



## Phylogenetic Overdispersion in Lepidoptera Communities of Amazonian White-sand Forests

Greg P.A. Lamarre<sup>1,2,3,6</sup>, Diego Salazar Amoretti<sup>4</sup>, Christopher Baraloto<sup>2,5</sup>, Frédéric Bénéuz<sup>3</sup>, Italo Mesones<sup>4</sup>, and Paul V.A. Fine<sup>4</sup>

<sup>1</sup> Université Antilles-Guyane, UMR Ecologie des Forêts de Guyane, Campus agronomique de Kourou, 97310 Kourou, French Guiana

<sup>2</sup> INRA, UMR Ecologie des Forêts de Guyane, Campus agronomique de Kourou, 97310 Kourou, French Guiana

<sup>3</sup> Société Entomologique Antilles-Guyane (SEAG), 18 lot Amaryllis, 97354 Rémire-Montjoly, French Guiana

<sup>4</sup> Department of Integrative Biology, University of California, 1005 Valley Life Sciences Building #3140, Berkeley, CA 94720, U.S.A.

<sup>5</sup> Department of Biological Sciences, International Center for Tropical Botany, Florida International University, Miami, FL 33199, U.S.A.

### ABSTRACT

In the Amazon basin and the Guiana Shield, white-sand (WS) forests are recognized as a low-resource habitat often composed by a distinct flora with many edaphic endemic plants. Small patches of nutrient-poor white-sand forests can pose a series of challenges to plants and animals. For plants, these challenges have been shown to function as strong filters that in turn drive taxonomic, functional and phylogenetic plant composition. However, very little is known about animal communities in WS forest and the effect that low-resource availability may have on higher trophic levels. Here, we investigate the diversity of both taxonomic and phylogenetic diversity of three Lepidoptera families' (Nymphalidae, Saturniidae, and Sphingidae) assemblages between low-resource (White-Sand Forest) and two adjacent high-resource habitats, *terra firme* clay and seasonally flooded forests. We found no clear effect of habitat type on taxonomic composition although butterfly and moth species abundance differed among the three contrasted habitats. The WS forest Lepidoptera community is significantly more phylogenetically overdispersed than expected by chance. We suggest that these low-resource habitats filter the number of plant lineages which, in turn, creates a bottom-up control structuring Lepidoptera phylogenetic structure. We recommend long-term sampling on Lepidoptera community both at larval and adult stages that may complement this study and test hypotheses linking herbivore phylogenetic structure to plant resource availability and trophic cascade theory.

*Key words:* Amazon basin; bottom-up; Lepidoptera composition; moth, Nymphalidae; phylogenetic structure; *varillales*.

IN THE AMAZON BASIN AND IN THE GUIANA SHIELD, WHITE-SAND FORESTS ARE RECOGNIZED AS A LOW-RESOURCE HABITAT COMPOSED BY A DISTINCT FLORA WITH MANY EDAPHIC ENDEMIC PLANTS (Spruce 1908, Anderson 1981, Fine & Baraloto 2016). Nutrient-poor white-sand (hereafter WS) forests pose a series of physiological challenges to organisms. For plants, these challenges have been shown to function as strong filters that in turn drive taxonomic, functional, and phylogenetic plant composition (Fine *et al.* 2010, Fine & Kembel 2011, De Oliveira *et al.* 2014, Fortunel *et al.* 2014). For example, environmental filters have been shown to select for plant species with resource conservation strategies in WS forest — *i.e.*, long leaf life span, denser and thicker leaves (Fortunel *et al.* 2014) in addition to lower leaf production rates than high-resource habitats (Lamarre *et al.* 2014). The phylogenetic structure of plant communities is also affected by the low-resource availability of the WS habitat in the Amazon basin (Fine & Kembel 2011, Guevara *et al.* 2016). Fine and Kembel (2011) found overdispersed phylogenetic structure of tree communities in Peruvian WS, and reasoned that if traits that promote habitat specialization are convergent, this overdispersed pattern could be

caused by environmental filtering. However, very little is known about the community assembly of animal communities inhabiting WS forest (but see Saaksjarvi *et al.* 2006, Álvarez Alonso *et al.* 2013) and the possible effect that low-resource availability has on higher trophic levels.

For insect herbivores, the resource limitations present in WS could play a significant role on structuring herbivore community composition, either directly or indirectly. Given that insect herbivores have been shown to feed non-randomly and often on phylogenetically related sets of host plant species (Novotny *et al.* 2002, Ødegaard *et al.* 2005), one would expect that plant species composition should affect the taxonomic and phylogenetic structure of herbivore communities. For example, if herbivores are specialists restricted to a subset of phylogenetically closely related host plants, then the phylogenetic community structure of herbivores may mirror the phylogenetic community structure of plants. On the other hand, if herbivores in a community are largely generalists with regard to host plants, then the phylogenetic community structure of plants will not influence the herbivore community structure. Several studies have repeatedly shown direct effects of plant species composition on herbivores and other higher trophic levels (Blake *et al.* 2003, Schaffers *et al.* 2008, see Lewinsohn *et al.* 2005 for a review). Moreover, plant

Received 26 November 2014; revision accepted 8 September 2015.

<sup>6</sup>Corresponding author; e-mail: greglamarre973@gmail.com

phylogenetic diversity has been shown to correlate with the structure and the composition of herbivore community (Pellissier *et al.* 2013) and/or higher trophic classes (Dinnage *et al.* 2012). These results are also consistent with the idea of a strong bottom-up control of herbivores mediated by plant phylogenetic diversity (Schuldt *et al.* 2014).

Here, we explore the effect that a low-resource habitat has on the community composition of three Lepidoptera families including butterflies (Nymphalidae) and moths (Saturniidae and Sphingidae) that are often dominant herbivores found in both tropical and temperate ecosystems. To test the relationship between habitat type and community composition, we compared taxonomic and phylogenetic diversity of assemblages between low-resource (WS) and two adjacent high-resource habitats, *terra firme* clay (hereafter TF) and seasonally flooded forests (SF) in Peru and French Guiana. In particular, we address the following questions: (1) are WS forest Lepidopteran communities taxonomically distinct from adjacent habitats? (2) How are WS Lepidopteran communities structured phylogenetically compared to high-resource forest habitats? The answers to these questions contribute to the understanding of the mechanisms underlying community assembly of both herbivore and plants in megadiverse Amazonian forests.

## MATERIALS AND METHODS

**STUDY SITES.**—Lepidopteran communities from White-sand (WS), seasonally flooded (SF) and *terra firme* (TF) clay forest habitats were sampled in four sites in Loreto, Peru and two sites in French Guiana. Within a plot network of modified-Gentry plots of 0.5-ha from a previous study, we installed two types of insect traps (see below) in 12 plots that represent the entire range of variation in climatic, edaphic, and forest structure factors observed in the larger plot network (Baraloto *et al.* 2011). Two replicates of each habitat type were sampled in each country representing a total of four habitat replicates. In French Guiana, our study plots were located at the Laussat Conservation Area in the northwest (05°28' N, 053°35' W, 2471 mm annual rainfall) and in the massif of Petite Montagne Tortue in the northeast (04°19' N, 052°14' W, 4421 mm annual rainfall). In the Department of Loreto, Peru, plots were installed in the Allpahuayo-Mishana National Reserve (Rio Nanay basin, 85°46' S, 053°12' W, 2701 mm annual rainfall) and the Jenaro Herrera Center of Investigation (Rio Ucayali basin, 84°58' S; 059° 8W, 2664 mm annual rainfall). Each habitat plot was installed at least 1 km from each other to reduce collecting specimens from adjacent habitats (*e.g.*, tourists, see Ødegaard 2004). The Guianas have a strong dry season and the department of Loreto, Peru has a mostly aseasonal climate with a few drier periods (Marengo 1998, Wagner *et al.* 2011).

**LEPIDOPTERA SAMPLING METHODS.**—We sampled adults of butterfly (Nymphalidae) and moth communities (Saturniidae and Sphingidae) weekly from August to November 2010 in French Guiana and between May and September 2011 in Peru, corresponding to

drier periods in each region. We collected specimens with aerial fruit traps (FT) for butterflies and automatic light traps (ALT) for moths. The FT consisted of four bait traps installed in the mid-understory (between 2 to 5 m). A fermented mixture of sugar, rum, and banana juice was placed onto a platform (40 cm × 40 cm) into the device, surrounded by a mosquito net cylinder (100 cm high, 30 cm diam.). For moth collections, we installed a portable automatic light trap (ALT) with an 8-W back-light tube to collect all arthropods during each new moon of the study period ( $\pm 2$  d). We installed ALTs in the middle of each plot and performed a total of 24 sampling-nights. A weak source of light such as used for ALTs does not attract insects from long distances, resulting in samples that are highly representative of the local habitat and vegetation (Merckx & Slade 2014). Within the studied plots each trap was separated by at least 20 m avoiding spatial interferences between and within type of traps. We employed equal sampling effort in terms of number of day–night periods for each trap in each plot and in each country. All Lepidoptera specimens from ALT and FT were sorted, counted, and then identified at species level. Unidentified morphospecies are included in the analyses and are still pending identification.

**SPECIES ASSEMBLY ANALYSES.**—First we investigated whether the WS forest Lepidoptera community supports a unique taxonomic composition compared to adjacent SF and TF. First, species richness per each family was plotted among forest habitats using Venn diagrams and the differences in number of species and species abundance among habitats was tested with chi-squared contingency table tests. In addition, we performed an index of habitat association using the same analyses as a recent study on tropical arthropods (Wardhaugh *et al.* 2012). This index of habitat association ( $S_m$ ) assigned species as habitat specialists ( $S_m > 0.9$ ), habitat facultative specialists ( $0.5 < S_m < 0.9$ ), or generalists ( $0.33 > S_m > 0.5$ ). Second, we compared the relative abundance of collected Lepidoptera family for each plot. We constructed a NMDS ordination using Bray–Curtis matrix similarities to visualize differences in Lepidoptera assemblages. We then tested for Lepidoptera dissimilarity among habitats and between countries with Kruskal–Wallis rank sum tests on the scores of the NMDS axes. Statistical analyses for taxonomic approach were conducted with *Vegan* (Oksanen *et al.* 2013) and *VenDiagram* (Chen & Boutsos 2011) packages in R.

**PHYLOGENETIC TREE AND ANALYSIS.**—To build a phylogenetic hypothesis for the collected lepidopteran species, we searched in GenBank for common sequences among our samples. We decided to work with the COI marker given that 61 percent of all of our study species and 98 percent of our study genera had this gene sequenced and submitted to GenBank. For specimens with no COI match in GenBank we substituted sequences of congeneric species. Congeneric species without sequences were added as polytomies to the final tree. To download the sequences from GenBank and to perform a preliminary alignment we used PhyloGenerator (Pearse & Purvis 2013). The alignment was performed using MUSCLE, MAFFT, and Clustal-Omega (Edgar

2004, Sievers *et al.* 2011, Katoh & Standley 2013). After using METAL to assess the SPP (Sum of Pairs Score) distances between alignments we selected the MUSCLE (Blackburne & Whelan 2012) alignment. We trimmed the alignment manually using ClustalX (Thompson *et al.* 1997) and we constructed our phylogenetic hypothesis with RaxML (Stamatakis 2014). We used Maximum Likelihood (GTR-GAMMA) with 50 runs and 200 replicates. Given that we only had the COI gene to build our phylogeny we also used a topological constraint tree grouping in polychromies for all members of the three families of Lepidoptera collected. Finally, we added Trichoptera (*Hydroptila* spp.) as an outgroup. This insect order forms a well-supported molecular and morphological monophyletic group with Lepidoptera (superorder Amphiesmenoptera) where Trichoptera presents more plesiomorphic characters (Wheeler *et al.* 2001). The resulting tree included 96 percent of all sampled Lepidoptera species (Fig. 4).

We used the *Picante* R package (Kembel *et al.* 2010) to calculate Phylogenetic Diversity (PD, *sensu* Faith 1992). This matrix is based on the sum of the total phylogenetic distances for a specific community sample. To assess the phylogenetic structure of the lepidopteran community, we calculated the Nearest Taxon Index (NTI) and Net Relatedness Index (NRI) per habitat and for each sampled plot. The NTI index calculates the mean distance to the closest taxon for every species in a given community. This index is especially sensitive to changes in the phylogenetic structure at the tips of the target phylogeny. The NRI index calculates the mean pairwise distance between all species in the community. Given that distances between species increase exponentially at deeper nodes of the phylogeny, this index is especially sensitive to phylogenetic patterns at the deeper nodes of the target phylogeny. Both indices are calculated using a permutation-randomization approach to assess: (1) the standardized effect size of the target sample against the expectation of randomly build null communities; and (2) the probability that this value is due to chance (*P*-value). For both indices, positive values represent communities that are more phylogenetically dispersed than expected

by chance (species are less related than expected), and negative values represent communities that are more phylogenetically clustered (species are more closely related) than expected by chance. Every analysis was performed with both presence-absence and abundance data.

## RESULTS

**TAXONOMIC COMMUNITY STRUCTURE.**—The total number of lepidopterans collected in the three forest habitats in the two countries comprised 756 individuals representing 70 Nymphalidae, 84 Saturniidae, and 27 Sphingidae species (Table 1). The largest difference among habitats in species richness among the three studied families occurred in Saturniidae with nearly twice as many species collected in TF as in WS (Fig. 1). Nymphalidae species richness did not differ significantly among the three forest types. Overall, there was relatively low overlap in composition across habitats (Fig. 1). For instance, the number of shared species across all habitats for Nymphalidae, Sphingidae, and Saturniidae was relatively small (9.1%, 7.7%, and 7.4%, respectively). Nevertheless, only Saturniidae differs significantly in species richness with low overlap between habitats ( $\chi^2 = 13.83$ , *df* = 2, *P* = 0.0009). We also found very little species overlap for each lepidopteran family between French Guiana and Peru (see Fig. S1). Overall, a higher abundance of lepidopterans was found in TF clay forests with 2.4 and 1.97 times more individuals than SF and WS, respectively. Chi-squared tests showed significant differences in species abundance among habitats for Nymphalidae ( $\chi^2 = 42.3$ , *df* = 2, *P*-value < 0.001), Saturniidae ( $\chi^2 = 90.6$ , *df* = 2, *P*-value < 2.2e-16), and Sphingidae ( $\chi^2 = 11.8$ , *df* = 2, *P*-value = 0.0027). SF habitat supports lower abundance of lepidopteran with 159 individuals collected. Most of the species showed either a distributional preference ( $0.5 < S_m < 0.9$ ) or are specialized to a habitat ( $S_m > 0.9$ ) with TF clay forest showing the greatest percentage of species specialized and/or have a clear preference to a forest type (Fig. 3). Our result does not show a greater number of specialized species in WS forest although the

TABLE 1. Overall Lepidopteran community description (per plot) showing Fisher-alpha and Simpson diversity indexes in addition to Phylogenetic diversity measures.

Country	Site	Plot	Habitat	Species richness	Overall abundance	Fisher-alpha	Simpson	Phylogenetic diversity
French Guiana	Petite Montagne	G11	WS	33	68	27.1	0.955	6,21
		G14	TF	43	95	30.3	0.967	7,10
	Tortue	G17	SF	27	43	31.1	0.957	5,63
		G3	WS	29	43	49.8	0.956	5,46
	Laussat	G4	TF	41	133	20.3	0.938	7,3
		G8	SF	29	42	47	0.957	5,72
Loreto, Peru	Jenaro Herrera	P13	WS	24	66	13.6	0.877	3,96
		P16	TF	34	87	20.5	0.948	6,22
		P19	SF	17	37	13.8	0.917	3,00
	Porvenir	P10	TF	28	79	16.5	0.949	4,58
		P3	WS	13	26	10.3	0.86	3,19
		P5	SF	16	37	13.8	0.922	3,30

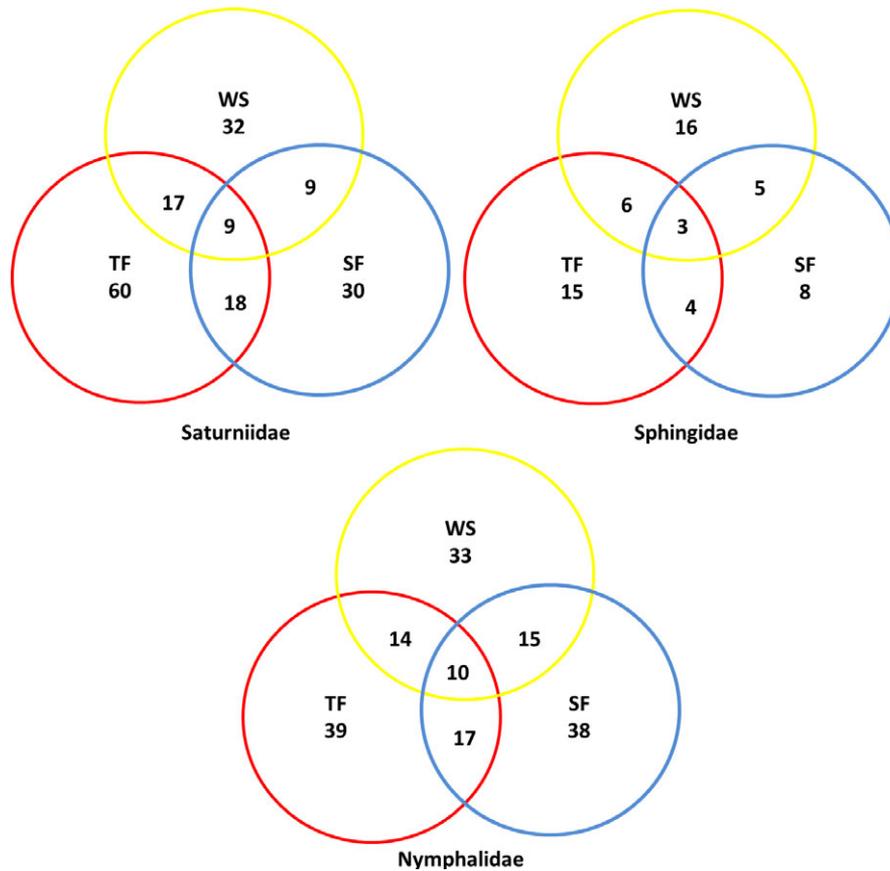


FIGURE 1. Venn diagrams illustrating the number of species in each Lepidoptera family between White-sand forest (WS, yellow), seasonally flooded forest (SF, blue), and *terra firme* clay forest (TF, red).

proportion is equally important than SF habitats (Fig. 3). A strong similarity in taxonomic community structure among habitats is observed in the NMDS ordination (Fig. 2) although it showed considerable differences in species composition between studied plots. Additionally, this ordination illustrated a strong turnover in Lepidoptera assemblage between French Guiana and Peru suggesting that continental scale geographic distance is the main factor affecting species taxonomic composition (Table 2; Fig. S1). Finally, our collection of Lepidoptera includes one exceptional moth species that were recently described as new for science (Saturniidae: Hemileucinae: *Hyperchiria mesones*) with three of the four paratypes collected in Peruvian WS forest (Bénéluz & Lamarre 2014).

**PHYLOGENETIC COMMUNITY STRUCTURE.**—The Lepidopteran community in WS showed high levels of phylogenetic overdispersion compared to TF and SF communities as well as compared to random expectation models (positive standard effect size (SES) value ( $SES > 0$ ) and high  $P$ -values ( $P > 0.95$ )). This result indicates that phylogenetic distances among co-occurring species are greater than expected by chance (Fig. 3; Table 3). Furthermore, phylogenetic overdispersion was higher for the deeper nodes of

the phylogeny of the local Lepidopteran community (mean phylogenetic distances, NRI) than for the tips of the phylogeny (mean nearest taxon index, NTI; Table 3). No significant non-random patterns of phylogenetic community structure were found for the other two habitats (Table 3).

## DISCUSSION

In spite of an apparent lack of dispersal barriers and high geographical proximity to other habitat types, the WS Lepidopteran fauna was the only habitat to show significant phylogenetic structure. Our results indicate that the Lepidopteran community found in WS is more phylogenetically overdispersed than expected by chance. This suggests that the traits that determine whether or not lepidopteran herbivores can be found in WS are not phylogenetically conserved. Based on what is currently known about the WS habitats (low-resource availability and non-random patterns of plant defense traits and phylogenetic structure), we hypothesized that these labile traits are related to the ability of Lepidoptera species to feed upon low-nutrient foliage, and the increased physical and chemical defenses from WS host plants (Fine *et al.* 2010).

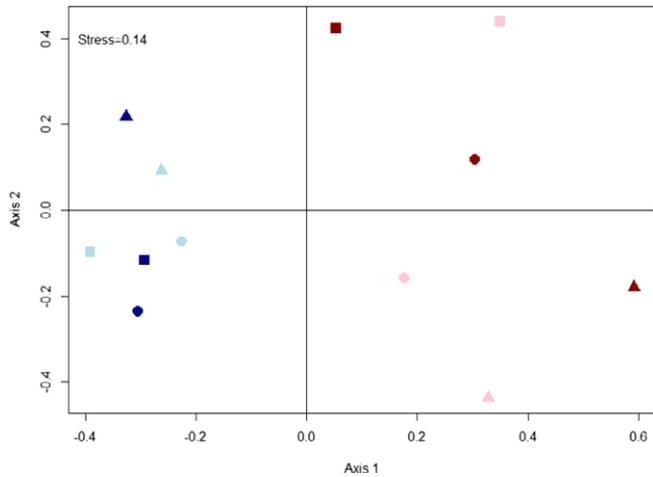


FIGURE 2. Non-metric Dimensional Scaling (NMDS) ordinations illustrating community similarity in Lepidoptera taxonomic composition among plots of different habitats, regions, and countries. French Guiana plots are colored in blue and Peruvian plots in red. Habitats include *terra firme* clay forest (circle), white-sand forest (triangle), and seasonally flooded forest (square). Light and dark blue symbols represent the Laussat and Regina regions of French Guiana, respectively. Light and dark red symbols represent the Porvenir and Jenaro Herrera regions of Peru, respectively.

TABLE 2. *Kruskal–Wallis correlation chi-squared test (with associated P-value) between the scores along the two ordination axes extracted from NMDS and habitat/country.*

Factors	Index	NMDS axis 1		NMDS axis 2	
		$\chi^2$	<i>P</i>	$\chi^2$	<i>P</i>
Habitat	Bray	0.269	0.874 <sup>NS</sup>	1.077	0.584 <sup>NS</sup>
Country		8.308	0.004 <sup>***</sup>	0.026	0.873 <sup>NS</sup>
Habitat	MPD	1.385	0.500 <sup>NS</sup>	0.615	0.735 <sup>NS</sup>
Country		3.103	0.078	0.103	0.749 <sup>NS</sup>

\*\*\* *P*<0.01 significant, NS. not significant.

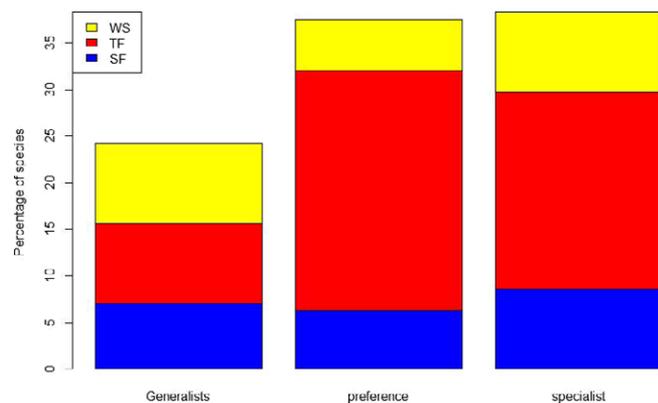


FIGURE 3. *Sm* index of habitat association. White-sand forest (WS, yellow), seasonally flooded forest (SF, blue), and *terra firme* clay forest (TF, red).

TABLE 3. *Results of Phylogenetic community structure analysis (MPD and MNPD) for the three studied habitats.*

Mean Pairwise Distance (MPD)			
Habitat	Number of taxa	Standardized effect size	
		(mpd.obs.z)	<i>P</i> -value (mpd.obs.p)
SF	72	−0.2331413	0.39
TF	113	−0.2912147	0.32
WS	80	2.5729103	1

Mean Nearest Taxon Distance (MNTD)			
Habitat	Number of taxa	Standardized effect size	
		(mntd.obs.z)	<i>P</i> -value (mntd.obs.p)
SF	72	0.131695	0.56
TF	113	0.1822968	0.56
WS	80	1.3420377	0.9

LEPIDOPTERA COMMUNITY COMPOSITION.—White-sand soils in Amazonian lowland forest have been found to harbor a unique tree species composition, but surveys of higher trophic levels have been limited to Ichneumonidae wasps (Saaksjarvi *et al.* 2006) and birds (Álvarez Alonso *et al.* 2013, Borges *et al.* 2016). We predicted that WS would support a significantly different assemblage of adult Lepidoptera compared to neighboring habitat types. Indeed, we found low overlap in species composition between WS and other habitats, although beta-diversity was high among all sampled sites, not only in WS- and non-WS comparisons (Figs. 1 and 2).

The low overlap in herbivore species composition between highly contrasted habitats may be explained by changes in host plant species composition and differences in habitat structure (such as canopy openness, see Dáttilo & Dyer 2014). Previous studies have shown that plant species composition shows strong turnover among local communities (Tuomisto *et al.* 2003) and across different habitats (Fine *et al.* 2010). Although our study suggests that environmental filtering among Lepidoptera communities was much weaker than geographic turnover (Fig. 2), we speculate that turnover in plant resources (*i.e.*, leaf, roots, and flower) could strongly influence herbivore community patterns among habitats. Thus, both geographic and habitat effects are likely to structure lepidopteran fauna. In addition, our sampling design collected only adult Lepidoptera which are very mobile organisms (*e.g.*, the ‘tourist issue’, see Ødegaard 2004). Environmental factors may not be the main driver of lepidopteran community structure, as is often assumed for tropical plant communities (Condit *et al.* 2002, Tuomisto *et al.* 2003). It is likely that some large body-sized moths such as some Sphingidae and Saturniidae could have been attracted from adjacent habitats. We therefore recommend long-term sampling in multiple seasons among these three major Amazonian forest habitats (DeVries *et al.* 2012) to enhance this analysis, and increasing the collection to sample larvae.

In spite of small sample sizes (see Fig. S2), our results showed that *terra firme* clay forests support nearly twice as many individuals than WS, a pattern consistent with predictions derived from the resource availability hypothesis (e.g., the growth-defense allocation tradeoffs, see Coley *et al.* 1985, Fine *et al.* 2006, Lamarre *et al.* 2012). Because plants growing in *terra firme* clay forests likely have more available resources, they could invest in less physical and chemical defenses, thus plant resources may be more palatable and attractive for herbivores. A study on the herbivore fauna of *Protium subserratum*, a species complex that has ecotypes on white-sand and *terra firme* forests also found significantly higher herbivore abundances in *terra firme* forests compared to white-sand forests (Fine *et al.* 2013).

Contrasting geological and edaphic histories between French Guiana and Loreto, Peru, are likely to influence the evolution and historical biogeography of the regional biota (Hoorn *et al.* 2010). Our results do show evidence of strong geographic turnover in Lepidoptera community composition between French Guiana and Peru (Fig. 2; Fig. S1). Furthermore, it is possible that both edaphic and climatic factors could act as important determinants in structuring Lepidoptera community assemblages at large geographical scales. Contrasts in soil profiles between ancient soil of the Guiana shield and young and fertile soil of Peru in addition to steep seasonality differences may influence the geographical turnover found in our studied plots (Fig. 2), a pattern already shown in Scarab beetle communities (Radtke *et al.* 2007).

**PHYLOGENETIC STRUCTURE IN WHITE-SAND LEPIDOPTERAN COMMUNITY.**—Our study revealed an unexpected pattern of phylogenetic overdispersion in the WS community of Lepidoptera. The pattern was consistent across all three sampled lepidopteran clades (Fig. 4). We predicted that the local plant phylogenetic community structure should shape herbivore community structure (Dinnage *et al.* 2012, Pellissier *et al.* 2013 and Schuldt *et al.* 2014). Our results show that this is not the case for the Lepidoptera community in WS forests. The study of Fine and Kembel (2011) that was in part conducted in the same sites found that the WS plant community was significantly phylogenetically clustered at the deeper nodes of the plant phylogeny (NRI) when relative abundance was included, an inverse pattern to the strong phylogenetic overdispersion found in this study. Insect herbivores, and specially moths and butterflies, are thought to have non-random phylogenetic feeding patterns (Ehrlich & Raven 1964, Janzen 1988, Novotny *et al.* 2002, Ødegaard *et al.* 2005). Because of this non-random plant host association, it has been proposed that changes in the plant phylogenetic structure of the plant community will be mirrored by changes in the herbivore phylogenetic community composition (Dinnage *et al.* 2012, Pellissier *et al.* 2013, Schuldt *et al.* 2014). Although we do not present plant community data, our data and findings from the same studied plots in Peru suggest that the phylogenetic structure at deeper nodes of the WS Lepidoptera community is not consistent with the phylogenetic structure of the deeper nodes of the plant community (Fine & Kembel 2011). One possible explanation for this

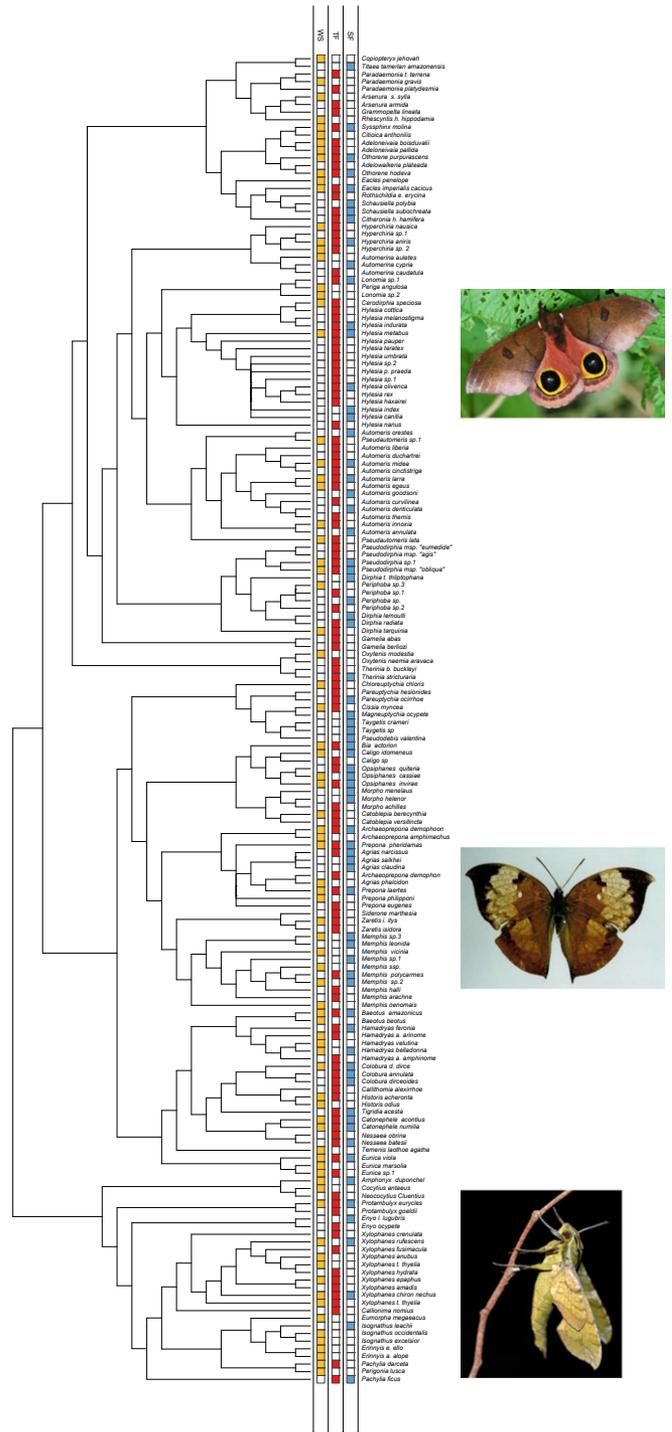


FIGURE 4. Phylogenetic tree of Tropical butterfly (Nymphalidae) and moths (Sphingidae and Saturniidae) and the habitat where they occur colored as follows: White-sand forest (WS, yellow), seasonally flooded forest (SF, blue), and *terra firme* clay forest (TF, red). Pictures represent an *Automeris innoxia* (Saturniidae), *Xylophanes pluto* (Sphingidae), and *Zaretis iys* (Nymphalidae).

contradictory pattern is that the strong habitat filtering that WS impose on the plant community (see Fine *et al.* 2005, Fine & Kembel 2011) might have selected over time convergent func-

tional traits across the Lepidoptera phylogeny (Ehrlich & Raven 1964, but see host shifts, Fordyce 2010).

White-sand forests are characterized by vegetation with pronounced sclerophylly, low diversity, and high endemism (Medina *et al.* 1990, Fine & Baraloto 2016) forging a distinct plant functional community (Fortunel *et al.* 2014). The low-resource environment is likely filtering the number of lineages of host plants available with focal tree lineages exhibiting strong habitat association (Fine *et al.* 2005, Lamarre *et al.* 2012). Only a small assemblage of herbivorous caterpillars might be able to feed on the thicker and tougher leaves exhibited by WS tree dominants (Fortunel *et al.* 2014). Similar patterns were found in hemipteran sap-suckers that modify their stylet to feed on relatively tough tissue (Pollard 1968). Moreover, studies conducted in species-rich Brazilian Cerrado presenting pronounced sclerophylly and related defenses attributes (Diniz & Morais 1997) similar to WS forest have shown a high number of host specialists (Morais *et al.* 2011), a pattern we might have predicted for WS herbivores. However, these studies did not assess phylogenetic structure among Lepidoptera assemblage. In turn, convergent adaptation in herbivores to feed on less palatable or better defended plants is likely to generate phylogenetic overdispersion found in our study. Nevertheless, other ecological interactions can also create an overdispersed pattern in herbivores.

The most important factors that would affect herbivore community structure are environmental filters and/or biotic interactions such as top-down and bottom-up control (Hunter & Price 1998, Price 2002). Nevertheless, given the scale of the local environmental factors in our study (the relative short distance between WS, SF, and TF), these are not likely to directly cause an overdispersed pattern on mobile organisms, as confirmed with our taxonomic approach. Top-down interactions are also not likely to promote overdispersed patterns because predators and parasitoids will not likely discriminate between closely related herbivores especially for adult butterflies that are not exposed to parasitism (see Jeffries & Lawton 1984 for the enemy-free space hypothesis). Finally, bottom-up control is likely to have the greatest effect on herbivore community structure (Ribeiro & Basset 1999, Price 2002). The relative strengths of bottom-up forces with plants as primary producers influencing associated higher trophic levels in tropical regions are thought to be much stronger than the influence of top-down effects (Price 2002). The low-resource environment of WS forests would directly affect higher trophic level because primary consumers have to deal with low-nutrient foliage quality, sclerophylly (Ribeiro & Basset 2009), resource conservation strategies (Fortunel *et al.* 2014), and a slow turnover of plant tissues (Lamarre *et al.* 2014).

We acknowledge the fact that the present study represents only a fraction of the total lepidopteran fauna (around 60% based on rarefaction curves, see Fig. S2), and that the small sample size makes it more difficult to interpret our results. Nonetheless, we also believe that a larger sample size would not likely change the patterns found for the phylogenetic structure of the WS lepidopteran fauna for three main reasons. First, the patterns of overdispersion were consistent between presence/absence and

abundance-weighted analyses which suggests that singletons (one of the major problems of small sample size for community data, see Ødegaard 2004) are not unduly influencing the patterns found here. Second, the overdispersed pattern is apparent on all major clades of the sampled insects (Fig. 3) suggesting that this pattern is robust to sampling effort, confirmed by the permutation-randomization approach of our phylogenetic analysis. Finally, the fact that this pattern was stronger at the deeper nodes of the phylogeny suggest that these results are not likely to be significantly altered by the addition of unsampled species as these would add tips to the branches of the tree rather than the deeper nodes.

## CONCLUSION

Our data suggest that the phylogenetic structure of the Lepidoptera community found in WS may result from the combination of a specific array of plant defense traits and local habitat filtering processes affecting plant functional diversity. Given the close relationship that herbivores share with their host plants (Ehrlich & Raven 1964), we expect that the presence or absence of compatible host plants (and their functional attributes that have evolved throughout the lepidopterans multiple time) in a given habitat will greatly determine the presence or absence of plant herbivores (Weiblen *et al.* 2006). Theory and some empirical data have suggested that plants in WS forests are not only phylogenetically clustered but also that are likely to be less palatable for herbivores than in other forest habitats (Coley *et al.* 1985, Fine *et al.* 2004). Although we did not collect data on herbivore feeding preferences at the larval stage, we believe that the filtering effect that the WS forests exert on the plant community and the distribution of defense traits could be a plausible explanation for the phylogenetic overdispersed pattern found in this study.

## ACKNOWLEDGMENTS

We thank the many colleagues that participated in the fieldwork in Peru, especially Julio Sanchez, Julio Grandez, Magno Vasquez Pilco, Milagros Ayarza Zuniga and Cyrus Harp, and in French Guiana, Anthony Percevaux, Xavier Leroy, Benjamin Leudet, Benoit Burban, Jean-Yves Goret and Jocelyn Casal. We also thank taxonomist Mohamed Benmesbah for his help in butterfly identification. We thank the Direction of Areas Naturales Protegidas y Fauna Silvestre (INRENA) which provided necessary permits for study and exportation of specimens and the Jefatura de la Reserva Nacional Allpahuayo-Mishana (N°005-2011-SER-NANP-RNAM-J, permiso de exportación N°006736). We thank César A. Delgado and José Álvarez Alonzo from Instituto de Investigaciones de la Amazonia Peruana (IIAP) for logistical support and help to work in and around the Estaciones Allpahuayo-Mishana and the Centro de Investigación Jenaro Herrera. Research was supported by a collaborative NSF grant (DEB-0743103/0743800) to C. Baraloto and P.V.A. Fine, the Fond Social Européen (FSE) to G.P.A. Lamarre and an INRA Package grant to C. Baraloto. This work has benefited from an

“Investissement d’Avenir” grant managed by Agence Nationale de la Recherche (CEBA, ANR-10-LABX-25-01).

## SUPPORTING INFORMATION

Additional Supporting Information may be found with online material:

FIGURE S1. Venn diagrams illustrating the number of species in each Lepidoptera family between countries.

FIGURE S2. Rarefaction curves for each type of forest habitat using Chao rarefaction extrapolation.

## LITERATURE CITED

- ÁLVAREZ ALONSO, J., M. R. METZ, AND P. V. A. FINE. 2013. Habitat specialization by birds in Western Amazonian White-sand forests. *Biotropica* 45: 365–372.
- ANDERSON, A. B. 1981. White sand vegetation of Brazilian Amazonia. *Biotropica* 13: 199–210.
- BARALOTO, C., S. RABAUD, Q. MOLTO, L. BLANC, C. FORTUNEL, B. HERAULT, N. DAVILA, I. MESONES, M. RIOS, E. VALDERRAMA, AND P. V. A. FINE. 2011. Disentangling stand and environmental correlates of above-ground biomass in Amazonian forests. *Glob. Chang. Biol.* 17: 2677–2688.
- BÉNÉLU, F., AND G. P. A. LAMARRE. 2014. Description of a new Hyperchiria (Lepidoptera: Saturniidae, Hemileucinae) from Loreto region, Iquitos, Peru. *Eur. Entomol.* 6: 199–204.
- BLACKBURNE, B. P., AND S. WHELAN. 2012. Measuring the distance between multiple sequence alignments. *Bioinformatics* 28: 495–502.
- BLAKE, S., D. I. MCCracken, M. D. EYRE, A. GARSIDE, AND G. N. FOSTER. 2003. The relationship between the classification of Scottish ground beetle assemblages (Coleoptera, Carabidae) and the National Vegetation classification of British plant communities. *Ecography* 26: 602–616.
- BORGES, S. H., C. CORNELIUS, M. MOREIRA, C. C. RIBAS, M. COHN-HAFT, J. M. G. CAPURUCHO, C. VARGAS, AND R. ALMEIDA. 2016. Bird Communities in Amazonian White-Sand Vegetation Patches: Effects of Landscape Configuration and Biogeographic Context. *Biotropica* 48: 121–131.
- CHEN, H., AND P. C. BOUTROS. 2011. VennDiagram: A package for the generation of highly-customizable Venn and Euler diagrams in R. *BMC Bioinformatics* 11: 35.
- COLEY, P. D., J. P. BRYANT, AND F. S. CHAPIN. 1985. Resource availability and plant Anti-herbivore defense. *Science* 230: 895–899.
- CONDIT, R., N. PITMAN, E. G. LEIGH JR., J. CHAVE, J. TERBORGH, R. B. FOSTER, P. NÚÑEZ, S. AGUILAR, R. VALENCIA, G. VILLA, H. C. MULLER-LANDAU, E. LOSOS, AND S. P. HUBBELL. 2002. Beta-diversity in tropical forest trees. *Science* 295: 666–669.
- DÁTILLO, W., AND L. DYER. 2014. Canopy openness enhances diversity of ant-plant interactions in the Brazilian Amazon rain forest. *Biotropica* 4: 712–719.
- DE OLIVEIRA, A. A., A. VICENTINI, J. CHAVE, C. D. T. CASTANHO, S. J. DAVIES, A. M. Z. MARTINI, R. A. F. LIMA, R. R. RIBEIRO, A. IRIBAR, AND V. C. SOUZA. 2014. Habitat specialization and phylogenetic structure of tree species in a coastal Brazilian white-sand forest. *J. Plant Ecol.* 7: 134–144.
- DEVRIES, P. J., L. G. ALEXANDER, I. A. CHACON, AND J. A. FORDYCE. 2012. Similarity and difference among rainforest fruit-feeding butterfly communities in Central and South America. *J. Anim. Ecol.* 81: 472–482.
- DINIZ, I. R., AND H. C. MORAIS. 1997. Lepidopteran caterpillar fauna of Cerado host plants. *Biodivers. Conserv.* 6: 817–836.
- DINNAGE, R., M. W. CADOTTE, N. M. HADDAD, G. M. CRUTSINGER, AND D. TILMAN. 2012. Diversity of plant evolutionary lineages promotes arthropod diversity. *Ecol. Lett.* 15: 1308–1317.
- EDGAR, R. C. 2004. MUSCLE: A multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5: 1–13.
- EHRlich, P. R., AND P. H. RAVEN. 1964. Butterflies and plants: A study in coevolution. *Evolution* 18: 586–608.
- FAITH, D. P. 1992. Systematics and conservation: On predicting the feature diversity of subsets of taxa. *Cladistics* 8: 361–373.
- FINE, P. V. A., AND C. BARALOTO. 2016. Habitat endemism in white-sand forests: Insights into the mechanisms of lineage diversification and community assembly of the Neotropical flora. *Biotropica* 48: 24–33.
- FINE, P. V. A., D. C. DALY, G. VILLA MUÑOZ, I. MESONES, AND K. M. CAMERON. 2005. The contribution of edaphic heterogeneity to the evolution and diversity of Burseraceae trees in the western Amazon. *Evolution* 59: 1464–1478.
- FINE, P. V. A., R. GARCIA-VILLACORTA, N. PITMAN, I. MESONES, AND S. W. KEMBEL. 2010. A floristic study of the white sand forests of Peru. *Ann. Mo. Bot. Gard.* 97: 283–305.
- FINE, P. V. A., AND S. W. KEMBEL. 2011. Phylogenetic community structure and phylogenetic turnover across space and edaphic gradients in western Amazonian tree communities. *Ecography* 34: 552–565.
- FINE, P. V. A., I. MESONES, AND P. D. COLEY. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. *Science* 305: 663–665.
- FINE, P. V. A., M. R. METZ, J. LOKVAM, I. MESONES, J. M. AYARZA ZUÑIGA, G. P. A. LAMARRE, M. V. PILCO, AND C. BARALOTO. 2013. Insect herbivores, chemical innovation, and the evolution of habitat specialization in Amazonian trees. *Ecology* 94: 1764–1775.
- FINE, P. V. A., Z. J. MILLER, I. MESONES, S. IRAZUZTA, H. M. APPEL, M. H. H. STEVENS, I. SAAKSJARVI, L. C. SCHULTZ, AND P. D. COLEY. 2006. The growth defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* 87: S150–S162.
- FORDYCE, J. A. 2010. Host shifts and evolutionary radiations of butterflies. *Proc. R. Soc. B* 277: 3735–3743.
- FORTUNEL, C., C. E. T. PAINE, P. V. A. FINE, N. J. B. KRAFT, AND C. BARALOTO. 2014. Environmental factors predict community functional composition in Amazonian forests. *J. Ecol.* 102: 145–155.
- GUEVARA, J. E., G. DAMASCO, C. BARALOTO, P. V. A. FINE, M. C. PEÑUELA, O. BÁNKI, C. CASTILHO, A. VINCENTINI, D. CÁRDENAS, F. WITTMANN, N. TARGHETTA, O. PHILLIPS, J. STROPP, I. AMARAL, P. MAAS, A. MONTEAGUDO, E. M. JIMENEZ, R. THOMAS, R. BRIENEN, A. DUQUE, B. MAGNUSSON, C. FERREIRA, E. HONORIO, F. F. DE ALMEIDA MATOS, F. RAMIREZ AREVALO, J. ENGEL, P. PETRONELLI, R. VASQUEZ, AND H. TER STEEGE. 2016. Low Phylogenetic Beta Diversity and Geographic Neo-endemism in Amazonian White-sand Forests. *Biotropica* 48: 34–46.
- HOORN, C., F. P. WESSELINGH, H. ter STEEGE, M. A. BERMUDEZ, A. MORA, J. SEVINK, I. SANMARTÍN, A. SANCHEZ-MESEGUER, C. L. ANDERSON, J. P. FIGUEIREDO, C. JARAMILLO, D. RIEF, F. R. NEGRI, H. HOOGHIEMSTRA, J. LUNDBERG, T. STADLER, T. SÁRKINEN, AND A. ANTONELLI. 2010. Amazonia through time: Andean uplift, climate change, landscape evolution, and biodiversity. *Science* 330: 927–931.
- HUNTER, M. D., AND P. W. PRICE. 1998. Playing chute and ladders: Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. *Ecology* 73: 724–732.
- JANZEN, D. H. 1988. Ecological characterization of a Costa Rican dry forest caterpillar fauna. *Biotropica* 20: 120–135.
- JEFFRIES, M. J., AND J. H. LAWTON. 1984. Enemy free space and the structure of ecological communities. *Biol. J. Linn. Soc.* 23: 269–286.
- KATOH, K., AND D. M. STANDLEY. 2013. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* 30: 772–780.
- KEMBEL, S. W., P. D. COWAN, M. R. HELMUS, W. K. CORNWELL, H. MORLON, D. D. ACKERLY, S. P. BLOMBERG, AND C. O. WEBB. 2010. Picante: R tools for integrations phylogenies and ecology. *Bioinformatics* 26: 1463–1464.
- LAMARRE, G. P. A., C. BARALOTO, C. FORTUNEL, N. DAVILA CARDOZO, I. MESONES, J. GRANDEZ RIOS, M. RIOS, E. VALDERRAMA, M. V. PILCO, AND P. V. A. FINE. 2012. Herbivory, growth strategies and habitat

- specialization in tropical tree lineages: Implications for Amazonian beta-diversity. *Ecology* 93: S195–S210.
- LAMARRE, G. P. A., I. MENDOZA, P. V. A. FINE, AND C. BARALOTO. 2014. Leaf synchrony and insect herbivory among tropical tree habitat specialists. *Plant Ecol.* 215: 209–220.
- LEWINSOHN, T., V. NOVOTNY, AND Y. BASSET. 2005. Insects on plants: diversity of herbivore assemblages revisited. *Annu. Rev. Ecol. Evol. Syst.* 36: 597–620.
- MARENGO, J. A. 1998. Climatología de la zona de Iquitos, Perú. In R. Kalliola, and S. P. Flores (Eds.). *Geoecología y desarrollo amazónico: Estudio integrado de la zona de Iquitos, Perú*, pp. 35–57. *Annales Universitatis Turkuensis Ser A II. Turun Yliopisto, Turku.*
- MEDINA, E., V. GARCIA, AND E. CUEVAS. 1990. Sclerophylly and oligotrophic environments: relationships between leaf structure, mineral nutrient content, and drought resistance in tropical rain forests of the upper Rio Negro region. *Biotropica* 22: 51–64.
- MERCKX, T., AND E. M. SLADE. 2014. Macro-moth families differ in their attraction to light: implications for light-trap monitoring programmes. *Insect. Conserv. Divers.* 7: 453–461.
- MORAIS, H. C., E. R. SUJII, M. ALMEIDA-NETO, P. S. DE-CARVALHO, J. D. HAY, AND I. R. DINIZ. 2011. Host specialization and species turnover of caterpillars among hosts in the Brazilian Cerrado. *Biotropica* 43: 467–472.
- NOVOTNY, V., Y. BASSET, S. E. MILLER, P. DROZD, AND L. CIZEK. 2002. Host specialization of leaf chewing insects in a New Guinea rainforest. *J. Anim. Ecol.* 71: 400–412.
- ØDEGAARD, F. 2004. Species richness of phytophagous beetles in the tropical tree *Brosimum utile* (Moraceae): The effects of sampling strategy and the problem of tourists. *Ecol. Entomol.* 29: 76–88.
- ØDEGAARD, F., O. H. DISERUD, AND K. OSTBYE. 2005. The importance of plant relatedness for host utilization among phytophagous insects. *Ecol. Lett.* 8: 612–617.
- OKSANEN, F. J., G. BLANCHET, R. KINDT, P. LEGENDRE, P. R. MINCHIN, R. B. O'HARA, G. L. SIMPSON, P. SOLYMO, M. H. H. STEVENS, AND H. WAGNER. 2013. *Vegan: Community Ecology Package*. R package version 2.0-10.
- PEARSE, W. D., AND A. PURVIS. 2013. *PhyloGenerator: An automated phylogeny generation tool for ecologists*. *Methods Ecol. Evol.* 4: 692–698.
- PELLISSIER, L., C. NDIRIBE, A. DUBUIS, J. N. PRADERVAND, N. SALAMIN, A. GUISAN, AND S. RASMANN. 2013. Turnover of plant lineages shapes herbivore phylogenetic beta diversity along ecological gradients. *Ecol. Lett.* 16: 600–608.
- POLLARD, D. G. 1968. Stylet penetration and feeding damage of *Eupteryx melissae* Curtis (Hemiptera, Cicadellidae) on sage. *Bull. Entomol. Res.* 58: 55–71.
- PRICE, P. W. 2002. Resource-driven terrestrial interactions webs. *Ecol. Res.* 17: 241–247.
- RADTKE, M. G., C. R. V. FONSECA, AND G. B. WILLIAMSON. 2007. The old and young Amazon: Dung beetle biomass, abundance, and species diversity. *Biotropica* 39: 725–730.
- RIBEIRO, S. V., AND Y. BASSET. 2009. Gall-forming and free-feeding herbivory along vertical gradients in a lowland tropical rainforest: The importance of leaf sclerophylly. *Ecography* 30: 663–672.
- SAAKSJARVI, I., K. RUOKOLAINEN, H. TUOMISTO, S. HAATAJA, P. V. A. FINE, V. VARGAS, G. CARDENAS, AND I. MESONES. 2006. Comparing composition and diversity of parasitoid wasps and plants in an Amazonian rainforest mosaic. *J. Trop. Ecol.* 22: 167–176.
- SCHAFFERS, A. P., I. P. REMAKERS, K. V. SÝKORA, AND C. J. F. ter BRAAK. 2008. Arthropod assemblages are best predicted by plant species composition. *Ecology* 89: 782–794.
- SCHULDT, A., M. BARUFFOL, H. BRUELHEIDE, S. CHEN, X. CHI, M. WALL, AND T. ASSMANN. 2014. Woody plant phylogenetic diversity mediates bottom-up control of arthropod biomass in species-rich forests. *Oecologia* 176: 171–182.
- SIEVERS, F., A. WILM, D. DINEEN, T. J. GIBSON, K. KARPLUS, W. LI, R. LOPEZ, H. McWILLIAM, M. REMMERT, J. SÖDING, J. D. THOMPSON, AND D. G. HIGGINS. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol. Syst. Biol.* 7: 539.
- SPRUCE, R. 1908. *Notes of a botanist in the Amazon and Andes*. MacMillan, London.
- STAMATAKIS, A. 2014. RAXML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312–1313.
- THOMPSON, J. D., T. J. GIBSON, F. PLEWNIAC, F. JEANMOUGIN, AND D. G. HIGGINS. 1997. The CLUSTAL X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 25: 4876–4882.
- TUOMISTO, H., K. RUOKOLAINEN, M. AGUILAR, AND A. SARMIENTO. 2003. Floristic patterns along a 43-km long transect in an Amazonian rain forest. *J. Ecol.* 91: 743–756.
- WAGNER, F., B. HÉRAULT, C. STAHL, D. BONAL, AND V. ROSSI. 2011. Modeling water availability for trees in tropical forests. *Agric. For. Meteorol.* 151: 1202–1213.
- WARDHAUGH, C. W., N. E. STORK, W. EDWARDS, AND P. S. GRIMBACHER. 2012. The overlooked biodiversity of flower-visiting invertebrates. *PLoS One* 7: e45796.
- WEIBLEN, G. D., C. O. WEBB, V. NOVOTNY, Y. BASSET, AND S. E. MILLER. 2006. Phylogenetic dispersion of host use in a tropical insect herbivore community. *Ecology* 87: 62–75.
- WHEELER, W. C., M. WHITING, Q. D. WHEELER, AND J. CARPENTER. 2001. The phylogeny of the extant hexapod orders. *Cladistics* 17: 113–169.