

RESEARCH

Host Plant Associations and Parasitism of South Ecuadorian *Eois* Species (Lepidoptera: Geometridae) Feeding on *Peperomia* (Piperaceae)

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ABSTRACT. The very species-rich tropical moth genus *Eois* Hübner (Lepidoptera: Geometridae) is a promising model group for studying host plant specialization and adaptive radiation. While most *Eois* species are assumed to be specialized herbivores on *Piper* L. species, records on other plant taxa such as *Peperomia* Ruiz & Pavón (Piperaceae) are still relatively scarce. Moreover, little is known about life history traits of most species, and only a few caterpillars have been described so far. We collected caterpillars associated with *Peperomia* (Piperaceae) host plants from June 2012 to January 2013 in three elevational bands of montane and elfin rainforests on the eastern slopes of the Andes in southern Ecuador. Caterpillars were systematically searched and reared to the adult stage. We were able to delimitate ten species of *Eois* on *Peperomia* by comparison of larval and adult morphology and by using 658 bp fragments of the mitochondrial COI gene (barcode sequences). Three of these species, *Eois albosignata* (Dognin), *Eois bolana* (Dognin), and *Eois chasca* (Dognin), are validly described whereas the other seven taxa represent interim morphospecies, recognized unequivocally by their DNA barcodes, and their larval and adult morphology. We provide information about their host plants, degree of parasitism, and describe the larval stages in their last instar. Additionally, caterpillars and moths are illustrated in color plates. This is the first comparative study dealing with *Eois* moths whose caterpillars feed on *Peperomia* hosts.

Key Words: Andes, caterpillars, host plants, larval morphology, mountain rainforest

While quantitative studies on tropical insects at the community level have proliferated in the last decades, e.g., to estimate their global species richness (Novotny and Miller 2014) or to investigate insect–plant interactions (Dyer et al. 2007, Miller and Dyer 2009, Novotny et al. 2010), documentation of the bionomics of individual species has experienced far less attention. As a result, there is still a substantial lack of high quality data about insect life history traits (Miller and Dyer 2009), even though species traits become ever more important in biodiversity studies (e.g., Barbaro and van Halder 2009, Brehm et al. 2013, Frenette-Dussault et al. 2013). For example, species trait data are indispensable for rigorous tests of hypotheses on insect functional biodiversity or evolutionary ecology.

Since the beginning of this century, the pantropically distributed genus *Eois* Hübner has attracted increasing interest with regard to ecological and evolutionary research. In the Neotropics, 211 species of this genus have thus far been validly described (Brehm et al. 2011), but estimates by Rodríguez-Castañeda et al. (2010) and Brehm et al. (2011) suggest that the total number to be expected is likely to be several times higher. *Eois* is a morphologically highly diverse genus (Strutzenberger et al. 2010), and is known for its distinct host plant specialization (Dyer and Gentry 2002, Connahs et al. 2009, Janzen and Hallwachs 2009, Rodríguez-Castañeda et al. 2010, Bodner et al. 2012, Dyer et al. 2015). However, despite a growing number of studies on *Eois* in recent years, baseline host plant data and records of early stages, essential for many further investigations, are still lacking for most species. For instance, caterpillars and host-plant relationships remain unknown for the majority of more than 200 *Eois* species recorded from just a small focal area in southern Ecuador (Seifert 2014).

A common notion in literature on *Eois* is that plants from the diverse genus *Piper* form the basic resource utilized by the caterpillars of these moths (Connahs et al. 2009, Rodríguez-Castañeda et al. 2010, Wilson et al. 2012). Records from other host plant taxa are scarce. We aim to document the life history traits of ten *Eois* species from montane and

elfin forests in southern Ecuador whose caterpillars are associated with an alternative host taxon, viz. the genus *Peperomia* (Piperaceae).

Peperomia is a predominantly tropical, species-rich genus of basal angiosperms containing about 1,600 species (Isnard et al. 2012). Like in the sister genus *Piper*, most species occur in the Neotropics (Wanke et al. 2006). In contrast to *Piper*, the genus *Peperomia* comprises exclusively small epiphytous or terrestrial herbs but no woody plants (Isnard et al. 2012). Furthermore, their leaves are often smaller and thicker than those of *Piper*.

In the framework of this study, we give information about the respective *Peperomia* host plants of 10 *Eois* taxa, their parasitoids, and describe their larval morphology in the last instar. Additionally, photographs of caterpillars and moths are provided for the first time.

Materials and Methods

From June 2012 to January 2013, caterpillars of *Eois* were collected in three elevational bands of tropical montane and elfin rainforest in southern Ecuador, situated on the eastern slopes of the Andes. Most samples were obtained in near-natural montane rainforest in the ‘Reserva Biológica San Francisco’ (RBSF) between 1,800 and 2,300 m a.s.l., in proximity to the San Francisco research station (03°58'18"S, 79°04'45" W). Furthermore, caterpillars were sampled in elfin forest at Cajanuma (2,700 to 3100 m a.s.l.; c. 04°06'58" S, 79°10'19" W) and in lower montane forest at Bombuscaro (about 1,000 m a.s.l.; c. 04°06'53"S, 78°57'57"W), two areas situated within Podocarpus National Park. For further information about the study area, the major vegetation types and their ecological characteristics see Beck et al. (2008), Gradstein et al. (2008), and Homeier et al. (2010).

Caterpillars were searched systematically by visually scanning potential host plants in various habitats, ranging from valley sites (‘quebradas’, riversides), characterized by tall trees, high nutrient availability and humidity, to dryer locations (ridges), characterized by smaller trees, little nutrient availability and lower humidity (Homeier

et al. 2010). In the course of this sampling, all encountered morphospecies of *Peperomia*, about 25 in total, were searched for characteristic feeding traces of *Eois* caterpillars (i.e., scraped leaf undersides, leaf holes, and peripheral leaf blade damage). Terrestrial and epiphytally growing *Peperomia* plants were sampled on the ground and in the forest understory up to 3 m height. All plant individuals showing conspicuous feeding traces were then investigated more closely.

Caterpillars were reared individually in small plastic boxes lined with paper towels and provided with fresh host plant foliage at least every other day. Until emergence of the moths, pupae were left in the boxes and sprayed lightly with water every 2–3 d. For later DNA barcoding, caterpillars which died prematurely as well as crippled pupae were preserved in ethanol (96%), while hatched moths were frozen at -18°C and later spread. Each caterpillar individual received a unique sample-ID code. For each individual, we recorded the collection date, the precise locality, the host plant morphospecies, and dates of pupation and moth emergence. All caterpillars and moths were photographically documented with a Nikon D70s camera (Nikon Corporation, Japan) and a Sigma 105 mm F2.8 EX DG macro lens (Sigma Corporation, Japan). For descriptions, the lengths of caterpillars in their last instar are given. Furthermore, if rearing was successful, larval development time and duration of pupal stage are provided. Host plant data are only given when the caterpillars were observed feeding on the respective *Peperomia* species for at least 2 d and reached the pupal stage. The risk of recording erroneous host plant affiliations due to stray larvae resting away from their true food source is thus minimized.

Because by far most Neotropical *Eois* are yet undescribed (Brehm et al. 2011), identification was limited to morphospecies level in most cases. Species delimitation of all determined individuals was aided by DNA barcoding ($n = 65$, Tables 1 and 2). DNA extraction and sequence generation followed the protocol by Strutzenberger et al. (2011). Barcode data were entered into the BOLD database (Ratnasingham and Hebert 2007, <http://www.boldsystems.org/>).

For barcoding, ethanol samples of early stages (caterpillars and pupae) as well as single legs of the dried moths were used. A 2% sequence divergence threshold was chosen for species delimitation (Strutzenberger et al. 2011). Especially in cases of multiple undescribed species in combination with a low coverage and thus limited knowledge of intraspecific morphological variability, this approach has proven efficient in handling the problem of species demarcation, with good performance in the family Geometridae (deWaard et al. 2011, Hausmann et al. 2011, Brehm et al. 2013, Rajaei et al. 2013).

Furthermore, species delimitation was assisted by comparison of wing patterns, where no intersexual differences were observed, and larval morphology. Terminology of larval characters follows Stehr (1987). Every genetically distinguishable taxon obtained a unique Barcode Index Number (BIN, Ratnasingham and Hebert 2013) within ‘Bold

Systems’ (<http://www.boldsystems.org/>), enabling an allocation of trait information to species in advance of their formal taxonomic description. The suitability of this procedure has successfully been proven for geometrid moths (e.g., Hausmann et al. 2013). To enable comparison with previous studies, we also provide our unique database numbers of geometrid moth species in southern Ecuador as well as informal morphospecies names hereafter. Informal morphospecies names are marked with the abbreviation ‘spnr’ for ‘species near’. Clade allocations are based on the classification by Strutzenberger et al. (2010). All moth vouchers are currently deposited in the research collection of Gunnar Brehm and will eventually be deposited in the Phyletisches Museum (Jena, Germany).

Herbarium specimens were collected and photographs were taken of all *Peperomia* morphospecies with evidence of feeding damage inflicted by *Eois* caterpillars. *Peperomia* morphospecies were allocated by two botanical specialists, M.-S. Samain and S. Wanke, to species affiliations (‘aff.’). If this was not possible, they received a unique identification number. Herbarium vouchers are deposited at Ghent University, Belgium.

Results and Discussion

During our field surveys we found 82 caterpillars of *Eois* associated with *Peperomia* plants of which 19 could be reared to adulthood. Sixty-five individuals (79.3%) could be either clearly identified as an already described species (17 individuals, 3 species) or unequivocally matched to a genetically distinct but undescribed *Eois* species (48 individuals, 7 species; Tables 1 and 2). Mean pairwise genetic distances of COI barcode sequences between species ranged from 2.3 to 11.3% (Table 1 and Supp Table 1 [online only]). The observed genetic variation of the COI barcodes within single morphospecies ranged between 0.00 and 0.15% (Supp Table 2 [online only]), supporting the appropriateness of a 2% threshold for species demarcation. For the remaining 17 individuals an unambiguous identification was not possible either because not enough tissue was left in parasitized caterpillars or DNA sequencing was unsuccessful. However, by comparison of larval morphology, eight of them could be assigned to the *E. spnr violada* complex, consisting of three cryptic sister taxa (Strutzenberger et al. 2010, Seifert 2014) which can best be separated through DNA barcoding (Table 1). Three species, *Eois albosignata* (Dognin), *Eois bolana* (Dognin), and *Eois chasca* (Dognin) are validly described taxa (type localities: *E. albosignata*—Colombia, Yuntas, near Cali; *E. bolana*—Ecuador, Loja; *E. chasca* Ecuador, Loja; see Scoble 1999) whereas seven other morphospecies most probably represent undescribed taxa (Table 1).

Ten *Eois* species feeding on *Peperomia* in just one small Andean study area is a remarkable extension of the known host plant affiliations of the genus. With a similar sampling approach, Blies (2014) observed 13 additional *Eois* species feeding on *Hedyosmum* spp. (Chloranthaceae). None of the observed *Eois* species whose caterpillars live on *Peperomia* plants are known to feed also on other host plant genera. Hence, even though plants in the genus *Piper* continue to rank as the most important host resource for larvae of *Eois* (Strutzenberger et al. 2010, Seifert 2014), our results indicate that radiation of this mega-diverse moth genus also implicated some larger host shifts. Furthermore, *Eois* species recorded on *Peperomia* belong to four phylogenetically distinct clades (*chasca* clade, *odatis* clade, *trillista* clade, and *sagittaria* clade; Seifert 2014) as described by Strutzenberger et al. (2010) and one unknown clade assignment (Table 2). Hence, host shifts to *Peperomia* appear to have occurred frequently and, considering with additional host plant information taken from Strutzenberger et al. (2010), at least five times independently (Seifert 2014). The close phylogenetic relationship between *Peperomia* and *Piper* supports the assumption of broad phytochemical commonalities between these sister genera, which might have facilitated independent intergeneric host shifts.

Nine out of ten of the *Eois* taxa collected as larvae on *Peperomia* plants had also been observed in light-trap samples in the same study area (Table 2), while one morphospecies (*Eois spnr vinosata_02*) had

Table 1. Mean pairwise genetic distances (%) of all observed *Eois* spp. feeding on *Peperomia* under Kimura’s 2-parameter substitution model (below the diagonal) and standard errors (above the diagonal) estimated by the bootstrap method (1,000 replications)

Taxon	N	1	2	3	4	5	6	7	8	9	10
1 <i>E. albosignata</i>	5	1.2	1.4	1.2	1.1	1.1	1.2	1.1	1.3	1.3	
2 <i>E. bolana</i>	5	9.7	1.3	1.2	1.2	1.2	1.2	1.2	1.3	1.3	
3 <i>E. chasca</i>	1	11.0	9.7	1.1	1.4	1.2	1.3	1.2	1.4	1.4	
4 <i>E. spnr antiopata</i>	7	9.8	9.9	7.2	1.3	1.2	1.4	1.2	1.3	1.3	
5 <i>E. spnr concatenata</i>	1	9.0	9.5	10.5	9.8	1.0	1.1	1.1	1.1	1.2	
6 <i>E. spnr violada_01</i>	10	8.3	9.0	7.9	9.4	7.2	0.7	0.7	1.2	1.2	
7 <i>E. spnr violada_02</i>	2	10.0	9.5	9.3	11.3	7.6	3.7	0.7	1.2	1.2	
8 <i>E. spnr violada_03</i>	4	8.7	9.5	8.7	10.0	7.9	4.1	4.0	1.2	1.2	
9 <i>E. spnr vinosata_01</i>	6	10.6	10.7	10.6	11.0	8.4	8.2	8.5	8.7	0.6	
10 <i>E. spnr vinosata_02</i>	1	10.8	10.4	10.6	11.2	9.2	8.7	9.1	9.1	2.3	

Only COI barcode sequences (658 bp) without contaminations and with correlating contigs were considered ($n = 42$, see Supp Table 1 [online only]). Analyses were carried out in MEGA6 (Tamura et al. 2013).

Table 2. Life history information about observed *Eois* species

Taxon	BOLD BIN and database number	Observed host plant(s)	Diet	Loc	Observed elevational distribution	N
<i>Eois</i> trillista clade						
<i>E. albosignata</i> (Dognin, 1911)	AAW4736 No. 2292	<i>P. viracochana</i> Trel. aff.	Mono	CJ (CJ)	2790–2835 (2900–2920)	8 (4)
<i>E. bolana</i> (Dognin, 1899)	AAW4735 No. 2283	<i>P. pilicaulis</i> C.DC. aff. <i>P. quadrifolia</i> (L.) Kunth aff. <i>P. viracochana</i> Trel. aff.	Oligo	CJ (RBSF, CJ)	2725–2835 (2110–2920)	8 (9)
<i>Eois</i> chasca clade						
<i>E. chasca</i> (Dognin, 1899)	AAF0381 No. 392	<i>P. tetraphylla</i> Hook. & Arn. aff.	Mono	RBSF (RBSF)	1820 (1800–2290)	1 (13)
<i>E. spnr antiopata</i> (Warren, 1904)	AAAY8000 No. 2279	<i>P. pilicaulis</i> C.DC. aff. <i>P. quadrifolia</i> (L.) Kunth aff.	Oligo	CJ (CJ)	2770–2835 (2900–2920)	13 (2)
<i>Eois</i> sagittaria clade						
<i>E. spnr concatenata</i> (Prout, 1910)	ABU9945 No. 2285	<i>P. eburnea</i> Sodiro aff.	Mono	RBSF (RBSF)	≈1950 (1800–1950)	1 (13)
<i>Eois</i> odatis clade						
<i>E. spnr violada_01</i> (Dognin, 1899)	AAI5253 No. 2356	<i>P. glabella</i> (Sw.) A.Dietr. aff. <i>P. sp. 041</i>	Oligo	RBSF (RBSF, CJ)	1810–2020 (1380–2920)	16 (91)
<i>E. spnr violada_02</i> (Dognin, 1899)	AAI5238 No. 2360	<i>P. glabella</i> (Sw.) A.Dietr. aff.	Mono	RBSF (RBSF)	1820–1980 (1800–1900)	5 (5)
<i>E. spnr violada_03</i> (Dognin, 1899)	AAW5637 No. 403	<i>P. glabella</i> (Sw.) A.Dietr. aff.	Mono	RBSF (BC, RBSF)	1815–1910 (1025–2010)	6 (77)
Unknown clade assignment						
<i>E. spnr vinosata_01</i> (Warren, 1907)	ABW9383 No. 709	<i>P. curtipes</i> Trel. aff.	Mono	RBSF (RBSF)	1990–2020 (1800–1973)	6 (11)
<i>E. spnr vinosata_02</i> (Warren, 1907)	ACH2043 (—)	<i>P. curtipes</i> Trel. aff.	Mono	BC (—)	≈1000 (—)	1 (—)

Diet: Mono = monophagous on one *Peperomia* species, Oligo = oligophagous on more than one *Peperomia* species; location: BC = Bombuscaro, RBSF, CJ = Cajanuma; as well as elevation and number (N) of sampled caterpillar individuals. Additionally, collection localities, elevational range and number of moth specimens sampled in previous years by light trapping are given in parentheses.

not been recorded previously. Unfortunately, the morphology of the imago remains unknown in this latter species because rearing was not successful. For all other collected *Eois* species whose caterpillars feed on *Peperomia* plants, illustrations of adult moths are given in Figs. 1 and 2.

Life History Notes and Descriptions of Caterpillars

For most recognized *Peperomia* feeding *Eois* species, a monophagous diet was observed. Just for three taxa, *E. bolana* (Dognin), *E. spnr antiopata*, and *E. spnr violada_01*, more than one *Peperomia* species were unambiguously confirmed to serve as host plants (Table 2). On average, recorded *Eois* species fed on 1.40 ± 0.70 SE species of *Peperomia* (Table 2). This high host plant specialization is in accordance to congeneric moth species living on other host plant genera (Dyer and Gentry 2002; Connahs et al. 2009; Janzen and Hallwachs 2009; Bodner et al. 2010, 2012; Blies 2014; Dyer et al. 2015). Furthermore, five out of eight *Peperomia* morphospecies, which serve as host plants, harbor at least two *Eois* taxa (mean number of *Eois* species per *Peperomia* species: 1.75 ± 0.71 SE; Table 2). Caterpillars of all 10 *Eois* species associated with *Peperomia* live solitarily. This life history trait seems to be dominant for this genus as gregarious feeding was only reported for occasional cases on *Piper* (F.B. and G.B., unpublished data, Wilson et al. 2012). Furthermore, clumped occurrence of *Eois* caterpillars was never observed on *Peperomia* plants. This might be one reason that, in contrast to some *Piper* feeding *Eois*, leaf skeletonization (Salazar et al. 2013) or complete defoliation of individual plants (Connahs et al. 2009) was never observed on *Peperomia*.

The caterpillars of all species fed on leaves and in some cases also on sprouts and infructescences of their *Peperomia* host plants. Feeding damage on leaves attained three different forms: 1) scraping from the underside of the leaves, sparing the upper epidermis, so that damaged leaf parts appear transparent (UF = underside feeders); 2) chewing

irregular holes inside the leaf blades (HF = hole feeders), and 3) feeding from the margin of the leaf blade (MF = margin feeders). In the laboratory, caterpillars of all species fed during day and night time, so no distinct time schedule of foraging activity was observed.

In captivity, duration of the fourth instar ranged between 4 and 7 d, and the L5 stage from 5 to 9 d. Pupation of all species occurred in flimsy cocoons, which is a common form within Geometroidea (Scoble 1992), between plant parts or in the paper towel lining. The duration of the pupal stage was recorded for four species. In *E. bolana*, *E. spnr violada_01* and *E. spnr violada_02*, time until moth eclosion ranged from 14 to 17 d. The elfin forest species *E. spnr antiopata* showed a distinctly longer pupal time, varying between 19 and 25 d.

Parasitism by tachinid flies (14 cases), and more rarely by ichneumonid (5 cases) and braconid wasps (1 case) was observed only in the *E. spnr violada* species complex. All tachinid flies and most ichneumonid wasps lived solitarily and, in contrast to observations reported by Connahs et al. (2009), tachinid larvae emerged from the final caterpillar instar. Altogether, as many as 57.1% of caterpillars from the *E. spnr violada* complex, which represented 24.4% of all *Eois* caterpillars found on *Peperomia*, turned out to be infested by parasitoids. This overall parasitism rate is nearly identical to the observed rate of ≈24% for *Eois* spp. whose caterpillars feed on *Piper* in the study area (F.B., unpublished data) whereas Connahs et al. (2009) reported merely 12.0% parasitism for Ecuadorian *Eois* spp. However, the observed parasitism rate of *Eois* in the study area is higher than for Geometridae in general (16.6%, Bodner et al. 2010), indicating a high incidence of parasitoids attacking these caterpillars. Because priority was given to an unambiguous species demarcation at least by DNA barcoding, 31 preimaginal stages (nonmature caterpillars, prematurely deceased caterpillars or crippled pupae) were preserved in ethanol before possible parasitism could have become apparent. Thus, the true parasitism rate might have been even higher. Unequivocal cases of parasitism were only recognized in the area of RBSF, i.e., at midelevations. The observed decrease

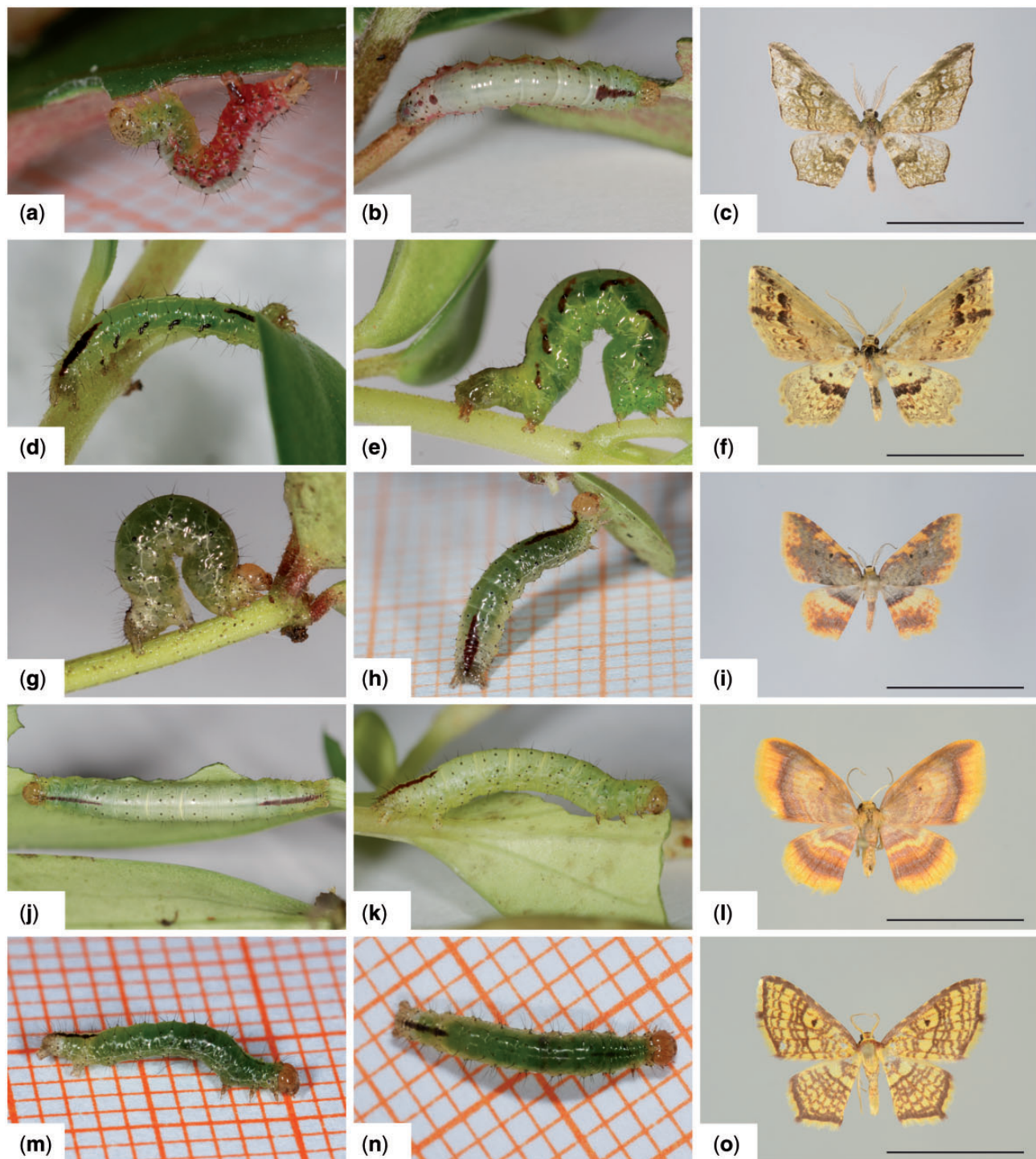


Fig. 1. Caterpillars (L5 stage) and moths of five *Eois* species associated with *Peperomia* plants in the study area: (a–c): *E. albosignata* (Dognin, 1911); (d–f): *E. bolana* (Dognin, 1899); (g–i): *E. chasca* (Dognin, 1899); (j–l): *E. spnr antiopata* (Warren, 1904); (m–o): *E. spnr concatenata* (Prout, 1910). Photographs c and i by G. Brehm, m and n by A. Broadbent; all other photographs by C. L. Seifert. Scale bar = 1 cm.

of parasitism rate with increasing elevation is congruent to the results by Connahs et al. (2009).

Young larvae, until the third instar, were not distinguishable to morphospecies level and in most cases showed a monochrome green coloration. In the fourth and fifth instars they developed more characteristic maculations and are marked with black circular pinacula bearing single setae. For all species but *E. spnr antiopata*, especially pinacula of seta SD 1 are, in contrast to the others, distinctly developed at least from A2 to A5. In the last instar, a determination to species level or at least an affiliation to a species complex based on caterpillar morphology was

possible in all cases. In combination with other ecological data, such as the elevational distribution and host plant species (see Table 2), only the cryptic sister species complex of *E. spnr violada* could not be distinguished further without resorting to DNA barcode data.

1 *Eois trillista* Clade

Eois albosignata (Dognin, 1911)
(Fig. 1a and b)

Length: 12 mm. The stocky larva is colorful with well-developed black pinacula. Head capsule brownish gray with darker spots. Thorax

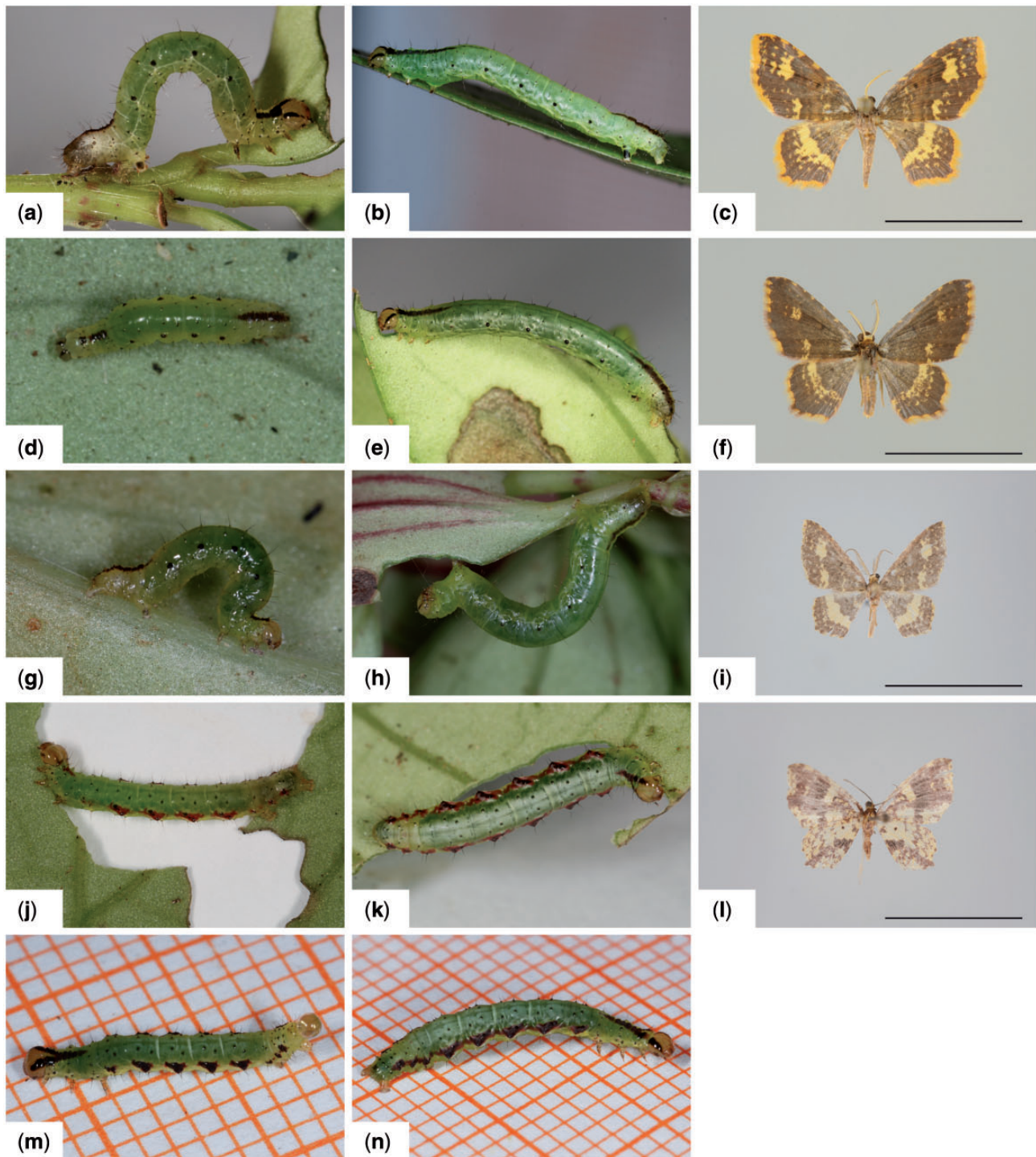


Fig. 2. Caterpillars (d, g: L4, all others: L5 stage) and moths of five *Eois* species associated with *Peperomia* plants in the study area: (a–c): *E. spnr violada_01* (Dognin, 1899); (d–f): *E. spnr violada_02* (Dognin, 1899); (g–i): *E. spnr violada_03* (Dognin, 1899); (j–l): *E. spnr vinosata_01* (Warren, 1907); (m–n): *E. spnr vinosata_02* (Warren, 1907). Photographs i and l by G. Brehm, m and n by A. Broadbent; all other photographs by C. L. Seifert. Scale bar = 1 cm.

segments laterally green, successively grading into a reddish coloration on the abdominal segments. Dorsally whitish, with a continuous brown dorsal line from T1 to A1, absent from segments A2 to A5, and reduced to 2–3 dots from A6 up to A9. In contrast to other larvae of *Peperomia* feeding *Eois* spp., the first part of the middorsal line broadens from T1 to A1. The prolegs are laterally darkish colored. Caterpillars were found at locations with a low canopy cover (20–40%), e.g., more open habitats beside broader forest trails. Host plants grew as epiphytes on trees at heights from 40 to 200 cm above ground. Feeding type: MF, UF.

Eois bolana (Dognin, 1899)

(Fig. 1d and e)

Length: 14–16 mm. Caterpillars are with bright green body coloration and little black pinacula 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 mostly interrupted (see Fig. 1d). If dorsal line is interrupted, then just present from T1 to A1 and A5 to A9. Lateral on A2–A6 five diagonal, reddish brown to black streaks which can be at least partially connected with the dorsal line, if present. The

degree of canopy cover at sample localities varied from 20 to 95%. Caterpillars were found on epiphytes growing from 30 to 210 cm above ground. Feeding types: MF, UF. Larvae also feed on shoot axis.

2 *Eois chasca* Clade

Eois chasca (Dognin, 1899)

(Fig. 1g and h)

Length: 11 mm. Coloration of the head is yellowish brown. The body is stocky and dark green with prominent black pinacula. A broad dorsal line of reddish brown coloration is present from T1 to A2 and A5 to A10. The host plant grew at a height of 85 cm at a very shady place, where canopy cover was about 95%. Feeding type: UF.

Eois spnr antiopata (Warren, 1904)

(Fig. 1j and k)

Length: 12–13 mm. The larva is greenish blue with black pinacula spots. The head is colored yellow brown without spots. The reddish brown dorsal line is present from T1 to A1 and from A6 to A10. In lateral view without any prominent characteristics. In all cases the host plants grew as epiphytes on trees at heights between 80 and 180 cm. Canopy cover above host plants ranged from 20 to 100% depending on the site. Feeding types: MF, UF. Larvae also feed on infructescences.

3 *Eois sagittaria* Clade

Eois spnr concatenata (Prout, 1910)

(Fig. 1m and n)

Length: 15 mm. The body is dark green with black pinacula spots; head is orange brown. The middorsal line is only weakly developed from T2 to A1, but well-marked from A6 to A9 with a dark brown color. One single caterpillar was found on *Peperomia eburnea* aff., which grew epiphytically 50 cm above ground. Feeding type: UF.

4 *Eois odatis* Clade

Eois spnr violada_01 (Dognin, 1899)

(Fig. 2a and b)

Length: 15 mm. The head capsule is yellowish brown. Larvae show dark-green body coloration with an interrupted dark-brown to black dorsal line which is only present from T1 to A1 and again from A6 to A10. The anterior part of the dorsal line can be continued as a bifurcated, acuminate line along the sides of the head capsule (Fig. 2f and g). Pinacula of SD 1 on segments A1 (A2)-A5 are always prominently developed. Prolegs of A6 can laterally show a black spot (Fig. 2f and g). Larvae feed at least on two different *Peperomia* spp., which grow both on the ground and as epiphytes in places with a canopy cover of 85% and higher. Caterpillars were found exclusively on epiphytic plant individuals up to 120 cm above ground. Feeding type: MF, UF.

Eois spnr violada_02 (Dognin, 1899)

(Fig. 2d and e)

This species is morphologically indistinguishable from *Eois spnr violada_01* and *Eois spnr violada_03*. Larvae feed on *Peperomia 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. Feeding type: MF, UF.

Eois spnr violada_03 (Dognin, 1899)

(Fig. 2g and h)

This species is morphologically indistinguishable from *Eois spnr violada_01* and *Eois spnr violada_02*. Larvae likewise feed on plants of *Peperomia 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. Feeding type: MF, UF. Larvae also feed on infructescences.

5 Unknown Clade Assignment

Eois spnr vinosata_01 (Warren, 1907)

(Fig. 2j and k)

Length: 13 mm. The body color is dark green with setae inserted in prominent black pinacula. A reddish dorsal line is just present from

T1 to T3 and bifurcates on T1 towards the head, in some individuals continuing on the head capsule in two rounded black bands (Fig. 2b). Single individuals show a relict dorsal line from A6 to A8 which is reduced to three much brighter reddish dots. Dorsally the larvae vary from greenish body color to white. Laterally, the segments from A1 (A2) onwards to A8 are dark red with black dots, sometimes with white coloration below and above. These lateral markings can be merged or interrupted between the segments, but are always well distinct on A2–A5. They are accompanied by broad, short humps giving caterpillar body a jagged appearance in dorsal view. Larvae were observed feeding on *Peperomia curtipes* aff. which occurred in humid and very shady habitats with dense canopy cover. Feeding type: HF, MF.

Eois spnr vinosata_02 (Warren, 1907)

(Fig. 2m and n)

Length: 13 mm. 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 within both species. This species is closely related to *E. spnr vinosata_01*, with a barcode distance (2.3%) just above the threshold accepted here. This species was, however, collected at a lower elevation in a very different forest type. Found in one single individual as larva only. The host plant also belongs to *Peperomia curtipes* aff. Feeding type: HF, MF.

In conclusion, our study has shown that the speciose moth genus *Eois* is less strongly bound to *Piper* than previously assumed. Further investigations on trophic interactions should start with surveying plant genera closely related to *Piper*. Particularly species of other Magnoliidae plant families (e.g., Winteraceae, Canellaceae, Lauraceae, Monimiaceae) and of Chloranthaceae appear promising candidates (Blies 2014, Seifert 2014). We further expect that intensive search on epiphytic *Peperomia* plants at greater tree heights will increase the number of *Eois* host plant records in that plant genus. Additionally, phytochemical investigations and a mapping of host plant interactions within a rigorous phylogenetic framework are required to better understand the ecology and evolution of host dynamics of *Eois* and could also help to identify further potential host plant taxa.

Descriptive data as presented in this study remain indispensable for further developing a phytophagous insect taxon, 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 histories of candidate taxa becomes available.

Supplementary Data

Supplementary data are available at *Journal of Insect Science online*.

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References Cited

- Barbaro, L., and I. van Halder. 2009.** Linking bird, carabid beetle and butterfly life-history traits to habitat fragmentation in mosaic landscapes. *Ecography* 32: 321–333.
- Beck, E., J. Bendix, I. Kottke, F. Makeschin, and R. Mosandl. 2008.** Gradients in a tropical mountain ecosystem of Ecuador. *Ecological Studies* 198, Springer Verlag, Berlin Heidelberg, Germany.
- Blies, D. 2014.** Caterpillar assemblages on *Hedyosmum* shrubs along an elevational gradient in a tropical mountain forest in south Ecuador. Diploma Thesis, University of Trier, Germany.
- Bodner, F., G. Brehm, J. Homeier, P. Strutzenberger, and K. Fiedler. 2010.** Caterpillars and host plant records for 59 species of Geometridae (Lepidoptera) from a montane rainforest in southern Ecuador. *J. Insect Sci.* 10: 1–22.
- Bodner, F., P. Strutzenberger, G. Brehm, and K. Fiedler. 2012.** Species richness and host specificity among caterpillar ensembles on shrubs in the Andes of Southern Ecuador. *Neotrop. Entomol.* 41: 375–385.
- Brehm, G., F. Bodner, P. Strutzenberger, F. Hünefeld, and K. Fiedler. 2011.** Neotropical *Eois* (Lepidoptera: Geometridae): checklist, biogeography, diversity, and description patterns. *Ann. Entomol. Soc. Am.* 104: 1091–1107.
- Brehm, G., P. Strutzenberger, and K. Fiedler. 2013.** Phylogenetic diversity of geometrid moths decreases with elevation in the tropical Andes. *Ecography* 36: 1247–1253.
- Connahs H., G. Rodríguez-Castañeda, T. Walters, T. Walla, and L. Dyer. 2009.** Geographic variation in host-specificity and parasitoid pressure of an herbivore (Geometridae) associated with the tropical genus *Piper* (Piperaceae). *J. Insect Sci.* 9: 1–11.
- deWaard, J. R., P. D. N. Hebert, and L. M. Humble. 2011.** A comprehensive DNA barcode library for the looper moths (Lepidoptera: Geometridae) of British Columbia, Canada. *PLoS One* 6: e18290.
- Dyer, L. A., and G. L. Gentry. 2002.** Caterpillars and parasitoids of a tropical lowland wet forest. (<http://www.caterpillars.org>) (accessed 10 July 2015).
- Dyer, L. A., J. S. Miller, S. B. Rab Green, G. L. Gentry, H. F. Greeney, and T. W. Walla. 2015.** Caterpillars and parasitoids of the eastern Andes in Ecuador. (<http://www.caterpillars.org>) (accessed 10 July 2015).
- Dyer, L. A., M. S. Singer, J. T. Lill, J. O. Stireman, G. L. Gentry, R. J. Marquis, H. F. Greeney, D. L. Wagner, H. C. Morais, I. R. Diniz, et al. 2007.** Host specificity of Lepidoptera in tropical and temperate forests. *Nature* 448: 696–699.
- Frenette-Dussault, C., B. Shipley, and Y. Hingrat. 2013.** Linking plant and insect traits to understand multitrophic community structure in arid steppes. *Funct. Ecol.* 27: 786–792.
- Gradstein, S. R., J. Homeier, and D. Gansert. 2008.** The tropical mountain forest—patterns and processes in a biodiversity hotspot. *Biodiversity and Ecology Series*, vol. 2. Universitätsverlag Göttingen, Göttingen, Germany.
- Hausmann, A., G. Haszprunar, and P. D. N. Hebert. 2011.** DNA barcoding the geometrid fauna of Bavaria (Lepidoptera): successes, surprises, and questions. *PLoS One* 6: e17134.
- Hausmann, A., H. C. J. Godfray, P. Huemer, M. Mutanen, R. Rougerie, E. J. van Niekerken, S. Ratnasingham, and P. D. N. Hebert. 2013.** Genetic patterns in European geometrid moths revealed by the Barcode Index Number (BIN) system. *PLoS One* 8: e84518.
- Homeier, J., S.-W. Breckle, S. Günter, R. T. Rollenbeck, and C. Leuschner. 2010.** Tree diversity, forest structure and productivity along altitudinal and topographical gradients in a species rich Ecuadorian montane rain forest. *Biotropica* 42: 140–148.
- Isnard, S., J. Proserpi, S. Wanke, S. T. Wagner, M.-S. Samain, S. Trueba, L. Frenzke, C. Neinhuis, and N. P. Rowe. 2012.** Growth form evolution in Piperales and its relevance for understanding Angiosperm diversification: an integrative approach combining plant architecture, anatomy, and biomechanics. *Int. J. Plant Sci.* 173: 610–639.
- Janzen, D. H., and W. Hallwachs. 2009.** Dynamic database for an inventory of the macrocaterpillar fauna, and its food plants and parasitoids, of Area de Conservación Guanacaste (ACG), northwestern Costa Rica. (<http://janzen.sas.upenn.edu>) (accessed 10 July 2015).
- Miller, J. S., and L. Dyer. 2009.** Special feature: diversity of insect-plant interactions in the eastern Andes of Ecuador. *J. Insect Sci.* 9: 1–3.
- Novotny, V., and S. E. Miller. 2014.** Mapping and understanding the diversity of insects in the tropics: past achievements and future directions. *Austral. Entomol.* 53: 259–267.
- Novotny, V., S. E. Miller, L. Baje, S. Balagawi, Y. Basset, L. Cizek, K. J. Craft, F. Dem, R. A. I. Drew, J. Hulcr, et al. 2010.** Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *J. Anim. Ecol.* 79: 1193–1203.
- Rajaei, H., J.-F. Struwe, M. J. Raupach, D. Ahrens, and J. W. Wägele. 2013.** Integration of cytochrome c oxidase I barcodes and geometric morphometrics to delimit species in the genus *Gnopharmia* (Lepidoptera: Geometridae, Ennominae). *Zool. J. Linn. Soc.* 169: 70–83.
- Ratnasingham, S., and P. D. N. Hebert. 2007.** BOLD: the barcode of life data system (www.barcodinglife.org). *Mol. Ecol. Notes* 7: 355–364.
- Ratnasingham, S., and P. D. N. Hebert. 2013.** A DNA-based registry for all animal species: the barcode index number (BIN) system. *PLoS One* 8: e66213.
- Rodríguez-Castañeda, G., L. A. Dyer, G. Brehm, H. Connahs, R. E. Forkner, and T. R. Walla. 2010.** Tropical forests are not flat: how mountains affect herbivore diversity. *Ecol. Lett.* 13: 1348–1357.
- Salazar, D., D. H. Kelm, and R. J. Marquis. 2013.** Directed seed dispersal of *Piper* by *Carollia perspicillata* and its effect on understory plant diversity and folivory. *Ecology* 94: 2444–2453.
- Scoble, M. J. 1992.** *The Lepidoptera*. Oxford University Press, New York.
- Scoble, M. J. 1999.** *Geometrid moths of the World: a catalogue*. vol. 2. CSIRO Publishing, Collingwood, Australia.
- Seifert, C. L. 2014.** Species diversity and phylogenetic position of *Eois* (Lepidoptera: Geometridae: Larentiinae) on *Peperomia* (Piperaceae) in a mountain rainforest in the south Ecuadorian Andes. M.Sc. thesis, University of Vienna, Vienna. (<http://othes.univie.ac.at/32993/>) (accessed 14 July 2015).
- Stehr, F. W. 1987.** *Immature insects*. Kendall/Hunt Publishing Company, Dubuque.
- Strutzenberger, P., G. Brehm, and K. Fiedler. 2011.** DNA barcoding-based species delimitation increases species count of *Eois* (Geometridae) moths in a well-studied tropical mountain forest by up to 50%. *Insect Sci.* 18: 349–362.
- Strutzenberger, P., G. Brehm, F. Bodner, and K. Fiedler. 2010.** Molecular phylogeny of *Eois* (Lepidoptera, Geometridae): evolution of wing patterns and host plant use in a species-rich group of Neotropical moths. *Zool. Scr.* 39: 603–620.
- Tamura, K., G. Stecher, D. Peterson, A. Filipowski, and S. Kumar. 2013.** MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* 30: 2725–2729.
- Wanke, S., M.-S. Samain, L. Vanderschaeve, G. Mathieu, P. Goetghebeur, and C. Neinhuis. 2006.** Phylogeny of the genus *Peperomia* (Piperaceae) inferred from the *trnK/matK* region (cpDNA). *Plant Biol.* 8: 93–102.
- Wilson, J. S., M. L. Forister, L. A. Dyer, J. M. O'Connor, K. Burls, C. R. Feldman, M. A. Jaramillo, J. S. Miller, G. Rodríguez-Castañeda, E. J. Tepe, et al. 2012.** Host conservatism, host shifts and diversification across three trophic levels in two Neotropical forests. *J. Evol. Biol.* 25: 532–46.

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