universität wien

MASTERARBEIT

Titel der Masterarbeit

Species diversity and phylogenetic position of *Eois* (Lepidoptera: Geometridae: Larentiinae) on *Peperomia* (Piperaceae) in a mountain rainforest in the south Ecuadorian Andes

verfasst von

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angestrebter akademischer Grad

Master of Science (MSc)

Wien, 2014

Studienkennzahl lt. Studienblatt:	A 066 879
Studienrichtung lt. Studienblatt:	Naturschutz und Biodiversitätsmanagement
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ABSTRACT

In the past, *Eois* larvae (Lepidoptera: Geometridae: Larentiinae) were commonly assumed to be predominantly monophagous on various *Piper* species. Only few feeding records indicated that some species may also exploit other host plants such as *Peperomia* (Piperaceae).

In this study I examined how many species of *Eois* in one focal area are affiliated with *Peperomia* and where these are located within the phylogeny of the genus. Furthermore, it was investigated, based on a tangled tree, if specific phylogenetic patterns exist between *Eois* moths and *Peperomia* plants. Additionally, for all observed *Eois* taxa larval stages are described and illustrated for the first time and information about their natural history is given.

The study took place in the south Ecuadorian Andes, spanning three elevational bands between 1000 and 3100m a.s.l. Specimens of *Eois* were collected between 2012 and 2013 by visually scanning plants of the species-rich genus *Peperomia*. *Eois* were identified to species level using DNA-barcoding. Trophic relationships to *Peperomia* spp. were established by the observation of feeding behaviour on their original host plant. Phylogeny hypotheses of *Eois* and *Peperomia* were generated by means of DNA sequence data and compared to each other to examine possible co-evolutionary relationships.

At least eight (out of 20-25) morphospecies of *Peperomia* plants were confirmed as host plants used by 10 genetically clearly distinguishable *Eois* species. Two of these moth species, *Eois albosignata* (Dognin, 1911) and *Eois bolana* (Dognin, 1899), are validly described while seven further taxa could merely be matched to undescribed morphospecies known from previous light trapping campaigns in the study area. One new species was encountered for the first time. The scattered placement of observed *Eois* species within the phylogeny of the moth genus clearly suggests that at least five convergent host switches to *Peperomia* have occurred. Multiple independent host shifts, probably always away from *Piper*, to another single plant genus are currently uniquely documented within *Eois*. Associations of the phylogenies show no evidence for co-speciation processes between *Eois* and *Peperomia*.

This work reveals that distinctly more *Eois* moth species feed on *Peperomia* plants than previously thought, and that *Eois* is less strictly associated with *Piper* than former assumptions had suggested.

Key words: *Eois*, host plant, Lepidoptera, life history, *Peperomia*, phylogenetic patterns, Piperaceae.

ZUSAMMENFASSUNG

Früher wurde angenommen, dass die Raupen der Nachtfaltergattung *Eois* (Lepidoptera: Geometridae: Larentiinae) überwiegend monophag an Pflanzen der Gattung *Piper* (Piperaceae) fressen. Einzelnachweise zeigten jedoch auf, dass auch andere Pflanzentaxa wie *Peperomia* (Piperaceae) genutzt werden.

In der vorliegenden Studie wurde untersucht, wie viele *Eois*-Arten in einem eng begrenzten Untersuchungsgebiet an der artenreichen Gattung *Peperomia* fressen und welche Stellung sie innerhalb der Phylogenie dieser Nachtfaltergattung einnehmen. Ob zwischen *Eois* und *Peperomia* enge ko-evolutionäre Beziehungen erkennbar sind, wurde anhand einer Gegenüberstellung der Phylogeniehypothesen geprüft. Abschließend wurden für alle nachgewiesenen *Eois*-Taxa Informationen zur Bionomie gegeben und ihre Raupenstadien erstmalig beschrieben und abgebildet.

Die Datenaufnahme erfolgte von 2012 bis 2013 im Andengebiet Süd-Ecuadors zwischen 1000 und 3100m ü.NN. *Peperomia*-Pflanzen wurden systematisch nach *Eois*-Raupen abgesucht und die so gesammelten Individuen mit Hilfe von DNA-Barcoding bestimmt bzw. aufgetrennt. Zur Erfassung der Wirtspflanzenbeziehungen wurden nur die im Freiland nachweislich genutzten *Peperomia*-Arten herangezogen.

Mindestens acht *Peperomia*-Morphospezies (von 20-25 untersuchten) wurden von 10 genetisch klar differenzierbaren *Eois*-Arten als Nahrungspflanze genutzt. Lediglich zwei dieser Nachtfalterarten, *Eois albosignata* (Dognin, 1911) und *Eois bolana* (Dognin, 1899), sind bislang taxonomisch beschrieben. Sieben weitere Arten konnten noch unbeschriebenen Morphospezies zugewiesen werden, welche bereits von früheren Lichtfängen im Untersuchungsgebiet bekannt waren, während eine weitere Art überhaupt erstmalig nachgewiesen wurde. Die Einordnung der gesammelten *Eois*-Arten in der Phylogenie lässt eindeutig erkennen, dass ein Wirtspflanzenwechsel hin zu *Peperomia*, vermutlich immer von *Piper* ausgehend, mindestens fünf mal konvergent geschah. Dieser mehrfach unabhängige Nahrungspflanzenwechsel hin zu einer anderen Pflanzengattung ist damit erstmalig für *Eois* nachgewiesen. Eine Gegenüberstellung der Phylogenien von Wirtspflanzen und Nachtfaltern wies keine Muster auf, die auf eine Kospeziation zwischen *Eois* und *Peperomia* hindeuten.

Diese Studie zeigt auf, dass deutlich mehr *Eois*-Arten an *Peperomia* leben als erwartet und dass demnach die trophische Bindung dieser Nachtfalter an *Piper* weniger stark ausgeprägt ist, als es bislang angenommen wurde.

INTRODUCTION

In recent years, interactions between tropical plants and herbivorous insects have been the subject of an increasing number of studies (e.g. Farrell et al. 1992, Coley & Barone 1996, Novotny & Basset 2005, Novotny et al. 2005, Lewinsohn et al. 2005, Lewinsohn et al. 2006, Dyer et al. 2007, Lewinsohn & Roslin 2008, Novotny 2009, Novotny et al. 2010). Among others, over time they led to a better understanding of evolutionary processes underlying these reciprocal interactions and ecological relations. For instance, the extent and dynamics of herbivore host plant specificity, its role during diversification processes (e.g. Janz et al. 2006, Hunt et al. 2007), or repercussions on distribution patterns (Slove & Janz 2013) are some examples of potential issues. In particular, the debate about the degree of host specificity in tropical as compared to temperate biota remains unsettled. Whereas Dyer et al. (2007) and Weiblen et al. (2006) observed a larger fraction of host specialist among tropical herbivorous insects, other studies did not arrive at the same conclusion (e.g. Novotny et al. 2002, Novotny et al. 2006). However, the extent of host specialism as well as the number of herbivore species affiliated per plant species remains central in considerations of global terrestrial species richness (Hamilton et al. 2010).

Despite many ongoing attempts to uncover functional relationships between insects and plants in the tropics (e.g. Dick et al. 2003, Oliveira & Freitas 2004, Weiblen et al. 2006, Novotny et al. 2010), one major obstacle is the paucity of data. There are basically two complementary approaches to shed light on plant-herbivore interactions across larger scales than individual focal species. On the one hand, one might opt for local community-wide surveys across a wide range of plants and insects that occur within selected ecosystems (e.g. Novotny et al. 2004, Novotny & Basset 2005, Dyer et al. 2007, Novotny et al. 2010). Alternatively, one may choose one or a few suitable herbivorous model organism groups and track their host-plant relationships across sites and regions (Braby & Trueman 2006, Mullen et al. 2011). To be considered suitable in this respect, candidate taxa have to be distinguishable at least to morphospecies level. Furthermore, basic information about their natural life history is desirable. Such data are available for most butterfly and some moth families like Sphigidae and Saturniidae. Depending on the specific research aim, model organism groups should be species rich, have a wide geographic distribution, and show specialization with regard to their respective host-plant.

Since the beginning of this century, the pantropically distributed, species-rich moth genus *Eois* Hübner (Lepidoptera: Geometridae: Larentiinae) has become such a focal organism group. The majority of species in this genus occurs in the Neotropical region, of which 211 species are validly described (Scoble 1999, Brehm et al. 2011). However, estimations by Rodríguez-Castañeda et al. (2010) and Brehm et al. (2011) suggest that the total number of species to be expected in this region alone is much higher. A DNA sequence-based study by Strutzenberger et al. (2011) recognized 166 genetically distinguishable species in just one small area in southern Ecuador, only 19 (≈ 11.4%) of which are currently described (Brehm et al. 2011). These figures and the ongoing recognition of new genetic entities (F. Bodner & G. Brehm, pers. communication) support this assumption.

Apart from this high local and regional species richness, *Eois* also make up a considerable fraction of individuals in Andean moth communities. For instance, preimaginal stages of *Eois* represent about 50% of the Lepidoptera community on selected shrubs in the study area (Bodner 2011, Bodner et al. 2012). Together with *Eupithecia* and *Psaliodes* they make up more than 60% of specimens and more than 70% of moth species sampled by light trapping (Brehm & Fiedler 2003) and therefore play an important role as herbivores as well as potential prey for higher trophic orders within the ecosystem.

Furthermore, *Eois* is known to show a high host plant specialization. On average, every *Eois* taxon uses 1.2 to 2.1 plant species as hosts (Connahs et al. 2009, Bodner et al. 2012). Based on a number of studies (e.g. Brehm 2002, Brehm & Fiedler 2005, Connahs et al. 2009), it was assumed that Neotropical representatives of this genus are almost exclusively associated with plants from the genus *Piper* (Piperaceae). This perception has changed in recent years, after some host plant records from other plant genera like *Peperomia* and *Hedyosmum* (Dyer et al. 2014, Janzen & Hallwachs 2014, Strutzenberger et al. 2010) had been obtained. However, *Piper* remains the quantitatively most important host genus for *Eois* caterpillars.

Morphological diversity, species-richness, host plant specificity and the suitability for barcode-based species demarcation (Strutzenberger et al. 2010) resulted in the usage of *Eois* as model organism group for evolutionary and ecological research in the Neotropics (e.g. Connahs et al. 2009, Rodríguez-Castañeda et al. 2010, Strutzenberger et al. 2010, Strutzenberger & Fiedler 2011, Wilson et al. 2012). Nevertheless, despite numerous studies about *Eois* in recent years, much basic information, essential for all further investigations, is lacking or incomplete. For instance, host plant data exist merely for about 38% of 230 observed *Eois* taxa in the study area (pers. communication G. Brehm & F. Bodner). Hence, concrete statements concerning e.g. the level of host plant specialization, the significance of single genera as host plants and specific distribution patterns of *Eois* are thus far possible to but a very limited extent. In order to broaden the current state of knowledge, the present study aims to investigate affiliations between *Eois* and *Peperomia* (Piperaceaea), the sister genus of *Piper* (Wanke et al. 2007). A continuous accumulation of such data is indispensable for more rigorous tests of hypotheses on the ecology and evolution of this highly speciose moth clade.

Peperomia, like *Piper*, is a predominantly tropical species-rich plant group of basal angiosperms including 1,500-1,700 described species (Wanke et al. 2006, Samain et al. 2007). Similar to *Eois,* the majority of species occurs in the Neotropics (Wanke et al. 2006). Many different types of growth and life forms are known, such as herbaceous geophytes, epiphytes and succulents which may be a result of long diversification time (Samain et al. 2009, Wanke et al. 2007). Because single larvae of *Eois* had previously been found on *Peperomia* (Dyer et al. 2014, Janzen & Hallwachs 2014; F. Bodner, pers. communication), this genus was chosen for a more in-depth investigation of its role as a potential host-plant. Given their species-richness in the Neotropics and especially their close phylogenetic relation to *Piper* (Wanke et al. 2007), it seemed likely that more intensive targeted sampling would result in uncovering host-plant relationships of additional *Eois* species.

The specific aims of this study are (i) to investigate which and how many species of *Eois* are associated with *Peperomia*, (ii) to assess their phylogenetic position within the moth genus, (iii) to examine whether specific phylogenetic patterns exist in the association of *Eois* with *Peperomia* , (iv) to assess whether most *Peperomia* clades occuring in the Andes are utilized equally as host by *Eois* caterpillars, (v) whether targeted surveys could reveal the existence of previously overlooked species, and (vi) to give short descriptive accounts of the larval stages of all observed *Eois* species as well as information about their natural history.

MATERIAL AND METHODS

Study sites

From June 2012 to January 2013, specimens of *Eois* and *Peperomia* were collected at three elevational bands of tropical mountain rainforest in southern Ecuador, situated on the eastern slopes of the Andes. Most samplings were carried out in near-natural montane rainforest in the "Reserva Biologíca San Francisco" (RBSF) between 1800 and 2300m a.s.l., near the research station Estación Científica San Francisco (3°58'18"S, 79°4'45"). Furthermore, caterpillars were sampled in cloud forest at Cajanuma (2700 to 3100m a.s.l.; S4°06'58", W79°10'19") and in lower montane forest at Bombuscaro (about 1000m a.s.l.; 4°06'53"S, 78°57'57"W), two areas within the Podocarpus National Park in proximity to RBSF. For further information about the study area, the major vegetation types and their ecological characteristics see Bendix et al. (2006), Beck et al. (2008) and Gradstein et al. (2008).

Taxon sampling and identification

Eois

To achieve the goal of recording as many *Eois* species as possible, my sampling strategy aimed at scanning a high number of different *Peperomia* species and individuals. Because of their diverse growth forms, a qualitative sampling approach was chosen. To this end, I routinely checked various micro-habitats ranging from high humidity ("quebradas", riversides) to dryer locations (ridges) in the three altitudinal belts mentioned above. Caterpillars were systematically searched by visually scanning potential host plants during the day. Especially the search for feeding traces on leaves turned out to be effectivee. Collected caterpillars were reared individually in small plastic boxes lined with humid paper and provisioned with fresh host plant foliage as needed, until emergence of the moth (see Bodner et al. 2010).

All caterpillars which prematurely died as well as crippled pupae were preserved in ethanol (96%) for later DNA barcode analyses. Hatched moths were killed with cyanide, frozen at -20 °C and later spread.

Each caterpillar received a unique sample-ID. Further recorded information were collection date, precise locality, provisional allocation to a host plant morphospecies, dates of moults if caterpillars were not in their final instar, dates of pupation and of moth emergence. Additionally, individuals were photographically documented using a Nikon D70s camera equipped with a Sigma 105mm macro lens and a Sigma EM-140 DG Macro flash. All caterpillar individuals were documented on the day of collection and after every moult.

Identification to species or, because most *Eois* are yet undescribed (see Brehm et al. 2011), to morphospecies level was mainly achieved by DNA barcoding of ethanol samples of early stages and in some difficult cases by using single legs of spread moths. Furthermore, species delimitation was assisted by comparison of wing patterns and larval morphology. For amplifying the 658 bp fragment of the mitochondrial cytochrome oxidase subunit I gene (the so-called barcode region: Hebert et al. 2003, Hebert & Gregory 2005, Hajibabaei et al. 2007) the established primer pair LepF (5'-ATTCAACCAATCATAAAGATATTGG-3')/ LepR (5'-TAAACTTCTGGATGTCCAAAAAATCA-3') (e.g. Hajibabaei et al. 2006, Prado et al. 2011) was used. For species delimitation within *Eois* application of a 2% sequence divergence threshold was chosen (Strutzenberger et al. 2011). Primarily in cases of multiple undescribed species in combination with a low coverage and thus limited knowledge of intraspecific morphological variability, this approach has proven effective in handling the problem of

species demarcation, with good performance especially in the family Geometridae (Hausmann et al. 2011, deWaard et al. 2011).

Every genetically distinguishable taxon obtained an identification number from a database maintained by G. Brehm (pers. comm.), a Barcode Index Number (BIN) from "Bold Systems" (www.boldsystems.org) and at least an informal morphospecies name for an unequivocal allocation (see Table 2). Hereafter, all informal morphospecies names are marked with the abbreviation spnr for "species near" between genus and species name to indicate the morphological similarity to a described *Eois* species, yet the informally named morphospecies was not identical to the taxonomically described species.

Peperomia

All morphospecies of *Peperomia* observed during the investigation period were photographed and leaf samples were preserved in silica gel to enable later DNA sequencing. All leaf samples received a unique sample ID to ensure an unequivocal assignment (see Table 1). Herbarium vouchers were collected of each host plant morphospecies with evidence of feeding *Eois* caterpillars. Plant determination based on photographs and herbarium vouchers was done by specialists (M.-S. Samain, Ghent University and S. Wanke, TU Dresden) and allowed either the allocation of a given sample to a described species or at least affiliation of that sample with a species group within the large genus *Peperomia*.

Herbarium vouchers were deposited at Ghent University, Belgium, whereas the leaf samples for sequencing were deposited at TU Dresden, Germany.

Description of larval morphologies

Coloration and size of the caterpillars were roughly described by reference to the photographs and measurements made during the rearing period. Due to the high degree of similarity among young caterpillars and because some species were found only once as fully grown individuals, only the last instar of each species is described below. In addition, two pictures showing the mature larva in dorsal and lateral view are given for all species.

Phylogenetic analyses - taxa selection, sequence data preparation and processing

Eois

In order to establish the phylogenetic position of Eois species whose larvae feed on *Peperomia*, all 112 taxa of *Eois* and 30 outgroup species belonging to the subfamilies Archiearinae, Larentiinae and Sterrhinae from the study of Strutzenberger et al. (2010) were used for a phylogenetic analysis. Additionally, five *Eois* species newly recorded during this study were included. Further information about the collection sites, Genbank accession numbers for COI and Ef1α genes and references of acquired data are given in Table 2. Because there is no remarkable effect on the relationships and support values (Strutzenberger et al. 2010), taxa with chimeric or incomplete sequences were not excluded. Primers used to sequence the complete COI gene and the nuclear marker Ef1α followed Strutzenberger et al. (2010).

Contig assembly was done with DNAStar Lasergene SeqMan Pro Ver. 7.1.0. Sequences were aligned manually by using PhyDE (Müller et al. 2005). Aligned COI and Ef1α sequences had a length of 1536 bp and 1225 bp, respectively. The combined alignment of both genes with a total length of 2761 bp was used for all further analyses.

Sequences were partitioned into six parts and adequate substitution model were applied following Strutzenberger et al. (2010). To assure compatability with the existing phylogeny of *Eois* calculated by Strutzenberger et al. (2010), the same values and parameter settings for Bayesian analyses using MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) were assumed. The tree was drawn and compiled using FigTree v1.4.0 and finally edited with Adobe Illustrator CS4.

Peperomia

Sequencing was realized with representative specimens of all sampled morphospecies. Both, morphospecies with and without evidence of *Eois* feeding damage were considered.

DNA isolation, amplification and sequencing of the *trnK* intron, *matK* gene and *trnK-psbA* spacer regions, as well as the use of primers followed an unpublished protocol by Frenzke et al. (pers. comm. S. Wanke, TU Dresden). All *Peperomia* sequences from this study were included in the alignment of Frenzke et al. (unpubl.). For an overview about the new specimens of this study see Table 1. In summary, 162 taxa including four outgroup taxa were employed for the analyses. The manual alignment and the exclusion of regions of uncertain sequence homologies (hotspots) were done with PhyDe (Müller et al. 2005). The phylogeny was generated applying Bayesian analyses with MrBayes 3.2.2 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) using the same settings as in the study by Frenzke et al. (unpublished). The tree was drawn, compiled and edited with FigTree v1.4.0 and Adobe Illustrator CS4.

Table 1 List of *Peperomia* specimens from this study, sampled in the three elevational bands in southern Ecuador, used for phylogenetic analyses. **Lab. No.**= used laboratory number for sequencing; **Sample ID**= unique leaf sample identification number for every specimen; **Collection site**: BC= Bombuscaro, SA= station area around RBSF, CJ= Cajanuma. * Because of failed sequencing, conspecific sequences from Frenzke et al. (unpublished) were obtained.

			Collection site		
Taxon	**Lab. No.**	**Sample ID**	**BC**	**SA**	**CJ**
***Peperomia acuminata* Ruiz & Pav. aff.**	Pe939	PepB21B	x		
***Peperomia acuminata* Ruiz & Pav. aff.**	Pe948	PepC31C			x
***Peperomia acuminata* Ruiz & Pav. aff.**	Pe955	PepX07X		x	
***Peperomia curtipes* Trel. aff.**	Pe977	Pep036		x	
***Peperomia curtipes* Trel. aff.**	Pe983	P21	x		
***Peperomia eburnea* Sodiro aff.**	Pe944	PepB26B	x		
***Peperomia eburnea* Sodiro aff.**	Pe950	PepX02X		x	
Peperomia glabella* (Sw.) A.Dietr. aff.	-	-		x	
***Peperomia hartwegiana* Miq. aff.**	Pe964	PepX16X		x	
***Peperomia juniniana* Trel. aff.**	Pe940	PepB22B	x		
***Peperomia lancifolia* Hook.**	Pe952	PepX04X		x	
***Peperomia persulcata* Yunck. aff.**	Pe947	PepC30C			x
***Peperomia pilicaulis* C.DC. aff.**	Pe969	P020			x
***Peperomia prostrata* Williams**	Pe938	PepB20B	x		
***Peperomia quadrifolia* (L.) Kunth aff.**	Pe945	PepC28C			x
***Peperomia quadrifolia* (L.) Kunth aff.**	Pe961	PepX13X		x	
***Peperomia quadrifolia* (L.) Kunth aff.**	Pe970	P025			x
***Peperomia quaesita* Trel. aff.**	Pe959	PepX11X		x	
***Peperomia rotundifolia* (L.) Kunth aff.**	Pe937	PepB19B	x		
***Peperomia rotundifolia* (L.) Kunth aff.**	Pe943	PepB25B	x		
***Peperomia rotundifolia* (L.) Kunth aff.**	Pe958	PepX10X		x	
***Peperomia* sp.**	Pe936	PepB18B	x		
***Peperomia* sp.**	Pe946	PepC29C			x
***Peperomia* sp.**	Pe949	PepX01X		x	
***Peperomia* sp.**	Pe951	PepX03X		x	
***Peperomia* sp.**	Pe953	PepX05X		x	
***Peperomia* sp.**	Pe954	PepX06X		x	
***Peperomia* sp.**	Pe957	PepX09X		x	
***Peperomia* sp.**	Pe962	PepX14X		x	
***Peperomia* sp.**	Pe963	PepX15X		x	
***Peperomia* sp.**	Pe965	PepX27X		x	
***Peperomia* sp.**	Pe968	P016			x
***Peperomia* sp.**	Pe974	P030			x
***Peperomia* sp.**	Pe975	P032			x
***Peperomia* sp.**	Pe979	P041		x	
***Peperomia* sp.**	Pe981	P044			x
***Peperomia tetraphylla* Hook. & Arn.**	Pe978	P038		x	
***Peperomia tetraphylla* Hook. & Arn. aff.**	Pe956	PepX08X		x	
***Peperomia tetraphylla* Hook. & Arn. aff.**	Pe960	PepX12X		x	
***Peperomia trinervis* Ruiz & Pav. aff.**	Pe942	PepB24B	x		
***Peperomia viracochana* Trel. aff.**	Pe967	P015			x

RESULTS

Peperomia feeding *Eois* species, their phylogenetic positions and host plant interactions

Overall, 82 individuals of *Peperomia* feeding *Eois* caterpillars were found during targeted search on at least 8 different plant species. In addition, characteristic signs on at least 12-17 further *Peperomia* morphospecies were observed, but despite close inspection no caterpillars could be located.

Of the 82 caterpillars, 65 could be clearly identified and matched to ten genetically distinct *Eois* species (see Table 2). For 17 individuals an unambiguous identification was not possible, but eight of them could be assigned to the cryptic *E.* spnr *violada* complex consisting of three *Peperomia*-feeding species in the study area. Identification was impossible either if the barcoding process failed, or if the caterpillars had been parasitized. Especially if infested by tachinid flies, not enough tissue remained for DNA extraction. However, none of the undetermined specimens showed an obviously different larval morphology to other recorded specimens, which would indicate the presence of additional species not represented in Table 2.

Two species, *Eois albosignata* and *Eois bolana*, belong to taxonomically described species (type localities: *E. albosignata* - Colombia, Yuntas, near Cali; *E. bolana* - Ecuador, Loja; see Scoble 1999). All other taxa were assigned preliminary morphospecies codes, also based on differences at the COI barcode sequence fraction (Table 3). Nine of the *Eois* species feeding on *Peperomia* had earlier been observed in the study area in light-trap samples (Brehm 2002, Hilt 2005, G. Brehm: pers. communication). One probably undescribed species, viz. *Eois* spnr *vinosata*_02, had not been recorded previously and is therefore new for the study area. For all but *Eois* spnr *vinosata*_02 the adult moths are known; they are illustrated in dorsal and ventral view in Figure 2.

The three species of the *E.* spnr *violada* complex form a monophyletic group of cryptic sister taxa (Figure 3). They are indistinguishable by external morphology (wing patterns) and could only be separated by barcoding (but possibly also by the study of the genitalia). That also applied to the *E.* spnr *vinosata* complex, because rearing of *E.* spnr *vinosata*_02 was not successful so the imago of this species remains unknown.

Table 2 Alphabetical list of all observed *Eois* taxa feeding on *Peperomia* in the study area in southern Ecuador. Undescribed species are associated to a similar, but named species by using spnr for "species near"; * new species to be included in phylogenetic analyses, not covered by Strutzenberger et al. (2010). **Bold BINs** taken from www.boldsystems.org; species **IDs** from species database of G. Brehm (pers. communication). **Code** was assigned to each sequenced specimen. If available, Genbank accession numbers for **COI** and **Ef1α** are cited. **Ref**: S, data taken from Strutzenberger et al. (2010); TS, newly collected specimens and data of this study.

Taxon	BOLD BIN	ID	Code	COI	Ef1α	Ref
Eois albosignata Dognin, 1911 *	AAW4736	2292	cs15	unpublished	unpublished	TS
Eois bolana Dognin, 1899 *	AAW4735	2283	cs26	unpublished	unpublished	TS
Eois spnr *antiopata* Warren, 1904 *	AAY8000	2279	cs08	unpublished	unpublished	TS
Eois spnr *concatenata* Prout, 1910	ABU9945	2285	Eo00238	GQ433563	GQ433442	S
Eois spnr *hermosaria* Schaus, 1901	AAF0381	392	Eo00096	GQ433537	GQ433416	S
Eois spnr *vinosata*_01 Warren, 1907 *	ABW9383	709	cs16	unpublished	unpublished	TS
Eois spnr *vinosata*_02 Warren, 1907 *	ACH2043	2595	cs23	unpublished	unpublished	TS
Eois spnr *violada*_01 Dognin, 1899	AAI5253	2356	Eo00034	GQ433512	GQ433391	S
Eois spnr *violada*_02 Dognin, 1899	AAI5238	2360	Eo00381	GQ433586	GQ433465	S
Eois spnr *violada*_03 Dognin, 1899	AAW5637	403	Eo00097	GQ433533	GQ433412	S

Eois species recognized to feed on *Peperomia* plants are scattered over the phylogenetic tree of this moth genus and can be allocated to four different clades as defined by Strutzenberger et al. (2010) (see Figure 3). *E. albosignata* and *E. bolana* belong to the "*trillista* clade" and are the first indications of *Peperomia* feeding *Eois* within this group. *E.* spnr *concatenata* is a member of the "*sagittaria* clade" and provides the first recognized host plant record for this entire clade. For the "*chasca* clade" including *E.* spnr *antiopata* and *E.* spnr *hermosaria*, and the "*odatis* clade" which includes the complexes of *E.* spnr *vinosata* and *E.* spnr *violada,* previous food plant records were available (Strutzenberger et al. 2010). Nevertheless, only two species of the "*odatis* clade", *E.* spnr *violada*_01 (Eo00034) and *E.* spnr *violada*_02 (Eo00381), had an unambiguous allocation to *Peperomia* before. A further record in the study of Strutzenberger et al. (2010) combined sequences of *E.* spnr *hermosaria* (Eo00096) with food plant records of a similar, but clearly distinguishable morphospecies from northeastern Ecuador. Hence, also for the sequenced specimen (Eo00096) a host plant affiliation was for the first time gathered in this study. Thus, for eight *Eois* spp. food plant data presented here are the first records ever obtained.

To the current state of knowledge at least 230 clearly identifiable *Eois* species (morphologically and/ or genetically) have thus far been recognized from the small study area in southern Ecuador alone (pers. communication G. Brehm). Only for 88 of these species (38.26%) we currently know their host plants (see Figure 1). While most *Eois* feed on *Piper*, the proportion of species whose larvae live on *Peperomia* amounts merely to 4.35%. Together with *Manekia* the plant family Piperaceae harbors 73 species of *Eois* moths registered in the study region. Of the remaining species, 13 are documented as feeding on *Hedyosmum* (Chloranthaceae), and two are associated with *Siparuna* (Siparunaceae, formerly often included in Monimiaceae).

The distribution pattern of *Peperomia* feeding *Eois* within the phylogeny is given in Figure 3. Together with additional host plant information taken from the study of Strutzenberger et al. (2010) this pattern allows the conclusion that host switches to *Peperomia*, probably from *Piper*, occurred at least five times independently.

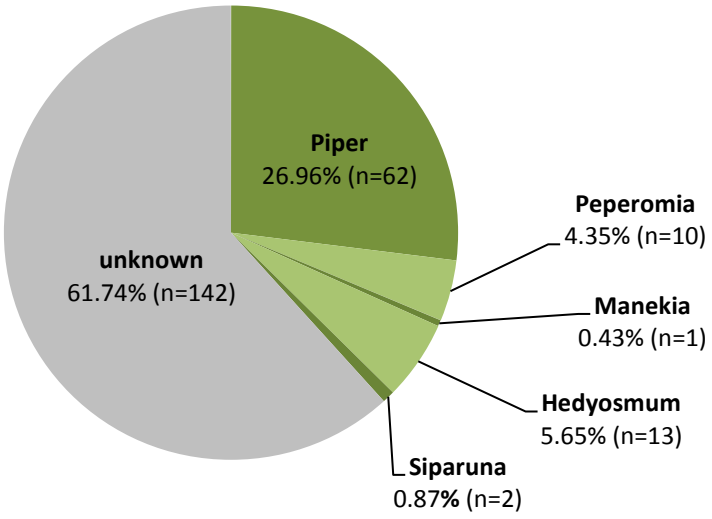


Figure 1 Percentage composition of host plant affiliations of all currently known *Eois* spp. (n=230) observed in the study area in southern Ecuador. Host plant records outside *Peperomia* from F. Bodner, D. Blies and G. Brehm (pers. communications).

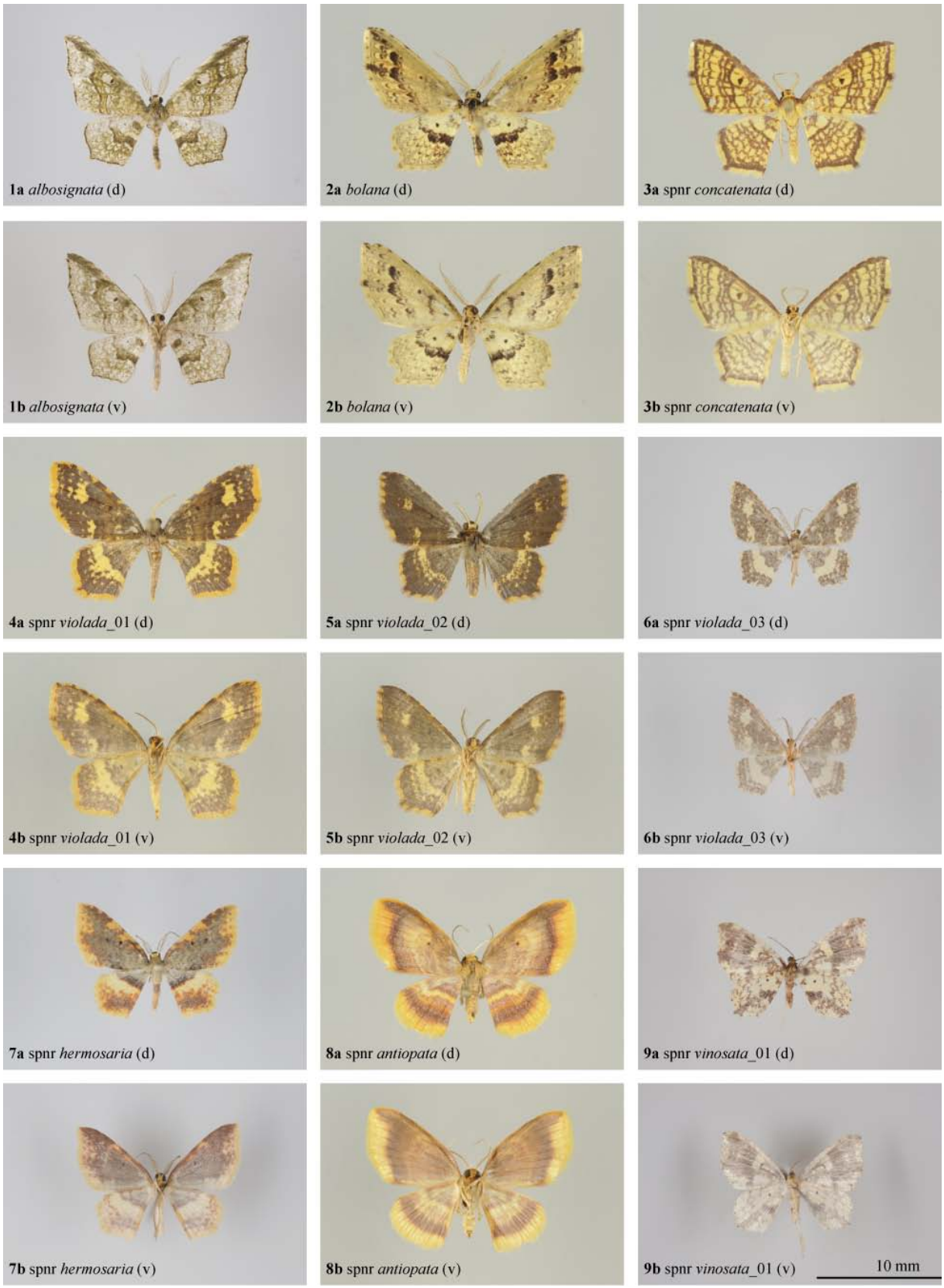
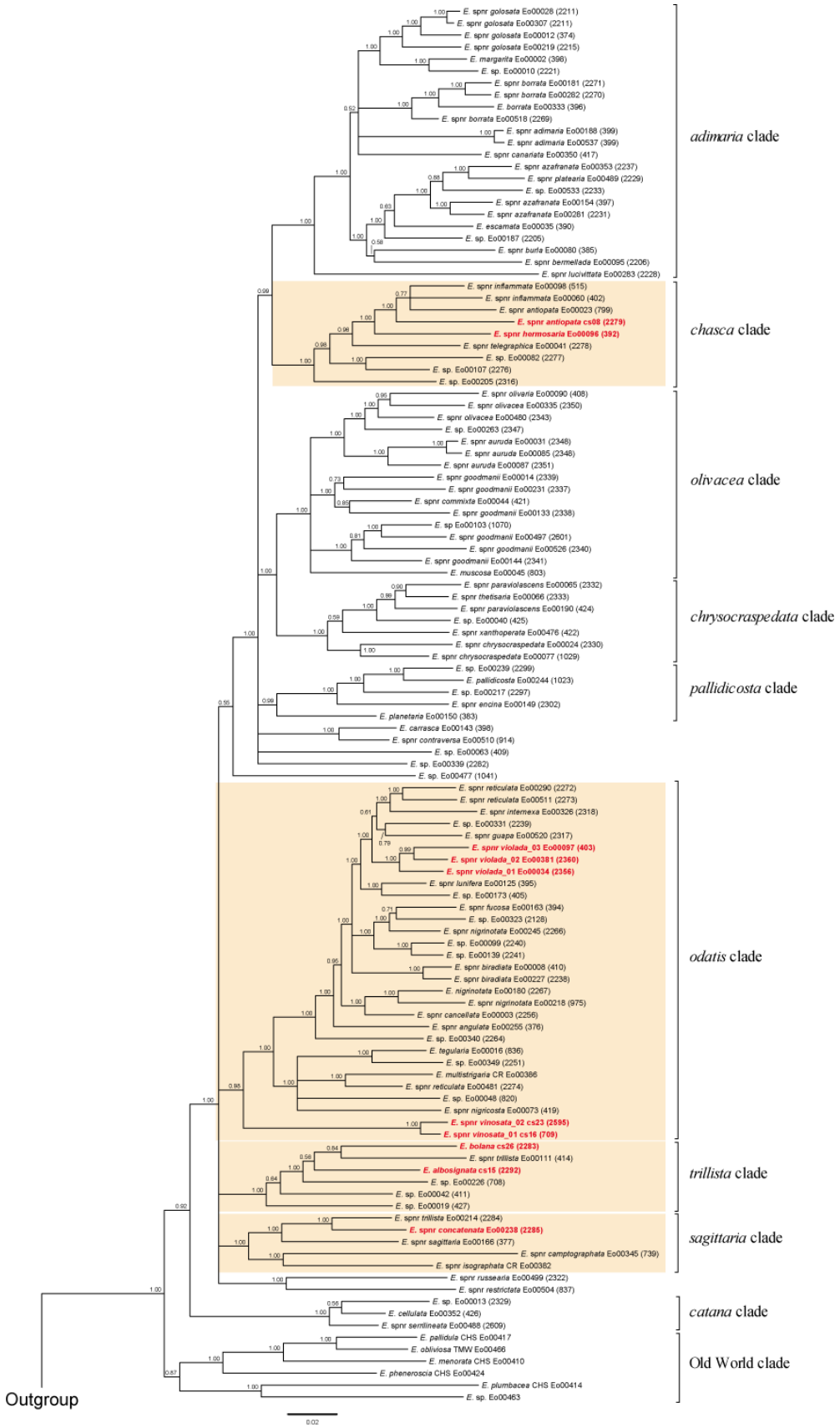


Figure 2 Dorsal (d) and ventral (v) view of adults of nine *Eois* moth species whose caterpillars feed on the plant genus *Peperomia*. **1-2** = "*trillista* clade", **3** = "*sagittaria* clade", **4-6**, **9** = "*odatis* clade" and **7-8** = "*chasca* clade". (Illustrations **1**a,b; **6**a,b; **7**a,b and **9**a,b by G. Brehm; all other photographs: C. L. Seifert). Scale bar valid for all pictures.



[Figure 3 (continued next page)**]**

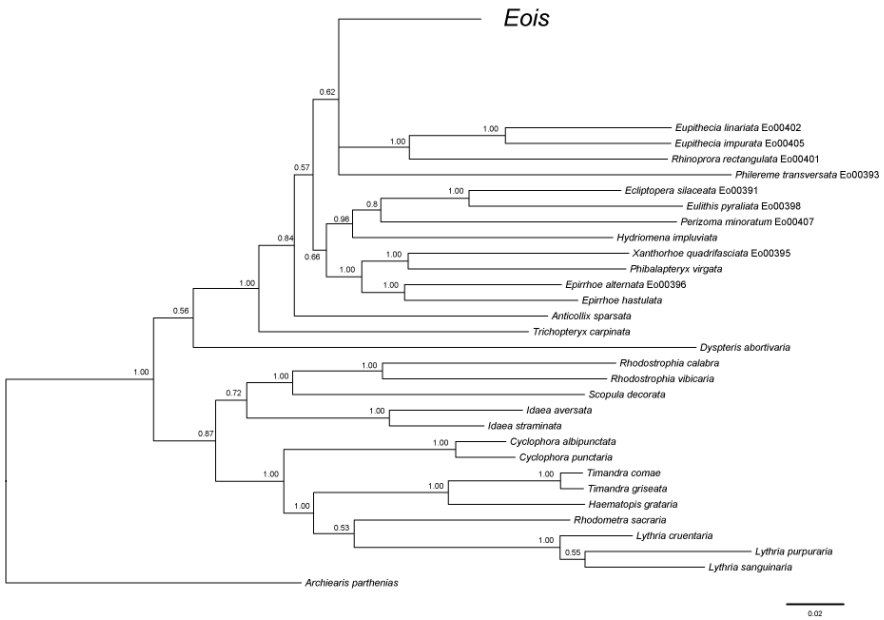


Figure 3 Bayesian 50% consensus tree of *Eois* and outgroup taxa with Bayesian posterior probabilities. Informal clade names are taken from Strutzenberger et al. (2010). Unique species ID numbers, given in parentheses, and also the taxon names of *Eois* species and morphospecies are from the database of G. Brehm (pers. communication). Names of *Peperomia* host plant taxa are printed in red, while the respective *Eois* clades are marked with a colored background. If possible, undescribed moth species are associated with a named species by morphological characteristics, using spnr for species near; otherwise they are only termed *E*. sp.

Table 3 Mean pairwise genetic distances of all observed *Eois* spp. under Kimura's 2-parameter substitution model (below the diagonal) and standard errors (above the diagonal) estimated by the bootstrap method. Only barcodes (658bp) without contaminations and with correlating contigs were considered (n=42).

	Taxon	n	1	2	3	4	5	6	7	8	9	10
1	*E. albosignata*	5	-	0.012	0.013	0.012	0.014	0.014	0.014	0.012	0.013	0.012
2	*E. bolana*	5	0.099	-	0.013	0.012	0.013	0.014	0.013	0.013	0.013	0.013
3	*E.* spnr *antiopata*	7	0.100	0.101	-	0.013	0.011	0.014	0.014	0.013	0.015	0.013
4	*E.* spnr *concatenata*	1	0.090	0.096	0.098	-	0.013	0.012	0.012	0.011	0.011	0.011
5	*E.* spnr *hermosaria*	1	0.113	0.099	0.074	0.106	-	0.014	0.014	0.012	0.013	0.012
6	*E.* spnr *vinosata*_01	6	0.108	0.110	0.113	0.084	0.109	-	0.006	0.012	0.013	0.012
7	*E.* spnr *vinosata*_02	1	0.111	0.106	0.115	0.092	0.109	0.023	-	0.012	0.013	0.012
8	*E.* spnr *violada*_01	10	0.084	0.092	0.097	0.071	0.080	0.084	0.089	-	0.007	0.007
9	*E.* spnr *violada*_02	2	0.103	0.097	0.117	0.076	0.096	0.087	0.092	0.037	-	0.008
10	*E.* spnr *violada*_03	4	0.089	0.097	0.102	0.078	0.089	0.089	0.093	0.041	0.040	-

The connections between single *Eois* species and their respective host plant taxa are shown in Figure 4. About 20–25 *Peperomia* morphospecies distributed over 6 plant clades were searched for *Eois* caterpillars. For at least eight plant species belonging to four clades evidence of *Eois* herbivory was obtained (see Figure 3). Utilized *Peperomia* clades are "Micropiper", "Exmicropiper", "Extildenia 2" and "Leptorhynchum". The first two harbor most *Eois* moth taxa with six and three species, respectively. Larvae of *E. bolana* and *E.* spnr *antiopata* were found feeding on plant species in both these *Peperomia* clades. For all other *Eois* species only one single *Peperomia* clade was recognized as host plant source.

The tangled phylogenetic trees reveal no obvious evidence for specific patterns, especially regarding co-speciation processes between *Eois* and their host plant genus *Peperomia*.

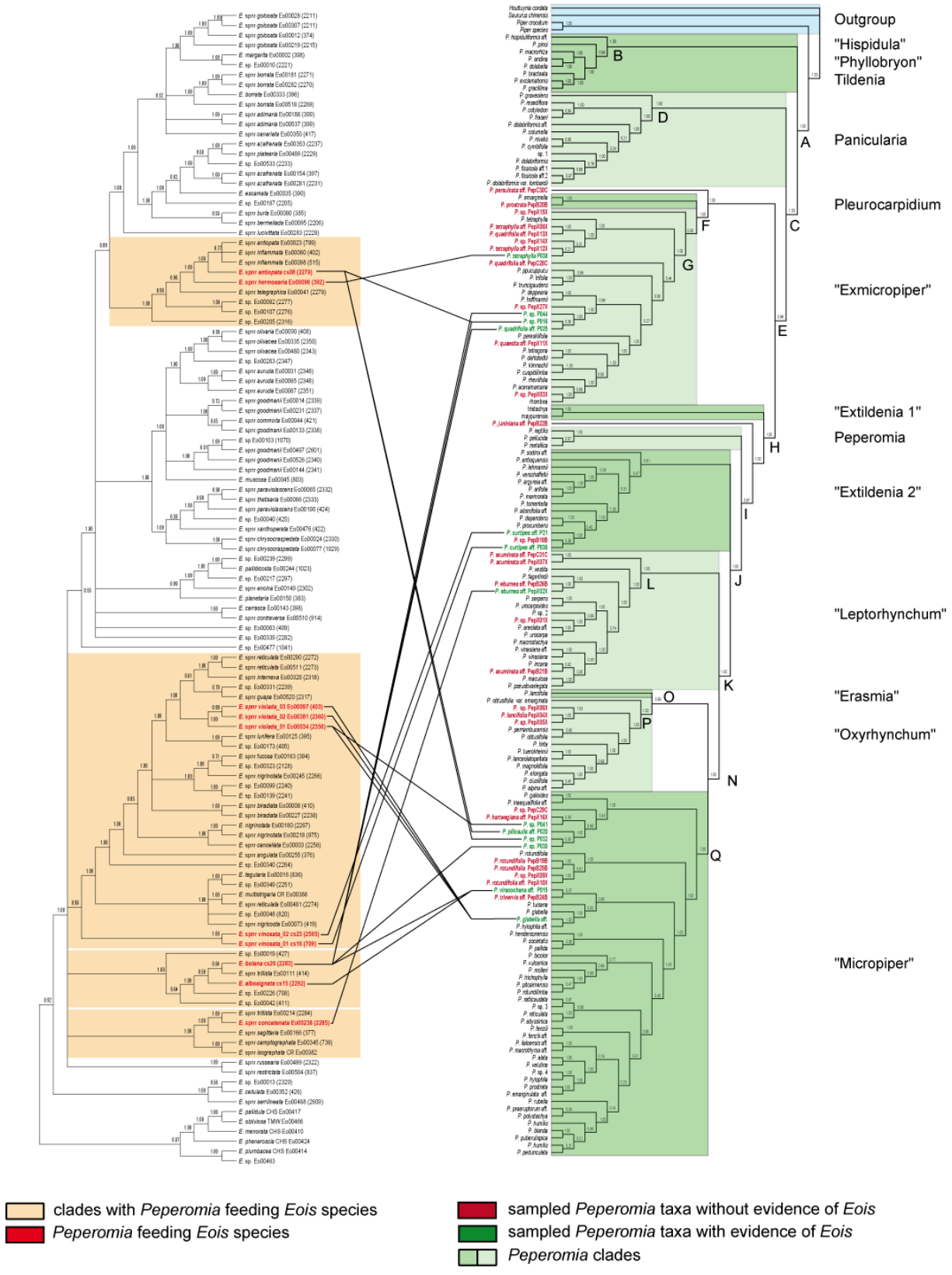


Figure 4 Comparison of Bayesian consensus trees of *Eois* moths and of the plant genus *Peperomia* to illustrate patterns of host use. Posterior probabilities are given for both phylogenies. Clade classification of *Eois* followed Strutzenberger et al. (2010), node and clade names of *Peperomia* are based on an unpublished study of Frenzke et al. (S. Wanke, pers. communication).

Natural history of *Peperomia* feeding *Eois* and descriptions of their caterpillars

For most recognized *Peperomia* feeding *Eois* species, a monophagous diet could be observed. Just for three species, *E. bolana*, *E.* spnr *antiopata* and E. spnr *violada*_01, more than one *Peperomia* species were unambiguously confirmed to serve as host plants (see Table 4). All ten *Eois* species live solitarily on their food plant as caterpillars. Young larvae are not distinguishable from one another and in most cases show a monochrome green coloration which is sometimes marked with black tubercles bearing setae. In the last instar a determination to species level or at least an affiliation to a species complex based on caterpillar morphology appears to be possible in many cases. In combination with other data such as the elevational distribution and host plant species (see Table 4) only the cryptic sister species complex of *E.* spnr *violada* could not be distinguished. The caterpillars of all species feed on leaves and in some cases also on sprouts and infructescences of their *Peperomia* host plants. Feeding damage on leaves can attain different forms. Three types were observed:

- Rasping from the underside of the leaves, sparing the upper epidermis, so the leaves seem transparent (**UF**= underside feeders).
- Chewing holes inside the leaf blades (**HF**= hole feeders).
- Feeding from the margin of the leaf blade (**MF**= margin feeders)

Parasitism by tachinid flies (14 cases), and more rarely by ichneumonid (4 cases) and braconid wasps (1 cases) was observed only in the *E.* spnr *violada* complex. Altogether, 54.3% of caterpillars from that species complex (and 23.2% of all *Eois* found on *Peperomia*) turned out to be infested by parasitoids.

Table 4 Observed *Eois* species with information about their **host plant/** host plant affiliation (only determined taxa are considered); **diet**: mono= monophagous, oligo= oligophagous; **location**: BC= Bombuscaro, SA= area around RBSF, CJ= Cajanuma; as well as altitude and numbers of sampled individuals. Additionally**, elevational** range, collection **localities** and **numbers** of moth specimens sampled in previous years by light trapping are given in parentheses, data from G. Brehm (pers. communication).

Taxon	Host plant	Diet	Loc.	elevation	No. Ind.
Eois albosignata	*P. viracochana* Trel. aff.	mono	CJ (SA, CJ)	2790-2835 (2670-2920)	8 (5)
Eois bolana	*P. quadrifolia* (L.) Kunth aff. *P. viracochana* Trel. aff.	oligo	CJ (SA, CJ)	2725-2835 (2110-2920)	8 (9)
Eois* spnr *antiopata	*P. pilicaulis* C.DC. aff.	oligo	CJ (CJ)	2770-2835 (2895-2920)	13 (2)
Eois* spnr *concatenata	*P. eburnea* Sodiro aff.	mono	SA (SA)	≈ 1950 (1800-1955)	1 (2)
Eois* spnr *hermosaria	*P. tetraphylla* Hook. & Arn.	mono	SA (SA)	1820 (1800-2180)	1 (63)
***Eois* spnr *vinosata*_01**	*P. curtipes* Trel. aff.	mono	SA (SA)	1990-2020 (1800)	6 (1)
***Eois* spnr *vinosata*_02**	*P. curtipes* Trel. aff.	mono	BC (-)	≈ 1000 (-)	1 (-)
***Eois* spnr *violada*_01**	*P. glabella* (Sw.) A.Dietr. aff.	oligo	SA (SA, CJ)	1810-2020 (1380-2920)	16 (42)
***Eois* spnr *violada*_02**	*P. glabella* (Sw.) A.Dietr. aff.	mono	SA (SA)	1820-1980 (1800-1850)	5 (5)
***Eois* spnr *violada*_03**	*P. glabella* (Sw.) A.Dietr. aff.	mono	SA (SA)	1815-1910 (1345-2000)	6 (53)

Eois albosignata Dognin, 1911

(Fig. 5a, b)

The stocky larva is colorful with black spots. Fully grown it reaches about 18 mm in length. Head capsule brownish grey with darker spots. Thorax segments laterally green, successively grading into a reddish coloration on the abdominal segments. Dorsally whitish, with a continuous brown dorsal line from T1-A1, interrupted on segments A2-A5, and reduced to in total 2 to 3 dots from A6 up to A9. In contrast to other larvae of *Peperomia* feeding *Eois* spp. the first part of the mid-dorsal line gets broader from T1 to A1. The prolegs are laterally darkish colored.

Feeding types: MF, UF.

An oligophagous species recorded only from high elevation cloud forest between 2670–2920m a.s.l. Caterpillars were found at locations with a low canopy cover (20–40%), e.g. more open habitats beside broader trails. Host plants grew as epiphytes on trees at heights from 40–200 cm above ground. A less abundant species, whose larvae were found just on two sample days and moths occurred at light only in very few individuals (pers. communication G. Brehm).

Eois bolana Dognin, 1899

(Fig. 5c, d)

Larvae up to 22 mm in length, with a bright green body color and little dark spots. Head capsule bright yellow brown with darker spots. Dorsal line is colored reddish brown to black, may be continuous from T1 to A9, but sometimes also broken (see Fig. 5c). If dorsal line is broken, then just present from T1-A1 and A5-A9. Lateral from A2-A6 five diagonal, reddish brown to black lines including the spiracles. These lines can be at least partially connected with the dorsal line, if present.

Feeding types: MF, UF. Larvae also feed on shoot axis.

Known to occur in a rather broad elevational extent of montane into cloud forest from 2110–2920m a.s.l. The degree of canopy cover varied from 20–95%. Caterpillars are oligophagous and were found on epiphytes growing from 30–210 cm above ground. A more common species with up to 7 individuals observed in one light trapping night (pers. communication G. Brehm).

Eois* spnr *antiopata Warren, 1904

(Fig. 5i, j)

Larvae are about 16 mm long, greenish blue with dark spots. The head is colored yellow brown without spots. The reddish brown dorsal line is present from T1-A1 and from A6-A10 and appears thin. In lateral view without any prominent characteristics.

Feeding types: MF, UF. Larvae also feed on infructescences.

This species occurs in high elevation cloud forest from 2770–2920m a.s.l. Larvae are oligophagous. In all cases the host plants grew as epiphytes on trees at heights between 80–180 cm. Canopy cover above host plants ranged from 20–100% depending on their habitat. This species was commonly found as larva, but thus far only two individuals had been attracted by light (pers. communication G. Brehm).

Eois* spnr *concatenata Prout, 1910

(Fig. 5e, f)

Length of fully grown larva is about 15 mm. The body is dark green with an orange brown head. The dorsal line is only weakly developed from T2-A1, but well marked from A6-A9 with a dark brown color.

Feeding type: UF.

This species is very rare and was so far recognized in montane rain forest in a narrow elevational range between 1800–1955m a.s.l. Only one single caterpillar was found on *P. eburnea* aff., which grew epiphytically 50 cm above ground.

Eois* spnr *hermosaria Schaus, 1901

(Fig. 5g, h)

Length of the adult larva is about 12 mm. Coloration of the head is yellowish. The body is stocky and dark green with well visible black cuticular rings. A broad dorsal line of a reddish brown coloration is present from T1-A2 and A5-A10.

Feeding type: UF.

Known occurrences of this species range from 1800–2180m a.s.l. The most common *Peperomia* feeding species of this moth genus observed at light traps (pers. communication G. Brehm), but just one single caterpillar was found. The host plant hung at a height of 85 cm down from a rock at a very shady place, where canopy cover was about 95%.

***Eois* spnr *vinosata*_01** Warren, 1907

(Fig. 6a, b)

The stocky caterpillars are colorful and variable; they reach a length of about 13-15 mm. The body color is green with setae inserted in black cuticular rings. A reddish dorsal line is just present from T1-T3 and bifurcates on T1 towards the head, in some individuals continuing on the head capsule in two rounded black bands. Single individuals show a relict dorsal line from A6-A8 which is reduced to three much brighter reddish dots. Dorsally the larvae vary from greenish body color to white. The segments from A1 (A2) onwards to A8 are dark red with black dots which include the spiracles and sometimes with white coloration below and above. These lateral markings can be merged or separated between the segments, but are always well distinct from A2-A5.

Feeding type: HF, MF.

Moths and caterpillars of this rare species were recognized in montane rainforest between 1800 and 2020m a.s.l. Larvae were observed feeding monophagously on a species of *P. curtipes* aff. which occurred in wetter and shady habitats with dense canopy cover.

***Eois* spnr *vinosata*_02** Warren, 1907

(Fig. 6c, d)

This species is not morphologically distinguishable by their larvae from *E.* spnr *vinosata*_01. No differences in size, coloration and their variability could be observed. Differences of coloration in the illustrations do not indicate diagnostic characteristics, but merely represent variation in both species.

Feeding type: HF, MF.

This species is closely related to *E.* spnr *vinosata*_01 and was just found in one single individual as larva. No records from light traps known thus far (pers. communication G. Brehm). The host plant species belongs also to *P. curtipes* aff. To date, this is the only *Peperomia* feeding *Eois* sp. of the study area recognized in lower montane rain forest at about 1000m a.s.l.

***Eois* spnr *violada*_01** Dognin, 1899

(Fig. 6e, f)

Fully grown caterpillars have a length of 20-22 mm. The head capsule is yellowish brown in color. Larvae show dark-green body coloration with an interrupted dark-brown to black dorsal line which is only present from T1-A1 and again from A6-A10. The anterior part of the dorsal line can be continued

as a bifurcated, acuminated line along the sides of the head capsule. Spiracles on segments A1 (A2)-A5 are always distinctly framed in black.

Feeding type: MF, UF.

A very common species with an extremely broad elevational range from 1380–2920m a.s.l. Larvae feed at least on two different *Peperomia* spp., growing both on the ground and as epiphytes at places with a canopy cover of 85% and higher. Caterpillars were found on epiphytic plant individuals up to 120 cm above ground.

***Eois* spnr *violada*_02** Dognin, 1899
(Fig. 6g, h)

This species is morphologically not distinguishable from *Eois* spnr *violada*_01 and *Eois* spnr *violada*_03.

Feeding type: MF, UF.

The rarest species of the *E.* spnr *violada* complex which only occurs in the montane forest zone between 1800–1980m a.s.l. Larvae feed monophagously on *P. glabella* aff. and were found on plants up to a height of 70 cm with a preference for shady places with at least 75% canopy cover.

***Eois* spnr *violada*_03** Dognin, 1899
(Fig. 6i, j)

This species is morphologically not distinguishable from *Eois* spnr *violada*_01 and *Eois* spnr *violada*_02.

Feeding type: MF, UF. Larvae also feed on infructescences.

Very common from 1345–2000m a.s.l. Larvae feed monophagously on plants of *P. glabella* aff. growing in deep shade. They were found on epiphytic plants at heights up to 150 cm as well as on plant individuals growing on the ground.

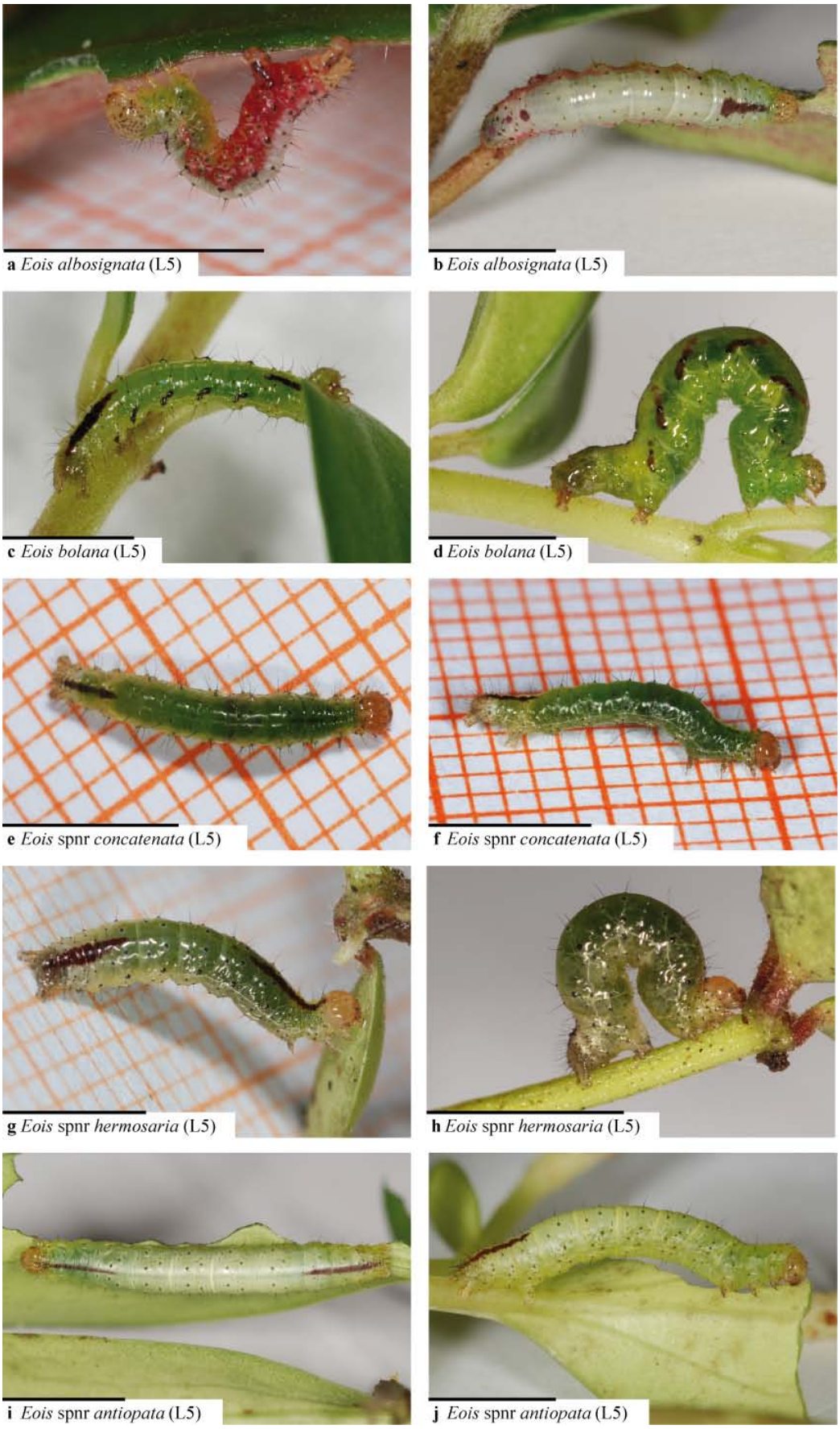


Figure 5 Caterpillars of recognized *Peperomia* feeding *Eois* species; **a-d** = "*trillista* clade"; **e, f** = "*sagittaria* clade"; **g-j** = "*chasca* clade"; scale bar = 5mm. (Illustrations **e** and **f** by A. Broadbent, all other photographs: C. L. Seifert).

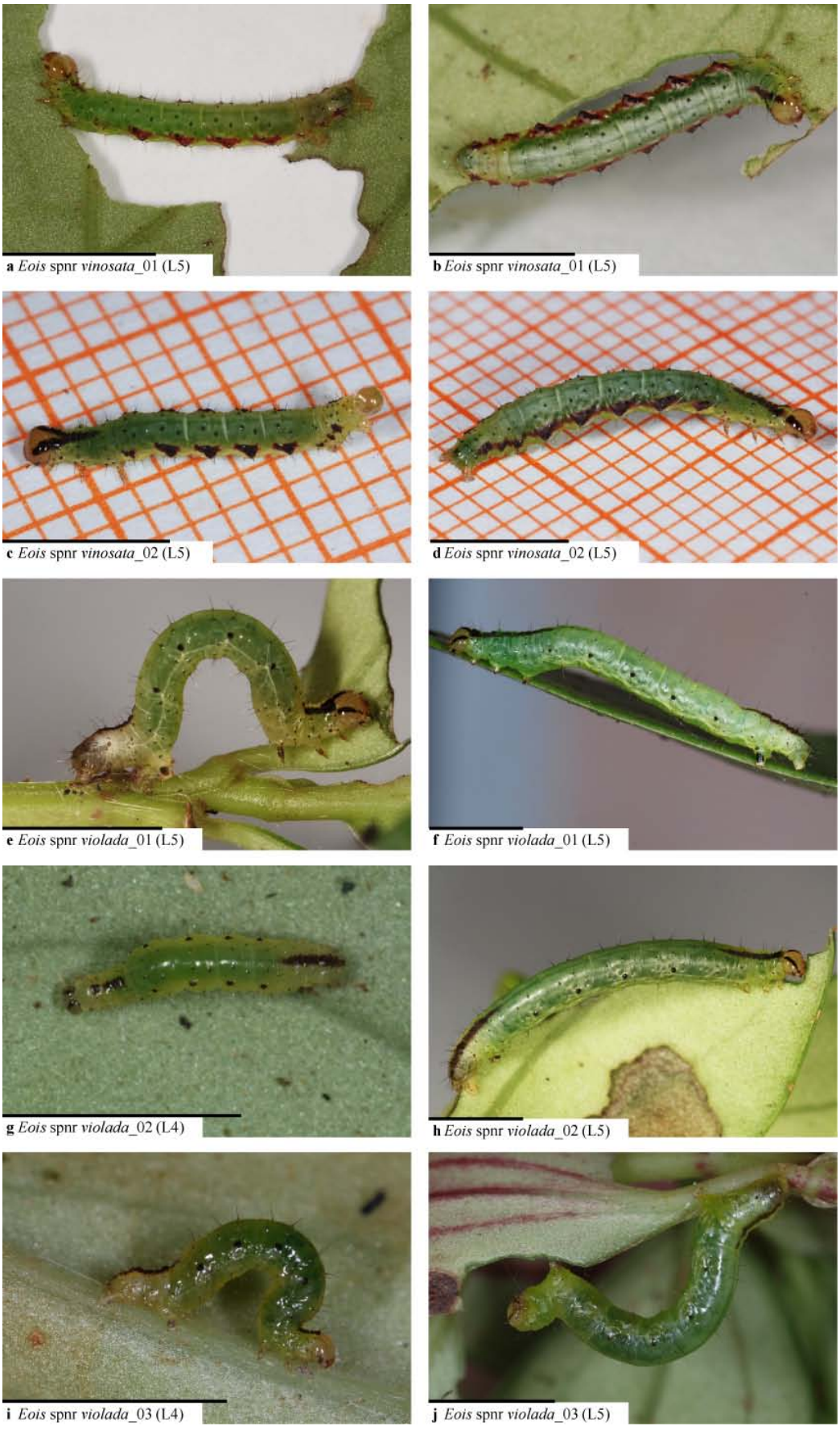


Figure 6 Caterpillars of recognized *Peperomia* feeding *Eois* species. All illustrated species belong to the "*odatis* clade"; scale bar = 5 mm. (Illustrations **c** and **d** by A. Broadbent, all other photographs: C. L. Seifert).

DISCUSSION

The significance of *Peperomia* as larval host plant genus of *Eois* moths

This study revealed that distinctly more *Eois* species in the study area in the southern Ecuadorian Andes feed on the plant genus *Peperomia* than previously recognized. Because some species were found only as single individuals or on one sampling occasion throughout the investigation period, the 10 *Eois* species feeding on *Peperomia* plants still very likely reflect but a part of the total number to be expected. This conclusion is further corroborated by observations of feeding damage on a wide range of additional *Peperomia* species. Even though no caterpillars could be detected on these occasions, it is highly probable that at least some of these instances hint to the existence of further, still unrecognized *Eois* species thriving on *Peperomia* host plants.

A rigorous survey of *Peperomia* plants was only possible to a limited extent because of their variable growth forms ranging from high-growing epiphytes to cushion-forming species at the ground. Therefore, semi-quantitative sampling techniques like branch beating, as applied for *Piper* (e.g. Bodner et al. 2012), or a visual search employing a fixed time limit, were inappropriate. Accordingly, a qualitative survey method was chosen. This renders it impossible to apply species accumulation methods to safely estimate the total number of *Eois* expected to be affiliated with *Peperomia* host plants in the study area.

Nevertheless, 10 *Eois* species observed to feed on *Peperomia* are a remarkable increase in the documentation of host plant relationships in this species-rich moth genus, especially when considering the limited investigation time in combination with the aforementioned methodological difficulties of sampling. These new data make up more than 11% of all *Eois* species with host plant records from the study region, with much of the other information resting on far more extensive and temporally replicated surveys (Bodner 2011). Furthermore, the sampling can be regarded as representative in its coverage of the genus Peperomia. DNA sequence data showed clearly that the surveyed plant specimens are spread over the whole phylogenetic diversity of *Peperomia* to be found in the study area (Figure 4).

On three morphospecies of *Peperomia*, viz. *P. tretraphylla*, *P. eburnea* aff. and "*P.* sp. P041", only single species of herbivorous *Eois* were detected. At least four other host plant morphospecies apparently harbored more than one *Eois* species (Figure 4). *Peperomia viracochana* aff. and „*P.* sp. P016"were each occupied by two *Eois* species. *P. glabella* aff. served as host plant for three taxa, all from the *Eois* spnr *violada* complex. The phylogenetically distinct host plants "P21" and "P036", both termed as *Peperomia curtipes* aff. were morphologically indistinguishable in the field and therefore treated as one plant morphospecies. This morphospecies served as host plant for the two sister species in the *Eois* spnr *vinosata* complex. For the species complexes of *P. pilicaulis* aff. and *P. quadrifolia* aff., each represented by three tested plant specimens, no clear statement is possible.

Nearly all surveyed *Peperomia* species showed traces of insect herbivore feeding damage. They could possibly stem from *Eois* species, but also from other herbivorous insects, especially from other Lepidoptera. For instance, caterpillars of other Larentiinae and Arctiinae (Erebidae) moths were sometimes found during my surveys. Hence, feeding traces alone will not allow a precise estimation of *Eois* species numbers affiliated with *Peperomia* host plants.

My surveys on *Peperomia* and parallel studies on *Hedyosmum* (Chloranthaceae; Blies 2014) in the southern Ecuadorian Andes show very clearly that the larvae of a considerable proportion of *Eois* species feed on plant genera other than *Piper* (Figure 1). Further feeding records exist for *Siparuna* (Siparunaceae). Recent evidence for such more varied host plant relationships comes also from other

locations in the Neotropical region (Wilson et al. 2012, Dyer et al. 2014, Janzen & Hallwachs 2014). Considering only verifiable entries (proven by available pictures at the databases and/or barcodes in BOLD), Janzen & Hallwachs (2014) also recorded *Hedyosmum*, *Piper* and *Peperomia* as host plants in northwestern Costa Rica. Additionally, they recorded three larvae on the fern *Pleopeltis polypodiodes* (Polypodiaceae) and one specimen of another *Eois* taxon on the invasive weedy shrub *Lantana camara* (Verbenaceae), both representing host plant families far outside of Piperales. Dyer et al. (2014) recorded *Piper*, *Peperomia* and *Hedyosmum* as well as *Siparuna* from the eastern Andes of northern Ecuador as host plant taxa. Furthermore they found one *Eois* species feeding on the genus *Alloplectus* (Gesneriaceae). From the area around "La Selva Biological Station" in north-eastern Costa Rica, Dyer & Gentry (2002) recorded *Eois* only feeding on *Piper*.

It is remarkable that all *Eois* species purportedly found on Verbenaceae, Gesneriaceae and Polypodiaceae also feed on one of the three well established host genera *Piper*, *Peperomia* or *Hedyosmum*, where they were mostly found in much higher abundances. Thus, the few *Eois* specimens on *Pleopeltis*, *Lantana* and *Alloplectus* are most likely stray larvae found away from their original host plant. Furthermore, misidentification of both, plants and caterpillars, as well as mistakes during data entry are conceivable sources of error. Hence, unless confirmed by novel evidence these affiliations of *Eois* caterpillars with plants in the families Polypodiaceae, Verbenaceae and Gesneriaceae are deemed suspect. Nevertheless, the increasing number of unambiguous host plant data paired with newly recorded plant taxa outside *Piper* and even Piperales show very clearly that the former assumption of *Eois* being monophagously associated with *Piper* (e.g. Brehm 2002, Brehm & Fiedler 2005, Connahs et al. 2009) is no longer tenable.

Yet, with more than 70% of all host plant records in the study area, *Piper* is according to current knowledge the prime host plant taxon. About 45 *Piper* species are currently known to occur between 1800 to 3150m a.s.l. (Link 2014). A given *Piper* species in this elevational band can be expected to harbor approximately 3.8 species of *Eois* (pers. communication F. Bodner). Given the apparent high degree of host specialization among *Eois* (i.e. 1.2 host plants per species; Bodner et al. 2012), this would suggest more than 140 *Eois* taxa associated with Piper in the area around "RBSF" alone.

Nevertheless despite a more intensive sampling on *Piper* in recent years (Bodner et al. 2010, Bodner 2011, Bodner et al. 2012), merely 27% of collected *Eois* species could be observed to feed on this genus. Furthermore, about 62% of recognized *Eois* species in the study area are without any host plant record. This underlines the incompleteness of available food plant associations and supports the assumption that other plant families besides Piperaceae might also play an important role as hosts. Notably, Magnoliidae and their sister clade Chloranthaceae (Soltis et al. 2011, Massoni et al. 2014) include the only unambiguous host plant genera of *Eois* moths (i.e. *Piper*, *Peperomia*, *Manekia*, *Siparuna* and *Hedyosmum*). Therefore, these two plant clades are worthwhile candidates for future investigations. Also, *Peperomia* should be scanned more intensively in the future. The species richness of this plant genus and its phylogenetic proximity to *Piper* give reason to expect a high number of associated *Eois* taxa. Furthermore *Peperomia* seems to favor the same environmental conditions as *Eois,* e.g. occurrence in a broad elevational gradient in mostly wet to moist, shady forest habitats. Since host switches to phylogenetic related plant taxa are common in herbivorous insects (e.g. Forister et al. 2009, Graves & Shapiro 2003, Hawkeswood & Monaghan 2007), it is conceivable that yet unconsidered plant genera within Magnoliidae and Chloranthaceae such as *Ocotea* and *Persea,* also harbor some *Eois* species and are therefore of interest for further sampling. Both belong to the species-rich family of Lauraceae and are represented in the study area with 14 and 12 recognized species, respectively (Homeier & Werner 2007).

Host specificity, abundance and parasitism rate of *Eois* species affiliated with *Peperomia*

For all ten *Eois* species reported here, only a monophagous or at most narrowly oligophagous larval diet could be observed, which is in accordance to *Piper* feeding congeneric moth species (Connahs et al. 2009, Dyer & Gentry 2002, Dyer et al. 2014, Janzen & Hallwachs 2014, Bodner et al. 2012). Table 4 shows that seven species probably live monophagously whereas two species, *E.* spnr *violada*_01 and *E.* spnr *antiopata*, appear to feed on at least on two *Peperomia* species. For *E. bolana* a total of three different host plants were recognized. This leads to an average of 1.4 host plant morphospecies per *Eois* taxon. The recognized mean value of utilized *Piper* morphospecies for the study area amounts to 1.2 (Bodner et al. 2012) while Connahs et al. (2009) ascertained an average of about 1.8-2.1. The deviation may is a result of the different extent of data collection and elevational range (Bodner et al. 2012). *Eois* which were sampled on *Hedyosmum* in the study area, exploit 1.3 host plant morphospecies on average (pers. communications D. Blies and F. Bodner). However, it seems that host specificity is quite similar within *Eois* and all values support the hypothesis of a highly specialized moth genus.

Furthermore, no *Eois* species recognized as feeding on *Peperomia* in the study area has thus far been recorded on other plant genera. Dyer et al. (2014) recorded one species feeding on *Peperomia* as well as on *Alloplectus* (Gesneriaceae). If this should proove to be a recurrent observation, it would represent an exception for *Eois* together with the aforementioned records for *Pleopeltis* and *Lantana*, because nearly all species of *Eois* feed on just one single host plant genus (Connahs et al. 2009, Dyer & Gentry 2002, Dyer et al. 2014, Janzen & Hallwachs 2014, Wilson et al. 2012). Apart from these doubtful records (see above), all *Eois* species thus far have to be characterized as monophagous on plant genus level and at most narrowly oligophagous on plant species level.

This high level of specialization supports Dyer et al. (2007) who observed increasing host plant specialization of Lepidoptera with decreasing latitude. While Novotny et al. (2002) suggested that communities of herbivorous insects in the tropics are dominated by low host plant specialization in general, a more recent study showed that host specificity varies across feeding guilds (Novotny et al. 2010). Especially the guild of larval leaf-chewers, which includes externally feeding larvae of moths and thus *Eois,* comprises a high proportion of specialists. Weiblen et al. (2006) also reported high clade specificity among tropical Lepidoptera concerning their larval host plants.

In the study area in southern Ecuador, the ratio between generalists and specialists within the family Geometridae seems to vary across moth clades. For instance, many genera of Larentiinae (e.g. *Eupithecia*, *Hagnagora* and probably also *Psaliodes*) are narrow specialists similar to *Eois,* while most representatives of Ennominae (e.g. *Oxydia*, *Isochromodes*, and *Sabulodes)* are known as generalists (Brehm 2003, Bodner et al. 2010, Bodner 2011, pers. communications G. Brehm and F. Bodner). This is congruent with reports of Novotny et al. (2010) that host specificity can differ distinctly within the same feeding guild between individual taxonomic lineages.

Some *Eois* whose larvae feed on *Piper* live gregariously (pers. communication F. Bodner, Wilson et al. 2012), a life history trait which could not be observed for species with *Peperomia* as host plant. Accordingly, clumped distributions of *Eois* caterpillars on *Peperomia* never occurred. This might be one reason that, in contrast to some *Piper* feeding *Eois*, leaf skeletonization (pers. communication F. Bodner, Salazar et al. 2013) or, like in some other areas, complete defoliation of individual plants (Connahs et al. 2009) was not observed on *Peperomia*. Generally speaking, a severe impact as pests on *Peperomia* seems unlikely given the scarcity of *Eois* caterpillars on these plants. This applies to *Eois* on *Hedyosmum* shrubs as well (pers. communications D. Blies and F. Bodner). Overall, larval abundances of *Eois* sampled on *Peperomia* were quite low, ranging from 1-16 individuals per plant.

However, if light trapping results are considered, not all of these species can be regarded as 'rare' species. Especially *Eois* spnr *hermosaria*, *E.* spnr *violada*_01 and *E.* spnr *violada*_03 were relatively abundant throughout about 60 light trap records, respectively (see Table 4). Nevertheless, many *Eois* species collected in the area are less abundant; some have been collected with only a few individuals among thousands of specimens (pers. communication G. Brehm). This relative rarity of *Eois* species in the study area is not a specific characteristic of this genus, but rather a common observation among Geometridae (pers. communication G. Brehm). Merely a few single geometrid moths are high abundant species in the study area (pers. communication G. Brehm & F. Bodner), for instance *Psaliodes cedaza* (Brehm 2002) and *Microxydia* spnr *ruficomma* (Bodner et al. 2012, pers. communication F. Bodner) .

Apart from *E.* spnr *vinosata*_02 which had not been recorded before in the study area, all *Eois* taxa had previously been encounterd during light trapping (pers. communication G. Brehm, see Table 4), and all but *E.* spnr *vinosata*_01 in more than one specimen. It is currently not possible to deduce which sampling strategy might be more effective to locate individual species in their distribution area, because none of them showed up in larger numbers. For instance, *Eois* spnr *hermosaria* was sampled in 63 individuals by light trapping while merely one single caterpillar was found. In contrast, *Eois* spnr *antiopata* was collected only twice as adult moth whereas 13 larvae were located. Nevertheless, my caterpillar surveys might indicate that some *Peperomia* feeding *Eois* species have narrow elevational activity ranges, so that they are likely to be seen at light traps only if these are placed in close proximity to their host plants, or if sampling effort is high.

If light-trapping data were considered in combination with caterpillar surveys, three *Eois* species affiliated with *Peperomia* occur only above 2000m a.s.l., whereas just one was found at ≈1000 m a.s.l. (Table 4). All the rest was mostly sampled between 1800 and 2000m a.s.l. This is approximately congruent to the elevational distribution patterns of *Eois* in Costa Rica (Brehm et al. 2011) where the majority of species occurs around 1700m a.s.l. and richness declines at upper and lower elevations. In the present context, only two species, *Eois albosignata* and *E.* spnr *antiopata* were located exclusively above 2600m a.s.l. Consequently, the data presented here gives no clear support to the hypothesis of Brehm et al. (2013) according to which predominantly *Eois* species from higher altitudes (above 2590m a.s.l.) may have switched to host plants other than *Piper*. The inclusion of species living on *Hedyosmum* does not change this pattern (pers. communications D. Blies and F. Bodner).

Parasitism was only observed in the station area around 1800-2000m a.s.l. In this elevational band, the parasitism rate was largely similar to *Piper* feeding *Eois* (pers. communication F. Bodner). In the higher elevations of Cajanuma, no unambiguous cases of parasitoid infestation were recognized for *Eois* larvae on *Peperomia*. The significant decrease of parasitism rate with increasing elevatioin is congruent to the results of Connahs et al. (2009) and applies also to *Piper* feeding *Eois* in the study area. Furthermore, parasitism was observed in all Neotropical areas where studies with *Eois* were realized (Connahs et al. 2009, Wilson et al. 2012, Janzen & Hallwachs 2014). Thus, parasitoids are natural predators for *Eois* caterpillars in all elevational ranges but their impact seems to decline with increasing altitude.

Phylogenetic implications

Except for *Eois* spnr *violada*_02, for which no imago was available, every single species could be assigned by adult morphological characteristics (mainly wing patterns) to the same clade as they were classified by phylogenetic analysis of sequence data. This supports the results of Strutzenberger

et al. (2010), according to which most *Eois* species could be allocated by their wing patterns to one of the nine clearly distinguishable Neotropical clades. In contrast to the study by Wilson et al. (2012), all species could be identified based on a 2% divergence threshold in their respective barcode sequences, without the necessity to include life history traits for the identification of cryptic species. Behavior, elevational range, host plants and larval morphology of all *Eois* species observed to feed on *Peperomia* plants were fully consistent with species delimitations by COI haplotypes. This further supports the usefulness of DNA barcoding to recognize and delimit species in the genus *Eois* (Strutzenberger et al. 2011, 2012). In particular, barcoding was essential to detect cryptic species diversity, e.g. in the poorly differentiated *E.* spnr *violada* complex.

The scattering of *Peperomia* feeding *Eois* over the whole phylogeny of these moths (Figure 3) showed that a secondary host shift, probably always away from *Piper* to *Peperomia,* evolved convergently at least five times. In contrast, host switches to other plant genera like *Hedyosmum* or *Manekia*, for which unambiguous records of *Eois* are available (Strutzenberger et al. 2010, Dyer et al. 2014, Janzen & Hallwachs 2014), seem not to have evolved multiple times independently (see Strutzenberger et al. 2010). Host shifts of Lepidoptera are more common between closely related plant taxa (Janz & Nylin 1998) and are facilitated most likely by their similar phytochemical compounds (Ehrlich & Raven 1964, Wahlberg 2001, Braby & Trueman 2006, Janz 2011, Ferrer-Paris et al. 2013, Nylin et al. 2014). To test if this is the relevant trait for specialization of *Eois*, comprehensive phytochemical studies concerning their host plants are necessary. The close phylogenetic relationship between *Peperomia* and *Piper* (Wanke et al. 2007, Smith et al. 2008), however, supports the assumption of broad phytochemical commonalities between these sister genera.

Most *Eois* species whose larvae feed on *Peperomia* form monophyletic groups (e.g. *E.* spnr *violada* complex, *E.* spnr. v*inosata* complex). Partially, they include moth taxa without host plant records (e.g. *E.* spnr *trillista* [Eo00111] within the *E. bolana* - *E. albosignata* group). It seems reasonable to assume that such missing host plant records result from incomplete sampling of *Peperomia,* viz. the "missing" species can be expected to also be herbivores of *Peperomia*. This hypothesis implies that such clusters result from host conservatism (McKenna & Farrell 2005, Stone et al. 2008), i.e. descendants derived through speciation from an ancestral *Peperomia* feeding species likely retained this host plant association on genus level. That would mean that an adaptation of one species to a novel host plant genus can lead to a monophyletic cluster affiliated with the same host plant genus in the course of speciation processes. The "*sagittaria* clade" of the genus *Eois* is one example for such a case with host-use clustered on *Peperomia*. If the doubtful record, according to which *E. chasca* (Eo00096) (now *E.* spnr *hermosaria*) was found on Gesneriaceae (see Strutzenberger et al. 2010), is excluded, *Peperomia* remains the only verified host plant genus for the "*chasca* clade". Especially for these two clades, it is conceivable that both harbor mainly or exclusively *Eois* species, whose larvae feed on *Peperomia*. A similar case is known from the "*adimaria* clade", where most species with host plant records are now proven to feed on *Hedyosmum* (Strutzenberger et al. 2010, Blies 2014). These conclusions are further supported by records obtained from online databases (Janzen & Hallwachs 2014, Dyer et al. 2014). Judging from available pictures, all *Peperomia* feeding species listed there matched to "*chasca* clade", while all specimens found on *Hedyosmum* and *Siparuna* belong to the "*adimaria*" and "*chrysocraspedata* clade", respectively. Thus, *Eois* species recorded in NW Costa Rica and NE Ecuador to feed on *Hedyosmum*, *Peperomia* and *Siparuna* belong to the same clades as the non-*Piper* feeding *Eois* moths in southern Ecuador.

Wilson et al. (2012) revealed no evidence of true co-speciation processes between *Eois* and *Piper*. The same seems also apply to species living on *Peperomia*. The *Eois* spnr *violada* complex consists of

three closely related species that all occur in the same area and all feed at least partially on the same host plant. On one occasion larvae of *E. violada*_01 and *violada*_02 were even found together on the same host plant individual. Hence, for these three species a diversification process driven by host plant speciation can be excluded. Another sister taxon group with 2.3% sequence divergence (Table 3) seems to feed on the same host plant (*P. curtipes* aff.), but these two moth species occur in different elevational ranges with a gap of about 800m according to the current state of knowledge (Table 4). Judging from these observations, speciation mediated through diversification of their host plants seems unlikely. Thus, both these examples do not suggest a strict co-speciation process between *Eois* moths and *Peperomia* plants. Also the tangled trees (Figure 4) provide no evidence for such a process. This is not surprising, because co-speciation events between plants and their herbivorous insects, seems to be quite rare (Forister & Feldmann 2011, Percy et al. 2004, Vienne et al. 2013).

Instead, diversification processes in phytophagous insect groups are driven by the exploitation of a new host plant genus or family which contains different phytochemical compounds (Braby & Trueman 2006, Kergoat et al. 2005). Thus, the multiple independent switching of an ancestral *Eois* species to *Peperomia* and the following radiation may well be a reason for the observed species richness of this genusI in the Neotropics.

This study in the Andes of southern Ecuador has shown that the moth genus *Eois* is less strongly bound to *Piper* than previously assumed. In all likelyhood, additional plant genera not yet recognized as trophically linked to this megadiverse moth clade will be identified as potential hosts. Further investigations on trophic interactions should concentrate on plant genera within Magnoliidae and Chloranthaceae, but also on currently unconsidered plant taxa. Additionally, phytochemical investigations are required to better understand the apparent host conservatism of *Eois.* Such knowledge could also help to identify further potential host plant taxa. In summary, descriptive data as presented in this study remains essential and indispensible for further developing a phytophagous insect group, like *Eois*, into a model group in evolutionary ecology. Ultimately, accurate statements about specialization processes and food web structures will be possible only if comprehensive information on the life history of candidate taxa becomes available.

ACKNOWLEDGEMENTS

I am deeply grateful to my supervisor Univ.-Prof. Mag. Dr. Konrad Fiedler for the opportunity to participate in this project as well as for helpful discussions and comments earlier manuscript drafts. Furthermore I thank PD Dr. Stefan Wanke (TU Dresden, Germany) and Ass.-Prof. Dr. Marie-Stéphanie Samain (Ghent University, Belgium) for generating the *Peperomia* phylogeny and determination of sampled plant specimens, respectively. I thank Dr. Harald Letsch for support in processing the phylogenetic analyses of *Eois*. For worthwhile suggestions and providing some of their data I thank Dr. Gunnar Brehm (Friedrich-Schiller-University Jena, Germany), Dr. Florian Bodner, Dr. Christian H. Schulze, Patrick Strutzenberger (Senckenberg Institute Dresden, Germany), Darinka Blies (University Trier, Germany) and Marc-Oliver Adams. Furthermore, I would like to thank Dr. Brigitte Gottsberger, Lisamarie Lehner, Anita Freudmann and Petra Bartl-Binder for sequencing *Eois* specimens, Markus Günther (TU Dresden) for sequencing *Peperomia* specimens, and Arthur Broadbent to carry out a pre-study. Many thanks also to the managers and staff of the Estación Científica San Francisco in Ecuador.

This study was financially supported by the German Research Foundation (DFG), FOR 816, subproject Fi 547/ 10-2 and the University of Vienna. Research permit (No. 023-2011-IC-FLO-DPL-MA) was kindly granted by the Ministerio del Medio Ambiente del Ecuador. The foundation Nature and Culture International (Loja, Ecuador) allowed access to the study sites and provided logistic support.

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