

Mesophyll cell-sucking herbivores (Cicadellidae: Typhlocybinae) on rainforest trees in Papua New Guinea: local and regional diversity of a taxonomically unexplored guild

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Abstract. 1. Host specificity of a guild of sucking insects tapping leaf mesophyll cells (Auchenorrhyncha: Typhlocybinae) was surveyed for the first time in the tropics, on 65 woody species from a lowland rainforest in Papua New Guinea (PNG).

2. Typhlocybinae species were host specific, feeding on 1–3 (median 1) plant species. Their assemblages did not functionally connect populations of different plant species, as an overwhelming majority (> 99%) of tree species pairs coexisting in the same forest did not share any typhlocybinae species.

3. Cell-sucking typhlocybines were more specialised than phloem- and xylem-sucking Auchenorrhyncha. Typhlocybines were also more specialised in PNG than on trees in temperate Europe, even after standardisation for different phylogenetic diversity of tropical and temperate trees.

4. The cell-sucking guild was species poor, with 0–5 (median 1) typhlocybinae species per tree species. Their distribution among tree species conformed to a Poisson distribution, suggesting that tropical typhlocybinae assemblages are not saturated with species.

5. Early succession plants supported a higher number of typhlocybinae species than primary forest hosts but this preference could not be explained by successional trends in specific leaf area, foliar nitrogen content, wood density, tree abundance, or tree size.

6. The effective specialisation of typhlocybines on 65 plant species $E=0.79$ was extrapolated to the entire known flora of PNG and used to estimate that there may be at least 2775 typhlocybinae species in PNG, in comparison to the global total of only 4508 taxonomically described species, including merely 40 from PNG.

Key words. Auchenorrhyncha, effective specialisation, food webs, Papua New Guinea, plant–insect interactions, species diversity.

Introduction

Herbivore guilds are highly variable in their local species diversity and host specificity (Novotny *et al.*, 2010). We can expect similar variance also in the size of their regional species pools, determined by plant species diversity, herbivore diversity

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per plant species, and host specificity (Novotny *et al.*, 2012). Unfortunately, regional species pools are virtually unknown for insect herbivorous guilds in tropical regions. Our estimates of global species richness also remain tentative and based mostly on a single, leaf-chewing, guild (Hamilton *et al.*, 2013).

Host specificity can determine the potential impact of herbivores on their host plants species. Specialised herbivores may have density dependent impacts on their host plant species and thus maintain plant diversity in tropical forests (Wright,

1983; Dyer & Letourneau, 2003) while generalists can mediate apparent competition among their shared hosts (Van Veen *et al.*, 2006). The latitudinal trend of increasing host specialisation of insect herbivores from temperate to tropical forests could explain a parallel trend in their species richness, but empirical evidence remains controversial, and limited to leaf-chewers; higher host specificity in the tropics was found for Neotropical Lepidoptera (Dyer *et al.*, 2007) but not for Palaeotropical leaf-chewers (Novotny *et al.*, 2006).

Verification of host species, requiring feeding experiments and rearing to adults, and quantifying the damage caused to plants is more difficult in sap-sucking insects than in chewing herbivores. A disproportionate number of tropical studies have thus focused on leaf-chewing guilds, particularly caterpillars and beetles, whereas sap-sucking insects, although species-rich in tropical forests (Hodkinson & Casson, 1991), have been especially neglected (Novotny & Basset, 2005). Sap-sucking insects can be classified into four guilds defined by a combination of feeding mode and target plant tissue, namely insects feeding by (i) tapping phloem vascular elements (Sternorrhyncha and a majority of Auchenorrhyncha), (ii) tapping xylem vascular elements (Auchenorrhyncha: Cicadoidea, Cercopoidea and Cicadellidae: Cicadellinae), (iii) dissolving and sucking leaf tissues (Heteroptera), and (iv) piercing and sucking individual leaf mesophyll cells (Auchenorrhyncha: Cicadellidae: Typhlocybinae) (Novotny & Wilson, 1997; Andrew & Hughes, 2005).

The mesophyll cell-sucking guild comprises a single clade of small and taxonomically difficult insects, often requiring dissection of genitalia for morphological identification of species. It is thus often ignored in ecological studies in the tropics, even in those focused specifically on the Auchenorrhyncha (e.g. Wolda, 1980). This is unfortunate as Typhlocybinae represent an important part of the entire Auchenorrhyncha diversity in tropical communities (e.g. 18% of species in a New Guinea rainforest; Basset & Novotny, 1999), as well as temperate zone regional faunas (e.g. 25% of species in Great Britain; Stewart, 2013; no tropical faunas have been sufficiently documented).

Mesophyll cell-suckers constitute an ecologically interesting guild, feeding on the same plant tissue as several leaf-chewing guilds (Novotny *et al.*, 2012). Temperate zone studies, particularly on the taxonomically well-described European fauna, revealed ecological parallels between mesophyll cell-suckers and miners, as both guilds are highly host-specific and species-poor (Claridge & Wilson, 1981a, 1981b, 1982). While at least one quantitative study of plant-miner food webs is available from tropical forests (Lewis *et al.*, 2002), the present study is to our knowledge the first analysing assemblages of the mesophyll cell-suckers on tropical trees.

Our survey of Typhlocybinae assemblages on rainforest trees, in combination with the data available from temperate forests, is used to test the following hypotheses: (1) host specialisation and species richness of Typhlocybinae increase in parallel from the temperate to tropical areas, as high host specificity facilitates the coexistence of higher number of species in the tropics, (2) host specialisation of Typhlocybinae increases from highly dynamic secondary to more stable primary vegetation in the tropics, in parallel to similar trends documented from temperate zone vegetation (Novotny, 1994, 1995), and (3) high host specialisation

of Typhlocybinae combined with high species diversity of plants in tropical forests leads to a high, and largely undocumented, species diversity of Typhlocybinae in the tropics.

Materials and methods

Focal plant taxa

The field work was situated in an approximately 10 × 20 km mosaic of primary and secondary lowland forests, centred on the forests near Ohu, Baitabag, and Mis Villages in the Madang Province of Papua New Guinea (PNG). The annual rainfall in the study area is 3558 mm, with a moderate dry season from July to September; the mean air temperature is 26.5 °C and varies little throughout the year (McAlpine *et al.*, 1983). The field work was completed in 6 months from May to November 2006, thus including both dry and rainy seasons.

Typhlocybinae larvae were sampled from 65 woody plant species, including closely related species (12 *Ficus*, 5 *Macaranga*, 4 *Syzygium*, 3 *Psychotria*, and 2 *Tabernaemontana* species) as well as representatives of 44 genera and 27 families from all major plant lineages, including gymnosperms, basal angiosperms, monocots, rosids, and asterids (APG, 2009) (Table S1). Plant phylogenetic relationships were reported in Novotny *et al.* (2010), based on 1285 base pairs of aligned chloroplast DNA encoding ribulose-1,5-bisphosphate carboxylase (rbcL) and assuming the monophyly of families and orders classified according to angiosperm phylogeny (APG, 2009). Plant vouchers are deposited in the PNG Forest Research Institute in Lae.

Each focal tree species was characterised by local abundance, succession optimum, maximum individual size, wood density, specific leaf area, and foliar nitrogen, i.e. the traits shown or hypothesised as important for the composition of other folivorous herbivores as they determine the amount or quality of resources available for herbivores (Kennedy & Southwood, 1984; Basset & Novotny, 1999; Whitfield *et al.*, 2012). All individuals with a diameter at breast height (DBH) > 1 cm from the focal species were surveyed within 50 forest plots 20 × 20 m each (2 ha in total) at our study sites. The plots were situated both in primary and secondary forests. The latter were 15- to 30-year-old forest resulting from natural succession after swidden agriculture (Leps *et al.*, 2001; Whitfield *et al.*, 2012). The mean basal area (m² ha⁻¹) for primary and secondary forests was used as an index of local abundance for each focal plant species. Further, the succession preference of each plant species was derived from its distribution between primary and secondary forest plots of the same size, as the percentage of basal area present in the primary forest. This Succession Index [$S_I = B_p / (B_p + B_s) \times 100$ where B_p and B_s is the basal area, in m² ha⁻¹, for the studied plant species in respectively primary and secondary forests] ranges from 0 to 100 for tree species entirely confined to secondary and primary forest, respectively. Species with $S_I < 50$ were classified as secondary forest species, the remaining ones as primary forest species. The maximum DBH was recorded for each tree species as an index of tree size. The presence or absence of latex outflow from damaged leaves was recorded for all studied species.

Specific leaf area (SLA), defined as leaf area divided by dry leaf mass ($\text{cm}^2 \text{g}^{-1}$), was estimated from leaf discs 2.3 cm in diameter punched from multiple individuals for all species from fully expanded, mature leaves without obvious signs of pathogen or herbivore damage. Further, foliar nitrogen (% of dry leaf mass) was measured in leaf samples taken from three different individuals for each tree species. The details for SLA and nitrogen measurements are reported in Whitfield *et al.* (2012). Wood density (g cm^{-3}) was estimated as wood dry biomass divided by volume, obtained from wood cores taken from three individuals of each tree species.

Focal herbivore taxa

Each focal plant species was sampled with equal effort, which amounted to 1500 m² of foliage area per species, removing potential effects owing to variations in host plant abundance. There were > 150 individual plants from each species sampled for herbivores, and > 300 sampling events (i.e. a particular plant sampled at a particular time) per plant species. Sampling focused on understory and mid-storey for trees which could be easily climbed.

Typhlocybinae nymphs were hand-collected from the foliage of the focal tree species, using an aspirator. They were brought to the laboratory where they were individually placed into rearing vials with a section of leaf from the plant species from which they were collected. Only reared adult males were used in the analysis; in Typhlocybinae, the taxonomy is based mostly on the morphology of male genitalia whereas females are often impossible to identify morphologically. All males were dissected and identified to morphospecies. Insect vouchers have been deposited at the Entomology Institute of the Academy of Sciences of the Czech Republic.

Statistical analysis

In this context, a herbivore assemblage was defined as all Typhlocybinae feeding partially or exclusively on a particular plant species. Each herbivore assemblage on plant *i* was characterised by the number of species (S_i) and individuals (N_i) per plant species. The distribution of S_i values was tested against the Poisson distribution which would result from mutually independent plant colonisation events by herbivore species, when the probability of a plant species acquiring a new herbivore is not influenced by the number of herbivore species already present on that plant species.

The effective specialisation (E) was defined as the proportion of typhlocybinae species feeding on a particular host plant that was unique to that plant and estimated as $E = S/T$, that is, as the ratio of the total number of typhlocybinae species (S) found on vegetation comprising H plant species, divided by the number of their trophic interactions (T) involving these plant species (May, 1990).

The number of typhlocybinae species in PNG was estimated as $S_{PNG} = S_{avg} \times H_{PNG} \times E_{PNG}$ where S_{avg} is the mean number of typhlocybinae species per plant species, H_{PNG} is the number of plant species in PNG (from Hoft, 1992), and E_{PNG} is the effective

specialisation estimated for S_{PNG} plant species by extrapolation from the relationship between E and H for $H = 1-65$ tree species in our data set (Thomas, 1990).

Herbivore assemblages were analysed and their species diversity estimated separately for major plant clades including Asterids, Rosids 1 and Rosids 2, as well as a paraphyletic 'Basal' clade comprising the taxa basal to the Rosid-Asterid clade, including basal Eudicots, Monocots, and Magnoliids and Gymnosperms (plant phylogeny based on the APG, 2009) as many herbivorous taxa exhibit a strong association with only particular plant clades (e.g. Pellissier *et al.*, 2013). The extrapolation of species richness to PNG flora excluded 2705 species of Orchideaceae, a particularly species-rich family which is most likely entirely devoid of typhlocybines (V. Novotny, pers. obs.).

The accumulation of herbivore species with increasing number of plant species was described by the Mao Tau function, an analytical analogue of a species accumulation curve derived by amalgamation of samples in random order. The total number of species, including those missing from the samples, was estimated using the Chao 1 index. The Mao Tau function and Chao 1 index were calculated by the program EstimateS (Colwell, 2008), where they are also explained. The trends in herbivore species richness were extrapolated from local to regional plant species richness using a power function fitted to the species accumulation curve for 40–65 plant species (as in Novotny *et al.*, 2012). Plant-herbivore interactions supported by a single feeding individual were retained in the analysis of species richness but were excluded from the analysis of host specificity as poorly supported. The robustness of host specificity analyses was tested by filtering our data set, progressively excluding rare species, or rare plant-herbivore interactions, and recalculating host specificity parameters (Figure S1).

Relationships between plant and herbivore traits were explored by classic correlation using 65 plant species as independent data points as well as by independent contrasts analysis, based on the phylogenetic relationships among plant species detailed in Appendix S1 and calculated by Compare 4.6. software (Martins, 2003). The similarity in herbivore assemblages between two different plant species was quantified by Jaccard's similarity index. The correlation between herbivore assemblage similarity and the phylogenetic distance between their host plant species was tested on all pair-wise comparisons between plant species using the Mantel test.

Our tropical data were compared with an analogous data set on typhlocybines from trees in Great Britain, published by Claridge and Wilson (1981a). Only the herbivore assemblages on plants identified to species were used in this comparison, thus reducing the original matrix to 28 tree and 45 typhlocybinae species. Further, we used arboreal species from the regional fauna of Italy (Vidano & Arzone, 1981, 1987a, 1987b; Arzone & Vidano, 1987) and Germany (Nickel & Remane, 2002) for similar comparisons.

Results

We reared 450 adult males from 47 typhlocybinae species feeding on 65 plant species sampled. Typhlocybinae species richness

ranged from 0 to 5 (median 1) species per tree species, including 26 tree species which were not colonised by any typhlocybinae herbivores. This species diversity is an underestimate (Chao 1 estimate was 129 species for the entire data set). The distribution of species richness among host plant species followed a Poisson distribution (Fig. 1a).

The Rosid 1 species hosted the highest and the Asterid species the lowest number of typhlocybinae species (Fig. 2a). This pattern was determined primarily by the proportion of tree species colonised by typhlocybines in each clade (Fig. 2b).

The number of typhlocybinae species per tree species decreased with S_j , both in the classic analysis (Spearman's $r = -0.488$, $P < 0.01$) and the independent contrasts analysis (Spearman's $r = -0.365$, $P < 0.01$). This correlation was generated by the differential likelihood of tree species from secondary and primary forests being colonised by typhlocybines (Fig. 3a) as there was no correlation between S and S_j once the tree species with no typhlocybines had been removed from the analysis ($P > 0.05$).

The Rosid 1 clade had significantly lower S_j values, i.e. more secondary forest species, than the remaining clades (ANOVA, $F_{1,3} = 8.625$, $P < 0.01$). A combined analysis of the effects of plant lineage and S_j on the number of typhlocybinae species revealed the primary importance of their succession preference (generalized linear model with plant lineages as a categorical and S_j as a continuous explanatory variable, $F_{4,59} = 6.749$, $R^2 = 0.267$, $P < 0.001$). Thus there are more typhlocybinae species on early succession plant species, and there are more early succession plant species in Rosids 1 than in the other plant taxa.

The preference of typhlocybines for secondary forest species could not be explained by any of the remaining ecological variables of the tree species (specific leaf area, foliar nitrogen content, wood density, tree local abundance, and maximum tree size) as none of these variables were correlated with typhlocybinae species richness (Spearman's r , $P > 0.05$) or probability that a tree species will be colonised by typhlocybinae species (ANOVA, $F_{1,2} < 3.8$, $P > 0.05$) in classic as well as independent contrasts analyses. Further, the incidence of latex also did not affect the probability of plant colonisation by typhlocybines, although the statistical power of the test was low owing to the small number of plant species with latex (Fig. 3b).

Within the 65 plant species studied, typhlocybinae species fed on 1–3 plant species from up to three different families, but typically (83% of species) they were specialised on a single host species (Fig. 4a). The percentage of monophages remained high, 79%, even when only the plant genera with > 1 species sampled were included in the analysis to make sure that there was always at least one closely related, potential alternative host sampled for each herbivore. In this analysis, there were 15 monophagous species from the total of 19 species. The narrow host specificity was robust with regard to sample size, as even species sampled by ≥ 10 individuals were predominately monophagous (Figure S1).

There was no difference in host specificity between plants reaching the highest abundance in the secondary forest (i.e. with $S_j < 50$), which hosted 27 typhlocybinae species including 22 monophages, and the primary forest species ($S_j > 50$) with 10

typhlocybinae species, nine of them monophagous (Fisher's exact test, $P > 0.05$).

The similarity between herbivore assemblages, estimated by Jaccard's index, was negatively correlated with phylogenetic distance between their host tree species, but this correlation explained only $< 2\%$ of the variability in community similarity (Pearson's $r = 0.130$, $P = 0.006$, Mantel test). Among the 2080 pairs which can be formed from the 65 plant species, 1339 did not share any typhlocybinae species because one or both plant species in the pair were not colonised by typhlocybines. An additional 725 pairs between plant species with typhlocybines also did not share any species, whereas only 16 pairs, i.e. $< 1\%$ of the total, shared at least one species.

The entire typhlocybinae assemblage on 65 plant species was characterised by an effective specialisation value of $E = 0.79$. The E value decreased with the number of plants sampled H , after a power function (Fig. 5). We used this function to extrapolate E values for the number of plant species known from PNG in the main plant lineages as well as for the entire flora (excluding orchids) of 10 662 plant species at $E_{PNG} = 0.30$ (Table 1). The expected number of typhlocybinae species feeding on all plant species in PNG was then estimated using the extrapolated E_{PNG} values, multiplied by the mean number of typhlocybinae species per plant species (Fig. 2a) and the number of plant species in PNG (Table 1). These calculations lead to the estimate of 2775 typhlocybinae species summed across the main four plant clades, and to 2855 species when all plant species were combined into a single extrapolation irrespective of differences in typhlocybinae species richness among clades (Table 1).

Discussion

The Poisson distribution of species richness among plant species suggests that colonisation events can be seen as mutually independent, and also independent of the number of herbivore species already present on the colonised plant species (Novotny *et al.*, 2012). This, together with a large number of plant species predicted as well as observed to be devoid of typhlocybines, suggests that mesophyll cell-sucking assemblages are not saturated with species.

Temperate zone typhlocybinae assemblages appear to be more species rich than tropical ones (Fig. 1) but this may be partly or entirely as a result of a larger geographic area sampled by the temperate study over a longer period of time, combined with under-sampling of tropical assemblages. The Poisson distribution predicted a large number of non-colonised tree species in tropical, but not on temperate zone hosts. No explanation is thus needed for the absence of typhlocybines on some tree species, other than the generally low species diversity of this herbivore taxon on tropical trees. This paucity of species is difficult to explain by ecological factors, such as the amount of resources available. It is probably determined by the speciation dynamics and limited size of the regional species pool, rather than local ecological processes (Ricklefs, 2004). In particular, the mesophyll cell-sucking guild comprises a single clade, Typhlocybinae, which has undergone extensive speciation as it now

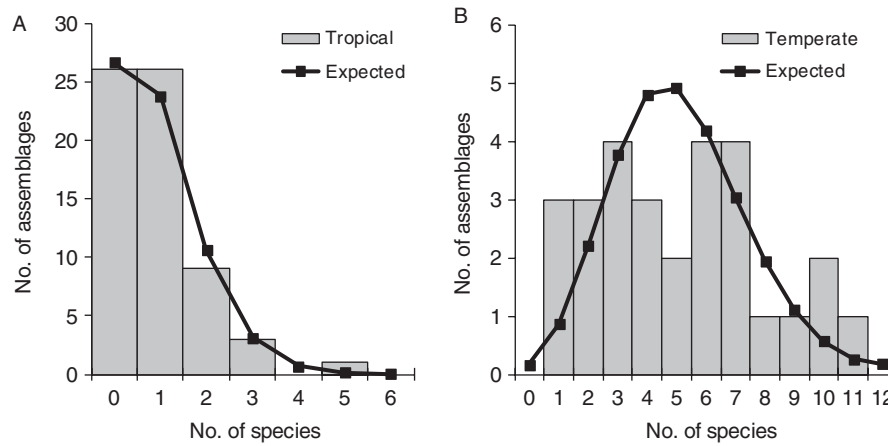


Fig. 1. Distribution of species richness values (number of species on individual plant species) for mesophyll cell-sucking Typhlocybinae on tropical (a) and temperate zone (b) tree species. The observed values are not significantly different from a Poisson distribution (line; $P > 0.50$ for tropical and $P = 0.0501$ for temperate data sets, χ^2 test).

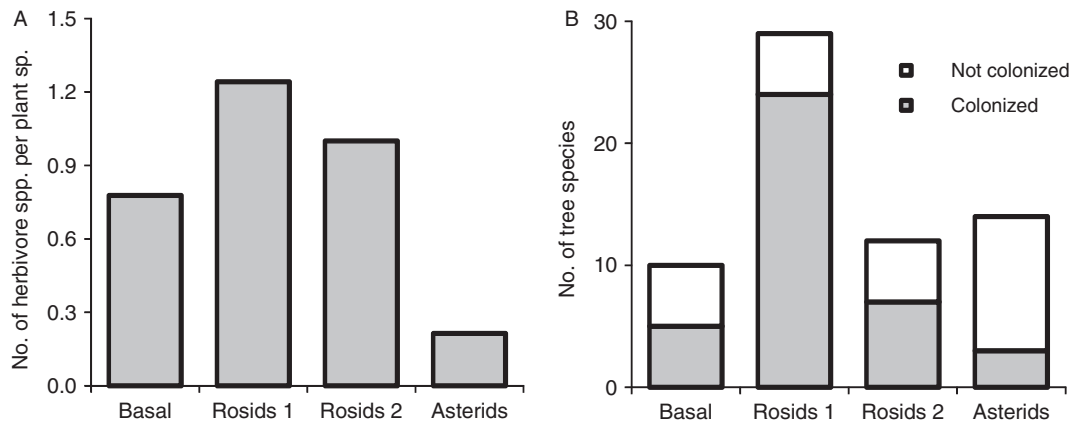


Fig. 2. The mean (\pm SE) number of typhlocybine species feeding on plant species from different clades (a) and the number of trees colonised and not colonised by typhlocybines in these clades (b). Basal is the clade basal to the rosid – asterid clade, including basal Eudicots, Monocots, Magnoliids, and Gymnosperms; Rosids 1, Rosids 2, and Asterid clades follow APG (2009) classification. The values for Rosid 1 and Asterid clades were significantly different (ANOVA $F_{1,3} = 3.918$, $P = 0.013$, Tukey's post-hoc tests, $P = 0.007$ for the mean number of typhlocybine species and the Kruskal–Wallis median test with multiple comparisons, $P = 0.0017$ for the number of colonised species). See Table S1 for the list of plant species in each clade.

represents one of the most diverse subfamilies of Auchenorrhyncha (e.g. second most diverse in Great Britain, a taxonomically well-known region; Stewart, 2013). However, even a large regional species pool results in low-diversity communities on particular plant species for highly host-specific guilds, such as the one studied here (Novotny *et al.*, 2012).

The pattern of colonised and non-colonised tree species was not entirely random, as early succession plant species were colonised more than twice as often as those from the primary forest. There are sound theoretical reasons for this difference as secondary forest species tend to be less well defended and more palatable to herbivores than the primary forest species (Brown, 1985; Herms & Mattson, 1992). Rather disappointingly, we were not able to explain the difference in typhlocybine herbivores by SLA, wood density or foliar nitrogen values. The first two parameters are closely correlated with growth rates of tropical trees and thus presumably with their

anti-herbivore defence, whereas nitrogen defines the quality of plant resources for herbivores (Turner, 2001). Latex, an important defence against leaf-chewing herbivores (Agrawal & Konno, 2009), proved to be irrelevant to mesophyll-sucking typhlocybines, as expected considering that their feeding mode enables them to avoid it in the leaf tissue.

Temperate zone typhlocybines are known for their narrow host specificity (Claridge & Wilson, 1981a). We found even higher specificity in tropical forests, where mesophyll cell-feeding was one of the most specialised herbivore guilds (Novotny *et al.*, 2012). The proportion of monophagous species on New Guinea trees was higher than on tree species in Great Britain and Italy (Fig. 4). This difference remained significant when only plant species from the Rosid 1 and Rosid 2 clades were considered in all data sets, in order to control for differences in phylogenetic diversity between tropical and temperate trees. In addition to a higher proportion of monophagous species, broad generalists

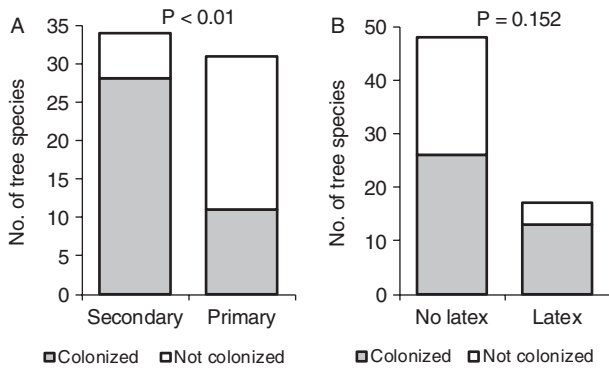


Fig. 3. The number of tree species with and without mesophyll cell-sucking typhlocybines among those preferring primary forest ($S_T > 50$) and secondary forest ($S_T < 50$) (a), and plant species with and without latex (b). Mesophyll cell-sucking typhlocybines species colonised more readily tree species from secondary forests ($P < 0.01$, Fisher's exact test), while they were indifferent to latex presence in the leaves ($P > 0.15$, Fisher's exact test).

feeding on plants from > 10 species and > 5 families were absent from the tropical data set while they were present in temperate ones. The higher host specialisation in the tropical compared with the temperate species was also supported by the host specificity patterns in the regional fauna of arboreal Typhlocybinae in Germany (Fig. 4a). Specialisation reduces inter-specific competition which facilitates species coexistence. This leads to the expectation of narrow specialisation in highly diverse communities of tropical herbivores (Dyer *et al.*, 2007). This theory is however not applicable to typhlocybines as their tropical assemblages in lowland rainforests are not markedly more diverse per tree species than those in the temperate zone. The present tentative, pair-wise comparison of communities between temperate Europe and tropical New Guinea needs to be replaced by a systematic study of host specificity trends along contiguous latitudinal gradients.

In the temperate zone, early succession vegetation dominated by unpredictable annual herbaceous species hosts less specialised leafhopper assemblages than more permanent plants in

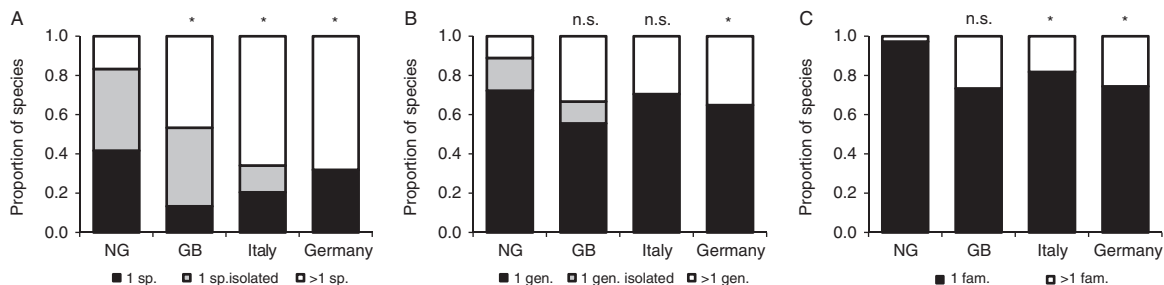


Fig. 4. The host specificity of typhlocybines species on rainforest trees in New Guinea (NG), temperate tree species in Great Britain (GB, Data from Claridge & Wilson, 1981a) and Italy (Data from Vidano & Arzone, 1987a,b; Arzone & Vidano, 1987), and in the temperate regional fauna of Germany (Data from Nickel & Remane, 2002) with respect to plant species (a), genera (b) and families (c). (a) Species feeding on a single plant species were separated into those with (black) and without (grey) another congenic plant species sampled as a potential alternative host; this distinction was not available for Germany. (b) Species feeding on a single plant genus were separated into those with (black) and without (grey) another congenic plant species sampled (except for Germany); (c) all species feeding on a single plant family (black) had plant species from other families sampled. The proportion of specialists (black and grey combined) in NG was compared with the three European data sets using Fisher's exact test with Bonferroni's correction for multiple comparisons. The significance of results (* for $P < 0.017$) is reported in each graph.

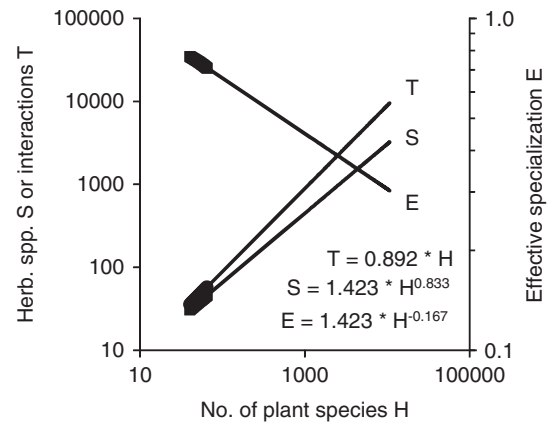


Fig. 5. The trend in the number of plant–herbivore trophic interactions (T), the number of herbivore species (S), and the herbivore effective specialisation ($E = S/T$) with increasing number of plant species (H). Herbivore assemblages on increasing number of plant species were combined in random order. The results for 40–65 plant species are shown and extrapolated to the 10 662 plant species for New Guinea (excl. orchids, see Table 1) using a linear function for T ($R^2 = 1.00$), and a power function for S ($R^2 = 0.999$) and E ($R^2 = 0.998$).

the later stages of succession (Novotny, 1994, 1995). This trend was not replicated along the rainforest succession as even pioneer tropical plant species tend to be long-lived woody species, and therefore sufficiently apparent (*sensu* Feeny, 1976) even for specialised herbivores (Leps *et al.*, 2001, Vlasanek *et al.*, 2013).

Species-poor and specialised assemblages of typhlocybines do not functionally connect populations of different plant species by their feeding as an overwhelming majority of tree species pairs coexisting in the same forest do not share a single typhlocybines species. In theory, they may have the potential to cause density-dependent mortality of their hosts, thus contributing to the high plant species diversity in tropical forests (Leigh *et al.*, 2004). However, the feeding damage they cause is likely to be minor, corresponding to their low density and small body size. Although many sap-feeding insects harm their hosts also by

Table 1. The number of mesophyll cell-sucking typhlocybinae herbivores feeding on the four main plant clades, as well as the entire flora of Papua New Guinea flora (S_{PNG}).

| | Basal | Rosids 1 | Rosids 2 | Asterids | Total | All plants |
|-----------|-------|----------|----------|----------|--------|------------|
| S_{avg} | 0.7 | 1.2 | 1.0 | 0.2 | – | 0.9 |
| H | 10 | 29 | 11 | 14 | 64 | 65 |
| H_{PNG} | 2804 | 2188 | 1549 | 4121 | 10 662 | 10 662 |
| E_{PNG} | 0.38 | 0.39 | 0.42 | 0.35 | – | 0.30 |
| S_{PNG} | 743 | 1072 | 647 | 313 | 2775 | 2855 |

S_{avg} , the mean number of mesophyll cell-sucking species per plant species estimated from sampling H plant species; H_{PNG} , the number of plant species in PNG (from Hoft, 1992, excluding orchids); E_{PNG} , the effective specialisation estimated for H_{PNG} plant species richness using the power function in Fig. 5. Plant clades are defined in Fig. 2.

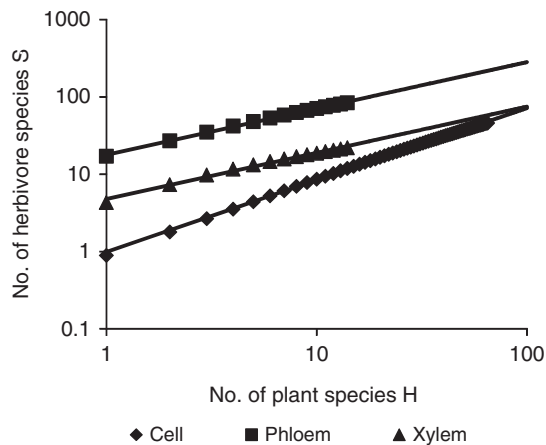


Fig. 6. Species accumulation in three sap-sucking guilds. Mao Tau species accumulation curves are shown for the mesophyll cell-sucking guild on 65 plant species, as well as the phloem- and xylem-sucking guilds studied on 14 plant species in the same forest by Dem *et al.* (2013) (cell-suckers $S = 0.986 \times H^{0.931}$, phloem-suckers $S = 17.814 \times H^{0.600}$, xylem-suckers $S = 4.786 \times H^{0.597}$, $R^2 \geq 0.99$ for all guilds).

pathogen transmission, this appears not to be the case for the cell-sucking guild (Nault & Ammar, 1989).

While the mesophyll cell-feeding guild represents a small portion of all herbivores feeding on any particular tree species, its relative importance increases on diverse vegetation as its highly specialised species accumulate rapidly with plant diversity. This is illustrated by the accumulation of species diversity in the mesophyll cell-, phloem- and xylem-sucking Auchenorrhyncha studied in the same lowland rainforest in this study and by Dem *et al.* (2013) (Fig. 6). While the mesophyll cell-sucking guild is the least diverse of the three on a single plant species, its diversity on 100 plant species already matches that of the xylem-feeders, while it would match also the diversity of phloem-feeders on approximately 6250 plant species, which is less than the known PNG flora.

The data set used here is based on relatively large samples in terms of leaf area, but produced a limited number of insect individuals. However, our analysis showed that the conclusion of high host specificity is robust, remaining valid when only

better sampled species and/or trophic interactions are included (Figure S1). In contrast, we have not sampled the entire species diversity in the studied assemblages so that the following regional diversity calculation is likely to be an underestimate.

The local data on typhlocybinae diversity and host specificity were used for a highly tentative estimation of the total diversity of typhlocybines on the flora of PNG. In this extrapolation, the effective specialisation is the most difficult parameter to estimate. The number of herbivore species can be described as a power function of the number of plant species, $S = c \times H^E$ (Novotny *et al.*, 2004). The number of trophic interactions increases linearly with the number of plant species as $T = k \times H$ where k is the mean number of herbivore species per plant species. This implies that effective specialisation, $E = S/T$, should be also decreasing with H as a power function, $E = c/k \times H^{-1}$ (Fig. 5). Local effective specialisation values are thus unsuitable for the extrapolation of herbivore diversity on large geographical scales. This was empirically demonstrated by Thomas (1990) on local and regional data for Heliconiinae butterflies and their Passifloraceae host plants, one of a few plant–herbivore systems that were well documented on both local and regional scales in the tropics (note that x and y parameters in Thomas, 1990 correspond respectively to local and regional effective specialization estimates, although the author did not use this term).

Such long-range extrapolations, relying on incompletely known flora and relatively limited samples of herbivores, are bound to be inaccurate but could at least provide an order of magnitude estimate for an exceptionally poorly known taxon such as typhlocybinae leafhoppers. This estimate ignores typhlocybinae species turnover (β -diversity) on the same plant species across PNG, which is however likely to be rather low, at least in the lowlands (Novotny *et al.*, 2007). Further, we sampled only woody plant species which may have higher typhlocybinae diversity than the herbaceous species included in our estimate (other than orchids, which were excluded from the analysis). Finally, the list of known plant species by Hoft (1992) does not represent the entire Papua New Guinean diversity, which was estimated at 15–20 000 species by Sekhran and Miller (1996), whereas Roos *et al.* (2004) predicted 13 858 plant species for the whole of New Guinea.

Our estimate of at least 2775 species in PNG suggests there is a large pool of taxonomically unknown species in PNG, and elsewhere in the tropics, as only 4508 Typhlocybinae species have been described globally, and only 40 of them from PNG (Dmitriev & Dietrich, 2011). Note that this estimated pool of unknown species is based on classic morphological taxonomy (including genitalia morphology). A similar gap between the number of described and expected species in tropical forests was reported for other small-bodied and taxonomically difficult taxa, such as hymenopteran parasitoids (Veijalainen *et al.*, 2012).

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Supporting Information

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Figure S1. Sensitivity of host specificity estimates to the abundance of typhlocybinae species. Mean (\pm SE) number of host plant species estimated for data sets comprising (i) species with minimum abundance from 1 to 10 individuals (solid squares), and (ii) plant-herbivore trophic interactions supported by at least 1–10 herbivore individuals (solid triangles). The number of herbivore species included in the analysis is shown for data filtered by minimum abundance per species (empty squares) and per trophic interaction (empty triangles).

Appendix S1. Phylogenetic relationships among the studied plant species in the Newick format (from Novotny *et al.*, 2010).

Table S1. Plant species sampled for herbivores, their taxonomy and ecological traits.

References

- Agrawal, A.A. & Konno, K. (2009) Latex: a model for understanding mechanisms, ecology, and evolution of plant defense against herbivory. *Annual Review of Ecology, Evolution, and Systematics*, **40**, 311–331.
- Andrew, N.R. & Hughes, L. (2005) Diversity and assemblage structure of phytophagous Hemiptera along a latitudinal gradient: predicting the potential impacts of climate change. *Global Ecology and Biogeography*, **14**, 249–262.
- APG (Angiosperm Phylogeny Group) (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society*, **161**, 105–121.
- Arzone, A. & Vidano, C. (1987) Typhlocybinae of broadleaved trees and shrubs in Italy. 3. Corylaceae. *Bollettino dell'Istituto di Entomologia della Universita degli Studi di Bologna*, **51**, 269–276.
- Basset, Y. & Novotny, V. (1999) Species richness of insect herbivore communities on *Ficus* in Papua New Guinea. *Biological Journal of the Linnean Society*, **67**, 477–499.
- Brown, V.K. (1985) Insect herbivores and plant succession. *Oikos*, **44**, 17–22.
- Claridge, M.F. & Wilson, M.R. (1981a) Host plant associations, diversity and species – area relationships of mesophyll-feeding leafhoppers of trees and shrubs in Britain. *Ecological Entomology*, **6**, 217–238.
- Claridge, M.F. & Wilson, M.R. (1981b) Species richness of mesophyll-feeding leafhoppers and leafminers of trees in Britain. *Acta Entomologica Fennica*, **38**, 19–20.
- Claridge, M.F. & Wilson, M.R. (1982) Insect herbivore guilds and species – area relationships: leafminers on British trees. *Ecological Entomology*, **7**, 19–30.
- Colwell, R.K. (2008) *EstimateS: Statistical Estimation of Species Richness and Shared Species From Samples*. University of Connecticut, Storrs, Connecticut [WWW document]. URL www.purl.oclc.org/estimates [accessed on 28 April 2013].
- Dem, F., Stewart, A.J.A., Gibson, A., Weiblen, G.D. & Novotny, V. (2013) Low host specificity in species-rich assemblages of xylem- and phloem-feeding herbivores (Auchenorrhyncha) in a New Guinea lowland rain forest. *Journal of Tropical Ecology*, **29**, 467–476.
- Dmitriev, D. & Dietrich, C. (2011) 3i interactive keys and taxonomic databases, subfamily Typhlocybinae [WWW document]. URL <http://www.catalogueoflife.org/annual-checklist/2012/details/database/id/84> [accessed on 26 July 2013].
- Dyer, L.A. & Letourneau, D. (2003) Top-down and bottom-up diversity cascades in detrital vs. living food webs. *Ecology Letters*, **6**, 60–68.
- Dyer, L.A., Singer, M.S., Lill, J.T., Stireman, J.O., Gentry, G.L., Marquis, R.J. *et al.* (2007) Host specificity of Lepidoptera in tropical and temperate forests. *Nature*, **448**, 696–700.
- Hamilton, A.J., Novotny, V., Waters, E.K., Basset, Y., Benke, K.K., Grimbacher, P.S. *et al.* (2013) Estimating global arthropod species richness: refining probabilistic models using probability bounds analysis. *Oecologia*, **171**, 357–365.
- Hermes, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or defend. *Quarterly Review of Biology*, **67**, 283–335.
- Hodkinson, I.D. & Casson, D. (1991) A lesser predilection for bugs: Hemiptera (Insecta) diversity in tropical rain forests. *Biological Journal of the Linnean Society*, **43**, 101–109.
- Hoft, R. (1992) *Plants of New Guinea and the Solomon Islands: Dictionary of the Genera and Families of Flowering Plants and Ferns*. Handbook No. 13. Wau Ecology Institute, Wau, Papua New Guinea.
- Kennedy, C.E.J. & Southwood, T.R.E. (1984) The number of species of insects associated with British trees: a re-analysis. *Journal of Animal Ecology*, **53**, 455–478.
- Leigh, E.G.J., Davidar, P., Dick, C.W., Puyravaud, J.-P., Terborgh, J., ter Steege, H. *et al.* (2004) Why do some tropical forests have so many species of trees? *Biotropica*, **36**, 447–473.
- Leps, J., Novotny, V. & Basset, Y. (2001) Habitat and successional status of plants in relation to the communities of their leaf-chewing herbivores in Papua New Guinea. *Journal of Ecology*, **89**, 186–199.
- Lewis, O.T., Memmott, J., Lasalle, J., Lyal, C.H.C., Whitefoord, C. & Godfray, H.C.J. (2002) Structure of a diverse tropical forest insect–parasitoid community. *Journal of Animal Ecology*, **71**, 855–873.
- Martins, E.P. (2003) *COMPARE 4.6. Computer Programs for the Statistical Analysis of Comparative Data*. Department of Biology, Indiana University, Bloomington, Indiana.
- May, R.M. (1990) How many species? *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **330**, 293–304.
- McAlpine, J.R., Keig, R. & Falls, R. (1983) *Climate of Papua New Guinea*. CSIRO and Australian National University Press, Canberra, Australia.
- Nault, L.R. & Ammar, E.D. (1989) Leafhopper and planthopper transmission of plant viruses. *Annual Reviews of Entomology*, **34**, 503–529.
- Nickel, H. & Remane, R. (2002) Check list of the planthoppers and leafhoppers of Germany, with notes on food plants, diet width, life cycles, geographical range and conservations status (Hemiptera, Fulgoromorpha and Cicadomorpha). *Beiträge zur Zikadenkunde*, **5**, 27–64.
- Novotny, V. (1994) Association of polyphagy in leafhoppers (Auchenorrhyncha, Hemiptera) with unpredictable environments. *Oikos*, **70**, 223–232.
- Novotny, V. (1995) Relationships between life histories of leafhoppers (Auchenorrhyncha, Hemiptera) and their host plants (Juncaceae, Cyperaceae, Poaceae). *Oikos*, **73**, 33–42.
- Novotny, V. & Basset, Y. (2005) Host specificity of insect herbivores in tropical forests. *Proceedings of the Royal Society of London Series B*, **272**, 1083–1090.

- Novotny, V. & Wilson, M.R. (1997) Why are there no small species among xylem-sucking insects? *Evolutionary Ecology*, **11**, 419–437.
- Novotny, V., Basset, Y., Miller, S.E., Kitching, R., Laidlaw, M., Drozd, P. *et al.* (2004) Local species richness of leaf-chewing insects feeding on woody plants from one hectare of a lowland rainforest. *Conservation Biology*, **18**, 227–237.
- Novotny, V., Drozd, P., Miller, S.E., Kulfan, M., Janda, M., Basset, Y. *et al.* (2006) Why are there so many species of herbivorous insects in tropical rainforests? *Science*, **313**, 1115–1118.
- Novotny, V., Miller, S.E., Hulcr, J., Drew, R.A.I., Basset, Y., Janda, M. *et al.* (2007) Low beta diversity of herbivorous insects in tropical forests. *Nature*, **448**, 692–695.
- Novotny, V., Miller, S.E., Baje, L., Balagawi, S., Basset, Y., Cizek, L. *et al.* (2010) Guild-specific patterns of species richness and host specialization in plant-herbivore food webs from a tropical forest. *Journal of Animal Ecology*, **79**, 1193–1203.
- Novotny, V., Miller, S.E., Hrcek, J., Baje, L., Basset, Y., Lewis, O.T. *et al.* (2012) Insects on plants: explaining the paradox of low diversity within specialist herbivore guilds. *American Naturalist*, **179**, 351–362.
- Pellissier, L., Ndiribe, C., Dubuis, A., Pradervand, J.-N., Salamin, N., Guisan, A. *et al.* (2013) Turnover of plant lineages shapes herbivore phylogenetic beta diversity along ecological gradients. *Ecology Letters*, **16**, 600–608.
- Ricklefs, R.E. (2004) A comprehensive framework for global patterns in biodiversity. *Ecology Letters*, **7**, 1–15.
- Roos, M.C., Kessler, P.J.A., Gradstein, S.R. & Baas, P. (2004) Species diversity and endemism of five major Malesian islands: diversity–area relationships. *Journal of Biogeography*, **31**, 1893–1908.
- Sekhran, N. & Miller, S.E. (1996) *Papua New Guinea Country Study on Biological Diversity*. PNG Department of Environment and Conservation, Waigani, Papua New Guinea.
- Stewart, A.J.A. (2013) Checklist of British species of Auchenorrhyncha [WWW document]. URL <http://www.ledra.co.uk/species.html> [accessed on 15 June 2013].
- Thomas, C.D. (1990) Fewer species. *Nature*, **347**, 237.
- Turner, I.M. (2001) *The Ecology of Trees in the Tropical Rain Forest*. Cambridge University Press, Cambridge, U.K.
- Van Veen, F.J.F., Morris, R.J. & Godfray, H.C.J. (2006) Apparent competition, quantitative food webs, and the structure of phytophagous insect communities. *Annual Review of Entomology*, **51**, 187–208.
- Veijalainen, A., Wahlberg, N., Broad, G.R., Erwin, T.L., Longino, J.T. & Saaksjarvi, I.F. (2012) Unprecedented ichneumonid parasitoid wasp diversity in tropical forests. *Proceedings of the Royal Society of London Series B*, **279**, 4694–4698.
- Vidano, C. & Arzone, A. (1981) Typhlocybininae of broad-leaf trees in Italy. 1. Alnus. *Acta Entomologica Fennica*, **38**, 47–49.
- Vidano, C. & Arzone, A. (1987a) Typhlocybininae of broadleaved trees and shrubs in Italy. 2. Betulaceae. *Bollettino dell'Istituto di Entomologia della Universita degli Studi di Bologna*, **51**, 257–267.
- Vidano, C. & Arzone, A. (1987b) Typhlocybininae of broadleaved trees and shrubs in Italy. 4. Fagaceae. *Redia*, **70**, 171–189.
- Vlasanek, P., Sam, L. & Novotny, V. (2013) Dispersal of butterflies in a New Guinea rainforest: using mark–recapture methods in a large, homogeneous habitat. *Ecological Entomology*, **38**, 560–569.
- Whitfield, T.J.S., Novotny, V., Miller, S.E., Hrcek, J., Klimes, P. & Weiblen, G.D. (2012) Predicting tropical insect herbivore abundance from host plant traits and phylogeny. *Ecology*, **93**, S211–S223.
- Wolda, H. (1980) Seasonality of tropical insects: I. Leafhoppers (Homoptera) in Las Cumbres, Panama. *Journal of Animal Ecology*, **49**, 277–290.
- Wright, S.J. (1983) The dispersion of eggs by a bruchid beetle among *Scheelea* palm seeds. *Ecology*, **64**, 1016–1021.

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