornuti, ca. 0.5 times length of phallus; apex of phallus with teardrop-shaped, pointed process.

Female. *Head* and *Thorax*: Essentially as described for species complex. *Abdomen*: Genitalia (Fig. 49) with papillae anales semi-ovate; sterigma a simple, weakly sclerotized lateral band; ductus bursae extremely short, ca. 0.65 width of sterigma, with narrow colliculum immediately before junction with corpus bursae; corpus bursae oblong, with most of posterior 0.65 with sclerotized inner wall, anterior margin with small rounded sclerites, two irregular lines of which extend posterad near mid-venter; short, inverted J-

shaped signum on left side of corpus, with anteriormost extension comprised of numerous slender spines; basal process of signum extending beyond outer wall of corpus bursae; an irregularly linear patch of long, slender spines in arch from signum to opposite side of corpus bursae.

Distribution and biology. This species is known from a series of males collected in Alto Paraná [ca. 190 m], and a small series from Ybycui National Park [ca. 70], the latter of which may not be conspecific. All specimens were collected in April. In contrast to most Neotropical *Eois* species, *E. altoparana* appears to be confined to the lowlands. Nothing is known of the biology.

Etymology. The specific epithet refers to the department of Alto Paraná, Paraguay.

Eois heppneri Doan, new species

Figs. 24, 41

Types. Holotype ♂, Venezuela, Lara, Yacambú National Park, 13 km SE Sanare, 4800’ [1463 m], 28–31 Jul 1981, J. Heppner, USNM slide 154,600, USNM ENT 01906915 (USNM)

Remarks and diagnosis. *Eois heppneri* is described from a single male from Yacambú National Park in Venezuela, where it is sympatric with at least two other species of the complex. Because the specimen is rather worn (Fig. 24), it is difficult to compare its forewing maculation with that of other species. However, the male genitalia are remarkably divergent from those of all other species in the group, distinguished by long valvae with the costa conspicuously bent near the middle and the presence of an

extremely large cornutus (ca. 0.75 length of phallus) in the vesica, surrounded by two clusters of stout cornuti.

Description. Male. *Head*: Essentially as described for species complex. *Thorax*: Essentially as described for species complex, except forewing length 8.0 mm (n = 1); forewing (worn) ground color nearly uniform grayish green, lacking antemedial and postmedial lines; brown discal spot present, small; costal margin weakly suffused with reddish brown; termen with faint, narrow, maroon-brown marginal line. [Fringe lacking.] Forewing underside pale grayish green, suffused with pale grayish brown in costal region. Hindwing concolorous with forewing. [Fringe lacking.] Hindwing underside pale gray green without suffusion. *Abdomen*: Genitalia (Fig. 41) with lacina only slightly attenuate distally, upturned, ca. 0.4 length of costa of valva, rounded apically; valva comparatively long, slender, with costa weakly angled downward near middle; sacculus narrow, ca. 0.3 length of valva, relatively straight throughout; ventral margin of valva beyond sacculus only weakly curved; membrane surrounding phallus with two dense fields of short spines converging basally; vesica with subtriangular, saw-toothed plate, two large clusters of moderate sized, slender cornuti, and a single large cornutus ca. 0.75 times length of phallus; apex of phallus with semi-sclerotized, triangular, pointed process.

Female. Unknown.

Distribution and biology. This species is known from a single male from Yacambú National Park, Venezuela, at 1463 m elevation. Nothing is known of the biology.

Etymology. The species name is a patronym for John B. Heppner, who collected the holotype of this and several other Venezuelan species.

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Explanation of figures

FIGURES 1–4. Adults of *Eois*. 1. *E. olivacea* holotype (courtesy of Gunnar Brehm). 2. *E. goodmanii*, Costa Rica. 3. *E. muscosa* holotype (courtesy of Gunnar Brehm). Head of *E. goodmanii*.

FIGURES 5–12. Adults of *Eois*. 5. *E.* cf. *olivacea* from Colombia. 6. *E. tochensis* holotype. 7. *E. pseudolivacea* paratype. 8. *E. auruda* holotype. 9. *E. beebei* holotype (courtesy of Gunnar Brehm). 10. *E. espadera* holotype. 11. *E. braziliana* holotype. 12. *E. ocherata* holotype.

FIGURES 13–20. Adults of *Eois*. 13. *E. boliviensis* holotype. 14. *E. pseudoboliviensis* holotype. 15. *E. cochabamba* holotype. 16. *E. fallera* holotype. 17. *E. multilineata* paratype. 18. *E. sclerobursana* holotype. 19. *E. nubesilva* holotype. 20. *E. parumsimii* paratype.

FIGURES 21–24. Adults of *Eois*. 21. *E. pijao* holotype. 22. *E. dognini* holotype. 23. *E. altoparana* holotype. 24. *E. heppneri* holotype.

FIGURES 25–30. Male genitalia of *Eois*; phallus removed (below). 25. *E.* cf. *olivacea*, USNM slide 154,479. 26. *E. tochensis*, USNM slide 154,450. 27. *E. pseudolivacea*, slide 69575 (UNR); arrow indicates elongate lacina. 28. *E. auruda*, USNM slide 154,179. 29. *E. beebei* holotype. 30. *E. espadera*, USNM slide 154,651.

FIGURES 31–36. Male genitalia of *Eois*; phallus removed (below). 31. *E. braziliana*, USNM slide 154,458; arrow indicates rounded lower margin of valva beyond sacculus. 32. *E. boliviensis*, USNM slide 154,454; arrow indicates triangular lobe of sacculus. 33. *E. pseudoboliviensis*, USNM slide 154,666; arrow indicates rounded ventral margin of sacculus. 34. *E. cochabamba*, USNM slide 154,491. 35. *E. fallera*, USNM slide 154,179; arrow indicates rounded lower margin of valva beyond sacculus. 26. *E. multilineata*, USNM slide 154,477.

FIGURES 37–41. Male genitalia of *Eois*; phallus removed (below). 37. *E. parumsimii*, slide 75819 (UNR). 38. *E. pijao*, USNM slide 154,176. 39. *E. dognini*, USNM slide 154,647; arrows indicate rounded ventral margin of sacculus, and distal spine of phallus. 40. *E. altoparana*, USNM slide 154,495. 41. *E. heppneri*, USNM slide 154,660; arrow indicates bend in costa of valva.

FIGURES 42–49. Female genitalia of *Eois*. 42. *E. pseudolivacea*, slide 86478 (UNR). 43. *E. braziliana*, USNM slide 154,171. 44. *E. ocherata*, USNM slide 154,453. 45. *E. multilineata*, USNM slide 154,498. 46. *E. sclerobursana*, USNM slide 154,649. 47. *E. nubesilva*, USNM slide 154,494. 48. *E. parumsimii*, slide 75961 (UNR). 49. *E. altoparana*, USNM slide 154,496.

FIGURES 50–53. Early stages of *Eois*. 50. Egg of *Eois pseudolivacea*. 51. Second instar larva of *Eois pseudolivacea* from Ecuador. 52. Fifth instar larva of *Eois pseudolivacea* from Ecuador. 53. Pupa of *Eois pseudolivacea* from Ecuador.


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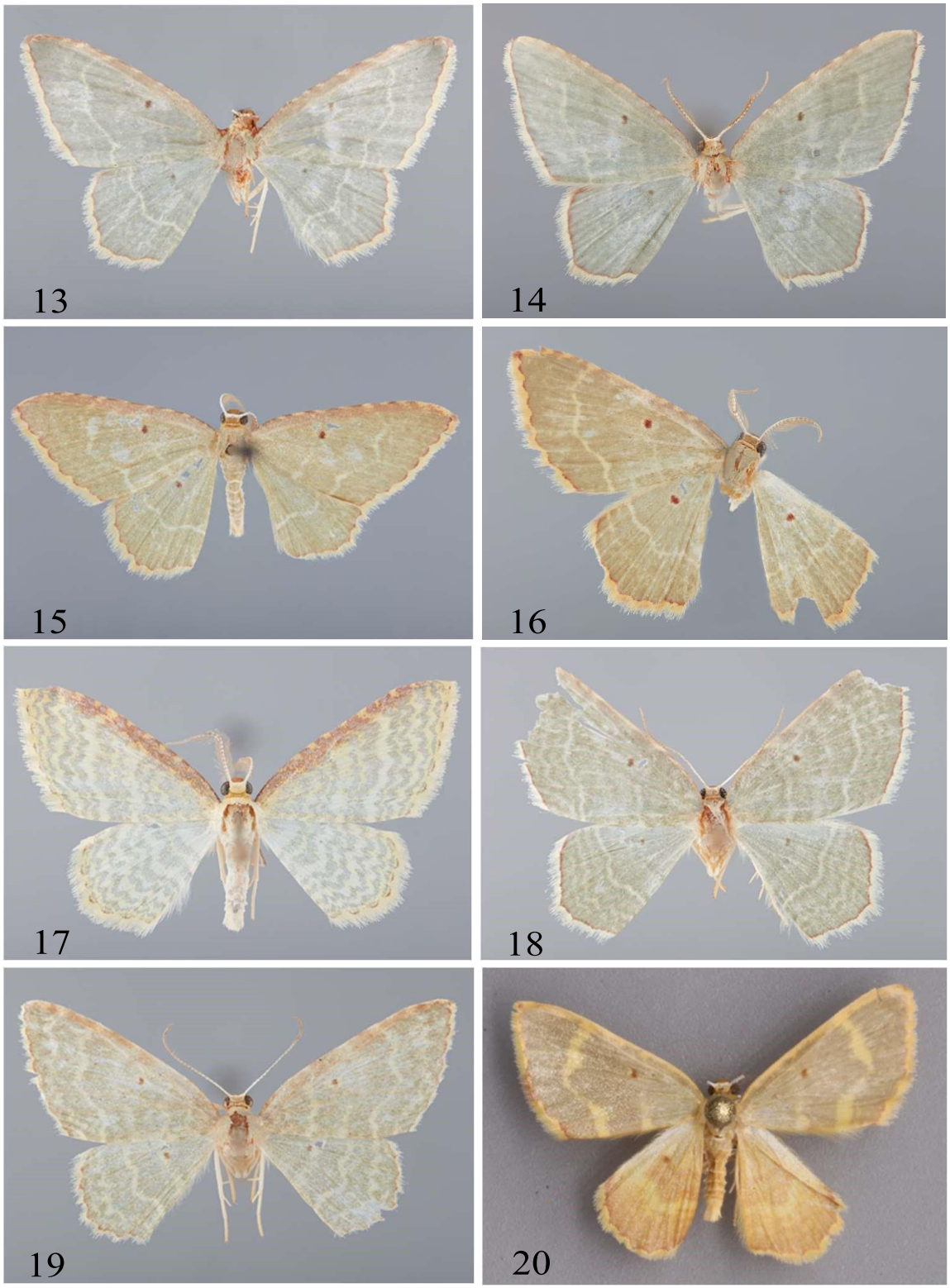
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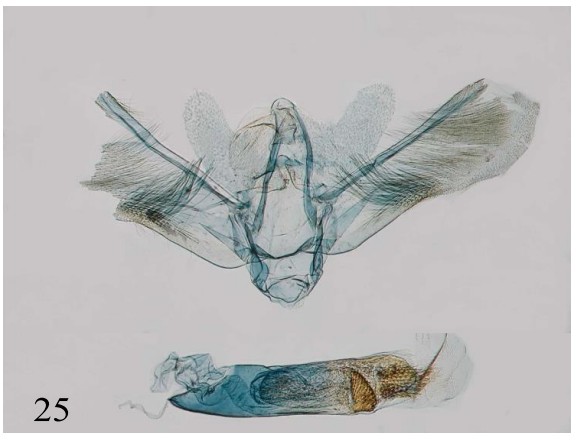
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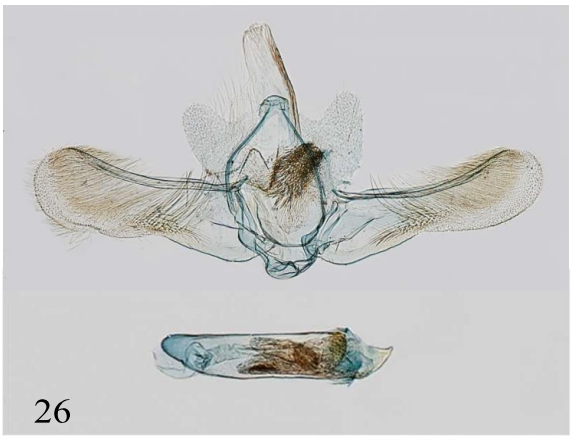
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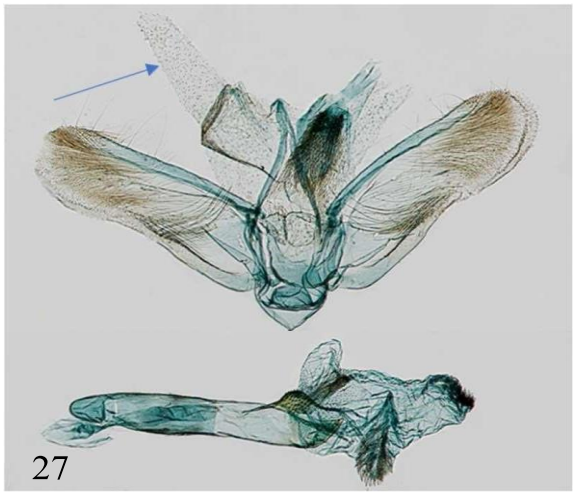
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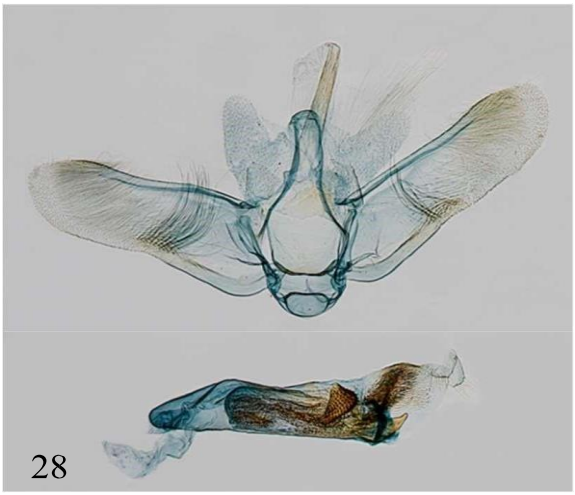
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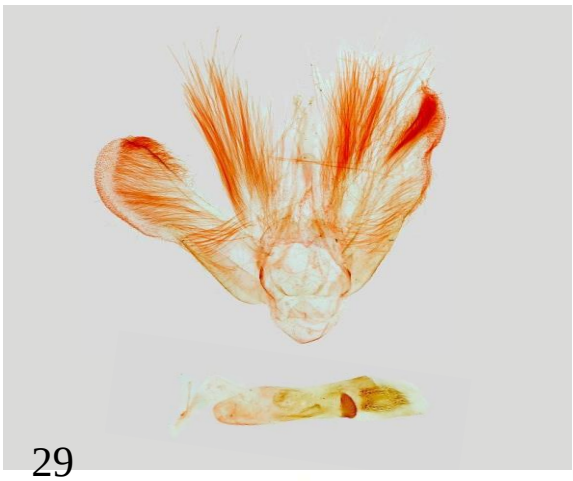
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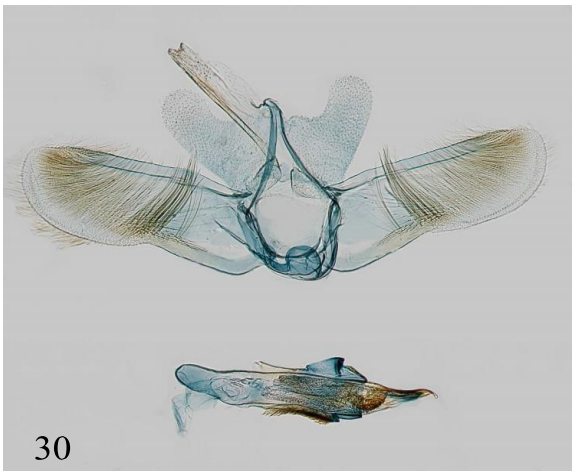
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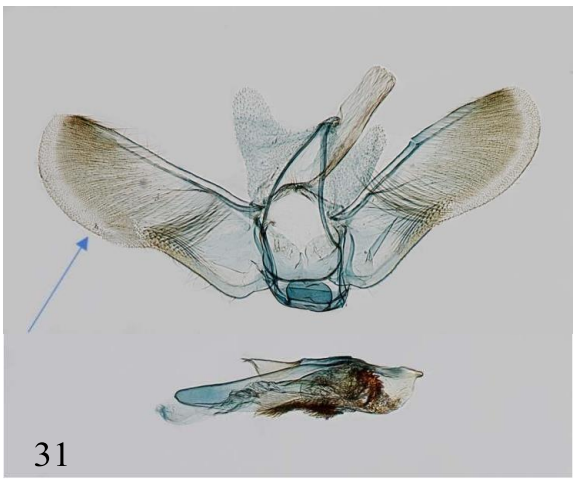
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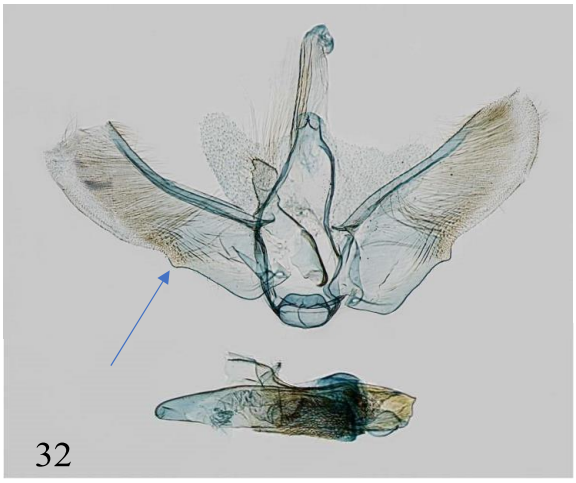
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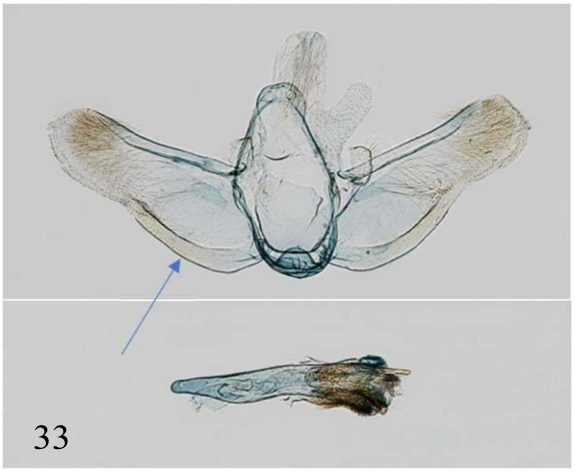
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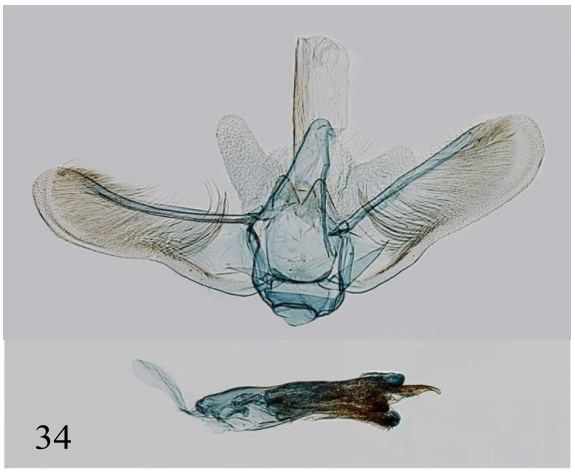
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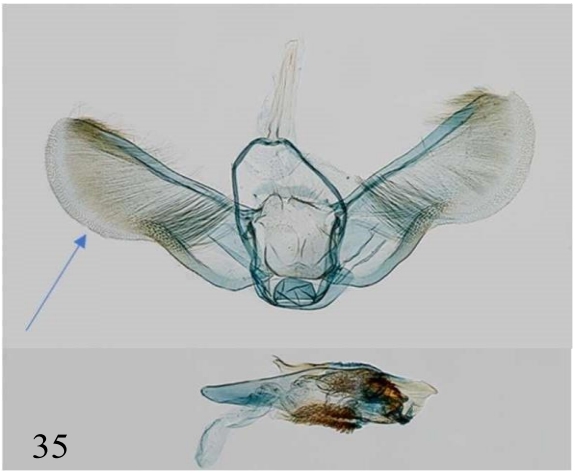
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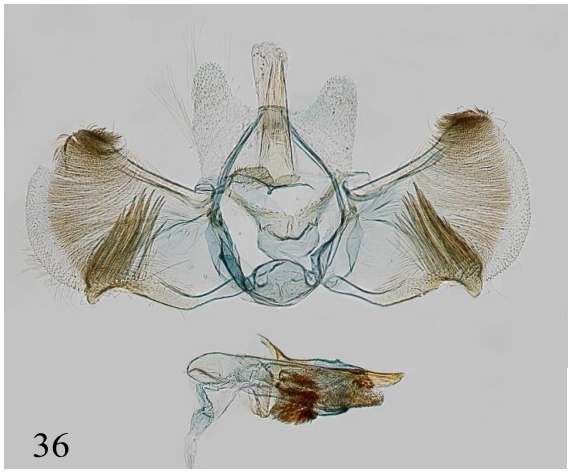
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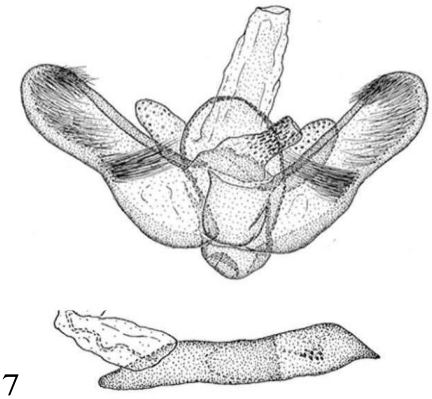

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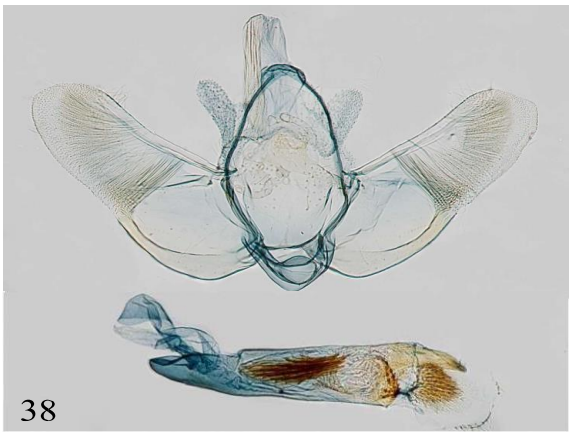

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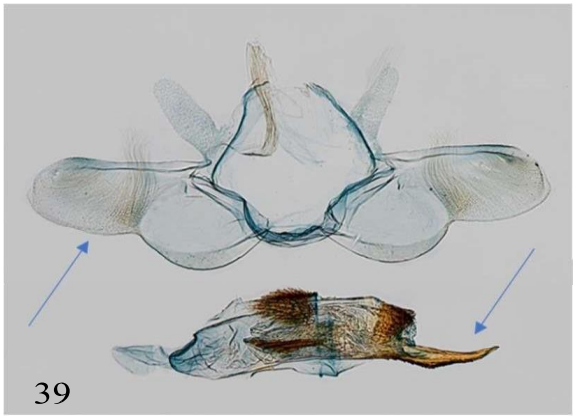

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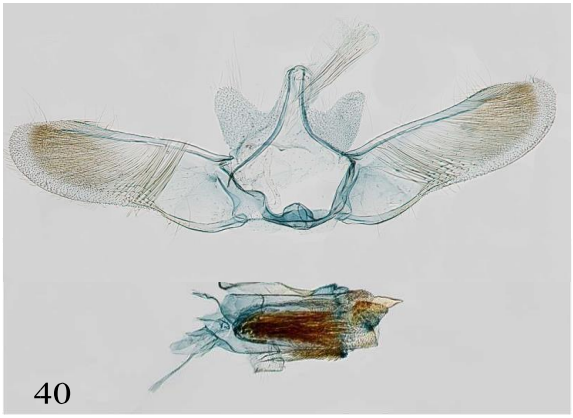

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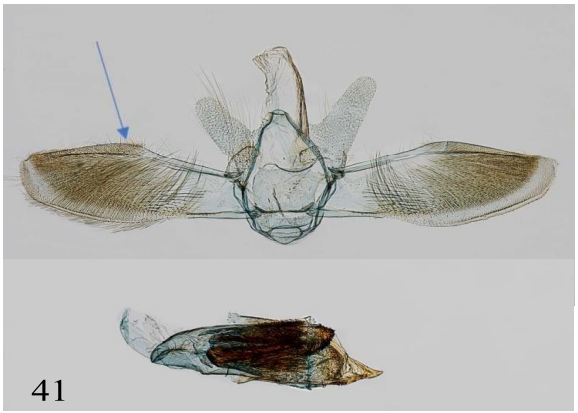

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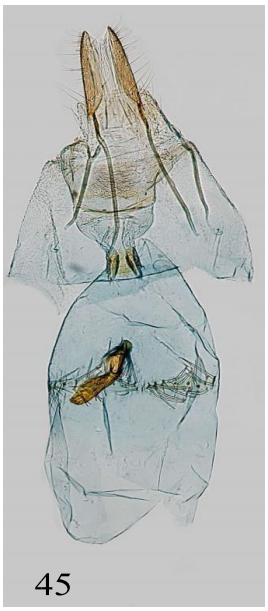
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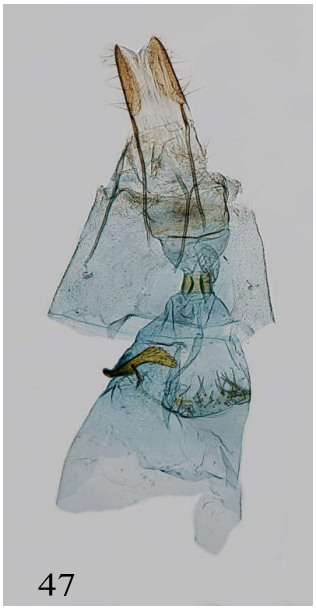
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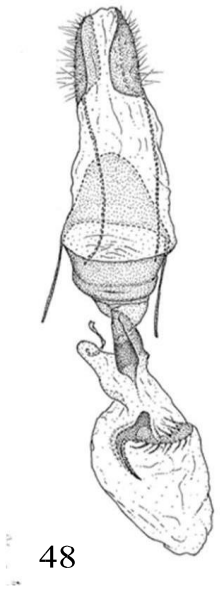
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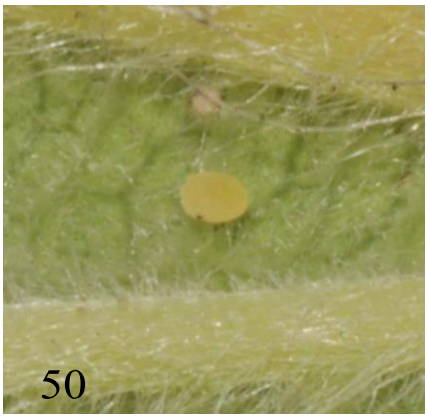
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Does shape matter? Evolution of male and female genitalia in *Eois* Hübner moths (Lepidoptera: Geometridae: Larentiinae)

Lydia M. Doan[1], James S. Miller[2], Matthew L. Forister[1], James A. Fordyce [3], Lee A. Dyer[1]

[1] Department of Biology, Ecology, Evolution and Conservation of Biology, University of Nevada, Reno, NV 89557, USA

[2] Entomology Department, American Museum of Natural History, New York, NY, 10024, USA (deceased)

[3] Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996, USA

Running head: Evolution of male and female genitalia in *Eois*

Abstract

The remarkable diversity of insect genitalia has inspired countless studies exploring their role in sexual selection and speciation. While most studies have focused on variation in male structures, an increasing number of studies on female genitalia have uncovered comparable diversity. In Lepidoptera, the complexity of male and female genitalia raises the possibility for various evolutionary pressures to be involved in the maintenance of inter- and intrasexual morphological diversity. In this study, we consider different mechanisms of trait evolution, including genetic drift, pleiotropy, female choice, cryptic female choice, male to male competition, sexual conflict, and the lock-and-key hypothesis. We examine the evolution of male and female genitalia in *Eois* Hübner, a diverse genus of moths in the family Geometridae. Based on a matrix of 107 morphological characters, we developed a phylogeny for a sample of 99 species (94 *Eois* and five outgroup taxa) and separate dendrograms based on male and female characters. We then examined congruence among trees, and discovered widespread discordance among the dendrograms based on male and female traits, suggesting at least partially independent evolution of traits between the sexes. Based on these findings, we conclude that the evolution of male and female genitalia in *Eois* cannot, at this time, be explained by a single hypothesis and is fertile ground for future work. Our research represents a first step in revealing patterns of male to female trait evolution in Lepidoptera and their possible functions.

Keywords: sexual selection, genetic drift, pleiotropy, female choice, male to male competition, sexual conflict, lock-and-key hypothesis, discordance

Introduction

Morphological features of the male genitalia of Lepidoptera have long been used for species circumscription and delineation and as characters in phylogenetic analyses because, for many species, the male genitalia are easily accessible, and their various complex structures can be homologized and their differences quantified[1-3], and they appear to diverge rapidly[4]. Because female genitalia are located mostly internally, their features have proven slightly more difficult to observe or quantify; as a result, they are less studied[1, 5]. For example, the paired structures known as "valvae," which males use to grasp females during copulation, are easily observed and typically well sclerotized, making them useful in diagnosing species and valuable for phylogenetic analyses[3, 6, 7, 8]. Because female Lepidoptera genitalia are sometimes more uniform in shape, or conservative at the generic level, they may not be as informative as male genitalia[2, 9, 10]. However, female genitalia can also be highly diverse, with many complex structures arranged in various orientations, sizes, and shapes, suggesting that they are also highly diversified and have the potential to reflect rapid or sustained evolution[5, 10]. While descriptions of the female genitalia in Lepidoptera are a standard component of species descriptions, the function of many of the described structures is still poorly understood. Such information is essential for understanding copulatory mechanisms in Lepidoptera, how female and male genitalia structures interact, evolve, and possibly evolve in concert, as well as their diversification [5].

There are numerous proposed mechanisms for the evolution of genitalia and diversity of male genital morphology, ranging from female choice and cryptic female choice, to sperm competition, male-female conflicts of interest, sexual conflict or

coevolution, and the lock-and-key hypothesis to potentially non-adaptive mechanisms such as genetic drift or pleiotropic effects[1, 2, 11-13]. These possibilities are too numerous and interconnected to describe in detail here, but it is interesting to note that Eberhard[9] initially proposed the mechanism of female choice in this context, in which females may exert preference for particular male genital morphologies, or mechanical or sensory components of male genitalia. Indeed, it has been shown that female genitalia can diverge between populations with different male genital preferences, resulting in correlated evolution of male and female traits[1, 14]. Eberhard[15] also introduced the concept of cryptic female choice where, depending on the species, females may use physical or chemical mechanisms to choose which sperm from different males to fertilize eggs, in both pre- and post-copulatory circumstances. This mechanism is difficult to observe or evaluate, especially when examining morphological structures in the absence of direct observations of behavioral and the physical interaction of male and female genitalia and their gametes[14, 16]. Another mechanism, male to male competition or avoidance of sperm competition, may involve the physical removal or displacement of rival sperm[1, 16]. Additionally, sexual conflict can occur in any mating system where a reproductive strategy that maximizes the fitness of one sex also decreases the fitness of the opposite sex[1, 17]. For example, female fitness may be improved by the use of sperm to provide greater copulatory stimulation, while male fitness might be increased by the removal or displacement of competing sperm from the female reproductive tract. Furthermore, traumatic copulation may occur when the male reproductive anatomy damages the female during copulation, and this is often evidenced by visible scarring of the female tract following copulation[17]. The last mechanism to consider is the lock-and-key hypothesis in

which morphological or mechanical differences between species act as a reproductive isolating barrier; in this hypothesis, male genitalia act as a "keys" to the appropriate female genitalia "locks;" i.e., species-specific structures have evolved to prevent copulation with heterospecific individuals owing to the lack of morphological fit or stimulation[18]. Therefore, a general expectation of the lock-and-key hypothesis could be a stepwise evolutionary pattern in which small changes in female genitalia would be closely tracked by changes in male genitalia[1]. However, until recently, female genitalia have been considered too simple to act as locks for many species[2, 4].

Without methods such as freezing specimens during different stages of copulation and dissecting females for evidence of anatomic damage, many of these proposed mechanisms for the evolution of genitalia are difficult to observe, especially in pinned museum specimens. However, in some genera of Lepidoptera morphological analyses may provide inferences about the course of genitalic evolution because characters of genital morphology are not only phylogenetically informative and useful in delineating species[3, 7, 8], but are themselves diverse and likely reflect function in their form. The genus *Eois* Hübner represents such a model group.

Eois is one of the most diverse genera in Geometridae accounting for up to 8.1% of all geometrid species, with 83% of known species occurring in the montane regions of the Neotropics[3, 19, 20-23]. There are a total of 267 described *Eois* species, 220 of which are found in South America and 47 of which are found in Africa and Southeast Asia[3, 19-21]. Moths in this genus specialize on shrubs and vines in the genus *Piper*, and studies have shown that diversification of *Eois* overlaps in complex ways with the diversification of *Piper*[22, 23]. *Eois* moths are generally small with wingspans ranging from 12 to 20 mm, and

with high diversity in wing pattern, including ground colors of yellows, greens, or browns[8].

Based on Moraes et al.[8] and Brehm et al.[3], there are diagnosable characteristics of genitalia morphology of *Eois* moths which include but are not limited to: absence of an uncus in males; a well-developed scaphium in males; valvae in males; vesica in males; various sized and positioned, sclerotized, horn-shaped signa in females; and multi-spined corpus bursae in females. To expand further on these structures for possible copulatory functions in *Eois*: valvae are two laterally paired appendages in male genitalia arising from the ninth abdominal segment that likely are used to grasp the external female abdominal surface[5]. The valvae may have hairs, spines, lobes, or androconia and vary greatly in size and shape among different species[5]. The vesica is an eversible membranous structure that lies internally in the male phallus, a cylindrical intromittent organ; the vesica bears sclerotized spikes or nub-like cornuti in *Eois* which may cause trauma to the female corpus bursa during copulation[5]. According to Galicia et al.[24], the sclerotized, horn-shaped signum in females may serve one or more of the following functions: 1) act as a complementary locking structure to the male phallus, stimulating ejaculation (either by breaking a physical barrier or stimulating a sensory structure in the male aedeagus); 2) protect the corpus bursae from damage inflicted by phallus structures during copulation; 3) hold the spermatophore, deposited by males during copulation, in place; 4) break open the spermatophore to release sperm[25]; or 5) filter particles of the degraded spermatophore that may block the sperm duct or the ductus seminalis. It is well documented (e.g., Meslin et al.[25]) that the multi-spined corpus bursae in females is a

copulatory pouch that receives, stores, and digests male ejaculate, such as the spermatophore.

The goal of the present study is to evaluate evolutionary patterns in *Eois* genitalia (Fig. 1) as a first step towards understanding these sexual traits within the genus. A naïve expectation would be that mechanisms of genetic drift or pleiotropic effects would result in more complicated patterns of genitalic evolution (potentially with non-congruent patterns in males and females), while mechanisms such as female choice, sexual conflict, or the lock-and-key hypothesis could result in a stepwise correlated evolutionary pattern, in which changes in female genitalia would be closely tracked by changes in male genitalia[1]. These possibilities motivate our study and raise the following questions: are male and female genitalia in *Eois* evolving in parallel? Or are genitalic traits in male and female *Eois* evolving independently of one another via mechanisms such as genetic drift or a combination of mechanisms? Does variation in male and female genitalia change at the same rate or extent across the phylogeny? In other words, might male and female reproductive structures be under different evolutionary pressures or constraints? All of these investigations represent an early stage of our work on the genus *Eois*, largely because knowledge of functional morphology of *Eois* genitalia is limited, as well as information on the role of different structures during copulation and the costs and benefits of mating for these insects.

Materials and Methods

We evaluated 94 species of *Eois* for which both male and females were available, and five outgroup species. Specimens (Fig. 2) were obtained from the following collections:

AMNH (American Museum of Natural History, New York, NY, USA); NHML (Natural History Museum, London, U.K.); UNR (Research collection of lepidoptera from the University of Nevada, Reno, Museum of Natural History, Reno, NV, USA); AME (McGuire Center for Lepidoptera and Biodiversity, Florida Museum of Natural History, Gainesville, FL, USA); and USNM (National Museum of Natural History, Smithsonian Institution, Washington, DC, USA).

Morphology

Dissection methods followed Robinson[26], except genitalic structures were slide mounted using Euparal or stored in glass micro-vials with glycerin. Images of adults and genitalia were photographed using a Canon EOS 40D digital SLR (Canon U.S.A., Lake Success, NY) mounted on a Visionary Digital BK Lab System (Visionary Digital, Palmyra, VA). Terminology for genital structures and forewing pattern elements follows Holloway[27], with descriptions of external and internal morphology derived from photographs and by viewing the specimens and genitalia structures under a Zeiss Stemi 2000-C Stereomicroscope with SCHOTT EasyLED Ring Light Illuminator. Figures were constructed in Adobe Photoshop (v24.6) and Illustrator (v27.6. 1) both part of the Adobe Creative Cloud 2023 (v5.11.0.522.1)[28].

For the 94 *Eois* and five outgroup species, we compiled a morphological data matrix of 107 characters (37 binary and 70 multi-state), including 16 external characters (characters 1–16), 58 male characters (characters 17–75), and 33 female characters (characters 76–107); see Appendix A for full details on individual characters. Species

were identified through the examination of photos of holotype or type photos (Fig. 2) and their genitalia.

Phylogenetic analyses

The data matrix (Tab. A1) was analyzed using both maximum parsimony and maximum likelihood methods. The character states in the matrix were treated as ordered where applicable. Parsimony analysis was executed in PAUP* v.4.0.a[29] as a heuristic search, with results summarized as both a strict consensus phylogeny of the most parsimonious trees, and as a 50% majority rule phylogeny. One hundred bootstrap replicates were generated for the 50% majority rule tree, but support was uniformly low and is not discussed further here. Maximum likelihood analysis was conducted using IQ-TREE v.1.6.12[30-33], and the most likely tree was retained for comparison with the results of the maximum parsimony analysis.

The morphological matrix contained both sex-specific traits (observed from only males or only females) and traits that are common to both sexes. The phylogenetic analyses, as described above, were conducted on the entire matrix, as well as matrix subsets of male-only and female-only traits. When analyzing male- and female-only matrices, we also investigated those matrices including characters common to both sexes. Trees resulting from maximum parsimony and maximum likelihood analyses were visualized using the plot.phylo function from the ape package in R[34], and congruence among trees was visually investigated (e.g., comparing a male-only tree with a female-only tree) using the cophylo function from the phytools package[35].

Throughout the text we use the term “phylogeny” to refer to trees using all characters, and the term “dendrogram” to refer to trees based on subsets of the characters (i.e., female-only traits and male-only traits), reflecting the fact that we are using the latter to investigate character evolution but not as hypotheses for the evolutionary history of lineages.

Results

Maximum parsimony and maximum likelihood phylogenies were largely congruent, but dendrograms of male- and female-only traits displayed varying levels of discordance with each other. Below we first discuss the extent to which the phylogeny based on all characters reflects our current understanding of *Eois* systematics, then we provide comparisons among trees estimated with different methods and different subsets (i.e., male- and female-only traits) of our data.

The parsimony analysis of the complete matrix resulted in 16 equally-parsimonious trees, which were summarized both as a strict consensus tree and as a 50% majority rule tree. Both the parsimony and maximum likelihood analyses appear to resolve most major species groups within this subset of *Eois* (Fig. 3), some of which correspond to groups identified by Strutzenberger[36, 20], Brehm[3], and Moraes et al[8] based on molecular analyses using two genes - the mitochondrial gene cytochrome oxidase subunit 1 (COI) and the nuclear gene Ef1-alpha. In both the parsimony and maximum likelihood results, the following clades, initially recognized by molecular analyses, were recovered as monophyletic using our morphological data: an *olivacea* clade, a *chrysocraspedata*[3] or *paraviolascens*[20] clade, and to a certain extent, a *pallidicosta* clade

(with *E. planetaria* falling outside of the clade in both trees) (Fig. 3). We also found that in some cases, particularly with *russearia* and *insignata*, specimens of a nominal species are very near to a nominal species but did not group together. For example, *russearia* and *pararussearia* did not form a monophyletic group, with distances between the two taxa differing between the parsimony and maximum likelihood results. A confounding factor here is the uncertainty concerning the identification of *E. russearia* because the type specimen is reported as lost. Nonetheless, our specimens of *E. russearia* and *E. binaria*, a species that bears similar wing pattern to *E. russearia*[8] formed a group in both analyses, corroborated by convincing similarities in genitalic characteristics.

Congruence of methods, discordance among dendrograms

The phylogenies generated by maximum parsimony and maximum likelihood analysis of the complete morphological matrix (including all characters, male and female) recovered trees that were largely congruent in topology (Fig. 3). As noted above, we are focusing on the most likely tree and summaries of the most parsimonious trees, while not reporting bootstrap values that were universally low. Also, despite general congruence between parsimony and likelihood phylogenies, it is notable that a small number of taxa appear in dramatically different locations in the two trees (Fig. 3). These results almost certainly reflect incomplete taxon sampling in an exceedingly complex genus, as we will discuss further below.

Despite the uncertainty inherent in reconstructions based on morphology, the overall structure of our analyses (e.g., recovering major *Eois* clades) based on the full matrix (Fig. 3) raises the possibility for strong inference with respect to our central

question of congruence in evolutionary patterns in male and female traits. We find a clear lack of congruence, such that patterns of evolution inferred from male traits are not mirrored in patterns of evolution inferred from female traits (Fig 4). The independence of evolutionary patterns in males and females is also reflected in the fact that dendrograms generated with each sex separately also show considerable lack of congruence with the overall (all character) phylogeny (Fig. 5 & 6).

For maximum likelihood (all characters) to male dendrogram comparisons, congruent clades include: clades of *E. nigrinotata*, *E. fragilis*, and *E. filiferata*; clades of *E. parumsimii*, *E. viridiflava*, and *E. peruviensis*; and clades of *E. griseicosta*, *E. diversicosta*, and *E. relaxaria* (Fig. 5). For comparisons of maximum likelihood to the female-only dendrogram, the following clades, among others, were congruent: clades with *E. obada, E. numida*, *E. sanguilineata*, *E. memorata*, *E. occia*, *E. mexicaria*, *E. insignata*, *E. veniliata*, and *E. lavendulan*, and clades with *E. pallidicosta*, *E. mediostrigata*, and *E. brunnea* (Fig. 6).

Isolated islands of congruence based on sex-specific traits

Despite the overwhelming signal of independent evolution in male and female genitalia (Fig. 4), isolated examples can be seen in which taxa cluster together in both male and female dendrograms (Fig. 7). Comparisons of male dendrogram to female dendrogram and maximum likelihood to male and female dendrograms, showed isolated islands of clades or taxa matching traits, in particular all comparisons that include *E. grataria*, *E. plumbacea*, and *E. discata* show congruence across analyses of subsets of the data (Fig. 4-7). For male to female dendrogram comparisons, *E. sanguilineata* and *E.*

memorata are sister species in both dengrograms, clades containing *E. veniliata, E. mexicaria, E. insignata* show congruence, and clades containing *E. pallidicosta, E. mediostrigata, E. encina*, and *E. brunnea*, show similar congruence (Fig. 7).

Discussion

Different evolutionary mechanisms, from genetic drift and female choice to the possible role of the "lock-and-key" isolating mechanism are still open questions in regards to factors that may drive evolution in lepidopteran genitalia. Answers to this issue will require future detailed investigations of reproductive attributes such as mating interactions, mating costs and benefits, functional morphology of interacting male and female genitalia traits, and rates of evolutionary divergence[1, 5]. In this preliminary study, we attempted to understand the patterns of variation and covariation of male and female genitalic traits, with the hope that any discordance or congruence revealed in our comparisons of male and female morphological analyses would allow us to speculate on what types of evolutionary mechanisms may have influenced genital evolution. It is unlikely that any one factor, such as genetic drift, pleiotropic effects, female choice, sexual conflict, or lock-and-key, would be responsible for generating patterns of male/female genitalic divergence across an entire genus, but it is possible that different mechanisms have contributed to patterns across *Eois* and that individual mechanisms are responsible for patterns within subsets of *Eois* taxa, and that different mechanisms might be in action in different parts of such a complex and hyperdiverse genus.

Our central finding is that the pattern of relationships inferred from male as compared to female characters reveals distinctly different and divergent patterns of

evolution. The lack of simple, congruent evolutionary patterns between sexes implies that multifarious factors, potentially including genetic drift or pleiotropic effects (or both), combined with other factors, might be driving the evolution of these traits that are diverse and of course central to the biology and evolution of sexual species. It is important to remember that great complexity in these issues derives from the fact that patterns in the evolution of male and female traits are inherently (and unavoidably) associated with observations on completely different structures in the two sexes. For male genitalia, there are multiple characteristics involving valvae, phallus, juxta, sacculus, and vesica that contribute variation to the morphological matrix, while the corpus bursae, signum, and papillae anales provide the majority of the female variation in our matrix.

It is also important to note that the discordance seen in our dendrograms could be an artifact of limited sample size, which is hard-won (speciemens were globally sourced from museums and extensive field efforts) but also limited relative to the diversity of the genus. Our study includes only 94 out of what is probably well over 1000 species for this genus, so the patterns we observe here may not mirror actual patterns from complete trees. Issues with incomplete taxon sampling in phylogenetic reconstruction and character evolution are ubiquitous and well-studied[37, 38]. Nevertheless, to achieve progress in understanding the evolution of genitalia in *Eois*, concentrating on subsets of taxa (e.g. *E. grataria*, *E. plumbacea*, and *E. discata*) that we identified with matching traits could allow for careful tests of existing hypotheses, such as lock-and-key or traumatic copulation. The subsets of taxa that showed greater congruence or trait matching in each of their respective comparisons are good candidates for examining how pre- and postzygotic reproductive isolating mechanisms or intersexual conflict might shape the

evolution of genitalia. These small subsets of taxa could profitably be pursued with population genetic or more fine-scale taxonomic comparisons.

Among other methodological limitations, it should be noted that the numbers of male and female traits were not the same. Not including external morphology of both sexes, there were 59 male characters versus 32 female characters. The discordance between the female dendrogram and the maximum likelihood phylogeny is less than that of the male tree (compared to the all-trait phylogeny), which could be due to the character matrix having more male than female traits or incomplete sampling. Interestingly, however, the tree inferred from the male characteristics has less structure overall which could suggest evolutionary lability and thus the possibility that male traits are subject to more diverse evolutionary pressures.

In future work, we hope to use morphological analyses in combination with molecular phylogenies to provide insight into rates of evolutionary divergence of morphological characters and how those rates of divergence can inform underlying mechanisms of evolution. Simmons[1] remarked that traits that rapidly evolve may have lower phylogenetic signals than those that evolve slowly[39], with the strength of phylogenetic signals depending on how genitalic traits are characterized[1]. In the case of *Gerris* (water striders), genitalic complexity displayed a strong phylogenetic signal, while quantitative variation in the shape of the characteristics did not, which suggests that different genitalic traits could be subject to different rates of evolutionary divergence and perhaps be subject to different forms of female choice[1, 40].

Another motivating force behind our study was to evaluate the informative nature of female genitalia for morphological taxonomy. Female genitalia in Lepidoptera have

been characterized as being “simple”— as their uniform shape is supposedly not as informative as male genitalia[2, 9, 10]. Although our morphological matrix has fewer female characters than male characters, the female traits are not inherently less informative. Rather this difference in total characters likely reflects a long tradition of character discovery in male genitalia which of course underlies our study as well. Moreover, Cordero and Baixeras[5] concluded that one genitalic structure could have multiple roles depending on its interaction with the genitalia of the opposite sex or other biological or chemical aspects. The dendrogram inferred from the female characteristics had more structure compared to the male trait dendrogram, thus this could be consistent with the situation in which one trait has multiple functions, in which case, further investigations involving mating interactions are needed to distinguish and understand the adaptive significance of traits[5].

Our morphological analyses have provided insight into the complexity of female genitalia in *Eois* and have highlighted the need for focusing on variation in female lepidopteran genitalia to not only understand mating interactions in lepidoptera, but functional morphology and evolution of interacting genitalic traits. To paraphrase the cliché, all journeys begin with a single step, and we hope that this study is many steps along what must be one of the most winding roads in organismal biology.

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Figures

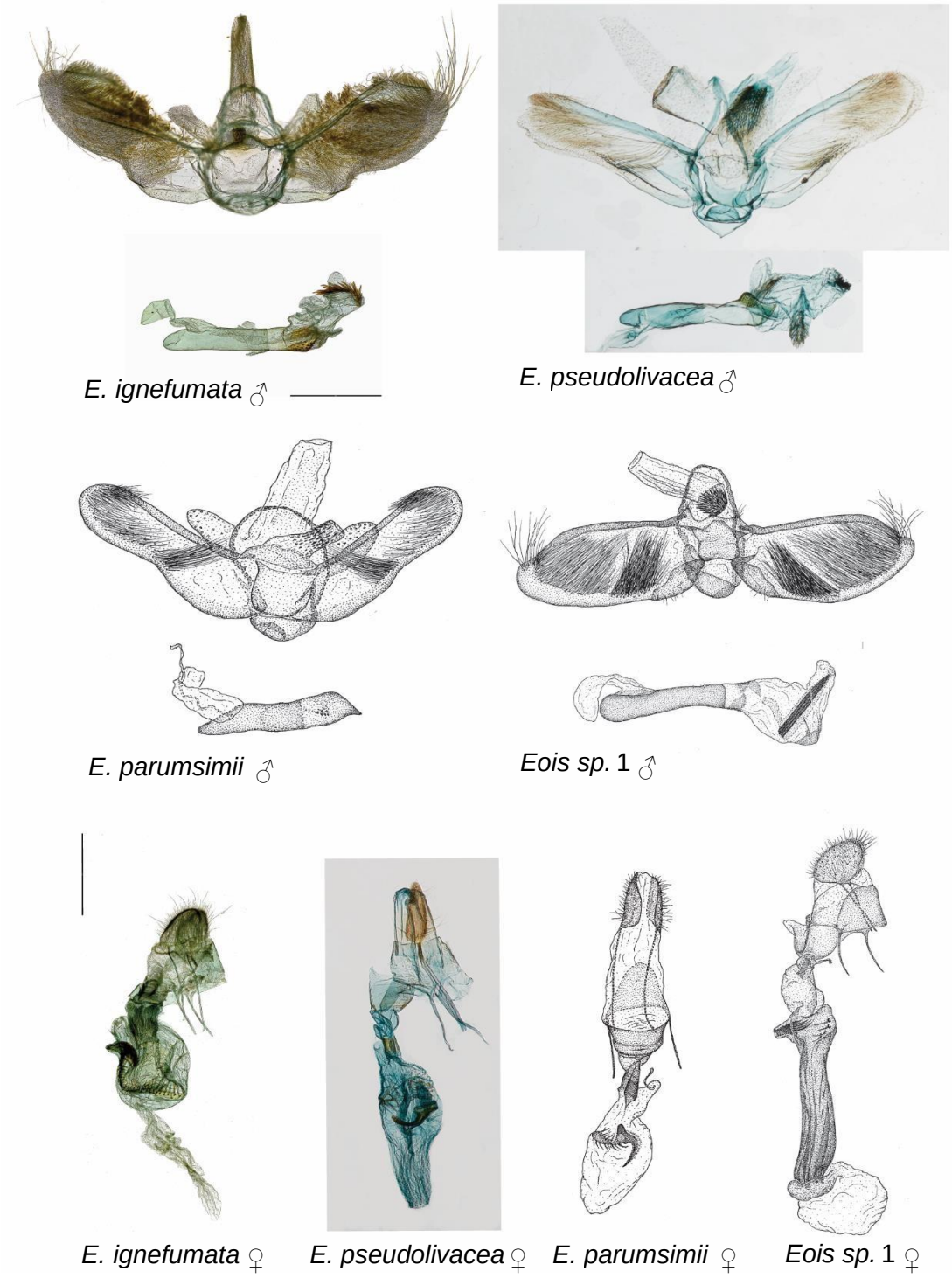

Figure 1. Male and female genitalia of 4 *Eois* species. The corresponding specimens are shown in Fig 2. Scale bar = 1 mm.

Figure 2. Sample of 40 *Eois* adult moths used in this study. Holotype, syntype, or near photos provided from the following: AMNH; UNR; and USNM. **1-5:** *E. agroica, E. ambarilla, E. apyraria, E. brunnea, E. brunneicosta;* **6-10:** *E. burla, E. carmenta*; *E. carnana carnana, E. cervina, E. coloraria;* **11-15:** *E. deleta, E. golosata, E. hermosaria, E. ignefumata, E. insignata;* **16-20:** *E. isographata, E. jifia, E. mexicaria, E. nigricosta, E. nr. viridiflava;* **21-25:** *E. numida, E. nympha, E. obada, E. occia, E. operbula;* **26-30:** *E. ops, E. pallidicosta, E. pararussearia, E. parva, E. parumsimii;* **31-35:** *E. peruviensis,*

E. planetaria, E. quardrilatera, *E. relaxaria, Eois sp. 1;* **36-40:** *E. rubiada, E. russearia, E. seria*, *E. xanthoperata, E. zorra*. Genitalia of 4 species are shown in Fig 1.

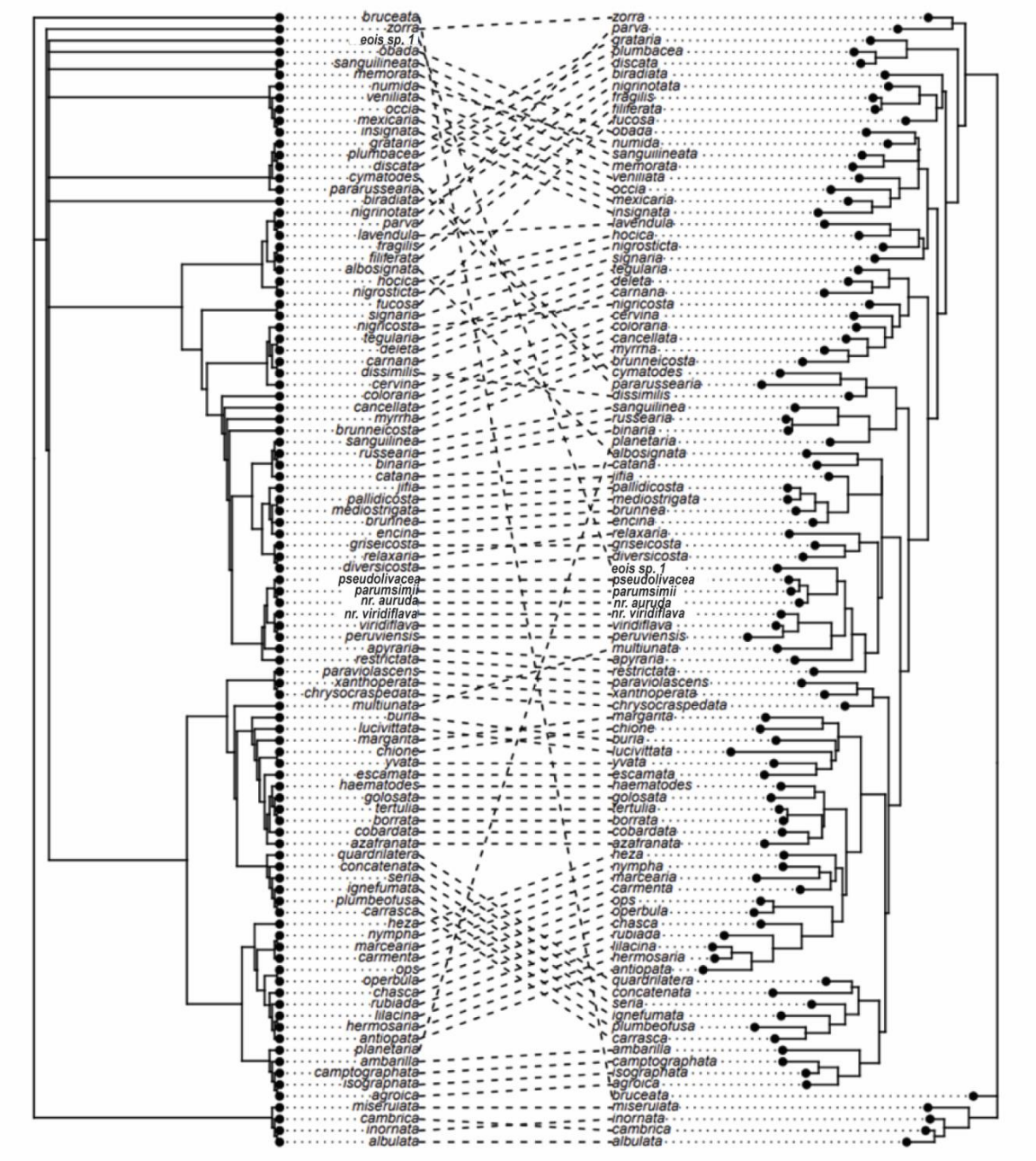

Figure 3. Comparison of results from 50% majority rule of 16 most parsimonious trees (left) and maximum likelihood tree (right) using all characters (male and female and common to both).

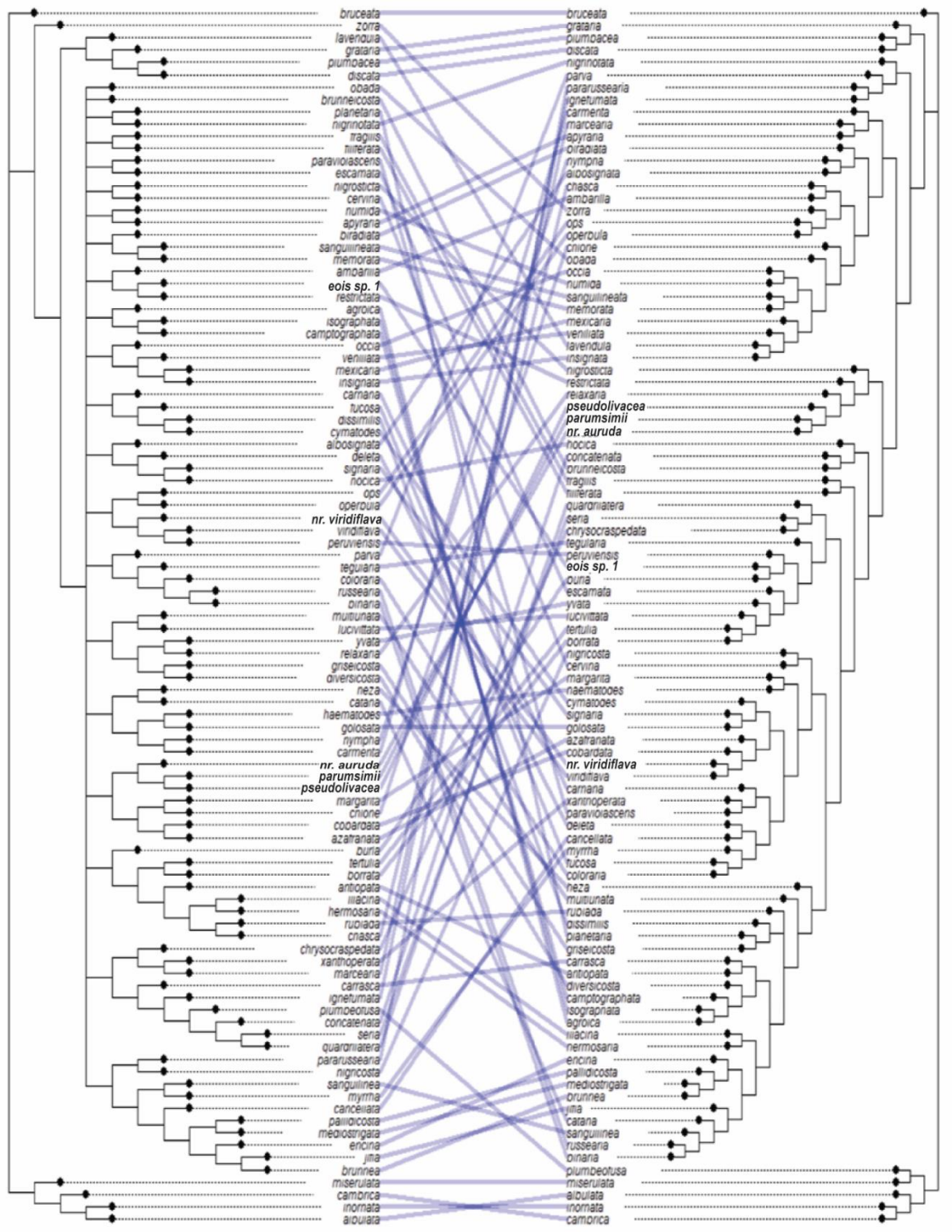

Figure 4. Comparison of maximum likelihood dendrogram of male-only traits (left) and maximum likelihood dendrogram of female-only traits (right).

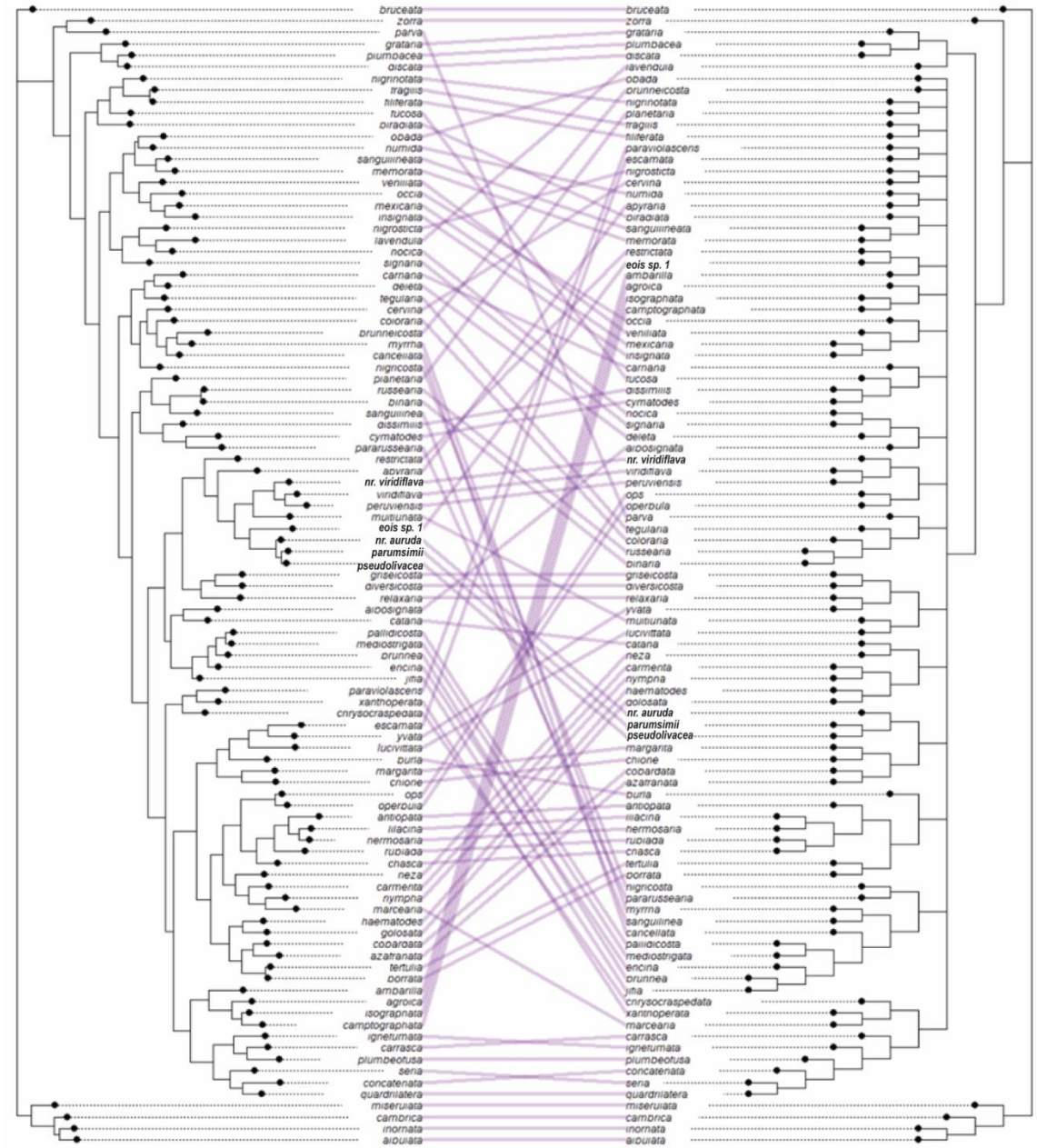

Figure 5. Comparison of maximum likelihood tree of all traits (left) and maximum likelihood dendrogram of male-only traits (right).

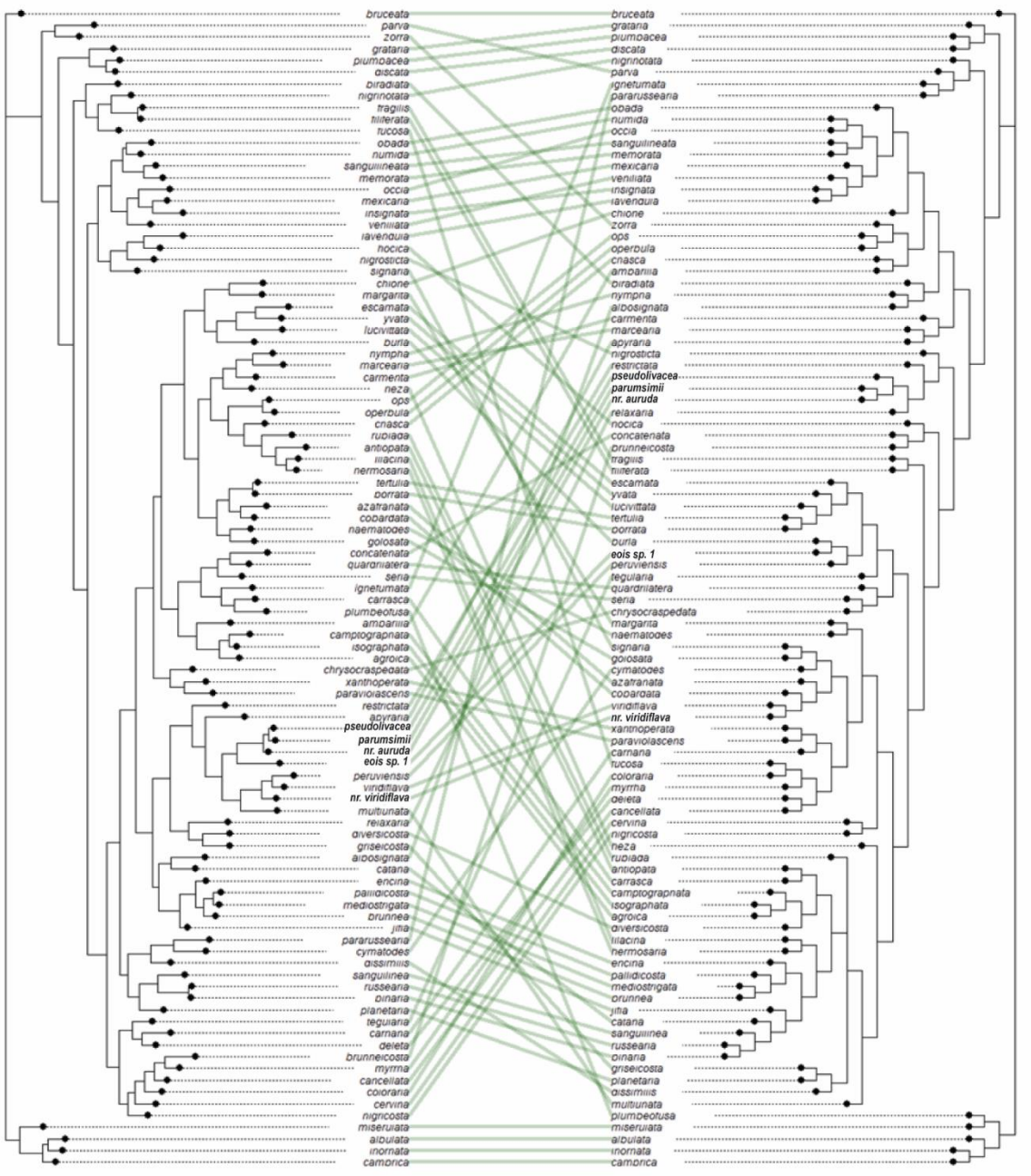

Figure 6. Comparison of maximum likelihood tree of all traits (left) and maximum likelihood dendrogram of female traits (right).

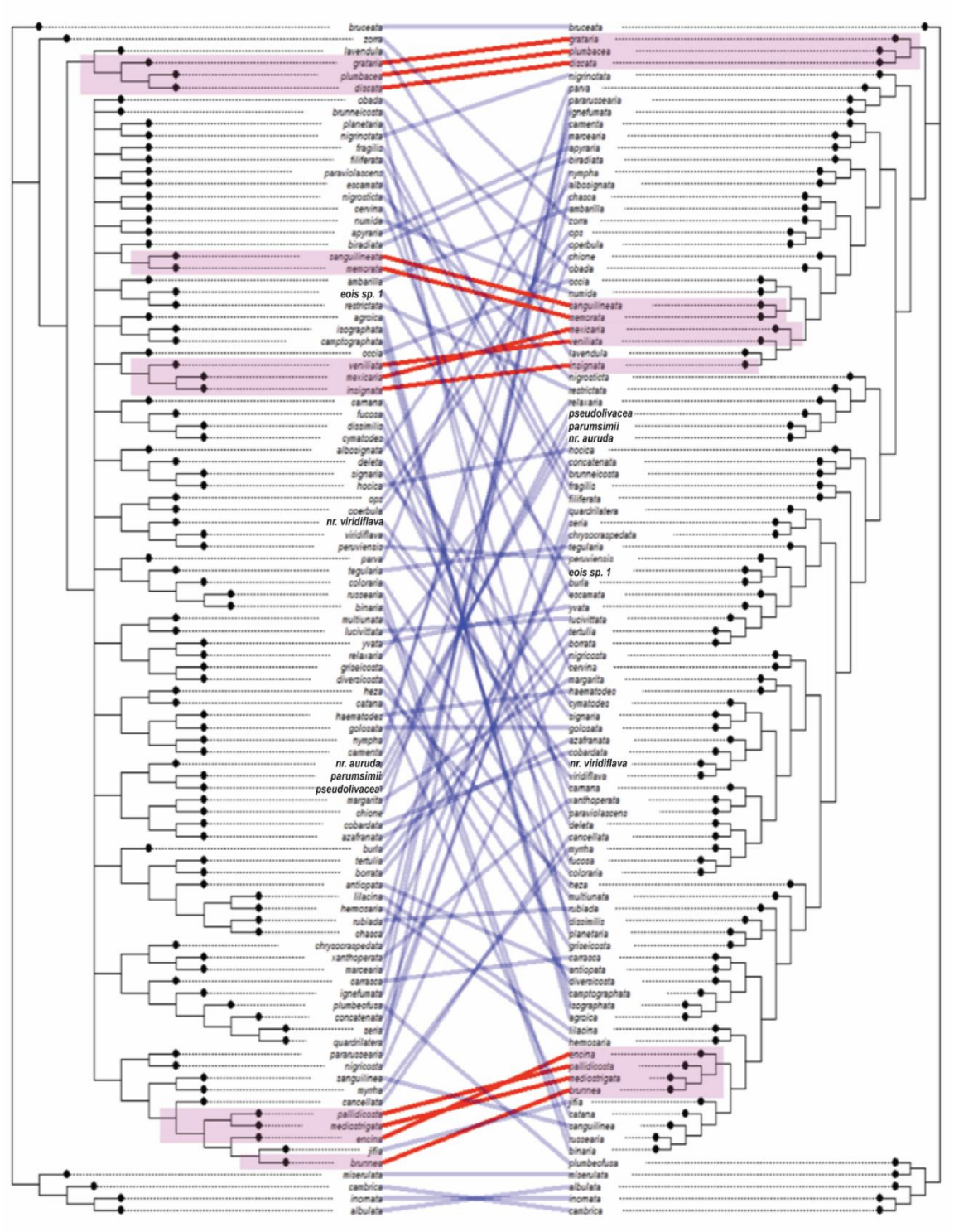

Figure 7. Comparison of maximum likelihood result for male-only traits (left) and maximum likelihood dendrogram of female-only traits (right). Red lines denote taxa that show similar patterns with both male and female traits.

Summary and Conclusions

The scientific study of biodiversity has always been a formidable challenge, and it has become even more so with the changing face of our planet, as climate and anthropogenic activities have altered the distribution and diversity of living organisms and ecosystems worldwide (Sánchez-Bayo & Wyckhuys 2019; Isbell et al., 2023). Unfortunately, there are simply not enough taxonomists being trained to handle the overwhelming task of documenting global biodiversity, and their numbers are declining, resulting in reduced output of taxonomic research and descriptions (Coleman & Radulovici, 2020). Ultimately, taxonomists are not being trained fast enough to be able to describe species before they go extinct. Thus, we are in a difficult and tragic position, for we cannot know all that we are losing if we do not know all that we have. Furthermore, it is not just new, undiscovered species that still persist in the remaining wild places, but the many undescribed species that are right under our noses in museum collections across the world.

One such example hidden among our research collections here at UNR is that of *Eois*, a hyperdiverse genus of moths. *Eois* is comprised of 267 valid species, however it is estimated that there are a thousand or more Neotropical species yet to be described (Brehm et al., 2011; Strutzenberger et al., 2017; Moraes et al., 2021). Not only is there a lack of accurate taxonomic descriptions, a paucity of distribution data for these moths, but also this genus undoubtedly harbors many undescribed cryptic species which leads to an underestimation of species richness (Strutzenberger et al., 2011; Moraes et al., 2021). All of these factors limit monitoring and conservation efforts of possibly vulnerable populations, and hinders investigations into ecological and evolutionary factors

underlying biodiversity of *Eois*. To begin to remedy shortcomings in our knowledge of this moth genus, my dissertation was focused on three different aspects of *Eois* systematics and evolution.

For the first section of my dissertation, I was interested in investigating diversity in *Eois*. Starting with the olivacea clade as a jumping off point as taxa in this clade are commonly reared specimens stored in the research collection of Lepidoptera from the UNR Museum of Natural History. Questioning the validity of the assigned identifications of these specimens, and interested in cryptic diversity, and a better understanding of actual species richness in *Eois*, I evaluated the relationships between genetic and genitalic variation with morphologically similar taxa within the *olivacea* species complex. I also investigated the extent to which ecological factors influence evolutionary patterns across populations and conducted phylogenetic analyses to interpret the pattern and timing of population divergence within the broader context of *Eois* diversification. I found that across 170 individuals sampled from different elevations and host plants at a single site within the Ecuadorian Andes, our population genetic analyses revealed that the samples can be assigned to four distinct taxa, with genetic divergence being associated with utilization of different hosts. The morphometric analysis, however, revealed that adult samples belonged to three distinct taxa, and the molecular dating analysis implied that these taxa formed a monophyletic clade that began diverging approximately five million years ago.

For the second section, after observing the diversity hidden among the olivacea species complex, I circumscribed and described 16 new species of the *Eois* olivacea clade based on traditional morphological techniques using specimens from various institutional

collections (UNR, AMNH, BMNH, USNM and AME), employing a data matrix of 107 morphological characters, initially developed by Dr. James Miller. I defined the clade based on wing pattern and other morphological features and provided detailed diagnoses and descriptions of each new species, as well as re-examining the four previously described species in the clade.

For the third section, to contextualize sexual traits and advance understanding of patterns of evolution of genitalia in *Eois*, I considered different driving forces of trait evolution (including genetic drift, pleiotropy, female choice, cryptic female choice, male to male competition, sexual conflict, and the lock-and-key isolation mechanism), to understand the evolution of male and female reproductive traits. Using the morphological data matrix mentioned above, I developed a phylogeny for a sample of 99 species (94 *Eois* and five outgroup taxa), using maximum parsimony and maximum likelihood methods, and separate dendrograms based on male-only and female-only characters. An examination among trees revealed discordance between dendrograms based on male-only and female-only traits, suggesting at least partially independent evolution of traits between the sexes to which I concluded that the evolution of male and female genitalia in *Eois* cannot, at this time, be explained by a single hypothesis. Moreover, morphological analyses provided insight into the complexity of female genitalia in *Eois*: dendrogram for female traits had more overall structure compared to the dendrogram for male traits, suggesting the influence of either our limited taxon sampling or the possibility of traits having multiple roles during copulation (Cordero & Baixeras, 2015). These findings highlight the need for further investigations on variation in female lepidopteran genitalia

to understand mating interactions in Lepidoptera, functional morphology, and evolution of reproductive traits.

Taken together, the results from my dissertation work demonstrates: 1) that diversity estimates for *Eois* are likely to increase partly because of cryptic species; 2) the importance of taxonomic circumscription and description of species; 3) and the appreciation for the complexities of genitalia that can help identify taxa and to disentangle patterns of genitalic evolution. With the sheer number of undescribed *Eois* species, it is an understatement to say that the work needed to investigate this genus is far from over.

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Appendix A - Adult Morphological Characters in *Eois*

This appendix contains additional methodology of *Eois* species identification, complete with a morphological guide based off of 94 *Eois* and 5 outgroup *Eois* species. The guide includes 107 characters (37 binary and 70 multi-state), with 16 external characters (characters 1–16), 58 male-only characters (characters 17–75), and 33 female-only characters (characters 76–107).

Methodology, terminology, and abbreviations

Examination of specimens was performed under a Zeiss Stemi 2000-C stereomicroscope with SCHOTT EasyLED ring-light illuminator. Species were identified through the examination of photos of holotype or type photos (Brehm unpublished data) and their genitalia. Terminology for genital structures and components of forewing pattern follow Holloway (1997) and Viidalepp (2011). With the exception of an unusual structure in the male genitalia of members of the several clades of *Eois*, that appears to lack a term. It is a flat, membranous, lateral flap of variable size and length attached to the sides of the tegumen and/or transtilla, which supports long, fine male scent scales or androconia. The term “lacina” is suggested for these structures.

In the guide below, brief descriptions of each morphological character are followed by a number/score, or character state, in brackets (e.g. **[1]**, **[2]**, **[3]**, etc.) which denotes variation of that character. Scores of 0, unless otherwise stated, are character states commonly observed in the outgroup taxa.

Abbreviations for morphological structures in the guide are as follows: Lp1 = labial palpus segment 1; Lp2 = labial palpus segment 2; Lp3 = labial palpus segment 3; FW = forewing; HW = hindwing; Tg7 = tergum 7; Tg8 = tergum 8; St7 = sternum 7; St8

= sternum 8; PVP = postvaginal plate; DB = ductus bursae; DS = ductus seminalis; CB = corpus bursae.

Eois Morphological Guide

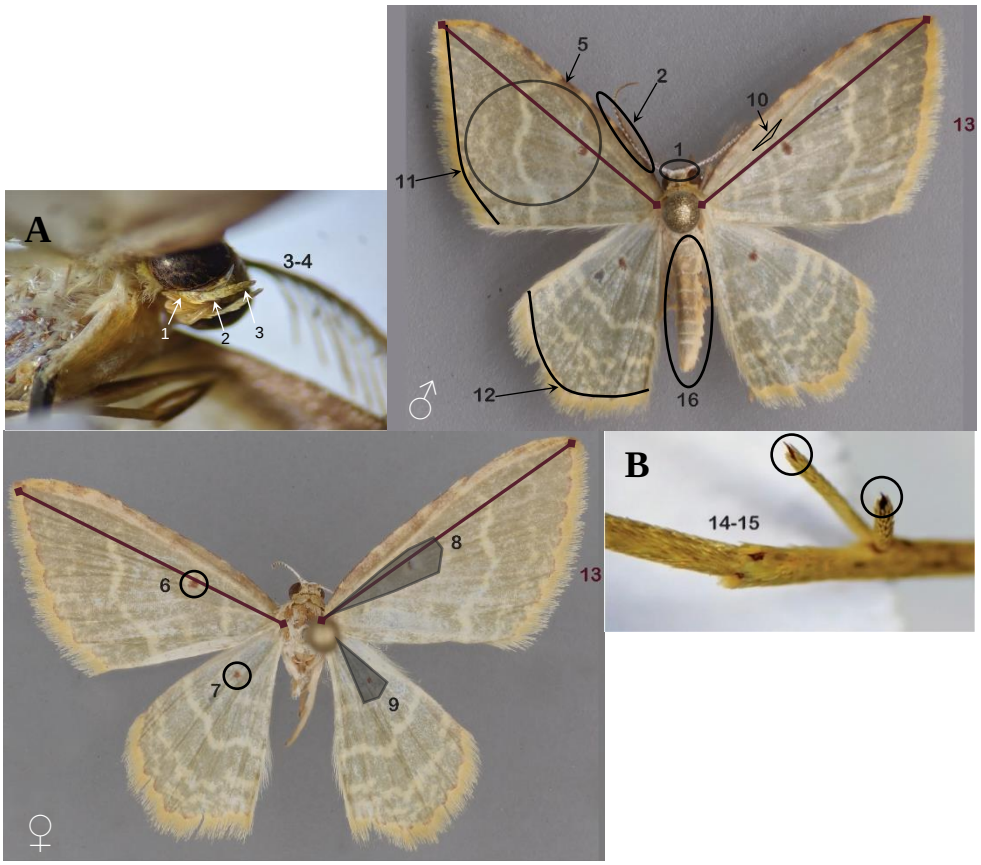

Figure A1. Images of male and female *Eois pseudolivacea* adults provided from the rearing collection from UNR. Accompanying the image of the adult are closeup images of mouth (A) and leg structures (B). Numbers assigned to morphological structures in each image correspond to external characters (1–16)* seen in both genders in this morphological guide.**Characters 1-16 can be discerned from either gender; for ease of viewing, some characters are displayed on either image of male or female in this figure. Character 13 is displayed twice on both images as this character compares forewing span of genders.*

External Characters of both genders

1. Scales between antennal bases concolorous with vertex of head **[0]**; a transverse band of snowy white scales located between antennal bases **[1]**; a narrow band of creamy yellow scales between antennal bases **[2]**.

2. Dorsum of antennal shaft/flagellum concolorous with remainder of head **[0]**; dorsum of antennal shaft white **[1]**; each antennal annulation mostly brown, edged with lighter scales **[2]**; dorsum of antennal shaft with alternating rings of light brown and creamy yellow scales **[3]**; each antennal annulation a mixture of brown and cream-colored scales **[4]**; mesal surface of antennal shaft white, lateral surface brown **[5]**.

3. Labial palpus segment 2 roughly half as long as Lp1 **[0]**; Lp2 roughly equal in length to, or slightly longer than, Lp1 **[1]**; Lp2 over twice as long as Lp1 **[2]**.

4. Labial palpus segment 3 small, approximately ¼ as long as Lp1 **[0]**; Lp3 somewhat elongate, nearly ½ as long as Lp1 **[1]**.

5. Forewing lacking shiny scales **[0]**; forewing with patches of glossy, metallic scales, especially grouped within dark-patterned areas **[1]**.

6. Forewing lacking a spot near apex of discal cell **[0]**; FW with a small brown spot near apex of discal cell **[1]**.

7. Hind wing lacking a spot near apex of discal cell **[0]**; HW with a brown spot near apex of discal cell **[1]**.

8. Forewing discal cell at least ½ wing length **[0]**; FW discal cell much shorter than ½ wing length **[1]**.

9. Hind wing discal cell at least ½ as long as wing, M3 and CuA1 veins arising separately from corner of cell **[0]**; HW discal cell short, approximately ⅓ as long as wing, M3 and CuA1 stalked **[1]**.

10. Forewing accessory cell present, moderate in length **[0]**; accessory cell present, long **[1]**; FW accessory cell absent **[2]**.

11. Forewing outer margin evenly convex **[0]**; FW outer margin with a projection at apex of vein CuA1 **[1]**.

12. Hindwing outer margin evenly convex **[0]**; HW outer margin with a projection at apex of vein M3 **[1]**.

13. Male forewing shorter than female FW **[0];** male forewing longer than female FW **[1].**

14. Sclerotized tips of tibial spurs short and delicate, simple **[0]**; sclerotized tips of tibial spurs elongate, simple **[1]**; sclerotized tips of tibial spurs extremely long, with blade-like lateral margins **[2]**; sclerotized tips of tibial spurs minute **[3]**.

15. Ventral margin of tibial spur simple **[0]**; each tibial spur with a sclerotized, scaleless seam running along ventral surface **[1]**.

16. Dorsum of abdomen a single, uniform color **[0]**; abdominal dorsum yellow with a reddish stripe on the posterior margin of each segment **[1]**; abdominal dorsum yellow with a wide transverse stripe on segments 3+4 and a second stripe on 7+8 **[2]**; abdominal dorsum with a single spot on anterior margin of each segment **[3]**; abdominal dorsum pink with a yellow mesal spot on segment 2 and one on segment 3 **[4]**; abdomen yellowish, with a brown dorsal spot on segment 2 **[5]**.

Male-Only Characters

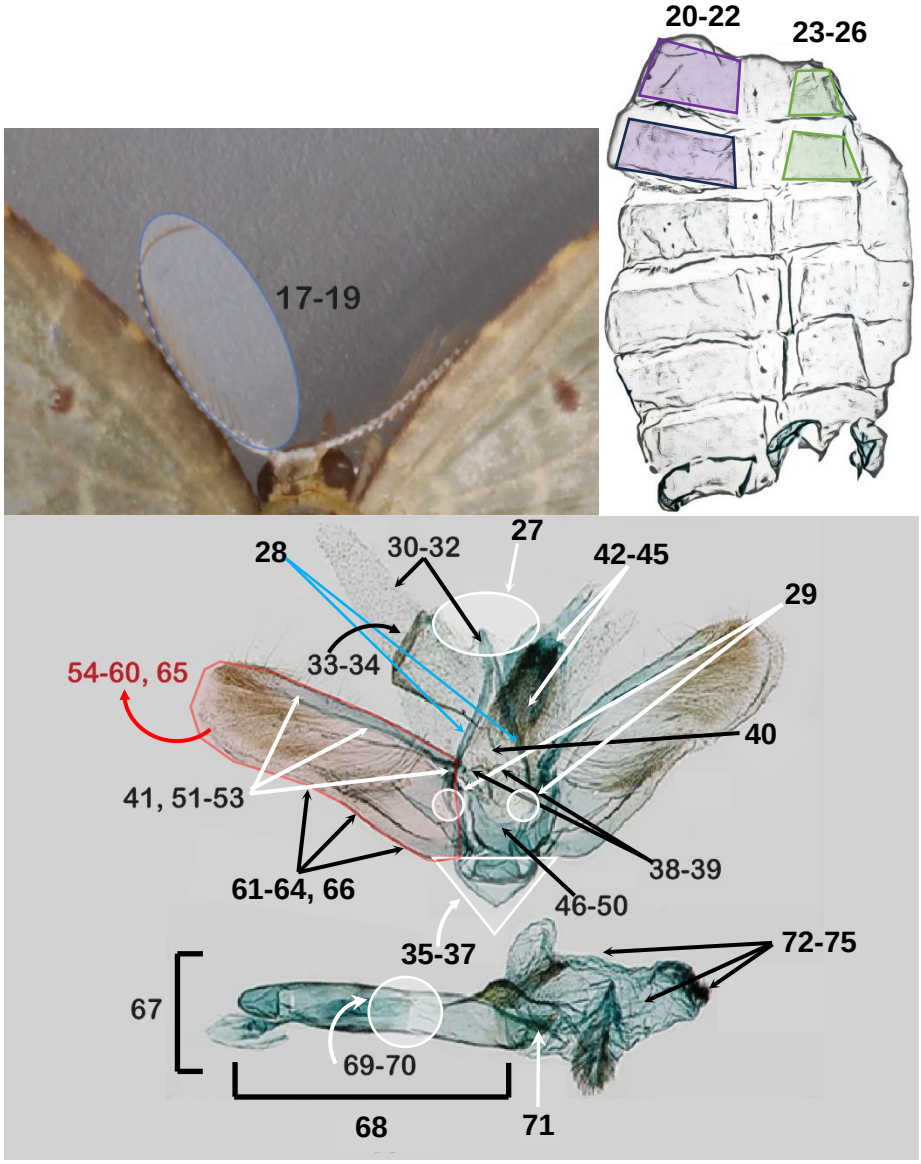

Figure A2. Images of antennae, male genitalia, and illustration of membranous abdomen of *Eois pseudolivacea* adult. Numbers assigned to morphological structures in this image correspond to the male-only genital characters 17–75).

17. Each antennal annulation, exclusive of rami, roughly cylindrical **[0]**; antenna fasciculate **[1]**.

18. Antenna bipectinate nearly to apex, rami becoming gradually shorter distally **[0]**; antenna bipectinate in basal ¾, simple in distal ¼ **[1]**; antenna bipectinate in basal ⅔, simple in distal ⅓ **[2]**; rami absent, antenna ciliate **[3]**.

19. Rami of antenna relatively short **[0]**; rami of antenna long and thin **[1]**; rami extremely short and flattened, apices bearing long bristles **[2]**; rami absent **[?]**.

20. Tergum 8 roughly rectangular **[0]**; Tg8 somewhat narrower posteriorly **[1]**; tergum 8 strongly tapered posteriorly **[2]**.

21. Tergum 8 roughly equal in width to Tg7 **[0]**; Tg8 much narrower than Tg7 **[1]**.

22. Posterior margin of tergum 8 simple **[0]**; posterior margin of tergum 8 bearing a row of golden, bristle-like scales **[1]**; posterior margin of Tg8 with a small, somewhat sclerotized upturned flange **[2]**.

23. Sternum 8 roughly rectangular **[0]**; sternum 8 slightly tapered posteriorly **[1]**; sternum 8 strongly tapered posteriorly **[2]**.

24. Sternum 8 equal in width to St7 **[0]**; Sternum 8 much narrower than St7 **[1]**.

25. Posterior margin of St8 transverse **[0]**; posterior margin of St8 with a shallow, U-shaped mesal excavation **[1]**; posterior margin of St8 with a deep, V-shaped mesal excavation **[2]**; St8 asymmetrical, divided into two asymmetrical parts **[3]**.

26. Posterolateral angles of St8 simple **[0]**; posterolateral angles of St8 acute **[1]**; posterolateral angles of St8 forming elongate prongs **[2]**.

27. Uncus present **[0]**; uncus absent **[1].**

28. Lateral portion of tegumen arms narrow **[0]**; lateral portion of tegumen arms extremely wide **[1]**.

29. Junction of tegumen and vinculum forming a shallow angle **[0]**; junction of tegumen and vinculum forming a deep notch **[1]**.

30. Dorsal part of tegumen, where arms meet at midline, relatively wide **[0]**; dorsal part of tegumen, where arms meet at midline, narrow, band-like **[1]**; dorsal part of tegumen extremely narrow **[2]**.

31. Dorsal portion of tegumen, where arms meet at midline, forming a rounded arch **[0]**; tegumen arms forming a somewhat triangular dorsal arch, curving slightly backward **[1]**; tegumen arms forming a narrow dorsal arch, curving strongly backward **[2]**.

32. Arms of tegumen fully sclerotized, androconia absent **[0]**; each arm of tegumen with a membranous section along posterior margin, this bearing a set of long androconia **[1]**; androconia on arms of tegumen attached to a short lacina (less than ½ as long as valva) **[2]**; androconia of tegumen attached to a long lacina, nearly ½ as long as valva **[3]**; lacina at least ⅔ as long as valva **[4]**.

33. Anal tube short, with a short longitudinal ventral sclerite **[0]**; anal tube long, ventral surface bearing a long, relatively wide sclerotized band **[1]**; anal tube extremely long, extending nearly to apex of valvae, ventral surface bearing a narrow longitudinal band **[2]**.

34. Surface of sclerite below anal tube simple **[0]**; sclerite below anal tube with a longitudinal furrow **[1]**.

35. Region of membrane below saccus simple **[0]**; region of membrane below saccus with a small pocket, bearing a set of spatulate androconia **[1]**.

36. Saccus deep, dorsal margin partially enclosing bases of valvae **[0]**; saccus shallow, dorsal margin transverse, valva bases exposed **[1]**.

37. Ventral margin of saccus broadly triangular **[0]**; ventral margin of saccus gently curved, band-like **[1]**; ventral margin of saccus forming a small, transverse-ovoid

pocket **[2]**; ventral margin of saccus forming a blunt conical pocket **[3]**; ventral margin of saccus forming an acute, triangular pocket **[4]**; ventral margin of saccus quadrate **[5]**.

38. Transtillar arms meet at midline to form a small U-shaped structure, pointing anteriorly **[0]**; transtillar arms forming a large, shelf-like, U-shaped structure anteriorly **[1]**; transtillar arms forming a small, V-shaped structure **[2]**.

39. Transtillar arms lacking projections (most *Eois*) **[0]**; each transtillar arm bearing an elongate, setose process ("labides") (outgroup character) **[1]**; transtilla absent **[?]**.

40. Area of manica posterior to tegumen simple **[0]**; area of manica posterior to tegumen bearing a pocket of long, bristle-like scales **[1]**.

41. Sclerite connecting base of valval costa to dorsum of juxta relatively narrow, simple **[0]**; sclerite connecting base of valval costa to juxta wide, elbowed **[1]**.

42. Membrane surrounding phallus base simple **[0]**; membrane surrounding phallus base bearing a small dorsal field of anteriorly-directed spines **[1]**; membrane surrounding phallus base with a large dorsal field of spines **[2]**.

43. Spines surrounding phallus base short **[0]**; spines surrounding phallus base relatively long **[1]**; spines surrounding phallus base extremely robust, thorn-like **[2]**; two lengths of spines surrounding phallus base **[3]**; spines absent at phallus base (outgroup characteristic) **[?]**.

44. A uniform field of spines surrounding phallus base **[0]**; spines in membrane surrounding phallus base arranged in a series of longitudinal rows **[1]**; spines surrounding phallus base arranged in two longitudinal rows **[2]**; a small group of lateral spines on either side of phallus, in addition to larger dorsal group **[3]**; spines absent at phallus base (outgroup characteristic) **[?]**.

45. Region of manica dorsal to spine-field simple **[0]**; region of manica dorsal to spine-field bearing a thin, Y-shaped sclerite **[1]**.

46. Juxta narrow at base **[0]**; juxta wide at base **[1]**.

47. Outer surface of juxta slightly convex or flat **[0]**; juxta bearing a pair of lateral depressions **[1]**.

48. Juxta gradually narrowing dorsally **[0]**; juxta abruptly narrowing in upper third **[1]**.

49. Dorsal margin of juxta relatively wide, with a V-shaped or U-shaped mesal excavation **[0]**; dorsal margin of juxta narrow but truncate, with a small down-curved lip **[1]**; dorsal part of juxta acute **[2]**; dorsal margin of juxta forming a sclerotized, down-curved, horn-like structure **[3]**.

50. Area between phallus and juxta simple **[0]**; a narrow, rod-like sclerite located between juxta and phallus **[1]**.

51. Costa of valva narrow and band-like **[0]**; costa extremely narrow, rod-like **[1]**; costa relatively wide **[2]**; dorsal margin of valva membranous, costa apparently absent **[3]**.

52. Costa long, extending nearly to valva apex **[0]**; costa somewhat shortened, falling well short of valva apex **[1]**.

53. Dorsal margin of costa simple **[0]**; dorsal margin of costa bearing a short, elbow-like process near apex **[1]**; dorsal margin of costa bearing a pair of large thorn-like processes **[2]**.

54. Valva relatively wide, dorsal and ventral margins roughly parallel **[0]**; valve wide, expanded toward apex **[1]**; valva extremely wide, forming a large oval **[2]**; valva abruptly narrowing toward apex **[3]**.

55. Valva apex rounded **[0]**; valva apex slightly acute, triangular **[1]**; valva apex strongly acute, apex blade-like **[2]**.

56. Inner surface of valva bearing setae for nearly its entire length **[0]**; inner surface of valva naked in basal half **[1]**.

57. Apex of valva with fine, hair-like setae, similar to those covering remainder of valva **[0]**; valva with a brush of bristle-like or spine-like setae near apex, contrasting with remaining setae **[1]**.

58. Inner surface of valva covered with hair-like setae only **[0]**; inner surface of valva covered with hair-like setae and pedicellate scales **[1]**.

59. Valva without an isolated set of long setae near apex of sacculus **[0]**; ventral margin of valva with a secondary group of robust setae near apex of sacculus **[1]**; setae at apex of sacculus extremely long, longer than width of valva **[2]**.

60. Valva lacking a row of setae along inner margin of sacculus **[0]**; valva with a group of long, hair-like setae along inner margin of sacculus **[1]**; valva with a dense group of bristle-like setae along inner margin of sacculus **[2]**.

61. Sacculus heavily sclerotized **[0]**; sacculus lightly sclerotized **[1]**; area of sacculus membranous, sacculus apparently absent **[2]**.

62. Sacculus approximately ½ as long as valva **[0]**; sacculus long, extending 2/3 or more the length of valva **[1]**; sacculus short, less than ⅓ as long as valva **[2]**; sacculus absent **[?]**.

63. Sacculus relatively narrow **[0]**; sacculus wide **[1]**; sacculus an extremely broad, somewhat ovoid triangle **[2]**; sacculus apparently comprising a thin, sclerotized rod **[3]**; sacculus absent **[?]**.

64. Ventral margin of sacculus roughly parallel to ventral margin of valva **[0]**; ventral margin of sacculus bowed outward **[1]**; ventral margin of sacculus with an elbow at base **[2]**.

65. Ventral margin of valva smoothly contiguous with outer margin of sacculus **[0]**; a shallow excavation formed along ventral margin of valva near sacculus apex **[1]**; a pronounced notch formed along ventral margin of valva near sacculus apex **[2]**.

66. Apex of sacculus simple **[0]**; sacculus bearing an apical spine **[1]**; sacculus bearing a transverse, apical flange **[2]**; sacculus with an acute, angled process at apex **[3]**; apex of sacculus bearing a large, spatulate process **[4]**; sacculus absent **[?]**.

67. Phallus moderate in width, distal opening narrow **[0]**; phallus wide, distal opening wide **[1]**; phallus narrow **[2]**.

68. Phallus moderate in length **[0]**; phallus elongate **[1]**; phallus short **[2]**.

69. Base of phallus gradually narrowing anteriorly, rounded **[0]**; phallus base broadly rounded **[1]**; base of phallus narrow, somewhat horn-shaped **[2]**.

70. Phallus base simple **[0]**; phallus base bearing a dorsal vertical flange **[1]**.

71. Apex of phallus simple **[0]**; apex of phallus bearing a hook-like ventral process **[1]**; apex of phallus forming a large, blade-like ventral process **[2]**; apex of phallus bearing a prominent, spatulate ventral process **[3]**; apex of phallus bearing a narrow, acute ventral process **[4]**.

72. Vesica comprising a single tube **[0]**; vesica bifurcate, comprising two appendices **[1]**.

73. Vesica lacking spine-like cornuti **[0]**; vesica bearing a single distal group of one or more coarse, spine-like cornuti **[1]**; vesica with two distal groups of spine-like cornuti **[2]**; vesica with a single, tiny nub-like distal cornutus **[3]**; distal group of spine-like cornuti on vesica short, together forming a ratchet-like structure **[4]**;

vesica bearing spine-like cornuti on dorsal appendix, and minute denticles on ventral appendix **[5]**.

74. Main duct of vesica simple at base **[0]**; main duct of vesica minutely scobinate at base **[1]**; main duct of vesica with a patch of spines near base **[2]**; main duct of vesica with a thorn-like process at base **[3]**.

75. Vesica lacking scobinate sclerites at base **[0]**; vesica with a pair of narrow, curved scobinate sclerites at base **[1]**; scobinate sclerites large **[2]**; vesica with a single, large scobinate sclerite at base **[3]**.

Female-Only Characters

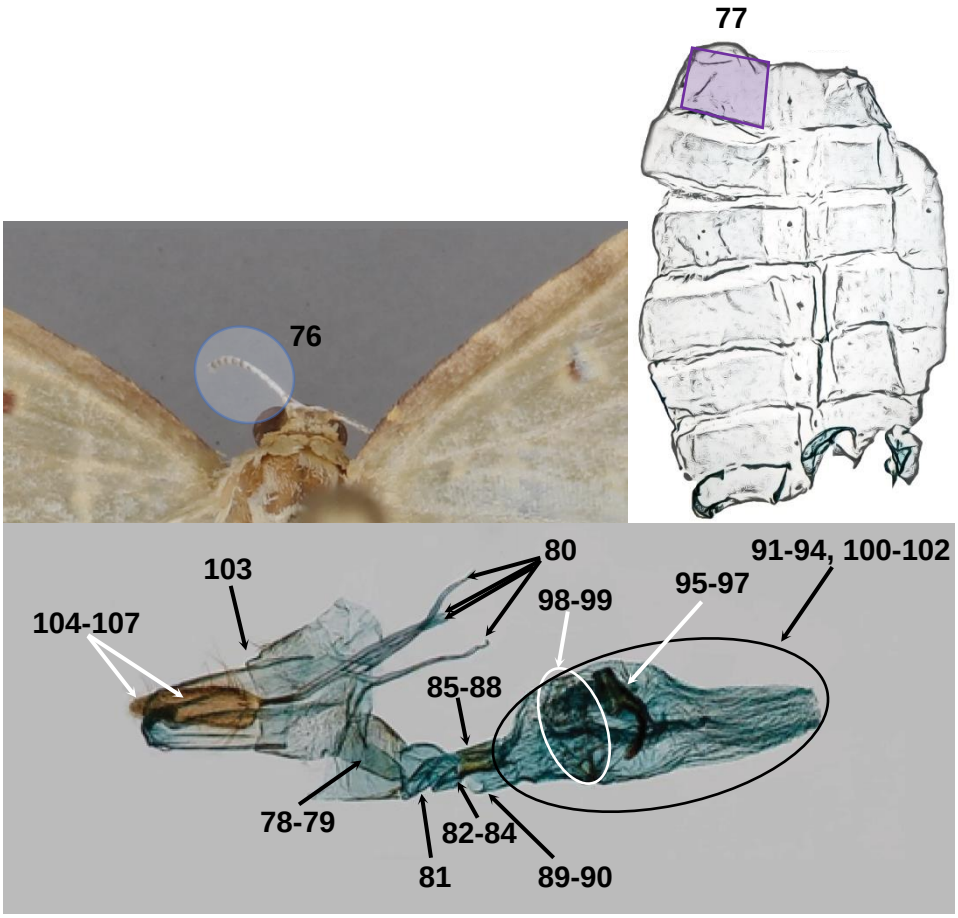

Figure A3. Images of antennae, female genitalia, and illustration of membranous abdomen of *Eois pseudolivacea* adult. Numbers assigned to morphological structures in each image correspond to the female-only genital characters (77-107).

76. Antenna ciliate **[0]**; antenna bipectinate **[1]**; antennal cilia long and bristle-like **[2]**.

77. Tergum 8 triangular, posterior margin gradually narrowed **[0]**; tergum 8 broadly triangular **[1]**; tergum 8 quadrate, posterior margin transverse **[2]**; tergum 8 a narrow, U-shaped band **[3]**.

78. Ventral surface of A8 in area of postvaginal plate membranous or lightly sclerotized, PVP apparently absent **[0]**; postvaginal plate present, simple **[1]**; postvaginal plate large, somewhat quadrate **[2]**; postvaginal plate short, strap-like **[3]**; postvaginal plate convex **[4]**; postvaginal plate a triangle, tapering posteriorly **[5]**.

79. Region of postvaginal plate smooth **[0]**; region of PVP bearing transverse striations **[1]**.

80. Anterior apophyses shorter than posterior apophyses **[0]**; anterior apophyses elongate, equal in length to posterior apophyses **[1]**.

81. Ostium large and funnel-shaped **[0**]; ostium forming a large, dorso-ventrally compressed, vase-like structure **[1]**; ostium comprising a narrow, transverse band **[2]**; ostium forming a large, concave structure, its dorsal wall striate **[3]**; region of ostium membranous **[4]**.

82. Region between ostium and ductus bursae moderate in length **[0]**; region between ostium and DB long **[1]**; region between ostium and ductus bursae short **[2]**; no membranous region between ostium and ductus bursae **[3]**.

83. Region between ostium and ductus bursae simple **[0]**; region between ostium and ductus bursae broadly membranous **[1]**; region between ostium and ductus bursae spiculate **[2]**; region between ostium and ductus bursae sclerotized **[3]**. region between ostium and ductus bursae absent **[?]**.

84. Region between ostium and ductus bursae simple **[0]**; region between ostium and ductus bursae bearing a ventral appendix **[1]**; region between ostium and ductus bursae bearing a small knob-like ventral process **[2]**; region between ostium and ductus bursae absent **[?]**.

85. Ductus bursae moderate in length **[0]**; ductus bursae short **[1]**; ductus bursae elongate **[2]**; ductus bursae apparently absent **[3]**.

86. Ductus bursae relatively narrow **[0]**; ductus bursae wide **[1]**; ductus bursae extremely narrow **[2]**; ductus bursae absent **[?]**.

87. Ductus bursae heavily sclerotized **[0]**; ductus bursae lightly sclerotized **[1]**; ductus bursae membranous **[2]**; ductus bursae absent **[?]**.

88. Lateral margins of ductus bursae simple **[0]**; lateral margins of ductus bursae rolled upward, ductus U-shaped in cross section **[1]**; lateral margins of ductus bursae rolled strongly inward, meeting near mid-line **[2]**; ductus bursae absent **[?]**.

89. Ductus seminalis arising from a small narrow appendix at base of CB **[0]**; DS arising from an elongate, triangular appendix at base of CB **[1]**; DS arising from a large, sac-like appendix at base of CB **[2]**.

90. Ductus seminalis arising ventrally and curling to moth's right **[0]**; ductus seminalis arising laterally on right side **[1]**; ductus seminalis arising dorsally and curling to the left **[2]**; DS arising laterally on left side and curling right **[3].**

91. Base of corpus bursae spineless **[0]**; base of corpus bursae bearing a group of internal spines immediately beyond ductus seminalis **[1]**; base of corpus bursae bearing an irregular, sclerotized plate **[2]**.

92. Area of corpus bursae basal to signum mostly membranous **[0]**; area of CB basal to signum bearing longitudinal striae **[1]**; area of CB basal to signum sclerotized **[2]**.

93. Base of corpus bursae rounded, contiguous with remainder of corpus **[0]**; base of corpus bursae forming a separate neck-like constriction **[1]**.

94. Surface of corpus bursae lacking a covering of internal spicules (most of ingroup *Eois*) **[0]**; entire surface of corpus bursae with a dense covering of internal spicules (most of outgroup) **[1]**; basal 2/3 of corpus bursae covered with internal spicules **[2]**.

95. Signum comprising an ovoid patch of short spines **[0]**; signum horn-like, its base partially protruding from CB **[1]**; horn seemingly reduced, barely projecting above surface of bursa **[2]**; signum absent **[3]**.

96. Signum located ventrally **[0]**; signum located laterally on right side of CB **[1]**; signum located laterally on left side of CB **[2]**; signum located dorsally **[3]**; signum absent **[?]**.

97. Internal part of horn-like signum narrow, curved, dentate along lateral margin **[0]**; internal part of horn-like signum wing-shaped, lateral margins serrate **[1]**; internal part of horn-like signum spatulate, lateral margins smooth **[2]**; internal part of horn-like signum forming a huge, claw-like structure **[3]**; internal part of horn-like signum comprised of long, curved spines **[4]**; internal part of horn-like signum smooth, horn-like **[5]**; horn-like signum absent **[?]**.

98. Corpus bursae lacking a sclerotized crescent surrounding signum (both outgroup, most ingroup *Eois*) **[0]**; a sclerotized crescent, variable in size, present in CB membrane surrounding signum **[1]**.

99. Corpus bursae without a sclerite arising from signum **[0]**; a spinose sclerite arising from signum, sclerite narrow, strap-like, wrapping around CB **[1]**; spinose sclerite becoming broad and plate-like **[2]**; signum sclerite a large ovoid plate, its outer

margin bordered with long, curved spines **[3]**; signum sclerite reduced, thin, spines few or absent **[4]**; signum sclerite short, covered with a mass of fine internal bristles **[5]**.

100. Central area of corpus bursae simple, without modifications beyond signum **[0]**; central area of CB with a large melanized area, covered in longitudinal striae **[1]**; area of CB beyond signum sclerite with an inset, well-defined rugose area **[2]**; central area of CB with a rugose, transverse fold **[3]**; corpus bursae with a smooth, inset sclerite in addition to signum sclerite **[4]**; central area of CB with a single, dentate pocket **[5]**; central area of CB bearing a pair of deep, spinose pockets **[6]**; central area of corpus bursae with a transverse sclerotized band **[7]**; central area of CB broadly sclerotized **[8]**.

101. Corpus bursae single-parted, distal appendix absent **[0]**; CB composed of two parts, distal portion broadly attached to remainder of corpus **[1]**; CB composed of two parts, distal appendix with a relatively narrow, cylindrical attachment to remainder of corpus **[2]**; secondary appendix with a narrow, neck-like attachment to CB **[3]**.

102. Distal appendix of corpus bursae smooth **[0]**; distal appendix of corpus bursae minutely wrinkled **[1]**; membrane of distal appendix delicate, fragile **[2]**; distal appendix absent **[?]**.

103. Dorsal membrane between Tg8 and papillae anales simple, with a small membranous invagination **[0]**; membrane between Tg8 and papillae anales bearing a large dorsal sac **[1]**.

104. Papillae anales roughly triangular in shape, distal portion rounded **[0]**; papillae anales elongate, distal portion rounded or acute **[1]**; papillae anales short, trapezoidal **[2]**; papillae anales sickle-shaped, acute at apex **[3]**; papillae anales extremely narrow **[4]**.

105. Papillae anales lacking an apical hook **[0]**; papillae anales with a tiny apical hook **[1]**.

106. Papillae anales evenly setose, bristles absent **[0]**; base of papillae anales with a series of longitudinal striae, a few scattered bristles present **[1]**; base of papillae anales heavily striate, with a dorsal corona of long, down-curved bristles **[2]**.

107. Surface of papillae anales simple **[0]**; surface of papillae anales covered with a series of longitudinal striae **[1]**.