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INVESTIGATING PROCESSES OF NEOTROPICAL RAIN FOREST TREE DIVERSIFICATION BY EXAMINING THE EVOLUTION AND HISTORICAL BIOGEOGRAPHY OF THE PROTIEAE (BURSERACEAE)

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Andean uplift and the collision of North and South America are thought to have major implications for the diversification of the Neotropical biota. However, few studies have investigated how these geological events may have influenced diversification. We present a multilocus phylogeny of 102 Protieae taxa (73% of published species), sampled pantropically, to test hypotheses about the relative importance of dispersal, vicariance, habitat specialization, and biotic factors in the diversification of this ecologically dominant tribe of Neotropical trees. Bayesian fossil-calibrated analyses date the Protieae stem at 55 Mya. Biogeographic analyses reconstruct an initial late Oligocene/early Miocene radiation in Amazonia for Neotropical Protieae, with several subsequent late Miocene dispersal events to Central America, the Caribbean, Brazil's Atlantic Forest, and the Chocó. Regional phylogenetic structure results indicate frequent dispersal among regions throughout the Miocene and many instances of more recent regional in situ speciation. Habitat specialization to white sand or flooded soils was common, especially in Amazonia. There was one significant increase in diversification rate coincident with colonization of the Neotropics, followed by a gradual decrease consistent with models of diversity-dependent cladogenesis. Dispersal, biotic interactions, and habitat specialization are thus hypothesized to be the most important processes underlying the diversification of the Protieae.

KEY WORDS: Amazon lowlands, *Crepidospermum*, diversity-dependent cladogenesis, habitat specialization, *Protium*, *Tetragastris*.

Neotropical rain forests harbor the highest concentration of plant diversity on the planet, with more than 90,000 seed plant species, including record totals of species richness at both local and regional scales for trees, shrubs, lianas, epiphytes, and herbs (Gentry 1982, 1988; Gentry and Dodson 1987; Antonelli and Sanmartín 2011a). Recent studies suggest that the past 23 million years have represented a particularly important period for the generation of high Neotropical diversity, and the importance of geological events in promoting diversification has been heavily emphasized (Antonelli et al. 2009; Hoorn et al. 2010a, 2010b; Hughes et al.

2013). At the same time, biotic factors such as competition and natural enemy attack are likely to influence the diversification of Neotropical plant lineages during their entire history (Schemske et al. 2009). There are two tectonic events that had major implications for the diversification of the Neotropical biota: Andean uplift and the collision of the North and South American plates (Raven and Axelrod 1974; Gentry 1982; Hoorn et al. 2010a, 2010b). However, few studies have investigated in detail how these geological events may have caused elevated diversification rates or how well they coincided with the timing of Neotropical plant radiations.

The approach and eventual collision of Central America with South America may have promoted Neotropical diversification by increasing the opportunities for dispersal and colonization between these two biogeographic regions. Raven and Axelrod (1974) hypothesized that this would have permitted the mixing of two separate tropical rain forest floras, the northern "Laurasian" and southern "Gondwanan" floras, resulting in a large, cumulative increase in total Neotropical diversity. Indeed, while the exact date of collision may be as recent as 3 million years ago (but see Farris et al. 2011; Montes et al. 2012), the close proximity of the two continents over the past 10–20 million years could have allowed dispersal between the continents via island-hopping and/or long-distance dispersal at various times during that period (Gentry 1982; Hughes et al. 2013). Recent phylogenetic, paleobotanical, and biogeographic studies have revised and refined our understanding of the history of Neotropical rain forests as well as updated the assignment of Raven and Axelrod's (1974) biogeographic affinities. Many previously categorized "Gondwanan" groups actually appear to be Laurasian immigrants to South America from Eurasia or Africa by the boreotropical dispersal route via North and Central America (Tiffney 1985; Davis et al. 2002; Weeks et al. 2005); these include many of the most dominant and species-rich lineages, such as *Guatteria*, *Ocotea*, *Inga*, and the Moraceae, among many others (Chanderbali et al. 2001; Richardson et al. 2001; Pennington and Dick 2004, 2010; Zerega et al. 2005; Erkens et al. 2007). Thus, there may have been multiple waves of migration for different taxa in either or both directions, causing the introduction of new lineages as well as spurring speciation within immigrant lineages via radiations of founder taxa.

The uplifting Andean mountains have created physical barriers that can sunder populations, promoting geographic isolation and thus increasing the opportunities for allopatric speciation (Chapman 1926). Indeed, geographic isolation has clearly been important for the diversification of Neotropical montane taxa (Gentry 1982; Särkinen et al. 2007) as well as inter-Andean valley dry forest taxa (Särkinen et al. 2012). However, several studies suggest it is of limited importance for Neotropical lowland rain forest lineages (Pennington and Dick 2010; Cavers and Dick 2013).

A second way that Andean uplift may have influenced Neotropical diversification is by influencing the rates of habitat specialization and ecological speciation. The erosion of new geological substrates, together with repeated marine incursions, created novel soil types in the Western Amazon (Hoorn 1993, 2010a). In addition, the Andean uplift had a major effect on the hydrology, flooding regimes, and the extent of flooded habitats in the entire Amazonian region (Hoorn et al. 2010a, 2010b). This event likely caused habitat specialization to the new soils and flooded habitats, which can lead to speciation depending on the spatial heterogeneity of the habitats and strength of selection across the environmental gradient (Moritz et al. 2000). Indeed, Gentry (1982) hypothesized that an important mechanism of diversification in Amazonian plant clades was repeated habitat specialization to different soil types, flooding regimes, and seasonal drought regimes. Species-rich Neotropical clades such as *Hirtella* (Chrysobalanaceae), the Sapotaceae, *Passiflora* (Passifloraceae), and the Bignonieae (Bignoniaceae), for example, have extensive fossil records and/or phylogenies estimating pre-Miocene ages in Neotropical forests and include many habitat-specialist species restricted to unique soils, flooding, and/or drought regimes (Gentry 1981; Jaramillo et al. 2010; Lamarre et al. 2012; Bardon et al. 2013; Lohmann et al. 2013).

Finally, biotic interactions are a prominent feature of tropical rain forests and influence plant speciation (Schemske et al. 2009). Studies of lineage-through-time plots have often noted patterns of initial acceleration of diversification rates followed by a decline. This pattern has been interpreted as a lineage entering a new biogeographic region and filling an empty niche, promoting diversification, followed by a slowdown as competition among close relatives increases as the niche becomes filled (Simpson 1953; Rabosky and Lovette 2008). A similar pattern could arise due to the coevolutionary arms race between plants and their natural enemies, with lineages possessing novel defenses gaining an evolutionary advantage and spurring speciation rates followed by counteradaptation by specialist enemies that would then slow down plant diversification (Ehrlich and Raven 1964). Alternatively, if clades not have yet exhausted their colonization of new habitats or biogeographic regions, or if natural enemies have not yet evolved counteradaptations to plant defense, the prediction would be to find no such decline, but rather a constant rate of diversification (Derryberry et al. 2011).

Molecular phylogenetic studies of extant taxa, integrated with fossil evidence, can help resolve where lineages originated, patterns of biogeographical spread, and the timing of diversification events (e.g., De-Nova et al. 2012; Hughes et al. 2013). The tribe Protieae (Burseraceae), currently comprising the genera *Protium*, *Crepidospermum*, and *Tetragastris*, is one of the most important Neotropical tree lineages in terms of its diversity and abundance and can serve as a model system to understand the relative

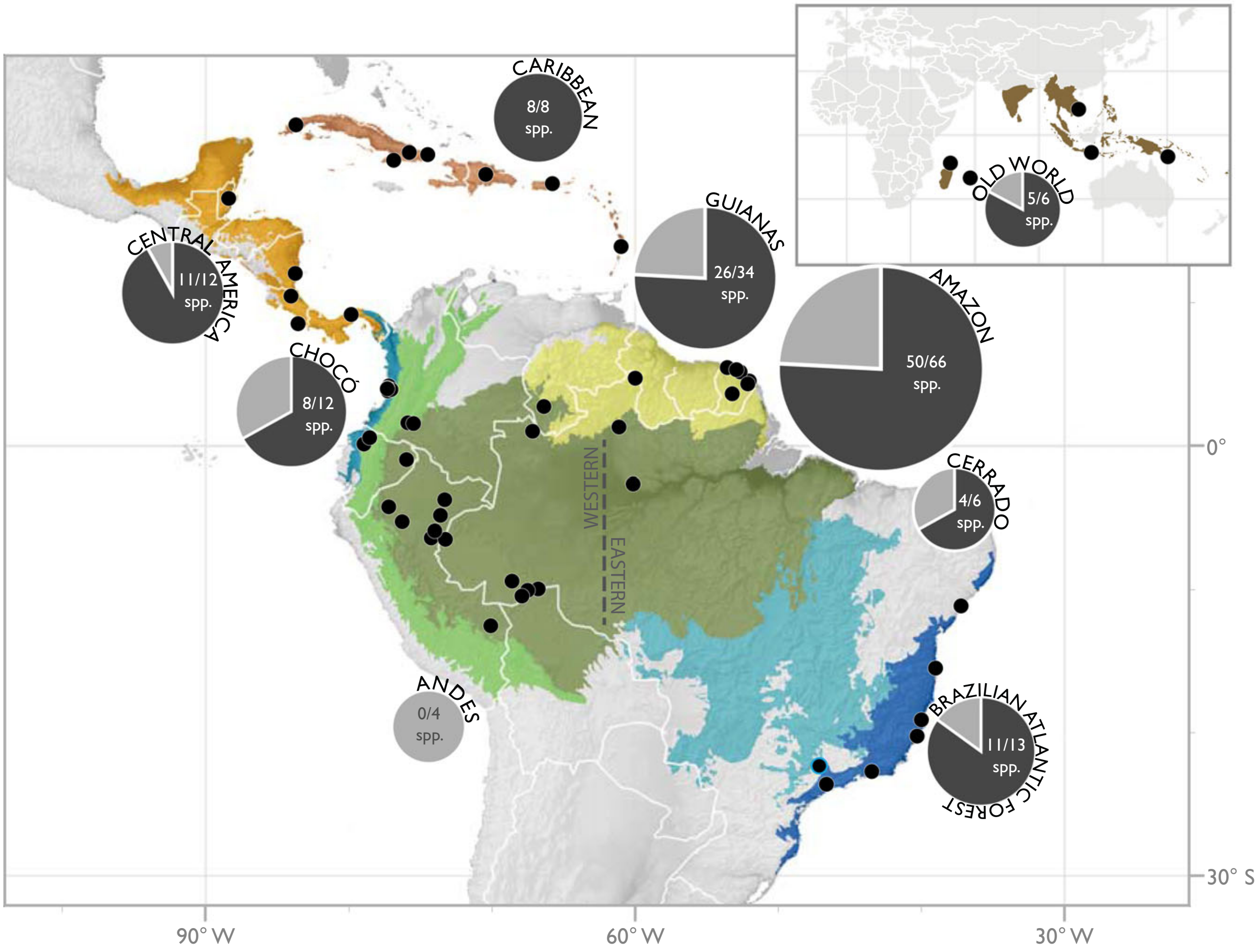

Figure 1. Map of the biogeographic regions in which Protieae occur; shaded areas represent the geographic distribution of the Protieae. Points denote localities in which specimens were collected for the molecular phylogenetic study. Each biogeographic region's total number of Protieae species is listed on a pie chart, with the total number of species for that region that is included in the phylogenetic study, demonstrating that our sampling effort is distributed evenly across biogeographic regions. Only published names are included in the pie charts; these totals do not include the several new species and subspecies that are also included in our phylogenetic study (see Appendix S1).

importance of dispersal, vicariance, habitat specialization, and biotic factors in driving Neotropical diversification. The tribe consists of small to large trees that are almost entirely confined to moist and wet tropical rain forests: 134 of its 140 published species occur in the Neotropics, and 86 of these are found in the Amazon basin and the Guianas (Appendix S1). In the Amazon, *Tetragastris* and *Protium* are both ranked in the top seven genera for most individuals in the Amazon Tree Diversity Network comprising 1170 plots across the Amazon basin (ter Steege et al. 2013). The Protieae comprise 4.2% of all individuals in the plots, and as a lineage they represent the second most abundant group after *Eschweilera* (Lecythidaceae). The alpha diversity of this clade is high in many places in Amazonia: more than 29 species have been found in a network of sixty-seven 0.1 ha plots in the Allpahuayo-Mishana Reserve near Iquitos, Peru (Fine et al. 2005), more than 24 species in 25 ha of Amazonian Ecuador (Valencia et al. 2004), and 35 species in 50 ha north of Manaus, Brazil (Rankin-de-Mora 1992). The majority of Protieae diversity is found in the Amazon basin and the Guianas but there are smaller numbers of species occurring in other biogeographic realms, including Central America, the Caribbean, the Chocó, Andean montane forests, the Brazilian Cerrado, the Brazilian Atlantic Forest, and the Paleotropics (Fig. 1). In addition, the Protieae include a variety of habitat specialists in white sand and flooded forests within the Amazon basin and Guianas (Fine et al. 2005; Lamarre et al. 2012), and in restinga and cerrado habitats in eastern and central Brazil, respectively. Significantly, the Protieae and many of its closest relatives contain at least some fossil records, allowing a fossil calibration of their molecular phylogeny and an analysis of the timing of diversification events. Finally, the taxonomy of the

Protieae has been well studied, allowing a high level of confidence in species delimitation and patterns of geographic distribution to be achieved (Daly 1987a,b, 1989, 1990, 2007; Daly and Fine 2011).

A deeper understanding of the origins of Neotropical rain forest diversity depends on the reconstruction of the biogeographic history of large, challenging, and ecologically dominant plant lineages like the Protieae. We asked:

(1) How old are the Protieae and when did they undergo diversification? Do most diversification events coincide with Andean uplift or continental collision? When have there been significant increases or decreases in diversification rate? To answer these questions, we reconstructed a fossil-calibrated multilocus phylogeny of 102 species of the Protieae and analyzed lineage-through time plots.

(2) What is the relative importance of vicariance versus dispersal, and when in the evolutionary history of the Protieae did these events occur? To answer these questions, we reconstructed ancestral biogeographic distributions, and characterized geographic structure of the Protieae phylogeny using community phylogeny methods.

(3) Is habitat specialization associated with cladogenesis? Are habitat specialists generally each other's closest relatives or is habitat switching associated with cladogenesis events? To answer these questions, we combined our phylogeny with habitat association data and modeled ancestral habitat association.

(4) Do lineage-through-time plots show a pattern consistent with diversity-dependent cladogenesis with an initial increase followed by a slowdown? To answer this question, we constructed lineage-through-time plots and modeled diversity-dependent cladogensis with and without extinction.

Methods and Materials

TAXON AND DNA SAMPLING

The tribe Protieae (Burseraceae) forms a strongly supported clade on morphological grounds, particularly based on fruit characters: a compound dehiscent drupe; pericarp fleshy to coriaceous or rarely sublignified, septicidally and acropetally dehiscent by 1–5 valves (= number of pyrenes developed); locules separated by a columella; pyrenes 1–5, each enveloped in a sweet, pulpy, white (rarely red) pseudaril and on dehiscence tenuously suspended from the fruit apex by an inverted V-shaped structure (Daly et al. 2011). It consists of ca. 140 species divided into three genera. For this study, 90 species of *Protium* (not including eight additional published subspecies or varieties), seven species of *Tetragastris*, and five species of *Crepidospermum* were sampled (102 total species; Appendix S1). All morphological and ecological variation shown in the tribe, as well as all major biogeographical regions of distribution, have been represented with this sampling. We conducted an initial analysis using more than one specimen per species whenever possible for a total of 157 tips in the phylogeny. In general, individuals from the same species formed monophyletic groups, but intraspecific relationships did not exhibit high posterior probabilities (Fig. S1). Furthermore, diversification analyses (see below) required that tips in the phylogeny represented individual species (i.e., independent evolutionary lineages), so we trimmed 46 of the 157 tips in our matrix to include only one individual per species. We only included multiple accessions per species when these accessions came from recognized taxonomic subspecies (and thus may represent independent evolutionary lineages), or when intraspecific variation may have warranted species recognition (e.g., Fine et al. 2013a). All specimens used in this study were vouchered and identified to species/subspecies by comparison to reference herbarium material. Representatives of five other genera of the Burseraceae, plus two species from Anacardiaceae comprised the outgroup.

We sequenced the nuclear ribosomal internal and external transcribed spacers (ITS and ETS, respectively), a fragment of exon 1 in the nuclear phytochrome C gene (phyC), and the chloroplast rps16 intron and trnL-F intergenic spacer. All sequences are deposited in GenBank (Appendix S1). For the outgroups, we used previously published sequences available in GenBank (www.ncbi.nlm.nih.gov/genbank). DNA extraction, PCR amplification, sequencing, and sequence editing/assembly followed Daly and Fine (2011) and Fine et al. (2013a). For nuclear markers, we cloned and sequenced 3–5 clones for all ITS amplifications, and 3–5 clones for ETS and phyC whenever direct sequencing revealed polymorphisms within individuals for these two markers. In general, clones formed clades, and we did not detect paralogous copies in any of the nuclear markers used in this study.

ALIGNMENT AND PHYLOGENETIC ANALYSES

Multiple sequence alignment for each locus was carried out in MAFFT 6.864 (Katoh et al. 2009). We used PartitionFinder (Lanfear et al. 2012) to concurrently infer an optimal partition scheme and model of nucleotide substitution. We ran Bayesian phylogenetic analyses for each locus and of the concatenated matrix using Mr. Bayes version 3.2 (Ronquist et al. 2012), and used a majority rule consensus tree (MRCT) to summarize the posterior probability distribution of trees. Full details of phylogenetic analyses are presented in Appendix S2. The final alignment has been deposited in TreeBase (submission ID 15484) and the Dryad Digital Repository (doi:10.5061/dryad.d71c1).

CALIBRATION AND DIVERGENCE TIME ESTIMATION

We used Burseraceae fossils to set age constraints on the phylogenetic tree and generate a chronogram to estimate absolute divergence times within the Protieae. We took into account the morphological characters observed on the fossils to assess the relationships of the fossils to the extant taxa in our phylogeny, and corroborated these relationships with those reported in other studies of Burseraceae (Weeks et al. 2005; Becerra et al. 2012; De-Nova et al. 2012). Fossil endocarps attributed to *Canarium* (Gregor and Goth 1979), the fossil *Protocommiphora europea*, which can be assigned to *Commiphora* or *Bursera* subgenus *Elaphrium* (Reid and Chandler 1933), and the fossil *Bursericarpum aldwickense* (Reid and Chandler 1933; Chandler 1961) assigned to the tribe Protieae were used to provide minimum age constraints for the fossil-calibrated analyses (see Appendix S2 for more details).

We used a combination of fossil and secondary calibrations with a relaxed molecular clock approach to simultaneously estimate phylogenies and divergence times in the face of uncertainty in phylogenetic relationships, evolutionary rates, and calibration times using BEAST (Drummond et al. 2012). We assigned age constraints on four internal nodes: node A, the most recent common ancestor (MRCA) of all taxa within the family Burseraceae; node B, the MRCA of *Canarium* and *Santiria* (tribe Canarieae); node C, the MRCA of *Bursera* and *Commiphora* (tribe Bursereae); and node D, the MRCA of all taxa within the Protieae. Because we did not have fossils available to set an age constraint for node A, we used a secondary calibration derived from an earlier study (De-Nova et al. 2012), which we assigned to the crown group defined by this node (i.e., crown Burseraceae). Because the fossils we used for the other nodes are fragmentary (see Appendix S2), it was not possible to be certain that any of those fossils possess features that would place them in the crown groups defined by any of the nodes. Therefore, we took a conservative approach and used them as minimum calibrations of the stem groups in nodes B–D (see also Forest 2009). We carried out three dating analyses: in analysis 1, we used the ages reported by De-Nova et al. (2012) for nodes A–D, and we used normal distributions as priors on the ages of these nodes. In analyses 2 and 3, we used the age reported by De-Nova et al. (2012) for node A parameterized with a normal prior, and for nodes B–D we used the fossil ages parameterized with prior exponential distributions (analysis 2) and prior log-normal distributions (analysis 3). We used the maximum clade credibility tree (MCCT) to summarize the posterior probability distribution of chronograms. Full details of dating analyses are presented in Appendix S2.

Finally, we evaluated lineage-specific shifts in diversification using the MEDUSA algorithm (Alfaro et al. 2009), which fits a series of birth–death (BD) models with an increasing number of rate shifts, estimates the maximum-likelihood value for each set of BD parameters, and uses stepwise AIC to select the model with the highest likelihood to describe the data. We predicted that we would find a significant increase in diversification rate during the Miocene that would coincide with Andean uplift and continental collision.

BIOGEOGRAPHIC ANALYSES—ASSESSING THE ROLE OF DISPERSAL AND VICARIANCE IN THE BIOGEOGRAPHIC HISTORY OF THE PROTIEAE

To evaluate the role of geography in the diversification of the Protieae, we inferred the biogeographic history of the species and inferred the patterns of dispersal and directionality of movement among geographic regions through time. For this analysis, we employed maximum-likelihood inference of geographic range evolution using the dispersal, extinction, and cladogenesis (DEC) model (Ree et al. 2005; Ree and Smith 2008) implemented in Lagrange version 0.1β and estimated split and ancestral states concurrently. To carry out this analysis, we assigned each species to one or more of the following nine biogeographic areas: Paleotropics (PT), Caribbean (CB), Central America (CA), Chocó (CH), Western Amazon (WA), Eastern Amazon (EA), Guianas (GU), Cerrado (CE), and Atlantic Forest (AF) (Fig. 1). We were not able to sample any of the four published Andean species. These biogeographic areas were delimited on the basis of tropical American paleogeography (Antonelli et al. 2009; Antonelli and Sanmartín 2011a), extant Neotropical floristic regions, and current distribution of extant species. Due to the large number of taxa and biogeographic areas used in this study, to complete the analysis in a reasonable amount of time, we constrained the maximum number of ancestral areas to three. Moreover, 95% of the extant Protieae taxa in our sample were distributed in three or fewer biogeographic areas. To study geographic range evolution through time, we first defined a null hypothesis in which there was no constraint on movement between areas at any time; thus, the rate of dispersal had a value of 1 among all areas. We contrasted this hypothesis with an alternative hypothesis reflecting the likely paleogeographic history of tropical America from the Oligocene forward (Antonelli et al. 2009; Antonelli and Sanmartín 2011a). Because it has been suggested that Protieae likely colonized the Neotropics from the Paleotropics via the northern hemisphere (i.e., the boreotropical connection hypothesis [Weeks et al. 2005]), we also contrasted the null hypothesis against an alternative hypothesis where South American species are descendants of Central American species. Because these three models have the same number of free parameters (i.e., dispersal and extinction) and are not nested (they differ in having additional parameters fixed at a boundary value), we used a difference outside the confidence window of two log-likelihood units (Edwards 1992) to select the best-fitting model (Ree and Smith 2008). Full details of these biogeographic analyses are presented in Appendix S2.

If the emergence of geographic barriers through geological time (e.g., mountain uplift, rivers, lakes) influenced diversification via vicariance, we would predict strong geographic phylogenetic structure, roughly coinciding with the timing of major geological events. Alternatively, if geological events promoted dispersal into new biogeographic regions (e.g., collision of tectonic plates), we predict weak geographic phylogenetic structure.

ASSESSING DISPERSAL WITH COMMUNITY PHYLOGENETIC METHODS

We also assessed the role of geography in the diversification of Protieae by evaluating the community phylogenetic structure within biographic regions to test for patterns of geographic structure at a regional scale (Lavin 2006). To carry out these analyses, we asked whether species occurring within the nine biogeographic areas were more, or less, related on average than species drawn from random (Hardy 2008). To test for this association, we estimated the mean pairwise distance (MPD, a metric more sensitive to phylogeny-wide patterns) and the mean nearest taxon distance (MNTD, a metric more sensitive to patterns in the tips of the phylogeny; for details on these metrics, see Webb et al. 2002) between all species in each biogeographic area, and we assessed the statistical significance of these metrics under null models generated by randomizing the tip labels across the tips of the phylogeny 999 times using the package picante (Kembel et al. 2010) in R 2.15.3 (R Development Core Team 2013). If the Protieae lineage has experienced frequent long-distance dispersal throughout its history, we predict random patterns with respect to relatedness and biogeographic region. Alternatively, if speciation most often occurs within biogeographic regions and dispersal is rare among regions, we predict strong patterns of geographic structure and thus significant patterns of phylogenetic clustering.

EVOLUTION OF HABITAT ASSOCIATION

To investigate whether habitat specialization has promoted lineage divergence within Protieae, we reconstructed ancestral habitats and investigate the pattern of habitat evolution (colonization or extinction) in ancestral species, and the inheritance of habitat states by daughter species at cladogenesis. To carry out this analysis, we employed maximum-likelihood inference of habitat evolution using the DEC model (Ree et al. 2005; Ree and Smith 2008) implemented in Lagrange version 0.1β. Analogous to geographic range, if an ancestor is a habitat generalist and occurs in two or more habitats, speciation can happen in two ways with respect to habitats: daughter species can either be one split into one habitat specialist and one generalist lineage, or the ancestor can split into two specialists in two different habitats (for details, see Ree and Smith 2008). To examine the evolution of habitat association, we assigned all species to one or more of the following six habitats based on extensive fieldwork (Daly 1987a,b, 1989, 1990, 1992, 2007; Fine et al. 2005; Baraloto et al. 2011; Daly and Fine 2011; Lamarre et al. 2012): Cerrado (C) (Werneck et al. 2012); montane forest, between 500 and 1000 m in elevation (M) (Young 1995); seasonally flooded forest (SFF), in which the water table remains at soil surface (or higher) for at least two months (Baraloto et al. 2011); terra firme forest (TFF), including relatively nutrient-rich clayey soils that characterize much of the tropical moist forests in Central America, the Chocó, and the Western Amazon (Hoorn et al. 2010a), in addition to brown sandy soils from the Western Amazon (Fine et al. 2005) and sand–silt–clay mixtures from the Guianas and Brazilian Atlantic Forest (Baraloto et al. 2011); white-sand/restinga forest (WSF), including nutrient-poor white-sand forests of Amazonia and the Guianas (Baraloto et al. 2011) and nutrient-poor sandy-soil restinga forests from the Brazilian Atlantic forest (Lacerda et al. 1993); and seasonal dry tropical forest (SDTF) (Pennington et al. 2010). We did not apply any constraint on movements between habitats.

If habitat specialization contributes to diversification, we predict that habitat specialists to a particular habitat type (e.g., SFF) will not be each other's closest relatives. Instead, we would expect to see that species are commonly most closely related to sister taxa from different habitat types. Moreover, if geological factors foster diversification by promoting habitat specialization, we predict that whenever ancestors occur across multiple habitats, these habitat generalist ancestors should not persist over multiple cladogenesis events, and instead should quickly split into habitat specialist lineages.

DIVERSITY-DEPENDENT CLADOGENESIS

To evaluate whether the Protieae show a pattern of diversification through time consistent with diversity-dependent cladogenesis versus a constant rate of diversification, we examined temporal variation in diversification rates. For this analysis, first we studied the distribution of waiting times, and evaluated whether diversification rates changed through time. If rates changed, we then assessed whether diversity-dependent diversification models could explain the data better than alternative diversification models. To evaluate changes in diversification rate, we used the R package laser (Rabosky 2006) to compute the γ-statistic (Pybus and Harvey 2000). Under a constant rate diversification process with no extinction (Yule 1924), γ follows a standard normal distribution (mean $= 0$), and a decline in diversification rates is inferred when $\gamma < 0$ (Pybus and Harvey 2000). To account for incomplete species sampling potentially biasing the estimation of γ-values, we created a null distribution for γ values (1000 simulations) using the estimated diversification rate under a pure birth (PB) process (Yule 1924) for the total number of known species randomly pruned to the sample size used in this study, and we compared the observed gamma value against this distribution. Because the missing species are almost certainly randomly distributed across the phylogeny (see Appendix S1), we also simulated missing speciation events (Cusimano et al. 2012) under the same PB process, and created 1000 datasets with complete species sampling in R using TreeSim (Stadler 2011). For each simulated dataset, we computed the γ-statistic and report the mean and standard deviation (SD).

Because we rejected a model of constant diversification (see Results), we compared the fit of different likelihood-based models of lineage diversification (Rabosky, 2006; Rabosky and Lovette 2008). Specifically, we fit the following models: PB, BD, logistic (DDL), and exponential (DDX) diversification, and three models that varied either speciation rate (SPVAR), extinction rate (EXVAR), or both (BOTHVAR) through time. We compared the fit of the best rate-variable model and best rate-constant model using the ΔAIC (difference between AIC score for the best rate-constant model and the AIC score of the best rate-variable model). To account for incomplete species sampling, we estimated the maximum-likelihood speciation and extinction rates under a constant rate BD model, and simulated 1000 datasets with complete sampling using TreeSim (Stadler 2011) in R. For each simulated dataset, we computed the AIC values, ΔAIC scores, and inferred parameters for each likelihood-based model.

Extinction can affect the pattern of lineage accumulation through time (LTT) and thus impair our ability to confidently infer diversification models (Quental and Marshall 2010), therefore we evaluated the fit of models that can accommodate extinction (Etienne et al. 2012). Because the best likelihood-based model was DDL (see Results), we fitted a DDL model plus linear dependence in extinction (DDL + E) and contrasted it against a DDL model without extinction (DDL − E) and a DDL model plus linear dependence in speciation and extinction (DDL + SE). For these analyses, we used the library DDD (Etienne and Haegeman 2013) in R.

We predict that we will find an increase in diversification in Protieae that coincides when the lineage enters into new biogeographic zones. After colonizing a new area, if diversification in the Protieae has been influenced by competition among close relatives inhabiting similar niche space and/or coevolution with natural enemies, we predict that a model of density-dependent diversification will best fit the data. Alternatively, if the Protieae lineage is still in the process of filling niches within biogeographic regions, or if natural enemies have not yet evolved to detoxify defenses, we expect to find no slowdown, but instead, a constant rate of diversification.

Results

PHYLOGENETIC ANALYSES

The tribe Protieae is monophyletic with 1.0 posterior probability, and it is sister to tribe Bursereae (Fig. 2). All published sections within the Protieae (*Protium* sections *Icica*, *Icicopsis*, *Marignia*, *Protium*, *Sarcoprotium*, *Pepeanthos*, *Papilloprotium*) are monophyletic with 1.0 posterior probability. The genera *Tetragastris* and *Crepidospermum* are also monophyletic with 1.0 posterior probability but are nested within *Protium*. Statistics for the aligned markers can be found in Table S1. Phylogenetic analyses for each locus supported the same evolutionary history for the Protieae (Figs. S2–S4). The topology of the MRCT summarizing the analysis of the concatenated matrix (Fig. S5) was consistent with the MCCT of the dating analyses (Figs. 2, S6, S7).

DIVERGENCE TIME ESTIMATION

All the estimated MRCT chronograms and Mr. Bayes analyses resulted in the same topology (Figs. 2, S5–S7). Comparing the three dating analyses, we found that the highest posterior densities (HPDs) for the age estimates of all the nodes within the crown Protieae, the focus group of this study, overlapped (Table 1; Figs. 2, S6, S7). In all analyses, the estimated age of crown Protieae falls in the late Oligocene (33–26 Mya), and most of the diversification events within this tribe occur after the mid-to-late Miocene (over the last 15 million years). Because the estimated ages did not differ among dating analyses (i.e., HPDs overlap), and the estimated mean ages in analysis 2 fall between those in analyses 1 and 3, we used the chronogram resulting from this analysis for all subsequent analyses (Fig. 2).

When we allowed diversification rates to vary among lineages using MEDUSA, we found support for a PB (Yule) model with a single rate shift from the background diversification ($r = 0.04$, $\varepsilon = 0$) at the base of the Neotropical Protieae approximately 25 Mya (range: 32–17 Mya, $r = 0.15$, $\varepsilon = 0$, AICc = 650.54; Fig. 2).

BIOGEOGRAPHIC ANALYSES: EVALUATING VICARIANCE AND DISPERSAL, AND DIRECTION OF MOVEMENT IN THE PROTIEAE

The unconstrained hypothesis fit the data better than the alternative hypotheses ($-\ln(L)$: 288.256 null model; $-\ln(L)$: 300.679 model A; $-\ln(L)$: 292.07 model B). The most likely ancestral range for the Protieae was a widespread range including Western Amazon + Paleotropics + Caribbean; however, there was uncertainty in this reconstruction, the splitting event at this node and several internal nodes (Figs. 3, S8). We define uncertainty as when multiple ancestral states and splitting events are within two log-likelihood scores and exhibit relative probabilities < 0.5. With this in mind, we restrict our description of results and discussion only to the most likely reconstructions (relative probability > 0.5). After the isolation of the Paleotropical lineages, a range contraction restricted the MRCA of the crown Neotropical Protieae to the Western Amazon.

The most likely model shows no support for vicariance causing strong geographic structure in the phylogeny. Instead, we see pervasive patterns of dispersal. Within Neotropical Protieae, the overall pattern of range evolution includes 26 range

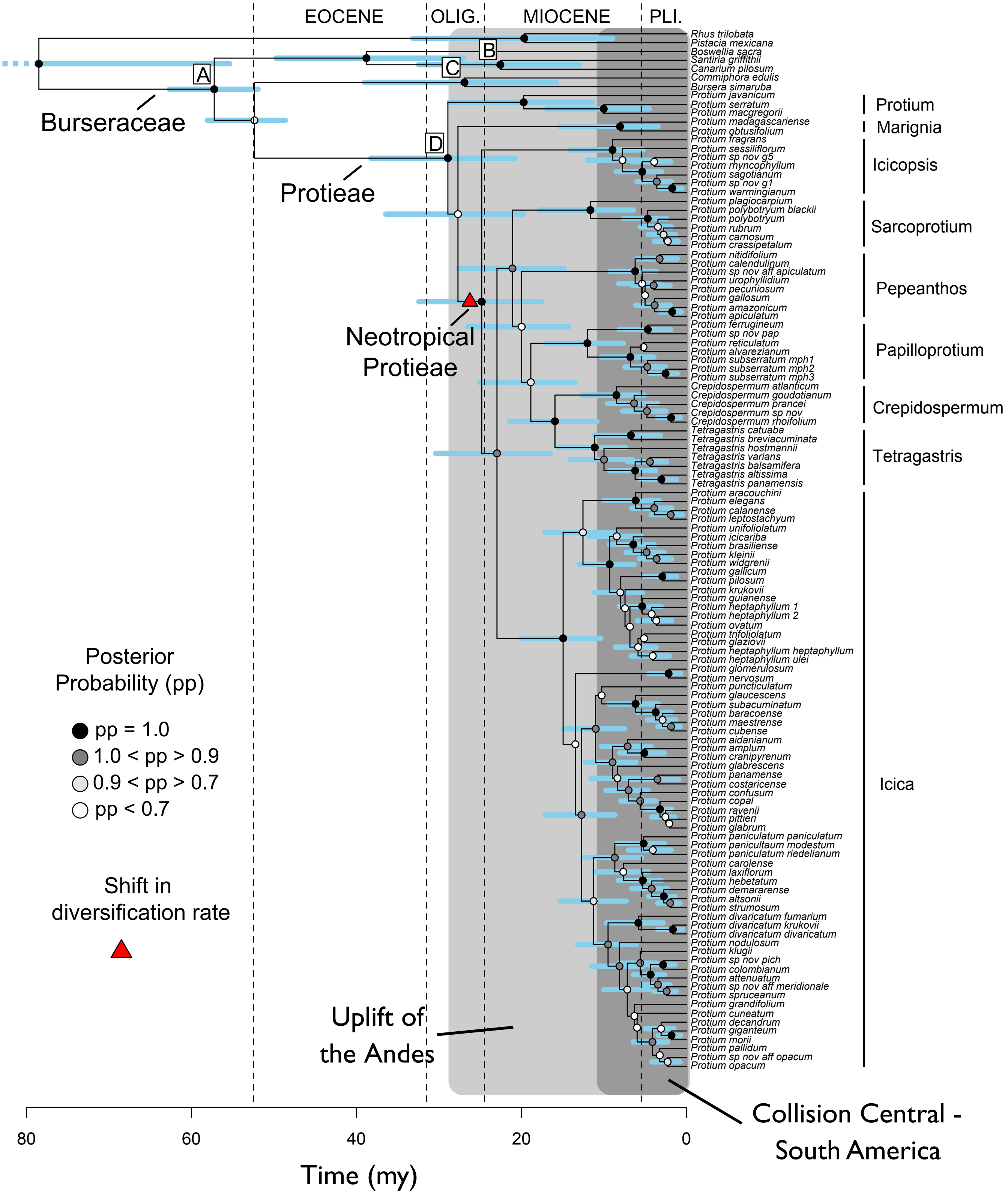

Figure 2. Molecular phylogeny of the tribe Protieae. Maximum clade credibility tree summarizing results of Bayesian dating analysis 2 with a normal probability prior on node A, and exponential probability priors on nodes B–D. Taxonomic sections within the Protieae indicated to the right of the clades. The red triangle indicates an inferred significant shift in diversification rate. The fossil-calibration age estimates for each node are presented in blue, with a time scale on the x-axis, with gray shading corresponding to the likely time frame of Andean uplift and continental collision.

Table 1. Age estimates (in Mya) for the Burseraceae and particular nodes within the tribe Protieae under three calibration scenarios using different prior probabilities to account for uncertainty in calibration points.

	Analysis 1	Analysis 2	Analysis 3
Clade	Mean age (95% HPD)	Mean age (95% HPD)	Mean age (95% HPD)
Burseraceae—crown	63.41 (66.54–60.43)	57.3 (62.66–51.92)	54.22 (59.72–48.84)
Protieae—stem	63.2 (66.8–59.8)	53.43 (59.24–48.60)	48.62 (51.57–46)
Protieae—crown	32.59 (43.1–23.16)	29.13 (38.25–20.78)	25.71 (33.58–18.05)
Tetragastris	12.53 (17.38–8.1)	11.3 (15.51–7.4)	9.89 (13.56–6.32)
Crepidospermum	9.762 (14.21–5.6)	8.68 (12.64–5.11)	7.64 (11.04–4.51)

For description of analyses, see Appendix S2.

expansions, most of them involving expansions from the Western Amazon to the Guianas with subsequent speciation occurring within broad ranges. In contrast, when range expansions occurred from Western Amazon to Brazil's Atlantic Forest, Eastern Amazon, Central America, Chocó, and the Caribbean, speciation largely occurred between areas of the ancestral range, indicating that in these cases range expansions led to allopatric divergence. Most of the remaining speciation events occurred within single areas, mainly in the Western Amazon.

EVALUATING DISPERSAL WITH COMMUNITY PHYLOGENETIC STRUCTURE

The MPD metric revealed significant regional phylogenetic clustering for Central America and the Caribbean (Table 2, Fig. S10), meaning that the phylogenetic distance among all the species within these geographic regions is smaller than the null expectation. The MNTD metric exhibited significant regional phylogenetic clustering for the Chocó, the Guianas, and Eastern and Western Amazon and marginal significance for Central America (Table 2), suggesting recent speciation within these regions. Overall, only Brazil's Atlantic Forest and the Cerrado exhibited no evidence of geographic structure.

EVOLUTION OF HABITAT ASSOCIATION

TFF is the most likely ancestral habitat of the Protieae (Figs. 3, S9). Similarly, at most internal nodes optimal reconstructions were also TFF, but in a few cases other ancestral habitats or combination of habitats were also statistically plausible (within two log-likelihood scores, and high relative probabilities). Overall, we found 13 habitat transitions, all involving habitat expansions from TFF to other habitats, mainly forests on sandy soils (WSF), with widespread ancestors usually persisting for short periods of time before allopatric or parapatric speciation between habitats. There was only one case of habitat extinction from TFF accompanied by dispersal to flooded forests (SFF) in *P. unifoliolatum.* Of the 11 strict habitat specialists to WSF and SFF, 8 of the 9 WSF and both of the 2 SFF specialists were mostly closely related to a habitat specialist from a different habitat, or a habitat generalist. Most sandy soil and flooded soil habitat specialists are found in the Western Amazon.

DIVERSIFICATION ANALYSES

The pattern of LTT is presented in Figure 4. The value for the γ-statistic is -3.8 ($P < 0.05$) indicating that the PB (Yule) model is rejected and that diversification rates have declined over time. This result is robust to incomplete sampling [γ-statistic (simulation of sample size) $= -2.55$, $P < 0.01$; mean γ-statistic (simulation of speciation events) $= -2.57 \pm 0.01$, mean $P < 0.01$)]. The likelihood-based model analyses (Tables 3, S2) show that the data fit the logistic diversity-dependent diversification (DDL) model the best, even when we accounted for incomplete species sampling (99.6% of the 1000 simulated datasets preferred this model), and extinction (Akaike weights: DDL + E = 0.005, DDL − E = 0.76, DDL + SE = 0.23; Table S3). Therefore, our data strongly indicate a slowdown in diversification rates since the Miocene in the tribe Protieae.

Because rate heterogeneity among lineages can confound inferences on changes in diversification rates through time (Rabosky 2010), we repeated all diversification analyses only for the Neotropical Protieae (given the MEDUSA results; see above). These analyses were consistent with the analyses of the full dataset and showed that the DDL diversification model best fit the data, even accounting for incomplete species sampling and extinction (Table S4).

Discussion

BIOGEOGRAPHIC HISTORY OF THE PROTIEAE

Our fossil-calibrated phylogeny and biogeographical reconstructions show that although the Protieae stem age dates to the early Eocene, the crown group dates to the late Oligocene/early Miocene (38–21 Mya), with all of the diversification events within the Neotropical Protieae coming after the end of the Oligocene. The great majority of these cladogenesis events occurred within the last 10 million years and thus broadly coincide with both

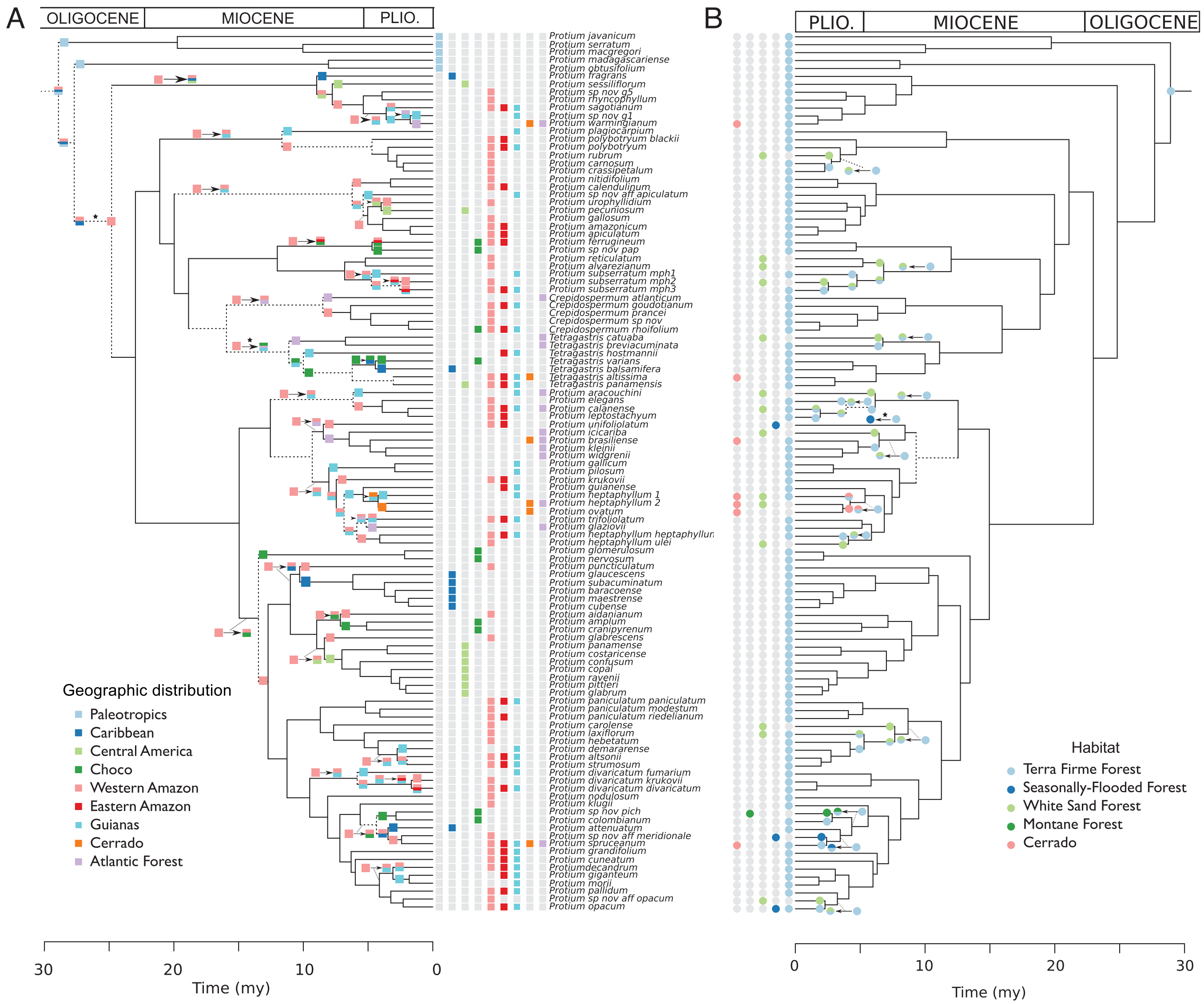

Figure 3. **(A) Maximum-likelihood reconstruction of geographic range evolution under the unconstrained hypothesis. Ancestral ranges are shown at the nodes; ranges inherited following cladogenesis events are shown at the vertices of daughter branches. Dotted-line braches indicate those for which the relative probability of the most likely reconstruction is <0.5 (horizontal branches for alternative ancestral states, vertical branches for alternative inheritances). Range transitions along branches show the inferred sequence of dispersal (arrow) or extinction (star) events. Internal nodes and branches without color indicate no changes in ancestral range. For each species, the extant geographic range is represented by colored boxes.**

(B) Maximum-likelihood reconstruction of habitat evolution. Ancestral habitats are shown at the nodes; habitats inherited following cladogenesis events are shown at the vertices of daughter branches. Dotted-line braches indicate those for which the relative probability of the most likely reconstruction is <0.5 (horizontal branches for alternative ancestral states, vertical branches for alternative inheritances). Habitat transitions along branches show the inferred sequence of dispersal (arrow) or extinction (star) events. Internal nodes and branches without color indicate no changes in ancestral habitat. For each species, the extant habitat is represented by colored circles.

Andean uplift as well as the approach and collision of North and South America. Our results thus generally agree with the synthesis of Hoorn et al. (2010b), suggesting that the Miocene was an especially important time period for Neotropical diversification. However, it is worth noting that of the nine lowland plant clades included in the synthesis of Hoorn et al. (2010b), Protieae's crown age is dated much older than all of them except *Anaxagorea* (Annonaceae), a small group of 21 species (Scharaschkin and Doyle 2006). Other diverse (100+ taxa) Neotropical lineages such as *Inga* (Fabaceae; 300+ species), *Guatteria* (Annonaceae; 150+ species) and *Mimosa* (Fabaceae; 500+ species) that have been subjected to phylogenetic analysis have reported crown ages within the range of 10–5 Mya (Richardson et al. 2001; Erkens et al. 2007; Simon et al. 2009).

Table 2. Results of community phylogenetic structure within regions.

Region	*N*	Obs. MPD	SES MPD	*P*	Obs. MNTD	SES MNTD	*P*
AF	13	34.53	−1.34	0.106	17.50	−1.20	0.108
CA	10	30.12	**−2.22**	**0.032**	17.47	−1.58	0.059
CB	8	29.90	**−1.98**	**0.032**	19.27	−1.23	0.12
CE	6	37.74	−0.29	0.343	26.45	−0.19	0.390
CH	10	36.27	−0.66	0.241	12.30	**−2.64**	**0.003**
EA	32	37.28	−0.87	0.197	12.00	**−2.04**	**0.021**
GU	34	36.31	−1.46	0.083	11.13	**−2.50**	**0.009**
PT	5	46.21	1.24	0.916	22.37	−1.04	0.157
WA	56	37.98	−0.82	0.199	8.72	**−3.98**	**0.001**

Note: AF = Atlantic Forest; CA = Central America; CB = Caribbean; CE = Cerrado; CH = Choco; EA = Eastern Amazon; GU = Guyana; PT = Paleotropics; WA = Western Amazon.

N = number of taxa in each region; Obs. MPD = observed mean pairwise distance in areas; SES MPD = standardized effect size of mean pairwise distance versus null model; Obs. MNTD = observed mean nearest taxon distance; SES MNTD = standardized effect size of mean nearest taxon distance versus null model; *P* = *P*-value of observed MPD versus null model. Significant results in boldface.

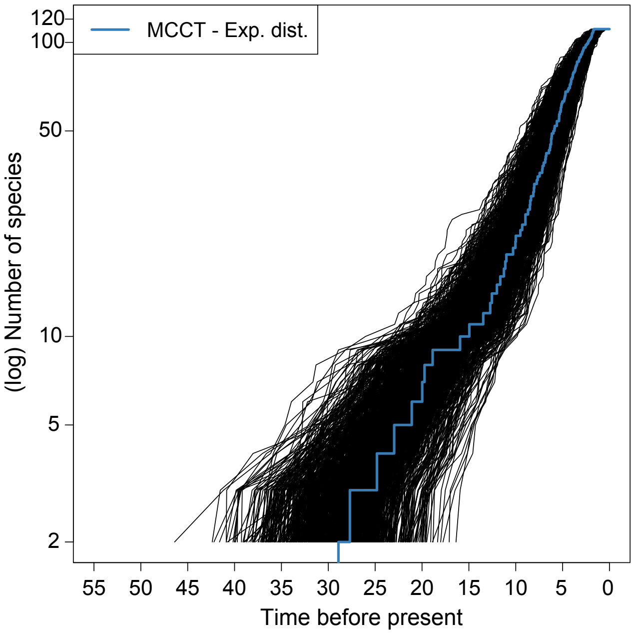

Figure 4. Lineage-through-time (LTT) plot obtained using dating analysis 2 (normal prior distribution on node A, exponential prior distribution on nodes B–D, see Fig. 2), along with the LTT plots obtained from 1000 random trees sampled from the posterior probability distribution.

Our biogeographic reconstruction results are largely consistent with previous work on the Burseraceae as well as other tropical plant lineages that have extant distributions in both the Paleo- and Neotropics but a fossil record in North America and/or Europe (Laurasia). A fossil-calibrated biogeographic reconstruction using molecular data of all of the major lineages of Burseraceae found that the likeliest scenario was a Paleocene North American origin (Weeks et al. 2005). Indeed, the Protieae's oldest fossil is from the London Clay dated 50–56 Mya and the next oldest fossil is a 44 Mya record from Oregon in North America of a fossil endocarp that closely resembles Protieae (Manchester 1994). We date the large split in the Protieae between the Paleotropics and Neotropics in our fossil-calibrated phylogeny as late Oligocene/early Miocene (32–17 Mya), when the world was becoming significantly cooler and drier and the high-latitude tropical forests contracted toward equatorial regions (Willis and McElwain 2002).

In addition, we found a significant increase in diversification rate at the base of the Neotropical Protieae clade in the late Oligocene/early Miocene, corresponding with the arrival of the clade to South America. This suggests that after Protieae inhabited North America and Europe during the thermal maximum in the Eocene, ancestors most likely dispersed across the sea separating southern North America from South America. This scenario matches interpretations by other studies that have sought to explain pantropical distributions in lowland rain forest Rubiaceae and Malpighiaceae lineages by finding evidence for dispersal to and from South America via Laurasia, termed the boreotropical connection hypothesis (Davis et al. 2002; Antonelli et al. 2009; Antonelli and Sanmartín 2011b).

Interestingly, however, our biogeographic reconstruction strongly suggests that the Neotropical Protieae that inhabit Central America and the Caribbean all derived from Amazonian ancestors. This is curious because the (admittedly scanty) fossil record of the Protieae is dominated by Central American, Caribbean, and North American records in the Oligocene and Miocene. The only Neotropical Protieae macrofossil was recently described from an amber deposit from the Dominican Republic dated 15–30 million years old (Chambers and Poinar 2013). The two oldest confirmed Protieae microfossils from the Neotropics are reported by Graham (1985) from the late Eocene of Panama and one reported by Jaramillo et al. (2014) from Panama from 11 Mya, as many previously reported Protieae fossil pollen records (e.g., those reviewed by Harley and Daly 1995) appear to be Sapotaceae (C. Jaramillo, pers. comm.).

DISPERSAL OF NEOTROPICAL PROTIEAE AND PATTERNS OF GEOGRAPHIC STRUCTURE

We found very little support for vicariance as a process influencing diversification in the Protieae. It is worth mentioning that we were not able to sample any of the four Andean montane species that may have arisen due to vicariance. In contrast, long-distance dispersal across biogeographic regions appears to have played

Table 3. Results of likelihood-based models of lineage diversification analyses showing the empirical AIC values for each model, the mean AICs (with SD), and percentage of the 1000 simulated phylogenies with complete sampling for which a particular model fit best.

	PB	BD	DDL	DDX	SPVAR	EXVAR	BOTHVAR
Empirical	−164.889	−162.889	**−178.779**	−165.726	−162.992	−160.749	−161.087
Simulation—mean	−378.003	−376.003	**−383.567**	−376.870	−374.343	−373.904	−372.421
Simulation—SD	6.95	6.95	**5.91**	6.59	6.68	6.96	6.67
Simulation—% best fitting	0.4	0	**99.6**	0	0	0	0

Boldface indicates preferred model. For model description, see Table S2.

a very important role for lowland taxa (which represent more than 96% of the Protieae). Taking into account the limited fossil record, we hypothesize that the Protieae immigrated to South America at least once in the Miocene either from Africa, the Caribbean, or Central America, underwent a significant radiation in the Amazon and dispersed from South America to Central America at least three separate times (5–10 Mya). Moreover, the eight endemic Caribbean species are the product of at least four different colonization events. Dispersal from the Amazon to the Brazilian Atlantic forest (5–10 Mya) and the Cerrado biome (3–5 Mya) each was accomplished at least three times. Such widespread dispersal has been achieved by means of the Protieae's small endozoochorous fruits, which are abundantly produced and attract a wide variety of birds, bats, and terrestrial mammal species (Daly 1987a). Thus, relatively recent migration between Central and South America appears to have been an important contributor to Protieae diversity, in accord with the hypothesis of Raven and Axelrod (1974). However, the direction of dispersal of the Protieae agrees with the prediction of Gentry (1982) that most Central American rain forest trees originated from Amazonian stock.

Patterns of geographic structure in phylogenies can suggest the timing and sequence of important dispersal events among regions (Pennington et al. 2006). We found significant patterns of regional phylogenetic structure for the Protieae in almost all of the Neotropical biogeographic regions (Table 2). Most of these significant results were from nearest-neighbor comparisons, which are heavily weighted by relatedness toward the tips of a phylogeny and are thought to reflect recent speciation within regions (Fine and Kembel 2011), meaning that within most regions we find co-occurrence of closely related species. Our results differ from phylogenetic community structure analyses of seasonally dry forest lineages, which typically exhibit deeper-level structure representing more ancient dispersal events among fragments of dry forest, followed by small in situ radiations, resulting in tree-wide phylogenetic clustering within regions (Lavin et al. 2004; De-Nova et al. 2012; Särkinen et al. 2012). By contrast, it is clear that all lineages of Neotropical Protieae have been capable of dispersing to all regions, resulting in very few tree-wide patterns of phylogenetic clustering within regions. For example, sections *Icica* and *Icicopsis* plus *Tetragastris* all include species occurring in the Caribbean, Central America, the Western and Eastern Amazon, the Guianas, and the Brazilian Atlantic Forest. However, unlike other lineages of Neotropical moist/wet forest plants (e.g., *Inga*, *Guatteria*, *Psychotria*, *Renealmia*), which have yielded no geographic structure whatsoever (Pennington and Dick 2010; Hughes et al. 2013), we find pervasive phylogenetic community structure toward the tips, suggesting that recent speciation has occurred within regions.

THE IMPORTANCE OF HABITAT SPECIALIZATION IN PROTIEAE EVOLUTION

We found that there are multiple, independent instances of habitat specialization related to different soil types, especially in Western Amazonia (Fig. 3). All habitat transitions occur in the absence of geographic barriers, thus our results are consistent with the hypothesis that parapatric speciation across edaphic and ecological gradients is an important driver of speciation, leading to the notable habitat specialization present in the Protieae (Fine et al. 2005, 2013a,b). Speciation by means of habitat specialization during the Miocene also is consistent with our finding of regional phylogenetic clustering toward the tips in Amazonia.

Recent integrative studies on the geological and biogeographic history of the Amazon have hypothesized that during the Miocene the formation and subsequent draining of the Pebas wetland was an important event that contributed to rates of biotic diversification (Hoorn et al. 2010b; Antonelli and Sanmartín 2011a; Roncal et al. 2013). As a result of the Andean uplift, almost the entire Western Amazon is hypothesized to have been covered by a massive wetland system in the middle Miocene, from approximately 10 to 20 Mya, that drained 7–10 Mya and subsequently was covered by Andean-derived sediments (Hoorn et al. 2010a,b). During the Miocene flooding, plant and animal species not adapted to wetland conditions would have been driven into *terra firme* refuges, which likely would have elevated extinction rates but could have also fragmented previously contiguous populations and encouraged some allopatric speciation (Antonelli et al. 2009). With the draining of the swamps during the late Miocene, vast areas of *terra firme* habitat would have been available for recolonization, presumably spurring diversification.

An additional consideration is that the new sediments laid down in the late Miocene represented a novel type of habitat for the Amazon basin because of their relatively high soil fertility (Hoorn 1993; Fine et al. 2005). Although sand and clay soils have always been present in Northern South America, Andean uplift and volcanism and the subsequent erosion of large amounts of sediment resulted in large areas of clay soils that had a high cation exchange capacity and nutrient availability that had not previously existed in the region for perhaps a hundred million years or more.

Moreover, the fact that these new high-resource sediments occurred within a mosaic of older, nutrient-poor soil types (including white sands) could have increased divergent selection across habitat boundaries and thus increased habitat specialization and speciation. This is because plants experience strong trade-offs in growth and defense allocation across resource gradients. Plants are hypothesized to be selected for high defense allocation in low-resource habitats because of the high cost of replacing tissue lost to natural enemies, whereas in high-resource habitats, fast growth may be able to outpace enemy attack (Coley et al. 1985). The larger the difference in resource availability between high- and low-resource habitats in a region, the stronger the trade-off should be, thus the stronger divergent selection leading to habitat specialization.

Recent studies have noted that Western Amazonian soils exhibit larger contrasts between low- and high-resource soil types (Quesada et al. 2009; Baraloto et al. 2011) than the Eastern Amazonian or Guianan regions, which contain *terra firme* areas with extremely low-nutrient sandy soils adjacent to low-to-medium fertility clay soils. In accord with this pattern, Fine et al. (2006) found that white sand and clay habitat specialists exhibited divergent growth and defense allocation in a reciprocal transplant experiment near Iquitos, Peru, while a similar experiment found no such trade-off in the Rio Negro basin of northern Brazil where nutrient-rich clay soils are absent (Stropp et al. 2014). Similarly, Central America and the Caribbean do not have large areas of extremely nutrient-poor soils, nor large areas covered by seasonally flooded habitats, thus habitat specialization to oligotrophic or seasonally flooded environments may be a very rare occurrence in these regions. We thus speculate that the high regional diversity of Protieae in Amazonia compared to other Neotropical regions is in part due to the presence of multiple habitats that span a large range of resource availability.

DIVERSIFICATION OF THE PROTIEAE THROUGH TIME

Colonization of the Neotropics by the Protieae was accompanied by an increase in diversification rate at approximately 25 Mya (range 32–17 Mya; Fig. 2). After the rate shift in the late Oligocene–early Miocene, a decline in diversification rate occurred (Fig. 4, Table 3). This is consistent with the hypothesis of increased diversification with ecological opportunity upon arrival to a new geographic region (i.e., South America), and gradual deceleration in diversification either because niches have become filled (Simpson 1953), or natural enemies have adapted to detoxify novel plant defenses (Ehrlich and Raven 1964). It is possible that Protieae have by now reached their “carrying capacity” or no longer have effective defenses against specialist enemies in the Neotropics, accounting for the more recent slowdown in diversification. Moreover, the studies that have been undertaken to date to characterize defense chemistry in the Protieae have revealed remarkable variation within the clade as well as between sister taxa (Fine et al. 2006, 2013b). This diversity in chemical defense is consistent with the hypothesis that interactions with natural enemies have been important drivers of adaptation in these plants and could be consistent with a decline in diversification toward the recent.

Rabosky and Lovette (2008) have suggested that an alternative hypothesis to the ecological opportunity model is the indirect effect that other factors may have on diversification, such as geographic range size (Rosenzweig 1995). Previous work has shown that biotic interactions may exert a direct effect on the geographic and ecological setting of species within the Protieae by promoting local adaptation in the Western Amazon (Fine et al. 2013b). If these interactions limit geographic range size and thus increase extinction rates, a pattern of decline in diversification rate through time would be expected. To further test these hypotheses, it will be necessary to include detailed information on the area of each species’ geographic distribution to test the relationship of range size and diversification rate.

CONCLUSIONS

The molecular phylogeny of the Protieae that we have reconstructed provides new insights into the biogeographic history of an extremely diverse and ecologically dominant group of rain forest trees and of Amazonia in general. Our results demonstrate that dispersal to South America appears to be the most significant event for the radiation of the Protieae, and this colonization appears to substantially predate collision of North and Central America. Once in South America, the clade diversified to a great degree in the Amazon basin, during the window of time in which Andean uplift was most likely to have been important in generating novel habitats. Next, after diversifying in the Amazon, the Protieae dispersed to many other biogeographic regions within the past 10 Mya and speciated, precipitating a secondary wave of diversification toward the recent even as the entire clade experienced a slowdown, consistent with diversity-dependent cladogenesis. We conclude that long-distance dispersal, biotic interactions, and habitat specialization are thus the most important processes underlying the diversification of this hyperdiverse clade of Neotropical trees.

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DATA ARCHIVING

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Appendix S1. List of recognized Protieae species, their taxonomic section, their biogeographic range, and their voucher and herbarium information and GenBank accession numbers.
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