Mangroves are an overlooked hotspot of insect diversity despite low plant diversity

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Abstract

We here compare the tropical arthropod fauna across a freshwater swamp and six different forest types (rain-, swamp, dry-coastal, urban, freshwater swamp, mangroves) based on 140,000 specimens belonging to *ca.* 8,500 species. Surprisingly, we find that mangroves, a globally imperiled habitat that had been expected to be species-poor for insects, are an overlooked hotspot for insect diversity despite having low plant diversity. Mangroves are very species-rich (>3,000 species) and distinct (>50% of species are mangrove-specific) with high species turnover across Southeast and East Asia. Overall, plant diversity is a good predictor for insect diversity for most habitats, but mangroves compensate for the low number of phytophagous and fungivorous species by supporting an unusually rich community of predators whose larvae feed in the productive mudflats. For the remaining habitats, the insect communities have diversity patterns that are largely congruent across guilds. The discovery of such a sizeable and distinct insect fauna in a globally threatened habitat underlines how little is known about global insect biodiversity.

Keywords

Insect biodiversity, Mangroves, NGS barcoding, species discovery, beta-diversity, global insect decline, Southeast Asia

Introduction

Insects are currently experiencing anthropogenic biodiversity meltdowns with declines having attracted much attention[1–4] and controversy[5–10]. The controversy is largely due to the paucity of high-quality data for arthropods, which is also responsible for imprecise estimates of global animal species richness[11,12] and understanding species turnovers[13–15]. These knowledge gaps are also likely to threaten the health of whole ecosystems given that arthropods provide a large number of important ecosystem services[3,16–19], contribute much of the animal biomass[20] and are yet frequently ignored in habitat assessments. The lack of baseline data is particularly worrisome at a time when tropical ecosystems are heavily impacted by habitat conversion and global change[21].

The situation is particularly dire for the species-rich tropics, for which so few comprehensive surveys have been conducted[22–24] that only three of the 73 studies in a recent review of insect declines involved tropical sites[8]. Furthermore, tropical insect surveys have traditionally focused on tropical rainforests[24], with other tropical habitats being largely neglected. Mangrove forests are a prime example of a tropical habitat for which the insect fauna is poorly characterized. Mangroves used to cover more than 200,000 km^2 of the global coastline[25], but have been experiencing an annual area loss of 1-2%[25,26]. Indeed, the losses of mangroves far exceed those of more high-profile ecosystems such as rainforests and coral reefs[26]. Unfortunately, these losses are further exacerbated by climate change[27], with some simulations predicting a further reduction by 46–59% for all global coastal wetlands by the year 2100[28]. This is a particularly worrying trend as mangrove ecosystems have been found to be sequestrate more carbon per hectare than tropical dryland forests[29]. These changes will not only endanger entire ecosystems that provide essential ecosystem services[30–32], but also threaten the survival of numerous mangrove species with unique adaptations. Mangrove specialists with such adaptations are well known for vertebrates and vascular plants[33,34], but the invertebrate diversity is largely unknown.

One reason why the mangrove insect fauna is likely to have received little attention is the low plant diversity in mangroves. Tropical arthropod diversity is usually positively correlated with plant diversity[23,24,35] which implied that mangroves would provide few insights into understanding whether insect herbivores drive high plant diversity in the tropics [36–38] or high plant diversity was responsible for high insect diversity [22,39]. Arguably, the traditional focus on addressing this question had the undesirable side-effect that the insect fauna of habitats with low plant diversity received comparatively little interest. Yet, many of these habitats are threatened with destruction, with mangroves being a good example. The few existing studies of mangrove insects focused on specific taxa[40–42], only identified specimens to higher taxonomic levels[43–45], and/or lacked quantitative comparison with the insect fauna of adjacent habitats. Given these shortcomings, these studies yielded conflicting results[44,46,47] with some arguing that high salinity and/or low plant diversity[33,44,46] were responsible for a comparatively poor insect fauna, while others found high levels of species diversity and specialization[47].

Here, we present the results of a comprehensive study of species richness and turnover of arthropods across multiple tropical habitats. The assessment is based on >140,000 specimens collected over >4 years from mangroves, rainforests, swamp forests, disturbed secondary urban forests, dry coastal forests, and freshwater swamps in Singapore (Fig. S1). In addition, we assess the species richness and turnover of mangrove insects across East and Southeast Asia by including samples from Brunei, Thailand, and Hong Kong. Specifically, our study (1) estimates mangrove insect diversity, (2) evaluates the distinctness in reference to five different forest habitats, (3) analyzes the biodiversity patterns by ecological guild, and (4) determines species turnover across larger geographic scales. Most of the work was carried out in Singapore because it has a large variety of different habitats that occur within 40km on a small island (724 km^2) that lacks major physical barriers. In addition, all habitats have experienced similar levels of habitat degradation or loss (>95%

overall loss of original vegetation cover[48]; *ca.* 90% loss of rainforest[49]; *ca.* 93% loss of swamp forest[50]; 91% loss for mangroves[51]).

A thorough assessment of insect biodiversity requires dense sampling over an extended period of time[52–54]. We sampled 107 sites using Malaise traps and subsequently processed specimens for 16 arthropod orders (Fig. S2) typically found in Malaise traps. The samples were typical in that Diptera and Hymenoptera comprised >75% of all specimens (Fig. S2) and these orders were therefore subsampled by taxon and ecological guild (Table S2). More than 140,000 specimens were NGS-barcoded[55] and grouped into putative species, which allowed for species richness and abundance estimates[56–58]. Contrary to expectations, we demonstrate that mangrove forests have a very distinct and rich insect fauna. In addition, the species turnover for all habitats in Singapore and the different mangrove sites in Asia is very high.

Results

Species delimitation based on NGS barcodes

We obtained 143,807 313-bp *cox1* barcodes, which were grouped into 8256–8903 molecular operationally taxonomic units (mOTUs, henceforth referred to as species) using objective clustering[59] at different p-distance thresholds (2–4%; Table S5). An alternative species delimitation algorithm, USEARCH[60], yielded similar species richness estimates of 8520–9315 species using the identity (--id) parameters 0.96–0.98. Most species boundaries were stable, with species numbers only varying by <12% across species delimitation techniques and parameters. We hence used the species generated via objective clustering at 3% p-distance for the analyses (see supplementary data Fig. S3 for results obtained with 2% and 4%).

Alpha-diversity across habitats

We rarefied the species richness curves by sample coverage[61] (Fig. 1) for each habitat, as well as by the number of specimens processed (Fig. S3). In addition, we only included trapping sites that had at least 100 barcoded individuals to prevent poorly-sampled sites from artificially inflating site dissimilarity. Alpha-diversity comparisons were made at the rarefaction point with the lowest coverage/number of specimens (i.e., swamp forest in Fig. 1, *top*). Our initial analysis compared the Alpha-diversity of rainforest, swamp forest, urban forest, freshwater swamp and coastal forest habitats and mangroves with all sites being grouped as a single habitat type. The species diversity of mangroves (1102.5 ± 10.8 species) is ca. 50-60% of the rarefied species richness of adjacent tropical primary/secondary forest (2188.4 ± 42.6 species) and swamp forest sites (1809 species) (Fig. 1a), but a site-specific analysis also revealed that two of the major mangrove sites in the study (PU & SB) have similar species richness as the freshwater swamp site after rarefaction (Fig. 1b). The species richness of a third mangrove site (SMO) was lower and more similar to the richness of an urban forest site. A newly regenerated mangrove (SMN), adjacent to an old-growth mangrove (SMO) had much lower species richness.

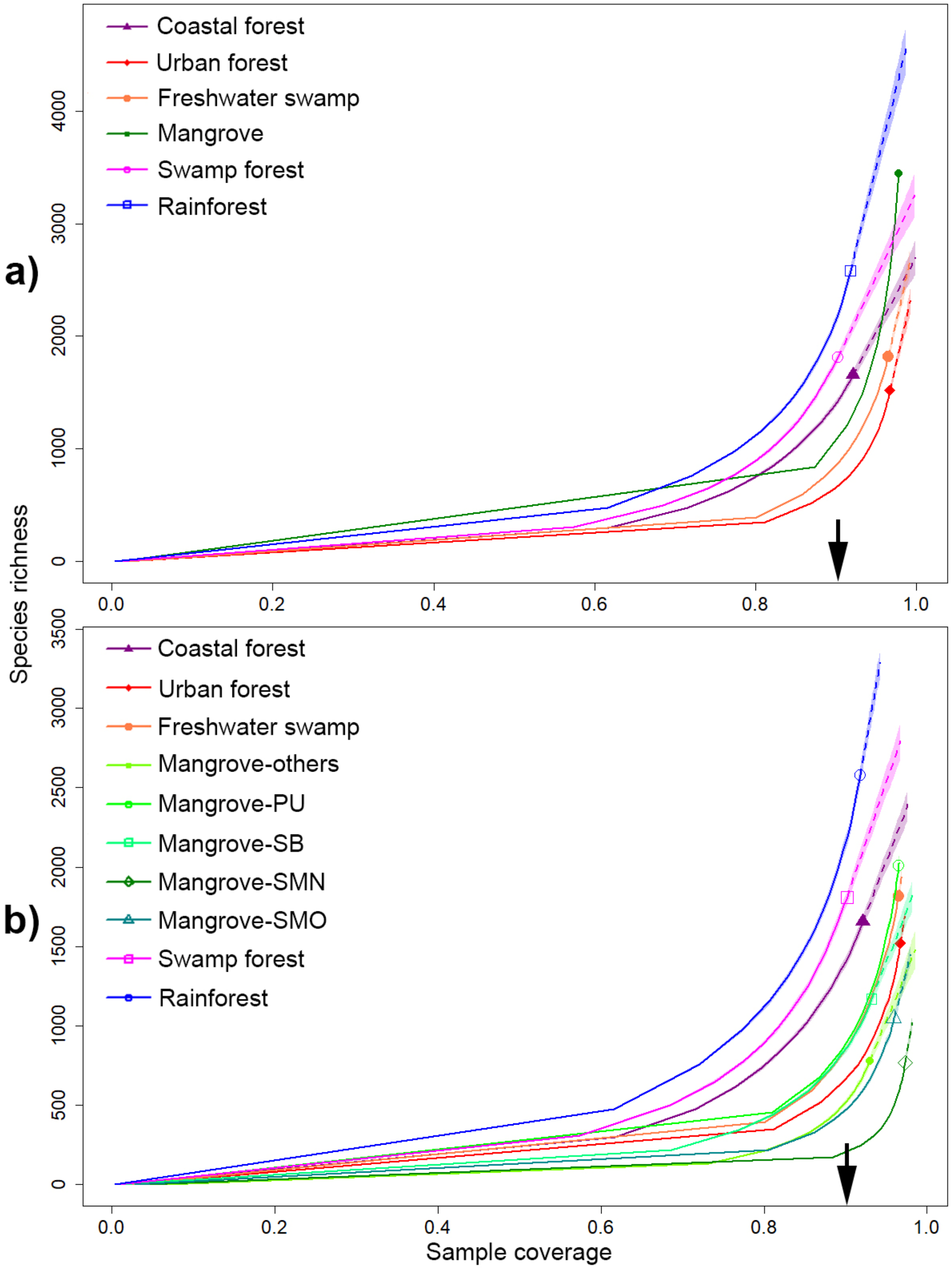

Figure 1. Insect alpha-diversity across tropical forest habitats. (a) Mangroves treated as one habitat; (b) Comparison of mangrove sites: Pulau Ubin (PU), Sungei Buloh (SB), Pulau Semakau old-growth (SMO), Pulau Semakau new-growth (SMN); solid lines = rarefaction; dotted = extrapolations. The arrow on the x-axis indicates the point of rarefaction at which species richness comparisons were made.

Species turnover across habitats

Mangrove arthropod communities are very distinct from those of the other habitats, with the communities from most habitats being well separated on NMDS plots (Fig. 2) even though several mangrove sites (PU, SB, SM) are geographically further from each other (>30 km) than from the other habitat types (Fig. S1). These patterns are also observed when the data are split into three taxon sets: (1) Diptera, (2) Hymenoptera, (3) remaining arthropod taxa (Fig. 2b). These results are also robust to the removal of rare species (Fig. 2a). Only 48 (0.6%) of the 8572 putative species in the species turnover analysis are found in all habitat types while 5989 (69.9%) are only in a single type (Table S6); within the mangroves, 50.2% of the 3557 species are only known from the mangrove habitat. The habitat type the mangroves share the most species with is the coastal forest (873 of 3557 species, 24.5%). When rare species are removed (<10 specimens), 481 of the remaining 1773 species (27.1%) are found in a single habitat while only 48 (2.7%) are found in all (Table S6); i.e., even after excluding rare species, a large proportion of the insect communities are putative habitat specialists.

Dissimilarity of the habitat-specific communities was confirmed with ANOSIM tests (Table 1A), which find significant differences between communities in both global (P = 0.001, R = 0.784) and pairwise habitat comparisons (P = 0.001 – 0.019, R = 0.341 – 0.983). The only exception are the coastal and urban forests (P = 0.079, R = 0.172) which may be due to the close proximity of Pulau Ubin coastal forest sites to urban settlements (Fig. S1). Note that a SIMPER analysis (Table 1B) finds a substantial number of shared species between the rainforest and swamp forest sites (13.88%). Both sites are in close geographic proximity (<5km; Fig. S1) and the within-habitat values for both sites are fairly high (rainforest = 29.59%, swamp forest = 31.10%). ANOSIM and SIMPER results are again robust to the removal of rare species (Tables S7 & S8) and the ANOSIM p-values for most comparisons are significant even according to re-defined statistical criteria for unexpected or new results ($p < 0.005$)[62]. The observed dissimilarity was largely due to species turnover with the turnover component (0.898) greatly outweighing nestedness (0.048; Table 1C & S9). This was similarly observed in most pairwise comparisons of habitats (turnover = 0.704 – 0.956, nestedness = 0.001 – 0.102). The only exception was mangroves and coastal forests (turnover = 0.658, nestedness = 0.254) which are in close geographic proximity on Pulau Ubin (Fig. 1).

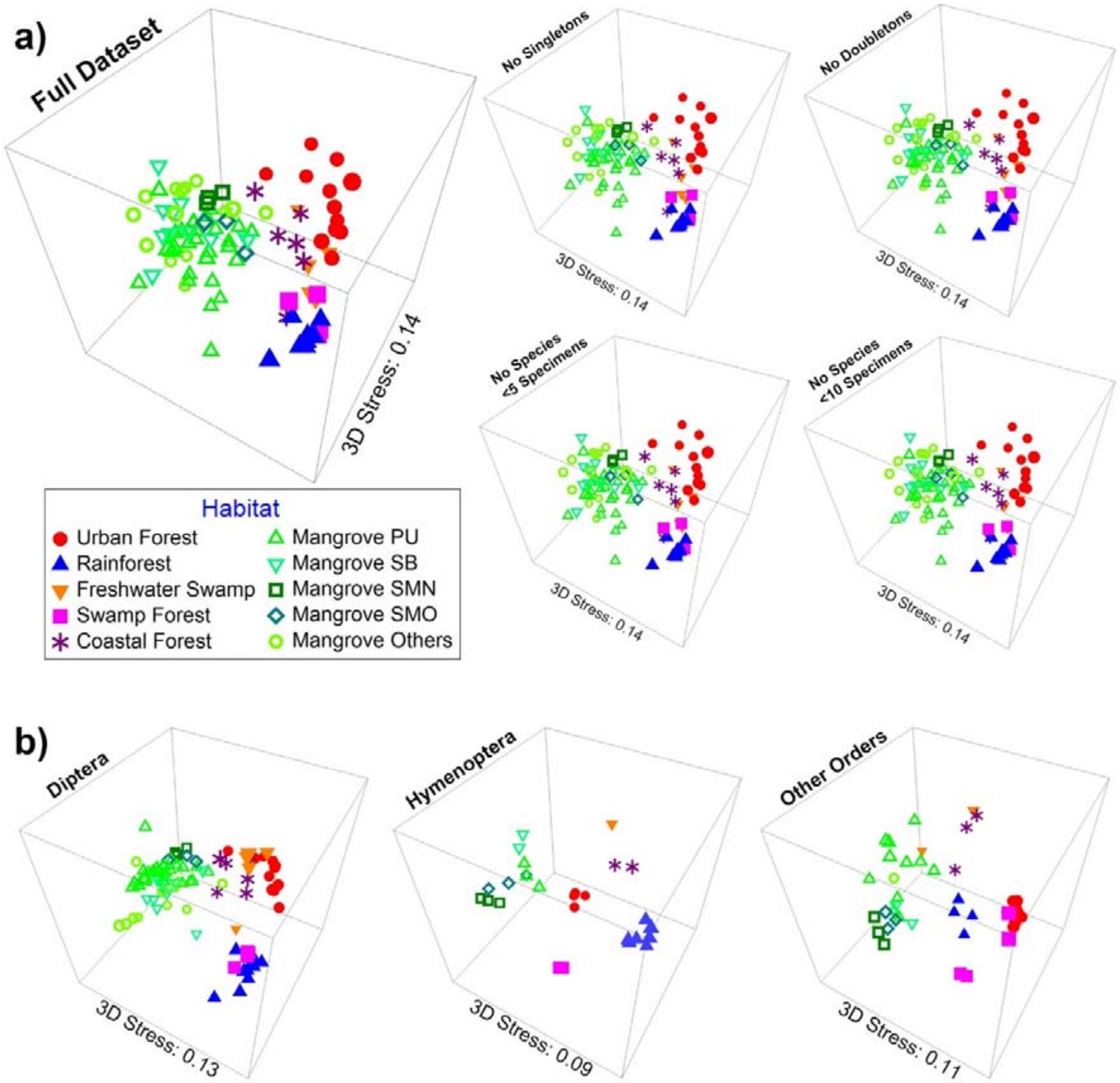

Figure 2. Insect communities across tropical forest habitats are distinct based on Bray-Curtis distances illustrated on 3D NMDS plots, regardless of whether (a) rare species are removed or (b) the data are split into different taxonomic groups.

Table 1. Species turnover across habitats. (A) Distinctness of communities in each habitat type as assessed with ANOSIM (pairwise p-value below and R-statistics above diagonal. (B) Distinctness of communities in each habitat type as assessed with SIMPER. (C) Species turnover and nestedness analysis (pairwise turnover values below and nestedness above diagonal).

A)

Overall P: 0.001 **Overall R:** 0.784

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.817	0.983	0.953	0.973	0.955
Urban forest	0.001		0.759	0.815	0.575	0.172
Swamp forest	0.001	0.001		0.934	0.769	0.893
Mangrove	0.001	0.001	0.001		0.856	0.546
Freshwater swamp	0.001	0.001	0.008	0.001		0.341
Coastal forest	0.001	0.079	0.005	0.001	0.017	

B)

	Within habitat (%)	Between habitats (%)					
		Rain-forest	Urban forest	Swamp forest	Mangrove	Fresh-water swamp	Coastal forest
Rainforest	29.59						
Urban forest	12.91	3.20					
Swamp forest	31.10	13.88	2.94				
Mangrove	12.25	1.62	3.09	1.98			
Freshwater swamp	17.29	2.13	4.69	4.10	2.74		
Coastal forest	12.09	3.82	9.41	4.00	6.08	9.05	

C)

Overall Dissimilarity: 0.946 **Overall Turnover:** 0.898 **Overall Nestedness:** 0.048

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.011	0.072	0.054	0.007	0.021
Urban forest	0.916		0.028	0.097	0.005	0.102
Swamp forest	0.710	0.922		0.092	0.027	0.001
Mangrove	0.914	0.819	0.878		0.062	0.254
Freshwater swamp	0.956	0.891	0.932	0.878		0.093
Coastal forest	0.908	0.704	0.940	0.658	0.756	

Relationship between insect and plant richness

Compared to mangroves (ca. 250 plant species), rainforest and swamp forest sites have 4.6 or 7.6 times the number of recorded plant species based on checklists for the sites (Table S4). This higher species richness is also confirmed by plot data for the rainforest[63] (839 species in 52 plots of $100m^2$) and swamp forest[64] (671 species in 40 plots of $400m^2$). However, the insect biodiversity of the rainforest and swamp forest sites is only 1.64 – 1.98 times higher than in the mangroves after rarefaction.

Analysis of ecological guilds and correlation between insect and plant diversity

For this analysis, we focused primarily on the Diptera and Hymenoptera which occupy a broad range of ecological guilds and dominate Malaise trap samples (see Brown 2005[65] & Hebert et al. 2016[66]). We also excluded trapping sites that were sampled for fewer than 6 months. We assigned insect species with known family/genus identities to ecological guilds (42,092 specimens belonging to 2,230 putative species) in order to understand how different habitats maintain insect diversity. After stepwise refinement of a multivariate ANCOVA model, the final model was defined as: *insectdiv ~ habitat + guild + plantdiv + guild:plantdiv* (*insectdiv*: rarefied insect species richness, *plantdiv*: plant species richness). The type-II sum of squares test reveal that guild and the interaction term between guild and plant diversity are highly significant factors ($p < 0.001$), while plant diversity ($p = 0.063$) and habitat ($p = 0.468$) are not. This suggests guild and plant diversity together have an important role in determining insect diversity but the precise relationship warranted further testing. Single variable linear regressions (*insectdiv ~ plantdiv)* were performed on each guild separately (Fig. 3) and plant diversity was found to only be highly significantly and positively correlated with the alpha-diversity of phytophagous and fungivorous insects ($p < 0.001$, $R^2 = 0.992$ and 0.990, $p = 0.886$ and 0.943 respectively).

After rarefaction, the different habitat types vary in composition (Fig. 4, see Table S10). Rainforest and freshwater swamp forest sites have higher numbers and proportions of phytophagous and fungivorous insect species (see also Figs S4 & S5). The insect communities of mangroves, however, are characterized by an unusually high proportion of predatory species while the urban forest sites are dominated by parasitoids. With regard to species turnover, communities are separated by habitat for most guilds and pairwise comparisons (Fig. 5, Tables S11 & S12).

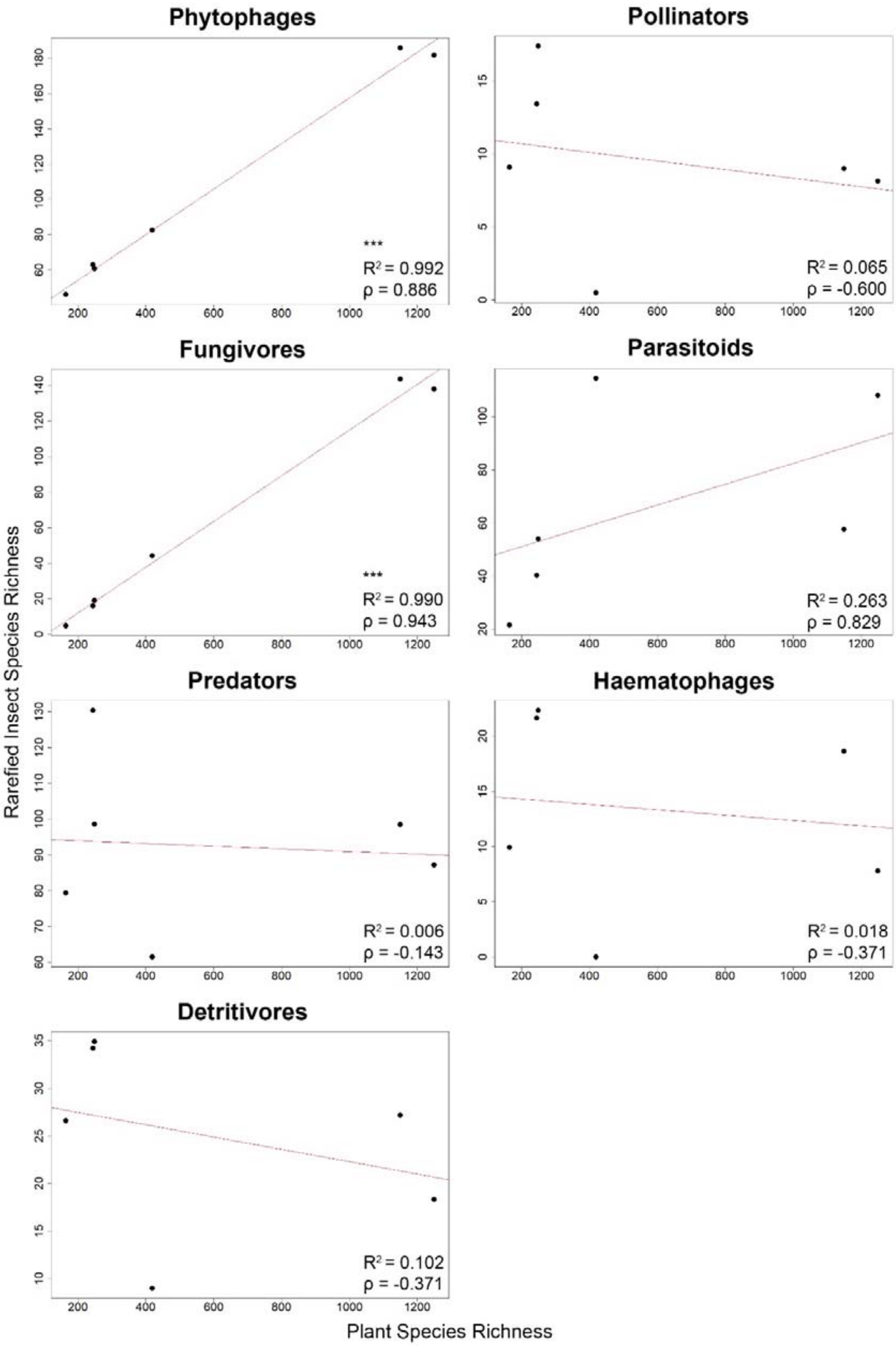

Figure 3. Only the diversity of phytophagous and fungivorous insects is correlated with plant diversity based on a linear regression model using rarefied insect species richness (*: ≤0.05, **: ≤0.01, ***: ≤0.001).

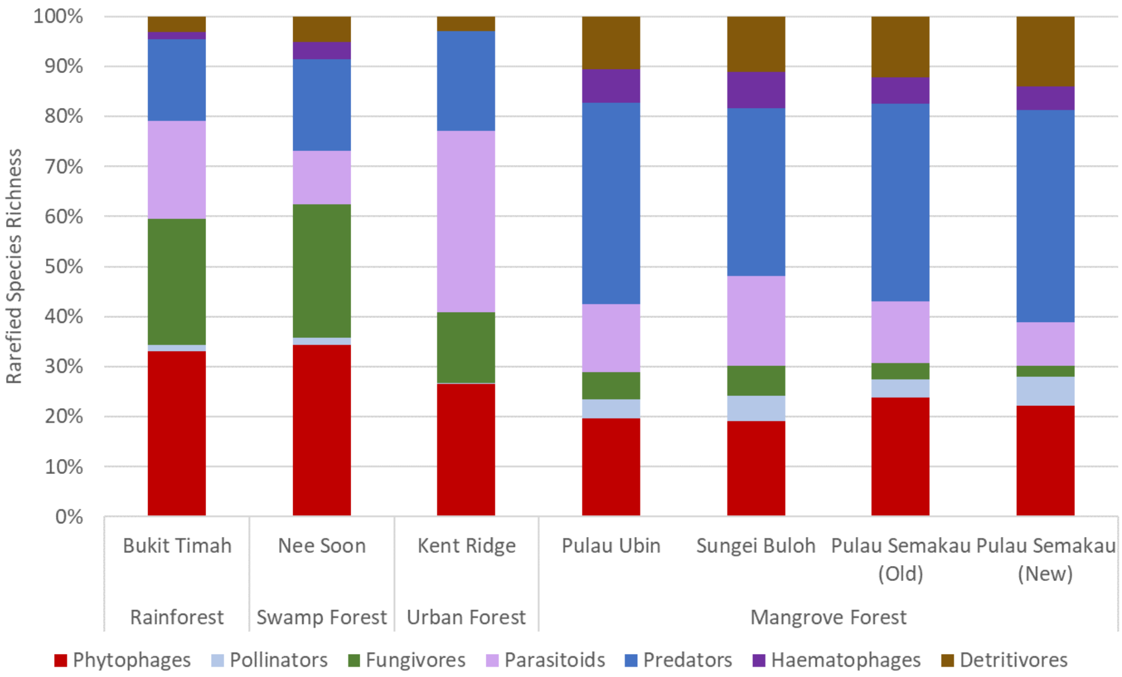

Figure 4. Distribution of insect guilds across habitats. Phytophages and fungivores dominate in rain and swamp forest, predators in mangroves and parasitoids in the urban forest (rarefied samples).

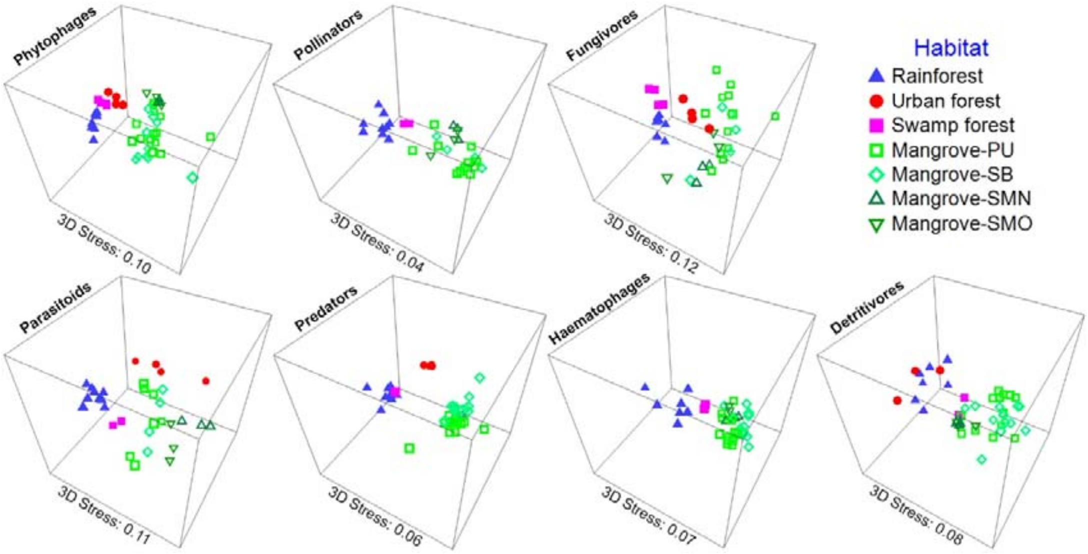

Figure 5. Habitat differentiation by insect guilds (3D NMDS plot of Bray-Curtis distances for habitats with >2 sites).

Species turnover across Asian mangroves

The specimens from Hong Kong belonged to 109 dolichopodid, 129 phorid, and 25 mycetophilid species. The corresponding number for Brunei were 96 and 76 species for dolichopodids and phorids, with too few mycetophilids being available for evaluation (Table S3). The southern Thai dolichopodids belonged to 74 species. We find high species turnover between Hong Kong, Brunei and Singapore, even after rarefying the specimen sample sizes (Fig. S6). Approximately 90% of all dolichopodid and phorid species are unique to each region and <1% are shared across all regions. Species turnover is even higher for the mycetophilids of Hong Kong and Singapore (>95%). Species turnover for the dolichopodids of Southern Thailand and Singapore is again high with only 11.5% of all species shared between both countries.

Discussion

Discovery of a largely overlooked, predator-enriched insect community in mangroves

It is often assumed that the insect diversity in mangroves is low because high salinity and low plant diversity are thought to interfere with insect diversification[23,67,68]. However, we here show that mangroves are species-rich despite low plant diversity (<250 species: [69–71]). In addition, the fauna of mangroves is very unique. More than half of its species are not found in other habitats, even though coastal forests are adjacent to mangroves. Indeed, after adjusting for sampling effort, the species diversity in Singapore's premier rainforest reserve (Bukit Timah Nature Reserve: 1.64 km^2) and largest swamp forest remnant (Nee Soon: 5 km^2) is only 50% higher than the diversity of major mangrove sites (PU: 0.904 km^2, SB: 1.168 km^2, SM: 0.174 km^2). The high diversity encountered in the mangrove sites was particularly unexpected because the rainforests of Bukit Timah Nature Reserve have been protected for more than 50 years[72,73] and have very high plant diversity (e.g., 1,250 species of vascular plants[63] including 341 species of trees[74] in a 2 ha plot of the Centre for Tropical Forest Science). Moreover, we extensively sampled the insect diversity in the reserve by placing multiple Malaise traps in primary, maturing secondary, and old secondary forests. Similarly, we expected the insect diversity of Singapore's largest swamp forest (Nee Soon) to greatly exceed the number of species found in the mangrove sites because the swamp forest is also known for its high species richness (e.g., 1,150 species of vascular plant species[75]).

A guild-level analysis reveals how mangroves maintain high species diversity. They are impoverished with regard to phytophagous and fungivorous species, but are home to a disproportionally large number of predatory species (Fig. 4) whose larvae develop in sediments (Empidoidea and Tabanidae). This suggests that the high insect diversity in different tropical habitats may be achieved by having larger proportions of species developing in the biologically most productive microhabitats – plants and fungi for many forest habitats and the rich and productive mud flats for mangroves.

In addition to finding high alpha-diversity in mangroves, we also document that the mangrove insect communities are very distinct. This conclusion is supported by a multitude of analyses (NMDS, ANOSIM & SIMPER). It is furthermore insensitive to the removal of rare species (Fig. 2) and driven by high species turnover rather than nestedness (see Table 1C). This stratification by habitat is still evident even when the two dominant insect orders in Malaise trap samples (Diptera and Hymenoptera) are removed (Fig. 2). Comparatively high overlap is only observed between mangroves and coastal forests (860 shared species) which is likely due to close proximity of the habitats on Pulau Ubin (Fig. S1) where back mangroves and coastal forests are contiguous. The uniqueness of the mangrove insect community is likely due to the unusual environmental conditions characterized by extreme daily fluctuations in salinity, temperature, and inundation. These extreme conditions likely require physiological and behavioural adaptations that encourage the emergence of an evolutionarily distinct fauna. What is surprising, however, is that we find no evidence for an adaptive radiation of particular clades. Instead, a large number of independent colonization events seems more likely given that the mangrove species usually belong to genera that are also known from other habitats (e.g., Dolichopodidae). This challenges the view that high salinity is a potent colonization barrier for invertebrates[67,68].

Mangrove regeneration is pursued in many countries, with mixed success in restoring the original plant diversity[76,77], but it remains poorly understood whether the regenerated mangroves harbour the original arthropod biodiversity. Our preliminary data based on 311 Malaise trap samples from one regenerated site suggests that this may not be the case. The regenerated mangrove (SMN) was replanted with a monoculture of *Rhizophora stylosa*[71] which replaced old-growth mangroves that had been cleared during reclamation work (1994–1999[51]). The restored site (SMN) has markedly lower insect species richness than all other mangrove sites, including a neighbouring old-growth mangrove (SMO; Fig. 1). This highlights once more the need for holistic habitat assessments that goes beyond plants and vertebrates[78].

Mangrove insect communities are not only rich and distinct in Singapore. Within Asia, we reveal a 92% species turnover between Singapore and Hong Kong (2,500 km north; Fig. S1) for taxa representing different guilds (Dolichopodidae–predators: 483 species, Mycetophilidae–fungivores: 67 species, Phoridae–mostly saprophagous: 591 species). While climatic differences could be advanced as a potential explanation, comparisons with the mangroves in the geographically close and tropical Borneo (Brunei) confirm a high species turnover of 85% (see also Grootaert 2019[79]). Further evidence for high regional species turnover in mangroves emerges when the dolichopodid fauna of Singapore's and Brunei's mangroves are compared with the fauna of Southern Thailand (coasts of South China and Andaman seas). Only 34 and 10 of the 74 known Thai species are shared with Singapore and Brunei respectively; These data suggest that a significant proportion of the global insect diversity may reside in mangroves. Based on the data from Singapore, it appears that much of the diversity may still be intact, given that we find no evidence that the insect diversity in Singapore's mangroves is depressed relative to what is found in the more pristine sites in Brunei or Hong Kong. This suggests that the loss of species diversity for small, flying insects in Singapore may not have been as dramatic as what has been documented for vertebrates and larger invertebrates[48,80,81].

Discovering a new insect hotspot with NGS barcoding

Global insect declines have recently received much attention by the scientific community[2] and public[82]. Obtaining relevant data is very difficult since quantifying insect diversity using conventional techniques is slow and expensive. This is because too many specimens have to be sorted into too many species before a holistic habitat assessment can be carried out[83]. In our study, this problem is overcome via sorting based on NGS barcodes which differ from traditional barcodes by costing only a fraction of barcodes obtained with Sanger sequencing. Based on previous tests, we find that species delimited with NGS barcodes have >90% congruence with species-level units delimited with morphological data[56,57,84,85]. This suggests that large-scale species discovery with NGS barcoding yields sufficiently accurate information on species abundance and distribution for habitat assessments[55,56]; i.e., NGS barcodes can be used for quickly revealing hidden hotspots of insect diversity in countries with high diversity and limited funding. We estimate that the ~140,000 specimens in our study could today be sequenced for <USD25,000 using 350 manpower days whereas a similar study based on morphology would require >150 manpower years[86]; i.e. some of the traditional obstacles to understanding insect biodiversity caused by the taxonomic impediment are finally disappearing.

Concluding remarks

We here document that the insect fauna inhabiting mangroves is not only rich, but also distinct when compared to many other tropical forest habitats. The discovery of such an unexpectedly rich and distinct insect community highlights how little we know about insect diversity. We predict that advances in sequencing technology will facilitate the discovery of numerous additional insect diversity hotspots in tropical and temperate habitats. Mangroves will likely be only one of many future additions to the growing list of habitats that have only recently been recognized as containing a large proportion of the global biodiversity (e.g., dry forests[87,88], forest savannahs[89,90]). Our study highlights that accelerating species discovery is a pressing task given that many of these habitats are disappearing at a much faster rate than tropical rainforests.

Methods

Sampling site, sample collection, and processing

Singapore has a large number of tropical habitat types that are all within 40 km of each other without being separated by major physical barriers. This allowed us to sample rainforests (from early secondary to mature secondary forest), urban-edge forests, mangroves, swamp forests, freshwater swamps and dry coastal forests. The freshwater swamp habitat differs from swamp forests by largely lacking tree-cover, while the dry coastal forests are distinct from the mangroves by lacking typical mangrove tree species. Note that the habitats had experienced similar levels of habitat degradation or loss due to urbanization (>95% loss of original vegetation cover[48]; *ca.* 90% loss for rainforests[49]; *ca.* 93% loss of swamp forest[50]; 91% loss for mangroves[51]). We sampled these habitat types using 107 trapping sites (Fig. S1). The mangrove sites were located primarily along the North-western and Southern coasts of the mainland, as well as on offshore islands in the south and northeast. The major mangrove sites were on Pulau Ubin (PU), Sungei Buloh (SB) and Pulau Semakau (SM), the last of which is represented by an old-growth (SMO) and a newly regenerated mangrove fragment (SMN).The swamp forest site (Nee Soon) was Singapore's largest remaining freshwater swamp remnant which is known for a rich insect fauna[91], overall high species richness, and level of endemism[92,93]. Bukit Timah Nature Reserve was selected as the tropical rainforest site given its high species diversity and protected status[72]. This reserve consists of forests in various stages of succession and hence we sampled different forest types with three sites each being in primary forest, old secondary forest, and maturing secondary forest. The "urban secondary forest" sites were located along a disturbance gradient ranging from the campus of the National University of Singapore (NUS) through several urban parks and forest edges in Central and South Singapore. The freshwater swamp site is located primarily in Kranji, a freshwater marsh at the flooded edge of a reservoir. The "coastal forest" sites were dry secondary forests adjacent to the coast at Labrador Park and Pulau Ubin, which are also close to urban settlements.

All specimens were collected between 2012–2019 (Table S1) using Malaise traps. These traps are widely used for insect surveys because they are effective sampling tools for flying insects and allow for standardized, long-term sampling. Note that the use of Malaise traps in our study was appropriate because the canopy height was comparable for most habitats given that we compared mature mangroves (PU, SB and SMO) with a wet swamp forest site, and different kinds of secondary forests (pers. obs.). Only the canopy height of some sites in Bukit Timah Nature Reserve (BTNR) was higher, but for BTNR we also included secondary forests and several traps were placed on steep slopes that would be able to sample canopy-active fauna from a lower elevation. With regard to the habitat patches, the fragments were larger for the rainforest and swamp forest than for any of the mangrove sites (tropical rainforest: 1.64 km^2; swamp forest: 5 km^2, mangrove forest fragments: 0.904 km^2 [PU], 1.168 km^2 [SB], 0.174 km^2 [SM][51]). Malaise traps in the mangroves were set up in the intertidal zone. Each Malaise trap sample consisted of one-week's worth of insects preserved in molecular grade ethanol. After an ethanol change, the specimens were sorted to order/family level by para-taxonomists, and specimens from 16 arthropod orders were extracted for barcoding (Fig. S2): Araneae, Blattodea, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Megaloptera, Neuroptera, Orthoptera, Phasmida, Plecoptera, Psocodea, Strepsiptera and Trichoptera. Diptera and Hymenoptera were the dominant orders in the Malaise traps (Fig. S2: >75% of specimens) and sorted further to family and genus-level where possible (Table S2), either based on morphology or based on DNA barcodes identified using the Global Biodiversity Information

Facility (GBIF: www.gbif.org) or the Barcode of Life Data (BOLD: www.boldsystems.org) databases. Only matches above 95% and 97% similarity were considered sufficiently precise for family- and genus-level matches respectively. The mangrove specimens from Hong Kong were collected by 24 Malaise traps installed between October 2017 to October 2018, while those from Brunei were collected by six Malaise traps from July to November 2014. Note that the mangrove forests in Brunei were less affected by urbanization than those in Singapore. The dolichopodid specimens from Thailand were obtained by different techniques including sweep-netting from 42 mangrove sites over a period of 15 months from Mar 2014 – Dec 2015.

Putative species sorting with NGS barcoding

NGS barcoding combines the advantages of cost-effective sequencing with Illumina with the approximate species-level resolution provided by DNA barcodes. The molecular procedures can be learned in hours and several hundred specimens can be processed per person and day. The overall barcode costs are now <10 cents per specimen if Illumina Novaseq is used for sequencing (2 cents/barcode based on USD 6,900 per 250-bp PE flow cell yielding 800 million reads: https://research.ncsu.edu/gsl/pricing). We used NGS barcoding to amplify and sequence a 313-bp fragment of the cytochrome oxidase I gene (*cox1*) using a protocol described in Meier et al.[55]. Direct-PCR[94] was conducted for specimens collected early in the study; during this phase, we used 1-2 legs of the specimen as template for obtaining the amplicon with the primer pair mlCO1intF: 5’-GGWACWGGWTGAACWGTWTAYCCYCC-3’[95] and jgHCO2198: 5’-TANACYTCNGGRTGNCCRAARAAYCA-3’[96]. For samples processed later, the whole specimen was immersed in Lucigen QuickExtract solution or HotSHOT buffer[97] and gDNA extraction was conducted non-destructively. The gDNA extract was then used as a PCR template with the afore-mentioned reagents and protocol. The primers used were labelled with 9-bp long barcodes that differed by at least three base pairs. Every specimen in each sequencing library was assigned a unique combination of labelled forward and reverse primers, which allowed the Illumina reads to be binned according to specimen. A negative control was prepared and sequenced for each 96-well PCR plate. Amplification success rates for each plate were assessed via gel electrophoresis for eight random wells per plate.

The amplicons were pooled at equal volumes within each plate and later pooled across plates. Equimolarity was estimated by the presence and intensity of bands on gels. The pooled samples were cleaned with Bioline SureClean Plus and/or via gel cuts before outsourcing library preparation to AITbiotech using TruSeq Nano DNA Library Preparation Kits (Illumina) or the Genome Institute of Singapore (GIS) using NEBNext DNA Library Preparation Kits (NEB). Paired-end sequencing was performed on Illumina Miseq (2x300-bp or 2x250-bp) or Hiseq 2500 platforms (2x250-bp) over multiple runs, thereby allowing troubleshooting and re-sequencing for specimens which initially failed to yield a sufficiently large number of reads. Some of the specimens were also sequenced on the MinION (Oxford Nanopore) platform using primers with a slightly longer tags (13-bp) and following the protocol described in Srivathsan et al.[98,57]. Raw Illumina reads were processed with the bioinformatics pipeline and quality-control filters described in Meier et al.[55]. A BLAST search to GenBank's nucleotide (nt) database was also conducted to identify and discard contaminants by parsing the BLAST output through *readsidentifier*[99] and removing barcodes with incorrect matches at >97% identity.

To obtain putative species units, the *cox1* barcodes were clustered over a range of uncorrected p-distance thresholds (2–4%) typically used for species delimitation in the literature[100]. The clustering was performed with a python script that implements the objective clustering algorithm of Meier et al. 2006[59] and allows for large scale processing. USEARCH[60] (cluster_fast) was used to confirm the results by setting -id at 0.96, 0.97 and 0.98. To gauge how many of our species/specimens matched barcodes in public databases, we used the "Sequence ID" search of the Global Biodiversity Information Facility (GBIF). We then determined the number of matches with identity scores <97. We then counted the number of matches to barcodes with species-level identifications.

Diversity analyses

For analysis of species richness and turnover, we excluded 11 trapping sites which had <100 specimens per site in order to prevent poor sampling from inflating site distinctness. To assess the species richness of the six major habitat types, samples were rarefied with the *iNEXT*[101] R package (R Development Core Team) using 1,000 bootstrap replicates in order to account for unequal sampling completeness. The rarefaction was performed by coverage[61] in the main analysis (Fig. 1) and by specimen count in the supplementary (Fig. S3). Site comparisons were carried out by comparing species diversity post-rarefaction to the lowest coverage/smallest number of specimens. The habitat type “mangrove” was treated both as a single habitat as well as separate sites (PU, SB, SMN, SMO, others) in separate analyses.

In order to study species turnover, we determined the distinctness of the communities across habitats using non-metric multidimensional scaling (NMDS) plots that were prepared with PRIMER v7[102] using Bray-Curtis dissimilarity. Plots were generated for each habitat type and the different mangrove sites; Bray-Curtis was chosen because it is a preferred choice for datasets that include abundance information. The dataset was split into three groups: the dominant orders (Diptera and Hymenoptera) and all others combined, in order to test if the results were driven by the dominant orders. Analysis of similarities (ANOSIM) and similarity percentages (SIMPER) were performed in PRIMER under default parameters in order to obtain ANOSIM p-values and R-statistics for both the entire dataset and the pairwise comparisons between habitat types. The SIMPER values were calculated for within and between-habitat types. The ANOSIM p-values can be used to assess significant differences while the R-statistic allows for determining the degree of similarity, with values closer to 1 indicating greater distinctness. We also used the *betapart*[103] R package to examine if the observed dissimilarity (Bray-Curtis) was due to species turnover or nestedness. The *beta.multi.abund* and *beta.pair.abund* functions were used to split the global and pairwise dissimilarity scores into turnover and nestedness components. Lastly, the robustness of the results was tested by removing singleton, doubleton and rare species (<5 and <10 individuals) from the datasets. The pruned datasets were subjected to the same analyses as the full dataset. For the guild-specific datasets, traps with fewer than three species were excluded in the species turnover analyses because large distances driven by undersampling can obscure signal.

To examine species turnover across larger geographic scales, dolichopodid, phorid, and mycetophilid specimens from Singapore were compared with those from Hong Kong (Dolichopodidae: 2,601; Phoridae: 562, Mycetophilidae: 186), and Brunei (Dolichopodidae: 2,800; Phoridae: 272), and data for the dolichopodids of Southern Thai mangroves (942 specimens). Since Singapore was more extensively sampled, the Singaporean dataset was randomly subsampled (10 iterations in Microsoft Excel with the RAND() function) to the number of specimens available for the other two countries (Table S3). The species diversity after rarefaction was then compared (with 95% confidence intervals for the rarefied data).

Ecological guild and plant diversity analyses

For the guild-level analysis, we focused primarily on the two dominant orders Diptera and Hymenoptera, which comprised of species from a large variety of ecological guilds. As splitting the dataset into smaller guild-level partitions would create low-abundance subsets, we excluded trapping sites that were sampled for <6 months, resulting in a dataset consisting of 62,066 specimens from 9 rainforest, 4 swamp forest, 4 urban forest, and 32 mangrove sites (Fig. S1). In order to test for an overall correlation between plant and insect diversity, we obtained data for the plant diversity in the respective sites from checklists and survey plots (Table S4). In order to further examine the correlation between plant and insect diversity across multiple ecological guilds, we assigned the identified Diptera and Hymenoptera families and genera non-exclusively to ecological guilds (phytophages, pollinators, fungivores, parasitoids, predators, haematophages and detritivores) based on known adult and larval natural history traits for the group (Table S2). Taxa with different adult and larval natural histories are placed in both guilds. Taxa lacking sufficient information or with highly variable life-history strategies were assigned to the "Others/Unknown" category and excluded from analysis.

Barcodes from each guild were separately aligned and clustered at 3% p-distance. These subsets were used for further analysis by randomly subsampling (10 iterations in Microsoft Excel with the RAND() function) the same number of specimens at the site with the smallest number of specimens (urban forest site, 2,543 specimens). For taxa that have adults and immatures with different natural histories (i.e., belong to two distinct ecological guilds), the species counts were halved and placed into both guilds when calculating rarefied species abundance and richness. Species turnover for the guild-specific subsets were analysed with PRIMER to generate NMDS plots, as well as ANOSIM and SIMPER values. The rarefied species richness values were also used for a multivariate model analysis. An ANCOVA model was constructed in R[104] with the *lm* function: *insectdiv ~ site * habitat * guild * plantdiv*, with insectdiv representing rarefied insect alpha-diversity and plantdiv representing plant species counts. The "site" factor was excluded due to collinearity and the model was refined via stepwise removal of factors starting with the most complex (interaction terms) and least significant ones. At each stage, the *anova* function was used to assess loss of informational content and the final model was derived when the reported p-value was significant ($p < 0.05$). The model's residuals were examined to ensure the data were normal. Subsequently, the *Anova* function from the *car* package[105] was used to obtain type-II test statistics. Finally, single-variable linear regression was performed in R with the *lm* function: *insectdiv ~ plantdiv* for each guild separately to obtain significance, multiple R-squared and Spearman's rho values.

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Competing interests

None

Table S1. Collection periods and trap localities; M = mangroves, SF = swamp forest, UF = urban forest, TF = tropical rainforest, CF = coastal forest, FS = freshwater swamp.

Sampling period	Location	Habitat type	GPS coordinates	No. of traps	Total no. of weekly samples	Used for guild-level analyses
Singapore						
Apr 2012 – Mar 2014	Pulau Ubin	M	1°24'36.3"N 103°59'25.5"E	3	72	Y
	Pulau Semakau original	M	1°12'17.6"N 103°45'37.7"E	3	72	Y
	Pulau Semakau replanted	M	1°12'03.1"N 103°45'45.4"E	3	72	Y
	Sungei Buloh Wetland Reserve	M	1°26'46.3"N 103°43'49.9"E	2	48	Y
	Nee Soon freshwater swamp	SF	1°23'00.3"N 103°48'46.5"E	2	48	Y
May 2014 – Jun 2014	Mandai Nature Park	M	1°26'18.3"N 103°45'49.7"E	3	6	N
	Pulau Tekong	M	1°25'47.3"N 104°03'46.3"E	3	6	N
	Sarimbun	M	1°25'59.1"N 103°41'21.8"E	3	6	N
Nov 2014 – May 2015	Nee Soon freshwater swamp	SF	1°23'00.3"N 103°48'46.5"E	2	14	Y
Apr 2015 – Sep 2015	NUS	UF	1°17'49.6"N 103°46'35.7"E	4	24	Y
Mar 2016 – Aug 2016	Pulau Ubin	M	1°25'11.64"N 103°56'6.25"E	10	60	Y
	Sungei Buloh Wetland Reserve	M	1°26'43.20"N 103°43'5.10"E	10	60	Y
	Labrador Park	M	1°16'13.3"N 103°48'10.1"E	3	18	N
	Labrador Park	CF	1°16'05.4"N 103°48'16.2"E	2	18	N
Aug 2016 – Oct 2017	Bukit Timah Nature Reserve primary forest	TF	1°21'13.90"N 103°46'47.57"E	3	45	Y
	Bukit Timah Nature Reserve old secondary forest	TF	1°21'17.96"N 103°46'54.01"E	3	45	Y
	Bukit Timah Nature	TF	1°21'4.57"N 103°46'53.80"E	3	45	Y

	Reserve maturing secondary forest					
Apr 2017 – 20 Sep 2017	NUS	UF	1°17'49.6"N 103°46'35.7"E	4	18	N
Sep 2017 – Dec 2017	NUS	UF	1°17'45.3"N 103°46'13.8"E	3	16	N
May 2018	Bishan-Ang Moh Kio Park	UF	1°21'35.7"N 103°50'49.9"E	2	2	N
	Enabling Village	UF	1°17'13.6"N 103°48'53.3"E	1	1	N
	Esplanade Theatre	UF	1°17'26.4"N 103°51'17.9"E	1	1	N
Mar 2018 – Jun 2018	Sungei Buloh Wetland Reserve	M	1°26'52.45"N 103°43'24.16"E	4	16	N
	Kranji Marshes	FS	1°25'0.56"N 103°43'43.50"E	3	12	N
	Lim Chu Kang	M	1°26'48.80"N 103°42'35.71"E	2	8	N
	Mandai Nature Park	M	1°26'37.96"N 103°45'59.70"E	4	16	N
	Pulau Ubin	CF	1°24'26.3"N 103°57'16.3"E	3	12	N
	Pulau Ubin	M	1°24'32.2"N 103°57'12.1"E	8	32	N
	Labrador Park	M	1°16'13.3"N 103°48'10.1"E	5	20	N
	Labrador Park	CF	1°16'05.4"N 103°48'16.2"E	4	20	N
Mar 2019 – Jun 2019	Coney Island	M	1°24'37.3"N 103°55'23.1"E	5	15	N
	Kranji Marshes	FS	1°25'11.0"N 103°43'54.3"E	4	15	N
	Pulau Ubin	CF	1°25'34.7"N 103°56'29.2"E	1	15	N
	Pulau Ubin	M	1°25'05.3"N 103°56'06.5"E	7	15	N
Hong Kong						
Nov 17 – Dec 17, May 18 – Jul 18	Ha Pak Nai	M	22°25'31.48"N 113°56'20.11"E	6	30	Y
	Hang Mei	M	22°15'9.83"N 113°52'5.84"E	5	25	Y
	Ho Chung	M	22°21'13.18"N 114°15'7.45"E	6	30	Y
	Lai Chi Wo	M	22°31'37.63"N 114°15'43.63"E	5	25	Y
	Nam Chung	M	22°31'31.62"N 114°12'28.94"E	5	25	Y
	Sai Keng	M	22°25'13.48"N 114°16'4.66"E	5	25	Y
	Sam A Chung	M	22°30'29.84"N 114°16'20.93"E	5	25	Y

	Sam A Tsuen	M	22°30'55.22"N 114°16'16.36"E	5	25	Y
	Sha Tau Kok	M	22°32'4.34"N 114°12'39.78"E	10	50	Y
	Sheung Pak Nai	M	22°27'7.09"N 113°57'45.11"E	5	25	Y
	Shui Hau	M	22°13'9.70"N 113°55'8.33"E	5	25	Y
	So Lo Pun	M	22°32'17.20"N 114°15'21.49"E	5	25	Y
	Tai O	M	22°15'28.44"N 113°51'48.96"E	6	30	Y
	Tai Tam	M	22°14'46.10"N 114°13'24.02"E	3	15	Y
	Tai Tan	M	22°26'18.85"N 114°19'59.77"E	1	5	Y
	To Kwa Peng	M	22°25'43.07"N 114°19'59.30"E	5	25	Y
	Tsim Bei Tsui	M	22°29'20.47"N 113°59'53.95"E	5	25	Y
	Tung Chung	M	22°16'52.50"N 113°55'44.04"E	6	30	Y
	Wong Chuk Wan	M	22°23'44.27"N 114°17'10.21"E	5	25	Y
	Yim Tin Tsai	M	22°22'32.74"N 114°18'5.76"E	5	25	Y
Brunei						
Jul 14 – Nov 14	Pulau Berambang	M	4°54'7.44"N 115°1'17.94"E	2	10	Y
	Labu Forest Reserve	M	4°51'41.75"N 115°6'59.69"E	2	10	Y
	Tutong Forest	M	4°46'9.54"N 114°36'20.64"E	2	10	Y

Table S2. Diptera and Hymenoptera species used in the guild-level analyses are identified to higher taxonomic levels where possible and assigned to ecological guild based on known natural history traits.

Taxon		Ecological Guild							
Family	**Genus**	**Phytophages**	**Pollinators**	**Fungivores**	**Parasitoids**	**Predators**	**Haematophages**	**Detritivores**	**Others/Unknown**
Diptera									
Agromyzidae		✓							
Anthomyiidae									✓
Asilidae						✓			
Asteiidae				✓					✓
Athericidae						✓			✓
Bombyliidae			✓			✓			
Calliphoridae									✓
Canacidae		✓							
Chloropidae	*Anacamptoneurum*	✓							
Chloropidae	*Cadrema*							✓	
Chloropidae	*Chlorops*	✓							
Chloropidae	*Chloropsina*	✓							
Chloropidae	*Conioscinella*	✓							
Chloropidae	*Dasyopa*								✓
Chloropidae	*Gampsocera*								✓
Chloropidae	*Gaurax*								✓
Chloropidae	*Lasiambia*					✓			
Chloropidae	*Liohippelates*	✓							
Chloropidae	*Malloewia*								✓
Chloropidae	*Olcella*	✓							
Chloropidae	*Oscinella*	✓							
Chloropidae	*Polyodaspis*					✓			
Chloropidae	*Pseudogaurax*					✓			
Chloropidae	*Pseudopachychaeta*	✓							
Chloropidae	*Rhodesiella*							✓	
Chloropidae	*Thaumatomyia*					✓			
Chloropidae	*Thyridula*								✓

Chloropidae	*Tricimba*						✓	
Clusiidae		✓						✓
Coelopidae		✓						
Cryptochetidae				✓				
Culicidae						✓	✓	
Diastatidae							✓	
Diopsidae		✓					✓	
Dolichopodidae					✓			
Drosophilidae	*Apenthecia*	✓						
Drosophilidae	*Chymomyza*	✓						
Drosophilidae	*Colocasiomyia*	✓						
Drosophilidae	*Dichaetophora*		✓					
Drosophilidae	*Drosophila*							✓
Drosophilidae	*Gitona*	✓						
Drosophilidae	*Hirtodrosophila*		✓					
Drosophilidae	*Hypselothyrea*	✓						
Drosophilidae	*Leucophenga*	✓						
Drosophilidae	*Liodrosophila*	✓						
Drosophilidae	*Luzonimyia*							✓
Drosophilidae	*Microdrosophila*	✓						
Drosophilidae	*Mycodrosophila*		✓					
Drosophilidae	*Paramycodrosophila*		✓					
Drosophilidae	*Scaptodrosophila*	✓						
Drosophilidae	*Scaptomyza*	✓						
Drosophilidae	*Stegana*	✓						
Drosophilidae	*Zaprionus*	✓						
Empididae					✓			
Ephydridae	*Allotrichoma*						✓	
Ephydridae	*Atissa*						✓	
Ephydridae	*Brachydeutera*	✓						
Ephydridae	*Cerobothrium*							✓
Ephydridae	*Ceropsilopa*	✓						
Ephydridae	*Discocerina*	✓						
Ephydridae	*Donaceus*							✓

Ephydridae	*Glenanthe*					✓
Ephydridae	*Hecamedoides*					✓
Ephydridae	*Hydrellia*	✓				
Ephydridae	*Limnellia*					✓
Ephydridae	*Nostima*	✓				
Ephydridae	*Notiphila*				✓	
Ephydridae	*Ochthera*			✓		
Ephydridae	*Orasiopa*					✓
Ephydridae	*Paralimna*	✓				
Ephydridae	*Placopsidella*			✓		
Ephydridae	*Polytrichophora*					✓
Ephydridae	*Ptilomyia*					✓
Ephydridae	*Rhynchopsilopa*					✓
Ephydridae	*Trimerogastra*					✓
Ephydridae	*Trypetomima*					✓
Ephydridae	*Zeros*					✓
Hybotidae				✓		
Keroplatidae		✓	✓			
Lauxaniidae		✓				
Lonchaeidae		✓				
Lygistorrhinidae		✓	✓			
Megamerinidae				✓		
Micropezidae						✓
Milichiidae	*Aldrichiomyza*					✓
Milichiidae	*Leptometopa*				✓	
Milichiidae	*Milichia*					✓
Milichiidae	*Milichiella*				✓	
Milichiidae	*Neophyllomyza*	✓				
Milichiidae	*Paramyia*					✓
Milichiidae	*Phyllomyza*				✓	
Muscidae						✓
Mycetophilidae		✓	✓			
Neriidae		✓				✓
Odiniidae						✓

Periscelididae		✓							
Phoridae									✓
Pipunculidae					✓				
Platypezidae				✓				✓	
Platystomatidae									✓
Psilidae		✓							
Pyrgotidae					✓				
Rhagionidae						✓	✓		
Rhiniidae						✓			
Sarcophagidae									✓
Sciaridae		✓		✓					
Sphaeroceridae									✓
Stratiomyiidae		✓						✓	
Syrphidae	*Allobaccha*		✓			✓			
Syrphidae	*Allograpta*		✓			✓			
Syrphidae	*Asarkina*		✓			✓			
Syrphidae	*Ceriana*		✓					✓	
Syrphidae	*Eosmallota*		✓					✓	
Syrphidae	*Eristalinus*		✓					✓	
Syrphidae	*Eristalis*		✓					✓	
Syrphidae	*Eumerus*		✓					✓	
Syrphidae	*Graptomyza*		✓					✓	
Syrphidae	*Ischiodon*		✓			✓			
Syrphidae	*Microdon*		✓			✓			
Syrphidae	*Paragus*		✓			✓			
Syrphidae	*Psilota*		✓					✓	
Syrphidae	*Spheginobaccha*		✓						✓
Syrphidae	*Syritta*		✓					✓	
Syrphidae	*Volucella*		✓					✓	
Tabanidae						✓	✓		
Tachinidae					✓				✓
Tephritidae		✓							
Ulidiidae									✓
Xenasteiidae									✓

Xylomyidae					✓
Hymenoptera					
Aphelinidae			✓		
Apidae		✓			
Bethylidae			✓	✓	
Braconidae			✓		
Ceraphronidae			✓		
Chalcidae			✓		
Chrysididae			✓		
Colletidae		✓			
Crabronidae					✓
Diapriidae			✓		
Dryinidae			✓		
Eulophidae			✓		
Eupelmidae			✓		
Evaniidae			✓		
Figitidae			✓		
Formicidae	*Acropyga*				✓
Formicidae	*Anochetus*			✓	
Formicidae	*Anoplolepis*			✓	
Formicidae	*Aphaenogaster*				✓
Formicidae	*Brachyponera*			✓	
Formicidae	*Camponotus*				✓
Formicidae	*Cardiocondyla*				✓
Formicidae	*Carebara*			✓	
Formicidae	*Cataulacus*				✓
Formicidae	*Chronoxenus*				✓
Formicidae	*Colobopsis*				✓
Formicidae	*Crematogaster*				✓
Formicidae	*Cryptopone*				✓
Formicidae	*Diacamma*			✓	
Formicidae	*Discothyrea*			✓	
Formicidae	*Dolichoderus*				✓
Formicidae	*Echinopla*				✓

Formicidae	*Ectomomyrmex*		✓	
Formicidae	*Euponera*		✓	
Formicidae	*Euprenolepis*			✓
Formicidae	*Gauromyrmex*			✓
Formicidae	*Hypoponera*		✓	
Formicidae	*Iridomyrmex*			✓
Formicidae	*Leptogenys*			✓
Formicidae	*Lioponera*		✓	
Formicidae	*Mayriella*			✓
Formicidae	*Meranoplus*			✓
Formicidae	*Mesoponera*			✓
Formicidae	*Monomorium*			✓
Formicidae	*Myrmecina*		✓	
Formicidae	*Nylanderia*			✓
Formicidae	*Odontomachus*			✓
Formicidae	*Odontoponera*			✓
Formicidae	*Oecophylla*			✓
Formicidae	*Paraparatrechina*			✓
Formicidae	*Paratopula*			✓
Formicidae	*Paratrechina*			✓
Formicidae	*Pheidole*			✓
Formicidae	*Philidris*	✓		
Formicidae	*Platythyrea*		✓	
Formicidae	*Polyrhachis*	✓		
Formicidae	*Ponera*			✓
Formicidae	*Prenolepis*			✓
Formicidae	*Prionopelta*			✓
Formicidae	*Proatta*		✓	
Formicidae	*Probolomyrmex*			✓
Formicidae	*Pseudoneoponera*		✓	
Formicidae	*Strumigenys*		✓	
Formicidae	*Rhopalomastix*		✓	
Formicidae	*Solenopsis*			✓
Formicidae	*Stigmatomma*		✓	

Formicidae	*Strumigenys*				✓	
Formicidae	*Tapinoma*					✓
Formicidae	*Technomyrmex*					✓
Formicidae	*Tetramorium*				✓	
Formicidae	*Tetraponera*	✓				
Formicidae	*Vollenhovia*					✓
Halictidae			✓			
Ichneumonidae				✓		
Megachilidae			✓			
Mymaridae				✓		
Platygastridae				✓		
Pompilidae				✓		
Pteromalidae				✓		
Scoliidae				✓		
Sphecidae				✓		
Sphecidae					✓	
Tiphiidae				✓		
Trichogrammatidae				✓		
Vespidae			✓		✓	

Table S3. Number of specimens from Singapore, Hong Kong and Brunei, as well as the size of the randomized subsample from Singapore.

	No. of Specimens				
Taxon	**Singapore**	**Singapore (Rarefied)**	**Hong Kong**	**Brunei**	**Thailand**
Dolichopodidae	17860	2800	2563	2798	924
Phoridae	2134	560	562	272	-
Mycetophilidae	223	180	186	-	-
Total	**20217**	**3540**	**3311**	**3070**	**924**

Table S4. Number of species of vascular plants for each sampling site in Singapore from checklist data.

Sampling Site	**Habitat**	**No. of Plant Species**	**Reference**
Nee Soon freshwater swamp	Freshwater swamp forest	1150	Wong et al., 2013[1]
Bukit Timah Nature Reserve	Rainforest	1250	Ho et al., 2019[2]
Kent Ridge	Urban-edge/disturbed forest	420	Tan et al., 2019[3]
Pulau Ubin	Mangrove	245	Lee et al., 2003[4]
Sungei Buloh Wetland Reserve	Mangrove	249	Tan et al., 1997[5]
Pulau Semakau	Mangrove	165	Teo et al., 2011[6]

Table S5. Number and distribution of mOTUs delimited using different thresholds (144,865 barcoded specimens)

Habitat/Country	**No. of Barcodes**	**No. of mOTUs from Objective Clustering**			**No. of mOTUs from USEARCH**		
		2%	**3%**	**4%**	**id=0.98**	**id=0.97**	**id=0.96**
Singapore full dataset							
Mangroves	67239	3557	3437	3320	3710	3524	3436
Rainforest	15669	2625	2573	2539	2669	2603	2570
Swamp forest	9464	1843	1804	1753	1895	1828	1795
Urban forest	20323	1552	1515	1478	1616	1549	1510
Freshwater swamp	21994	1881	1812	1744	1988	1878	1805
Coastal forest	9118	1707	1667	1627	1755	1691	1664
Total	**143807**	**8903**	**8572**	**8256**	**9315**	**8821**	**8520**
Subset used for guild-level analysis							
Mangroves	37641	1778	1720	1673	1828	1744	1702
Rainforest	9212	1525	1490	1474	1545	1503	1483
Swamp forest	5893	1090	1052	1030	1105	1070	1048
Urban forest	9320	919	898	885	941	908	893
Total	**62066**	**4169**	**4002**	**3917**	**4298**	**4098**	**3994**
Southeast and East Asian datasets							
Dolichopodidae							
Singapore	17860	263	254	248	280	259	249
Hong Kong	2601	111	109	104	115	110	106
Brunei	2800	98	96	95	107	98	95
Thailand	924	80	74	72	93	80	73
Total	**24185**	**480**	**453**	**426**	**543**	**482**	**447**
Phoridae							
Singapore	2134	293	281	278	300	285	280
Hong Kong	562	137	129	125	138	130	129
Brunei	272	76	76	75	77	76	75
Total	**2968**	**453**	**429**	**417**	**467**	**437**	**431**
Mycetophilidae							
Singapore	223	45	44	43	45	44	44
Hong Kong	186	26	25	25	26	25	25
Total	**409**	**69**	**67**	**67**	**70**	**67**	**67**

Table S6. Common and rare species found in only 1, 2, 3, 4, 5 or all habitats.

	No. of species				
	Full dataset	**No singletons**	**No doubletons**	**No species with <5 specimens**	**No species with <10 specimens**
Species in **mangroves** only	1788	880	638	441	256
Species in **rainforests** only	1569	638	415	243	91
Species in **swamp forests** only	875	342	200	102	39
Species in **urban forests** only	509	166	101	58	25
Species in **freshwater swamps** only	794	360	237	127	56
Species in **coastal forests** only	454	153	71	33	14
Species in **two habitats**	1580	1580	1253	887	555
Species in **three habitats**	565	565	565	494	350
Species in **four habitats**	274	274	274	265	230
Species in **five habitats**	116	116	116	116	109
Species in **all habitats**	48	48	48	48	48
Total	**8572**	**5122**	**3918**	**2814**	**1773**

Table S7. Species turnover ANOSIM analysis results indicate distinct communities in each habitat type, whether with singletons and doubletons removed, or species with less than 5 and 10 specimens. Pairwise p-value outputs are displayed in the bottom-left of the pairwise matrix while the R-statistics are displayed at the top-right.

No Singletons

Overall P: 0.001 **Overall R:** 0.777

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.809	0.981	0.948	0.972	0.951
Urban forest	0.001		0.747	0.815	0.571	0.173
Swamp forest	0.001	0.001		0.927	0.756	0.893
Mangrove	0.001	0.001	0.001		0.852	0.541
Freshwater swamp	0.001	0.001	0.008	0.001		0.347
Coastal forest	0.001	0.083	0.005	0.001	0.017	

No Doubletons

Overall P: 0.001 **Overall R:** 0.774

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.803	0.980	0.946	0.972	0.954
Urban forest	0.001		0.735	0.816	0.563	0.179
Swamp forest	0.001	0.001		0.922	0.750	0.889
Mangrove	0.001	0.001	0.001		0.849	0.538
Freshwater swamp	0.001	0.001	0.008	0.001		0.331
Coastal forest	0.002	0.072	0.005	0.001	0.019	

No Species <5 Specimens

Overall P: 0.001 **Overall R:** 0.767

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.795	0.970	0.941	0.971	0.954
Urban forest	0.001		0.720	0.817	0.559	0.180
Swamp forest	0.001	0.001		0.913	0.750	0.885
Mangrove	0.001	0.001	0.001		0.843	0.533
Freshwater swamp	0.002	0.001	0.008	0.001		0.331
Coastal forest	0.002	0.061	0.005	0.001	0.017	

No Species <10 Specimens

Overall P: 0.001 **Overall R:** 0.759

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.779	0.959	0.934	0.967	0.952
Urban forest	0.001		0.701	0.819	0.548	0.178
Swamp forest	0.001	0.002		0.904	0.738	0.877
Mangrove	0.001	0.001	0.001		0.837	0.526
Freshwater swamp	0.002	0.001	0.008	0.001		0.331
Coastal forest	0.001	0.062	0.005	0.001	0.017	

Table S8. Species turnover SIMPER analysis results indicate distinct communities in each habitat type, whether with singletons and doubletons removed, or species with less than 5 and 10 specimens.

No Singletons

	Within habitat (%)	Between habitats (%)					
		Rain-forest	Urban forest	Swamp forest	Mangrove	Fresh-water swamp	Coastal forest
Rainforest	33.65						
Urban forest	13.70	3.57					
Swamp forest	35.74	15.86	3.31				
Mangrove	12.78	1.80	3.26	2.22			
Freshwater swamp	18.80	2.36	5.06	4.57	2.93		
Coastal forest	12.98	4.27	10.04	4.50	6.44	9.82	

No Doubletons

	Within habitat (%)	Between habitats (%)					
		Rain-forest	Urban forest	Swamp forest	Mangrove	Fresh-water swamp	Coastal forest
Rainforest	35.83						
Urban forest	14.15	3.79					
Swamp forest	38.05	17.12	3.55				
Mangrove	13.14	1.91	3.36	2.39			
Freshwater swamp	19.61	2.52	5.30	4.90	3.07		
Coastal forest	13.62	4.57	10.44	4.86	6.68	10.37	

No Species <5 Specimens

	Within habitat (%)	Between habitats (%)					
		Rain-forest	Urban forest	Swamp forest	Mangrove	Fresh-water swamp	Coastal forest
Rainforest	38.68						
Urban forest	14.86	4.13					
Swamp forest	40.06	18.89	3.93				
Mangrove	13.65	2.08	3.50	2.65			
Freshwater swamp	20.84	2.76	5.68	5.46	3.29		
Coastal forest	14.49	4.99	11.08	5.39	7.03	11.15	

No Species <10 Specimens

	Within habitat (%)	Between habitats (%)					
		Rain-forest	Urban forest	Swamp forest	Mangrove	Fresh-water swamp	Coastal forest
Rainforest	42.79						
Urban forest	15.91	4.79					
Swamp forest	42.93	21.56	4.49				
Mangrove	14.55	2.41	3.75	3.04			
Freshwater swamp	22.41	3.25	6.28	6.25	3.63		
Coastal forest	15.95	5.83	12.09	6.19	7.65	12.16	

Table S9. Species turnover and nestedness analysis reveal that the high dissimilarity is due more to turnover rather than nestedness, whether with singletons and doubletons removed, or species with less than 5 and 10 specimens. Pairwise turnover values are displayed in the bottom-left of the pairwise matrix while the nestedness values are in the top-right.

No Singletons

Overall Dissimilarity: 0.944 **Overall Turnover:** 0.894 **Overall Nestedness:** 0.051

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.013	0.075	0.058	0.009	0.020
Urban forest	0.911		0.031	0.099	0.005	0.107
Swamp forest	0.693	0.918		0.098	0.030	0.002
Mangrove	0.908	0.816	0.871		0.063	0.263
Freshwater swamp	0.953	0.889	0.928	0.876		0.098
Coastal forest	0.905	0.695	0.936	0.648	0.748	

No Doubletons

Overall Dissimilarity: 0.944 **Overall Turnover:** 0.892 **Overall Nestedness:** 0.052

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.015	0.078	0.060	0.010	0.020
Urban forest	0.909		0.033	0.099	0.004	0.112
Swamp forest	0.685	0.915		0.102	0.032	0.003
Mangrove	0.906	0.816	0.868		0.064	0.268
Freshwater swamp	0.952	0.889	0.926	0.875		0.101
Coastal forest	0.903	0.687	0.934	0.643	0.744	

No Species <5 Specimens

Overall Dissimilarity: 0.944 **Overall Turnover:** 0.891 **Overall Nestedness:** 0.054

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.017	0.081	0.063	0.011	0.020
Urban forest	0.905		0.037	0.099	0.004	0.118
Swamp forest	0.677	0.912		0.107	0.035	0.004
Mangrove	0.904	0.817	0.862		0.064	0.274
Freshwater swamp	0.950	0.889	0.922	0.875		0.103
Coastal forest	0.902	0.679	0.931	0.638	0.741	

No Species <10 Specimens

Overall Dissimilarity: 0.945 **Overall Turnover:** 0.888 **Overall Nestedness:** 0.057

	Rainforest	Urban forest	Swamp forest	Mangrove	Freshwater swamp	Coastal forest
Rainforest		0.023	0.082	0.069	0.014	0.018
Urban forest	0.897		0.041	0.099	0.003	0.128
Swamp forest	0.665	0.907		0.115	0.038	0.005
Mangrove	0.898	0.818	0.856		0.065	0.282
Freshwater swamp	0.944	0.890	0.917	0.876		0.105
Coastal forest	0.899	0.665	0.927	0.632	0.742	

Table S10. Rarefied species richness values and confidence intervals of data subset that could be assigned to ecological guilds.

Habitat Type	**Rainforest**		**Freshwater Swamp Forest**		**Urban Forest**		**Mangrove Forest**							
Site	**Bukit Timah**		**Nee Soon**		**Kent Ridge**		**Pulau Ubin**		**Sungei Buloh**		**Pulau Semakau (Old)**		**Pulau Semakau (New)**	
Ecological Guild	**Mean**	**C.I.**	**Mean**	**C.I.**	**Mean**	**C.I.**	**Mean**	**C.I.**	**Mean**	**C.I.**	**Mean**	**C.I.**	**Mean**	**C.I.**
Phytophages	181.80	±2.64	185.95	±3.25	82.50	NA	62.75	±2.23	60.60	±1.43	51.15	±1.89	31.15	±1.63
Pollinators	8.15	±0.71	9.00	±0.87	0.50	NA	13.45	±1.28	17.40	±0.76	7.40	±1.41	9.10	±1.12
Fungivores	137.95	±2.58	143.55	±2.09	44.00	NA	16.00	±1.62	19.05	±0.73	6.20	±1.05	3.10	±0.72
Parasitoids	108.15	±2.22	57.60	±2.2	114.50	NA	40.25	±2.31	53.90	±1.52	29.90	±2.68	13.75	±1.62
Predators	87.15	±2.71	98.50	±1.05	61.50	NA	130.40	±3.23	98.60	±1.63	85.95	±1.94	59.80	±2.64
Haematophages	7.80	±0.76	18.65	±0.76	0.00	NA	21.65	±0.67	22.35	±0.83	12.05	±0.86	5.95	±0.43
Detritivores	18.30	±1.37	27.15	±1.17	9.00	NA	34.20	±1.29	34.90	±0.87	26.65	±1.24	19.05	±1.01

Table S11. Species turnover ANOSIM analysis results indicate distinct communities in each habitat type for each ecological guild. Pairwise p-value outputs are displayed in the bottom-left of the pairwise matrix while the R-statistics are displayed at the top-right.

Phytophages

Overall P: 0.001 **Overall R:** 0.588

	Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest		1.000	0.706	0.721
Urban forest	0.001		1.000	0.468
Swamp forest	0.001	0.029		0.665
Mangrove	0.001	0.003	0.001	

Pollinators

Overall P: 0.001 **Overall R:** 0.836

	Rainforest	Swamp forest	Mangrove
Rainforest		0.387	0.915
Swamp forest	0.127		0.853
Mangrove	0.001	0.004	

Fungivores

Overall P: 0.001 **Overall R:** 0.351

	Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest		1.000	0.726	0.435
Urban forest	0.001		1.000	0.088
Swamp forest	0.001	0.029		0.432
Mangrove	0.001	0.206	0.001	

Parasitoids

Overall P: 0.001 **Overall R:** 0.758

	Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest		0.962	0.925	0.793
Urban forest	0.001		1.000	0.736
Swamp forest	0.018	0.067		0.711
Mangrove	0.001	0.001	0.006	

Predators

Overall P: 0.001 **Overall R:** 0.906

	Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest		1.000	0.414	0.954
Urban forest	0.001		1.000	0.916
Swamp forest	0.109	0.067		0.913
Mangrove	0.001	0.001	0.002	

Haematophages

Overall P: 0.001 **Overall R:** 0.905

	Rainforest	**Swamp forest**	**Mangrove**
Rainforest		0.435	0.957
Swamp forest	0.139		0.791
Mangrove	0.001	0.002	

Detritivores

Overall P: 0.001 **Overall R:** 0.853

	Rainforest	**Urban forest**	**Swamp forest**	**Mangrove**
Rainforest		0.613	0.487	0.949
Urban forest	0.008		1.000	0.904
Swamp forest	0.056	0.100		0.614
Mangrove	0.001	0.001	0.002	

Table S12. Species turnover SIMPER analysis results indicate distinct communities in each habitat type for each ecological guild.

Phytophages

	Within habitat (%)	Between habitats (%)			
		Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest	30.46				
Urban forest	27.72	4.93			
Swamp forest	34.21	18.38	5.18		
Mangrove	12.37	1.29	4.13	1.67	

Pollinators

	Within habitat (%)	Between habitats (%)		
		Rainforest	Swamp forest	Mangrove
Rainforest	41.27			
Swamp forest	48.30	28.15		
Mangrove	26.01	0.88	3.57	

Fungivores

	Within habitat (%)	Between habitats (%)			
		Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest	32.01				
Urban forest	31.71	4.48			
Swamp forest	36.88	19.40	4.61		
Mangrove	10.58	1.87	8.26	1.34	

Parasitoids

	Within habitat (%)	Between habitats (%)			
		Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest	27.47				
Urban forest	10.13	3.18			
Swamp forest	59.26	10.76	1.14		
Mangrove	12.00	2.40	2.43	2.84	

Predators

	Within habitat (%)	Between habitats (%)			
		Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest	29.22				
Urban forest	34.03	4.60			
Swamp forest	64.88	19.72	3.62		
Mangrove	22.78	0.28	1.35	1.20	

Haematophages

	Within habitat (%)	Between habitats (%)		
		Rainforest	Swamp forest	Mangrove
Rainforest	18.86			
Swamp forest	56.42	10.55		
Mangrove	27.40	0.61	9.27	

Detritivores

	Within habitat (%)	Between habitats (%)			
		Rainforest	Urban forest	Swamp forest	Mangrove
Rainforest	14.99				
Urban forest	20.18	7.12			
Swamp forest	52.77	9.49	1.67		
Mangrove	18.87	0.37	1.33	6.91	

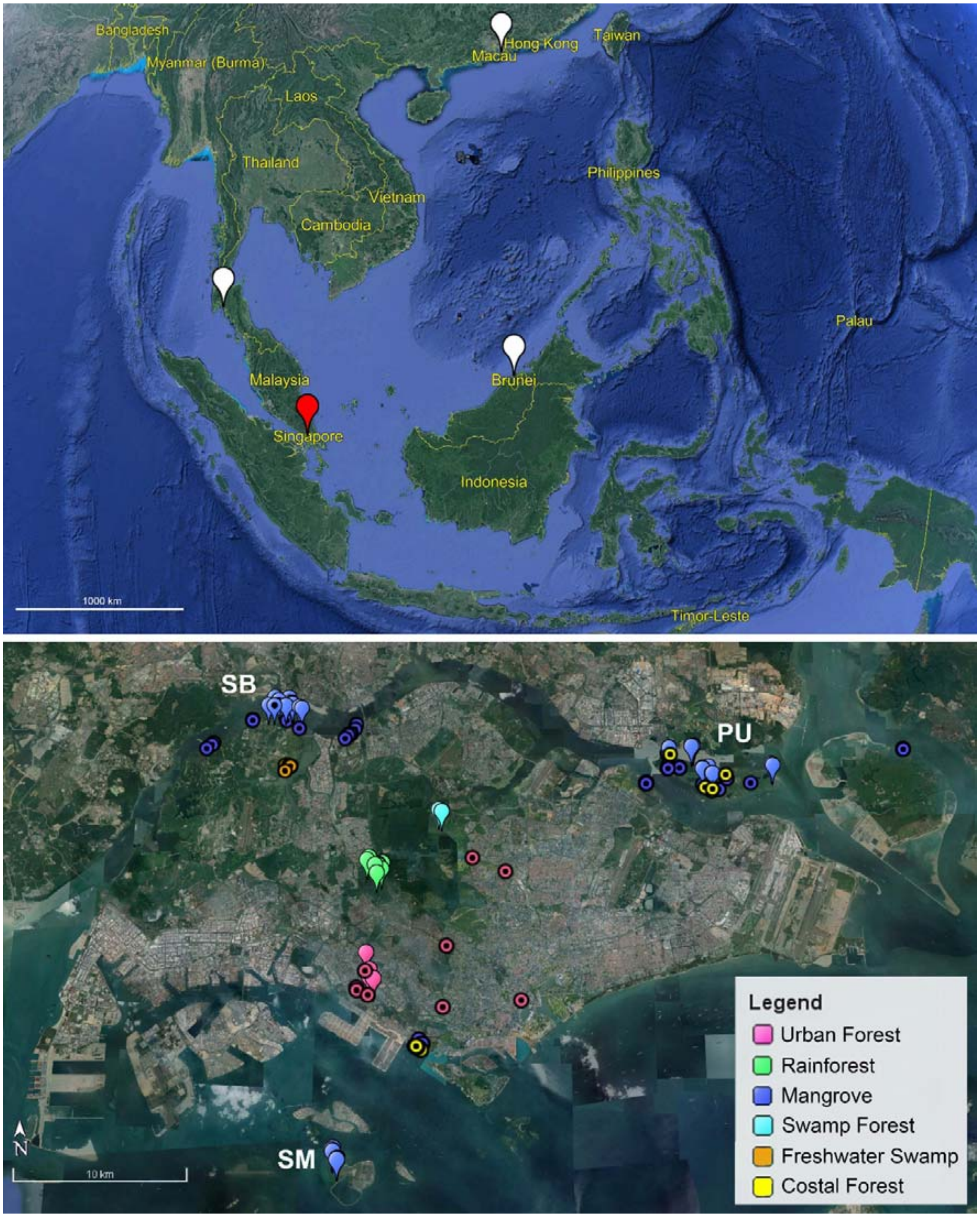
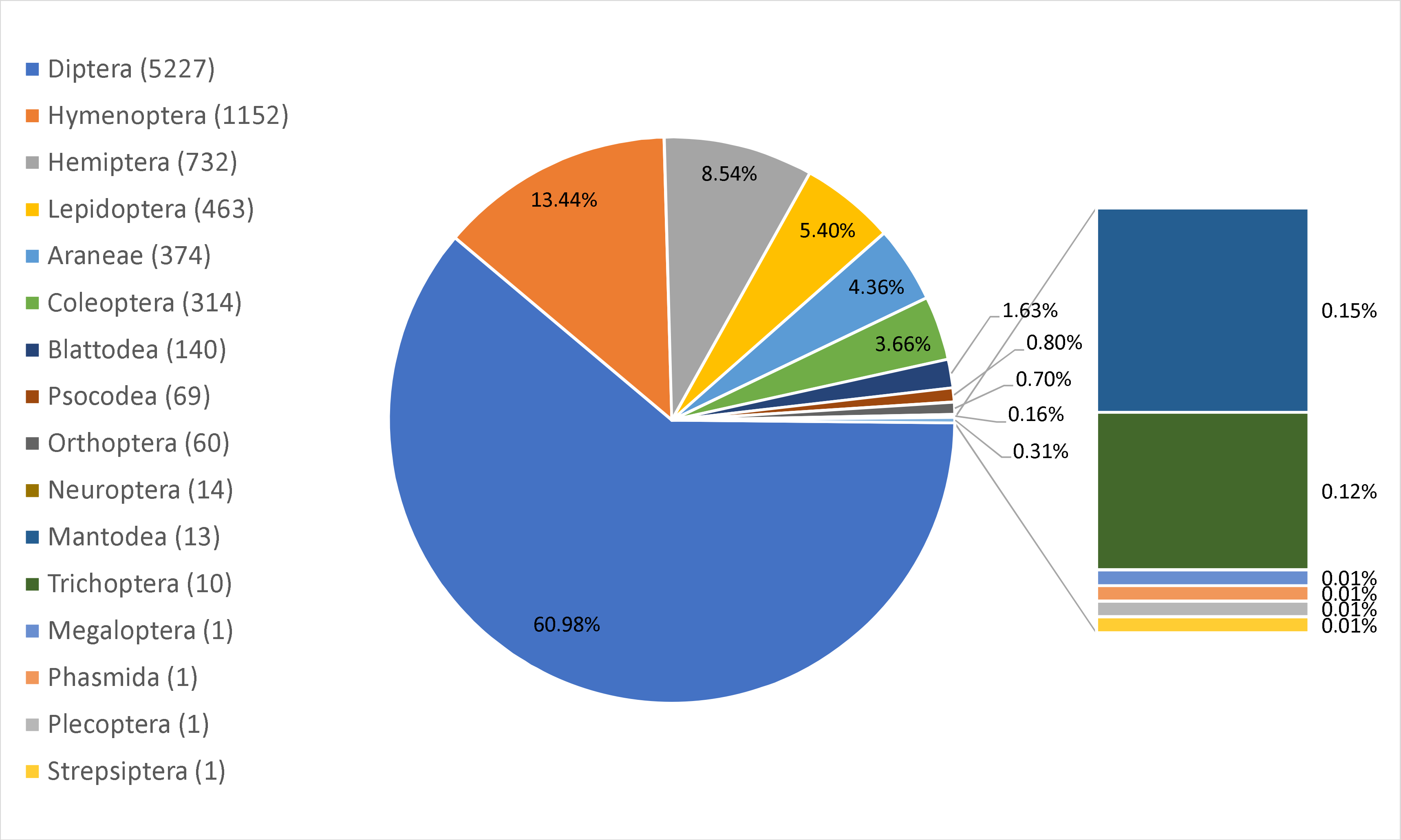


Figure S1. Sampling localities in the Oriental Realm (*top*: Singapore, red; other countries, white) and within Singapore (*bottom*: circular markers indicate trapping sites excluded from the species turnover analyses; pin markers with dot indicate traps excluded from guild-level analyses).

Figure S2. Arthropod orders sampled with Malaise traps in this study and their species proportions. The number beside each order indicates the number of species sampled based on 3% p-distance objective clustering.



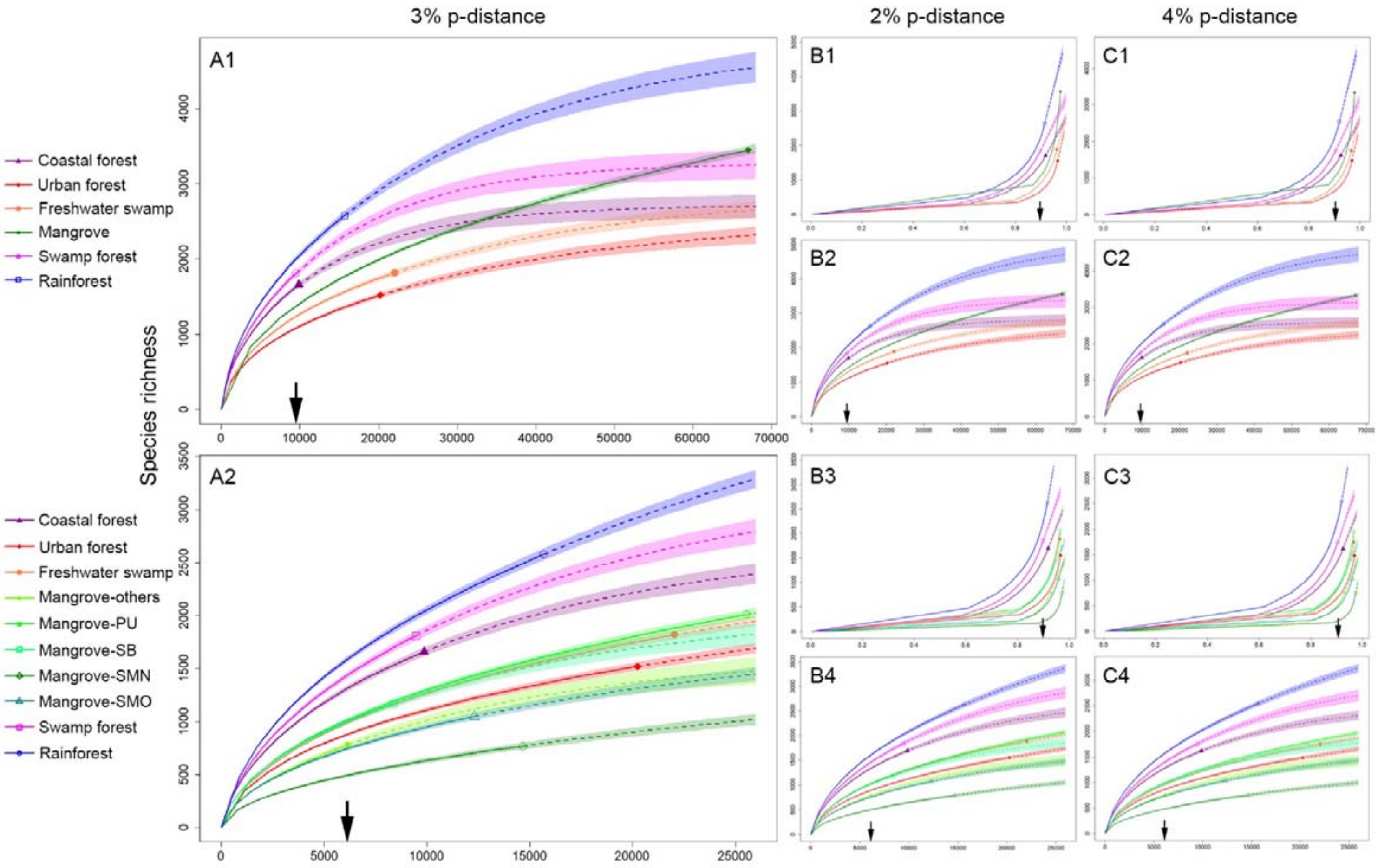


Figure S3. Insect alpha-diversity across tropical forest habitats rarefied by specimens (A1 & 2, B2 & 4, C2 & 4) and coverage (B1 & 3, C1 & 3), for 2% (B1 – 4), 3% (A1 – 2) and 4% (C1 – 4) p-distances mOTUs. Mangroves are treated as a single habitat (top) and split by site in a separate analysis (bottom): Pulau Ubin (PU), Sungei Buloh (SB), Pulau Semakau old grove (SMO), Pulau Semakau new grove (SMN); solid lines = rarefaction; dotted = extrapolations. The arrow on the x-axis indicate the point of rarefaction at which species richness comparisons were made.

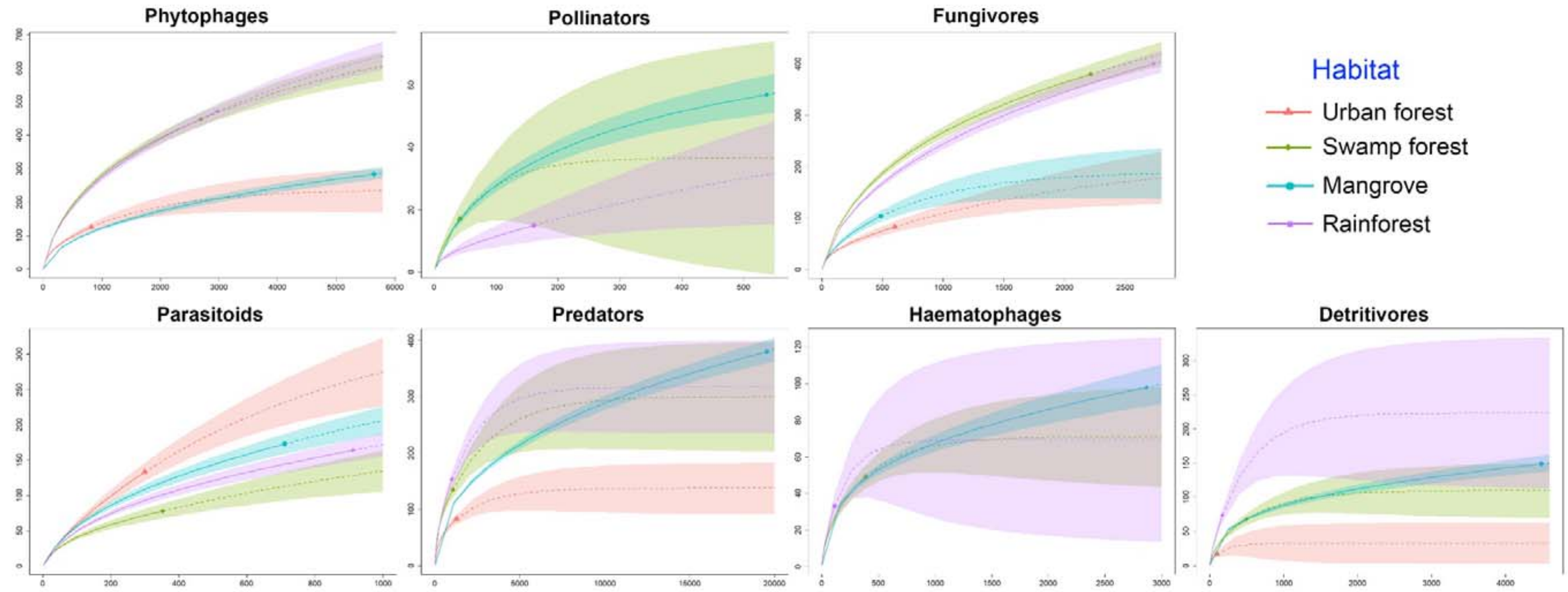


Figure S4. Comparison of species diversity across habitats (3% p-distance mOTUs) split by ecological guild. Curves were plotted for the mangrove sites as a single habitat type. The full lines represent rarefactions, while the dotted lines extrapolations and the point between the lines as actual observed values.

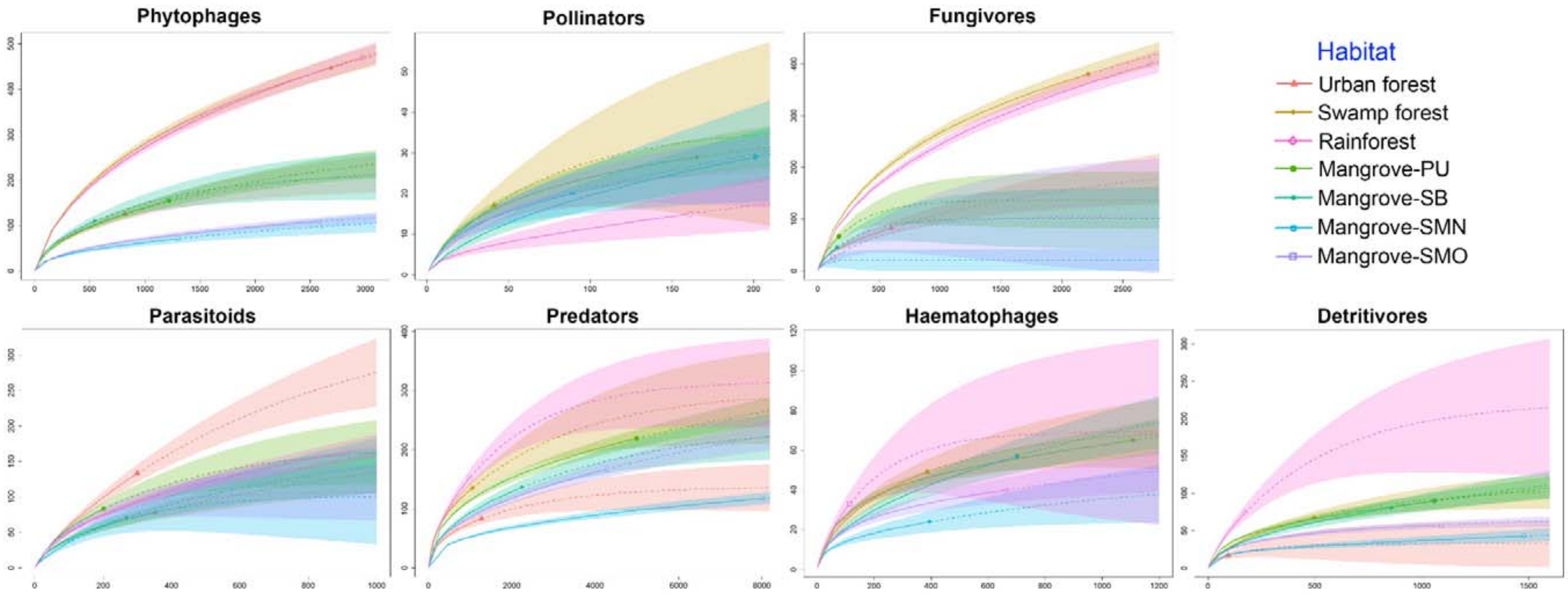


Figure S5. Comparison of species diversity across habitats (3% p-distance mOTUs) split by ecological guild. Mangrove sites are represented by Pulau Ubin (PU), Sungei Buloh (SB), Pulau Semakau old grove (SMO), Pulau Semakau new grove (SMN). Curves were plotted for the mangrove sites as separate sites. The full lines represent rarefactions, while the dotted lines extrapolations and the point between the lines as actual observed values.

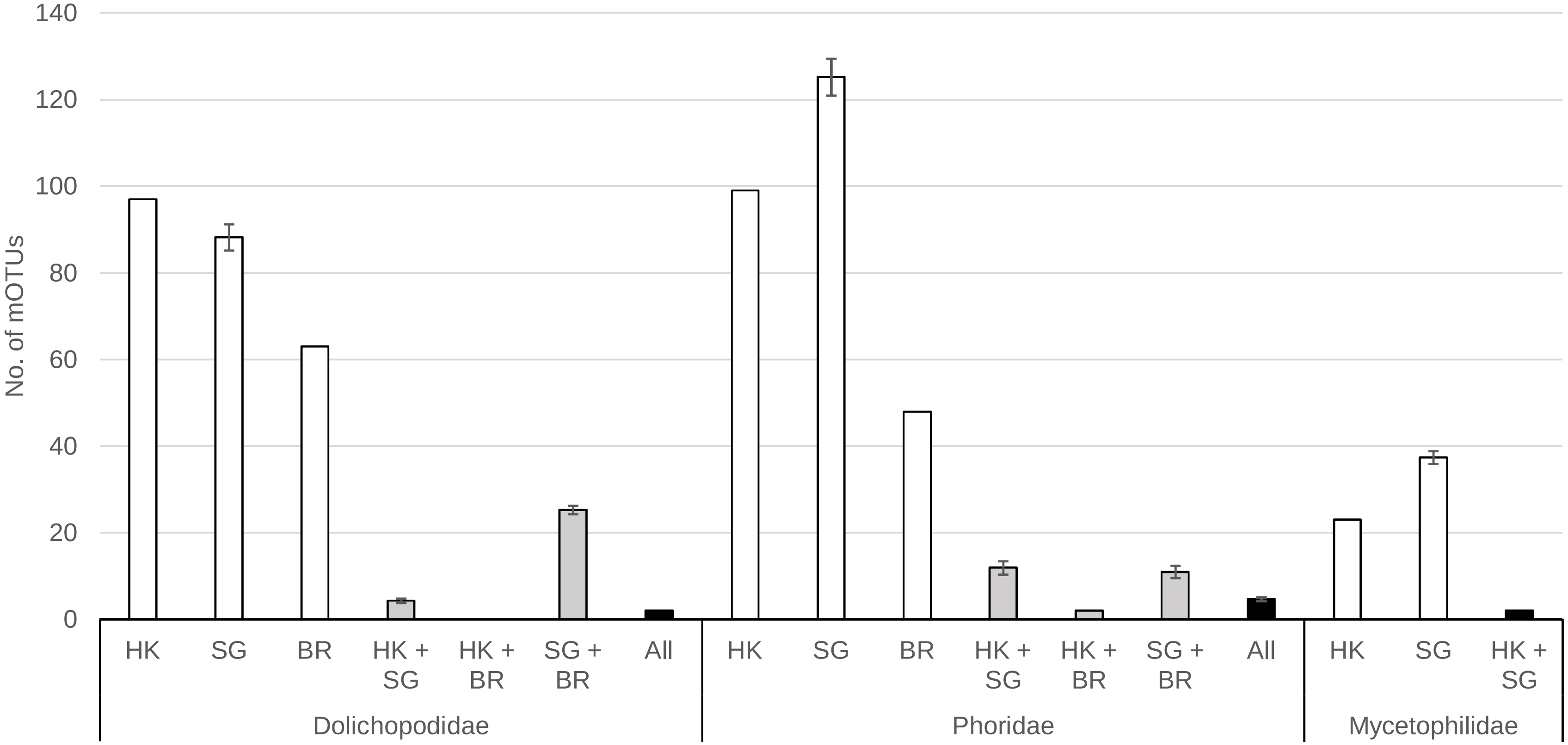


Figure S6. High species diversity and turnover for mangroves from Singapore, Brunei, and Hong Kong based on three Diptera families. Singapore data are rarefied to specimen numbers from Brunei and HK (error bars = 95% confidence intervals).

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