

Diversity and phylogenetic community structure of ants along a Costa Rican elevational gradient

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The diversity and phylogenetic community structure of many organisms is negatively affected by factors that covary with elevation. On the Pacific slope of the Cordillera Guanacaste within Area de Conservación Guanacaste (ACG) in northwestern Costa Rica we found a negative relationship between elevation and ant diversity on each of three volcanos. This pattern was evident when diversity was measured through molecular operational taxonomic units (MOTU) or by phylogenetic diversity (PD) based on DNA barcodes or a multi-gene phylogeny. We observed an asymmetrical mid-elevation peak at approximately 600–800 m and we found high species turnover between sites on the same mountain and among the three mountains. At the highest elevation cloud forest sites we found evidence of significant phylogenetic clustering, the expected result of environmental filtering. The narrow elevational range of each species, coupled with the high diversity at each sampling point, emphasizes that climate change will bring strong changes in the location and composition of biodiversity on these mountains. The structure and composition of the hyperdiverse communities present at any one elevation is extremely vulnerable to a changing climate.

It is a common observation that arrays of ant species in high elevation cloud forests are comparatively species poor when compared with lower elevation rain forest communities (Janzen 1973a, Fisher 1996, 1999, Longino and Colwell 2011). Declines in ant diversity in montane forests are likely to be the consequence of reduced radiant energy with increasing elevation and more uniform yearround daily temperatures (Olson 1994). Waterlogged substrates warm slowly in a nearly perpetual cloud bank (Hunt et al. 1999), and thus the lifestyle of any organism confined to solid substrates is like living in a refrigerator (Janzen 1983). However, that environment is changing. Models of climate change on tropical mountains predict a 600 m upslope shift in temperature isotherms in the coming decades (Christensen et al. 2007). Using the Intergovernmental Panel on Climate Change estimated median regional rate of change for the next century of 3.2°C (Christensen 2007), recent modeling exercises in Costa Rica explored the effects that such a change would have on biological communities (Colwell et al. 2008). Given that many tropical ant species appear to have an elevational range smaller than this (e.g. 500 m - Olson 1994), a 600 m elevational movement of an isotherm is particularly significant. Specifically in the case of the isolated northwestern volcanic mountains of Costa Rica,

even small changes in elevation (and therefore temperature and moisture) can have a proportionally larger effect than what one might expect on temperate mountains of similar heights (Janzen 1967, Rosner 2013).

Understanding the effect of factors that covary with elevation on diversity is an area of macroecological investigation with a long history (for a recent review see McCain and Grytnes 2010). Recently, our understanding of diversity across elevational gradients has benefited from a growing literature where phylogenetic methods are used to elucidate ecological patterns. To date, while the botanical community has used DNA barcodes (Hollingsworth et al. 2009) to conduct phylogenetic analyses of community structure, animal studies have tended to use an approach which capitalises on published phylogenies, GenBank sequences, or their own derived (but non-DNA barcode) sequences. For example, Machac et al. (2011) used three studies of ant communities along elevational gradients in North America and Europe, supplemented with publicly accessible DNA sequences from GenBank (Benson et al. 2008), to create their own phylogenies and found evidence of phylogenetic clustering high-elevation communities (phylogenetic among clustering - expanded upon below - is the prevalence of closely related taxa in a particular environment). Lessard et al. (2009) analysed 12 published studies of ant distributions and demonstrated strong phylogenetic structuring within communities that had not been invaded by 'tramp' species (e.g. Linepithema humile, the Argentine ant) - while

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within those communities which contained invasive species this structuring was not present. Graham et al. (2009) used a Bayesian phylogeny of three nuclear and two mitochondrial markers (not including cytochrome *c* oxidase I) to reveal evidence of phylogenetic clustering at both high and low elevation populations of Ecuadorian hummingbirds. Gonzalez-Caro et al. (2012) examined the same dataset to reveal increasing degrees of phylogenetic clustering with elevation using three methods of measuring diversity. Forest et al. (2007) used one of the plant barcoding loci (rbcl - Hollingsworth et al. (2009)) to compare and contrast diversity patterns evident across the South African Cape and found that the diversity measured using species (taxonomic) richness or phylogenetic diversity were 'decoupled'. Kress et al. (2009) used DNA barcodes (rbcL) to reveal evidence of phylogenetic clustering in Panamanian swamp forests and overdisperson in plateau forests. Whitfeld et al. (2012) examined the phylogenetic community structure of New Guinean forest along a disturbance gradient using one of the standard plant barcoding loci and found that secondary forests displayed significant clustering while primary forests were characterised by a phylogenetically overdispersed communities. One common element to studies of the phylogenetic diversity and structure of natural communities is uncovering evidence for phylogenetic clustering (Cavender-Bares et al. 2009, Vamosi et al. 2009). Here, we examined the ant communities along a Neotropical elevational gradient to test for evidence of phylogenetic clustering when phylogenetic measures were calculated using the standard animal DNA barcoding locus (cytochrome *c* oxidase I or COI).

We measured the patterns of diversity for ant species in the Area de Conservación de Guanacaste (ACG - <www. acguanacaste.ac.cr>) on an elevational transect from sea level to the cloud forest peaks of three volcanoes. We asked whether ACG ant species had a 500 m elevational range (Olson 1994) and whether using DNA-based methods of delimiting those species increased or decreased this range. Are communities at each sampling elevation simply a random draw from the total ACG species fauna, or are they influenced by competition or environmental filtering? For instance, do the island-like, elevation-restricted cloud forest ant assemblages exhibit phylogenetic clustering? Mechanisms that can produce non-random structure on habitat islands (Janzen 1973b) include competition (assemblages should be phylogenetically over-dispersed if competition is common among close relatives) or colonization (assemblages should be phylogenetically clustered if a lineage possesses biological traits that permit resource exploitation at a particular elevation) (although see Mayfield and Levine 2010). We mapped our observed species occurrence matrices onto a published multi-gene phylogenetic tree (Moreau et al. 2006) to test whether DNA barcode derived measures of phylogenetic diversity (PD) are a reasonable proxy for multi-gene phylogenetic measures of PD.

Long-term standardized arthropod sampling through continuous, elevational gradients, are often used to test macroecological hypotheses such as the relative importance of habitat filtering/selection or the competitive exclusion of closely related species (Vamosi et al. 2009), and to documenting changes expected with a changing global climate. While the largest effects of a changing climate are likely to occur at high latitudes, the most immediate (and damaging to biodiversity) will occur in the tropics at high elevation (Mora et al. 2013). Thus, understanding the evolutionary history resident within (and arriving at) these cloud forests is of critical import. While phylogenetic diversity measures have been used in ant community ecology in the past (Smith et al. 2005, 2009, Smith and Fisher 2009, Machac et al. 2011), here we extend its use by comparing the patterns evident with genera and elevation using newly published DNA barcodes and a published ant phylogeny.

Methods

The study area is the Pacific side of the 120 000 ha terrestrial portion of the Area de Conservación Guanacaste (ACG) in northwestern Costa Rica – a protected area that includes three distinct types of tropical forest (tropical dry forest, rain forest and cloud forest) and forest that has been cleared but is currently being allowed to regenerate (Janzen 2000, Janzen and Hallwachs 2011). To accommodate these discontinuities, we analysed our findings both across the sealevel to summit elevational range, and also across the continuously forested range from each volcano (~500–1500 m). Distance-based phylogenetic measures using these DNA barcodes were then compared with calculations of phylogenetic diversity (Faith 1992) derived from a published multi-gene phylogeny (Moreau et al. 2006).

Ant collections were made from sea level to the tops of three volcanos (Orosi, Cacao and Rincon de la Vieja) over 103 collection days from 2008–2011 (Fig. 1). Within the ACG, we considered 'high' elevation sites to be between 1300 and 1600 m, while 'low' elevation roughly corresponds to the range from sea-level to 300 m. The collection methodology was adapted from the ALL protocol (Agosti and Alonso 2000) to be amenable for one investigator with a focus on the leaf-litter community of ants in the so called brown food web (Swift et al. 1979) and is detailed in Supplementary material Appendix 1. Briefly, diversity at each site was sampled with pit-fall traps, leaflitter sifting (Davis sifters), mini-Winkler sifters, bait (cookie and tuna), active searching and Malaise traps. Malaise traps ((Townes 1962) - <www.santetraps.com>) were maintained throughout the year at low-elevation dry forest sites (Naranjo 10 m, Santa Rosa 330 m) and at three elevations on Volcan Cacao (1000, 1200, and 1500 m) and were emptied fortnightly. Upon collection (either active or passive) ants were preserved in 90% ethanol. The habitat at each locality at each collection visit was documented at ground-level in the forest understory with high-resolution panoramic photographs captured using a GigaPan system (<www.gigapan.org>), and are publically available (<www. gigapan.com/galleries/10092/gigapan> – Fig. 2).

Ant specimens from each location and trap type were sorted to genus and morphospecies using available taxonomic literature and online resources (Longino 2012a). Representatives of each morphospecies (both workers and reproductives) were point-mounted, photographed and tissue sampled for DNA extraction (generally a single leg; however for very small ants, all the point-side legs were



Figure 1. Map of Area de Conservación Guanacaste (ACG), in northwestern Costa Rica. Three terrestrial ecosystems: dry forest (yellow); rain forest (green); cloud forest (blue). Sampling localities are indicated by red triangles.



Figure 2. Example high-resolution panoramic images of habitats sampled from 10–1500 m along Volcan Cacao. The location of each sampling site is portrayed by a red dot over a black shape of the ACG that is superimposed on each panorama. Each GigaPan (<www.gigapan.org>) panorama is a composite image of, on average, 925 photographs (total – 7620 images). The total images are 36.3 gigapixels (average = 4.5 gigapixels) and have been viewed more than 170 000 times. More than five dozen habitat panoramas completed while sampling for ants can be viewed in a gallery at <www.gigapan.com/galleries/10092/gigapan>.

removed). DNA extracts were prepared using a glass fibre protocol (Ivanova et al. 2006). Extracts were re-suspended in $30-40 \,\mu\text{l}$ of dH₂O, and a 658 bp region near the 5' terminus of the cytochrome c oxidase 1 (COI) gene was amplified using standard protocols (Smith et al. 2006, 2007, 2008, Fisher and Smith 2008). When initial amplification was not successful composite, or shorter, sequences were generated using internal primers. Primer information for individual sequences can be retrieved from BOLD using the Process IDs detailed in Supplementary material Appendix 2, but primers and the full details of the methodology are detailed elsewhere (Smith et al. 2006, 2007, 2008, Fisher and Smith 2008). All collection information and accessions (BOLD and GenBank) are available on Barcode of Life Data System (BOLD - <www.barcodinglife.org>) in the public dataset: Elevational Changes in Formicidae Diversity (ACG) (<http://dx.doi.org/10.5883/DS-ASACG2>).

Sequence diversity for specimens that produced a COI fragment longer than 350 bp were compared across a range of divergence thresholds (1, 2, 3 and 4%) in order to calculate molecular operational taxonomic units (MOTU – (Floyd et al. 2002)) using the program jMOTU (Jones et al. 2011). Diversity accumulation curves, and an estimate of shared diversity (Jaccard classic index), were calculated for each of these sites (and pairs of sites) for each MOTU % divergence scenarios using EstimateS ver. 8.2.0 (Colwell 2005). Here, a 'site' is the aggregation of samples taken from a sampling locality at a particular elevation. Sequence divergences were calculated using p-dist and the Kimura 2 Parameter (K2P) distance model (Kimura 1980), and a neighbor-joining (NJ) tree (Saitou and Nei 1987) was used to visualise these distances.

Diversity terms

Classical measures of diversity use some combination of richness and abundance based on entities most often delineated at the taxonomic level of species (Magurran 2011). Here, we measured diversity in two ways. Firstly, we calculated the richness and abundance of MOTU based on the 'entities' described by genetic thresholds of similarity ranging from one and four percent, and is therefore analogous to classical measures where the diversity of a site was measured based on the richness (total number) and abundance of taxonomically described units (most often species). Alternatively, we also calculated diversity as the phylogenetic diversity (PD) (Faith 1992) for each sampling site. PD is the branch length spanned by the set of species at each elevation but does not include the root of the larger phylogeny. If close relatives exhibit greater ecological similarity than distant relatives (Cavender-Bares et al. 2009), then PD is expected to be an important measure of community assembly. PD and MOTU are often, but not always, correlated (contrast Forest et al. 2007, Smith and Fisher 2009).

Phylogenetic clustering and dispersion were measured using the nearest taxon index (NTI) (Webb et al. 2002) as calculated using Phylocom (ver. 4.2) (Webb et al. 2008) and in R (R Core Team) using the ape (Paradis et al. 2004) and Picante (Kembel et al. 2010) packages. NTI quantifies the extent of phylogenetic terminal clustering within particular sampling elevations and tests whether patterns are significantly different from chance expectations (Webb 2000, Webb et al. 2002). NTI measures the mean phylogenetic distance among ant species found at a particular elevation, relative to the mean and standard deviation of possible elevational associations distributed randomly across the phylogeny.

$$NTI = -1 \times \left(\frac{mn(Y_{obs}) - mnY(n)}{sdY(n)}\right)$$

Where Y_{obs} is the phylogenetic distance to the nearest cooccurring taxon in the phylogeny, $mn(Y_{obs})$ is the mean of all possible pairs of n taxa, mnY(n) and sdY(n) are the mean and standard deviation respectively expected for n taxa randomly distributed across the phylogeny (Webb et al. 2002). To test whether this measure of phylogenetic clustering of elevational associations across the community phylogeny was significantly different from chance, under a null model of random association, we performed 1000 permutations of elevational-associations to simulate a distribution of NTI for each community. The significance of the observed values was examined with a two-tailed test of significance (p = 0.05).

We first tested how phylogenetic measures of community structure (PD and NTI) differed with elevation using linear regression. We then compared the DNA barcodebased measures of phylogenetic diversity to the patterns observed when we measured PD using a large-scale ant phylogeny (Moreau et al. 2006). Since the Moreau et al. phylogeny is predominantly at the genus level, we reduced our incidence data to genus-level per elevation and volcano and then trimmed the Moreau et al. phylogeny in R (using ape and Picante) so that the Moreau et al. phylogeny contained only those genera found at those elevations on those volcanoes. While the phylogeny is archived on Treebase (Piel et al. 2003) (S1573), we used the tree that included branch lengths which is available on Moreau's website: <www.moreaulab.org/resources.html>.

Results

Between 2008 and 2011 inclusive over 103 collection days, an estimated 24 000 ants (an average of 20 ants in each of 1200 collections) were collected. Of these, 7714 were point mounted, photographed (3575) and a DNA extract was prepared from their tissue (principally a single leg). Mitochondrial DNA from the 5' region of COI (the standard animal barcode region) was generated for 5808 of these specimens. Typical of ants, the mtDNA was highly AT biased with an average GC content of 31.57% (SE = 0.037). All specimen information, georeferenced collection data and DNA data is publicly available at: ">http://dx.doi.org/10.5883/DS-ASACG2>.

Diversity, phylogenetic diversity and phylogenetic disperson

We estimated the number of species at different elevations using 4 arbitrary thresholds of molecular variability (1, 2, 3, 4%) (Fig. 3 and Fig. 4A, Supplementary material





Figure 3. Molecular and phylogenetic diversity and community structure of ants along a Neotropical elevational gradient illustrated with a NJ tree made with one sequence from each of the provisional species identified as 2% Molecular Operational Taxonomic Unit (MOTU) from six elevations on the continuously forested section of Volcan Cacao (560–1500 m) (NJ phenograms of the entire dataset (all volcanoes and elevations and specimens) can be made on BOLD). The branch tips are coloured by collection localities (as in Fig. 4B) to help illustrate the diversity of each site (i.e. the frequency of each colour) and the narrow elevational range of each MOTU (i.e. very few extend beyond one or two adjacent colour bars). Dark blue is 500 m, purple is 700 m, light blue is 1000 m, red is 1100 m, green is 1300 m and pink is 1500 m. The dashed line box surrounds the species of *Adelomyrmex* and *Stenamma*.

Figure 4. (A) Accumulation curve calculated by EstimateS (Colwell 2005) for the set of specimens collected on the continuously forested section of Volcan Cacao (560–1500 m, DNA sequence > 350 bp) using 1, 2, 3 and 4% MOTU calculated using jMOTU (Jones et al. 2011). The 95% upper and lower confidence intervals (dashed lines) are included for 1 and 4% respectively. (B) Phylogenetic diversity (PD) for specimens collected along the entire elevation gradient (6-1500 m) - collection localities on Volcan Cacao coloured as in Fig. 3. (C) PD and elevation for specimens collected along the continuously forested sites between 560 and 1500 m on Volcan Cacao, Orosi and Rincon de la Vieja. (D) Nearest Taxon Index (NTI) and elevation for specimens collected from all sampling sites across three volcanoes and the pairwise difference in elevation. Clustering is evident at both high and low elevations (clustering is significant when coloured in red), while mid-elevation values tended to display less clustering.

Appendix 3). In other studies, where all available data was used to determine species, a 2% MOTU in the barcode region yielded the tightest congruence to patterns of diversity measured using classical taxonomy (Smith et al. 2005, Smith and Fisher 2009).

Ant diversity (whether measured via PD or MOTU) in the continuously forested areas between ~500 and 1500 m declined with elevation but an asymmetrical midelevational peak was evident when sites from sea-level to the highest elevations were considered (Fig. 4B). PD declines with elevation from 550–1500 m on all three volcanoes (Fig. 4C). The trend between elevation and MOTU was congruent to that between elevation and PD (Supplementary material Appendix 3, Fig. 1).

We found a very high rate of species turnover from elevation to elevation on the same volcano and among volcanoes as well. Seventy percent of the species/MOTU sampled were found at only one elevation and, on average, there is little similarity (Jaccard $\sim < 0.1$) of MOTU composition between sampling sites more than 300–400 m in elevation apart (Supplementary material Appendix 3, Fig. 2, 3). We found that shared diversity decayed with elevational distance (Jaccard Classic on 2% MOTU) where more than 80% of the variation in MOTU similarity is explained by the difference in elevation (Fig. 5).

The communities sampled from sea-level to the highest elevations at each volcano were phylogenetically clustered (as measured by NTI) with increasing elevation. Localities that displayed significant clustering (p < 0.05) (indicated in red on Fig. 3E) included all high elevation sites. Patterns of high elevation phylogenetic clustering were particularly driven by species in genera such as *Adelomyrmex* and *Stenamma* where species were found nearly exclusively above 1300 m (Fig. 3 dashed box). Significant clustering is evident at high elevations while mid-elevation values tended to display less clustering (Fig. 4D), however, individual NTI values from mid-elevation sites were generally not found to be significant (p > 0.05).

Comparison of phylogenetic diversity measures derived from DNA barcodes to Moreau et al. phylogenetic measures of PD

When we compared the distribution of species at elevations on Volcan Cacao as based on the large-scale genus-level phylogeny of Moreau et al. to the larger dataset comprised of DNA barcode sequences/species NJ phylogeny (Fig. 6A, B) we found the same negative relationships between increasing elevation and diversity on the volcanoes and a mid-elevation peak in diversity when sites from sea level up were included. We found that 85.9% of the variation in the trend measured using the Moreau et al. phylogeny was captured in the simple neighbor-joining tree based on distance measures of the DNA barcode (Fig. 6C).

Discussion

Our analysis of leaf litter ant diversity along this Neotropical elevational gradient revealed a very high rate of turnover, both between elevational sites on the same volcano, and among volcanoes, such that complementarity decayed significantly with increasing elevational distance. We found that cloud forest ant arrays were phylogenetically clustered and that phylogenetic community structure measures based on the single mitochondrial COI DNA barcode region paralleled those derived from a large-scale multi-gene phylogeny. The congruence between phylogenetic measures based on DNA barcodes and a multi-gene phylogeny allows 1) the use of DNA barcodes in ecophylogenetic analyses, 2) a phylogenetic approach to be used without having to barcode specimens (as long as morphological identifications are accurate) and 3) reinforces the utility of barcode data within a phylogenetic approach if using standardized DNA barcodes are produced in environmental barcoding (Hajibabaei et al. 2012) with nextgeneration sequencing technologies.



Figure 5. Decay curve for the effects of elevation on MOTU similarity (Jaccard). More than 80% of the variation in similarity is explained by differences in elevation. Sites farther than 300 m apart in elevation are always less than 0.1 (Supplementary material Appendix 3, Fig. 2, 3).



Figure 6. Comparing the distribution of species at elevations on Cacao using either the large-scale genus phylogeny or the larger dataset comprised of DNA barcode sequence/species NJ phylogeny. (A) Genera present at different elevations along Volcan Cacao plotted on Moreau et al. (2006). (B) Species present at the same sampling sites plotted on the neighbor-joining tree (K2P distance). (C) Significant relationship between PD measured using a distance based NJ phylogeny calculated solely on the DNA barcode region and PD calculated based on the same samples placed on the large scale multi-gene Moreau et al. phylogeny.

Tropical arthropod communities change with elevation (Janzen 1973a, Janzen et al. 1976, Wolda 1987, McCoy 1990, Hodkinson 2005). With ants across tropical and temperate systems, there is either a linear decline (Brühl et al. 1999, Hågvar 2005, Glaser 2006, Lessard et al. 2007) or a mid-elevational peak (Andersen 1997, Samson et al. 1997, Fisher 1998, van der Hammen and Ward 2005, Longino and Colwell 2011). We found reduced phylogenetic diversity and significant phylogenetic clustering at high elevation sites (as predicted by a hypothesis of severe environmental filtering) and that diversity peaked at midelevation.

We have demonstrated that PD measured using DNA barcodes is strongly linked to the gain or loss of deeper evolutionary history. Our results parallel earlier observations of the reduction of PD with elevation among temperate ant species (Machac et al. 2011) but do not support the expectation that ants within 500 m of each collection event will be members of a similar community (Olson 1994). There was little overlap between communities at a series of arbitrarily chosen elevations on the same mountain communities often separated by only 300-400 m (see Fig. 3 and the frequency of colour bars at tips that demonstrate elevational range). Our findings emphasize the unique and otherwise cryptic vulnerability of the array within each elevational band. It furthermore suggests that with climate change the membership of an elevational band will not remain constant. A changing climate dramatically alters species ranges and the ecology of communities across the globe. Neotropical species will experience rapid and drastic changes over a few hundreds of meters of elevation (Colwell et al. 2008, Rosner 2013) as compared to the dramatic alterations that temperate and arctic will experience over hundreds of kilometers of latitudinal change (Parmesan 2006, Mora et al. 2013). These tropical changes will result in the attrition of low elevation species at a given geographic point (for whom there is no possible migration from an even warmer/lower elevation) (Colwell et al. 2008), or high elevation mountain top extinction (where species are pushed upwards beyond their physiological tolerance) as cold and cloudy high elevation ecosystems are eliminated (Pounds et al. 1999). For example, the species from two genera, Adelomyrmex and Stenamma are known to occur with near exclusivity in high elevation ACG forests (Longino 2012b). This habitat specificity – evident across all members of the genera observed here contributed significantly to our observation of phylogenetic clustering observed in cloud forests (Fig. 3) – and furthermore reinforces the concern for maintaining this diversity in light of a changing climate. Climate models for northwestern Costa Rica predict that high elevation regions will receive significantly less precipitation during the dry season, while the frequency of dry events during the rainy season will increase (Karmalkar et al. 2008) a result of changing rainfall patterns and a rising cloud base. Learning the degree to which evolutionary history is significantly clustered in these forests emphasises the impending upheaval posed by this drying.

A frequent characteristic of the low(er) elevation communities within the ACG (and other localities (Smith and Fisher 2009)) is an increased prevalence of tramp and invasive species. If the expected upslope cloud forest immigrants include such tramp species, then we anticipate that these arrivals could remove the existing phylogenetic clustering and structure – as has been demonstrated within other ant communities by Lessard et al (2009).

The congruence of a distance-based single-gene phylogeny with a larger multi-gene phylogeny reinforces our use of phylogenetic community ecology measures such as PD and NTI with DNA barcodes. Characterizing a community based solely on its constituent species might miss potentially significant trends in the evolutionary history captured in a measure of phylogenetic diversity (Forest et al. 2007, Davies et al. 2011). We used a measure of PD for COI defined as the sum of all phylogenetic branches connecting species together within a community. In this sense, it is differentiated from more classical presentation of Faith's PD where the sum of the branch lengths present at a site would include a reference to a larger regional phylogeny. Cadotte et al. (2010) categorized how we measured PD as 'Community PD' (the sum of the branch lengths in a community without regard to any larger regional species pool) the more appropriate measure of PD for assessing species coexistence since community processes operate on extant members (Cadotte et al. 2010). We used a small sub-set of the ever increasing number of methods of phylogenetic reconstruction to measure PD and while testing the effect of method of phylogenetic reconstruction upon the estimation of PD will be an interesting and profitable arena of investigation - it is not our intent to complete these investigations here. Indeed, recently it has been shown that the particular algorithm used to generate the phylogeny does not alter qualitative PD results (Cadotte et al. 2009a, b).

Phylogenetic diversity and DNA barcoding

Our phylogenetic analysis was parameterized with the field-collected specimens for which we could account for their entire history, we sequenced multiple specimens/ species and we verified the morphology-based identifications thus avoiding the post hoc problems of debatable species lists (Vamosi et al. 2009) and uncertain linkages between named specimens and un-curated genetic data (Bridge et al. 2003, Harris 2003, Nilsson et al. 2006). In addition, we tested whether there was a difference between phylogenetic diversity calculated using barcodes or using a multi-gene phylogeny. We found no differences between estimates of richness (MOTU) and phylogenetic diversity and phylogenetic clustering evident at high elevations. Studies that examined the phylogenetic diversity of natural communities across a landscape-level variable frequently uncover evidence of phylogenetic clustering (Cavender-Bares et al. 2009, Vamosi et al. 2009). While this can be interpreted as evidence for some manner of physiological filtering by the environment or habitat - it could be due to a number of alternative explanations (Mayfield and Levine 2010). For example, environmental filtering could cause phylogenetic overdispersion if the traits that promoted high elevation habitat specialization had convergently evolved. Alternatively, competitive exclusion could produce phylogenetic clustering if, historically,

competitive exclusion had already eliminated all but the most efficient competitors in this refrigerated environment. Finally, it should be noted that while the multi-gene phylogeny we used is currently accepted by the community as the gold-standard, it is best parameterised at the genus level and thus the topology may not reflect the same topology of a multi-gene species level phylogeny.

Idiosyncrasies of the barcoding locus for phylogenetic community ecology

Because NTI measures the average minimal distance between species of ants that are found at a sampling elevation (terminal clustering independent of deep level clustering) it is a particularly useful measurement when analyzing phylogenies derived from single gene mitochondrial DNA barcode data. Due both to the fact that the DNA barcode is a single mitochondrial marker (Ballard and Whitlock 2004, Ballard and Rand 2005), and the saturation of third base pair substitutions that is evident in insect (and specifically ant) mitochondrial DNA (Clare et al. 2008) - the deeper phylogenetic signal evident in COI is likely to be limited. However, the pattern of phylogenetic diversity derived using computationally simple phylogenetic analyses (such as neighbor-joining (Saitou and Nei 1987)) was similar to the pattern found with large-scale multi-gene phylogenetic analysis. For ants, DNA barcode data served as a useful proxy in the measurement of phylogenetic diversity.

While testing the pattern of NTI declining with elevation from mid to high elevations, it is important to note which specific individual cases are actually significant (Fig. 4E). In measuring the trend of NTI across elevational ranges collected from transects along three volcanos in northwestern Costa Rica we found a significant relationship between elevation and phylogenetic clustering. This conclusion is further supported by the fact that of the individual site comparisons – those that were statistically significant when compared to a null distribution of phylogenies – are also the high-elevation sites (Fig. 4E). This supports our conclusion that there is some manner of habitat filtering occurring at these high elevation sites.

Why the small community boundaries in northwestern Costa Rica?

We found very little overlap in sites we sampled and therefore, not only was diversity greater than we had previously anticipated – the restricted elevational distribution of each of these species and communities renders each more vulnerable to local extinction events. Historically, the drop in diversity at low-elevations was attributed to the increased frequency of desiccating conditions due to increased rates of evaporation resulting from high temperatures and abundant sunlight while the drop in diversity at higher elevations (cloud forest) was as a result of the increased likelihood of cooler temperatures and continuously web substrates combined with reduced solar radiation (Olson 1994). In Olson's work (1994) he found that 73% of species examined had an elevational range of 500 m or less, while here we find that 70% of our species had a range of 200 m (only one elevational sampling area). Longino and Colwell (2011) found that their low and mid-elevation sites (sites between 50 and 500 m) were more similar in species composition than their high elevation sites (sites from approximately 1000 to 2000 m). Our results suggest that in the small volcanoes of the ACG, not only the high-elevation communities are narrow - all community boundaries are narrow. While further work, over a greater geographic range is required to determine the generality of these conclusions, it is important to note that any a priori determination of elevational zonation would have precluded our capacity to discover patterns such as those revealed here. The important conservation message from our diversity measures is a reinforced understanding of the unique nature of the community at each elevational site. The extremely high levels of local endemism demonstrated here warrant consideration when dealing with Central American ant fauna with apparently wide distributions. If such extreme endemicity is the norm rather than the exception (Andersen and Ashe 2000, Andersen 2010, Rubinoff and Schmitz 2010) many areas of isolated forest, or elevational gradients host significant portions of the region's evolutionary history. These findings reinforce a conservation lesson that has been learned and re-learned many times - that nothing is too small to protect (Rubinoff and Schmitz 2010). Protecting any portion of the elevational gradient would help preserve the evolutionary history of this hyperdiverse group. Furthermore, this narrow range suggests that these tropical species will be more likely to experience the 'range-shift' gaps when an isotherm shifts upslope (Colwell et al. 2008).

Comparison to other Central American genera

Several Neotropical genera have been recently reviewed or revised where these analyses included specimens collected in the ACG from which COI was generated and made publically available on GenBank. This warrants a comparison between these taxonomic and phylogeographic treatments and the work reported here. The examples of *Adelomyrmex, Azteca* and *Tatuidris* are not isolated comparisons but serve as exemplar genera to the ample opportunities for hypotheses to be tested regarding parapatric speciation along the mountaintops of Central America.

Adelomyrmex

Adelomyrmex is a small, litter-inhabiting ant with many species known to be range-restricted, mountain-top endemics for whom climate change poses a clear threat (Colwell et al. 2008, Longino 2012b). In a recent revision, Longino (2012b) concluded that there were numerous isolated mountain-top species. Preliminary results from DNA barcoding specimens collected from across Central America (Leaf Litter Arthropods of Meso-America (LLAMA) <http://llama.evergreen.edu/>) suggested there were deep genetic divisions between geographically separate populations of what had been morphologically considered to be a single species. Such results could indicate a lineage with extremely low capacity for dispersal and therefore gene flow (though these mountaintops were better connected as recently as 20 000 yr ago). However, within the ACG, there are apparently five individual species of Adelomyrmex co-occuring at a single, high-elevation cloud forest (Volcan Cacao 1500 m). The sympatric occurrence of such deep divergences suggests that the genetic diversity observed in the LLAMA collections may not only be due to a pattern of isolation-by-distance within a single species; but in some cases corresponds to valid (if identified) species boundaries. However, the high degree of sympatric diversity evident within the ACG strengthens the conclusions of the regional analysis; Adelomyrmex species are particularly vulnerable to mountain-top extinction from a changing climate (Colwell et al. 2008) due to such fine-scale microsite segregation. Indeed, the species of Adelomyrmex and Stenamma observed in our collections are strongly associated with the significant phylogenetic clustering we observed at high elevations in the ACG (Fig. 3, 4E) and thus our analyses add a phylogenetic perspective to this vulnerability - if the species of Adelomyrmex and Stenamma we have documented in the ACG cloud forest become locally extinct – the phylogenetic community structure of this forest will likely become more dispersed (not only through the addition of newly arriving species, but through the extinction of resident species specialised to that environment). Mountain-top extinction threatens not only the diversity of these skyislands, but also the evolutionary history resident there.

Tatuidris

The Neotropical genus *Tatuidris* was recently revised in a study that included COI DNA barcodes from the ACG (Donoso 2012). Donoso reported deep variability within this single species and 37% of this surprising variation was explained by geographic separation. He concluded that this was most parsimoniously explained as *T. tatusia* being an allopatrically distributed single species that is undergoing isolation by distance (Donoso 2012).

Azteca

Pringle et al. (2012) surveyed Azteca across the Neotropics and their study included samples taken from within the ACG. Samples from one species, Azteca pittieri, displayed evident and significant isolation by distance (IBD) across the Neotropics (Supplementary material Appendix 3, Fig. 4). However, this taxonomic name also contained four evident genetic clusters within 1-200 km of each other (the ACG population contains two synonymous base pair differences that are sympatric). Is A. pittieri another case, as in Tatuidris, where there is evident intraspecific mitochondrial divergence distributed along a geographic cline or a case such as in Adelomyrmex where evident mitochondrial variants exist in sympatry. Alternatively - does the truth lie somewhere in the middle - where the current name contains both inter- and intra-specific variation. Longino (1996, 2007), considered the A. pittieri complex as a single taxonomic unit, but called attention to the potential for the existence of cryptic species within its range (Mexico - Panama) particularly as there are two morphological size classes of queen.

Future work and predictions

Hölldobler and Wilson (1990) characterized competition as the hallmark of ant ecology. We found that in the cloud

forests of a Neotropical elevational gradient, apparently severe environmental filtering plays a greater role in community composition than does competition. It appears inevitable that ant species from lower elevations will arrive at these cloud forest localities as Neotropical mountaintop cloud forest dries (Colwell et al. 2008). If these arriving species are from communities more defined by a different selective history (that might include competition or r radically different physiological limitations), they will bring an entirely new set of selective pressures to bear on the residents of the cloud forest (including colony size (more numerous), colony defense and competition (more vigorous)). The arriving species will likely also not comprise a broad representation of lower elevation community, but are more likely to be a subset of opportunistic, habitat generalists. Arrivals of this kind are likely to be the change that creates a tipping point for functional collapse. If our predictions are correct, such shifts will not only result in a translocation of lower elevation communities, but in the homogenization of unique and phylogenetically clustered diversity through the quick transmission of highly mobile, 'weedy' species. Documenting these changes requires ongoing field inventories.

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We dedicate this paper to the memory of Canadian zoologist W. W. Judd (1913–2011). Bill Judd was responsible for inspiring generations of Canadian biologists and naturalists and for working towards the conservation of rare habitats in southern Ontario at a time when both actions were rarely rewarded.

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Supplementary material (Appendix ECOG-00631 at </br><www.oikosoffice.lu.se/appendix>). Appendix 1–3.

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