



## Species tree phylogeny and biogeography of the Neotropical genus *Pradosia* (Sapotaceae, Chrysophylloideae)



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### ABSTRACT

Recent phylogenetic studies in Sapotaceae have demonstrated that many genera need to be redefined to better correspond to natural groups. The Neotropical genus *Pradosia* is believed to be monophyletic and includes 26 recognized species. Here we reconstruct the generic phylogeny by a species-tree approach using \*BEAST, 21 recognized species (36 accessions), sequence data from three nuclear markers (ITS, ETS, and RPB2), a relaxed lognormal clock model, and a fossil calibration. We explore the evolution of five selected morphological characters, reconstruct the evolution of habitat (white-sand vs. clayish soils) preference, as well as space and time by using a recently developed continuous diffusion model in biogeography. We find *Pradosia* to be monophyletic in its current circumscription and to have originated in the Amazon basin at ~47.5 Ma. Selected morphological characters are useful to readily distinguish three clades. Preferences to white-sand and/or clay are somewhat important for the majority of species, but speciation has not been powered by habitat shifts. *Pradosia brevipes* is a relative young species (~1.3 Ma) that has evolved a unique geoxylic life strategy within *Pradosia* and is restricted to savannahs. Molecular dating and phylogenetic pattern indicate that *Pradosia* reached the Brazilian Atlantic coast at least three times: at 34.4 Ma (*P. longipedicellata*), at 11.7 Ma (*P. kuhlmannii*), and at 3.9 Ma (weakly supported node within the red-flowered clade).

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### 1. Introduction

Neotropical rainforests have higher plant species diversity than any other place on Earth (Richardson et al., 2001; Antonelli and Sanmartín, 2011; Baraloto et al., 2012; Fouquet et al., 2012b). The Amazon region may contain approximately 16,000 species of trees (ter Steege et al., 2013), and more than 250 species of trees may be found in just a single hectare in Central Amazon (Oliveira and Nelson, 2001). However, the evolutionary history of such high diversity in flowering plants remains poorly understood, particularly because there are still few species-level phylogenetic studies for the Amazonian flora (Pennington et al., 2006b; Hopkins, 2007; Fiaschi and Pirani, 2009; Hoorn et al., 2010).

The plant family Sapotaceae is widely distributed in the tropics (Bartish et al., 2005; Swenson et al., 2008a,b) and is an important component of rainforests of South America (ter Steege et al.,

2000). In a single hectare of lowland forest in the Central Amazon, near Manaus, one may encounter 44 trees (DBH > 10 cm) from 23 different species of Sapotaceae, making it one of the most abundant and species-rich families in such forests (Milliken, 1998; Oliveira and Daly, 1999; Oliveira and Nelson, 2001). However, classification of Sapotaceae has been notoriously difficult and different systems have been proposed (Baehni, 1938, 1965; Lam, 1939; Aubréville, 1964; Pennington, 1990, 1991), leading to unnatural and practically inconvenient classifications, mainly due to high degrees of morphological homoplasy (Swenson and Anderberg, 2005; Swenson et al., 2008a,b). Furthermore, circumscriptions of genera and species are prone to large uncertainties (Terra-Araujo et al., 2012b; Gomes et al., 2013), while conservation assessments have suggested that many species of Neotropical Sapotaceae are extinct, threatened to extinction, or endangered (IUCN Red List, 2013).

Pennington (1990) revised Sapotaceae for the Neotropics, but over the last decade numerous additional species have been described (Pennington, 2006; Alves-Araújo and Alves, 2011, 2012a,b; Morales, 2012; Terra-Araujo et al., 2012a, 2013). Most of these belong to the subfamily Chrysophylloideae (Swenson

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and Anderberg, 2005), but the relationships among the groups in the subfamily are still poorly understood, and some of the genera sensu Pennington (1990) are polyphyletic in their current circumscription (Swenson et al., 2008a). Recent efforts based on an increased number of sampled taxa and nuclear sequence data have confirmed that the larger genera *Pouteria* and *Chrysophyllum* are unnatural assemblages, but that *Pradosia* is monophyletic if the African species *P. spinosa* (Ewango and Breteler, 2001) is excluded (A.D. Faria, pers. comm.). However, these studies included only two or three species of *Pradosia* and given the fact that previous generic limits across the subfamily have not corresponded to natural groups, testing the monophyly of *Pradosia* requires broader taxon sampling.

*Pradosia* comprises 26 species and is distinguished by a single morphological character: a drupaceous fruit with a thin cartilaginous endocarp (Fig. 1L and M), which is absent in the rest of the subfamily (Terra-Araujo et al., 2013). The genus varies in habit from geoxylic shrubs (one species) to medium-sized and large trees. It is endemic to South America with a single species (*P. atrovioleacea*) extending into Panama and Costa Rica (Pennington, 1991). Species are distributed across a wide variety of habitats, including savannahs and evergreen to deciduous forests in the Amazon region, the Brazilian Atlantic coast, and the Andes. The Amazon and Atlantic coast are the two major centers of diversity for Sapotaceae (Pennington, 1990; Alves-Araújo and Alves, 2011; Terra-Araujo et al., 2013), where 80% of the species occur. The biogeographical connections between these regions are still poorly understood and, to date, only a handful of studies have estimated divergence times between taxa from these regions, finding that organisms in the two areas have been isolated from each other for several million years (Vicentini, 2007; Fiaschi and Pirani, 2009; Pellegrino et al., 2011; Fouquet et al., 2012a,b). Furthermore, *Pradosia* includes species inhabiting nutrient-poor white-sand forests, others that are restricted to forests on clay, and yet others that occur in periodically flooded forests. This is an interesting abiotic distribution since studies have proposed that habitat diversity in the Amazon contributes to plant species diversification (Levin, 2004; Fine et al., 2005), however, this has never been explored in Sapotaceae.

The goal of the present study was to test the monophyly and estimate a phylogenetic species tree for *Pradosia* using nuclear data (ETS, ITS and RPB2). Next, for clade diagnostic purposes, a selection of morphological traits considered useful in distinguishing species within the genus was mapped onto the phylogeny. In addition, we investigated the evolution of habitat preference to the species and explored the biogeographic connections between the Amazon and the Atlantic coast by estimating divergence times within the genus. Ancestral areas of common ancestors in the phylogeny were optimized using both a discrete approach (Lemey et al., 2009), as well as a continuous character on the posterior distribution of species trees (Nylinder et al., 2014).

## 2. Material and methods

### 2.1. Taxon sampling

This study included samples for 18 of the 23 species of *Pradosia* recognized by Pennington (1990), plus three recently described species (Alves-Araújo and Alves, 2012a; Terra-Araujo et al., 2012a, 2013). Ten of the 21 species were represented by two or more accessions (i.e. *P. atrovioleacea*, *P. cochlearia*, *P. decipiens*, *P. granulosa*, *P. kuhlmannii*, *P. lactescens*, *P. ptychandra*, *P. restingae*, *P. schomburgkiana* and *P. verrucosa*). We opted for sampling accessions from distant geographic locations for widespread taxa. Material of four species was unavailable, including *P. argentea*

and *P. glaziovii*, two narrow endemics known only from the type collections with doubtful collection sites dating back 100 and 180 years, respectively; and *P. beardii* and *P. huberi* for which DNA samples could not be obtained. Outgroups were chosen based on previous phylogenetic findings that the putative closest relatives of *Pradosia* is *Chromolucuma*, *Micropholis* or *Pouteria* (Swenson et al., 2008a). All taxa, voucher information, and accession numbers are listed in Appendix A.

### 2.2. DNA sequencing and alignment

Genomic DNA was extracted from ca. 20 mg of silica dried leaf material for 41 accessions, and from seven additional herbarium specimens. Extractions were obtained using the Qiagen DNeasy Plant Mini kit using manufacturers protocol, with an additional elution for old herbarium material. Vouchers were deposited in the INPA, NY, and S herbaria. We also included two additional published accessions of *Pradosia ptychandra* of which only ITS sequences are available (Gonzalez et al., 2009).

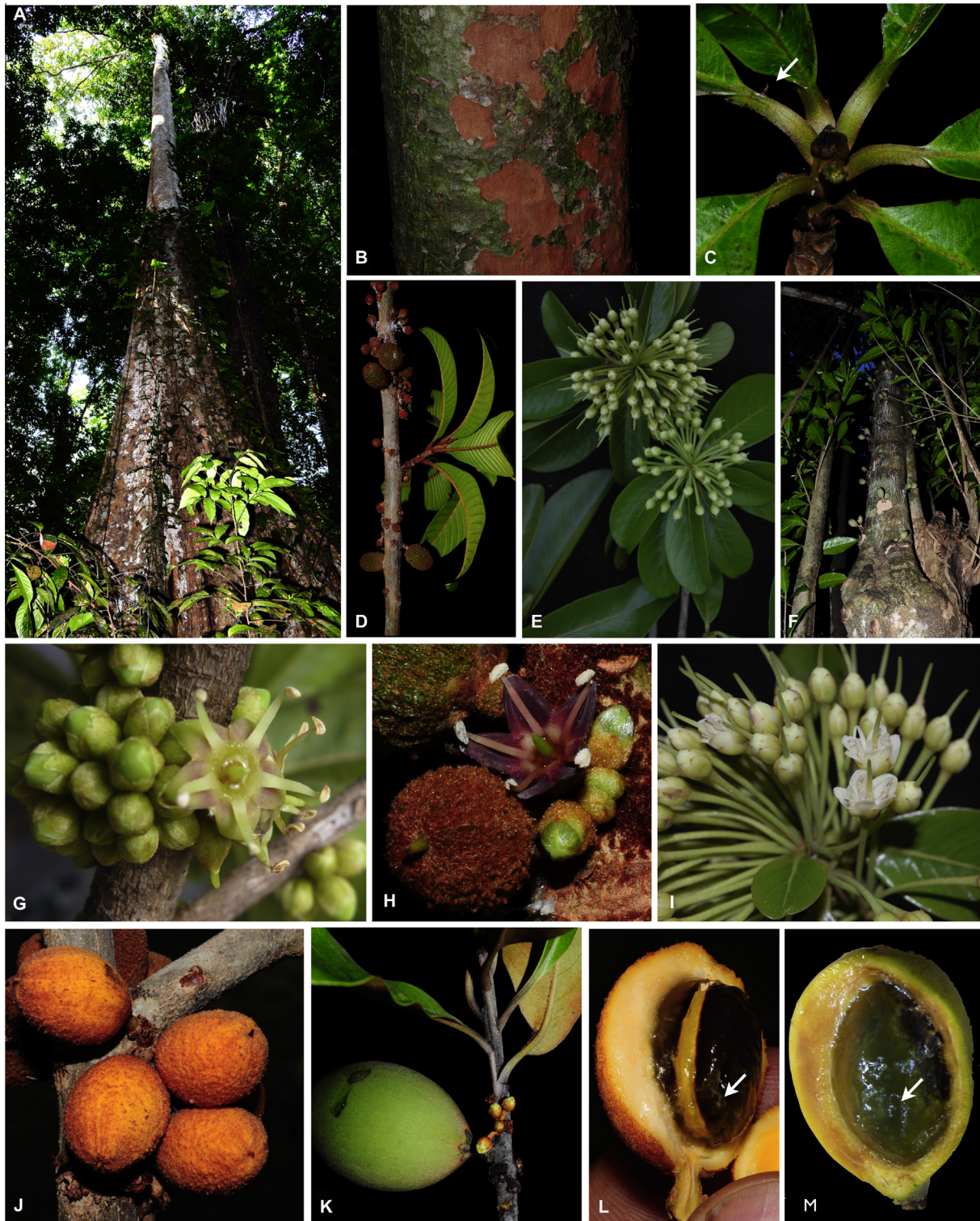
Molecular sequence data for three different nuclear regions was extracted: the external transcribed spacer (ETS), the internal transcribed spacers 1 and 2 (ITS), and the low copy nuclear gene RPB2 (Oxelman and Bremer, 2000). These markers were selected since they have previously been proven useful for phylogenetic inference in Sapotaceae (Bartish et al., 2005; Swenson et al., 2008a,b, 2013). Extraction, amplification, and primers of ETS, ITS, and RPB2 followed the protocol reported by Swenson et al. (2013), but see also Swenson et al. (2008a) for ETS and Bartish et al. (2005) for ITS. The PCR products were purified using the ExoSap clean-up kit (Fermentas, St. Leon-Rot, Germany), and sequenced on an ABI 3730 Capillary Electrophoresis Genetic Analyzer with ABI BigDye Terminator v3.1 Cycle Sequencing chemistry (Applied Biosystems Inc., Foster City, CA). Sequences were assembled using the Staden software packages Pregap4 and Gap4 (Staden et al., 1998). Alignments were performed in MEGA vs. 5.05 using MUSCLE (Edgar, 2004). A 45 base pair segment of ITS was excluded due to ambiguous alignment.

Each marker was individually tested for the best-fit substitution model using jModelTest 0.1.1 (Posada, 2008) under the Akaike Information Criterion (AIC; Akaike, 1974). Estimated parameters values and log-likelihood scores for the different models were calculated based on three schemes of model substitutions optimized on an ML tree. The best fit model for each of ITS, ETS and RPB2 was the HKY + G. Unambiguous gaps of two or more base pairs in length were coded as additional simple binary characters following the guidelines of Simmons and Ochoterena (2000), treated as a separate partition, and analyzed under a simple substitution model.

### 2.3. Node calibration

To calibrate the *Pradosia* phylogeny we adapted the same fossils used by Bartish et al. (2011) who estimated divergence times for Chrysophylloideae. *Psilatricolporites maculosus* is a microfossil that appears in a sequence of sediments from the Paleocene/Eocene transition (~55 Ma) in the Maracaibo Basin of Western Venezuela (the oldest reported dated representative of Chrysophylloideae from the New World), and from the Early Eocene in Colombia (Rull, 2000; Jaramillo and Dilcher, 2001). The second fossil is a leaf from the Cerrejón Formation in Colombia (Wing et al., 2009), dated to the Late Paleocene (~58 Ma) and is suggested to belong to Sapotaceae. Since generic limits in Neotropical Sapotaceae are still largely unclear (Swenson et al., 2008a), but all taxa in the area together with some African species constitute a monophyletic group (Bartish et al., 2011), we believe the safest calibration was to constrain the root age to 58 Ma,





**Fig. 1.** Field images of *Pradosia* (Sapotaceae, Chrysophylloideae). (A) *P. cochlearia*, a canopy tree growing in rainforests of the Amazon region; (B) bark of *P. verticillata* showing a typical pattern for the genus; (C) scales on the petioles of *P. lahoziana*; (D–F) flowers appear in fascicles on branches (D), shoot apices, (E) or trunks (F); rotate flower of *P. restingae* (G), *P. granulosa* (H) and cup-shaped flowers of *P. longipedicellata* (I); fruits of *P. granulosa* (J) and *P. cochlearia* (K); (L and M) transection of fruits showing the half-transparent cartilaginous endocarp in (L) *P. granulosa* and (M) *P. surinamensis*. Photos: Anderson Alves-Araújo (G), Ricardo I. Barbosa (M), Jomar Jardim (E, I), and Mário H. Terra-Araujo (A–D, F, H, J–L).

modeling the uncertainty in emergence of the group by an exponential decay (mean 1.68, upper 97.5% credibility interval: 64.2 Ma). These parameter values are congruent with the age of

Neotropical Chrysophylloideae (Clade G) estimated by [Bartish et al. \(2011\)](#). No other reliable internal calibration is presently advisable.

## 2.4. Operational areas

In order to obtain full distributions of each taxon, herbaria material from Brazil (ALCB, CEPEC, CVRD, HRB, INPA, IPA, MBML, MG, PEUFR, RB, SP, UEFS), USA (CA, PH, NY, US), and Europe (S) was scored for collection localities. A geo-reference database was assembled from label information of 275 specimens, which then was used to generate distribution maps by using the MAPS packages in R (R Core Team, 2012). This enabled the identification of six main areas of distribution clusters: (1) Amazon basin, (2) Brazilian Atlantic coast, (3) Central Brazil–Eastern Paraguay, (4) Ecuador–Peru, (5) Chocó region, and (6) North South America–Panama–Costa Rica. All areas are well delimited geographically as well as ecologically and only one species, *P. atrovioleacea*, is present in more than one area (the Amazon and Panama/Costa Rica). Species in the Amazon basin and in the Brazilian Atlantic coast (from Pernambuco in the north to the Paraná State in the south) occur almost exclusively in wet lowland forests, but a northeast–southwest drier climatic barrier, the *Cerrado-Caatinga* biome, separates these areas. Two endemic species (i.e. *P. montana* and *P. cuatrecasii*) are found in two distinct areas: (1) *P. montana* occurs in tropical deciduous forests along the Pacific coast of Western Ecuador and Northwestern Peru, an important zone of endemism (Linares-Palomino et al., 2010); (2) *P. cuatrecasii* is only known from wet lowland forests in the Chocó, a region that is isolated from the Amazon by the Western Cordillera of Colombia (Burnham and Graham, 1999; Frasier et al., 2008). Lastly, in the northern range of the distribution, the area of South America–Panama–Costa Rica, species of *Pradosia* occur in wet montane or deciduous forests. This area is separated from the Amazon by a dry climatic zone similar to the *Cerrado* (Llanos) and by the Eastern cordillera of the Andes in Colombia that reaches an altitude of 2000 m above the sea level, and from the Ecuador–Peruvian area by the North Andes.

## 2.5. Species tree inference

Phylogenetic relationships and support between species were estimated with Bayesian inference (BI; Rannala and Yang, 1996; Yang and Rannala, 1997), using the multi-species coalescent module implemented in BEAST 1.7.5. (Heled and Drummond, 2010; Drummond et al., 2012). Each locus (ITS, ETS and RPB2) was treated as a unique partition using a relaxed lognormal clock model, best fitting substitution models, and a Yule tree prior (Yule, 1925). Weakly informative priors for substitution rates of all included genes were set using exponential distributions with mean 0.1. The prior on mean species tree population size was set in a similar fashion to the priors on substitution rates but with an exponential distribution with mean 1.0. Since the standard solution for species tree inference implemented in BEAST (Heled and Drummond, 2010) is conditioned on pre-defined terminals, all included specimens were assigned to species according to individual taxonomic assessments. The Monte Carlo Markov chains (MCMC) were set to run three times for 50 million generations each, logging tree parameters every 25,000 generations (BEAST input file supplied as Supplementary data). Chain mixing and convergence were checked in Tracer v.1.5 (Rambaut and Drummond, 2009) to confirm that the estimated sample sizes (ESS) values for all parameters were >200. The posterior distribution of trees from the three runs were combined after removal of a proportion of each run as burn-in, and a maximum clade credibility (MCC) tree was calculated in TreeAnnotator (part of the BEAST package). The MCC tree with 95% highest posterior density (HPD) intervals of divergence time estimates were prepared in FigTree v.1.3.1. (Rambaut, 2009).

## 2.6. Discrete ancestral area, habitat and morphological character reconstruction

To reconstruct the biogeography pattern of *Pradosia*, ancestral states of habitat and morphology, an analysis file was set up in BEAUTi 1.7.5 (part of the BEAST package). We preferred to optimize morphological traits and geography as discrete units on the species phylogeny rather than the gene trees in order to preserve the relationships between the diagnostic characters and species, rather than specimens. In order to do this, and since the current implementation of species tree inference in BEAST does not allow for observing how nodes in the species trees are updated in the MCMC, optimization had to be performed on the empirical posterior distribution of inferred species trees. Geographic area, morphological character states and habitat preference, were added to the analysis as discrete traits each modeled by an individual symmetric rate matrix with equal probability of transition between any two states (Lemey et al., 2009). For characters with more than four states we used the option to optimize the rate matrices by Bayesian Stochastic Search Variable Selection (BSSVS) using default Poisson priors. To count the number of minimum changes for each trait across all sampled topologies we used a stochastic mapping approach (Minin and Suchard, 2008), under the assumption of non-informative prior information on character change rate. The traits were run three times in BEAST for 10 million generations, sampling parameters every 5000 generations, and the result was visualized as described above.

Morphological characters and habitat preferences were assembled from field observations combined with published data (Pennington, 1990, 1991, 2006) (Table 1). The five morphological characters are: bark property, midvein characteristics, distribution of fascicles, size of flowers, and flower colour. Several species of *Pradosia* have a sweet tasting inner bark, and some species are indeed locally known as *casca-doce* (“sweet-bark”) (Ducke, 1942). The midvein character is scored according to being flat/raised or sunken. When sunken, it may be below the level of the adjacent lamina. Fascicles of flowers are scored whether they appear along the trunk (cauliflorous); axillary; along the branches (ramiflorous) on old wood below the leaves without any adjacent leaf scar; or at the apices of the branches (terminal) (Fig. 1D and E). Flower size is scored according to corolla length, with an arbitrary limit set to 4 mm to distinguish between small and larger flowers. We also scored the colour of the corolla as a final character (Fig. 1G–I).

Habitat was divided into four classes reflecting the range of different ecological and vegetation adaptations: (1) forests dominated by deciduous trees, which includes open formations of higher altitude (0–1900 m.a.s.l.), ranging from tall to stunted humid-dry forests to shrublands and grasslands, usually with pronounced seasonality in rainfall, interrupted by several months of drought; (2) tropical savannah (i.e. *Cerrado* forest), comprising areas of dense forest with closed canopy as well as open scrubland with few trees or grassland with scattered shrubs, usually on sandy

**Table 1**

Characters states used in the ancestral reconstruction of habitat and selected morphological traits of *Pradosia* (Sapotaceae, Chrysophylloideae).

Characters states
1. Bark having a (0) sweet taste, (1) non-sweet taste
2. Leaf midvein on the adaxial surface (0) flat/raised, (1) sunken
3. Fascicles of flowers being (0) cauliflorous, (1) axillary, (2) ramiflorous, (3) terminal
4. Length of corolla (0) <4 mm, (1) >4 mm
5. Corolla (0) white, (1) reddish, (2) greenish
6. Habitat being (0) deciduous forest, (1) savannah, (2) rainforest on sandy soils, (3) rainforest on clayish soils



soils, and with annual precipitation ranging from 800 to 1.200 mm; (3) tropical rainforest on sand, a type of vegetations with a relatively open canopy represented by small trees or shrubs to open scrubland, growing on nutrient-poor white-sand soils with low pH (i.e., *campina*, *campinarana* and *restingae* forests), annual rainfall > 3.000 mm without a distinct dry season; (4) tropical rainforest on clay or *terra-firme* forest (=dry soil), characterized by tall, dense, evergreen forest with a canopy usually taller than 30 m, an annual rainfall of >3.000 mm without a distinct dry season (Pennington et al., 2006a; Oliveira-Filho, 2009; Portillo-Quintero and Sánchez-Azofeifa, 2010).

### 2.7. Estimation of ancestral area using continuous diffusion

As an alternative to estimate ancestral ranges based on discrete pre-defined areas, we also explored a recent approach to estimating ancestral areas in a species tree environment under the assumption of continuous diffusion of species over time by means of Brownian motion (Nylinder et al., 2014). Continuous diffusion is used in population genetics (Lemmon and Lemmon, 2008; Lemey et al., 2010), but is adaptable to species level by allowing the tip locations to be sampled from potentially disjunct representations of species distributions. To set up the analysis we followed the guidelines described in Nylinder et al. (2014), and shaped the geographic distribution of each species in Google Earth (Google Earth, 2014) by connecting the most extreme outliers for each species so that all examined collections fall within the boundaries. An exponential prior on the standard deviation of the lognormally distributed rate distribution was set to 2.712, corresponding to an upper 97.5% credibility interval of 10.0, which allows the diffusion rate to deviate up to ten times from the mean. Analyses were run in BEAST 1.8.1 (Drummond et al., 2012) three times for 50 million generations each, sampling parameters every 50,000 generations (see the BEAST input file in Supplementary data section). The diffused posterior distribution of trees was summarized according to the same procedure described above and visualized using SPREAD v.1.0.5 (Bielejec et al., 2011), with ancestral distributions shown as the default 80% HPD of sampled node locations.

## 3. Results

### 3.1. Species tree

The aligned matrices comprise 2532 characters, of which 2503 are from nucleotide sequences and 29 binary coded indels (Table 2). The species tree analysis support *Pradosia* as monophyletic with a posterior probability (PP) of 0.99, with *P. longipedicellata* as sister to all remaining species in the genus (Fig. 2; species trees are supplied separately as Supplementary data). *Pradosia* can be further subdivided into three main lineages, all of which are strongly supported and correlated with morphological, geographical and ecological characters (Fig. 3). The first clade (1.0 PP), here labeled “Montane”, includes species with a narrow distribution in the northeast of Colombia, north of Venezuela, and Ecuador (*Pradosia caracasana*, *P. colombiana* and *P. montana*). The second clade (1.0 PP), here labeled “sweet-bark”, includes almost exclusively species from the Amazon region, except for *P. kuhlmannii*

that occurs along the Brazilian Atlantic coast. *Pradosia kuhlmannii* falls out as sister to a strongly supported group (0.99 PP) consisting of *P. schomburgkiana*, *P. cochlearia* and *P. decipiens*. The third clade (1.0 PP), here named “red-flowered”, includes species from the Amazon region (eight species), the Brazilian Atlantic coast (three species), the savannahs of Central Brazil (*P. brevipes*), and the Chocó region on the Pacific Coast in Colombia (*P. cuatrecasii*). Within the red-flowered clade, except for the groups comprising *P. cuatrecasii*, *P. atroviolacea* and *P. grisebachii* (0.85 PP), and *P. verticillata*, *P. surinamensis*, *P. lactescens*, *P. restingae*, *P. granulosa*, *P. brevipes* and *P. verrucosa* (0.98 PP), the relationships between the species remain ambiguous with low node support.

### 3.2. Divergence time estimation

The crown node of *Pradosia* was dated to the late Eocene/early Oligocene at 34.4 Ma (27.4–41.4 Ma) with a subsequent split at 32.4 Ma (38.9–25.9 Ma) in the Eocene–Oligocene boundary. The split between sweet-bark and red-flowered taxa occurred 27.7 Ma (34.2–21.2 Ma) with following speciation events within these clades occurring during the Miocene and Pliocene. No speciation younger than ~0.6 Ma was identified and speciation appears to be constant throughout the history of the genus.

### 3.3. Morphological traits and habitat

The five selected diagnostic morphological features (Table 1) and habitat preference optimized on the species phylogeny are reported in Fig. 3A–F. The ancestral taste of the inner bark condition is non-sweet, with at least four evolutionary transitions to sweet taste in the sweet-bark and red-flowered clades. The flat/raised midvein feature occurs in *Pradosia longipedicellata* and is fixed in the sweet-bark clade (Fig. 3B). Terminal flowers are present only in *P. longipedicellata*, which is sister to rest of the genus, whereas fascicles along the branches below the leaves (ramiflorous) is the ancestral state and is shared by most species of *Pradosia* (Fig. 3C). The two other conditions, fascicles born on the trunk (cauliflorous) and in leaf axils, appear to have evolved at least twice, respectively, from ramiflorous ancestors. Apart from the rather large flowers in *P. longipedicellata*, small flowers (<4 mm) are predominant in the first order sister clades, but large flowers are then fixed in all species except *P. surinamensis* in the red-flowered clade (Fig. 3D). Shifts in flower colour occur only within the red-flowered clade where the probably red ancestral flower evolves into a green or greenish flower (Fig. 3E), sometimes with a conserved red shade (Fig. 1G).

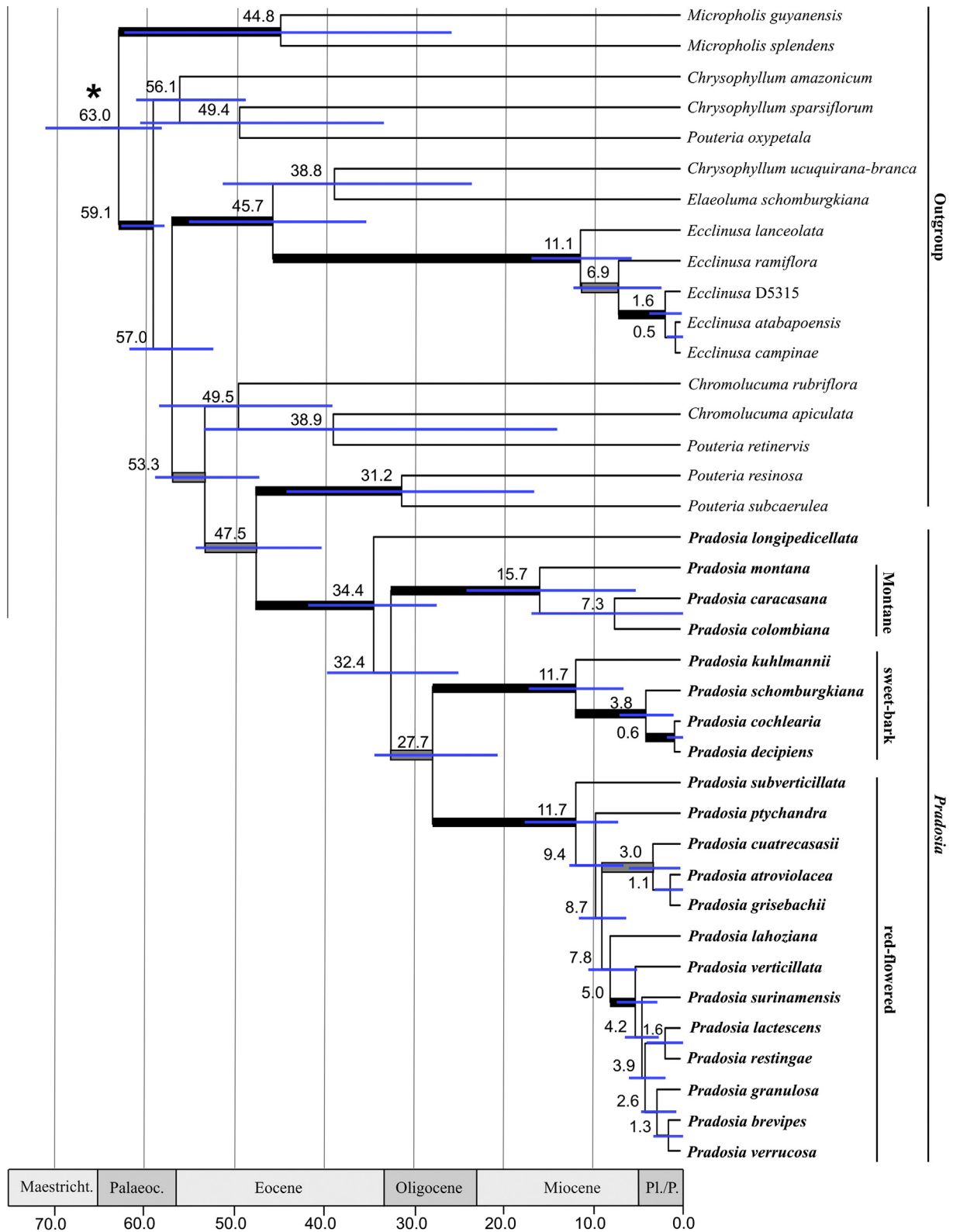
Reconstruction of habitat preference indicates that rainforest on clay has the highest probability of being the ancestral habitat for the genus, with one or two shifts to deciduous forests in the Montana clade (*P. colombiana* and *P. montana*), another to savannah in the red-flowered clade (*P. brevipes*), and at least three shifts in the genus to forests on white-sandy soils (Fig. 3F). Several species occur, however, on both white-sand and clayish soils.

### 3.4. Biogeographical reconstruction

The continuous diffusion analysis identifies a broad ancestral area for the *Pradosia* stem node at ~47.5 Ma lying in a NW–NE axes including most of Amazonia, Central Brazil and the Atlantic coast (Fig. 4A, node 1). The generic progenitor may have reached the Brazilian Atlantic coast for the first time ~34.4 Ma ago. The genus established a clade in northern South America during Late Eocene and Oligocene between 32.4 and 15.7 Ma of which the Pacific coast of Ecuador seems to have been the first area colonized (Fig. 4A, node 2). The reconstruction further indicates that *Pradosia* reached the Atlantic coast of Brazil a second time in Miocene

**Table 2**  
Characteristics of the nrDNA ITS, ETS and RPB2 regions from 52 accessions of *Pradosia* and outgroups examined in this study.

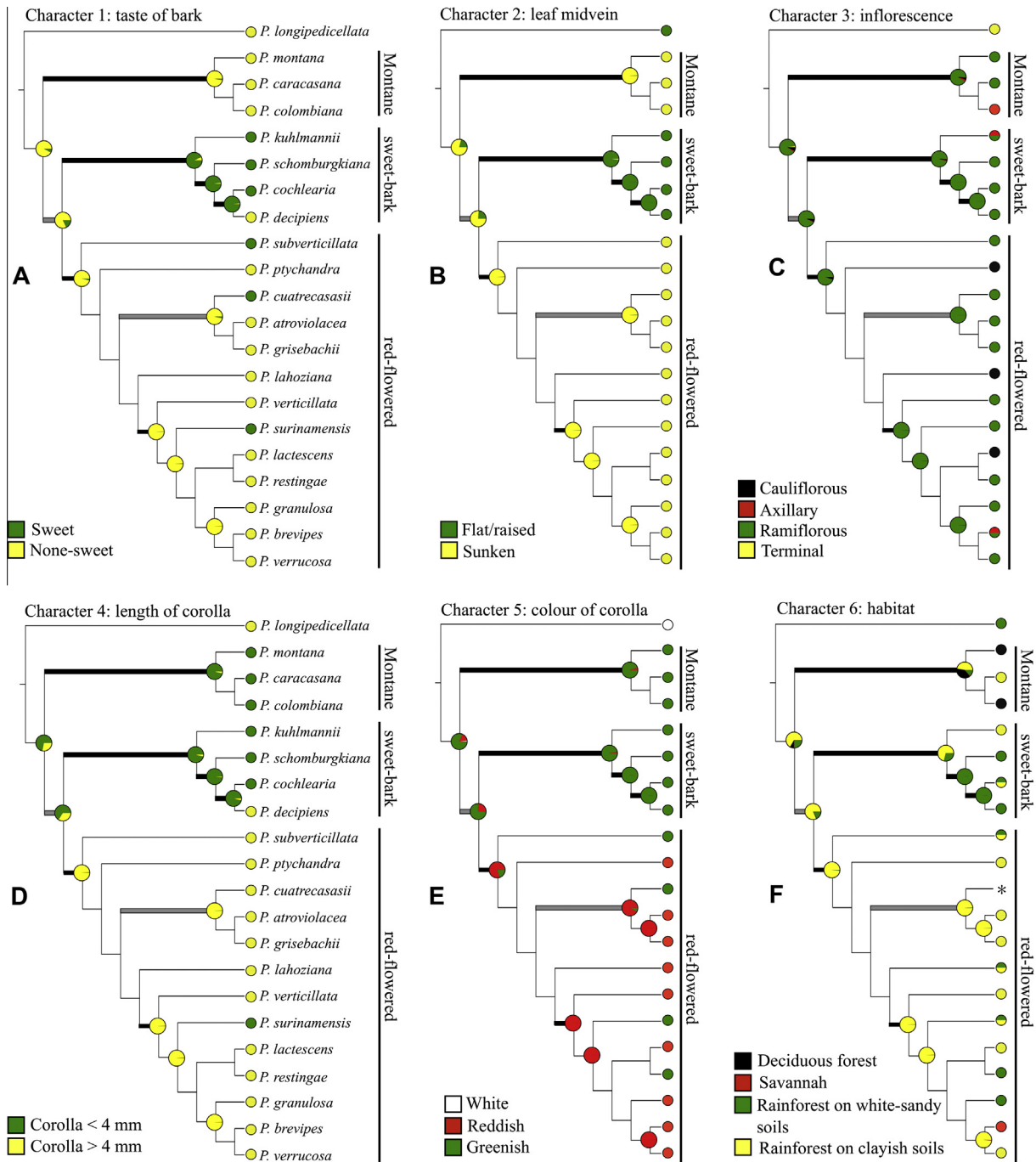
Characteristic	ITS	ETS	RPB2
Total size range (bp)	860	388	1255
Parsimony informative	145	114	137
Coded indels	22	5	2



**Fig. 2.** Maximum clade credibility (MCC) species tree drawn as a chronogram of *Pradosia* and outgroup (Sapotaceae, Chrysophylloideae) obtained from the BEAST analysis using fossil calibration (asterisk). Numbers above branches are median ages and blue bars give the 95% highest posterior density (HPD) intervals for age estimates. Posterior probabilities (PP) are indicated as black (PP > 0.95) and grey (0.85 > PP < 0.95) branches. The geological time scale is in million years and adapted from Walker and Geissman (2009). Abbreviations: Pl./P. = Pliocene/Pleistocene. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(*P. kuhlmannii*; Fig. 4B, node 3) while the remaining lineage in Amazonia continued to diversify (*P. schomburgkiana*, *P. cochlearia* and *P. decipiens*; Fig. 4B, node 4). A second dispersal event from the Amazon to the northwest of South America also occurred after

8.7 Ma, represented by divergence of *P. cuatrecasasii*, which is the only species occurring in the Chocó region in Colombia (Fig. 4C, node 6). Contemporaneously, diversification in the Central and Northern Amazonia continued (Fig. 4D; node 7), followed by



**Fig. 3.** Pruned phylogeny showing *Pradosia* (Sapotaceae, Chrysophylloideae) and the ancestral character state reconstructions of five morphological features (taste of bark, leaf midvein, inflorescence position, length of corolla and colour) and habitat obtained from BEAST analyses. Posterior probabilities (PP) are indicated as black (PP > 0.95) and grey (0.85 > PP < 0.95) branches. Pie charts at internal nodes indicate posterior distributions of ancestral character states. Asterisk indicates species not scored for character state. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

another range expansion to the Brazilian Atlantic coast at ~3.9 Ma (Fig. 4D, node 8) and a single independent transition into dryer savannah of Central Brazil ca. 1.3 Ma (4.6–0.6 Ma) by what we today call *P. brevipes*.

## 4. Discussion

### 4.1. Phylogeny and morphological apomorphies

Our results corroborate previous studies (Swenson et al., 2008a; Bartish et al., 2011) that *Pradosia* forms a well-supported clade with some closest relatives in a non-monophyletic *Pouteria*

(Fig. 2). Most of the members are distributed in three main clades: the Montane, sweet-bark, and the red-flowered clades.

Morphological characters used to recognize Neotropical Chrysophylloideae genera have multiple times been demonstrated to be homoplasious and of limited use (Swenson and Anderberg, 2005; Swenson et al., 2008b). However, the drupaceous fruit (Fig. 1L and M) is a very useful character being consistent and lending additional support to the monophyly of the group (Swenson et al., 2008a; Terra-Araujo et al., 2012a, 2013). Other features such as characteristics of the bark are also useful in the field at the species or subclade level, but not at the generic level as far is known. For example, a smooth bark of grey-brown or yellowish-grey

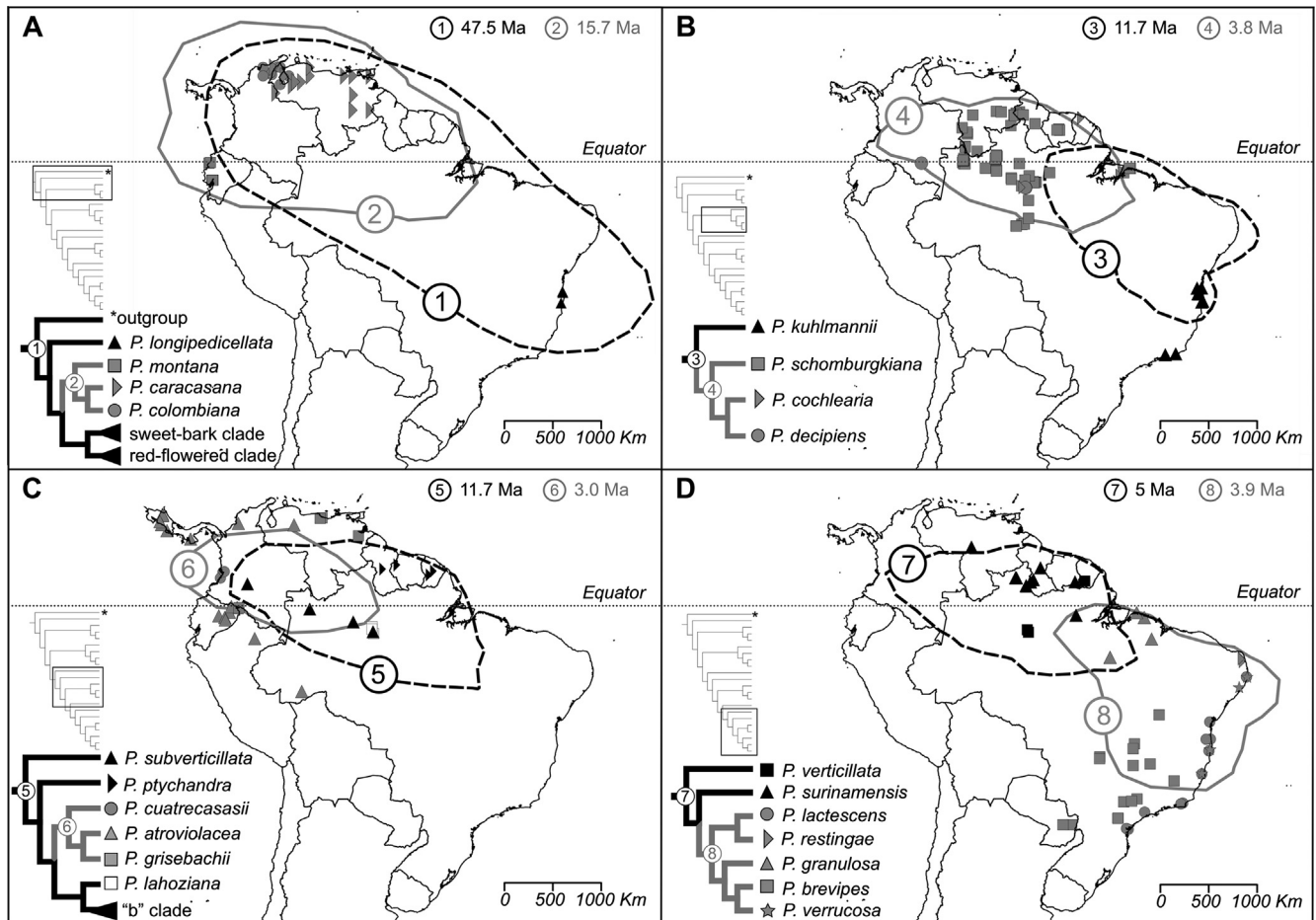


Fig. 4. Snapshots of estimated ancestral node areas from the MCC tree of *Pradosia* (Sapotaceae, Chrysophylloideae). Numbered nodes in the inserted phylogeny refer to the encircled area with the same number. Ages are given as median ages (Ma).

colour, sometimes bearing discolored scars (red or brown) and with a sweet taste is only found within *Pradosia*, and not in any other genera of Neotropical Sapotaceae (Fig. 1A and B). Ducke (1942) highlighted the sweet taste of the inner bark in *Pradosia* and attributed it to the presence of glycyrrhizin. Other studies have found flavonoids in the sweet bark of some species (e.g., *Pradosia huberi*; not included), which may be related to protection against injuries (Medeiros et al., 2010). The ancestral reconstruction of this character revealed that the sweet taste first appears in the sweet-bark clade with a subsequent loss in *P. decipiens*, and involved independently further three times in the red-flowered clade (Fig. 3A). Hence, sweet bark is not a unique character for the sweet-bark clade, but can be used in combination with the primary vein morphology to recognize it.

Patterns of leaf venation are problematic across Sapotaceae (Swenson and Anderberg, 2005; Swenson et al., 2008a), but provide many useful characters for the recognition of genera and species (Pennington, 1990, 1991), as demonstrated in a phylogenetic framework (Swenson et al., 2013). In *Pradosia*, patterns of secondary and tertiary venations vary, but the character whether the midvein is flat/raised or sunken provides some power to identify members of clades consistent with the phylogeny. A flat or raised midvein is present in *P. longipedicellata* and in the sweet-bark clade (Fig. 3B). Thus, combining sweet bark and a flat/raised midvein (not sunken) is diagnostic for all members of the sweet-bark clade except for *P. decipiens*.

The distribution of flowers may be under strong selective pressure due to pollinators (Warren et al., 1997). Flowers are fascicled

in Sapotaceae, a remarkably conserved feature throughout the family (Pennington, 1990), but the fascicles can be found on the trunk, along branches, in leaf axils, or being terminal. In *Pradosia*, fascicles are predominantly born along the branches and/or the axils of fallen leaves characterized as ramiflorous (Fig. 3C). True axillary fascicles with persistent leaves are only found in *P. colombiana*. Cauliflory has evolved at least twice in *Pradosia* and is a useful specific character. This character is widespread in the family, but never common, and in Neotropical Chrysophylloideae it is found in two species of *Chrysophyllum*, one in *Ecclinusa* and one in *Micropholis* (Pennington, 1990); and in Australasia, it is found in the New Guinean genus *Magodendron* (Vink, 1995) and in *Planchonella cauliflora* Munzinger & Swenson from New Caledonia (Munzinger and Swenson, 2009).

Some floral characters such as presence or absence of stamino-odes have been used as cardinal characters to distinguish genera in Sapotaceae (Pennington, 1990, 1991), but if used in a phylogenetic framework, several of these characters are homoplastic and must be reassessed (Swenson et al., 2008a,b). Sapotaceae flowers are sympetalous having a fused corolla tube and free corolla lobes. The fused part may vary in length, which influences the corolla shape. In *Pradosia*, flowers are frequently 5-merous, lack stamino-odes, and the tube is short, providing the basis for a rotate corolla shape. Pennington (1990) recognized a partition of four to five mm long corolla being useful for group-recognition in *Pradosia*, and this arbitrary length limit appears to be consistent with the recovered tree topology (Fig. 3D). Our analysis shows that a small corolla (<4 mm long) is probably a plesiomorphic feature followed by an



increased flower size in the red-flowered clade with only one exception (*P. surinamensis*). In terms of floral colour, white flowers are rare, only present in *P. longipedicellata* while the remaining species have either reddish or greenish flowers (Fig. 3E). In *Pradosia* the change from greenish to red flowers evolved once with three back-reversals to greenish. In summary, corolla colour shows high but not full congruence with the size of the corolla, with small flowers are usually greenish and larger flowers red and showy.

#### 4.2. Diffusion biogeography and habitat shifts

The subfamily Chrysophylloideae was proposed to have established in South America through a dispersal event from Africa in the Paleocene (~59 Ma) (Bartish et al., 2011), where it became very species-rich and widespread in the Eocene (Burnham and Johnson, 2004; Wing et al., 2009; Jaramillo et al., 2010). Based on a limited sample, Bartish et al. (2011) estimated the origin of the *Pradosia* stem node to some 57 Ma; an age estimate we refine here to the Middle Eocene (~47.5 Ma) that took place along a NW–NE axis in northern South America, an area which includes the sites of the oldest Chrysophylloideae fossils (Fig. 4A). The genus is mostly confined to lowland rainforests and our reconstruction suggests that preference for clayish soil is plesiomorphic within *Pradosia*. An initial split at ~32.4 Ma led to a lineage that colonized high altitudes plus dry areas in northern South America (Fig. 4A, node 2). This is the only time when *Pradosia* got established in dry deciduous forests, one species restricted to Ecuador and Peru (*P. montana*) and another distributed through the northwestern coast of Colombia and Venezuela up to 450 m altitude (*P. colombiana*). A third species (*P. caracasana*) appears to represent the remnant of the ancestral lineage that grew in lowland rainforest on clayish soil, but with a wide habitat amplitude, ranging from lowland rainforests at sea level to montane forests at 1900 m altitude. Colonization of high altitudes in the Middle/Late Miocene coincides with the uplift of the Eastern Cordillera of the northern Andes during the Miocene Climatic Optimum (MCO: ~15 Ma; see Zachos et al., 2008). Therefore, these results are consistent with the hypothesis that the ancestor of the Montane clade may have extended the ecological range to high altitudes when the climate was warmer.

Lowland rainforest in South America frequently occurs on either clayish or white-sand soils, resulting in completely different ecological conditions. The white-sand soil is nutrient-poor, have low pH and low water retention, and these areas usually form isolated islands scattered across the Guiana and Brazilian Shields, as well as the Amazon region; and they are usually surrounded by tall rainforests on nutrient-rich clay (Prance and Schubart, 1978; Anderson, 1981; Prance, 1996; Frasier et al., 2008). White-sand is therefore an extreme habitat in which preadaptation can facilitate colonization success (Pillon et al., 2010). Such harsh edaphic conditions have therefore been proposed to be an important component for speciation in the Amazon (Fine et al., 2013). Our analysis indicates that *Pradosia* managed to colonize white-sand soils early in its evolutionary history (*P. longipedicellata*). The most internal reconstructed ancestral node (32.4 Ma) indicates a 25% probability that the lineage was preadapted to white-sand, but some 70% that the ancestors occurred on clayish soil (Fig. 3F). Thirteen species are restricted to rainforests on either white-sand or clayish soils whereas four species are indifferent to soil type. Because of low node support, especially in the red-flowered clade, we find no strong evidence for the idea that habitat shifts has powered speciation in *Pradosia*.

*Pradosia* originated somewhere in tropical South America and reached the Atlantic coast of Brazil at least three times (Fig. 4). The earliest event (*Pradosia longipedicellata*) could have been around 34.4 Ma, followed by the split of *P. kuhlmannii* (Fig. 4B, node 3), and a clade of species with uncertain relationships in

Early Pliocene (Fig. 4D, node 8). Today, a northeast–southwest drier climatic barrier, the *Cerrado–Caatinga* biome, separates the evergreen forests of the Atlantic coast and the Amazon. However, the biogeographic pattern in *Pradosia* is particularly similar with other disjunct groups of plants and animals [e.g. for plants: the white-sandy specialist genus *Pagamea*; for animals: some anurans, including Bufonidae and Phyzelaphryninae, small lizards of the genus *Leposoma*, and groups of small mammals, such as species of the climbing rats and marsupials], indicating that these two large blocks of forests were connected through the Cenozoic (Costa, 2003; Vicentini, 2007; Fiaschi and Pirani, 2009; Pellegrino et al., 2011; Fouquet et al., 2012a,b). These historical connections through the dry corridor of open vegetation in central South America allowed the contact between the Atlantic coast and the Amazon several times through the evolutionary history of these groups and consequently some dispersal events and diversification in these forests.

Some biogeographical routes connecting the Amazon and the Atlantic coast have been proposed by Por (1992): (1) through the Paraná river basin; (2) through the coast of northeastern Brazil; and (3) through gallery forests crossing drier areas of Central Brazil. Despite weak clade support in the red-flowered clade, we find an indication of these migration routes. *Pradosia kuhlmannii* (northeastern Brazilian coast) is sister to species from northwestern and Central Amazon (Fig. 4B); the clade comprised by *Pradosia lactescens* and *P. restingae* are related to species from Central-East Amazon; and *P. verrucosa* is more closely related to *P. brevipes* of Central Brazil and Eastern Amazon (Fig. 4D). Given these relationships and distributions, *P. kuhlmannii*, *P. verrucosa* and the *P. lactescens* – *P. restingae* clade appear to represent a migration route through Central Brazil. The divergence times estimated for *P. kuhlmannii* and *P. lactescens* – *P. restingae* during the Late Miocene/Early Pliocene matches a time when the climatic barrier between Amazon and the Atlantic coast may have been more narrow (Hoorn et al., 2010), and such conditions may have allowed dispersal events between these two large blocks of rainforests. *Pradosia* colonized the *Cerrado* once in the Pliocene when the climate became progressively cooler, which favoured savannahs instead of rainforest in Central Brazil (Ledru, 2002). The adaptation to dry conditions of Central Brazil further involved a drastic change in the morphology of *P. brevipes*, which has a specialized subterranean (geoxylic) habit well adapted to the dry climate and periodic fires experienced by this species in *Cerrado* areas of southeast and Central Brazil (Pennington, 1990). Such condition is rare in the family, and it is only found in two American species, *P. brevipes* and in *Pouteria subcaerulea* Pierre ex Dubard, both occupying approximately the same geographical area.

*Pradosia cuatrecasii* is the only member of the genus that has colonized the Chocó region, an area in Colombia that became isolated from the Amazon in the Early Miocene by the uplift of the Western Cordillera as suggested by Burnham and Graham (1999). This isolation has led to the development of a distinctive flora with a high number of endemic species such as *Pagamea* in Rubiaceae (Vicentini, 2007) and *Potalia* in Gentianaceae (Frasier et al., 2008). *Pradosia cuatrecasii* is a very distinct species mainly characterized by large leaves with numerous sunken secondary veins and large flowers and fruits. Our finding suggests that the ancestor of *P. cuatrecasii* may have been dispersed across the Andes into the Chocó during Late Miocene some 8 Ma, and afterwards became geographically isolated and morphologically more distinct.

## 5. Conclusions

Our results confirm that *Pradosia* is monophyletic in its current circumscription and can be subdivided into three clades: Montane,

sweet-bark, and red-flowered. The Montane clade includes species distinguished by leaves with a sunken midvein and small (<4 mm long) greenish flowers. The sweet-bark clade has sweet bark combined with a flat midvein and small greenish flowers. Species of the red-flowered clade have leaves with a sunken midvein and usually large (>4 mm long) reddish flowers. The genus originated in the Middle Eocene at 47.5 Ma somewhere in the rainforests of South America, likely in the Amazon basin, and may have reached the Brazilian Atlantic coast for the first time around 34.4 Ma. This was followed by an expansion of the genus from the Amazon region to the northeast of South America in the Oligocene with two (or three) independent colonizations of the Brazilian Atlantic coast in Middle Miocene and Early Pliocene. Disjunctions between the Amazon region and the Brazilian Atlantic coast are concordant with several studies of plants and animals whose limits more or less coincide with those observed in *Pradosia*. Lastly, when examining the association between habitat and species, our analysis indicated that *Pradosia* early managed to colonize rainforests on white-sand soils. However, reconstruction of habitat preference indicates that the genus has ability to habitat shift rather than being preadapted to this poor soil. Nonetheless, although some studies (Fine et al., 2005, 2013) have suggested that habitat diversity has played an important role in species diversification in some Neotropical plant groups, we find no strong evidence that this is the case in *Pradosia*.

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## Appendix A

Taxa, voucher information, and GenBank accessions used in this phylogenetic study for *Pradosia* (Sapotaceae, Chrysophylloideae) with accessions published here in bold (extraction from herbarium material is indicated with an asterisk).

Taxon	Voucher Origin: Collector (Herbarium)	Nuclear Markers		
		ITS	ETS	RPB2
<i>Chromolucuma apiculata</i> Alves-Araújo & M. Alves	Brazil: Voeks 88 (CEPEC)	<b>KM042299</b>	n.s.	n.s.
<i>Chromolucuma rubriflora</i> Ducke	Brazil: Terra-Araujo 833 (INPA, S)	<b>KM042300</b>	<b>KM042359</b>	<b>KM042333</b>
<i>Chrysophyllum amazonicum</i> T.D. Penn.	Brazil: Assunção & Pereira 207 (INPA)	KJ399341	KJ453564	KJ453712
<i>Chrysophyllum sparsiflorum</i> Klotzsch ex Miq.	Brazil: Sothers & Silva 927 (INPA)	KJ399356	KJ453574	KJ453722
<i>Chrysophyllum ucuquiranabranca</i> (Aubrév. & Pellegr.) T.D. Penn.	Brazil: Nascimento & al. 777 (INPA)	KJ399357	KJ453575	KJ453723
<i>Ecclinusa atabapoensis</i> (Aubrév.) T.D. Penn.	Brazil: Costa 486 (INPA)	<b>KM042301</b>	<b>KM042360</b>	<b>KM042334</b>
<i>Ecclinusa campinae</i> Terra-Araujo & Costa	Brazil: Costa 1209 (INPA)	<b>KM042303</b>	<b>KM042362</b>	n.s.
<i>Ecclinusa</i> D5315	Brazil: Dávila 5315 (INPA)	<b>KM042304</b>	<b>KM042363</b>	n.s.
<i>Ecclinusa lanceolata</i> (Mart. & Eichler) Pierre	Brazil: Terra-Araujo 542 (INPA)	<b>KM042302</b>	<b>KM042361</b>	<b>KM042335</b>
<i>Ecclinusa ramiflora</i> Mart.	Suriname: Irwing & al. 55081 (S)	DQ246678	KJ453580	KJ453728
<i>Elaeoluma schomburgkiana</i> (Miq.) Baill.	Brazil: Keel & Coelho 243 (S)	DQ246679	KJ453583	KJ453731
<i>Micropholis guyanensis</i> (A. DC.) Pierre	Brazil: Hopkins & al. 1475 (INPA)	KJ399361	KJ453584	KJ453733
<i>Micropholis splendens</i> Gilly ex Aubrév.	Brazil: Assunção & Pereira 13 (INPA)	KJ399362	KJ453585	KJ453735
<i>Pouteria oxypetala</i> T.D. Penn.	Brazil: Bertoni & Geremias 293 (IAC)	KJ399417	KJ453658	KJ453780
<i>Pouteria resinosa</i> T.D. Penn.	Brazil: Faria & Ribeiro 2007/33 (INPA)	KJ399433	KJ453670	KJ453787
<i>Pouteria retinervis</i> T.D. Penn.	Brazil: Ribeiro & al. 1936 (INPA)	KJ399435	KJ453672	KJ453789
<i>Pouteria subcaerulea</i> Pierre ex Dubard in Lecomte	Brazil: Faria & Ribeiro 2008/3 (SPF)	KJ399441	KJ453678	KJ453796
<i>Pradosia atroviolacea</i> Ducke	Brazil: Terra-Araujo 738 (INPA, S)	<b>KM042305</b>	<b>KM042364</b>	<b>KM042336</b>
<i>Pradosia atroviolacea</i> Ducke	Costa Rica: Anderberg 52 (S)	<b>KM042306</b>	<b>KM042365</b>	n.s.
<i>Pradosia brevipes</i> (Pierre) T.D. Penn.	Paraguay/Brazil: Lindeman 6743 (U)	AY552158	KJ453687	n.s.
<i>Pradosia caracasana</i> (Pittier) T.D. Penn.*	Venezuela: Stergios 17109 (NY)	<b>KM042307</b>	<b>KM042366</b>	n.s.
<i>Pradosia cochlearia</i> (Miq.) Baill.	Brazil: Brito & al. 29 (INPA)	KJ399449	KJ453688	KJ453805
<i>Pradosia cochlearia</i> (Miq.) Baill.	Brazil: Terra-Araujo 855 (INPA, S)	<b>KM042308</b>	<b>KM042367</b>	<b>KM042337</b>

## Appendix A (continued)

Taxon	Voucher Origin: Collector (Herbarium)	Nuclear Markers		
		ITS	ETS	RPB2
<i>Pradosia colombiana</i> (Standl.) T.J. Ayers & Boufford*	Colombia: Haught 4303 (S)	<b>KM042309</b>	n.s.	<b>KM042338</b>
<i>Pradosia cuatrecasasii</i> (Aubrév.) T.D. Penn.*	Colombia: Cuatrecasas 13988 (K)	<b>KM042310</b>	n.s.	n.s.
<i>Pradosia decipiens</i> Ducke	Brazil: Brito & al. 1950 (INPA)	KJ399450	KJ453689	KJ453806
<i>Pradosia decipiens</i> Ducke	Brazil: Terra-Araujo 676 (INPA, S)	<b>KM042311</b>	<b>KM042368</b>	<b>KM042339</b>
<i>Pradosia granulosa</i> Pires & T.D. Penn.*	Brazil: Rios 841 (INPA)	<b>KM042312</b>	n.s.	<b>KM042340</b>
<i>Pradosia granulosa</i> Pires & T.D. Penn.	Brazil: Terra-Araujo 863 (INPA, S)	<b>KM042313</b>	<b>KM042369</b>	<b>KM042341</b>
<i>Pradosia grisebachii</i> (Pierre) T.D. Penn.*	Colombia: Styermark 90134 (NY)	<b>KM042314</b>	n.s.	n.s.
<i>Pradosia kuhlmannii</i> Toledo	Brazil: Terra-Araujo 763 (INPA, S)	<b>KM042315</b>	<b>KM042370</b>	<b>KM042342</b>
<i>Pradosia kuhlmannii</i> Toledo	Brazil: Terra-Araujo 770 (INPA, S)	<b>KM042316</b>	<b>KM042371</b>	<b>KM042343</b>
<i>Pradosia lactescens</i> (Vell.) Radlk.	Brazil: Alves-Araújo 1256 (UFP)	<b>KM042317</b>	n.s.	<b>KM042344</b>
<i>Pradosia lactescens</i> (Vell.) Radlk.	Brazil: Terra-Araujo 764 (INPA, S)	<b>KM042318</b>	<b>KM042372</b>	<b>KM042345</b>
<i>Pradosia lactescens</i> (Vell.) Radlk.	Brazil: Terra-Araujo 767 (INPA, S)	<b>KM042319</b>	<b>KM042373</b>	<b>KM042346</b>
<i>Pradosia lahoziana</i> Terra-Araujo	Brazil: Terra-Araujo 760 (INPA, S)	<b>KM042320</b>	<b>KM042374</b>	<b>KM042347</b>
<i>Pradosia longipedicellata</i> Alves-Araújo & M. Alves	Brazil: Terra-Araujo 769 (INPA, S)	<b>KM042321</b>	<b>KM042375</b>	<b>KM042348</b>
<i>Pradosia montana</i> T.D. Penn.*	Ecuador: Palacios & Rubio 9968 (NY)	<b>KM042322</b>	<b>KM042376</b>	<b>KM042349</b>
<i>Pradosia ptychandra</i> (Eyma) T.D. Penn.	French Guiana: Mori 25441 (S)	<b>KM042323</b>	n.s.	<b>KM042350</b>
<i>Pradosia ptychandra</i> (Eyma) T.D. Penn.	French Guiana: NH200016 (CAY)	FJ037897	n.s.	n.s.
<i>Pradosia ptychandra</i> (Eyma) T.D. Penn.	French Guiana: NL110310 (CAY)	FJ037899	n.s.	n.s.
<i>Pradosia restingae</i> Terra-Araujo	Brazil: Alves-Araújo 1373 (INPA, RB, S)	<b>KM042324</b>	n.s.	<b>KM042351</b>
<i>Pradosia restingae</i> Terra-Araujo	Brazil: Jardim 5588 (INPA)	<b>KM042325</b>	<b>KM042377</b>	<b>KM042352</b>
<i>Pradosia schomburgkiana</i> (A.D.C.) Cronquist	Brazil: Costa 184 (INPA, S)	<b>KM042326</b>	<b>KM042378</b>	<b>KM042353</b>
<i>Pradosia schomburgkiana</i> (A.D.C.) Cronquist	Brazil: Costa 541 (INPA, S)	<b>KM042327</b>	n.s.	<b>KM042354</b>
<i>Pradosia schomburgkiana</i> (A.D.C.) Cronquist	Brazil: Costa 1577 (INPA, S)	<b>KM042328</b>	n.s.	<b>KM042355</b>
<i>Pradosia subverticillata</i> Ducke	Brazil: Terra-Araujo 579 (INPA, S)	<b>KM042329</b>	n.s.	<b>KM042356</b>
<i>Pradosia surinamensis</i> (Eyma) T.D. Penn.	Guyana: Harris 1076 (U)	AY552157	KJ453700	KJ453808
<i>Pradosia verrucosa</i> Ducke*	Brazil: Oliveira 2201 (INPA)	<b>KM042330</b>	n.s.	n.s.
<i>Pradosia verrucosa</i> Ducke	Brazil: Terra-Araujo 780 (INPA, S)	<b>KM042331</b>	<b>KM042379</b>	<b>KM042357</b>
<i>Pradosia verticillata</i> Ducke	Brazil: Terra-Araujo 857 (INPA, S)	<b>KM042332</b>	<b>KM042380</b>	<b>KM042358</b>

## Appendix B. Supplementary material

The BEAST input files (.xml) and the time-calibrated species tree (.tree) are available in the Supplementary data section of this article. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ympev.2015.03.007>.

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