Editor’s Note

Another yearly volume of Treubia is published. I have only recently become involved in the publication of this journal and I can say that the research in this issue is increasingly interesting. I hope to remain actively involved in the publication of this journal and that we can continue to reach a larger audience as time goes on.

This volume of TREUBIA contains 5 papers of vertebrates and invertebrates. The contents of these papers vary widely from vocalizations of frogs to tropical forest spider communities. I can only hope in the future that we continue to receive interesting submissions from all areas of zoology of the Indo-Australian Archipelago.

Also this year two esteemed colleagues from LIPI retired from the service of science, Dr. Mas Noerdjito who studied the ecology of birds and Dr. Agustinus Suyanto who dedicated his life to the study of mammals.

Finally I would like to thank all of the co-editors, referees, computing assistants, secretaries and administrative assistants for their collaborative work without which this journal could not be published. I also acknowledge financial support from the Director of Research Center for Biology, LIPI to publish this essential journal.

Cibinong, December 2011

Chief Editor
ABSTRACT

Spiders were sampled from one-hectare tropical rainforest plots in three parks in northern Vietnam. Inventories were based on ecologically structured sampling employing five methods. A series of non-parametric estimators were used to extrapolate the true species richness from the samples for each locality and indicate the magnitude of sampling effort necessary to inventory a variety of protected Southeast Asian tropical forests. We investigated the Beta diversity between sites and explored the distinctness of the communities sampled by the various collecting methods. Our approach takes the incompleteness of our inventories into account and estimates the number of unobserved shared species. Rank sample abundance was positively correlated with number of sites observed. However, when sample abundance was scaled by incidence (as an index of detection probability), this relationship disappeared. This suggests no difference in the probability that abundant and rare species will be present in different sites even if the detection probability of rare species is low. The three sites differed in their observed and estimated point diversity with the lowest diversity site, Cuc Phuong, also having the least vertically-stratified spider community. The three sites, separated by 150–300 km and differing in vegetation community, elevation, geology, and other attributes, experience an estimated 65–85% turnover in species composition over differences of this magnitude. We discuss the rationale for using the non-parametric estimator approach and caution that estimates can be unreliable when samples contain an insufficient portion of the community.

Key words: Beta diversity, Chao-Jaccard index, community ecology, detection probability, ecological stratification, species accumulation curves, species richness estimators, structured sampling

INTRODUCTION

With over 42,000 described species, spiders are the world’s seventh largest order (Coddington & Levi 1991, Platnick 2011). They are...
ubiquitous and abundant predators, especially in tropical forest ecosystems. One of the challenges of working with such a group is that it is almost impossible to obtain a complete inventory where every species from a place is represented (Coddington et al. 2009, Coddington et al. 1991, Longino et al. 2002, Novotny & Basset 2000). This is because diverse tropical arthropod communities consist of a few common species and a very large number of rare ones (Colwell & Coddington 1994, Gotelli & Colwell 2001, Magurran 2004). Fortunately, statistical methods are available to extrapolate the actual number of species from structured samples (Colwell 2009, Colwell & Coddington 1994, Gotelli & Colwell 2001). This is particularly important in studies of beta diversity and other comparative community ecology studies (Magurran 2004).

Two different classes of non-parametric biodiversity estimators are available: abundance-based and incidence-based. Both types of estimators are driven by rare species although they differ in the way they count rarity. Abundance-based methods are driven by the total number of specimens in the collection, regardless of when they were collected. Species represented by a single individual are called singletons; by two individuals, doubletons. For incidence-based estimators, rarity is determined by the number of samples in which a species occurs, regardless of the actual number of specimens. Species found in exactly one sample are called uniques; in two samples, duplicates. All of these methods use the proportion of species in a sequence of rare abundance classes to extrapolate to the zero abundance class, which is the estimated number of species overlooked by the survey. This plus the observed species richness is the estimated total number of species (Colwell & Coddington 1994, Magurran 2004).

As with estimates of species richness from a single inventory, incomplete inventories are also a problem for comparative studies. Jaccard's (1901) classic index of community similarity is the proportion of observed species shared between two samples. However, incomplete inventories may fail to sample genuinely shared species from either site, or both. Because Jaccard’s index considers only the observed species, Chao et al. (2005) have proposed a correction to the Jaccard index. Chao's modification factors in an estimated number of unobserved species shared between two communities. As with other such estimators, the correction is driven by rare species in one or both communities.

This study compares tropical rainforest spider communities collected in three National Parks in northern Vietnam. We did not control for elevation, geology (Nam 1995), or similar variables. Instead, we focused on similarities and differences between in the spider community in different types of protected forest. Site attributes are given in Table 1. Point
diversity studies of tropical spider communities have a rich literature (e.g., Coddington et al. 2009, Coddington et al. 1991, Sørensen et al. 2002). All exhibit a similar species abundance distribution pattern: a few species are very abundant and many species are very rare. However, little work has been done on the beta diversity of spider communities as a whole.

Our study explored the relationship between sample abundance and the number of sites a species was observed in. Our objective was to explore whether or not abundant species are more likely to be shared between different sites. If so, this suggests that the few abundant species found in one forest tend to be successful generalists and differences in communities are concentrated in the medium to rare abundance classes. The alternative hypothesis is that both abundant and rare species in one forest habitat and location are equally likely to be present in a distant and characteristically different forest. This suggests a more mosaic quality to the spider community across the landscape. Testing this hypothesis is complicated by the relationship between sample abundance and detection probability. Species that are abundant in samples may also simply have a high probability of detection, making them more likely to be sampled in multiple sites. Teasing apart multiple site detection from sample abundance requires scaling by an estimate of detection probability. We used incidence, the number of samples a species was present in, as an index of detection probability. Incidence is less than or equal to abundance. So species with low incidence that are observed in multiple sites are emphasized by this approach.

This study relies on morphospecies concepts, the typical unit in tropical arthropod biodiversity studies (Erwin & Geraci 2009, Longino & Colwell 1997). The authors have experience sorting inventories of this kind to morphospecies (Toti et al. 2000, Coddington et al. 2009). We are currently generating DNA barcode data on a subset of the specimens to cross-check morphospecies. Problems with reliance on either morphospecies concepts or DNA barcode OTUs (operational taxonomic units) have been discussed in the literature (e.g., Krell 2004, Meyer & Paulay 2005, Olivier & Beattie 1996, Rubinoff et al. 2006, Will et al. 2005). Our ultimate solution will be to use a combination of both methods with the goal of exposing and resolving points of conflict. Any method can be misleading under some circumstances, but two independent methods are unlikely to be misleading in the same way. With that in mind, this dataset should be considered preliminary. We will focus here on the rationale and methodology behind the quantitative biodiversity framework and highlight the potential for hypothesis testing.
METHODS

Inventory

In September–October 2009, spiders were sampled from three localities in northern Vietnam: Vu Quang, Cuc Phong, and Cat Ba. We established a one-hectare square plot in a protected forest habitat at each location and conducted a structured inventory (Coddington et al. 2009, Coddington et al. 1991, Scharff et al. 2003, Sørensen et al. 2002). Five methods were employed for collecting spiders: 1) beating vegetation during the day (BED), 2) searching for spiders in the aerial stratum at night (LUN), 3) searching for spiders on the ground during the day (LDD), 4) searching for spiders on the ground at night (LDN), 5) extraction of sifted leaf litter using a Winkler trap (WIN; Fachgeschäft & Buchhandlung für Entomologie, Vienna, Austria, www.entowinkler.at). Searching and beating methods were conducted in one-hour blocks; leaf litter sifting was done in two hour blocks plus a minimum drying time of 48 hours. For statistical purposes, it would be ideal if the probability of sampling any individual spider in the hectare was equally likely, but this is never the case in such inventories (Gotelli & Colwell 2011). Each collecting method samples from a subset of the spider community. So a mixture of methods is used trying to cover as much of the community as evenly as possible.

We attempted to collect all spiders encountered (adults and juveniles) except aggregations of recently hatched siblings. When relying solely on morphology, it is typically only possible to determine spider species from adults, so juveniles are disregarded in inventories such as this (e.g., Coddington et al. 2009, Coddington et al. 1991). We collected juveniles in order to estimate the proportion of adult to juvenile spiders in the forest at the time of sampling.

Sorting and Data Management

The data consist of samples of specimens which represent a certain number of morphospecies, each with some abundance. Each sample represents a "collection event" (including the date and sampling method) from a locality (one of the three one-hectare plots). Specimens were sorted into vials, each containing one or more specimens representing one morphospecies, a locality label, a collection event label, and a morphospecies code with abundance. Finally, a unique record code label was added to each vial. All of this facilitates management of the data.

A single specimen was considered the voucher for each morphospecies, analogous to a holotype in taxonomy. Synonymization and splitting of morphospecies concepts occurred during the sorting process.
Correctly associating males and females of morphospecies is one critical operation where investigators must be diligent against errors. The difficulty of this is in part related to the degree of sexual dimorphism, which can be more or less pronounced in different spider lineages.

Point and Shared Diversity Estimates

The data were loaded in EstimateS version 8.2. In the Diversity Settings dialog box, we increased the number of randomization runs to 100 and ran the analysis. Following the documented recommendations, we repeated the analysis using classic formulas (as opposed to bias-corrected formulas, which is the EstimateS default) when prompted to do so and reported the larger estimate.

For the analysis of shared species, we selected the option to compute bootstrap standard errors for Chao indices and ran the analysis.

Community Stratification

We investigated the degree to which the five sampling methods used yielded similar or contrasting sets of morphospecies at each locality. We used an approach similar to the shared diversity estimates but compared the five methods to each other within each of the three locations. Based on the pattern that emerged from this analysis, we re-analyzed the data grouped into two classes: 1) methods that sampled from the ground stratum and 2) methods that sampled from the aerial stratum above the ground.

Visualizing Output

The species accumulation curve (Sobs Mao Tau) was plotted against Individuals. Two abundance-based (Chao 1 Mean, ACE Mean) and two incidence based estimators (Chao 2 Mean, and ICE Mean) were added, as were two upper 95% confidence curves (Chao 1 95% CI Upper Bound, Chao 2 95% CI Upper Bound), and four curves tracking rare species (Singletons Mean, Doubletons Mean, Uniques Mean, and Duplicates Mean), which along with observed species drive the non-parametric estimators.

For shared diversity estimates (both between localities and between methods or strata within localities), we plotted the estimated proportion of shared species between all pairs of localities (Chao-Jaccard-Est Abundance-based). We multiplied the standard deviation (Chao-Jaccard-EstSD Abundance-based) by 1.96 to get the 95% confidence interval and plotted these as error bars (Colwell 2009). The classic Jaccard index is shown as a dashed line. When displaying results in tabular form, we
report the Chao-Jaccard estimated proportion of shared species plus or minus the 95% confidence interval for each pairwise combination of the five sampling methods. Where the Chao-Jaccard estimate plus the 95% confidence interval crosses the 1.0 mark, two samples are considered to have been drawn from statistically indistinguishable underlying populations.

Statistical Analysis

The relationship between sample abundance, detection probability, and number of sites represented was tested using Spearman's rank correlation in R (R Development Core Team, 2001). The test was performed both on sample abundance against number of sites and also on sample abundance divided by incidence against number of sites. Singletons (106 species) were excluded from this analysis because singletons by definition cannot have been found in more than one site. Differences in the ratio of adults to juveniles in samples from the three sites were tested using the Pearson's chi-square test in R (R Development Core Team, 2001).

RESULTS

In total, 2010 adult spiders representing 262 morphospecies were collected from the three study sites. Of the three sites, the inventory at Cuc Phuong was the nearest to completion. Although fewer samples were taken, sampling yielded more adult specimens (683) than either of the two other sites (Table 2). The number of species observed (82) was the lowest for the three sites. Estimators were remarkably stable, suggesting that this inventory was 68–78% complete. Vu Quang had the most observed species (140) and was the most incomplete (54–62%). Estimator curves for Vu Quang and Cat Ba are still rising, another indication of the relative incompleteness of these inventories (Figure 1).

The spider communities at all three sites were distinct from each other with pairs of sites sharing only 28–31 observed species and 16 species observed at all three sites (Figure 2). Eighty-nine of the 262 observed species (34%) were found in more than one site. Rank sample abundance was positively correlated with the number of sites observed (Spearman's rank correlation, \( P<0.05 \)). However, this relationship disappeared when sample abundance was scaled by incidence (Spearman's rank correlation, \( P=0.7 \)).

In Cat Ba and Vu Quang, almost every sampling method yielded a distinct portion of the overall spider community. In Cuc Phuong, we found multiple methods sampling from indistinguishable underlying populations (Table 2). This pattern appears to be related to community
stratification. All five sampling methods focus either on the ground stratum or the aerial stratum above. Reanalysis of the data with methods pooled as ground sampling (LDD, LDN, WIN) or aerial sampling (BED, LUN) showed that the ground and aerial communities are drawn from statistically indistinguishable underlying populations, i.e., the community has low stratographic structure. This contrasts markedly with the significantly stratified pattern seen in Cat Ba and Vu Quang (Figure 3).

Overall, adult spiders accounted for about one third (36.4%) of the total spiders sampled. The highest proportion of adults (47.8%) was collected at Vu Quang, also the richest site in our data. The lowest proportion of adults (29.4%) was from Cat Ba, a site with intermediate diversity. The proportion of adults from low-diversity Cuc Phuong was 40.3%. The ratio of adults to juveniles among sites was significantly different (Pearson’s chi-squared test, P<0.05).

**DISCUSSION**

Biodiversity on a landscape scale comes down to two numbers: the number of species in a place and the rate at which the community changes across space. Decades of tropical arthropod diversity studies have concentrated on the first number (e.g., Coddington et al. 2009, Coddington et al. 1991, Erwin & Geraci 2009, Longino et al. 2002, Longino & Colwell 1997). It has only been within the last few years that statistical methods have become available to investigate the spatial dimension based on communities for which a substantial portion of the component species are unobserved (Chao et al. 2005, 2006, Colwell 2009).

Non-parametric biodiversity estimators are generally regarded as lower bound estimates (Gotelli & Colwell 2001, Shen et al. 2003). When estimator curves are rising, as they are for Vu Quang and Cat Ba (Figure 1), it seems probable that further sampling at these sites would lead to even higher estimates, at least initially. We expect the upper 95% confidence intervals on the Chao 1 and Chao 2 to be informative under these circumstances not as an estimator but as an upper bound; in 19 of 20 trials the actual species richness would be expected to fall below the upper 95% CI curves.

There are few data available on the rate at which diverse tropical spider communities (or any diverse tropical arthropod community for that matter) change over space (but see Hulcr et al. 2008, Miller et al. 2009, Novotny et al. 2005, Novotny et al. 2007, Novotny & Weiblen 2005, Wang et al. 2010). Our three sites are approximately 150–300 km apart and differ in forest type, geology, elevation, and other attributes. We
detected a roughly 65–85% turnover in the Southeast Asian forest spider community over differences of this magnitude. This suggests that additional sampling in the region, especially at finer spatial scales and controlling for variables such as vegetation community and elevation, could help elucidate the dynamics of change in the tropical spider community across the landscape.

The positive correlation between rank sample abundance and number of observed sites is not surprising since our ability to detect any given species must be related to its availability to our sample methods. What is elucidating is the proportion of species in moderately rare sample abundance classes that were detected in multiple sites (Figure 4). This illustrates a relationship between sample abundance and detection probability and suggests that more shared species would be detected in low abundance classes with greater sampling intensity. This undersampling bias as applied to shared species is precisely what the Chao-Jaccard index is designed to compensate for. When sample abundance was scaled by incidence as an index of detection probability, the effect of sample abundance on site number was removed. We conclude from this that the probability of a species being actually present in multiple sites is largely independent of their sample abundance.

The number of species expected in a community may be associated with niche space (Bastolla et al. 2005, MacArthur & Wilson 1967, Whittaker 1999). The unanticipated heterogeneity in community stratification among our sample sites could be related to species richness. If so, communities with narrow average niches (indicated by high fidelity of individual species to a particular sampling method) would be predicted to contain a relatively large number of species. This could explain the relatively low species richness and low community stratification in Cuc Phong.

Most tropical spider inventories focus almost exclusively on adults because, unlike juveniles, these can be sorted to morphospecies with a high degree of confidence. However, this understates the true abundance of spiders in the habitat. By collecting all spiders seen (except for aggregations of recently hatched siblings) regardless of their ontogenetic stage, we are gathering basic information that may be used to estimate the true number of spiders present. Our finding that the ratio of adults to juveniles differs significantly among our sites tells us something about the confidence we will be able to place on such estimates.

Non-parametric biodiversity estimators have a distinguished track record in studies of tropical arthropod biodiversity (see table 1, appendix 1 in Coddington et al. 2009) and have occasionally featured in studies of
other taxa (e.g., Chazdon et al. 1998, Dumbacher et al. 2011, Heyer et al. 1999, Novotny et al. 2007). Applying this approach is useful whenever there is a question about the degree of completeness of a structured inventory or when comparing inventories thought to be incomplete. However, these estimators have relatively low statistical power meaning that a reliable estimate of the true species richness may require that a substantial fraction of the community be sampled (Chao et al. 2009, Coddington et al. 2009).

Table 1. Attributes of the three study sites, including site location, latitude-longitude coordinates, elevation, sample dates, and general geology. All samples were from tropical rainforest habitats in National Parks.

<table>
<thead>
<tr>
<th>Study site</th>
<th>Coordinates</th>
<th>Elevation</th>
<th>Dates</th>
<th>Geology</th>
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<tbody>
<tr>
<td>Cuc Phuong National Park, Cay Dang Co Thu trail</td>
<td>20.299039°N 105.655756°E</td>
<td>200 m</td>
<td>7–13 October 2009</td>
<td>Mesozoic rocks, limestone</td>
</tr>
<tr>
<td>Vu Quang National Park, forest near Don Bien Phong [border station] 567</td>
<td>18.331306°N 105.439111°E</td>
<td>50 m</td>
<td>27 September–4 October 2009</td>
<td>Granitoid rocks</td>
</tr>
</tbody>
</table>

**ACKNOWLEDGMENTS**

Expedition support was generously provided by the Netherlands Centre for Biodiversity Naturalis. Thanks to the expedition participants Khuat Dang Long, Cees van Achterberg, Rob de Vries, Eric van Nieuwerkerken, Eulàlia Gassó Miracle, and Camiel Doorenweerd. Administrative support from Jan van Tol facilitated many aspects of this research. Thanks to Leiden University undergraduate student interns Manon de Waard and Rense Schelfhorst for their substantial contributions to this work. Thanks to Rhett Harrison and Qie Lan (Xishuangbanna Tropical Botanical Garden, China) for enlightening discussions on biodiversity analysis and help with R. We are grateful to the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology and its director Le Xuan Canh for generous assistance facilitating the field work overall. Finally, we thank Cat Ba National Park, Cuc Phuong National Park, Vu Quang National Park, and the staff and administrators of these institutions.
REFERENCES


Table 2. Observed proportion of shared species (Jaccard index) and Chao-Jaccard estimated proportion of shared species and 95% confidence interval for all pairwise comparisons of the five sampling methods for each locality. Classic Jaccard index in parentheses. Where the Chao-Jaccard estimate plus the 95% confidence interval crosses the 1.0 mark, two samples are considered to have been drawn from statistically indistinguishable underlying populations; such pairs are indicated in bold type. Methods that sample the aerial stratum above the ground are BED (beating vegetation during the day) and LUN (searching in the aerial stratum at night); methods that sample the ground stratum are LDD (searching for spiders on the ground during the day), LDN (searching for spiders on the ground during the night), and WIN (Winkler trap for extracting arthropods from drying sifted leaf litter). Number of observed species for each method by site reported in parentheses in the column headers. Number of replicates for each method within each locality reported in parentheses in the left column.

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<tr>
<th>Locality</th>
<th>BED (42)</th>
<th>LUN (61)</th>
<th>LDD (14)</th>
<th>LDN (23)</th>
<th>WIN (23)</th>
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<tr>
<td>Cat Ba</td>
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<td>BED (6)</td>
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<td>LUN (12)</td>
<td><strong>1±0.251 (0.32)</strong></td>
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<td>LDD (4)</td>
<td>0±0 (0)</td>
<td>0.013±0.029 (0.01)</td>
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<td>LDN (8)</td>
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<td>0.13±0.143 (0.06)</td>
<td>0.41±0.298 (0.28)</td>
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<td>0±0 (0)</td>
<td>0.666±0.325 (0.28)</td>
<td>0.174±0.220 (0.12)</td>
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<td>LUN (10)</td>
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<td>0.909±0.347 (0.25)</td>
<td>0.721±0.347 (0.24)</td>
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<td>LDN (27)</td>
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<tr>
<td>LUN (9)</td>
<td>0.463±0.251 (0.14)</td>
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<tr>
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<td>LDN (6)</td>
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<td>WIN (4)</td>
<td>0±0 (0)</td>
<td>0.009±0.016 (0.02)</td>
<td>0.207±0.276 (0.14)</td>
<td>0.072±0.131 (0.09)</td>
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Figure 1. Diversity curves for collections of spiders from three localities in Vietnam (Cat Ba, Cuc Phuong, and Vu Quang) plotted against accumulated number of individuals. Curves represent observed species (thick black line), four non-parametric estimators (Abundance based: Chao 1: dark line solid circles, ACE: light line solid circles; Incidence based: Chao 2: dark line empty circles, ICE: light line empty circles), upper 95% confidence interval for Chao 1 (dot short dash line) and Chao 2 (dot long dash line), and four curves tracking rare species (Singletons: dark short dash line, Doubletons: light short dash line, Uniques: dark long dash line, and Duplicates: light long dash line. Incidence-based estimators for Cat Ba from bias-corrected formula; all other estimators from classic formula.
Figure 2. Estimated proportion of shared species between pairs of localities. Box length indicates Chao-Jaccard estimate, error bar is the upper 95% confidence interval. Observed proportion of shared species (classic Jaccard index) is shown as a dashed line.
Figure 3. Estimated proportion of shared species for methods that sample from ground strata (LDD, LDN, WIN) vs. aerial strata (BED, LUN) for each locality. Conventions as in Figure 2. The Chao-Jaccard plus upper 95% confidence interval exceeds 1.0 for Cuc Phuong. This indicates weak stratographic structure in the Cuc Phuong spider community.
Figure 4. Summary figure showing the proportion of species observed in one, two, or all three sample sites pooled into octaves with singletons excluded. Octaves 1–8 represent sample abundances of 21–177, 11–20, 7–10, 5–7, 3–5, 3, 2, and 2, respectively. Rank sample abundance is positively correlated with number of sites observed. However, this effect disappears when sample abundance is scaled by incidence as an index of detection probability.
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5. Acknowledgements, if any, should be placed preceding the list of references

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