

# Tropical insect diversity: evidence of greater host specialization in seed-feeding weevils

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**Abstract.** Host specialization has long been hypothesized to explain the extraordinary diversity of phytophagous insects in the tropics. However, addressing this hypothesis has proved challenging because of the risk of over-looking rare interactions, and hence biasing specialization estimations, and the difficulties to separate the diversity component attributable to insect specialization from that related to host diversity. As a result, the host specialization hypothesis lacks empirical support for important phytophagous insect clades. Here, we test the hypothesis in a radiation of seed-feeding insects, acorn weevils (*Curculio* spp.), sampled in temperate and tropical regions (California and Nicaragua, respectively) with an equivalent pool of oak host species. Using DNA sequences from three low-copy genes, we delimited to species level 778 weevil larvae extracted from host seeds and assessed their phylogenetic relationships by Maximum Likelihood and Bayesian inference. We then reconstructed the oak-weevil food webs and examined differences in alpha, beta and gamma diversity using Hill numbers of effective species. We found a higher alpha, beta and gamma diversity of weevils in Nicaragua compared to California despite similar richness of host species at both local and regional level. By means of Bayesian mixed models, we also found that tropical weevil species were highly specialized both in terms of host range and interaction strength, whereas their temperate congeners had a broader taxonomic and phylogenetic host spectrum. Finally, in Nicaraguan species, larval body size was highly correlated with the size of the acorns infested, as would be expected by a greater host specialization, whereas in California this relationship was absent. Altogether, these lines of evidence support the host specialization hypothesis and suggest contrasting eco-evolutionary dynamics in tropical and temperate regions even in absence of differences in host diversity.

**Key words:** *beta diversity; Curculio; insect-plant interactions; latitudinal diversity gradient; phylogenetic MCMCglmm; phytophagous insects; Quercus.*

## INTRODUCTION

The latitudinal gradient of increasing biodiversity from the poles to the tropics is one of the most widely recognized and fascinating patterns of life. Nowadays, there is agreement that this pattern is in part due to an increase in productivity toward the tropics, as a higher productivity may allow more species to maintain viable populations in the tropics (Brown 2014). However, this explanation is insufficient because a productive ecosystem can support either a high diversity composed of a

relatively few individuals or a low diversity composed of many individuals. A high diversity is nonetheless expected in the tropics if resources are more finely partitioned, thus reducing niche overlap and facilitating coexistence among species (Hutchinson 1959, Brown 2014). The reasons why niches should be more finely partitioned in the tropics than in temperate regions remain unclear, although some hypotheses have been proposed. Resource specialization is for instance expected to be more common in tropical regions if their enhanced productivity, more benign climatology, and greater environmental stability increase the importance of biotic interactions over abiotic stress (Dobzhansky 1950, Schemske et al. 2009, Brown 2014). A predominance of biotic interactions should trigger important evolutionary processes generating ecological diversity, including character displacements, adaptive radiations and

Manuscript received 27 March 2017; revised 27 April 2017; accepted 10 May 2017. Corresponding Editor: Lee A. Dyer.

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co-evolutionary dynamics (Schluter 2000), and should also prevent niche shifts by enhancing biotic resistance (Brown 2014). Lower abiotic stress should reduce species' thermal tolerances, making geographic features, such as mountain ranges, stronger barriers to dispersal (Janzen 1967, Ghalambor et al. 2006, Salisbury et al. 2012, García-Robledo et al. 2016). This in turn should favor genetic subdivision of populations conducive to speciation, as well as lead to smaller distribution ranges and higher spatial turnover (Janzen 1967, Ghalambor et al. 2006, Brown 2014). According to this hypothetical model, therefore, ecological specialization driven by a preponderance of biotic interactions in the tropics would be the key mechanism underlying the latitudinal gradient of diversity (Schemske et al. 2009, Salisbury et al. 2012, Brown 2014).

Despite host specialization has long been hypothesized to explain the extraordinary diversity of phytophagous insects in the tropics (Lewinsohn and Roslin 2008), evidence have been controversial so far (Ollerton and Cranmer 2002, Novotny et al. 2006, Dyer et al. 2007, Schleuning et al. 2012). One important reason is that tropical regions tend to be insufficiently sampled, which may lead to over-look rare interactions and hence bias estimates of specialization (Ollerton and Cranmer 2002). Despite the difficulties, a global-wide comparative analysis has recently provided compelling evidence that phytophagous insects generally have narrower diet breadths at lower latitudes (Forister et al. 2015). Diet breadths, however, differ among insect guilds and we still lack information from important groups such as seed-feeding insects, which otherwise would help to generalize those findings (Novotny et al. 2010, Forister et al. 2015).

Evidence for the host specialization hypothesis is not only insufficient, but crucial aspects of the theory require further attention. One is the extent to which the higher specialization in the tropics is mediated by the richness of host species (Novotny et al. 2006, Lewinsohn and Roslin 2008). Tropical regions usually harbor a higher diversity of potential host plants at a regional and local level, and it is often believed that this would allow the niche of the consumer to be more finely partitioned (see Forister et al. 2015, and references therein). Yet if resource specialization is driven by interactions between competing insects, a higher diversity of phytophagous insects in the tropics may still occur even when the diversity of hosts does not differ from that found in temperate regions. Demonstrating that dietary specialization is higher in the tropics regardless of host richness would not only provide strong support for the hypothesis but also would offer a new perspective to understand the latitudinal gradient of biodiversity. Hence, the challenge is to be able to separate the diversity component attributable to host specialization from that related to differences in host diversity (Novotny et al. 2006, Lewinsohn and Roslin 2008). In their comprehensive analysis, Forister et al. (2015) addressed this issue indirectly by means of path analysis; they showed

that a higher host diversity contributes but does not fully explain the higher specialization of insects in tropical regions. Direct evidence is nonetheless lacking.

Here, we investigate the importance of host specialization in the latitudinal diversity gradient through a study of insect-host associations in the striking evolutionary radiation of acorn weevils (*Curculio*, Curculionidae, Coleoptera). Acorn weevil adults are poorly flying insects with specialized long noses used to drill holes in seeds of a variety of trees, primarily of the genus *Quercus* (Gibson 1969, 1977), in order to forage and lay their eggs. Unlike other groups of phytophagous insects, the radiation of their oak hosts (*Quercus* genus, L., 1758) does not peak in the equator but in Mexico, with a maximum of 160–165 species, and gradually decreases poleward (Nixon 2006). This allows conducting tropical-temperate comparisons of insect-plant associations within a single radiation while keeping constant the pool of potential hosts available. While the importance of host specialization in the diversity of seed-feeding insects has been previously highlighted (Janzen 1980), its contribution to the latitudinal gradient of diversity has never been evaluated. Incomplete taxonomic knowledge, particularly for immature life stages, is arguably the main barrier to gathering such data and acorn weevils are not an exception. To date, there is just one monograph describing 27 species for the whole Neotropical region (Gibson 1977), and the most comprehensive phylogeny of the genus lack any of these species (Hughes and Vogler 2004a). Hence, the evolutionary and ecological information available for this group is severely skewed biogeographically. Notwithstanding, DNA sequences from specific genes enables today species-level delimitations without formal morphological descriptions (Pons et al. 2006), thus allowing to fill this gap.

To investigate whether host specialization affects the latitudinal diversity gradient, we exhaustively sampled acorn weevils in two biogeographical regions -one temperate (California) and another tropical (Nicaragua)-which despite their remarkable difference in latitude contain a similar regional pool of oak species (Nixon 2002, 2006). First, we used Generalized Mixed Yule Coalescent models (GMYC) based on molecular information to delimit to species level weevil larvae reared from incubated acorns, thus additionally providing undisputed insect-host associations (Pinzón-Navarro et al. 2010, Muñoz et al. 2014). Once the weevil species from Nicaragua and California were well characterized, we estimated the regional pool of species and confirmed that alpha, beta and gamma diversity was higher in the tropical region (Nicaragua) than in the temperate one (California) region. Therefore, we asked whether the observed patterns could be explained by weevil species exhibiting a higher host specialization in the tropics than in temperate regions irrespective of their phylogenetic relationship. To this purpose, we constructed a molecular phylogeny for all the species sampled in both regions, and used phylogenetic-based methods to compare three

dimensions of host specialization -host range, interaction strength and the specialization index  $d'$  between these tropical and temperate regions. Additionally, by means of phylogenetic-based methods we also assessed mechanisms that may have influenced the evolution of host specialization in these insects. Second, we specifically examined the possibility that specialization might be associated with differences in the relationship between acorn size and weevils' body size. The larvae of *Curculio* complete their development in a single, sometimes multi-infested seed, and the lack of resources can severely restrict their growth (Bonal and Munoz 2008). This constraint may have important fitness consequences because body size at the end of larval development is strongly related with larval survival, adult size and future fecundity (Desouhant et al. 2000, Bonal et al. 2012). It is therefore plausible that much of the interspecific diversification of weevils may have been triggered by body size adaptations to exploit acorns of different size (Hughes and Vogler 2004b, Bonal et al. 2011). If the strength of biotic interactions varies along the latitudinal gradient (Schemske et al. 2009), the expectation is that the correlation between larval size and acorn size should be tighter in the tropics than in temperate regions.

## MATERIAL AND METHODS

### Study group

The species of the genus *Curculio* (Coleoptera, Curculionidae) feed on seeds of various plant families, being Fagaceae and particularly the genus *Quercus* their most common hosts (Gibson 1969, 1977). After mating, weevil females select a host tree and drill a hole through the seed coat with their specialized long rostrum, where they lay their eggs (Bonal and Munoz 2008). Larvae develop

inside the acorns feeding on the endosperm during 2–4 weeks and finally emerge and burrow in the topsoil to pupate, passing by the unfavorable season in diapause.

### Study areas and sampling method

Our study was conducted in the Central Valley of California and Las Segovias region in Nicaragua, where the oak richness is comparable with 20 and 16 species, respectively (Stevens et al. 2001, Nixon 2002). *Quercus* is a large genus divided in sections which represent different evolutionary lineages (Nixon 2002). California and Nicaragua both include representatives of the red oak (sect. *Lobatae*) and white oak (sect. *Quercus*) sections, indicating that the phylogenetic spread of potential hosts is also comparable. We selected 29 and 17 sampling sites in California and Nicaragua, respectively, sampling over an area of more than 58,000 km<sup>2</sup> and thus covering the distribution range of almost all of the oak species present at each region (Fig. 1, Appendix S1: Table S1). The landscape was a mosaic of pasture and oak woodlands circumscribed to an altitudinal belt ranging from 600–1800 m a.s.l. in California and 800–1,700 m in Nicaragua. We sampled during the period in which most of the oak species ripen their acorns: July–August (2007–2009) for Nicaragua and September (2010) for California. We used the same standard sampling method across sites. For 60 minutes at each sampling site, we gently shook branches of available focal trees and then three people actively searched the ground for fallen infested acorns. Infested acorns were easily distinguished by the black dots resulting from the punctures in the seed coat during oviposition. Adult weevils were picked up by hand when found in accessible branches. The oak host species were identified by visual inspection in the field and identifications were further confirmed by collecting plant material to be examined by plant experts from

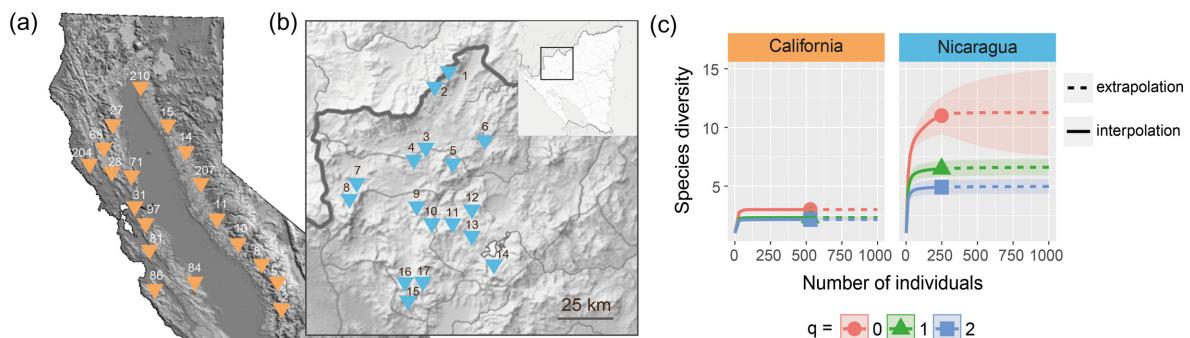


FIG. 1. Distribution of sampling sites in California (a) and Nicaragua (b), and species diversity accumulation curves of acorn weevils (*Curculio* sp.) based on Hill numbers (c). Species richness ( $q = 0$ ), Shannon diversity ( $q = 1$ ) and Simpson diversity ( $q = 2$ ). [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

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#### *Species delimitation and phylogenetic reconstruction*

Infested acorns were placed individually in plastic trays in the lab under constant conditions of light/dark, temperature and humidity. Larval emergence was monitored daily until it ceased and each larva was preserved in 1.5 ml plastic vials filled with 95% ethanol. To obtain a representative subset of all sites and all oak species, we selected weevil larvae coming from all host tree species and all sampling sites by balancing their numbers in proportion to the abundances in the field (see Appendix S1 for sample sizes). Following Gibson (1969), we identified some adult specimens from California as belonging to *Curculio pardus* (Chittenden 1927), *C. occidentis* (L., 1758) and *C. aurivestis* (Chittenden, 1927). Adult specimens from Nicaragua did not match any of the species described in Gibson (1977).

Weevil larvae do not present diagnostic organs or structures, so that taxonomic classification is not possible under a classic morphological framework. To circumvent this hindrance we used a species delimitation procedure based on molecular information. DNA was extracted from a 2 mm caudal portion of each larva (abdomen for adults) following a salt extraction protocol (Aljanabi and Martinez 1997). We amplified the mitochondrial genes cytochrome *c* oxidase subunit I (*cox1*) and cytochrome B (*cytb*) using the primers C1-J-2183 and L2-N-3014 (Loxdale and Lushai 1998), and CB1 and CB2 (Simon et al. 1994), and also a fragment of the nuclear gene elongation factor 1 $\alpha$  (*EF1 $\alpha$* ) using the primers EF1-R and EF1-F. The PCR procedures described in Hughes and Vogler (2004a) were followed for the three genes. Sequencing was done from both strands with the same primers used for the PCR using BigDYE 2.1 and an ABI PRISM 3730 analyzer (Applied Biosystems, Foster, USA). Sequence chromatograms were assembled and edited using SEQUENCHER 4.6 (Gene Codes Corp., Ann Arbor, USA). The sequences were trimmed to 711, 413 and 581 base pairs to reduce the proportion of missing data.

GMYC models were then used to delimit species-level entities based on the *cox1* haplotypes. This model tracks tree branching rates identifying transitions from coalescent (intra-specific) to speciation patterns, thereby delimiting independently evolving lineages or mtDNA clusters (Pons et al. 2006). After identical haplotypes were collapsed, we used all identified species to build a Maximum Likelihood (ML) phylogenetic tree applying a GTR+I+G substitution model with RAxML 7.0.4 (Stamatakis 2006). The phylogeny was made ultrametric with PAUP\* 4.0 (Swofford 2003), using the gall feeding *Curculio pyrrhoceras* as outgroup (Hughes and Vogler 2004a). The GMYC analysis was done with the “single-threshold” option in the R package SPLITS (Pons et al. 2006). The sequences of all Californian larvae matched

with those of the adult species previously identified, so that we were able to assign them their corresponding Linnaean names. For Nicaraguan specimens, however, we had to use preliminary numbers as operative names for each of the species-level entities delimited.

To establish the phylogenetic relationships among weevil species, the sequences (*cox1*, *cytb*, and *EF1 $\alpha$* ) of one individual per GMYC group were pooled with 15 additional temperate *Curculio* species and again with *C. pyrrhoceras* set as an outgroup (Hughes and Vogler 2004a). The three genes were aligned separately with CLUSTAL W (Larkin et al. 2007) and then concatenated. Best-fit models of nucleotide substitution for each gene were assessed by Akaike Information Criterion (AIC) using JMODELTEST 0.1.1 (Posada 2008). The reliability of tree topology and the strength of the nodes were assessed by ML and Bayesian inference. ML analyses were performed with RAxML 7.2.6 (Stamatakis 2006), and PHYML was used to assess the repeatability of the topology and the likelihood-ratio test of branch support (Guindon et al. 2010). Bayesian analyses were performed with MR. BAYES 3.2 (Ronquist et al. 2012) applying two parallel Markov chain runs of two million generations sampling every 1000 steps. Standard convergence diagnostics were checked to ensure that Markov chains reached stationarity. Trees were summarized using the “all-compatible” consensus with 25% burn-in (Appendix S1: Fig. S1).

#### *Diversity and host specialization measures*

To test for differences in local and regional diversity of weevil species between region, we used asymptotic diversity estimates based on Hill numbers of order  $q$ . Species richness ( $q = 0$ ), Shannon diversity ( $q = 1$ , exponential of Shannon entropy) and Simpson diversity ( $q = 2$ , inverse of Simpson concentration, Jost 2006) were calculated with a sample-size-based rarefaction and extrapolation procedure, using the R package “iNEXT” (Hsieh et al. 2016). Kruskal-Wallis tests were then used to assess differences among regions in (1) local species richness and diversity of weevil species, (2) number of oak hosts per sampling site, and (3) the ratio of weevils per host. Variation in the composition of weevil assemblages among sites ( $\beta$  diversity) was assessed by means of Sørensen index, which was partitioned into its species turnover and nestedness components with the R-package “betapart” (Baselga and Orme 2012). This procedure computes 3 multiple-site dissimilarities (species turnover  $\beta_{tu}$ , nestedness-resultant fraction  $\beta_{ne}$  and overall beta diversity,  $\beta_{ser}$ ) and resamples them for a subset of sites (parameters were 100 samples from 10 sites in each region).

To assess differences among regions in host specialization of weevil species, quantitative *Quercus-Curculio* trophic webs were drawn by means of the R-package “bipartite” (Dormann et al. 2008), following the framework described in Novotny et al. (2012). To avoid confounding specialization with rarity, we excluded three Nicaraguan species which were supported by just one or

two observations (Novotny et al. 2012). The rest of interactions were supported by at least 11 trophic interactions recorded in a minimum of six different sampling sites.

We considered three of the many dimensions of insect host-specificity. First, we measured host range as the number of oak hosts from which larvae of a given weevil species had been reared (Forister et al. 2015). Second, we estimated the interaction strength of insect species with their main host (i.e., the proportion of interactions between a weevil species and its main host relative to all interactions recorded, Bascompte et al. 2011). Third, we measured the specialization index  $d'$ , which expresses how specialized a given weevil species is in relation to oak host availability by calculating the extent to which a species deviates from a random sampling of available interacting partners (Blüthgen et al. 2008). Weighted by the overall availability of each host, the index  $d'$  ranges from 0 (no specialization) to 1 (perfect specialization). Additionally, the affiliation of *Quercus* species to *Lobatae* or *Quercus* sections was recorded as a rough measure of phylogenetic specificity to assess whether weevil host ranges were restricted to closely related species of one or alternatively of more than one oak genus sections (Appendix S1: Table S2).

#### *Morphological differences between tropical and temperate weevils*

If there were significant differences in host specialization between the studied regions, we expected the relationship between the size of weevil and the acorns they infested to be stronger in the region where host-specificity was higher. This is because larval performance should not differ among different oak hosts in the more generalist weevil species. To test this prediction, we assessed the relationship between the width of larval head (i.e., a proxy of larval body size and adult fitness, Bonal et al. 2011) and the volume of the acorns infested by each weevil species. After rearing the larvae, the width and length of all acorns were measured with a digital caliper and their volume derived assuming a geometric equivalence with a prolate spheroid. Each acorn was dissected to assess the remaining endosperm in order to discern if larvae had fed ad libitum, i.e., there was endosperm left when they finished their development, or if their growth was restricted by resource availability. Then, for each weevil species and sites, a subset of larvae grown ad libitum was photographed with a digital stereomicroscope (MOTIC DM-143, Xiamen, China) and the width of the head capsule was measured with a coupled imaging software (MOTIC IMAGE PLUS 2.0). We obtained information of the head of 178 larvae and the corresponding volume of the acorns from which they had emerged, 59 from California and 119 from Nicaragua.

#### *Phylogenetic-based comparative methods*

To investigate differences in traits related to host specialization between tropical and temperate weevil

species, we fitted phylogenetic generalized linear mixed models based on Markov chain Monte Carlo (MCMC), as implemented in the R-package “MCMCglmm” (Hadfield 2010). These types of models allow to explore the inclusion of complex covariance structures as random effects in linear models, including those arising from phylogenetic relationships. To increase sample size and phylogenetic breadth, we complemented our own data with published information of host range of eight additional North American *Curculio* species (henceforth referred as temperate or Nearctic species indistinctly) from Gibson (1969). To model variation in host range (number of hosts), interaction strength (the number of interactions between a weevil species and its main host relative to all interactions recorded) and the specialization index  $d'$  (log-transformed), we respectively implemented models with Poisson, Binomial and Gaussian distribution of errors. A general non-informative prior was used in all cases. Convergence diagnostic of chains was checked by means of Heidelberg-Welch tests (to assess that chains came from a stationary distribution) and by ensuring that the autocorrelation for both fixed and random effects was kept below 0.1 (Hadfield 2010). Phylogenetic signal of host range, interaction strength and specialization index was assessed by calculating the phylogenetic heritability of each trait as variance (phylogeny)/[variance (phylogeny) + variance (residual)] (Hadfield and Nakagawa 2010). This index is equivalent to Pagel's  $\lambda$  and ranges from 0 (no phylogenetic signal) to 1 (all the variation observed in a given trait is explained by phylogenetic distance among species).

Additionally, we used phylogenetic reconstructions through character stochastic mapping to visually inspect whether transitions from tropical to temperate regions and vice-versa were accompanied by changes in the phylogenetic breadth of their oak hosts (i.e., high phylogenetic specificity when oak hosts belong to a single *Quercus* genus section vs. low phylogenetic specificity when oak host belong to different *Quercus* sections). Given a phylogeny and discrete character states for extant species, stochastic character mapping applies a Monte Carlo algorithm to sample the posterior probability distribution of ancestral states of transitions on phylogenetic branches under a Markov process of evolution (Huelsenbeck et al. 2003, Lapiedra et al. 2013). Using the complete weevil phylogeny (Appendix S1: Fig. S1), we ran 1,000 simulations for each trait using the package “phytools” (Revell 2012).

We also used Gaussian MCMCglmm to investigate the relationship between the width of larval head and the volume of the acorns infested by each weevil species. Since our level of analysis here is individual larva, two sources of non-independence of the data may arise: (1) auto-correlation within species (if individuals within a species behave in similar ways), and (2) phylogenetic autocorrelation among close-relatives. To deal with this clustered structure we included phylogeny and species identity as random factors in the models (see Sol et al.

2014 for a similar analysis). As our prediction was that a relationship between larval head and acorn volume should only be clearly observed in the tropics, we ran models separately for Nicaragua and California. Singletons and rare species (i.e., those with <3 larvae grown ad libitum) were excluded from this analysis. Finally, differences in larval performance when developed in oak hosts belonging to different *Quercus* genus sections were analyzed, when possible, for each weevil species separately by means of linear models including head width as a response variable and oak genus section as factor. All analyses were performed with R (R Core Team 2015).

## RESULTS

### *Species delimitation and diversity patterns*

The final 778 *cox1* sequences obtained, 529 from California and 249 from Nicaragua, were grouped into 14 species-level clusters after the GMYC analysis. In California it was possible to assign all larvae either to *Curculio occidentis*, *C. pardus* or *C. aurivestis*. None of the 11 GMYC groups from Nicaragua matched any published sequence, hence they were tentatively considered as putative new species. Species accumulation curves reached an asymptote in California while in Nicaragua the estimated regional pool of species suggests that at least one more weevil species had yet to be discovered (Fig. 1 and Table 1). The consensus phylogenetic tree showed that most of the Nicaraguan species formed a well-supported clade independent of the lineages of temperate weevils (Appendix S1: Fig. S1).

Although the number of oak species in Nicaragua was lower than in California (Table 1), Nicaragua held a higher alpha diversity of weevil per site, whether

TABLE 1. Acorn weevil and oak host diversity in California and Nicaragua.

	California	Nicaragua
Sampled sites	29	17
Observed <i>Curculio</i> species	3	11
Gamma diversity		
Species richness	3.00 ± 0.0	11.25 ± 0.73
Shannon diversity	2.33 ± 0.05	6.61 ± 0.37
Simpson diversity	2.16 ± 0.05	4.99 ± 0.39
Alpha diversity		
Species richness	1.84 ± 0.13	3.82 ± 0.66***
Shannon diversity	1.61 ± 0.12	2.98 ± 0.36***
Simpson diversity	1.58 ± 0.11	2.61 ± 0.31**
Observed <i>Quercus</i> hosts	8	5
Oak hosts per site	1.86 ± 0.15	1.29 ± 0.11*
Weevil:Host ratio per site	1.12 ± 0.11	3.14 ± 0.41***

Species richness, Shannon and Simpson diversity indexes are asymptotic estimates based on Hill numbers ( $q = 0$ ,  $q = 1$  and  $q = 2$ , respectively). Differences between regions are Kruskal-Wallis tests and values are mean ± standard error when applicable.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

measured in terms of species richness ( $\chi^2 = 16.9$ ,  $P < 0.0001$ ), weevil-oak ratios ( $\chi^2 = 20.8$ ,  $P < 0.0001$ ), Shannon diversity index ( $\chi^2 = 12.1$ ,  $P < 0.001$ ) and Simpson diversity index ( $\chi^2 = 9.5$ ,  $P = 0.002$ ). Weevil communities in Nicaragua also showed a higher beta diversity ( $0.78 \pm 0.03$  vs.  $0.62 \pm 0.05$ ; mean of Sørensen index ± standard deviation), which was mostly due to a greater species replacement ( $\beta_{tu}$ ) from site to site (Fig. 2, Appendix S1: Table S1).

### *Host specialization and trait relationships*

The *Quercus-Curculio* trophic webs showed remarkable differences between regions in terms of host specialization (Fig. 3, Appendix S1: Table A2). Nicaraguan weevil species had significantly smaller host ranges (Poisson MCMCglmm: pMCMC <0.001, phylogenetic heritability = 0.72; Fig. 4a). In Nicaragua, the interaction strength of weevil species was higher than in California, with over 90% of larvae emerging from acorns of the main host (Binomial MCMCglmm: pMCMC = 0.032, phylogenetic heritability = 0.73; Fig. 4b). Differences in the specialization index pointed to the same direction, but in this case the difference was marginally non-significant (Gaussian MCMCglmm: pMCMC = 0.09, phylogenetic heritability = 0.62). According to phylogenetic reconstructions of host specificity, the transition from temperate to neotropical regions was associated with a trend for weevils to specialize on oak hosts that belong to a single *Quercus* genus section, i.e., to a narrower phylogenetic breadth of their hosts (Appendix S1: Fig. S2).

Larvae head size positively co-varied with the volume of the acorns from which they had emerged (Fig. 5 and Appendix S1: Table S3). However, the slope of this relationship was only significant for Nicaragua (Gaussian

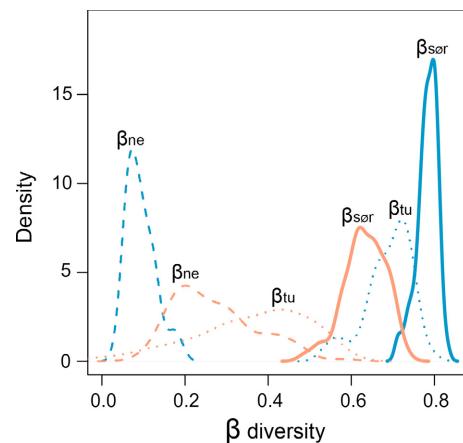


FIG. 2. Partition of Sørensen index of beta diversity ( $\beta_{sor}$  solid line) into species turnover ( $\beta_{tu}$ , dotted line) and nestedness resultant dissimilarity ( $\beta_{ne}$  dashed line) of Californian (blue) and Nicaraguan (orange) communities of acorn weevils (*Curculio* sp.). [Color figure can be viewed at wileyonlinelibrary.com]

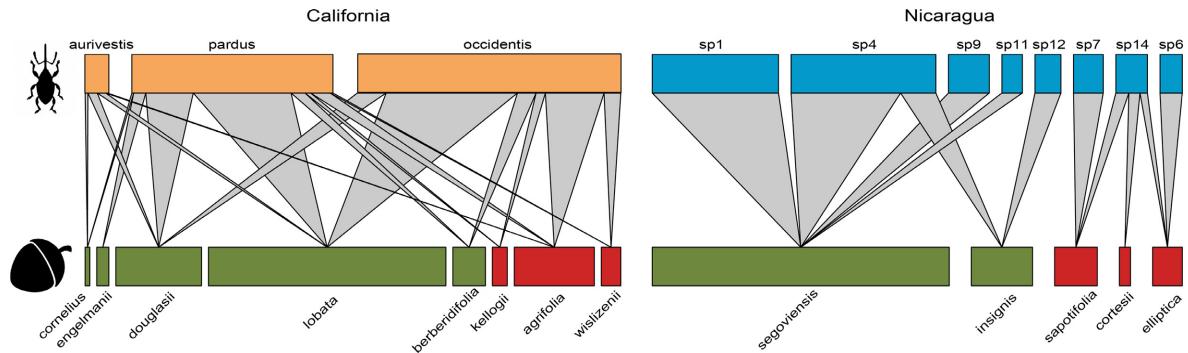


FIG. 3. Quantitative *Quercus-Curculio* trophic web for California and Nicaragua. Nicaraguan weevil species are molecularly delimited species-level entities designated with preliminary numbers (see text for further details). The width of nodes and links is relative to sample abundance. Green and red color shows oak species affiliation to *Quercus* or *Lobatae* genus sections respectively. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

MCMCgmm:  $pMCMC < 0.001$ ) but not for California ( $pMCMC = 0.116$ ). Consistent with the lack of specialization, larvae of Californian weevils emerged from acorns of hosts belonging to both red and white oak sections and reached similar body sizes when developed ad libitum irrespective of the oak section (*C. aurivestis*:  $F_{1, 15} = 0.08$ ,  $P = 0.79$ ; *C. occidentis*:  $F_{1, 20} = 0.16$ ,  $P = 0.69$ ; *C. pardus*:  $F_{1, 18} = 1.01$ ,  $P = 0.34$ ). In contrast, only two Nicaraguan larvae (out of 249) emerged from acorns of a host of the “non-preferred” *Quercus* section, and their head width was two standard deviations below their own species’ mean (0.9 mm vs.  $1.33 \pm 0.18$  and 0.8 mm vs.  $1.51 \pm 0.12$ ).

#### DISCUSSION

According to the ecological theory, the outstanding biodiversity of tropical regions may in part result from a greater subdivision of resources due to stronger or more specialized biotic interactions (Schemske et al. 2009, Brown 2014). Here, we report the first evidence supporting this hypothesis for the guild of insect seed predators. We found an outstanding alpha, beta and gamma diversity of acorn weevils (*Curculio* sp.) in Nicaragua, which could not be attributed to higher host availability either at a local or regional level. Most Nicaraguan weevils formed a separate evolutionary lineage with highly specialized species, whereas their temperate congeners had a broader taxonomic and phylogenetic host spectrum with a weaker interaction strength. Additionally, larval body size correlated with acorn size in Nicaragua but not in California, which is also consistent with the host-specialization hypothesis and suggests different ecological and evolutionary dynamics across latitudinal regions (Schemske et al. 2009, Brown 2014). Overall, these results support the view that host specialization promotes the diversity of phytophagous insects in tropical regions (Dyer et al. 2007, Forister et al. 2015).

Our weevils’ sampling covered almost the entire range of oak species in Nicaragua and California. The

estimated pools of species, calculated through extrapolations based on Hill numbers of effective species (Jost 2006, Hsieh et al. 2016), showed that it is likely that more surveys in Nicaragua would result in additional species found in the same oak hosts and localities. This remarkable and yet unknown diversity of acorn weevils in Nicaragua -the first account of this genus for the Neotropical region since Gibson (1977), representing a 40% increase of previous estimates of species richness- was not associated with a greater number of oak hosts in the tropics, neither at each sampling site nor at the regional level, thereby enabling to remove host availability as confounding factor (Lewinsohn and Roslin 2008).

In accordance with the host specialization hypothesis (Dyer et al. 2007, Forister et al. 2015), Nicaraguan weevils showed more exclusive interactions with their hosts than their temperate congeners. This finding supports the long-standing perception that resources are more finely apportioned among species in the tropics, which may promote species coexistence by reducing niche overlap (Hutchinson 1959, Salisbury et al. 2012, Brown 2014). Seed-feeding insects tend to show some degree of phylogenetic conservatism in their plant host range (Kergoat et al. 2005, Delobel and Delobel 2006), and this was the case for the acorn weevils considered in this study. However, even when controlling for phylogenetic effects, Nicaraguan species exhibited higher host specialization than Californian species than would be expected by their relative position along the phylogeny. This pattern suggests a trend towards host specialization in the transition from a temperate to a tropical distribution, a possibility supported by phylogenetic reconstructions with character stochastic mapping.

Previous work points out that oscillations between generalization and specialization have been common within lineages of phytophagous insects under appropriate selective pressures (Nosil 2002). Consistent with this suggestion, host range appeared to be a rather evolutionary labile trait among the weevils considered in our study (Fig. 4a). Additionally, temperate species showed

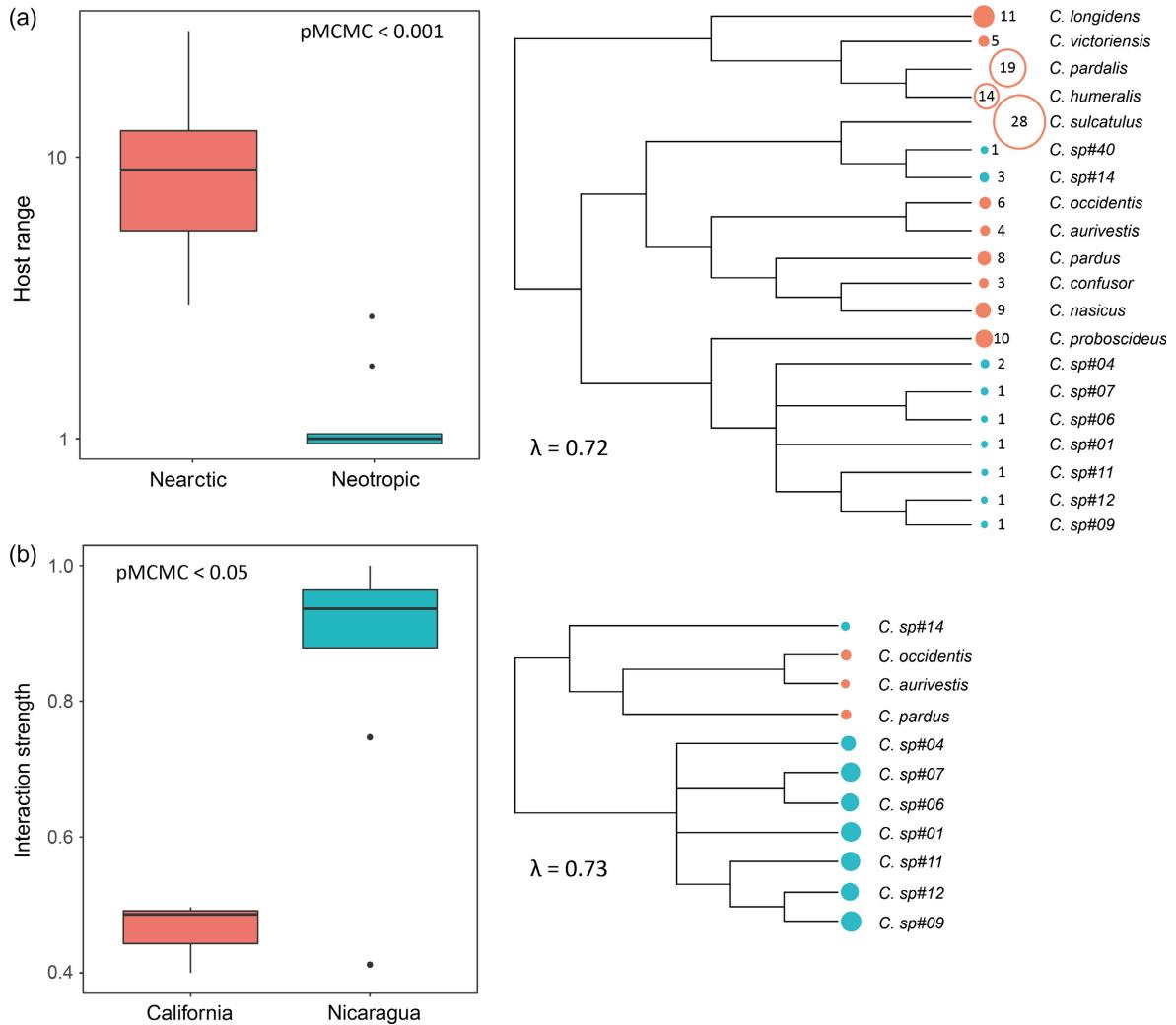


FIG. 4. Differences in host range (a) and interaction strength (b) between weevil species (*Curculio* sp.) from Nearctic/Californian and Nicaraguan distribution. Host range is the number of oak hosts per weevil species. Interaction strength is the number of larval emergences from the main host relative to all emergences. Values are medians, and first and third quartiles. Notches indicate 95% confidence intervals of median. Significance levels are pMCMC values according to phylogenetic GLMMs. Adjacent panels show the distribution along the phylogeny of trait values proportional to circle size, and  $\lambda$  denoting their phylogenetic heritability. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

a lower phylogenetic specificity, i.e., a higher phylogenetic distance among potential hosts (Barrett and Heil 2012), and the larvae of the Californian species performed equally well in acorns from both *Quercus* genus sections. In contrast, only two Nicaraguan larvae (out of 249) emerged from acorns of a host of the “non-preferred” *Quercus* section and they developed smaller heads despite having been grown feeding ad libitum. These differences in larval performance in non-preferred host acorns might reflect that Californian species have acquired more biochemical pathways to cope with the variety of compounds present in their broader acorn diets (Muñoz et al. 2014), a possibility that still awaits empirical confirmation.

Additional evidence in support of the host specialization hypothesis comes from the analysis of the

relationship between larval body size and acorn size of the host species. Larger larvae were associated with larger acorns in Nicaragua but not in California, fitting our prediction of a tighter matching associated with more specialized trophic interactions in the tropics. These results are remarkable considering that the morphological traits were not retrieved from the bibliography (e.g., Hughes and Vogler 2004b) but directly measured and that only larvae grown ad libitum were included, thus minimizing the potential effect of intra-specific phenotypic plasticity. Assuming that seed size is an important driver of morphological variation in weevils and that larval size is a good proxy of fitness, premises that are well supported by current evidence (Desouhant et al. 2000, Hughes and Vogler 2004b, Bonal et al. 2011), the existence of latitudinal differences in the relationship

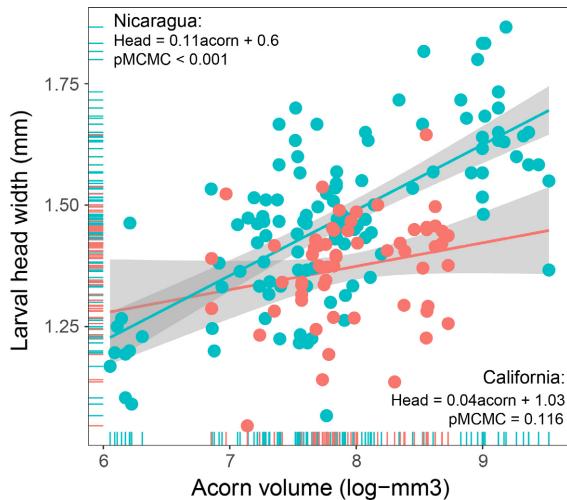


FIG. 5. Correlation between acorn volume (log-transformed mm<sup>3</sup>) and larval head width (mm) from California (orange) and Nicaragua (blue). The slopes are significantly different among regions according to phylogenetic GLMMs. [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

between seed and body size points to differences in selection pressures related to biotic interactions. That is, selecting for host specialization might result in a tighter acorn-body size matching to increase the efficiency of resource exploitation (Hughes and Vogler 2004b, Bonal et al. 2011). Instead, a generalist-feeding scenario might promote biochemical detoxification ability to improve larval performance on a wider set of host species (Muñoz et al. 2014).

The contrasting eco-evolutionary scenarios suggested by our analyses may also be affected by additional factors besides competition. The high beta diversity of acorn weevils in Nicaragua, particularly the strikingly high species turnover component (Fig. 2), suggests that the geographic distribution of these tropical weevils are smaller than those of their temperate congeners. Instead, the distribution of all North American weevil species often encompasses more than one state (Gibson 1969). As suggested for other animal lineages in the tropics, this pattern can be the result of population isolation followed by allopatric speciation (Salisbury et al. 2012). Fifty years ago Daniel Janzen (1967) formulated the hypothesis that in the tropics the relative environmental stability could have reduced species' thermal tolerances making mountain passes stronger dispersal barriers than in temperate regions. Since then, this hypothesis has received considerable support (Ghalambor et al. 2006), transcending its original form into a wider version that includes ecological specialization as a factor fostering higher diversification rates in the tropics (Salisbury et al. 2012). Insects living in tropical montane ecosystems have been found to be less tolerant to high temperatures than those restricted to lowlands (García-Robledo et al. 2016). Therefore, from the point of view of a tropical

montane, poorly flying insect, such as an acorn weevil, the warm and dry valleys can represent strong dispersal barriers. In addition, their oak hosts are also restricted to montane habitats so that weevil populations may experience high levels of isolation. In such situations, local adaptation to the available hosts, e.g., equivalent reductions of body size in sister species specialized on the same oak host, could be the by-product of parallel evolution (Nosil et al. 2002).

A drawback of studies like ours is the lack of information along the entire latitudinal gradient. Certainly, global and multiple-clades tests of the host specialization hypothesis are essential to improve statistical power and draw general conclusions, as recently demonstrated by Forister et al. (2015). However, a focus on particular radiations and specific regions may still provide important insight into mechanisms by allowing a better control of confounding factors and the generation of more specific hypotheses. We anticipate that the oak-weevil system, with its remarkable latitudinal mismatch between insect and plant diversity, will provide excellent opportunities to continue exploring the origin of latitudinal biodiversity patterns in the future. Research priorities include the analysis of phylogeographic relationships among weevil populations and species, necessary to infer their historical patterns of diversification, and the study of how biotic and abiotic factors combine to shape biodiversity. This research should be developed not only from the perspective of the insect but also from that of the host, which defies current theory by departing from the classic latitudinal biodiversity pattern. Increasing our knowledge of the links between ecological specialization, restricted dispersal and diversity of acorn weevils in both tropical and temperate regions, together with a better understanding of the evolutionary ecology of the host upon which they depend, will further contribute to understand why the tropics sustain such an extraordinary diversity of organisms.

#### ACKNOWLEDGMENTS

We thank Josué Urrutia, Bladimir Acuña (UNAN/Managua), Keith Gaddis (UCLA) and Belén Sánchez-Humanes for support during the fieldwork, and Indiana Coronado (UNAN/Leon and MOBOT) for the classification of Nicaraguan oaks. Jofre Carnicer provided valuable comments on an initial draft of the manuscript and Joan Maspons and Ferran Sayol were very helpful with the phylogenetic analyses. G.P was partly supported by a post-doctoral grant from the Ramon Areces Foundation and the European Research Council Synergy Project ERC-2013-SyG-610028 IMBALANCE-P. This research benefited from the IBERO-REDD+ network funded by CYTED. R.B was supported by a contract of the Atracción de Talento Investigador Programme (Gobierno de Extremadura TA13032) and the project AGL2014-54739-R.

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