

# Species Richness and Host Specificity among Caterpillar Ensembles on Shrubs in the Andes of Southern Ecuador

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## Abstract

Caterpillar ensembles were sampled on 16 species of shrubs from the family Asteraceae and the genus *Piper* (Piperaceae) in open and forest habitats in the Andean montane forest zone of southern Ecuador between August 2007 and May 2009. Trophic affiliations of caterpillars to the host plants were confirmed in feeding trials. Overall, species richness of herbivorous caterpillars was high (191 species across all plants), but varied strongly between ensembles associated with different plant species (2–96 lepidopteran species per shrub species). Ensembles on *Piper* species were characterized by low effective species numbers and high dominance of one or two species of the Geometridae genus *Eois* Hübner. Low species number and high dominance were also found on latex-bearing *Erato polymnioides*, whereas ensembles on two other Asteraceae species were far more diverse and less strongly shaped by a few dominant species. The observed diversity patterns fit well to the concept that anti-herbivore defenses of plants are the major factors regulating associated insect ensembles. Local abundance and geographic range of host plants appear to have less influence. Lepidopteran species feeding on Asteraceae were found to be more generalistic than those feeding on *Piper* species. We conclude that caterpillar ensembles on most, but not all, studied plant species are defined by a small number of dominant species, which usually are narrow host specialists. This pattern was more distinct on *Piper* shrubs in forest understory, whereas Asteraceae in disturbed habitats had more open caterpillar ensembles.

## Introduction

Tropical insect herbivore ensembles on plants remain in the focus of ecological research (Novotny & Basset 2005). Their diversity, host specificity and ensemble structure have been investigated at a number of sites around the world with varying scales and focus (e.g. Diniz & Morais 1997, Novotny *et al* 2002, Dyer *et al* 2007, Skippari *et al* 2009, Janzen & Hallwachs 2011). Among the topics of particular interest are the underlying mechanisms causing the overall high diversity of tropical insects (Novotny *et al* 2006), the total extent of their diversity (Hamilton *et al*

2010) and their vulnerability to habitat loss (Koh *et al* 2004). However, those studies are still comparatively few and they often arrived at contradicting conclusions. For example, Novotny *et al* (2002) characterized tropical herbivore ensembles on trees as “predictably simple” in that they were dominated by few abundant specialists with little spatial and temporal variability, while previous studies had emphasized high levels of randomness (e.g. Floren & Linsenmair 1998). On the matter of host specialization, Novotny *et al* (2006) concluded that tropical herbivorous insects are no more specialized than in temperate-zone ensembles, whereas Dyer *et al* (2007) observed significantly

higher specialization in tropical ensembles. Rodríguez-Castañeda *et al* (2010) have recently shown that patterns of host plant use may change with elevation, adding yet another dimension of complexity.

Further investigations into this field are obviously required to reveal the patterns and processes that shape tropical herbivore – host plant interactions. In particular, data from additional regions and plant taxa may be the cue to resolve conflicting conclusions and improve our understanding of mechanisms that drive herbivore ensembles on individual plant species in the tropics.

We here present results from a study on ensembles of herbivorous caterpillars on shrubs in a tropical montane rainforest in southern Ecuador. Herbivore ensembles on shrubs are assumed to be somewhat less species rich than those on trees (Lawton 1983), which are more commonly the focus of investigations into tropical host–herbivore interactions. Specifically, we studied the caterpillar ensembles of 16 shrub species from the families Asteraceae and Piperaceae, and address the following questions: (1) Do herbivorous caterpillar ensembles on Asteraceae and Piperaceae differ with regard to their observed or effective species richness or dominance structure?; (2) Is caterpillar species richness on shrubs correlated with the geographical range, local abundance or anti herbivory defense mechanisms of the shrub species?; (3) Does host specificity of herbivores differ between Piperaceae and Asteraceae?; and (4) Are the studied caterpillar ensembles “predictably simple” in their structure, as suggested by Novotny *et al* (2002)?

## Material and Methods

### Study area

We studied caterpillar ensembles in the Reserva Biológica San Francisco (RBSF), a nature reserve adjacent to Podocarpus National Park in southern Ecuador (province Zamora-Chinchipec), located on the eastern slope of the Andes. This area has been the target of intensive ecological research since 1997 (Beck *et al* 2008a), including studies on moth ensembles along elevational and disturbance gradients (e.g. Brehm & Fiedler 2003, Fiedler *et al* 2008, Hilt & Fiedler 2008). It has been identified as a global diversity hotspot of moths (Brehm *et al* 2005). RBSF is mostly covered by nearly pristine montane rain forest and by various types of succession vegetation of different stages after anthropogenic interventions (Beck *et al* 2008b, c, Homeier *et al* 2008).

Caterpillars were sampled in forest and neighboring open habitats between 1,800 and 2,050 m asl, in proximity to the Estación Científica San Francisco (3°58'S, 79°05'W). Sampling periods lasted from August 2007 to March 2008 and from December 2008 to June 2009.

### Study organisms

In both plant families, the major criteria for selection of target species were shrub-like growth form, availability of a sufficient number of replicate individuals, and their accessibility. Three species from different genera of the family Asteraceae were selected (*Ager-Ageratina dendroides*, *Baccharis latifolia* and *Erato polymnioides*), which are highly abundant in open and successional habitats in the RBSF (Beck *et al* 2008b). No other Asteraceae shrubs at our sites were sufficiently abundant to be included in this study.

Within the family Piperaceae, we selected 13 species of *Piper*, representing the most species rich (Homeier & Werner 2007) and abundant (own observations; see also Homeier *et al* 2008) genus of forest understory shrubs in the RBSF. Taxonomy of *Piper* is still poorly resolved (Jaramillo & Manos 2001). To the present, six of the 13 species from this study have been formally identified, while the remaining species are tentatively referred to by morphospecies numbers. The only *Piper* species often encountered in heavily disturbed, open or half-open areas in the RBSF was *Piper lenticellosum*. The other 12 species were confined to closed forest of various succession stages. The 13 studied *Piper* species comprised all representatives of the genus that were reasonably common along forest trails between 1,800 and 2,050 m asl.

We surveyed a total of 194 individuals from these 16 plant species. Not all shrub individuals were sampled throughout the entire study. Some died off or were destroyed by falling trees or flooding, while others were only selected later in the project to replace losses. To estimate the abundance of our target shrub species, we performed a preliminary inventory along the trail system in RBSF before the onset of herbivore sampling. While these counts were incomplete we accept them to largely reflect the relative abundances of targeted shrubs in the area.

All immatures of any Lepidoptera family encountered during sampling were collected. The only exceptions were leaf miners and stem borers. Miners were rarely encountered and require much effort to rear at high mortality in the laboratory (own observations). Stem borers cannot be sampled without destroying the target plant, which would have rendered resampling impossible.

### Field collections and laboratory rearing

Sample collection was accomplished by means of a two-staged sampling approach. This consisted of first visually searching the target shrub and secondly beating the shrub with a wooden stick over a white sheet of 1×1 m<sup>2</sup>. For both stages, sampling effort was standardized by an estimation of the shrub's crown volume. For details on the sampling method, see Bodner *et al* (2010b). Caterpillar sampling was performed every 6 weeks resulting in a total of eleven temporal replicates per shrub.

All collected specimens were stored in small plastic boxes and subsequently reared in the laboratory in larger plastic boxes lined with damp paper towels to provide constant high air humidity. Trophic associations were verified by feeding trials: caterpillars that did not immediately accept leaves or flowers of the shrub they originated from were first additionally offered dead leaves, foliose lichens and epiphyllic lichens and algae. If those were not accepted either, leaves from various other plants growing either in the close vicinity of the target shrub or known to be accepted by many polyphagous caterpillar species were offered as alternative. Caterpillars were kept in the laboratory until death or emergence of either the adult moth or parasitoids.

### Sorting and identification

All lepidopteran herbivores encountered were carefully sorted to morphospecies level through a multi-stage process. Species delimitation and identification was based on an integrative taxonomy approach (Schlick-Steiner *et al* 2010) using a combination of adult morphology, caterpillar morphology and DNA barcoding. Specimens that we managed to rear to adults were identified by comparison with photographs, with published literature, or identified by taxonomic experts. Identification of the remaining specimens was first attempted by comparison of photographs of the caterpillars with those of caterpillars that had developed into adults. If this was not possible, larvae were matched to adults by DNA barcodes provided that the specimens were still suitable for DNA extraction. The use of DNA sequences for identification of immature stages is well established (e.g. Miller *et al* 2005, Ahrens *et al* 2007, Strutzenberger *et al* 2011). The barcoding region (Hebert *et al* 2003) of the mitochondrial cytochrome-oxidase subunit I gene was sequenced using primers LepF/LepR (Hebert *et al* 2003), for details, see Strutzenberger *et al* (2011). We aligned our sequences with 1,993 reference sequences generated by the iBOL project ([www.boldsystems.org](http://www.boldsystems.org)) from adult Geometridae and Erebidae (Arctiinae) collected at the RBSF (G. Brehm, unpublished data). Species identification and delimitation was assisted by neighbor joining

trees. Trees were calculated with MEGA version 4.3 (Tamura *et al* 2007) using the Kimura two-parameter distance model (Kimura 1980). Sequences with a pairwise distance of less than 3% were considered conspecific. In total 409 barcodes were generated from both unidentified immatures and adult reference specimens (see Electronic Supplementary Material (ESM) Table S1 for a complete list of GenBank accession numbers). Samples that remained unidentified had to be discarded from all analyses. For the present study, only caterpillars belonging to species that were confirmed to feed on living plant tissues of the target shrubs were considered, viz. all caterpillars that turned out to feed on epiphylls, foliose lichens, dead plant material, or for which no trophic association could be established were excluded.

### Data analysis

Egg and larval numbers in large clutches and caterpillar groups were down-weighted to their cubic root. The reasoning for this was (a) their overrepresentation: egg clutches and large larval groups are comparatively easy to find, while solitary early instars are far more cryptic; and (b) raw numbers were overstating their apparent ecological importance. For example, in the case of *Altinote dicaeus albofasciata* Hewitson (Lepidoptera: Nymphalidae), the most prominent case of gregarious caterpillars in our data set, mortality of early instars was excessive and later instars did not nearly show up at comparable relative abundances in the dataset (own observations). Down-weighting to cubic root transformed the average values for egg clutches and caterpillar groups of this species to similar values as counts of survivors at early post-gregarious instars.

To assess herbivore species richness per plant species, we calculated Coleman rarefaction curves, Mao Tau species accumulation curves and the species richness estimators ICE (Colwell 2009) plus the abundance based versions of first, second and third order Jackknife estimators. We subsequently selected the species richness estimator most suitable for this range of coverage according to Brose & Martinez (2004). This procedure was only changed for *Piper* sp. VIII, as, due to data structure, second and third order Jackknife estimators returned meaningless values lower than observed species richness and were therefore ignored. Dominance was calculated as Berger–Parker indices—i.e. the contribution of the most abundant species to the dataset (first-order dominance). We also calculated modifications to additionally include the second and third most abundant caterpillar species per shrub (termed second and third order dominance hereafter). To additionally account for differences in the sampled crown volume per species, we re-scaled the x-axis of the Coleman rarefaction

curves for every shrub species by its average crown volume. This correction factor was also used in correlation analyses. As replicate units, we treated the collected samples, comprised of all herbivore specimens collected from one plant individual during one sampling event. We consider those samples to be sufficiently independent from those taken from the same plant 6 weeks earlier or later, as our sampling removed the majority of caterpillars from the shrubs (Bodner *et al* 2010b) and almost all overlooked caterpillars would have either died or completed their development before the next sampling event (own observations).

As a measure of herbivore diversity we calculated the bias-corrected exponentiated version of Shannon's entropy (hereafter termed bc-exp( $H_s$ )), which represents the effective number of species (Jost 2006), with the program SPADE (Chao & Shen 2009). For this purpose, we aggregated all available samples for each of the studied shrub species. Herbivore diversity measures for shrub species with small sample sizes (<50 caterpillars) must be treated with caution. All other standard statistical procedures, such as correlation analyses, were performed with the software Statistica 7.1 (Statsoft Inc 2005).

For analysis of the possible influence of plant geographic range on herbivore diversities, we extracted data on plant distributions from the online database of the Missouri Botanical Garden (<http://www.tropicos.org/>). We exported available records for those plant species identified to species level into Google Earth (Google Inc. 2010) and counted the number of occupied 1×1° latitude×longitude grids as a rough measure of species distribution areas.

To evaluate the degree of host specificity for every caterpillar species, we combined data from this study, own unpublished observations, and host plant affiliation data from Brehm (2003), Bodner *et al* (2010a), Dyer *et al* (2010) and Robinson *et al* (2010). Caterpillar species were then grouped by their host plant range on both plant family and plant species level. Following Fiedler (1998), we distinguished monophagous (reported from only one plant family/species), oligophagous (reported from two or three plant families/species) and polyphagous (reported from four or more plant families/species) caterpillar species. We restricted this analysis to Macrolepidoptera, as data on microlepidopterans from Ecuador is too scarce. Further, we excluded all herbivore species that could not be identified at least to genus level, to allow for a minimum of comparability with literature data. Finally, we excluded all herbivore species for which we could not obtain at least four records, as this is the minimum number where a species could theoretically rank as polyphagous using the aforementioned definition.

## Results

In total, 18,890 preimaginal specimens of Lepidoptera (8,929 caterpillars, 9,885 eggs and 76 pupae) were collected in the course of this study (see ESM Table S2 for a complete list of species). Of these, 477 (2.5%) were excluded as likely strays, since no trophic connection with the target shrubs or their epiphylls could be established. Another 1,396 (7.4%) were found to feed on epiphyllous or epiphytic lichens, algae or detritus. These were also excluded from analysis since they did not qualify as herbivores of the target shrubs in a strict functional sense. Caterpillars that feed on detritus or epiphylls in their early instars, but switch to living plant tissue of the target shrub species in their later instars, were retained in the dataset. Finally, another 295 caterpillars—mostly microlepidopterans and very early instars of Geometridae and Noctuidae—were excluded as they could not be reliably assigned to a (morpho)-species.

The largest part of the remaining dataset (16,722 individuals) comprised caterpillar groups (109 groups with 4,692 individuals) and egg clutches (43 clutches with 9,047 eggs) of gregarious *A. dicaeus albofasciata* on *E. polymnioides*. After down-weighting of larval groups and egg clutches, Nymphalidae was the second most prominent family in the dataset (16.1%) following Geometridae (70.1%). The majority of Geometridae were members of the genus *Eois* on *Piper* shrubs (47.2% of entire dataset), a moth genus known to be largely comprised of specialist herbivores of Piperaceae (Dyer *et al* 2010, Strutzenberger *et al* 2010).

Four of the surveyed *Piper* species (*densiciliatum*, *subscutatum*, VIII, XV) yielded less than 50 herbivorous caterpillars each and were consequently excluded from some of the analyses and subsequent discussion, as these low numbers may cause erratic behavior of diversity estimators and indices.

A total of 191 different herbivorous caterpillar species were encountered during this study. On individual plant species, their number ranged from only two on *P. lenticellosum* to 96 on *B. latifolia*. While observed herbivore species richness per *Piper* species averaged at 9.5 and never exceeded 18, herbivore richness was far larger on all studied Asteraceae species with a minimum of 40 (Fig 1, Table 1). On Asteraceae species, bc-exp( $H_s$ ) of herbivores ranged from 2.79 to 33.97. On Piperaceae species, it ranged from 1.07 to 9.11 with an average of 5.58. After exclusion of the four most scarcely populated *Piper* species, which ranked highest in bc-exp( $H_s$ ), the maximum value was reduced to 7.15, and the average to 4.44.

On most studied shrub species, estimated total caterpillar species richness was far higher than observed species numbers. Besides, the ensemble on *P. lenticellosum*, that

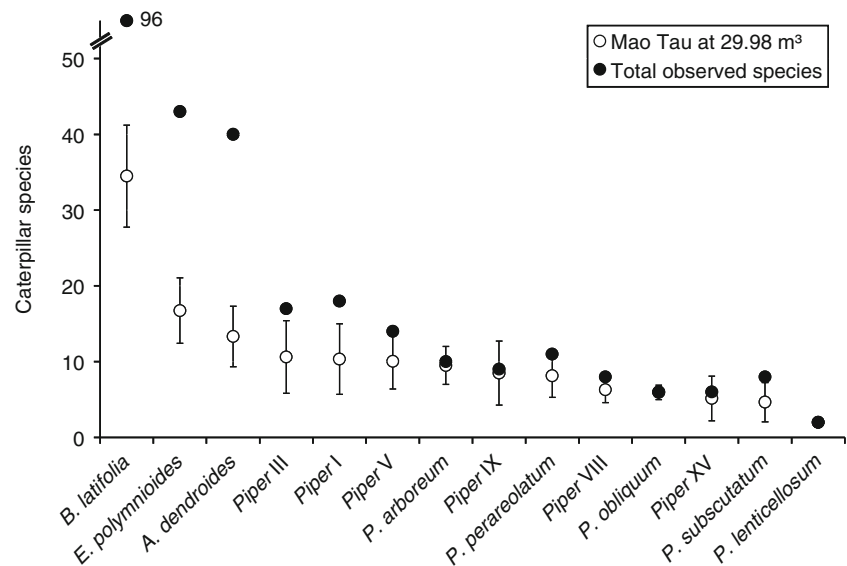


Fig 1 Rarefied species richness (Mao Tau) at 29.98 m<sup>3</sup> of sampled shrub volume (empty circles) with 95% confidence intervals (whiskers), and total observed species numbers (filled circles) for all shrub species with 29.98 m<sup>3</sup> or more total sampled shrub volume.

estimators predicted to be covered entirely by our samples, only the ensembles on four further shrub species (*Piper arboreum*, *Piper obliquum*, VIII, and XII) were estimated to be more than 53% complete. Mean coverage was 57.0% overall (Asteraceae, 46.6%; Piper, 59.4%).

Inspection of Coleman rarefaction curves confirms these results and yields further insights. The curve of *B. latifolia* starts off steeply and runs well above all others (Fig 2a), again emphasizing the far higher diversity of caterpillar ensembles on this shrub species as compared to all others covered in this study. The curves of most *Piper* species also feature a steep increase at the start (Fig 2b), but flatten off notably faster than those of *A. dendroides* and *E. polymnioides* (with the exception of *Piper densiciliatum*, see below), which soon overtake them. This means that a comparatively more substantial fraction of the caterpillar fauna of *Piper* shrubs is covered with a limited number of samples, while samples from *A. dendroides* and *E. polymnioides* continue to accumulate species. On most shrub species, the curves display the usual shape (e.g. Xuan Mao *et al* 2005) and are still far from approaching their asymptote. However, for some shrub species the geometry of the curves is notably different. The curves of *P. lenticellosum*, *P. obliquum* and *P. sp. XII* flatten off much more strongly than in all other plant species, indicating that the caterpillar inventory is close to completion. In contrast, the curve of *P. densiciliatum* increases steeply in an almost linear fashion with no sign of saturation.

We correlated the abundance of shrub species with their associated numbers of herbivore caterpillar species. These analyses were restricted to shrubs from the genus *Piper*, to avoid taxonomic bias. Absolute numbers of observed species correlated notably ( $r^2=0.572$ ,  $P=0.003$ ) with total sampled shrub volume of the specific plant species. Estimated total caterpillar numbers correlated

moderately with shrub abundance ( $r^2=0.395$ ,  $P=0.021$ ). Correlations based on rarefaction to the largest common total shrub volume for the 12 (excluding *P. densiciliatum*) and 11 (excluding *P. densiciliatum* and XII) best-sampled shrub species yielded similar trends, but no significant results ( $r^2 \leq 0.355$ ,  $P \geq 0.053$ ). Even weaker relationships were found between shrub abundance and bc-exp( $H_5$ ) values ( $r^2=0.203$ ,  $P=0.223$ ; calculated only for the nine *Piper* species with more than 50 caterpillar specimens).

Host plant specificity was evaluated for those 76 macrolepidopteran species that were identified at least to genus level and had at least four records associated with them (Fig 3). Overall, specificity of these herbivores on the level of attacked host plant families differed distinctly between the sets of species associated with the two studied plant families. While 48.8% of the caterpillar species found on Asteraceae are only known to feed on plants of this family, 74.4% of caterpillar species on Piperaceae were scored as monophagous at the plant family level. This difference in host plant specificity became even more distinct when considered at the plant species level: On Asteraceae, only 11.6% of the caterpillar species were monophagous and 53.5% were polyphagous, whereas on Piperaceae, 62.8% were monophagous and 25.6% polyphagous.

Berger–Parker dominance indices ranged from 0.170 (0.348 for top three species) on *A. dendroides* to 0.987 (1.000 for top two species, as only two were found) on *P. lenticellosum* (Fig 4). The median value for dominance was 0.450 for all shrub species, and did not differ much between Asteraceae (0.426; range, 0.170–0.839) and *Piper* species (0.475; range, 0.320–0.987). However, while all three dominance values on *E. polymnioides* ranked high, values for the other two Asteraceae were notably lower. *Ageratina dendroides* returned the lowest values for all three indices, while *B. latifolia* was intermediate for top



Table 1 Herbivore species richness and diversity values for the studied shrub species

	Spp.	N	Bc- exp( $H_S$ )	Rarefied species	Estimated total species	Coverage (%)	Total shrub volume (m <sup>3</sup> )	N shrubs
<i>A. dendroides</i>	40	112	33.97	13.9	85.5 <sup>a</sup>	46.8	161.57	21
<i>B. latifolia</i>	96	791.3	19.96	36.8	182.8 <sup>a</sup>	52.5	201.92	21
<i>E. polymnioides</i>	43	611.4	2.79	17.5	105.8 <sup>a</sup>	40.6	119.38	24
<i>P. arboreum</i>	11	61.5	7.15	9.5	14.0 <sup>c</sup>	78.8	47.78	10
<i>P. densiciliatum</i>	8	22	9.11	-	25.1 <sup>a</sup>	31.9	14.36	10
<i>P. lenticellosum</i>	2	313	1.07	2.0	2.0 <sup>d</sup>	100	29.98	7
<i>P. obliquum</i>	6	62	3.87	5.9	7.0 <sup>c</sup>	85.9	31.59	10
<i>P. perareolatum</i>	10	128	2.71	8.2	21.8 <sup>a</sup>	45.9	47.84	10
<i>P. subscutatum</i>	8	24	7.34	4.8	16.5 <sup>a</sup>	48.5	80.55	10
<i>Piper</i> I	18	541	5.14	10.7	36.9 <sup>a</sup>	48.7	96.78	11
<i>Piper</i> III	17	195.4	6.24	11.1	43.7 <sup>a</sup>	38.9	80.11	10
<i>Piper</i> V	14	108	6.22	10.1	26.8 <sup>a</sup>	52.2	62.53	10
<i>Piper</i> VIII	8	21	7.70	6.8	9.0 <sup>c</sup>	89.4	48.24	12
<i>Piper</i> IX	9	54	4.16	8.5	24.3 <sup>a</sup>	37.0	33.23	9
<i>Piper</i> XII	7	151.6	3.44	-	9.0 <sup>b</sup>	77.9	19.82	8
<i>Piper</i> XV	6	11	8.44	5.1	16.3 <sup>a</sup>	36.7	37.98	11

Rarefied species are based on a shrub volume of 29.89 m<sup>3</sup>; no values are available for *P. densiciliatum* and XII, as total sampled shrub volume was below this value. Shrub species with less than 50 caterpillars found in total are shaded in gray; here, any diversity estimates are hardly informative. *Spp.* number of caterpillar species; *N* number of caterpillar individuals (decimals due to down-weighting of groups); *Bc-exp(H<sub>S</sub>)* bias-corrected exponentiated version of Shannon's entropy.

<sup>a</sup>Total species number according to third-order abundance-based Jackknife estimator.

<sup>b</sup>Total species number according to second-order abundance-based Jackknife estimator.

<sup>c</sup>Total species number according to first-order abundance-based Jackknife estimator.

<sup>d</sup>No additional species predicted by estimators.

one species and third and second lowest for top two and three, respectively. The highest dominance values were those on *P. lenticellosum*, where the most abundant caterpillar species alone already achieved higher dominance (0.987) than the top three on any other of the shrub species. As with *E. polymnioides*, the contribution of the second most abundant caterpillar species was already minute. Most other *Piper* species also reached high dominance values of >0.7 for the three most abundant caterpillar species, but their dominance values increased in a more stepwise fashion.

## Discussion

### *Species richness, diversity and dominance structure*

We found the studied shrub species to overall harbor a rich and abundant caterpillar fauna and further sampling would likely still increase those numbers substantially. However, while most plant species harbored a similar number of species, there were notable outliers. The difference in terms of species richness between the poorest ensemble on *P. lenticellosum* and the most species rich ensemble on

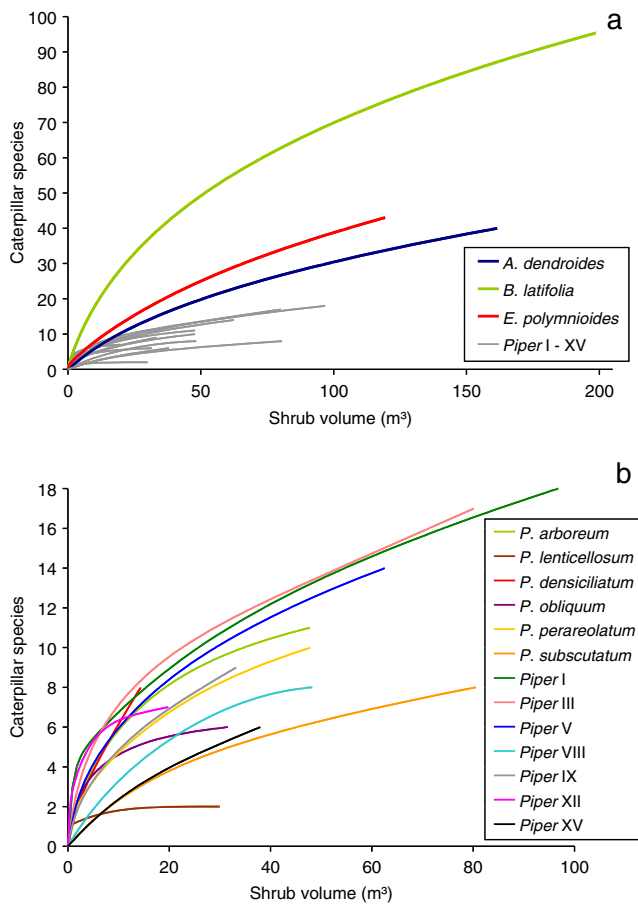


Fig 2 Coleman rarefaction curves for all shrub species with Asteraceae highlighted (a) and only for *Piper* spp. (b). Curves discussed in the main text are labeled. Color figures with all species indicated are shown in the online version.

*B. latifolia* was more than 15-fold for rarefied, and even more than 90-fold for estimated total caterpillar species numbers (Table 1).

Caterpillar ensembles on asteracean shrubs were notably more diverse than on *Piper* shrubs. All three Asteraceae ranked higher than any *Piper* species in total number of

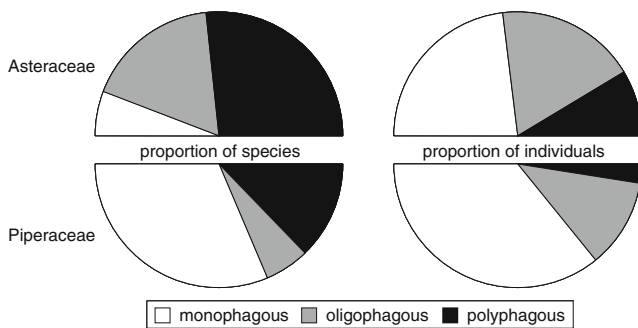


Fig 3 Proportion of species (left) and individuals belonging to species (right) considered as poly- (black), oligo- (gray), and monophagous (white) at plant species level on Asteraceae (top) versus *Piper* species (bottom).

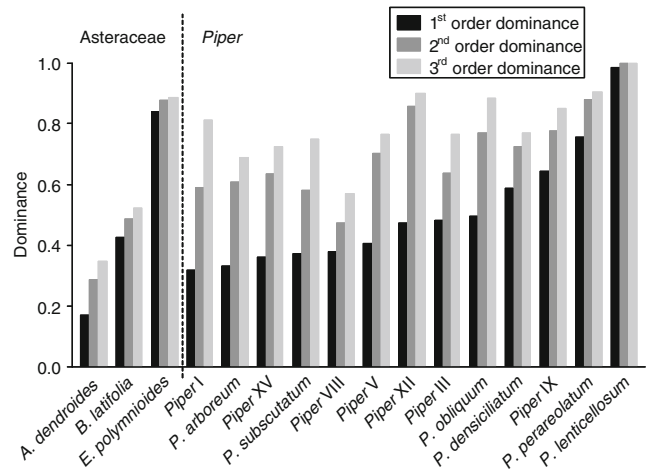


Fig 4 First (black), second (dark gray) and third (light gray) order dominance values of herbivores for all sampled shrub species. Shrub species sorted first by family, then by first order dominance.

both observed and predicted species. This rank order persisted after rarefaction to largest common total sampled shrub volume, although the differences between *A. dendroides* and *E. polymnioides* and the highest ranking *Piper* species vanished almost entirely and only *B. latifolia* retained a clear lead. Inspection of the shape of the species rarefaction curves also confirmed the higher herbivore richness on all three Asteraceae, as curves continue to ascend in a more steady way than even the most herbivore-rich *Piper* species.

Comparison of Shannon diversities, i.e. the effective number of species in an ensemble, and dominance values revealed similar patterns. In terms of effective species numbers, *A. dendroides* and *B. latifolia* retained their clear lead over all *Piper* species with sufficiently large data sets for comparison. Both feature comparatively low dominance values. The high Shannon diversity for *A. dendroides*, which reaches close to the total number of observed species, shows that there is no subset of real core herbivores of *A. dendroides* that would define its ensemble. This is also confirmed by the low dominance values and the low number of herbivore individuals on this shrub. Instead, *A. dendroides* harbors a substantial number of caterpillar species, each one rarely encountered. *Erato polymnioides*, to the contrary, scores far lower in Shannon diversity, while dominance values are far higher. With an effective species number of only 2.8, it ranks at the lower end of the range occupied by the *Piper* species. This indicates that, unlike on the other two Asteraceae and notably similar to most *Piper* species, the ensemble on *E. polymnioides* is defined by a very small number of specialist herbivores, the most prominent of which was the gregarious butterfly *A. dicaeus albofasciata*.

*Piper* species with sufficient data for detailed analysis featured low effective species numbers between 1.1 and 7.2. High levels of dominance with the three most

abundant species usually accounting for around 75% of the ensembles also indicate that their herbivore ensembles are defined by a small set of core species. Inspection of raw data for these nine *Piper* species revealed that those three most abundant caterpillar species were almost always members of the geometrid genus *Eois*, with few other taxa in third places. Only one non-*Eois* scored as second rank herbivore, namely on *P. lenticelloseum*, where only one *Eois* species was found. Most caterpillar observations on *Piper* spp. beyond the few dominant species resulted from accumulation of very rare species, or from generalists that likely utilize *Piper* only as minor host plants. Further sampling effort would likely increase their numbers, as suggested by unsaturated species accumulation curves and estimates of sampling coverage. To identify the core ensembles, on the other hand, in most cases a notably lower sampling effort would have sufficed, as rarefied species numbers for 30 m<sup>3</sup> of sampled shrub volume already surpassed effective species numbers for all shrub species with sufficient data with the only exception of *A. dendroides*.

Within the genus *Piper*, the highest Shannon diversity values were obtained for *P. densiciliatum* and XV. We excluded both from quantitative comparisons due to very small sample sizes, and they both featured a high fraction of species encountered in only a single individual (six of eight and four of six species, respectively). High proportions of singletons, a common feature of tropical herbivore ensembles, can pose problems for the study of host–herbivore interactions (Novotny & Basset 2000). It is often hard to establish whether they represent a naturally rare host association or tourist individuals. While true tourists were identified and excluded through our extensive feeding trials, the possibility still persists that a confirmed trophic association occurs but accidentally in nature.

Novotny et al (2003) reported on caterpillar ensembles from three *Piper* species in comparison with records from further 68 species of other woody plants from Papua New Guinea. The median of dominance values reported here (0.450) is close to that of 0.48 in Novotny et al (2003). Equally, dominance values for our *Piper* species lie in a similar range as those reported for one native (*Piper macropiper*) and two introduced (*Piper aduncum* and *Piper umbellatum*) *Piper* in the New Guinean study, although none rank as low as the 0.09 reported for the introduced *P. umbellatum*. We also calculated bias-corrected Shannon diversities from their data. Values for the two introduced *Piper* species (*P. aduncum*, 8.054; *P. umbellatum*, 25.728) ranked above all of our *Piper* species with the exception of *P. densiciliatum* and XV, both of which we excluded from our analysis due to their small data sets. In contrast, the native New Guinean *P.*

*macropiper* scored even lower than most of our Ecuadorian species (1.684).

### Factors shaping ensemble patterns

We expect that the observed diversity patterns are the consequence of a variety of factors. Some studies have reported a positive correlation between the extent of a plant's geographic range and the species richness of its associated herbivores (reviewed in Lewinsohn et al 2005). We therefore plotted rarefied herbivore species richness against plant geographic range (Fig 5). As species identifications are essential for assigning ranges, only the three Asteraceae species and five *Piper* species with assigned names and sufficient data could be included in this analysis. This limited dataset did not allow for statistical evaluation, but two different patterns are visible in the diagram. Species richness of caterpillar ensembles on Asteraceae appears to be positively influenced by plant geographic range, whereas no such trend is apparent in *Piper* species. We tentatively conclude that plant geographic range has only limited influence on herbivore diversity in our samples—if at all—and that such an influence is not consistent between different plant families.

The number of caterpillar species per plant species was moderately correlated with the plant's abundance in the study area. However, this relationship became ever more obscured the better the sampling effects in the data were controlled for. Hence, even though abundance of individual plant species can have a strong influence on the species richness of its associated insect herbivores (Kelly & Southwood 1999), this does not appear to be a strong driver in the herbivore ensembles of our study.

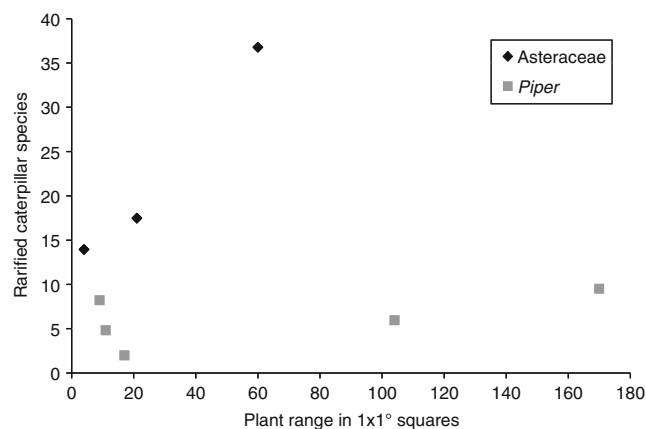


Fig 5 Rarefied species richness of herbivore communities plotted against estimated geographic range of the host plant species (obtained by counting 1×1° squares occupied with records from the Missouri Botanical Garden database). Only plant species that were identified to species level and with a sampled volume of ≥29.98 m<sup>3</sup> (rarefaction threshold) are included. Black diamonds Asteraceae, gray squares *Piper* spp.



Chemical defense properties of plant species strongly affect their herbivores, although not much is currently known on how they influence associated herbivore ensembles as a whole (Lewinsohn *et al* 2005). Unfortunately, there is hardly any data available on the toxicity of the plant species of this study for herbivorous insects. *Ageratina* is a member of the Eupatorieae, many of which produce pyrrolizidine alkaloids (Rizk 1991). Some *Ageratina* species are toxic to mammals (Fuller & McClintock 1986), while others are known to contain at least non-toxic pyrrolizidine alkaloids (Lang *et al* 2001). PAs are usually toxic to non-specialist herbivores and used as feeding deterrents by a number of plant clades (Trigo 2011). Essential oils of *B. latifolia* were tested for insecticidal activity against disease vectors (Laurent *et al* 1997, Chantraine *et al* 1998) with little success. *Erato polymnioides*, like other species from this genus (Moran & Funk 2006), produces milky latex which likely acts as anti-herbivore defense (Agrawal & Konno 2009). *Piper* species are known to be generally well defended against herbivores (Dyer & Palmer 2004); however, pertinent data for the species included in our study are mostly lacking. Bernard *et al* (1995) found that extracts from most of 14 tested Neotropical *Piper* species, including *P. obliquum*, significantly reduced growth rate and increased mortality when added to the diet of the highly polyphagous European corn borer *Ostrinia nubilalis* Hübner (Lepidoptera: Crambidae). Despite the lack of species-specific information, the general trend to be inferred from these phytochemical studies matches with the observed species richness of the individual herbivore ensembles: the usually well-defended *Piper* rank lowest, followed by latex-bearing *E. polymnioides* and the putative pyrrolizidine alkaloid plant *A. dendroides*, and all are greatly surpassed by *B. latifolia*. However, inferences and generalizations of this sort have to be considered with care and data on the chemical defense properties of the studied plant species would be necessary to further test this hypothesis.

#### Host specificity

Host plant specificity of caterpillar species found on *Piper* plants in this study was pronouncedly higher than of those found on Asteraceae. This was especially clear on the level of utilized plant species. Increased specificity was also visible for plant families, although almost half of the lepidopteran species found on Asteraceae were scored as monophagous at this level. This is in line with results by Vanin *et al* (2008), who reported “remarkably narrow feeding preferences” for *Piper* herbivores from both field and laboratory data.

Host plant data for Neotropical caterpillars—even after exclusion of particularly poorly studied microlepidopterans—is extremely incomplete, so conclusions

remain tentative. Furthermore, although data from a number of sources were included in our analysis, the majority of records used still stems from this study and therefore has an inherent bias towards the two studied plant families. However, considering as our study includes 13 *Piper* species and only three asteracean species, bias in this dataset would rather be expected to overemphasize specificity on the asteracean side. Hence, the discovery a disproportionately high fraction of specialist feeders on *Piper* bears some weight. A possible explanation is stronger chemical defense in *Piper* species (Bernard *et al* 1995) as compared to the studied Asteraceae, rendering Piperaceae less suitable to generalist feeders and promoting the evolution of specialists like the moth genus *Eois* (Strutzenberger *et al* 2010, Strutzenberger & Fiedler 2011).

Connahs *et al* (2009) studied geographic variation in host plant utilization by *Eois* species for various Neotropical sites. They concluded that *Eois* species in Ecuador utilize on average two *Piper* species as host plants, with a 95% confidence interval of ~1.7 to 2.2 (measured from their diagram). This is notably higher than our average of 1.2 *Piper* species utilized per *Eois* species. We assume that these differences are at least partly due to the larger size of their data set. Additionally, the much larger elevational range covered in Connahs *et al* (2009) may play a role, as *Eois* diet breadth is known to change with altitude (Rodríguez-Castañeda *et al* 2010). Irrespective of these minor differences, all these lines of evidence support the notion that *Piper* species generally support herbivorous caterpillar ensembles of comparatively low diversity and characterized by high host specificity.

#### Complexity of caterpillar ensembles

Novotny *et al* (2002) reported caterpillar samples from rain forest trees in Papua New Guinea as dominated by few specialized species with little variation in assemblage structure and concluded that herbivore ensembles were “predictably simple”. This characterization also applies to most of the caterpillar ensembles on Ecuadorian shrubs reported here. However, ensembles on *A. dendroides* and *B. latifolia* notably divert from this characterization. *B. latifolia* has one moderately dominant caterpillar herbivore, *Microxydia* sp. nr. *ruficomma* Prout (Lepidoptera: Geometridae), but beyond this there exists a rich and abundant caterpillar ensemble on that shrub. In contrast, ensembles on *A. dendroides* entirely lack any even moderately dominant species, but are composed of a substantial number of rare, unpredictable species.

We conclude that the studied caterpillar ensembles differ strongly and in various ways between the two plant families and individual plant species. Certain *Piper* species

were hardly colonized by herbivorous caterpillars at all. Local abundance and geographic range of the shrub species likely modulate the local diversity of their affiliated herbivores to some degree, but overall chemical anti-herbivore defense appears to be the most important factor. Hence, edibility of individual plant species must be taken into account when attempts are made to up-scale from herbivore ensembles of plant species to entire vegetation units. As in other studies from tropical lowland forest, most ensembles harbored only few dominant and therefore functionally relevant caterpillar species—usually specialists—that define them. Hence, caterpillar ensembles on shrubs in the montane forest zone of the Andes can be considered “predictably simple” in the sense of Novotny *et al* (2002), although clear exceptions exist. The differences in specificity of herbivore ensembles of common shrubs in different habitats might indicate that with the ongoing loss of natural tropical mountain forests also the diversity of plant–herbivore interactions will be affected (Koh *et al* 2004), at the cost of highly specific interactions that prevail amongst forest understory shrubs.

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