Insect Conservation and Diversity

Insect Conservation and Diversity (2015) **8,** 132–142 doi: 10.1111/icad.12090

Diversity in tropical ecosystems: the species richness and turnover of moths in Malaysian rainforests

LOUISE A. ASHTON,[1] HENRY S. BARLOW,[2] AKIHIRO NAKAMURA[3] and ROGER L. KITCHING[3,1] [1]Environmental Futures Research Institute and Griffith School of Environment, Griffith University, Nathan, Qld, Australia, [2]PO Box 10139, Kuala Lumpur, 50704, Malaysia and [3]Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Mengla, Yunnan, China

Abstract. 1. As a contribution to accurate estimation of arthropod alpha and beta diversities in tropical forests, we present results of some of the largest moth samples ever collected in the Malaysian region.

2. To estimate alpha diversity, light traps were run at three geographically distinct locations. We generated individual-based and coverage-based rarefaction curves to estimate sampling sufficiency and alpha diversity of the locations. Despite a large number of moths collected (67 282 individuals, from three locations), none of the rarefaction curves reached asymptote. The species accumulation curves based on the Chao1 richness estimator at each location suggested that, even when sampling yielded over 30 000 individuals, Chao1 could not reliably estimate the observed number of species. In one of the three locations, moths were collected systematically by light traps in 1979–1980 and 2000–2001. Despite over 160 trapping nights and 16 500 individuals collected in total, the estimated total number of species (2262) was well below the general collection conducted at the same location over a 35-year period [3921 species (1975–2013)].

3. Beta diversity was investigated using the samples collected at one location on two occasions over a 20-year period. The faunal composition has changed over the 20-year period, possibly as a result of extensive land-use change around the study location. We estimated the minimum sampling effort required to detect such changes by calculating type II errors. Unlike alpha diversity estimation, we found that only four replicate samples, each with only two trapping nights, would be sufficient to reliably detect changes in assemblage composition.

Key words. Alpha diversity, beta diversity, Lepidoptera, moths, richness estimators, tropical rainforest.

Introduction

It is beyond dispute that tropical rainforests contain massive numbers of insect species and that, numerically speaking, these faunas dominate terrestrial global biodiversity (Erwin, 1982; Stork, 1988, 1993; Wilson, 1992; Ødegaard, 2000; Novotny *et al.*, 2007; Hamilton *et al.*, 2010; Basset *et al.*, 2012). Insect species richness is dominated by four 'mega-Orders': the Coleoptera, Diptera, Lepidoptera, and Hymenoptera. Much effort over the last 30 years has been devoted to estimates of global species richness based on extrapolations from the results of a range of local rainforest surveys. Early predictions built on restricted beetle surveys of a single species of Neotropical tree arrived at immense totals centred on 30 million species globally (Erwin, 1982; Stork, 1988; May, 1990). More accurate estimates of host–plant specificity,

Correspondence: Roger L. Kitching, Key Laboratory of Tropical Forest Ecology, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Menglun, Mengla, Yunnan 666303, China. E-mail: R.Kitching@griffith.edu.au

Order-to-Order relativities, canopy-ground overlaps, taxonomic effort and sufficiency, and spatial turnover have led to a current consensus of about 6 million species (Basset *et al.*, 2012) within a confidence band of about 2–20 million (Hamilton *et al.*, 2013). Ultimately, all these estimates are constrained by the amount and accuracy of the data which are used as the basis for extrapolation.

As one of the four insect mega-Orders, the Lepidoptera afford the potential for yet more accurate estimates. As has been pointed out elsewhere (e.g. Kitching *et al.*, 2000; Ashton *et al.*, 2011), the Lepidoptera have several key advantages over some other insect orders as a choice for estimating diversity and understanding ecosystem function, *viz*.: (i) they are the best known of the large Orders taxonomically; (ii) a large proportion of them (the moths) are readily mass sampled using automated light traps; (iii) they are species rich which, coupled with (ii), gives statistical power in diversity analyses; and (iv) Lepidoptera predominately use plant materials for larval food (living and dead), which connects them functionally with the underlying botanical structure of their environments. Of course, for some purposes, the more trophically diverse Coleoptera provide complimentary opportunities for analysis (see, e.g. Basset *et al.*, 2012).

Basset *et al.* (2012) have presented estimates of the number of species that may occur in a half hectare of lowland tropical forest in Panama extrapolated from probably the largest and best structured baseline survey ever conducted (the 'IBISCA-Panama' project). They predicted about 6000 species of arthropods occurred within the 0.40 ha examined and this number rose to between 17000 and 40000 for the entire 6000 ha of which the study area was part. Remarkably, these authors found that about 66% of the entire regional rainforest fauna was likely to occur within just 1 ha of forest. The data generated by the IBISCA Panama project comprised a representative range of focal taxa constrained, in part, by the availability of expertise and taxonomic maturity within one taxon or another. This study estimated that about 1000 species of Lepidoptera occurred within the 6000 ha of the San Lorenzo Protected Area in lowland Panama. The number of moth species, however, may be underestimated, as other studies with more sampling effort often yield well over 1000 species of moths from one location (e.g. Barlow & Woiwod, 1989). The question remains as to how much sampling effort is required to estimate the species richness (alpha diversity) reliably at a given location.

The dipterocarp-dominated forests of south-east Asia are species rich even by global rainforest standards. They comprise three or four of the original 25 global diversity hotspots ('Indo-Burma', 'Sundaland', 'Wallacea', and the Philippines) (Myers, 1988; Mittermeier *et al.*, 2000; Myers *et al.*, 2000) where very high species richness and endemism combine with high levels of conservation vulnerability. The rainforests of south-east Asia are threatened by deforestation, driven primarily by conversion to oil palm (Koh & Wilcove, 2008). Malaysia and Indonesia are two of the biggest oil palm producers globally (Foster *et al.*, 2011). Other important drivers of forest loss in this region include logging and conversion to fibre plantation (Abood *et al.*, 2014). In the current period of habitat loss and fragmentation, the tools which permit accurate description of diversity and ecosystem services associated with the rainforest are essential for conservation and predictions of future responses to environmental pressures (Sodhi *et al.*, 2004). Light-trapped moths have been used to quantify ecological impacts of anthropogenic disturbances (e.g. Kitching *et al.*, 2013; Ashton *et al.* 2011, Beck *et al.*, 2010). To make a reliable assessment, ecologists often pool moths caught from more than one trap night to avoid undersampling which may cause inflation of the values of beta diversity. The number of pooled trap nights and replicated samples, however, have been generally selected without formal justification.

We present here results of analyses and extrapolations based on four extended light trap collections of moths from three Malaysian locations which together represent over 1000 trap nights and more than 70 000 specimens, all of which have been identified to species or morphospecies. A previous analysis of one of these data sets has been presented by Barlow and Woiwod (1989) who compared the diversity observed with that of other biogeographic regions. These huge sample sizes allow estimations of total species richness (alpha diversity), using standard extrapolation methods, to be used with greater confidence than any previous study of tropical Lepidoptera of which we have knowledge. The sampling at one of the locations was repeated after considerable landscape transformation had occurred, allowing us, further, to investigate changes in assemblage composition (beta diversity), and to estimate minimum sampling effort to detect such changes. In addition, at the same twice-sampled location, one of us (HSB) has maintained an inventory of the same target taxa from 1975 to 2013. This allows us to evaluate the accuracy of the extrapolations in ways which have not previously been possible.

Materials and methods

Moths were collected at three locations in Malaysia, two in Peninsula Malaysia and one in Sabah, Borneo (Fig. 1).

Sungai Halong, Belum, North Perak, Malaysia

Sungai Halong (referred to as 'Halong' hereafter) is located in primary forest at the Sungai Halong Base Camp, Belum, North Perak, Malaysia, 252 km north (352°) of Kuala Lumpur, Malaysia (5°22′19″N 101°23′4″E) at about 311 m above sea level (asl). The area is now a designated state forest (the Royal Belum State Park) and is part of the Belum-Temengor Forest Complex. The forest is so-called Hill Dipterocarp Forest and a general account of the natural history of the area is provided by Davison (1995). Temperatures in the region fluctuate over

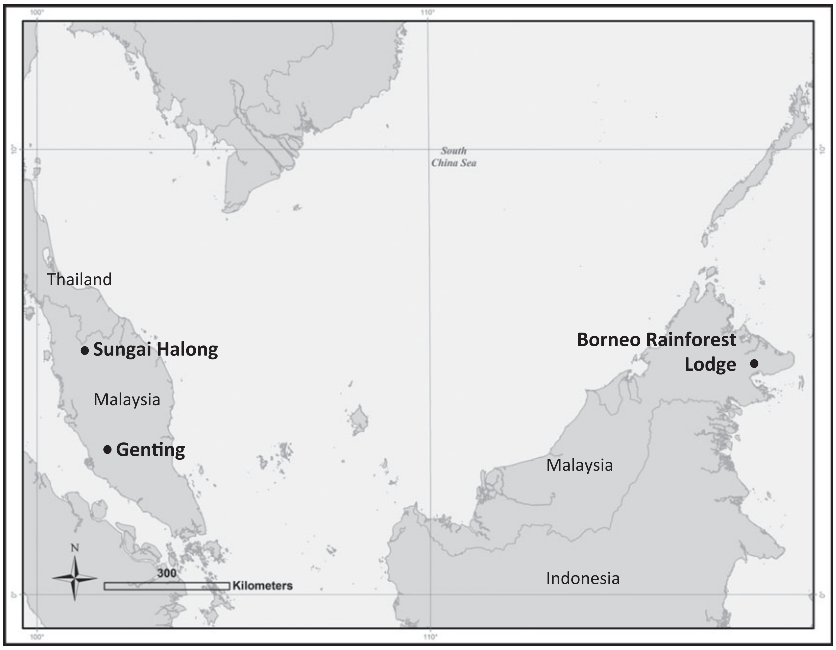

Fig. 1. Map of the Federation of Malaysia indicating the study locations at Genting Tea Estate, Sungai Halong and Borneo Rainforest Lodge.

a narrow range (23–33 °C) and the annual rainfall is about 3200 mm.

Genting Tea Estate, Selangor, Malaysia

The Genting Tea Estate ('Genting' hereafter) is located adjacent to the Genting Highlands, Genting Sempah, Pahang, 30 km NNE of Kuala Lumpur, Malaysia (3°20′60″ N 101°47′00″E). GTE is an overgrown tea estate, with 96 acres of advanced secondary rainforest and only very small patches (<2 acres) of primary forest, located at 650 m asl. Barlow and Woiwod (1989) present further details of the location. The area around this estate was dramatically changed between 1980 and 2000, as greater pressure for agricultural land lead to clearing. In 1980, the area surrounding GTE was a mosaic landscape, predominantly consisting of primary and secondary rainforest patches, with occasional clearings for vegetables. By 2000, extensive clean clearing of the surrounding hills had occurred, mainly for vegetable growing, including the very steep slopes. In 2000, very few patches of secondary forest remained, and primary rainforest was rare. The GTE property consists of 96 acres of advanced secondary rainforest, with only very small patches (<2 acres) of primary forest. This forested area at GTE is now the largest patch of rainforest in the immediate vicinity. Temperatures at GTE fluctuate between an annual minimum of about 18 °C and a maximum of 31 °C. Mean rainfall measured over 34 years between 1978 and 2011 was 2927 ± 124 mm.

Borneo Rainforest Lodge, Sabah, Malaysia

The Borneo Rainforest Lodge ('BRL' hereafter) (5°11′40″N 118°16′53″E) is located at about 58 m asl in the valley of the river Danum, Sabah, 260 km ESE of Kota Kinabalu, capital of Sabah State. The location is close to the Danum Valley Field Centre for which an abundance of background data is available. The 43800 ha Danum Valley Conservation Area (4°50′N – 5°00′N and 117°35′E – 117°45′E) preserves primary lowland dipterocarp forest. Situated within the 1 million hectare Yayasan Sabah logging concession, the primary forest reserve is surrounded by logged-over forest. The primary forest has a high density of dipterocarp trees frequently forming a closed canopy (Newbery *et al.*, 1992).

Trapping methods

The first surveys of light trapping at Halong, the BRL and Genting employed Rothamsted-style light traps (Williams, 1948; Colwell & Coddington, 1994; Southwood & Henderson, 2000) which were run from dusk to dawn on each trap night.

At Halong, two traps were run simultaneously from June 1993 to December 1994. Trapping was carried out regularly during that 17-month period producing a total of 264 trap nights of information. At Genting, moth collection occurred across 2-year long sampling periods, one in 1979–1980 ('Genting 1980' hereafter) and one in 2000–2001 ('Genting 2000'), producing a total of 118 and 46 trapping nights, respectively. At BRL a single trap was operated for a total of 171 nights between June 1997 and December 1998.

In total, about 28 families of Lepidoptera are represented in our samples. Three taxa, the family Geometridae and the superfamilies Noctuoidea (*sensu* Zahiri *et al*., 2011) and Pyraloidea dominated the samples. Within the Noctuoidea, memebers of the subfamily Arctiinae (family Erebidae) were particualrly abundant. The results from Genting 1980 have been analysed and presented by Barlow and Woiwod (1989).

In addition to the two annual periods surveyed at the Genting Tea Estate, moths were collected from 1975 to 2013 at a 200-w tungsten light situated on a whitewashed verandah. Collections were made from dusk until 0100 h and, occasionally, later. This collecting point was situated about 16 m above the locations of the Rothamsted traps and about 10 m distant horizontally. The whitewashed verandah looked out over secondary and primary forest and was not within line of sight of the Rothamsted traps. Moths have been collected at this site for about 275 nights each year from 1975 to 2013 to compile the 38-year record discussed here. Only macromoths were sampled during this period, therefore Pyraloidea and Thyridoidea were not included in comparative analyses of this 38-year collection.

Analysis

The alpha diversities of the three locations were investigated using individual-based, sample-based, and coverage-based rarefaction curves (Chao & Lee, 1992; Chao & Jost, 2012). Rarefaction curves permit us to judge the extent to which such a rarefied accumulation curve approaches the notional asymptote representing the size of the entire fauna susceptible to our trapping methods. Individual-based rarefaction curves plot rarefied species richness against a given number of individuals selected randomly from the observed samples, and sample-based rarefaction curves use samples instead of individuals for resampling (Colwell *et al*., 2012). Coverage-based rarefaction curves, on the other hand, plot rarefied species richness against a given sample completeness, which is the estimated proportion of the total number of individuals in a community represented by the species sampled (Chao & Jost, 2012). For all types of rarefaction curves, we used 100 replicate bootstrapping runs to estimate 95% confidence intervals. Rarefaction curves were also extrapolated by doubling the number of individuals (for individual-based and coverage-based rarefaction curves) or samples (sample-based rarefaction curves), as recommended by Colwell (2013). Building on this we can estimate, with increasing confidence as the total sample size increases, the proportion of the entire fauna susceptible to our trapping methods. Rarefaction curves were generated using the *iNEXT* package (Hsieh, 2013, ver. 1.0) in R.

The total species richness was estimated using the non-parametric Chao1 estimator (Colwell *et al*., 2012) as recommended by Hortal *et al*. (2006). Abundance-based estimators, such as Chao1, have been shown to be less biased than incidence-based estimators for simulated mobile organisms (Brose & Martinez, 2004). We plotted the value of the Chao1 estimator, with 95% confidence intervals, against a given number of individuals randomly selected from the entire pool of samples in the location. Randomisation was carried out 100 times to calculate 95% confidence intervals. Chao1 values were calculated using EstimateS ver. 910 (Colwell, 2013). We also used EstimateS to calculate Fisher's Alpha values for the entire assemblage at each location (with standard deviation). We calculated a range of other diversity metrics, including the inverse Simpson and exponential Shannon indices, as well as proportions of singletons (number of singleton species divided by the total number of species).

Given the difficulties encountered in the estimation of alpha diversity in particular locations (see below), it becomes important to test the effectiveness of attempts to detect spatial or temporal turnover (i.e. beta diversity). Accordingly, we tested how many trap nights per sample and how many samples were required to detect reliably changes in beta diversity between Genting in 1979–1980 and 2000–2001. We did this by calculating the type II error rate (i.e. the failure to reject a false null hypothesis) using combinations of different numbers of pooled trap nights (from 1 to 5 per sample), and the numbers of replicate samples (from 3 to 8 per treatment). We calculated F and P values to test for the differences in moth assemblage composition, using permutational multivariate ANOVA (PERMANOVA) using the *adonis* routine available within the *vegan* package (Oksanen *et al*., 2013) in R. Samples were randomly selected for any given numbers of trap nights per sample from within the data available from Genting 1980 (118 trap nights available) and Genting 2000 (46 trap nights), and PERMANOVAS were conducted. The randomisation was repeated 999 times and the number of cases where $P > 0.05$ (type II error) was recorded. Bray–Curtis (using log-transformed moth abundances), Sørensen and Raup-Crick (the latter two using binary presence/absence data) dissimilarity measures were used to quantify the differences in the compositions of moth assemblages. All singletons (moth species which were collected from one sample) were removed for each analysis. R code is available upon request from the corresponding author.

Results

Alpha diversity from the three locations

Table 1 summarises the results from the periods of light-trap sampling at Halong, BRL and Genting 1980 and 2000. A total of 70 529 moths comprised these samples. Halong had the highest number of species (2795

Table 1. Summary of moth data sets collected from three rainforest locations in Malaysia. The numbers in parentheses are 95% confidence intervals.

	Halong	BRL	Genting	
Collection year	1994	1998	1980	2000
Number of individuals sampled	33 449	19 002	14 831	3247
Number of species sampled	2795	2390	1593	791
Sample nights	264	171	118	46
Sample coverage (%)	97.3 (±0.1)	94.5 (±0.3)	96.0 (±0.3)	86.6 (±0.9)
Species richness at equal sample coverage*	1207 (±13)	1256 (±22)	781 (±12)	791 (±28)
Species richness extrapolated by two (based on total number of individuals)	3357 (±78)	3103 (±79)	1992 (±64)	1115 (±54)
Chao1 estimated species richness	3662 (±76)	3666 (±107)	2270 (±74)	1478 (±92)
Shannon exp (±mean)	464.8 (±1.9)	387.5 (±2)	269.4 (±1)	221.2 (±3)
Inverse Simpson conc (±mean)	61.5 (±4)	78.1 (±7)	47.6 (±0.4)	52.7 (±0.9)
Fisher's alpha (±mean)	821.7 (±8)	742.7 (±9)	469.7 (±7)	337.6 (±9.5)

*Species richness was standardised at the lowest sample coverage (86.6% from Genting 2000).

spp.), closely followed by BRL (2390 spp.), while Genting 1980 had significantly lower species richness (1593 spp.). Other diversity metrics – Shannon exponential and Fisher's alpha are of the same order (Table 1), with the exception of the inverse Simpson concentration, which was higher for the BRL assemblage. BRL also had the highest proportion of singletons (BRL = 0.43, Genting 1980 = 0.37, Halong = 0.32).

Individual-based rarefaction curves showed the characteristic deceleration associated with an approach to an asymptote, yet this asymptote was far from being reached for any of the three locations (Fig. 2a). We found similar trajectories between BRL and Halong, but extrapolating to twice the number of individuals showed slightly more species at BRL compared with Halong (Fig. 2a). Species richness for Genting 1980 was substantially lower than at other locations.

Coverage-based rarefaction curves showed that the observed number of moth individuals (i.e. up to the points indicated by the black circles in each case) indicate that 90% of individuals in the community belong to species in the samples (Fig. 2c). Extrapolation showed that doubling the number of observed individuals did not substantially improve sample coverage (Fig. 2c), with sample coverage for all locations remaining under 100% (Fig 2e). When the locations were compared using equal sample coverage (94.5% – the lowest coverage as observed at BRL), species richness was highest at BRL (2390 species), followed by Halong (2128) and, then, Genting 1980 (1391) (Fig. 2c; Table 1).

Sample-based rarefaction curves showed inconsistent results compared with those based on individual or sample coverage (Fig S1). When compared using equal numbers of samples ($N = 118$ – the lowest number of samples at Genting 1980), species richness was the highest at Halong, followed by BRL and Genting 1980. This reflects sampling efficiency and intensity at each location, showing that more moth species were collected per trap night at Halong (where two traps were run simultaneously) than at other two locations (one trap).

Species accumulation curves based on the Chao1 richness estimator (a lower bound of estimated richness) continued to increase even at the end of the curves (Fig. 3). Estimated species richness did not stabilise even for Halong, where over 30 000 moths were collected. The Chao1 estimates were around 500 species under the ultimate estimates, when half the number of individuals were included in the analysis, representing still considerable sampling effort (16 724 moths in Halong, 9501 in BRL, and 7415 in Genting 1980, Fig. 3).

Short- versus long-term surveys

We calculated Chao1 estimators for the Genting 1980 and 2000 data sets combined. This is then comparable with the species accumulation curve for the total of species collected informally at Genting over 38 years (Fig. 4). This shows that the combined estimate of species richness at this location (2262.4 ± 13 standard deviation) is an underestimation of the total number of species actually encountered in the long-term survey (3921 spp.). It is also important to note that the long-term Genting species richness line continued to increase steadily between 2000 and 2013, and no asymptote has yet been reached after 38 years of collecting (Fig. 4).

Comparing the two Genting surveys

The sampling intensity of Genting 2000 was lower than that of Genting 1980 (46 trap nights in 2000, compared with 118 in 1980). This resulted in a much lower number of species counts in 2000 (791) than in 1980 (1593), and a lower sample coverage in 2000 (87%) compared with 1980 (96%). However, the interpolated and extrapolated

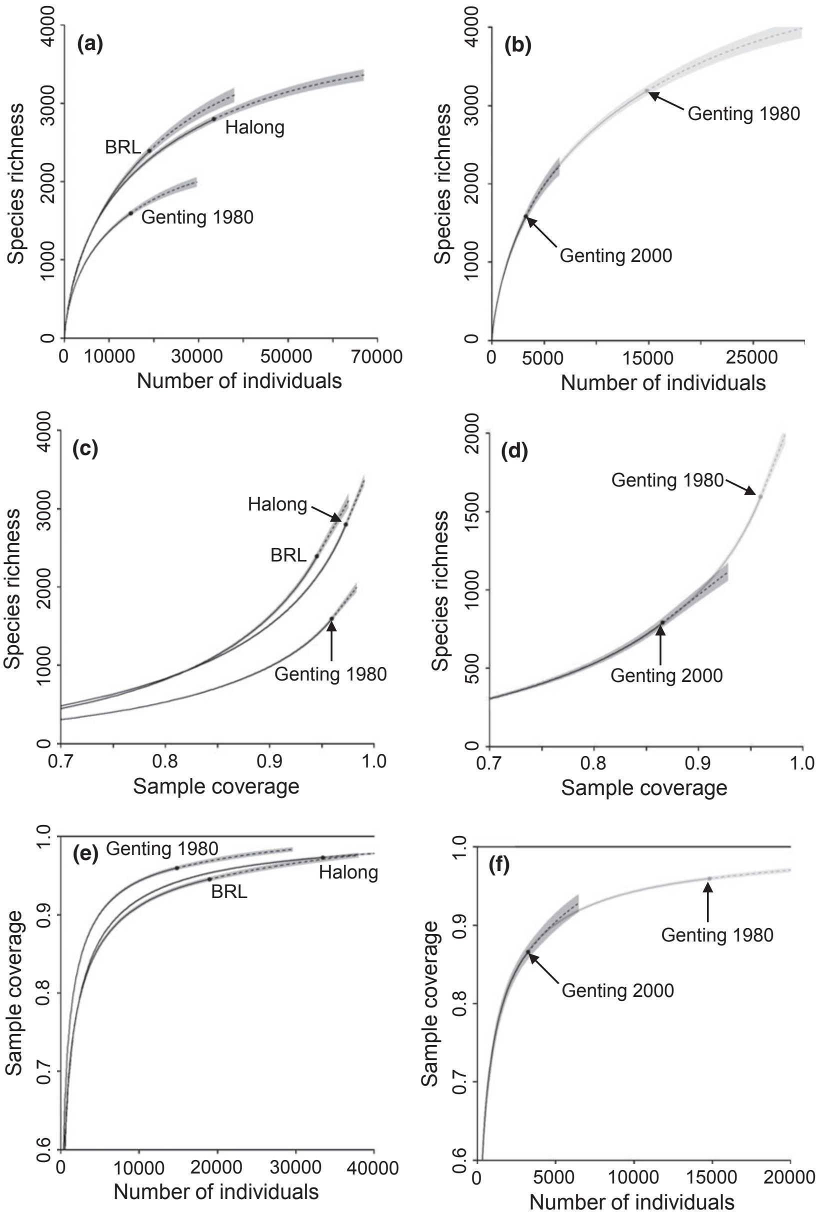

Fig. 2. Individual-, sample-, and coverage-based rarefaction and extrapolation curves for each location – Halong, BRL, and Genting 1980 and curves comparing Genting 1980 and Genting 2000 sampling. In all figures, black circles represent the sampling extent and dashed lines are extrapolations by a factor of 2 with 95% confidence intervals. In the Genting 1980 and 2000 comparisons (b, d, f), black curves show the Genting 2000 sampling and the grey curve represents Genting 1980 sampling. The curves show individual-based (a, b) and coverage-based (c, d) rarefaction curves, and sample coverage plotted against the number of individuals (e, f).

trajectories of rarefaction curves for these two sampling periods were very similar (Fig. 2b, 2d, d.f.), suggesting that the species richness may well have been the same if the same number of individuals (or sample coverage) had been obtained in the Genting 2000 samples.

Unlike species richness, however, the assemblage composition of moths was very different between Genting 1980 and 2000. Mean Bray–Curtis and Sørensen dissimilarity values were 90.3% (±3.82 standard deviation) and 91.5% (±3.49 standard deviation), respectively. To test just how many samples would be needed to detect differences between these two data sets, we examined Type II errors under different regimes of random resampling (see Methods) (Table 2). Due to the limited number of permutations possible, a significant difference was not detected when only three samples per data set were included in the

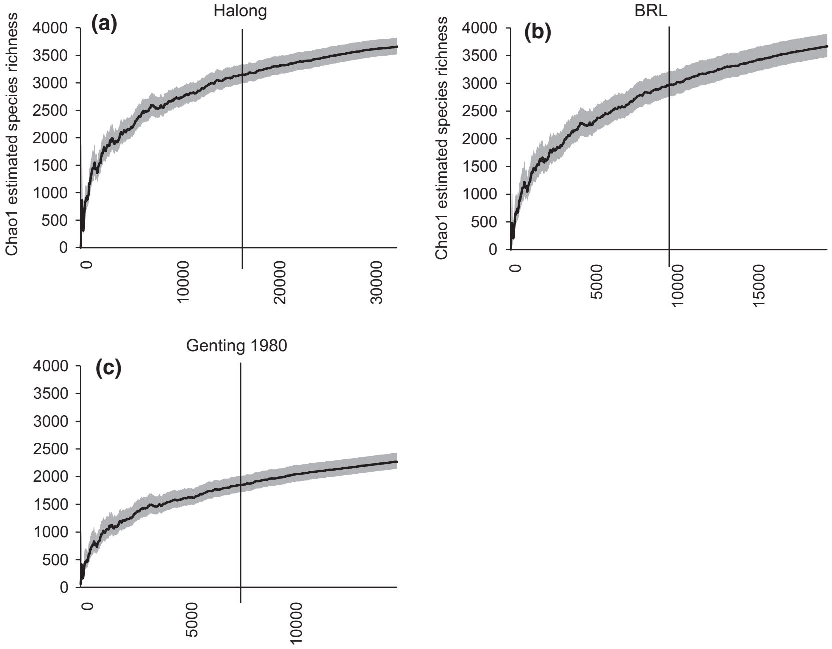

Fig. 3. Chao1 estimated species richness as a function of the number of individuals encountered at (a) Halong, (b) BRL, and (c) Genting 1980. Vertical lines are dawn where half the numbers of individuals is encountered.

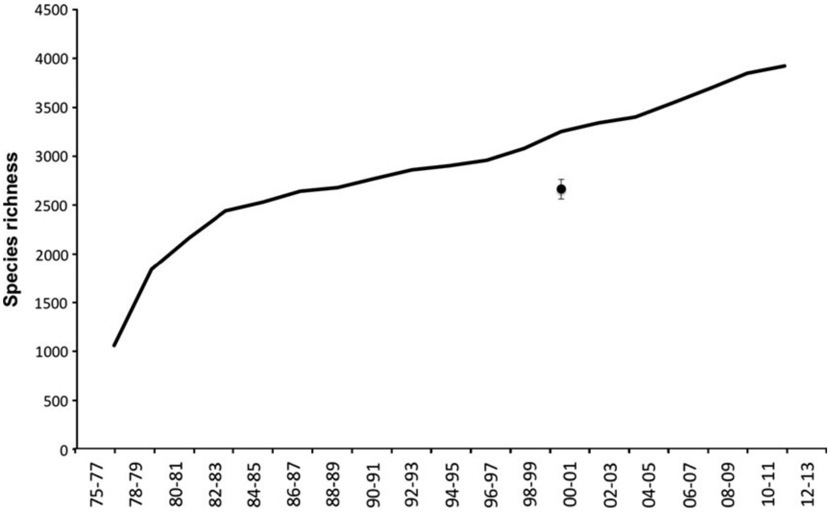

Fig. 4. Collectors' curves for all moths from the 1975–2013 collections from Genting Tea Estate, with an estimated total richness (Chao1 with 95% confidence intervals) from the two major sampling occasions combined (1980 and 2000). Chao1 value was plotted at the period (2000–2001) when the second survey (Genting 2000) was completed.

analysis. However, when sample size was increased to four or more, we found a very low rate of Type II errors. When only one trap night was included per sample, Type II errors were 1.9%, 2.1%, and 2.5% based on Bray–Curtis, Sørensen and Raup–Crick dissimilarity measures, respectively, for a sample size of 4 per occasion (Table 2). Type II error was almost negligible (<0.1%) when two or more trap nights were included per sample for the sample size of four or more (Table 2).

Discussion

How many species?

The challenging question of how many species of insect occur locally or globally in tropical rainforest has challenged many authors (see Basset *et al.*, 2012; and references in the Introduction). We are in a position to comment on the slightly more restricted question: how

Table 2. Summary of Type II error rates using moth assemblages collected from Genting 1980 and 2000. Type II errors (the number of failures to reject the null hypothesis) were calculated for a given number of trap nights per sample and the number of replicate samples per occasion. Assemblage similarities were calculated using Bray–Curtis and Raup–Crick indices (see text).

Number of samples per occasion	Number of trap nights per sample			
	1	2	3	4
Bray–Curtis				
3*	na	na	na	na
4	0.019	<0.001	<0.001	<0.001
8	<0.001	<0.001	<0.001	<0.001
Sørensen				
3*	na	na	na	na
4	0.021	<0.001	<0.001	<0.001
8	<0.001	<0.001	<0.001	<0.001
Raup–Crick				
3*	na	na	na	na
4	0.025	<0.001	<0.001	<0.001
8	<0.001	<0.001	<0.001	<0.001

*The number of possible permutations was too small to calculate *P* values.

many species of Lepidoptera are there in a tropical forest? The first point of note is that, like most earlier studies, we have not sampled all the Lepidoptera. Our light-trap samples targeted the traditional ‘macro’-moths plus Pyraloidea and Thyridoidea. Our samples did not include most of the families of ‘micro’-moths. Light traps, of course, sample very few butterflies. To convert any estimate from the protocols we have used to ‘all Lepidoptera’ require confident extrapolations based on wider estimates of faunal richness. For Borneo, Robinson and Tuck (1993) estimated a total richness of all moths at more than 8628 species using an extrapolation ratio of 1:1.39 of ‘macros’ to ‘micro’s. Holloway and Barlow (2011) updated Robinson and Tucks’ figure to 10 905 and added the known butterflies to produce a total figure for lepidopteran richness for all of Borneo at an impressive 11 841 (with minor corrections to their arithmetic). Turning to our estimates and assuming the Bornean ratio is regionally applicable, we obtain figures for the moths alone of 5090 for Halong, 3156 for Genting (based on the 1979–1980 sampling alone), and 5097 for the Borneo Rainforest Lodge (Chao1 estimate × 1.39 = estimated macro + micro). We have no separate estimates of butterfly richness from these locations. For the ‘casual’ collecting at Genting a similar multiplication with this ratio produces a total moth richness of 5422 species. Recent unpublished work by T. Whitaker, H. S. Barlow and S. L. Sutton (in ms, 2013) on the Pyraloidea and Thyridoidea of Borneo suggest that the earlier figures for richness for these regions (1500 species of Pyraloidea and 240 of Thyridoidea for all of South-east Asia, see Robinson *et al*., 1994) and hence the ratio of micros to macros may be substantially underestimated. Our figures, therefore, should be regarded as minimal estimates of local richness.

Inter-regional variation

The three local estimates of richness are based on different sampling sizes and, in the case of the Halong, different sampling intensities (two traps were run simultaneously in Halong). To compare species richness across the three locations, we used coverage-based and individual-based rarefaction curves. Comparisons of species richness at equal sample coverage and individuals showed that the Halong and BRL locations had very similar species richness. Comparing the Genting samples also showed a remarkable similarity in species richness between the two sampling periods 1980 and 2000.

Of the three locations, and corrected for sample coverage, the Bornean location (BRL) is clearly richer than either of the other two (73% richer than central peninsular location at Genting, 12% richer than Halong). The location at Sungai Halong close to the Thai border is, in turn, 53% richer than Genting. There are a number of likely reasons for these disparities. First, the Halong and BRL locations are at a lower elevation than Genting (311 m asl at Halong, 58 m asl at BRL, compared with 650 m asl at Genting). On first principles we might expect this to reduce endemism but increase overall richness. Second, in the case of Halong, there is a ‘peninsular effect’ within the theory of island biogeography suggesting that, all else being equal, locations further isolated along peninsulas will have fewer species than those at the base of peninsulas (see, e.g. Taylor & Regal, 1978; Milne & Forman, 1986). This last idea is further backed up by unpublished data collected by three of us (LAA, AN, and RLK) in the extreme south of Yunnan – the very ‘root’ of south-east Asia where moth diversities are even higher. This peninsular effect is both a reflection of the universal species–area relationship and the increased environmental heterogeneity which frequently correlates with it. Last, the Halong and BRL locations were surrounded by undisturbed primary dipterocarp forest, whereas in 1979-80 the Genting location sat within a patchwork of primary and secondary forest, with some horticultural development.

The extraordinary species richness of the island of Borneo is well known (Holloway 1983–2011). Holloway (1987) estimated a total of 4500 species of Macrolepidoptera for the island of Borneo. Holloway and Barlow (2011) discuss this richness, pointing out that in some highly diverse moth families, current known levels of endemism may be as high a 30%. In addition, the Bornean fauna has eastern connections to the Papuan and Australian regions which likely add to its richness.

Other environmental drivers of moth diversity, which we have not measured here and may have influenced the high diversity observed in both Halong and BRL, include precipitation (Beck *et al*., 2010) and tree phenological events (Intachat *et al*., 2001), which have been positively correlated with moth diversity in Borneo and Peninsular Malaysia.

Inter-temporal variation

The Genting location was sampled using the same light-trap methodology (but different sample sizes) in 1979–1980 and 1999–2000. Based on the rarefaction curves, we cannot detect changes in species richness across the two periods although there is dramatic change in the species composition (>90% Bray–Curtis and Sørensen dissimilarity values). In addition, regular casual collecting took place at the Genting location between 1975 and 2013. This 38-year record of casual collecting showed an acceleration in the collectors' curves (Fig. 4) in the mid-1990s. This point falls between the two periods of intensive light-trap collection and may well reflect the same qualitative change in faunal composition. The most parsimonious explanation for this change is that the environment and, in particular, the resources available to Lepidoptera, had changed during this period. We note that, in the period between the two surveys, substantial environmental modification (logging and conversion to agricultural land) took place around the sampling location and this may, at least in part, be responsible for the changes observed in the data. We stress, however, that there was no spatial replication in our sampling and these observations are speculative.

The estimation of alpha and beta diversity

Our results show clearly that the estimation of local alpha diversity, even when very large point samples are involved is challenging. The Halong data set, which comprised over 30000 individuals, for example, continued to show a rising rarefaction curve. Furthermore, employing these data as the basis for total species prediction using either the Chao1 estimator or simple extrapolation methods did not produce asymptotic values. Indeed, the extrapolation results show clearly that even doubling the Halong sample sizes (to over 60 000 individuals) would not have achieved this goal. The data sets we collected were year-long point samples with no spatial replication. If sampling takes place, with spatial replication, within a smaller, well-defined landscape unit, then lower sampling effort might be sufficient to obtain adequate estimates of alpha diversity. Such spatial replication would likely capture local environmental heterogeneity and increase the likelihood of detecting species that do not respond as well to light-trap stimuli. Such spatial replication has been a feature of previous studies of moth assemblages in the region (see, e.g. Intachat *et al*., 1999; Schulze & Fiedler, 2003; Beck *et al*., 2010).

In contrast, detecting changes in assemblage composition appears to be relatively straightforward. Samples considerably less than those employed here would have detected the significant differences observed between the two temporal surveys at Genting. As long as sufficient trap nights (a minimum of two in this case) are employed per replicate sample to overcome the inherent variability in individual catches by light traps, then as few as four samples may be sufficient to detect differences. Alternatively, as in Kitching *et al*. (2013), trapping up to a certain minimum number of individual insects per sample may be employed. In the case of the two Genting samples the effect size was so large that little statistical power was needed to detect it. The more subtle the changes in the assemblage difference between times (or locations), then the greater the sampling effort needed to detect such change.

Concluding remarks

The results discussed in this study underline the great species richness of moths in tropical rainforests and the efforts required to estimate this richness adequately. Inevitably extrapolation methods must be used (as in all attempts to estimate arthropod richness), but we show clearly that these are heavily influenced by the underlying sample size on which they are based. For the ecosystems and taxa studied here, anything less than a sample of 30 000 moths caught by light trapping, we suggest, is inadequate to allow confident predictions of total richness. We also demonstrate that there is no substitute for long-term 'old-fashioned' accumulation of information through collecting in a region which, inevitably, uncovers total species diversity albeit very slowly. We do show, however, that considerably less effort is required to detect changes in diversity confidently especially when these are well marked. Similar contrasts in the relative ability to estimate alpha and beta diversities have been found in the studies of ant assemblages (Tista & Fiedler, 2011). We have argued elsewhere (Kitching, 2006) that well-executed, large-scale, short-term surveys analysed comparatively, and involving spatial replication, can shed light on the relative distribution of species richness. In a rapidly changing, heterogeneous, natural world we probably have to settle for these.

Acknowledgements

HSB thanks J. D. Holloway, Alan Watson, the late Alan Hayes, Kevin Tuck, and the late Michael Schaffer for assistance with identification. For help in operating the light traps, we thank Foo Yee Min and Simon Hok Kim Loong at Genting, Barabara Helmund, Natalie Guerard and Marcus Erle at Sungai Halong, and Nasir Abdul Majid at Borneo Rainforest Lodge. We are grateful to Ian Woiwod for assistance in analysing early data from Genting and to James McBroom and Masatoshi Katabuchi for statistical advice. Mr Lim Kooi Fong provided invaluable assistance with data entry. The study has been much improved following the insightful comments of Chris Burwell and three anonymous referees.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: doi: 10.1111/icad.12090:

Figure S1. Sample-based rarefied and extrapolated species richness at (a) Halong, BRL, and Genting 1980 and (b) Genting 1980 and Genting 2000. Black circles represent the number of species encountered during the sampling; dashed lines represent an extrapolation by a factor of two.

References

Abood, S.A., Lee, J.S.H., Burivaloa, Z., Garcia-Ulloa, J. & Koh, L.P. (2014) Relative contributions of the logging, fibre, oil palm, and mining industries to forest loss in Indonesia. *Conservation Letters*, doi: 10.1111/conl.12103.

Ashton, L.A., Kitching, R.L., Maunsell, S.C., Bito, D. & Putland, D.A. (2011) Macrolepidopteran assemblages along an altitudinal gradient in subtropical rainforest – exploring indicators of climate change. *Memoirs of the Queensland Museum – Nature*, **55**, 375–389.

Barlow, H.S. & Woiwod, I.P. (1989) Moth diversity of a tropical forest in peninsular Malaysia. *Journal of Tropical Ecology*, **5**, 37–50.

Basset, Y., Cizek, L., Cuénoud, P., Didham, R.K., Guilhaumon, F., Missa, M., Novotny, V., Ødegaard, F., Roslin, T., Schmidl, J., Tishechkin, A.K., Winchester, N.N., Aberlenc, H.P., Bail, J., Barrios, H., Bridle, J.R., Castaño-Meneses, G., Corbara, B., Curletti, G., Darocha, W.D., Debaker, D., Delabie, J.H.C., Dejean, A., Fagan, L.L., Floren, A., Kitching, R.L., Medianero, L.E., Miller, S.E., Gama De Oliveira, E., Orivel, J., Pollet, M., Rapp, M., Ribeiro, S.P., Roisin, Y., Roubik, D.W., Schmidt, J.B., Sorensen, L. & Leponce, M. (2012) Arthropod diversity in a tropical forest? *Science*, **338**, 1481–1484.

Beck, J.A.N., Schwanghart, W., Khen, C.V. & Holloway, J.D. (2010) Predicting geometrid moth diversity in the heart of Borneo. *Insect Conservation and Diversity*, **4**, 173–183.

Brose, U. & Martinez, N.D. (2004) Estimating the richness of species with variable mobility. *Oikos*, **105**, 292–300.

Chao, A. & Jost, L. (2012) Coverage-based rarefaction and extrapolation: standardizing samples by completeness rather than size. *Ecology*, **93**, 2533–2547.

Chao, A. & Lee, S.M. (1992) Estimating the number of classes via sample coverage. *Journal of the American Statistical Association*, **87**, 210–217.

Colwell, R.K. (2013) EstimateS: Statistical estimation of species richness and shared species from samples. Version 9. Users guide and application published at < http://viceroy.eeb.uconn.edu/EstimateS > 3rd April 2014.

Colwell, R.K., Chao, A., Gotelli, N.J., Lin, S.-Y., Mao, C.X., Chazdon, R.L. & Longino, J.T. (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation, and comparison of assemblages. *Journal of Plant Ecology*, **5**, 3–21.

Colwell, R.K. & Coddington, J.A. (1994) Estimating terrestrial biodiversity through extrapolation. *Philosophical Transactions of the Royal Society London B*, **345**, 101–118.

Davison, G.W.H. (1995) *Belum: A Rainforest in Malaysia.* Malaysian Nature Society, Kuala Lumpur, Malaysia.

Erwin, T.L. (1982) Tropical forests: their richness in Coleoptera and other arthropod species. *Coleopterists' Bulletin*, **36**, 74–75.

Foster, W.A., Snaddon, J.L., Turner, E.C., Fayle, T.M., Cockerill, T.D., Ellwood, M.D.F., Broad, G.R., Chung, A.Y.C., Eggleton, P., Vun Khen, C. & Yusah, K.M. (2011) Establishing the evidence base for maintaining biodiversity and ecosystem function in the oil palm landscapes of South East Asia. *Philosophical Transactions of the Royal Society B*, **366**, 3277–3291.

Hamilton, A.J., Basset, Y., Benke, K.K., Grimbacher, P.S., Miller, S.E., Novotny, V., Samuelson, A., Stork, N.E., Weiblen, G.E. & Yen, J.E. (2010) Quantifying uncertainty in estimation of global species richness. *American Naturalist*, **176**, 90–95.

Hamilton, A.J., Novotný, V., Waters, E.K., Basset, Y., Benke, K.K., Grimbacher, P.S., Miller, S.E., Samuelson, G.A., Weiblen, G.D., Yen, J.D.L. & Stork, N.E. (2013) Estimating global arthropod species richness: refining probabilistic models using probability bounds analysis. *Oecologia*, **171**, 357–365.

Holloway, J.D. (1983–2011) *Moths of Borneo*, Southdene Sdn. Bhd., Kuala Lumpur, Malaysia (18 volumes frequently co-published with the Malayan Nature Journal).

Holloway, J.D. (1987) Macrolepidoptera diversity in the Indo-Australian tropics: geographic, biogeographic, biotopic and taxonomic variations. *Biological Journal of the Linnaean Society*, **30**, 325–341.

Holloway, J.D. & Barlow, H.S. (2011) The Moths of Borneo: building on the past with the benefit of fresh field sampling to construct a taxonomic foundation for the future. *Malayan Nature Journal*, **63**, 445–548.

Hortal, J., Borges, P.A. & Gaspar, C. (2006) Evaluating the performance of species richness estimators: sensitivity to sample grain size. *Journal of Animal Ecology*, **75**, 274–284.

Hsieh, T.C. (2013) iNEXT: iNterpolation and EXTrapolation for species diversity. R package version 1.0. < http://chao.stat.nthu.edu.tw/blog/software-download/ > 1st June 2014.

Intachat, J., Chey, V.K., Holloway, J.D. & Speight, M.R. (1999) The impact of forest plantation development on the population and diversity of geometrid moths (Lepidoptera: Geometridae) in Malaysia. *Journal of Tropical Forest Science*, **11**, 329–336.

Intachat, J., Holloway, J.D. & Staines, H. (2001) Effects of weather and phenology on the abundance and diversity of geometrid moths in a natural Malaysian tropical rain forest. *Journal of Tropical Ecology*, **17**, 411–429.

Kitching, R.L. (2006) Crafting the pieces of the diversity jigsaw puzzle. *Science*, **313**, 1055–1057.

Kitching, R.L., Ashton, L.A., Nakamura, A., Whitaker, T. & Khey, V.T. (2013) Distance driven species turnover in Bornean rainforests: homogeneity and heterogeneity in primary and post-logging forests. *Ecography*, **36**, 675–682.

Kitching, R.L., Orr, A.G., Thalib, L., Mitchell, H., Hopkins, M.S. & Graham, A.W. (2000) Moth assemblages as indicators of environmental quality in remnants of upland Australian rain forest. *Journal of applied Ecology*, **37**, 284–297.

Koh, L.P. & Wilcove, D.S. (2008) Is oil palm agriculture really destroying tropical biodiversity? *Conservation Letters*, **1**, 60–64.

May, R.M. (1990) How many species? *Philosophical Transactions of the Royal Society of London B*, **330**, 293–304.

Milne, B.T. & Forman, R.T.T. (1986) Peninsulas in Maine: woody plant diversity, distance and environmental patterns. *Ecology*, **67**, 967–974.

Mittermeier, R.A., Myers, N. & Mittermeier, C.G. (2000) *Hotspots: Earth's Biologically Richest and Most Endangered Terrestrial Ecosystems*. Conservation International, Washington, District of Columbia.

Myers, N. (1988) Threatened biotas: 'hotspots' in tropical forests. *The Environmentalist*, **8**, 187–208.

Myers, N., Mittermeier, R.A., Mittermeier, C.G., da Fonseca, G.A.B. & Kent, J. (2000) Biodiversity hotspots for conservation priorities. *Nature*, **403**, 853–858.

Newbery, D.M., Campbell, E.J.F., Lee, Y.E., Proctor, J. & Still, M.J. (1992) Primary lowland dipterocarp forest at Danum Valley, Sabah, Malaysia: structure, relative abundance and family composition. *Philosophical Transactions of the Royal Society of London Series B*, **335**, 341–356.

Novotny, V., Miller, S.E., Hulcr, J., Drew, R.A.I., Basset, Y., Janda, M., Setliff, G.P., Darrow, K., Stewart, A.J.A., Auga, J., Isua, B., Molem, K., Manumbor, M., Elvis Tamtiai, E., Martin Mogia, M. & Weiblen, G.D. (2007) Low beta diversity of herbivorous insects in tropical forests. *Nature*, **448**, 692–695.

Ødegaard, F. (2000) How many species of arthropods: Erwin's estimate revised. *Biological Journal of the Linnean Society*, **71**, 583–597.

Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2013) Vegan: Community Ecology Package. R package version 2.0-10. < http://CRAN.R-project.org/package = vegan > 1st June 2014.

Robinson, G.S. & Tuck, K.R. (1993) Diversity and faunistics of small moths (Microlepidoptera) in Bornean rainforest. *Ecological Entomology*, **18**, 385–393.

Robinson, G.S., Tuck, K.R. & Schaffer, M. (1994) *A Field Guide to the Smaller Moths of Southeast Asia*. Natural History Museum, London, UK.

Schulze, C.H. & Fiedler, K. (2003). Vertical and temporal diversity of a species-rich taxon in Borneo. *Arthropods of Tropical Forests: Spatio-temporal Dynamics and Resource Use in the Canopy*. (ed. by Y. Basset, V. Novotny, S.E. Miller and R.L. Kitching), Cambridge University Press, Cambridge, UK, pp. 69–85.

Sodhi, N.S., Koh, L.P., Brook, B.W. & Ng, P.K.L. (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology and Evolution*., **19**, 654–660.

Southwood, T.R.E. & Henderson, P.A. (2000) *Ecological Methods*, 3rd edn. Wiley, Chichester, UK.

Stork, N.E. (1988) Insect diversity: facts, fiction and speculation. *Biological Journal of the Linnean Society*, **35**, 321–337.

Stork, N.E. (1993) How many insect species are there? *Biodiversity and Conservation*, **2**, 215–232.

Taylor, R.J. & Regal, P.J. (1978) The peninsular effect on species diversity and the biogeography of Baja California. *American Naturalist*, **112**, 583–593.

Tista, M. & Fiedler, K. (2011) How to evaluate and reduce sampling effort for ants. *Journal of Insect Conservation*, **15**, 547–559.

Williams, C.B. (1948) The Rothamsted light trap. *Proceedings of the Royal entomological Society of London A*, **31**, 135–144.

Wilson, E.O. (1992) *Diversity of Life*. Harvard University Press, Cambridge, Massachusetts.

Zahiri, R., Kitching, I.J., Lafontaine, J.D., Mutanen, M., Kaila, L., Holloway, J.D. & Wahlberg, N. (2011) A new molecular phylogeny offers hope for a stable family level classification of the Noctuoidea (Lepidoptera). *Zoologica Scripta*, **40**, 158–173.

Accepted 17 August 2014

Editor/associate editor: Raphael Didham