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ORIGINAL ARTICLE

Beta diversity at multiple hierarchical levels: explaining the high diversity of scarab beetles in tropical montane forests

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ABSTRACT

Aim High gamma diversity in tropical montane forests may be ascribed to high geographical turnover of community composition, resulting from population isolation that leads to speciation. We studied the evolutionary processes responsible for diversity and turnover in assemblages of tropical scarab beetles (Scarabaeidae) by assessing DNA sequence variation at multiple hierarchical levels.

Location A 300-km transect across six montane forests (900–1100 m) in Costa Rica.

Methods Assemblages of Scarabaeidae (subfamilies Dynastinae, Rutelinae, Melolonthinae) including 118 morphospecies and > 500 individuals were sequenced for the *cox1* gene to establish species limits with a mixed Yule–coalescent method. A species-level phylogenetic tree was constructed from *cox1* and *rrnL* genes. Total diversity and turnover among assemblages were then assessed at three hierarchical levels: haplotypes, species and higher clades.

Results DNA-based analyses showed high turnover among communities at all hierarchical levels. Turnover was highest at the haplotype level (community similarity 0.02–0.12) and decreased with each step of the hierarchy (species: 0.21–0.46; clades: 0.41–0.43). Both compositional and phylogenetic similarities of communities were geographically structured, but turnover was not correlated with distance among forests. When three major clades were investigated separately, communities of Dynastinae showed consistently higher alpha diversity, larger species ranges and lower turnover than Rutelinae and Melolonthinae.

Main conclusions Scarab communities of montane forests show evidence of evolutionary persistence of communities in relative isolation, presumably tracking suitable habitats elevationally to accommodate climatic changes. Patterns of diversity on all hierarchical levels seem to be determined by restricted dispersal, and differences in Dynastinae could be explained by their greater dispersal ability. Community-wide DNA sequencing across multiple lineages and hierarchical levels reveals the evolutionary processes that led to high beta diversity in tropical montane forests through time.

Keywords
Biogeography, Costa Rica, community structure, diversity turnover, haplotypes, premontane communities, phylogenetic diversity, Scarabaeidae.

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INTRODUCTION

The insects of tropical forests constitute a large portion of global biodiversity (Prance, 1982; Wolda, 1987), but quantifying species richness and distributions remains problematic. A critical parameter for assessing the magnitude of tropical insect diversity is the degree and spatial scale of turnover among local species assemblages (beta diversity). To date, studies addressing this issue have come to contradictory conclusions. Whereas low turnover has been observed in lowland forests (Novotny *et al.*, 2007), studies in tropical highlands have revealed high species turnover (see e.g. Novotny & Missa, 2000; Axmacher *et al.*, 2004; Ødegaard, 2006; Hulcr *et al.*, 2008; Kumar *et al.*, 2009; García-López *et al.*, 2010, 2012). Direct comparisons also suggest higher diversity in montane than lowland forests (Rodríguez-Castañeda *et al.*, 2010). These results indicate the important contribution of highlands to the total number of insect species in tropical forests. However, studies of montane species richness to date have been conducted across a range of elevations or vegetation types, making it difficult to discern whether the high turnover is primarily due to habitat heterogeneity or whether it is a feature of highland forests per se.

In an attempt to avoid the confounding issue of elevational turnover and environmental variability, García-López *et al.* (2010) studied assemblages of Scarabaeidae by selecting sites with similar environmental conditions in montane forests of Costa Rica (see below). Despite the presumably low habitat heterogeneity and short geographical distances between study sites (maximum distance 300 km), only *c.* 50% of species were shared among any of these sites, suggesting that habitat differentiation alone does not explain the spatial turnover in these communities, but instead the dissimilarity may be due to physical isolation and the resulting limited exchange among sites. Moreover, the study revealed that compositional differentiation was not related to geographical distance among sites, contrary to the widely observed distance decay of community similarity expected if differentiation is mainly a function of dispersal between sampling sites (Nekola & White, 1999). This leaves the possibility that in these mountains the evolutionary diversification of lineages locally and the accumulation of diversity *in situ* is the cause for the observed turnover.

It has been postulated that communities in tropical mountain habitats are relatively stable because climatic cycles can be partly compensated for by short-range movements up or down mountain slopes (Darlington, 1970). Persistence of isolated population where dispersion is not required eventually leads to an increase of community differentiation among mountains (Colwell *et al.*, 2008), which increases the degree of beta diversity (Darlington, 1970; MacVean & Schuster, 1981). The effect could be enhanced in the tropics, as tropical communities appear to be more sensitive to climate variability compared with temperate zones and hence the temperature differences produce stronger barriers (Janzen, 1967; Sheldon *et al.*, 2011; Sunday *et al.*, 2011). Diversification therefore may be promoted by narrow climatic tolerances of species, which increase the chance for allopatric isolation, speciation and differentiation of communities in climatically stratified tropical montane regions (Cadena *et al.*, 2011).

How important are these processes for differentiation of Costa Rican mountain forest communities? Whole-community DNA sequencing provides a powerful approach to disentangling the complexity of evolutionary and contemporary factors affecting species distributions (Craft *et al.*, 2010; Papadopoulou *et al.*, 2011; Baselga *et al.*, 2013). By studying within-species variation, as well as higher-level phylogenetic relationships of species in local communities, the approach considers simultaneously the degree of connectivity among sites, the effect of speciation and extinction locally and regionally, and the phylogenetic history of clade diversification. If dispersion is limited, mutation and speciation will add local variants initially at the terminal level of phylogenetic trees, which increases local population differentiation due to drift and lineage extinction. If dispersion is suppressed over longer periods, turnover gradually affects deeper branches as well (Graham & Fine, 2008; Pavoine & Bonsall, 2011). In addition, differentiation and turnover of communities may be driven by ecological processes, such as habitat filtering and species interactions, which leave characteristic signatures on the phylogenetic community structure (Webb *et al.*, 2002; Múrria *et al.*, 2012). A key question then is whether the patterns of turnover are consistent across multiple hierarchical levels, which would indicate the longer-term stability and isolation of local montane forest communities and the constancy of evolutionary processes over time.

Here, we extend our studies of Costa Rican montane scarab communities (García-López *et al.*, 2010) to investigate whether the patterns of diversity and turnover indicate the persistence of communities in isolation, which may indicate the historical stability of populations. We analysed community structure at three hierarchical levels (haplotype, species and clades) using DNA sequences of multiple representatives of all species sampled at each study site. Analyses of turnover were also performed separately for different phylogenetic lineages present in the resulting phylogenetic tree, representing the subfamilies Dynastinae, Melolonthinae and Rutelinae, in order to test for clade-specific differences in diversity patterns. Finally, we assessed the phylogenetic composition of local communities (Webb *et al.*, 2002) with respect to the wider regional pool of all sampled species. By studying a large number of lineages and bringing together various hierarchical levels to bear on the question of evolutionary community assembly, we expected to elucidate the causes and dynamics of community differentiation and to provide a framework for explaining high species richness and local endemism in montane tropical forests.

MATERIALS AND METHODS

Study group

The family Scarabaeidae, and each of the subfamilies Dynastinae, Melolonthinae and Rutelinae (*sensu* Janssens, 1949), constitute a useful target group (*sensu* Moreno *et al.*, 2007) for evaluating biodiversity in tropical forests (Morón-Ríos & Morón, 2001; Morón-Ríos *et al.*, 2003), as they facilitate key functions of wood decomposition and nutrient recycling through their saproxylic and phytophagous habits (Ritcher, 1958). Despite their high diversity in Neotropical areas, Central American species are well known taxonomically because of their functional importance and their striking morphology (Kohlmann & Morón-Ríos, 2003; Ratcliffe, 2003; Ratcliffe & Cave, 2006). Adults of most genera of Dynastinae, Melolonthinae and Rutelinae feed on the foliage of trees and shrubs but some feed mainly on flowers. Larvae of Melolonthinae are phytophagous (subterranean feeders on roots), whereas Rutelinae and Dynastinae contain saproxylic species and also phytophagous species (Ritcher, 1966).

Study area and sampling methods

Specimens were taken from the earlier study of García-López *et al.* (2010), which was based on detailed collecting efforts at six primary forest sites along a north-west/south-east transect of 300 km in three mountain ranges (Guanacaste, Central and Talamanca) in Costa Rica (García-López *et al.*, 2010; see Appendix S1 in Supporting Information). These sites were situated in an elevational strip varying from 900 to 1100 m along the Atlantic slope and in the same life zone, the premontane rain forest of Holdridge (1947), sharing similar environmental characteristics. Eight field surveys were conducted using ultraviolet light traps each month on three consecutive nights (see García-López *et al.*, 2010). This initial sampling included 3758 specimens assigned to 118 species based on morphology (see García-López *et al.*, 2010).

DNA isolation and sequencing

A subset of 519 specimens was selected for DNA sequencing of the mitochondrial cytochrome *c* oxidase subunit I gene (*cox1*), including one representative of every species found at each of the sampled localities. Out of these, 152 specimens were also sequenced for the 16S rRNA gene (*rrnL*) to represent each of the coalescent groups (below).

DNA was extracted from leg tissue using the Promega 96-well plate kit. An 850-bp fragment of *cox1* was amplified using standard oligonucleotide primers C1-J-2183 (Jerry) and TL2-N-3014 (Pat) (Simon *et al.*, 1994) or Jerry and SPatR (Timmermans *et al.*, 2010). A 500-bp portion of *rrnL* was amplified using primers 16Sar (Palumbi, 1996) and 16SB2 (Xiong & Kocher, 1991). Amplification products were purified using Millipore Multiscreen 96-well plates (Millipore, Billerica, MA, USA) and sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified by ethanol precipitation and run on an ABI PRISM3700 DNA Analyzer (Applied Biosystems). Sequence chromatograms were assembled and edited using the SEQUENCHER 4.6 software (Gene Codes Corp., Ann Arbor, MI USA). New sequences have been submitted to GenBank under accession numbers JX963137–JX963398 (*cox1*) and JX982860–JX982947 (*rrnL*).

Estimate of species-level entities

The generalized mixed Yule–coalescent (GMYC) model (Pons *et al.*, 2006) was used to delimit species-level entities from the *cox1* haplotypes (henceforth 'GMYC groups'). This model tests for a change in branching rates at the species boundary to classify the observed waiting times between two diversification events in the tree as either interspecific (Yule) or intraspecific (coalescent) processes, to delimit independently evolving mtDNA clusters. This analysis was performed on a maximum likelihood tree obtained from all unique *cox1* sequences with RAxML 7.0.4 (Stamatakis, 2006) under a GTR+Γ substitution model. The resulting topology was made ultrametric using penalized likelihood as implemented in R8S 1.7 (Sanderson, 2003). The GMYC analysis was conducted using the R package SPLITS (http://r-forge.r-project.org/projects/splits/) with the 'single threshold' option.

Phylogenetic analysis

Relationships among the GMYC groups were established from the combined *cox1* and *rrnL* sequences, selecting one representative for each group. The latter sequences were aligned using the online version of MAFFT 5.8 under default parameters (Katoh *et al.*, 2005). The combined matrix was used for building a tree of the sampled species using Bayesian inference implemented in MRBAYES 3.1 using the software default options (Ronquist & Huelsenbeck, 2003). The most appropriate evolutionary models were SYM+I for *cox1* and HKY+I+G for *rrnL*, as selected with JMODELTEST 0.1.1 (Posada, 2008). Two independent Markov chain Monte Carlo (MCMC) runs of 10 million generations each were performed, using default parameters for the MCMC and sampling every 1000 generations. Standard convergence diagnostics (the standard deviation of split frequencies and the potential scale reduction factor, as implemented in MRBAYES) were checked to ensure that the Markov chain had reached stationarity. Posterior probabilities were calculated after discarding the first two million generations (20% of the sampled trees) as burn-in. All trees (post burn-in) were summarized using an 'all-compatible' consensus.

Beta diversity analyses

The Sørensen index of similarity for each site pair was used as an inverse measure of beta diversity across forest sites at both GMYC and haplotype levels. These values were used to examine the relationship between community similarity and geographical distances among forest sites by fitting exponential decay curves using non-linear regression (Nekola & White, 1999). In the same way, the PhyloSor index was used to analyse phylobeta diversity across the study area and its relationship with distance. Phylobeta diversity (Graham & Fine, 2008) is a measure of compositional change among communities taking into account phylogenetic distances (branch lengths) of the participating species. The PhyloSor index is a measure of the phylogenetic diversity (PD) between two communities based on the shared fraction of branch-length connecting taxa in these communities (Bryant *et al.*, 2008). PD was measured for each sampled community by calculating the sum of the branch length in the tree that connects all species in that community and the root (Faith, 1992). PD was also used to estimate the correlation with species richness.

In order to explore the significance of the observed patterns of diversity turnover, differences between the observed and the expected values of beta and phylobeta diversity were evaluated through a Kruskal–Wallis test and Bonferroni *post hoc* test using STATISTICA (http://www.statsoft.com/). The random expectations were calculated from 1000 random assemblages constructed using the 'independent swap' algorithm that randomizes species co-occurrence but keeps constant the sample richness per community and frequency of occurrence of each species across all communities (Gotelli & Entsminger, 2003). These analyses were carried out using the R packages PICANTE (Kembel *et al.*, 2010) and VEGAN (Oksanen *et al.*, 2011).

Community phylogenetic structure

We assessed communities for phylogenetic overdispersion (co-occurring species are more distantly related than expected by chance) or phylogenetic clustering (co-occurring species are more closely related than expected) relative to the combined analysis tree (Webb *et al.*, 2002; Cavender-Bares *et al.*, 2004). Phylogenetic relatedness of co-occurring taxa in local assemblages was quantified using the mean pairwise phylogenetic distance (MPD) and mean nearest taxon distance (MNTD) (Webb *et al.*, 2002). The observed MPD and MNTD were compared to the distribution of phylogenetic distances in 1000 randomized communities with the 'independent swap' algorithm (Gotelli & Entsminger, 2003) to generate null expectations of phylogenetic relationship (Webb *et al.*, 2002). The net relatedness index (NRI) and nearest taxon index (NTI) for each community was then calculated according to the method in Webb *et al.* (2002). Values of NRI or NTI > 0 indicate phylogenetic clustering and values < 0 indicate phylogenetic overdispersion.

RESULTS

DNA-based species delineation

DNA sequences for *cox1* were obtained for a representative sample of the 118 morphologically defined species and their geographical ranges. Of 519 specimens for which DNA extraction was attempted, 403 (78%) individuals representing 93 morphological species were sequenced successfully and produced 263 distinct haplotypes. GMYC analysis grouped these haplotypes into 113 separate entities. In 16 cases, a given morphospecies (García-López *et al.*, 2010) was split into additional GMYC groups, but no case of fusion of two morphospecies into a single GMYC group or any other type of incongruence was observed (Appendix S2). Notably, in the dynastine genera *Cyclocephala* and *Phyllophaga* we found seven and five species, respectively, which each exhibited two or three GMYC groups, indicating that these species-rich genera may include many more species that have yet to be recognized (Appendix S2). With a few exceptions, these alternate GMYC groups were allopatric. Hence, the number of GMYC groups per forest site closely matched the number of morphological species. However, due to PCR failures and lack of available specimens, between 11 and 17 morphospecies (26.5–39%) at a given site were not included in the DNA-based analysis (Table 1), although the proportion of coverage was high when pooling all sites (only five morphospecies with neither *cox1* nor *rrnL* sequences; Table 1). The three subfamilies Melolonthinae, Rutelinae and Dynastinae included 27, 32 and 54 GMYC groups, respectively (Table 1).

The phylogenetic tree incorporated 134 sequences of *rrnL* representing 85 morphospecies, and together with the *cox1* sequences the combined matrix included representatives of 106 morphological species and 126 GMYC groups (Table 1). The phylogenetic tree showed generally good agreement with the subfamily and genus level classification, although both the Melolonthinae and Rutelinae were paraphyletic (Fig. 1). The paraphyly of subfamilies was not unexpected based on recent findings from a wider taxon sampling of Scarabaeidae (Ahrens *et al.*, 2011). However, three highly supported clades included the great majority of species of each subfamily. These three clades correspond to the genera *Phyllophaga* (Melolonthinae) (Clade 1, 24 GMYC groups); *Anomala* and *Callistethus* (Rutelinae) (Clade 2, 24 GMYC groups); and *Cyclocephala* and seven smaller genera (Dynastinae) (Clade 3, 37 GMYC groups) (Fig. 1). These clades were selected to carry out analyses of clade-specific differences.

Community composition and beta diversity patterns

Species richness at the six sites varied between 22 and 49 GMYC groups, of which between 4 and 22 groups were unique to a given site (Fig. 1, Table 1). The geographical ranges of GMYC groups with regard to the six sampling sites (Fig. 1) followed roughly the expectation of a 'hollow curve'

Table 1 Species and haplotype richness of scarab communities (subfamilies Melolonthinae, Rutelinae and Dynastinae) in six localities in Costa Rica.

Site	Morphospecies (García-López *et al.*, 2010)	Haplotypes	GMYC (*cox1*)	GMYC (*cox1*) + *rrnL**	Morphospecies without sequence	GMYC in subfamilies (Mel./Rut./Dyn.)	Exclusive GMYC (*cox1*)+*rrnL** (%)	Exclusive haplotypes (*cox1*+*rrnL*) (%)
Orosilito	31	29	21	22	11	3, 3, 16	4 (18)	19 (65)
Heliconias	62	64	43	47	15	8, 16, 23	13 (28)	45 (70)
Isla Bonita	50	58	38	39	13	8, 9, 22	11 (28)	43 (74)
Montura	38	39	28	28	11	5, 7, 16	5 (18)	26 (67)
Copal	61	77	45	49	13	10, 17, 22	13 (26)	61 (79)
Bitárkara	62	61	46	49	17	11, 12, 26	22 (45)	48 (79)
Total in all the sampled forests	118	263	113	126	5	27, 32, 54	68 (54)	242 (88)

*Number of generalized mixed Yule–coalescent (GMYC) groups based on *cox1* haplotypes together with species from *rrnL* sequences.

distribution (Preston, 1948) of many narrowly and much fewer widespread species (Table 2, Fig. 1). About half of all species (68 GMYC groups) were recorded from a single site only, while the widespread species were mainly limited to the Dynastinae (Table 2). Where species occurred in multiple sites, they had a tendency to be present at adjacent localities (Fig. 1). For example, in 13 out of 31 species present at two sites, these sites were immediately adjacent. At the haplotype level, compositional similarity was very low, as between 65% and 79% of haplotypes were exclusive to one site (Table 1).

In accordance with the range sizes, the number of GMYC groups per site was small for Clade 1 and Clade 2, ranging between 3 and 10 groups per site, while the number of groups in Clade 3 varied between 13 and 20 (Table 3). The patterns of PD at the six sites closely mirrored the species richness, whether all species were considered together (Spearman's rank correlation $r = 0.99$, $P < 0.05$), or each of the three clades separately (Clade 1: $r = 0.85$, $P < 0.05$; Clade 2: $r = 0.93$, $P < 0.05$; Clade 3: $r = 0.94$, $P < 0.05$) (Table 3). The PD at each site was always higher in Clade 3 than in the other two clades, due to a greater number of species and the higher level of mean divergence among species that was evident from the longer branches in the tree (Fig. 1).

Pairwise compositional community similarity was generally low both when assessed for the whole community and for the three clades individually (Fig. 2a). Community similarity at the species level (Sørensen index for GMYC groups) ranged from 0.21 to 0.46 for the whole communities (mean: 0.38, SD: 0.07), and was 0.45 between the most closely adjacent sites (< 25 km apart). Values in Clade 3 ranged from 0.36 to 0.69 (mean: 0.54, SD: 0.08), and were considerably higher than values for Clade 1 (range 0–0.46; mean: 0.2, SD: 0.13) and Clade 2 (range 0–0.57; mean: 0.22, SD: 0.21). At the haplotype level, despite the low level of haplotype sharing (range 0.06–0.16; mean: 0.09, SD: 0.03), the patterns of turnover (Sørensen index for haplotypes) mirrored the findings at the GMYC level, showing similarity values higher for Clade 3 (range 0.04–0.23; mean: 0.13, SD: 0.05) than for Clade 1 (range 0–0.22; mean: 0.05, SD: 0.08) and Clade 2 (range 0–0.13; mean: 0.02, SD: 0.05). Compositional similarity of communities at the species (GMYC groups) and haplotype levels was not correlated with geographical distance (GMYC groups: $R^2 = 0.23$, $P > 0.05$; haplotypes: $R^2 = 0.08$, $P > 0.05$) for the entire community and even lower for separate clades (Fig. 2a).

Pairwise phylogenetic similarity (PhyloSor values) showed values that ranged from 0.41 to 0.43 for the whole set, and a remarkably narrow range of variation between pairs (mean: 0.42, SD: 0.01). Phylogenetic similarity was generally higher when the three clades were considered separately and the variance was higher than for the analysis of the entire community. Again, Clade 3 showed higher values (range 0.69–0.84; mean: 0.77, SD: 0.05) than Clade 1 (range 0.41–0.77; mean: 0.59, SD: 0.12) and Clade 2 (range 0.53–0.76; mean: 0.61, SD: 0.07) (Fig. 2b). Neither lineage showed geographical distance decay of similarity at the phylogenetic level ($P > 0.05$ in all cases).

The generally low level of structure by geographical distance in patterns of assemblage similarity was confirmed by the absence of significant deviation from the values expected

Figure 1 Phylogenetic tree showing the scarab species distribution in six sampling sites in Costa Rica. Numbers indicate the three clades used for the analyses. M: Melolonthinae; R: Rutelinae; D: Dynastinae. *Ambly*: *Amblyoproctus*; *An*: *Anomala*; *Anc*: *Ancognatha*; *Asp*: *Aspidolea*; *Call*: *Callistethus*; *Chlor*: *Chlorota*; *Chry*: *Chrysina*; *Coe*: *Coelosis*; *Cy*: *Cyclocephala*; *Dys*: *Dyscinetus*; *Enema*: *Enema*; *Hemiphil*: *Hemiphileurus*; *Heterog*: *Heterogomphus*; *Homophil*: *Homophileurus*; *Iso*: *Isonychus*; *Megac*: *Megaceras*; *Parapuc*: *Parapucaya*; *Pel*: *Pelidnota*; *Pha*: *Phalangogonia*; *Phil*: *Phileurus*; *Phy*: *Phyllophaga*; *Platy*: *Platycoellia*; *Puc*: *Pucaya*; *Spod*: *Spodochlamys*; *Spodi*: *Spodistes;* *Sten*: *Stenocrates*; *Strig*: *Strigoderma*; *Tom*: *Tomarus*. Complete references of the name and author of the species are listed in the Appendix S2. The scale bar of genetic distance indicates the estimated changes per nucleotide.

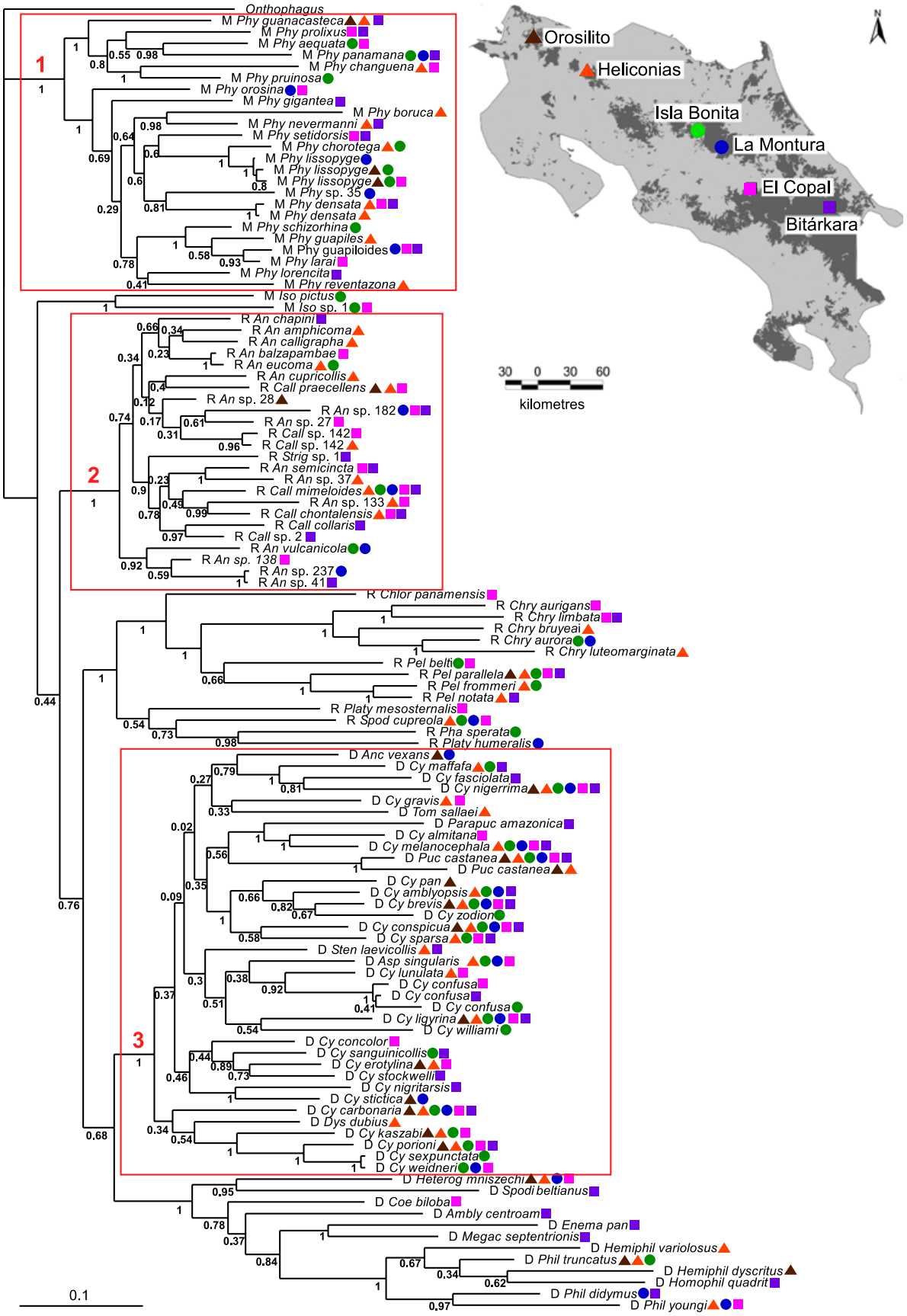

Table 2 Distributional ranges of scarab communities (subfamilies Melolonthinae, Rutelinae and Dynastinae) measured as the number of generalized mixed Yule–coalescent (GMYC) groups of each clade present at one to six forest sites in Costa Rica (n = 113).

	No. of forest sites					
	1	2	3	4	5	6
Clade 1 (Melolonthinae)	11	8	5	0	0	0
Clade 2 (Rutelinae)	16	4	3	0	1	0
Clade 3 (Dynastinae)	15	7	3	4	2	6
Total assemblage	58	26	13	6	4	6

from randomized sampling of the pool ($P > 0.05$; Kruskal–Wallis test on communities resampled from the total pool encountered at all sites). Only in a single case (Clade 2 in Orosilito) was beta diversity lower than expected from a random draw from the total pool ($P < 0.05$) (Appendix S2).

Phylogenetic structure of communities

The phylogenetic tree from the combined *cox1* and *rrnL* gene sequences was used to assess how communities are structured evolutionarily. The results of the analyses of the phylogenetic structure of forest communities varied depending on the specific test metric (NRI or NTI) and the site under investigation (Table 3), and no clear pattern of clustering or overdispersion was evident across the whole data set. However, in all cases, the indices for individual assemblages were significantly different from the null prediction ($P < 0.05$). When split for the three clades the signal of phylogenetic overdispersion and clustering at a site frequently deviated among the clades. Only Orosilito, being the least species-rich community with the fewest exclusive species, was characterized by phylogenetic clustering (Table 3).

DISCUSSION

Montane tropical forests are known to hold great species richness, but the evolutionary processes generating this diversity remain unclear. Our community-level DNA analysis provided a new approach to study this phenomenon, by subjecting a representative sample of specimens from a thorough collecting campaign to a multi-hierarchical analysis of beta diversity. We obtained a measure of population divergence for numerous co-distributed species, which established patterns of genetic diversity for various lineages. In addition, the sequence data were grouped into species-level entities to conduct analyses of community ecology, generating measures of richness and turnover at the species and phylogenetic levels. The study therefore applies the multi-hierarchical analysis of communities (Craft *et al.*, 2010; Papadopoulou *et al.*, 2011; Baselga *et al.*, 2013) to complex tropical lineages and provides new ways for studying these megadiverse ecosystems. We were particularly interested in testing existing biogeographical hypotheses about the community stability and isolation of tropical montane forest areas that may contribute to the current scenario of diversity turnover in these areas.

A key finding of the DNA-based analysis was that patterns at the species and haplotype levels mirror each other, both with regard to total diversity and turnover among communities. Intraspecific haplotype variation was highly structured geographically, with 87% of haplotypes present only at one site, compared with 54% of GMYC (species) groups (Table 1), and hence turnover among sites was much greater at the haplotype level. The correlation of haplotype and species level was most clearly evident when separating the study into the three subclades, which revealed that species and haplotypes were more widespread and turnover among sites was lower in Dynastinae than in the Rutelinae and Melolonthinae (Fig. 2a). Moving up the hierarchy, these differences in degree of turnover between the subfamilies were also evident at the clade level (phylobeta diversity), showing greater similarity among communities in Dynastinae (Fig. 2b). Only at the level of the entire assemblage, phylobeta diversity among sites was uniform in pairwise comparisons of local sites (Fig. 2b), as the major clades are widely distributed.

The correlation of species and genetic diversity within communities has been recognized previously, and may be attributable to processes (e.g. dispersal limitation) that determine simultaneously both the species richness in a given area and the genetic variation within populations (Vellend, 2005).

Table 3 Diversity measures at each sampling site in Costa Rica for the three main scarab clades (n = 113). In all cases indices were significantly different from the null prediction ($P < 0.05$).

	Clade 1				Clade 2				Clade 3				Total assemblage			
Site	GMYC	PD	NTI	NRI	GMYC	PD	NTI	NRI	GMYC	PD	NTI	NRI	GMYC	PD	NTI	NRI
Orosilito	3	0.44	2.09	1.05	2	0.47	1.73	1.49	13	1.92	1.21	0.12	22	3.75	1.52	0.2
Heliconias	9	1.25	−1.9	−1.65	10	1.07	0.7	0.54	20	2.5	−2.02	1.41	49	6.96	−1.46	−1.51
Isla Bonita	7	1.09	1	0.32	3	0.63	−0.75	−0.67	19	2.32	0.49	−1.39	38	5.65	0.82	−0.14
Montura	5	1.09	−1.15	−1.09	4	0.53	−2.18	−2.18	12	1.94	0.52	−0.69	26	5.04	−1.26	−0.18
Copal	9	1.35	−0.26	0.59	10	1.11	−0.4	0.21	18	2.21	−0.42	0.47	48	6.52	−0.13	1.96
Bitárkara	9	1.25	−0.38	0.32	9	1.01	−1.2	−0.38	18	2.62	−0.48	−0.5	44	6.95	−1.03	−0.24

GMYC, generalized mixed Yule–coalescent model; PD, phylogenetic diversity; NTI, nearest taxon index; NRI, net relatedness index.

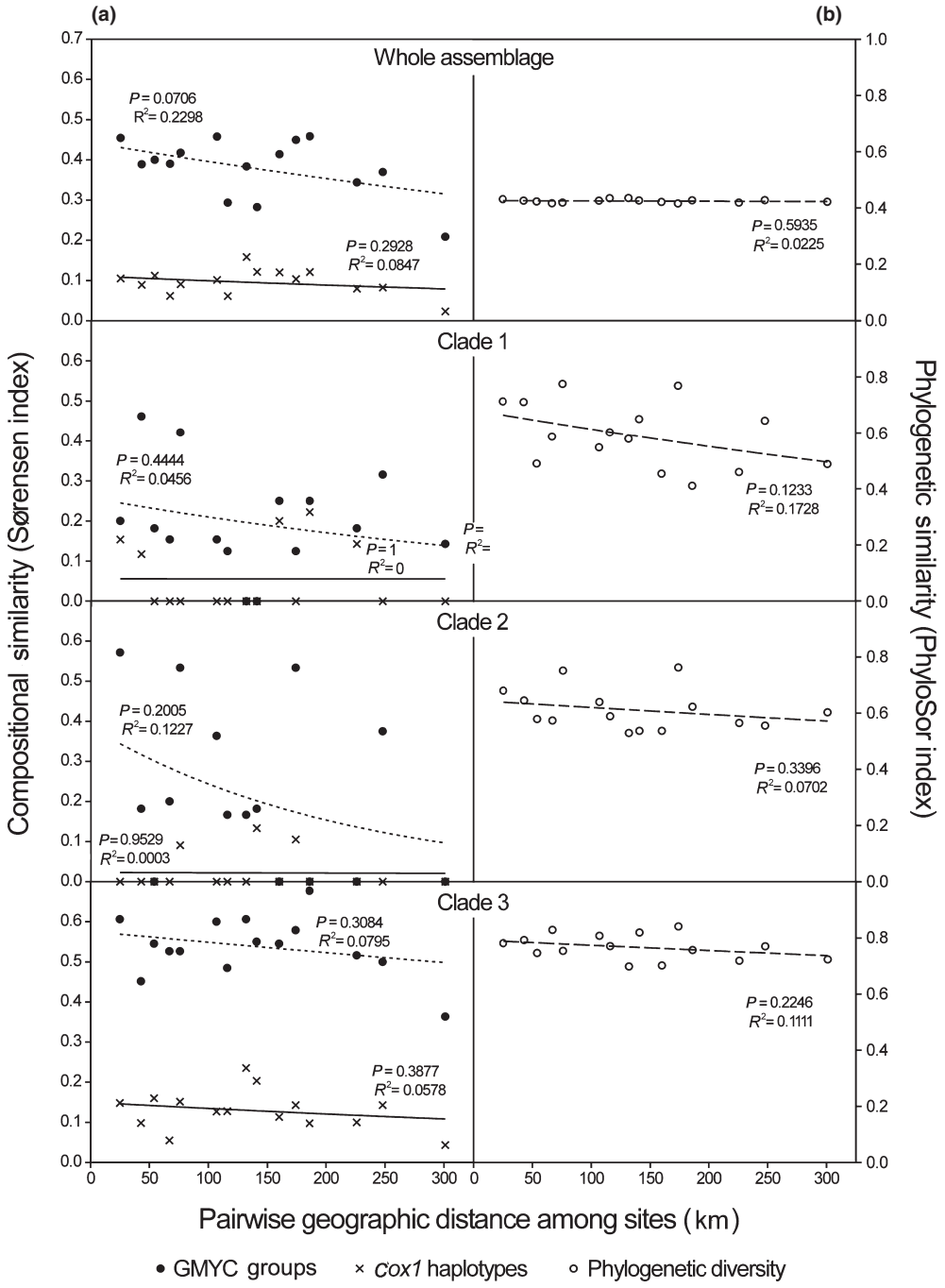

Figure 2 (a) Distance decay of compositional similarity (Sørensen index) of scarab communities at six sites in Costa Rica (n = 113) based on generalized mixed Yule–coalescent (GMYC) groups and *cox1* haplotypes as a function of geographical distance for total assemblage and the selected clades. (b) Distance decay of phylogenetic similarity (PhyloSor index) (n = 113) for total assemblage and the selected clades. All distance decays were fitted to an exponential curve.

This may also lead to a correlation of beta diversity at these hierarchical levels (haplotypes and species), as already described in communities of darkling beetles in the Aegean islands (Papadopoulou *et al.*, 2011) and water beetles across Europe (Baselga *et al.*, 2013). These findings suggest that the same process is responsible for patterns on both hierarchical levels, specifically that it is the degree of dispersal that determines the range size of a species as well as the gene flow within the species' range (and hence the turnover among sites). The pattern of turnover also extends to the clade level, which concludes a continuum of extremely high differentiation of assemblages at the tips (populations), through intermediate degrees of differentiation at the species and subclade levels, to a largely uniform distribution at deep levels, as the presumed effect of individual dispersal gradually becomes unimportant for the distribution of deep lineages. The steady

decrease of turnover from the haplotype level to species and clade levels is consistent with prolonged isolation of assemblages, which initially leads to differentiation at the population level through drift and ultimately the formation of new species. Local extinctions and 'ecological drift' (Hubbell, 2001) may then lead to a pattern of local presence and absence of higher-level clades.

If dispersal is implicated in community divergence, we can expect that lineage-specific differences in the size of geographical ranges and turnover between Dynastinae and Rutelinae/Melolonthinae differ consistently in the rate of movement between sites. Dispersal propensity of lineages may depend on habitat associations, including the stability of habitats (Papadopoulou *et al.*, 2011; Ribera & Vogler, 2000) or dispersal-related functional traits (Ikeda *et al.*, 2012), which may differ between these groups. In the case of the study group, the different trophic habits presented by the studied species could be associated with differences in their dispersion capability. However, feeding preferences are not uniform in these lineages and, moreover, none of the required resources (roots and decaying wood) are scarce in the studied forests. Therefore, differences in ecological associations are unlikely to be the primary reason for the differences in patterns. Alternatively, species of Dynastinae may have greater climatic tolerances that result in fewer constraints to dispersal in the climatically structured mountain landscape, which is supported by their greater elevational ranges compared to those in the two other subfamilies (García-López *et al.*, 2012).

As an alternative to dispersal-based processes, community assembly may be driven by species traits and interspecific competition. These factors are believed to leave a signature in the phylogenetic structure of local assemblages that are widely assessed with the NRI and NTI metrics (Webb *et al.*, 2002). In the analysis of NRI and NTI, in all cases the values revealed significant differences from null expectations of random phylogenetic composition (Fig. 1, Table 3). Yet, the signals differ based on the specific metric used and on the clade and site analysed (Table 3), and therefore the findings may simply indicate a slight deviation from randomly drawn assemblages due to stochastic effects (Emerson & Gillespie, 2008; Graham & Fine, 2008; Cavender-Bares *et al.*, 2009), or due to the mix of biogeographical and palaeoclimatic lineage histories represented by these large communities. Another possibility is that the phylogenetic structure could be obscured by the absence in the phylogeny of potential sister groups present in other areas that were not included in the current study. Hence, a more inclusive study of species present elsewhere in the region could be the next step towards understanding the biogeographical history of these groups in the mountains of Costa Rica.

Observed species distributions are consistent with a vicariance scenario from prolonged isolation among sites. The isolation of mountain habitats has already been identified as a cause of increased species richness in other taxa of the Mesoamerican mountains (Micó *et al.*, 2006) and elsewhere (Ribas *et al.*, 2007). Mountains are known to provide refuges for species under climate change because they allow species to track their environment up and down over short distances (Jansson & Dynesius, 2002; Colwell *et al.*, 2008; Cadena *et al.*, 2011). Given the well-documented changes in climate during glacial cycles of the Pleistocene in this region (Stuart, 1966; MacVean & Schuster, 1981), which strongly influenced diversity distribution through the creation and closing of dispersal corridors (Jansson & Dynesius, 2002), it may be surprising that these communities show the signature of habitat stability. However, while climate oscillations may change the local conditions greatly, these shifts are slow enough to permit habitat tracking on small spatial scales along elevational gradients (Múrria & Hughes, 2008; Carnaval *et al.*, 2009; Graham *et al.*, 2010). Hence, the expansion and compression of highland forests during climatic cooling (Bush *et al.*, 2004; Flenley, 1998) probably provided uninterrupted dispersal corridors for montane species (MacVean & Schuster, 1981). Subsequent warming in the Holocene caused species to track favourable conditions at higher elevations (Bush *et al.*, 2004; Flenley, 1998). This resulted in newly isolated populations (Colwell *et al.*, 2008), because tropical species affected by warming climates are far more likely to undergo upslope movements than latitudinal shifts (Janzen, 1967; Bush, 2002; Bush & Hooghiemstra, 2005). However, exchange among mountains may continue in species with high dispersal ability or climatic tolerance, such as the Dynastinae in our study.

This dispersal-based scenario could also explain the weak or non-existent relationship between the community similarity and geographical distances among the mountains they occupy (Fig. 2), as expected in a habitat matrix with limited rates of exchange among sites (Nekola & White, 1999). In a mountain setting where climate change opens and closes migration routes, conditions for exchange may vary drastically and distort any regular pattern expected from geography.

While climatic oscillations in high-elevation forest ecosystems can be tracked by most species of an assemblage over short spatial distances, this is different in tropical lowlands where climate variability causes landscape changes on a much larger spatial scale. Consequently, habitat tracking requires long-range dispersal which leads to large ranges of species and greater homogenization of species and haplotype distributions. In this sense, the difference between mountain and lowland tropical forests is analogous to the dichotomy of running (lotic) and standing (lentic) water habitats (Ribera & Vogler, 2000). Standing water bodies are ephemeral and populations associated with these habitats have to undergo regular dispersal that homogenizes the assemblages. This results in much larger ranges of species and individual haplotypes in standing water species. The lotic–lentic divide (and other habitat types that differ in stability; Papadopoulou *et al.*, 2011) can serve as a useful analogy to the processes affecting species ranges and turnover in lowland versus highland tropical forests.

An important finding from our approach of 'DNA profiling' (Monaghan *et al.*, 2009) of assemblages is the diversity of cases, from widespread species showing little genetic differentiation, to allopatric GMYC entities within a single morphologically distinguishable taxon, to allopatric sister lineages and deeply separated lineages exclusive to one site. Studying any one of those species, by conducting population genetics or phylogeographical analysis, would be likely to provide a biased image of the evolutionary history of these mountain ecosystems. Using communities as the unit of observation is in its infancy. The multi-hierarchical approach provides strong support for tropical highlands diversification through climate tracking, but additional taxa need to be studied to test the generality of the postulated processes.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Sampling sites in Costa Rica, from García-López *et al.* (2010).

Appendix S2 List of species present in the phylogenetic tree.

Appendix S3 Variation in observed and expected compositional beta diversity and phylobeta diversity results (Kruskal–Wallis test).

BIOSKETCH

Alejandra García-López conducted this work as part of her PhD at the University of Alicante (Spain). Her interest lies in the study of patterns and processes of species distribution and diversification, with particular emphasis on the group Scarabaeidae in tropical and Mediterranean forests.

Author contributions: E.M., A.G.L. and E.G. conceived the project, A.G.L. collected and identified the specimens, A.G.L., A.P.V. and C.M. conceived the community sequencing approach and analysed the data, A.G.L., E.M., C.M. and A.P.V. discussed the results and led the writing.

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