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A New Amazonian Section of Protium (Burseraceae) including both Edaphic Specialist and Generalist Taxa. Studies in Neotropical Burseraceae XVI.

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Abstract—Protium section Papilloprotium and Protium alvareziathanum are described and a key to the four species of the section provided. The section is distinguished principally by its papillate abaxial leaflet surface, and the petals saccate at base and villous adaxially. The rather widespread edaphic generalist P. ferrugineum shares a common ancestor with two small clades, one containing two white-sand specialists (P. reticulatum and P. alvareziathanum) that appear to have diverged from a common ancestor allopatrically via fragmentation of white-sand habitats, and the other exhibiting degrees of edaphic specialization among variants of P. subserratum. This scenario highlights the role of edaphic heterogeneity as a driver in the diversification of the Amazon flora.

Keywords—Amazonia, rain forest trees, South America, speciation, white-sand forests.

The Burseraceae comprise one of the most important tree families in the Amazon, and across the Andes in the Chocó biogeographic region, as has been made clear in numerous quantitative forest inventories (e.g. review by Daly et al. 2011). It is an excellent model system for studying biogeographic patterns of primary upland forests because it is usually not an important component of secondary forests (e.g. Lisboa 1989), nor of floodplain forests (e.g. Foster 1990; Campbell et al. 1992). Central Amazonia is where the family truly shines by all standard criteria (e.g. Matos and Amaral 1999; Lima Filho et al. 2001). For example, in an area of 70 ha inventoried prior to 1992 by the Biological Dynamics of Forest Fragments (BDFF) project near Manaus, Brazil, the family was one of the highest in relative density but also one of the most species rich. The Burseraceae placed second in relative diversity, with 49 species, and Protium Burm. f. was the most species-rich genus, with 35 species (Rankin-de-Merona et al. 1992). One species of Protium exhibited the highest relative density in the inventory.

Most Amazonian Burseraceae occur in upland forests on clay or sandy loam soils, but there is a significant number of habitat specialists as well, including the majority of the tribe Proteae (including the genera Cepidoespernum Benth. & Hook. f., Protium, and Tetragastris Gaertn.) in the Peruvian Amazon. Almost three-quarters of the thirty-five-four species encountered in the Peruvian Amazon by Fine et al. (2005) were restricted to only one of three edaphic habitats: white-sand forest, clay soil forest, or brown sand forest from eroded river terraces. Differences in soils and topography correlate with changes in species diversity, species composition, and forest structure; indeed, they often determine distinct forest types. In the mosaic of habitats one often encounters in regions of lowland humid forests, there are generalists in each component, but there are also edaphic specialists (e.g. Young and León 1989), and the compositional differences are often statistically significant (Guillaumet 1987; Tuomisto et al. 1995; Fine et al. 2010). The most extreme of the edaphic habitat types are white-sand forests, which are also known as campina, campinarana, varillal, or bana, among other local names (Fine et al. 2010). These low-canopy forests on partly exposed white sands occur sporadically throughout much of Amazonia as islands on the scale of hectares or square kilometers. White-sand forests have little in common floristically with the surrounding tall forests on clay soils (Guillaumet 1987; Fine et al. 2010), and they function as habitat islands, with their florals showing a higher proportion of species adapted for relatively long-distance dispersal than surrounding forests on clay soils (Macedo 1977; Macedo and France 1978; Anderson 1981). White-sand forests are associated with a high degree of endemism, even including some endemic genera (e.g., Struwe et al. 1997).

Here we describe a new section of Protium from northern South America; the four species that comprise it include a new species from white-sand formations in northern and western Amazonia. The biogeographic and habitat preferences of these four species, together with their phylogenetic relationships, suggest an important role for the edaphic heterogeneity of the Amazon basin in the diversification of this new section.

Materials and Methods

Morphological Studies—Both authors have observed and collected three of the four species in this new section in multiple localities, working with seedlings and saplings as well as sterile and fertile adult trees. We have also examined virtually all extant herbarium material of the group.

DNA Extractions—In Appendix I we list the collection numbers, herbaria where the specimens are deposited, geographic locality, and GenBank accession number for each of the samples included in our molecular analysis. The samples were either dried fresh in silica gel and stored at -80°C or taken from herbarium sheets. All leaf tissue was ground using BioSpec Products Inc. (Bartlesville, Oklahoma) mini beadbeater-16 with their 2.3 mm zirconia/silica beads. Extractions were carried out using the DNEasy plant mini kit (Qiagen, Valencia, California). The protocol for herbarium specimens was modified to include an additional step where 3–5 µl of Protease K were added to the sample in the lysis buffer and incubated on a horizontal shaker at 42°C for 12–24 hr.

PCR - Five Loci—We sequenced the ribosomal ITS and external transcribed spacer (ETS), a single copy nuclear locus, phytochrome C (phyC), and two chloroplast loci, rps16 and trnL-F to assess phylogenetic relationships within tribe Papilloprotium. The ITS region was broken into two regions, ITS1 and ITS2 (Table 1).

Polymerase Chain Reactions were carried out in 20 µl volumes of 1.0 µl 10 µM primer, two to five µl DNA, one µl 100% DMSO and Bioneer’s (Alamada, California) accupower standard or “high-fidelity TLA” PCR premix, which includes one U DNA polymerase, 250 µM dNTPs and two µl reaction buffer per 20 µl PCR reaction. The amplification products were visualized with UV light on 1% TBE agarose gels and cleaned using exonuclease I and shrimp alkaline phosphatase (USB Corporation, Cleveland, Ohio).
Sequencing and Alignment—Amplification products were cycle-sequenced using 10 µl reactions with the standard BigDye (Applied Biosystems, Foster City, California) protocol and afterwards cleaned again using an EDTA-ethanol precipitation step. Sequencing was performed on an ABI 3730 sequencer (Applied Biosystems). The resulting chromatograms were edited, and forward and reverse sequences were compiled using Geneious Pro 3.5.6 (BioMatters Ltd., Auckland, New Zealand). Initial sequence alignments were made using ClustalW (EMBL-EBI) with subsequent manual alignments in MacClade 4.05 (Maddison and Maddison 2002).

Taxon Sampling—For outgroup comparison we selected representatives from each of the named sections in the Protieae, which included the genera Crepidospermum and Tetragastris (Fine et al. 2005). In Fine et al. (2005) the closest lineage to the Papillocyprium taxa was a clade including members of Crepidospermum, Tetragastris, and sections Pepeanthos Daly, Icicopsis Engl., and Sarcoprotium Daly. Further analyses with more than 30 species of Protieae have identified Old World Protium (sections Marignia Swart and Protium) as comprising the outgroup to all New World Protieae (including Protium, Crepidospermum, and Tetragastris) (P. Fine and D. Daly, unpublished data). Thus, we used Protium madagascariense Engl. from Madagascar as our outgroup taxon to root the clade. We added one other distribution as selected by MrModeltest; and 2) running the Markov chain portion of invariable sites, and rates for variable sites following a gamma Table 1 of nucleotide substitution with estimated base frequencies, proportion of invariable sites, and rates for variable sites following a gamma distribution as selected by MrModeltest; and 2) running the Markov chain for 3 x 10^8 generations, sampling one tree every 300 generations for a total of 10,000 sampled trees. We analyzed two runs in parallel with four chains each (one cold and three heated), and assessed stationarity by examining the standard deviation of the split frequencies. At 3 x 10^8 generations, this value was below 0.003 and there was no trend in the log versus generation plot with sufficient mixing of the cold and heated chains. Thus, following the recommendations of Huelsenbeck and Ronquist (2001), we discarded the first 25% of all sampled trees as “burn in.” MrBayes computes a 50% majority-rule consensus tree of the retained trees. We also conducted a maximum likelihood analysis on the partitioned data set using a randomized accelerated maximum likelihood (RAxML) program on the CIPRES cluster at the San Diego Supercomputing Center (Stamatakis et al. 2005). This analysis comes with a rapid bootstrap heuristic that automatically determines the point at which enough bootstrapping replicates have been produced (Stamatakis et al. 2008).

Taxonomic Treatment


Turma naturalis a sectio Pepeanthos resina lactea plerumque sine pulvino laterali folioli venis selerado ramosissimis terminans cotyledone plano-convixis simile sed a caeteris speciebus congernibus his notulis differt: folioli pagina adaxialis plerumque dense papillata petali basis saccata et pagina adaxialis villosa antherae apex apiculatus.

Tortuous trees; outer bark thin, often rough due to raised lenticels, inner bark red; resin milky. Leaves usually lacking pulvini on lateral petiololes (present on Protium ferrugineum), present on terminal petiololes; leaflet margin (sub)seriate or entire; abaxial surface densely papillate (Fig. 1). Leaflet architecture (Fig. 2) with secondary vein fabric festooned-brochidodromous; some intersecondarys usually present on most leaflets, parallel to secondaries and often nearly as long but slightly deflected (zig-zag); tertiaries alternate-percurrent with some composite admedials near midvein; same pattern found in quaternaries but these also occasionally freely ramify; perpendicular epimedian tertiaries usually present; freely ending veinlets (FEVs) proliferating into highly branched sclereids; when teeth present both the tooth and superjacent sinus vascularized by a principal vein. Inflorescences pseudopodiaceous. Flowers 5-merous; calyx much taller than nectary disk; androecium pentadynamous, anthers apiculate (the connective extending beyond the thecae); pollen (from Harley and Daly 1996) 33–37 µm, endoapertures very small (Endoaperture Shape Index-ENSI 4.9); shape subprolate or prololate, rarely prolate-spherical [ratio polar axis length to equatorial diameter-P/E (1.12–)1.14–1.68], P 22–40(–22.6–39.2), E 17.2–31.1(–16–34) µm; angulaperturate; colpi narrow; costae pronounced; wall

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer Name (Forward/Reverse)</th>
<th>Primer Sequence</th>
<th>Citation</th>
<th>Substitution Model selected by MrModeltest</th>
</tr>
</thead>
<tbody>
<tr>
<td>ITS1</td>
<td>NY183 (F), NY109 (R)</td>
<td>CTTTATCATTAGGAAAGGAG (F), GTGAGCCCCGACGACG (R)</td>
<td>K. Wurdack (Fine et al. 2005)</td>
<td>GTR + G</td>
</tr>
<tr>
<td>ITS2</td>
<td>NY45 (F), NY 43 (R)</td>
<td>GCATCGATGAAGAAGTAGTGF (F), TATGCCTAAAYTACGGCCTG (R)</td>
<td>K. Wurdack (Fine et al. 2005)</td>
<td>GTR + G</td>
</tr>
<tr>
<td>ETS</td>
<td>ETS-I (F), ETS-18S-IGS (R)</td>
<td>TTCCGGTATCCGTTGCTTTAATC (F), GAGAAACGATGACTGACTCTG (R), GCCGAGGATCAACCAGG (R)</td>
<td>Weeks et al. 2005 (F), Baldwin and Markos 1998 (R)</td>
<td>HKY + G</td>
</tr>
<tr>
<td>phyC</td>
<td>phyC-smbF1, phyC-smbR1</td>
<td>GCCAYTGAARTAYAARCT (F), CCRCCCACTTACCTGTT (R)</td>
<td>Clayton et al. 2007</td>
<td>HKY + G</td>
</tr>
<tr>
<td>rps16</td>
<td>rps16 (F), rps16 (R)</td>
<td>GTGATGAAAAGCAACGCTT (F), TCGGGATGCACTACATGCGACAC (R)</td>
<td>Oxelman et al. 1997</td>
<td>GTR + G</td>
</tr>
<tr>
<td>TrnL-F</td>
<td>trnLF-C, trnLF-F</td>
<td>CGAAATCGTGAGCTCGAC (F), ATTGAACCTGGTGACACGAG (R)</td>
<td>Taberlet et al. 1991</td>
<td>GTR</td>
</tr>
</tbody>
</table>

Table 1. Sources of primers used in this study, and substitution model for each locus selected by MrModeltest 2.3. All model parameters were unlinked across loci.
thickness 1–2 µm; exine appears psilate; colpi with granular membranes; rarely (P. ferrugineum) the mesocolpium psilate and sparsely punctate and the apocolpium perforate; nectary disk surface pubescent (glabrous in P. ferrugineum, also rarely in P. subserratum); gynoecium with style longer than ovary and unbranched, stigma surface smooth. Fruit red to orange; pyrene bony or rarely (P. ferrugineum) thinly cartilaginous, the funicular scar usually ≤ halfway up ventral surface (3/4 in P. ferrugineum); cotyledons entire, plano-convex. Germination epigeal and cryptocotylar, seedlings with first eophylls opposite, 5–11-foliolate, serrate. Figures 1–4.

**Distribution and Ecology**—The distributions of the four species of sect. *Papilloprotium* range from rather widespread to regionally endemic (Fig. 3). *Protium subserratum* is widespread in Amazonia plus the Guianas, with a disjunct distribution in Antioquia, Colombia; *P. ferrugineum* (Engl.) Engl. occurs in western Amazonia and is disjunct across the Andes in Valle, Colombia; *P. alvarezianum* is found in northern and western Amazonia; and *P. reticulatum* (Engl.) Engl. is known only from the upper Rio Negro basin (tributaries of the Rio Negro and Rio Uaupés) in Venezuela and Brazil. *Protium subserratum* has a broad edaphic distribution; it has been collected in clay soil, eroded river terrace soil (brown sand), and white-sand forests. *Protium ferrugineum* has similarly been collected in a variety of terra firme soil types, although it has never been found in extreme (low canopy) white-sand forests, famous for their stressful environments of extreme soil infertility and poor drainage due to a perched water table. In contrast, *Protium alvarezianum* and *P. reticulatum* are both restricted to these low-canopy white-sand areas, and have
not been collected on other, more common, edaphic habitat types.

Within *Protium*, section *Pepeanthos* could most easily be confused with sect. *Papilloprotium* because of the milky resin, the usual lack of conspicuous pulvinuli on lateral petiolules, leaflets with freely ending veinlets terminating in highly branched sclereids, and the cotyledons plano-convex. These similarities and salient differences between the two sections are summarized in Table 2.

Characteristics unique to sect. *Papilloprotium* in tribe *Protieae* are the papillate abaxial leaflet surface, the petals saccate at base and villous adaxially, and the anther apex apiculate. Other notable characteristics are found elsewhere in the genus: milky resin (also in sect. *Pepeanthos*), pseudospicate inflorescences (also in sect. *Icicopsis*), and the FEVs terminating in highly branched sclereids (also in *Tetragastris*, *Crepidospermum*, and *Protium* sect. *Pepeanthos*); the great majority of *Protium* species have the FEVs one- or two-branched.

Papillate leaf surfaces, usually on the abaxial side, are characteristic of understory plants in moist tropical forests, but they may serve distinct functions depending on their morphology and arrangement, as discussed in Rollet et al. (1990). Papillae significantly increase surface area, so thin-walled papillae may serve to increase cuticular evaporation; they may also function as “collecting lenses” to enhance capture of diffuse understory light. On the other hand, thick-walled papillae that form a “wreath” around stomates create a calm air chamber above them, thereby reducing stomatal transpiration. The latter xeromorphic condition, with the papillae obscuring the stomates, is characteristic of *Protium subserratum* (Fig. 1A) except for an eastern Amazon variant (“morphotype 1”, see discussion below) that has a thick cuticle covering the whole surface other than the stomates. Meanwhile, *P. alvarezianum* and *P. ferrugineum* (Fig. 1B) have papillae that are mound-like or bulbous to subspherical, respectively, interspersed among stomates that are recessed within a raised chamber or collar. This xeromorphic condition fits logically with the high insolation and possible periodic water stress of white-sand habitats.

*Protium ferrugineum* stands apart in sect. *Papilloprotium* morphologically, and the molecular evidence indicates that it is sister to the common ancestor of the other three species. It is the only described species in the group that consistently has pulvinuli on the lateral leaflets, the pollen with the mesocolpium psilate and sparsely punctate and the apocolpium perforate, the nectary disk glabrous, the pyrene oblong to slightly obovoid (vs. ovoid), and the funicular scar three fourths (not < 50%) of the distance up the ventral face of the pyrene.
Fig. 4. *Protium alvarezianum* Daly & P. Fine. A. Fruiting branchlet, with inset showing detail of margin with tooth. B. Flowering branchlet, showing short subterminal inflorescences. C. Staminate flower, at right with calyx lobes and two petals removed. D. Longisection of staminate flower with perianth removed, showing pubescent nectary disk and pistillode with reduced locules and ovules; inset above showing antepetalous anther in lateral and ventral views. E. Pistillate flower, at right with calyx lobes and two petals removed. F. Longisection of pistillate flower with perianth removed, showing pubescent pistil with long style. G. Detail of infructescence, showing persistent bracteole bases. H. Pyrene in lateral and ventral views, showing funicular scar less than halfway to apex. A from Vásquez & Soto 11891 (NY), B-D from Daly & Acevedo 5116 (NY), E-F from Boom & Weitzman 5381 (NY), G-H from Cid Ferreira 9336 (NY).
Table 2. Salient characters linking and separating sect. Papilloprotium and sect. Pepeanthos.

<table>
<thead>
<tr>
<th>Character</th>
<th>sect. Papilloprotium</th>
<th>sect. Pepeanthos</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resin</td>
<td>milky</td>
<td>milky</td>
</tr>
<tr>
<td>Snail-shaped glands</td>
<td>absent</td>
<td>present</td>
</tr>
<tr>
<td>Lateral pulvinuli</td>
<td>absent or present</td>
<td>absent or inconspicuous</td>
</tr>
<tr>
<td>Leaflet margin</td>
<td>(sub) serrate or entire</td>
<td>entire</td>
</tr>
<tr>
<td>Leaflet abaxial surface</td>
<td>papillate (most taxa)</td>
<td>not papillate</td>
</tr>
<tr>
<td>FEVs (freely ending veinlets)</td>
<td>terminate in highly branched sclereids</td>
<td>terminate in highly branched sclereids</td>
</tr>
<tr>
<td>Inflorescences</td>
<td>pseudospicate (flowers/sub)sessile</td>
<td>open-paniculate</td>
</tr>
<tr>
<td>Petal morphology</td>
<td>narrowly subulate, saccate at base between K lobes</td>
<td>not saccate at base</td>
</tr>
<tr>
<td>Petal adaxial surface</td>
<td>villous</td>
<td>glabrous</td>
</tr>
<tr>
<td>Petal orientation</td>
<td>(sub) erect at anthesis</td>
<td>(sub) erect at anthesis</td>
</tr>
<tr>
<td>Stamen series</td>
<td>dimorphic (pentadynamous)</td>
<td>sometimes pentadynamous</td>
</tr>
<tr>
<td>Antesepalous stamens</td>
<td>not reflexed</td>
<td>often reflexed between petals at anthesis</td>
</tr>
<tr>
<td>Anther apex</td>
<td>short-italiculate (extension of connective)</td>
<td>not apiculate</td>
</tr>
<tr>
<td>Pollen</td>
<td>polar outline angulaperturate</td>
<td>polar outline fossaperturate</td>
</tr>
<tr>
<td>Disk surface</td>
<td>usually pubescent</td>
<td>glabrous or pubescent</td>
</tr>
<tr>
<td>Style</td>
<td>at least as long or ovary, unbranched</td>
<td>shorter than ovary, with 5 short branches</td>
</tr>
<tr>
<td>Stigma surface</td>
<td>smooth</td>
<td>caudiculate and spiculate</td>
</tr>
<tr>
<td>Pyrene funicular scar</td>
<td>usually below halfway point on ventral surface</td>
<td>well above halfway point</td>
</tr>
<tr>
<td>Pyrene thickness</td>
<td>usually bony</td>
<td>usually cartilaginous</td>
</tr>
<tr>
<td>Cotyledons</td>
<td>entire, plano-convex</td>
<td>entire, plano-convex</td>
</tr>
<tr>
<td>Germination</td>
<td>epigeal, cryptocotylar</td>
<td>epigeal, phanerocotylar</td>
</tr>
<tr>
<td>First eophylls</td>
<td>opposite, 5-11-foliolate, serrate</td>
<td>opposite, simple, entire or distally serrulate</td>
</tr>
</tbody>
</table>

**KEY TO THE SPECIES OF Proti um Section Papilloprotium**

1. Lateral petiolules 3–13 mm long, a distal pulvinulus usually visible; leaflets entire, rarely with a few scattered teeth ........................................... 2

2. Lateral leaflets (broadly) elliptic to slightly (ob)lanceolate; all anthers dorsifixed and sagittate; petals acute or acuminate, base truncate; (1) pyrene), apex narrowly and sharply short-acuminate, base cordate to truncate ........................................... P. ferrugineum

3. Lateral petiolules 1–8 mm long, without a distal pulvinulus; leaflets entire or more often coarsely serrate or with at least a few scattered teeth ........................................... 3

3. Leaflets coriaceous or less often chartaceous, the margin coarsely (sub)serrate or less often entire; pistillate inflorescences and infertesences 3.5–14 mm long; calyx lobes 0.7–1 mm long (staminate) or 1.1–1.3 mm long (pistillate, also often persistent and measurable on fruits); fruit 0.6–1 cm wide when >1 pyrene, apex acute or acuminate, base truncate .................... P. subseratrum

3. Leaflets thinly chartaceous, the margin entire or with a few scattered teeth; pistillate inflorescences and infertesences 2.3–3 cm long (4.5 cm with fruits); calyx lobes 2.2–2.6 mm (staminate) or 1.5–2 mm long (pistillate, also often persistent and measurable on fruits); fruit 0.8–1 cm wide (to 1.4 cm wide when >1 pyrene), apex narrowly and sharply short-acuminate, base cordate to truncate ........................................... P. alvarezianum

**Protium alvarezianum** Daly & P. Fine, sp. nov.—TYPE:
Brazil. Mun. São Paulo de Olivença, Estrada Bomfim, trail beyond road, ca. 6 km S of town center, 3°30’S, 68°57’W, 23 Nov 1986 (m fl), D. C. Daly, R. P. Lima, & V. S. Pinho 4414 (holotype: INPA; isotypes: AAU!, C!, CAS!, DUKE!, NY!, PORT!, TUR!, W!).

Arbor 5–15 m usque altus in sylvis in arena alba Amazoniae occidentalis ramosi graciles folioli parvi et chartae margine (sub)integer ab affinis ibi notulis differt: inflorescentia constantre brevis (3 cm usque, 4.5 usque in fructu) calyx longior bracteae inflorescentiae tumidae base fructu persistens stamina et staminodii longiores fructus parvior baviori cordata. Sparsely branched tree 5–15 m × 4–15 cm DBH. Inner bark red; sapwood white, 2–3 mm thick; heartwood reddish, soft without. Resin usually sparse, white, gummy and very thick (reportedly clear on Thomas 3178, “light latex” on Gentry & Stein 46997). Leaves 4–7-jugate, 30–54 cm long; petiole 7.3–15 cm long, interjuga 2.5–4.5 cm long, petiole and rachis with dense, straight, ascending to appressed, golden hairs to 0.5 mm long, also sparse to dense, fine, erect, golden hairs to 0.25 mm long; lateral petiolules 0.1–0.7 cm long, without pulvinuli, basal pair of leaflets 5.6–10.8 × 0.9–4.8 cm, lanceolate to ovate or elliptic, other laterals 6.2–16 × 2.2–4.5 cm, oblong-elliptic to slightly oblong-(ob)lanceolate, terminal petiolule 1.2–2 cm long, terminal leaflet 8–11 × 2.9–4.3 cm, leaflets thinly chartaceous, drying dark greenish brown, apex abruptly to gradually and narrowly long-acuminate, the acumens (0.3) 0.7–2 cm long, base asymmetric, basicopic side acute, acrosopic side truncate to obtuse, sometimes decurrent, margin entire or often some leaflets with a few scattered teeth, especially on the acumens; on abaxial surface the midvein prominent, the secondary prominent to prominulous, the higher-order veins prominulous to flat, subglabrous or toward base provided with sparse to scattered, ascending to flexuous hairs to 0.5 mm long (sometimes all along midvein); on adaxial surface the midvein narrowly prominulous, the secondaries and higher-order veins narrowly prominulous.
to flat, surface glabrous. Inflorescences conflated at branchlet apices, robust, staminate ones to 9 cm long, pistillate inflorescences and infructescences 2-3 cm long (~4.5 cm with fruits), pseudospicate; axes with dense, appressed to ascending, golden hairs to 0.3 mm long; bracts on primary axes 1.5–2 mm long, subulate to lanceolate, fleshy and persistent, bracteoles 0.5–1.3 mm long, very broadly ovate and sharply acuminate, the base semiclasping and swollen. Flowers congested along axes, calyx 2.5–3 × 2.8–3.6 mm overall, taller than the nectary disk, with dense to sparse hairs on axes; in staminate flowers the lobes 2.2–2.6 mm long, (narrowly) triangular and slightly acuminate; petals 4.8–6.1–1.3–1.4 mm, green without, out, subulate, distally spreading to reflexed, with an inrolled apiculum 0.35–0.5 mm long, slightly saccate at base, axillary surface with pubescence as on calyx (rarely scattered), adaxial surface with dense, ascending to floccuous, white hairs to 0.8 mm long; antepetalous staminodes 3.6–4 mm long with lanceolate anthers 0.6–0.75 mm long, the antepetalous ones 2.8–3.6 mm long with ovate anthers 0.4–0.5 mm long, filaments slightly compressed; nectary disk 0.3–0.5 mm tall and 0.2–0.5 mm thick, with dense, ascending hairs to 0.3 mm long; pistillode 0.35 mm × 0.5 mm wide, slightly taller than nectary disk, depressed-ovoid, with dense, ascending hairs to 0.4 mm long, provided with reduced locules and ovules, the style 0.2–0.35 mm long; pistillate flowers with calyx lobes 1.5–2 mm long; petals 4.4–5.5 × 1.4–1.85 mm, suberect at anthesis; antepetalous staminodes 2.2–2.8 mm long with lanceolate anthers 0.4–0.6 mm long, the antepetalous ones 1.4–1.85 mm long with ovate anthers 0.4–0.45 mm long; nectary disk 0.2–0.4 mm tall and 0.3–0.4 mm thick; pistillode 3.2 × 1.2–1.4 mm, with dense, ascending hairs to 0.4 mm long, ovary ovoid, style 1.6 mm long, the stigmata 0.4 mm tall, laterally compressed-globose. Fruit maturing dark red, 1.4–1.6 × 0.8–1 cm (to 1.4 cm wide when > 1 pyrene), obliquely ovoid, the apex narrowly and sharply short-acuminate, the base cordate to truncate, not stipitate, surface with sparse to scattered, flexuous hairs to 0.5 (–0.6) mm long, sometimes these persisting only at base and apex; on dehiscence the valves pink within; pseudaril white; pyrene 1.05–1.12 × 0.6–0.8 cm, in dorsiventral view ovate with acute to slightly acuminate apex and rounded base, in lateral view ovate with a shallow cleft slightly less than halfway to the apex corresponding to the funicular scar, the scar laterally oblong and covering ca. 20% of the anterior face, the surface glabrous and smooth. Cotyledons unknown, on seedlings the first eophylls 11-foliolate (Fine et al. 823, NY). Figure 4.

**Common Names, Uses, Etymology**—Brazil: “lacere” (Daly et al. 5116); Peru: “copal” (Vásquez & Soto 11891). It should be noted that these names are by no means species-specific; rather the Brazilian name is applied to various taxa having a milky resin, and the Peruvian name is applied to most Amazonian Burseraceae. No uses reported. The specific epithet acknowledges José Álvarez Alonso, conservation biologist, ornithologist, journalist, discoverer of and champion for white-sand forests in the Peruvian Amazon. He was the driving force behind the creation of the first reserve in Peru that protects white-sand forests, the Reserva Nacional Allpahuayo-Mishana.

**Distribution and Ecology**—The known distribution of the new species consists of four apparent disjunctions: (1) the upper Rio Negro basin, in southern Amazonas department in Venezuela and contiguous Amazonas, Brazil; (2) the Japarú basin in NW Brazil; (3) the middle Ucayali basin in Loreto, Peru; and (4) the Morona basin north of the Marañon River in Loreto, Peru. This is clearly a white-sand specialist, growing in sandy ultisols or podzols. It has been recorded from two similar vegetation types in Brazil and Peru (Fig. 3):

1. chlamidal / low campinarana / low bana — Dense poles, few exceeding 4–5 m, in soil with ca. 20 cm of litter/humus overlying ca. 30 cm of impermeable concretion overlying sand (e.g., Peru, Daly & Accedo 5116, 5118).
2. varillal / Amazonian caatinga / campinarana forest / tall bana, in white-sand on undulating terrain, the canopy discontinuous with occasional trees to 15 m tall, layer of litter and humus 10–30 cm deep (Peru, Dévila et al. 1171), or in caatinga alta, the canopy ca. 15 m tall and few emergents to 35 m (Brazil, Cid Ferreira 9336, Daly et al. 4414).

This species has been collected flowering Nov-Apr, and fruiting Feb-Jul (Oct).

**Additional Specimens Examined**—VENEZUELA. Amazonas: Depto. Rio Negro, Nebina Base Camp, Río Mavarrina, 00°50′N, 66°10′W, 140 m, 27 Jan 1985 (fl), Boom & Weitzman 5381 (AAU, AMAZ, MO, NY), 23 Feb 1985 (immat. fr), Boom & Weitzman 5948 (AAU, COL, MICH, MO, NY, US, W), 4–5 Jul 1984 (fl), Davide & Miller 26997 (NY), 22 Apr 1984 (immat. fr), Gentry & Stein 46874 (NY), 27 Apr 1984 (immat. fr), Gentry & Stein 46980 (NY), 20 Mar 1984 (immat. fr), Liesner 16486 (NY); S side of Río Mavarrina (= Río Baria), 00°49′50″N, 66°09′40″W, 23 Feb 1985 (immat. fr), Nee 31066 (NY, 2 sheets), near base camp, 00°50′N, 66°10′W, 23 Apr 1984 (immat. fr), Thomas 3178 (NY).

PERU. Loreto: Daly & Requena, Ditto. Sapuana, Centro de Investigaciones Jenaro Herrera, ca. 5 km E of Río Ucayali, 4°55′S, 73°45′W, 21 Apr 1987 (m fl), Daly & Accedo 5116 (NY), 21 Apr 1987 (immat. fr), Daly & Accedo 5118 (NY); Prov. Requena, Ditto. Curinga, near Ungurahulla, Río Blanco, 5°51′S, 73°46′W, 1 Nov 2004 (fl), Dévila et al. 1171 (NY); Prov. Datem de Marañón, Ditto. San Lorenzo, near community of Tierra Blanca, Río Morona, 4°16′S, 73°46′W, 2 Apr 2002 (seedling), Fine et al. 823 (NY), 24 Apr 2002 (fr), Fine et al. 832 (UC), 24 Apr 2002 (juvenile), Fine et al. 844 (AMAZ, MO, NY, UC), 5 Dec 2006 (fr), Mesones 1312 (NY, UC), 5 Dec 2006 (fl), Mesones 1316 (NY, UC), Jenaro Herrera, 4°35′S, 73°45′W, 130 m, 13 Mar 1989 (immat. fr), Nascimento et al. 155 (IAN, MG, 2 sheets).

In comparison to other species in sect. Papilloprotonium, *P. alvarezianum* is distinctive because of its slender branchlets; the leaflets are small and thinly chartaceous, with (sub)entire margin; the pistillate inflorescences are consistently short (to 3 cm long, 4.5 cm with fruits); the calyx is longer; the inflorescence bracts are swollen and at least the base persists in fruit; the stamens and staminodes are longer; and the fruits are smaller with a coritate or less often truncate base.


**Distribution and Ecology**—*Protium ferrugineum* is widespread in Amazonia north of the Rio Amazonas, with a trans-Andean disjunction in Valle, Colombia and an apparent disjunction to the south in Huimaitá, Amazonas, Brazil. It is also versatile ecologically, reported from tall moist forest in clay or sandy clay or white-sand soil; high caatinga, forest seasonally flooded by black-water rivers; and dwarf forest in quartzite deposits. The species occurs in two distinct habitats in the Ducke Reserve outside of Manaus, Brazil: baixio or...
forest on low-lying terrain in sandy soil with poor drainage, and vertente or well-drained slope forest in clay soil. In Peru, *P. ferrugineum* is not associated with low-canopy white-sand forest, although there are a few collections of juvenile individuals from high canopy white-sand forest (Fine et al. 2005).


**Distribution and Ecology**—This species has the most restricted distribution of the section, to date known only from the upper Río Negro basin in Venezuela and Brazil, but expected in contiguous Colômbia. It is usually a small tree, 4–16(–25) m × 5–30 cm DBH, reported explicitly from bana (= campina) vegetation and descriptively from low forests on poorly drained soils overlain by white sand, with one report from caatinga pedregosa or rocky caatinga at Tunuí on the Rio Uaupés; this is likely an analogous formation with grafitic outcrops.


**Distribution and Ecology**—This is by far the most broadly distributed species of the section, widespread in Amazonia, extending into Guyana and French Guiana, and disjunct in Antioquia, Colombia. In Amazonia the species is known from Brazil (Acre, Amapá, Amazônas, Maranhão, Pará, Rondônia), Colombia (Amazonas), Ecuador (Napo, Santiago-Zamora), Peru (Loreto), and Venezuela (Amazonas). It occurs in a range of habitats like that of *P. ferrugineum*.

*Protium subserratum* shows pronounced geographic variation, but thus far the species is considered to be a widespread and variable taxon. Any future assessment of the infraspecific taxonomy must take into consideration the variation that can be observed even within a single collection. For example, on the several duplicates of *Krukoff* 7063 (seen at BM, GH, K, NY), collected in the Rio Madeira basin, the leaflet size varies greatly from one specimen to another and the leaflet margin ranges from markedly serrate to completely entire. The morphological variants (morphotypes) of *P. subserratum* and their geographic distributions are presented in Table 3 and their putative relationships are shown in Fig. 5.

### Results

The Bayesian posterior probabilities were mapped onto a majority rule consensus of all 7,500 post burn-in trees (Fig. 5). The topology of this consensus tree for the ingroup (sect. *Papilloprotium*) exactly matched the best tree produced by maximum likelihood (RAxML). Both the Bayesian and maximum likelihood analyses suggest strong support (1.00 posterior probability, 100% bootstrap) for the monophyly of sect. *Papilloprotium*. Trees have been submitted to TreeBASE (study number S11442). *Protium ferrugineum*, the most divergent of the four taxa within sect. *Papilloprotium*, is sister to the other three species. The two taxa restricted to low-canopy white-sand forests, *P. reticulatum* and the new *P. alvezianum*, are sister to each other, and their common ancestor is sister to *P. subserratum*. Within *P. subserratum*, Peruvian Amazonian specimens are sister to the clade that is composed of the eastern specimens collected in French Guiana and Guyana, showing some amount of phylogeographic structure among populations across the continent. However, individuals from “morphotype 1” are nested within a grouping that includes individuals categorized as “morphotype 2” and “morphotype 3.” Therefore, these three morphotypes do not form monophyletic clades, consistent with the hypothesis that they do not warrant taxonomic status without more comprehensive sampling.

### Discussion

Infraspecific classification of the Burseraceae is approaching resolution (e.g. Thulin et al. 2008), although some groupings of genera based on molecular phylogenies obtained to date are difficult to justify on morphological grounds (Daly et al. 2011). Tribe Proteae (*Protium, Tetragastris, Crepidospermum*; e.g. Daly 1989) has survived intact despite rather drastic realignments in the rest of the family (e.g. Weeks et al. 2005), but it is clear that final resolution of generic limits in Proteae will require accessing DNA material of several as yet unsampled lineages. Still, it is remarkable that a number of

### Table 3. Morphological variants of *Protium subserratum* and their geographic distributions.

<table>
<thead>
<tr>
<th>Character</th>
<th>morphotype 1</th>
<th>morphotype 2</th>
<th>morphotype 3</th>
<th>morphotype 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaflet texture</td>
<td>chartaceous</td>
<td>coriaceous</td>
<td>coriaceous</td>
<td>markedly coriaceous</td>
</tr>
<tr>
<td>Leaflet margin</td>
<td>entire</td>
<td>teeth usually present</td>
<td>entire</td>
<td></td>
</tr>
<tr>
<td>Abaxial tertiaries</td>
<td>impressed</td>
<td>raised</td>
<td>raised</td>
<td></td>
</tr>
<tr>
<td>Abaxial color</td>
<td>(greenish) brown</td>
<td>usually brown</td>
<td>usually whitish, less</td>
<td></td>
</tr>
<tr>
<td>Adaxial surface</td>
<td>often glossy</td>
<td>dull</td>
<td>dull</td>
<td></td>
</tr>
<tr>
<td>Inflorescence secondary axes</td>
<td>often well-developed</td>
<td>sometimes well-developed</td>
<td>poorly-developed</td>
<td></td>
</tr>
<tr>
<td>Fruit apex</td>
<td>acuminated</td>
<td>acuminate</td>
<td>acute to acuminate</td>
<td></td>
</tr>
<tr>
<td>Voucher</td>
<td>Daly et al. 3764 (MG, NY) Palacios et al. 999 (MO, NY)</td>
<td>Clark 6672 (MO, NY)</td>
<td>Restrepo &amp; Matapi 365 (COAH, NY)</td>
<td></td>
</tr>
</tbody>
</table>
groups within *Protium* proposed as “natural” based on morphological evidence have been well-supported in molecular phylogenies (Fine et al. 2005); these include the neotropical sections *Icicopsis* (Daly 1989), *Sarcoprotium* (Daly 1992), and *Pepeanthos* (Daly 2007), plus African *Marignia*, and Asian *Protium* (Harley and Daly 1996), and now *Papilloprotium*.

*Tetragastris* and *Crepidospermum* are supported as distinct clades, although current evidence indicates they are nested within *Protium* (Fine et al. 2005; see Fig. 5). Characters almost unique to *Tetragastris* in Proteae include petals fused at least half their length and the anther base entire (not sagittate) in staminate flowers; the lack of lateral pulvinuli is unusual but not unique. The only character state unique to *Crepidospermum* in the tribe is the total lack of pulvinuli; characteristics rare elsewhere in the tribe include the presence of snail-shaped glands on most surfaces, flowers sometimes haplostemonous, and the cotyledons plano-convex and U- or J-shaped. Further analysis will be required to determine if these two genera should be subsumed within a more broadly defined *Protium*, perhaps as distinct sections.

Edaphic specialization has been implicated as a major contributor to the Neotropical flora (Gentry 1989). Fine et al. (2005) found that a great majority of Proteae in the Peruvian Amazon were restricted to a subset of edaphic habitats available in the region. Two of the four species in section *Papilloprotium*, *P. alvarezianum* and *P. reticulatum*, are restricted to rare, physiologically stressful, white-sand habitats on sites that are extremely nutrient-poor and often poorly drained due to a perched water table. Moreover, these are commonly small habitat islands often separated from similar habitats by hundreds (sometimes many hundreds) of kilometers.

The phylogenetic results, which indicate that *P. alvarezianum* and *P. reticulatum* are closest relatives, suggest that in the past the common ancestors became separated geographically, likely somewhere close to the vertex of Venezuela, Brazil, and Colombia in the upper Río Negro basin (Fig. 3). *Protium reticulatum* is found only in this area, while *P. alvarezianum* has a distribution ranging into Brazil and into Peru as far west as the Río Morona, but always in white-sand forests. Possible vicariant fragmentation of white-sand habitats in the last several million years in the western Amazon (Frasier et al. 2008) may have separated ancestors of *P. alvarezianum* and *P. reticulatum*, leading to an instance of allopatric speciation between white-sand islands. An alternative to this latter scenario is a long-distance dispersal event by ancestors of *P. alvarezianum* to white-sand forests to the west, initiating the speciation process that split this lineage within section *Papilloprotium*.

The other two species, *P. ferrugineum* and *P. subserratum*, have been collected in a variety of edaphic habitat types. As noted above, *P. subserratum* includes four main morphotypes, one of which is found only in white-sand forests. Interestingly, the phylogenetic results indicate that this
white-sand morphotype does not derive from the most basal nodes within the taxon, but instead appears to be derived from common ancestors that likely inhabited clay or brown-sand forests. Additional sampling is needed to further investigate the role of edaphic heterogeneity in driving morphological evolution within this species.

It is striking that colonization of white-sand forests has occurred at least twice in this small clade of four species: once in the common ancestor of *P. alvarezianum* and *P. reticulatum*, and again within *P. suberratum*. It is interesting to note that *P. ferrugineum* is only rarely found in white-sand forests, being much more common in clay or brown sand soils, even though it is sister to the other three species of the section. This suggests that colonization of white-sand forests has been a recent phenomenon for this section relative to the long history of the Protieae in the Amazon basin. This is curious, given the ancient presence of white sands in the region (Räsänen et al. 1987; Hoorn 1993; Hoorn 1994; Huber 1995; Struwe et al. 1997). Phylogenetic studies of other Amazonian angiosperm clades that include white-sand specialists have found the reverse pattern, with white-sand specialists appearing at a basal position within a clade and therefore likely representing the most ancient species, as in *Potalia* (Gentianaceae; Frasier et al. 2008).

The phylogenetic results suggest that diversification within this part of tribe Protieae has been promoted in two ways: vicariance or long-distance dispersal, and habitat shifts. First, the likely fragmentation of white-sand habitats in the last several million years in the western Amazon would have increased the chances for allopatric speciation to occur between white-sand islands, sundering *P. reticulatum* from *P. alvarezianum*. Second, coupled with the tribe’s remarkable tolerance of a variety of different substrates is a proclivity for subsequent edaphic specialization, as it appears has happened two times just in this small group of four species, notably within *P. suberratum*. Such examples highlight the importance of the edaphic heterogeneity of the Amazon in the diversification processes of its extraordinarily diverse flora.

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**Literature Cited**


Fine, P. V. A., F. Zapata, D. C. Daly, T. M. Misiewicz, I. Mesones, H. F. Cooper, and C. E. A. Barbosa. In press. The importance of environmental heterogeneity and spatial distance in generating phylogeographic structure in edaphic specialist and generalist species of *Protium* (Burseraceae) across the Amazon basin. *Journal of Biogeography*.


