Botanical Journal of the Linnean Society, 2017, **185**, 27–55. With 4 figures.

Towards a natural classification of Sapotaceae subfamily Chrysophylloideae in the Neotropics

APARECIDA DONISETE DE FARIA[1]*, JOSÉ RUBENS PIRANI[2], JOSÉ EDUARDO LAHOZ DA SILVA RIBEIRO[1], STEPHAN NYLINDER[3], MÁRIO HENRIQUE TERRA-ARAUJO[4], PEDRO PAULO VIEIRA[5] and ULF SWENSON[3]

[1]*Departamento de Biologia Animal e Vegetal, Centro de Ciências Biológicas, Universidade Estadual de Londrina, Campus Universitário, Rodovia Celso Garcia Cid, PR 445, Km 380, 86057-970, Londrina, PR, Brazil*
[2]*Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, 05508-9070, São Paulo, SP, Brazil*
[3]*Department of Botany, Swedish Museum of Natural History, PO Box 50007, SE-104 05 Stockholm, Sweden*
[4]*Instituto Nacional de Pesquisas da Amazônia, Avenida André Araujo 2936, Coordenação de Pesquisas em Botânica, 69060-000, Manaus, AM, Brazil*
[5]*Laboratório de Biologia Molecular, Fundação de Medicina Tropical, Av. Pedro Teixeira, 25, Dom Pedro, 69040-000, Manaus, AM, Brazil*

Received 17 January 2017; revised 15 May 2017; accepted for publication 2 June 2017

Generic limits of *Chrysophyllum* and *Pouteria* (Chrysophylloideae, Sapotaceae) have been found to be untenable. We here search for natural lineages in Neotropical Chrysophylloideae by sampling 101 terminals for molecular sequences of nuclear ribosomal DNA (external and internal transcribed spacer), the nuclear gene *RPB2* and 17 morphological characters. Data were analysed with Bayesian inference and parsimony jackknifing. Morphological traits were finally optimized onto the tree to identify the most coherent characters. The resulting phylogenetic tree suggests that the limits of the well-known genera *Chrysophyllum* and *Pouteria* must be amended. *Diploon*, *Ecclinusa* and *Elaeoluma* can be maintained and *Chrysophyllum* sections *Ragala* section *Prieurella* and the satellite genera *Achrouteria*, *Cornuella*, *Martiusella* and *Nemaluma* merit generic resurrection. *Lucuma* may be restored if the type species belongs to the clade. The accepted genera *Chromolucuma*, *Pradosia* and *Sarcaulus* gain strong clade support, but are embedded in a core clade of *Pouteria* and may be relegated to the subgeneric level if morphological studies cannot provide evidence concurring with narrow generic concepts. Circumscriptions of *Micropholis* and *Chrysophyllum* sections *Chrysophyllum* and *Villocuspis* remain unclear and must be explored by using an extended taxon sampling. We predict that yet-to-be-analysed species of *Pouteria* sections *Franchetella*, *Gayella*, *Oxythece* and *Pouteria* and members of the currently accepted genera *Chromolucuma*, *Pradosia* and *Sarcaulus* will fall inside the core clade of *Pouteria* when analysed.

ADDITIONAL KEYWORDS: BEAST – *Chrysophyllum* – classification – jackknife – *Pouteria* – South America.

INTRODUCTION

Sapotaceae are important wet forest components throughout the Neotropics, with some members also being found in drier biomes of the Brazilian Cerrado and Campo Rupestre. Important diagnostic characters of the family include the presence of latex, simple and entire leaves, malpighiaceous trichomes and flowers arranged in fascicles (except in *Sarcosperma* Hook.f.). The number of species has been estimated at *c*. 1250 (Pennington, 1991; Govaerts, Frodin & Pennington, 2001), but it is steadily increasing in tropical America (Pennington, 2006, 2007), Africa (Gautier *et al.*, 2016) and the Pacific region (Swenson, Munzinger &

*Corresponding author. E-mail: cidadefaria@uol.com.br

Bartish, 2007b; Munzinger & Swenson, 2009; Swenson & Munzinger, 2012, 2016). A new subfamilial classification was proposed by Swenson & Anderberg (2005), recognizing Chrysophylloideae (similar in circumscription to Chrysophylleae), Sarcospermatoideae and Sapotoideae, with the last now including four tribes (Isonandreae, Sapoteae, Sideroxyleae and Tseboneae; Smedmark, Swenson & Anderberg, 2006; Smedmark & Anderberg, 2007; Gautier *et al.*, 2013). The number of genera in the family has fluctuated according to the adopted generic concepts, from Aubréville's 122 (Aubréville, 1964a), to Baehni's 63 (Baehni, 1965) and Pennington's 53 (Pennington, 1991), all depending on the morphological features chosen to be indicative of natural relationships. Generic boundaries in the family are still not entirely clear, particularly in the Neotropical Chrysophylloideae (Swenson, Richardson & Bartish, 2008a).

Pennington (1990, 2006, 2007) recognized approximately 350 species in Chrysophylleae in the New World, distributed in nine genera: *Chromolucuma* Ducke (five species), *Chrysophyllum* L. (45 species), *Diploon* Cronquist (one species), *Ecclinusa* Mart. (11 species), *Elaeoluma* Baill. (four species), *Micropholis* (Griseb.) Pierre (39 species), *Pouteria* Aubl. (*c.* 200 species), *Pradosia* Liais (25 species) and *Sarcaulus* Radlk. (five species). He adopted wide generic concepts for *Chrysophyllum* and *Pouteria*, resulting in pantropical distributions. Phylogenetic reconstructions of Sapotaceae have, however, clearly demonstrated that these two genera are polyphyletic, that the type species are placed in clades restricted to the New World and that features traditionally used for their delimitation show extensive homoplasy that lead to untenable classifications (Swenson & Anderberg, 2005; Triono *et al.*, 2007; Swenson, Bartish & Munzinger, 2007a; Swenson *et al.* 2008a; Swenson, Nylinder & Munzinger, 2013). Thus, species of the non-staminodial genus *Chrysophyllum* in Malesia and New Caledonia (Vink, 1958) are better placed in *Amorphospermum* F.Muell., *Niemeyera* F.Muell. or *Pycnandra* Benth. (Swenson *et al.*, 2013) and are only distantly related to *Chrysophyllum* spp. in the New World (Swenson *et al.*, 2008a). Similarly, several Australasian taxa were included in *Pouteria* by Baehni (1942), transferred to *Planchonella* Pierre (van Royen, 1957), transferred back to *Pouteria* (Baehni, 1965), returned to *Planchonella* (Aubréville, 1967) and again united with *Pouteria* (Pennington, 1991). These changing taxonomic positions reflect results of taxonomic work done before molecular phylogenetic tools became widely used. Molecular phylogenetic analyses of the Australasian taxa clearly group species into monophyletic lineages concurring with *Planchonella*, *Pleioluma* (Baill.) Baehni, *Sersalisia* R.Br. and *Van-royena* Aubrév. (Swenson *et al.*, 2007a, 2013). The African *Pouteria* satellite genus *Donella* Pierre ex Baill. has been recently reinstated (Mackinder, Harris & Gautier, 2016), but *Aningeria* Aubrév. & Pellegr., *Gambeya* Pierre and *Malacantha* Pierre are also in need of resurrection; however, their circumscriptions have not yet been investigated (Swenson *et al.*, 2008b). Similarly, circumscription of the genera of Chrysophylloideae in the New World remains unclear.

Recent phylogenetic analyses of molecular data indicate that Neotropical Chrysophylloideae probably form a clade that could have originated in South America in the Palaeocene, *c.* 59 Mya (Bartish *et al.*, 2011). Some of the diagnostic characters used by Pennington (1990) to delimit the Neotropical genera are presence vs. absence of stipules, staminodes and endosperm, with the shape of the corolla (rotate/cup-shaped or cyathiform/tubular). Also, Pennington's (1990) dichotomous generic keys indicate that the genera could be unnaturally delimited; for example, the majority of *Pouteria* spp. possess staminodes, but some lack them, a character pertinent for other genera. Stipules, a diagnostic feature for *Chromolucuma* and *Ecclinusa*, are also found in *P. congestifolia* Pilz, *P. flavilatex* T.D.Penn. and *P. stipulifera* T.D.Penn., leading Alves-Araújo & Alves (2012a) to transfer *P. congestifolia* to *Chromolucuma*. *Chrysophyllum sensu* Pennington comprises species with pentamerous flowers, no staminodes (or with fewer than the corolla lobes) and copious endosperm, a combination of characters that is also suggestive of some *Pouteria* spp. We therefore do not exclude the possibility that some of the Neotropical species of *Chrysophyllum* and/or *Pouteria* belong to other genera. Since many new sapotaceous species have been recently described (Alves-Araújo & Alves, 2011, 2012a, b; Morales, 2012; Terra-Araujo, Faria & Vicentini, 2012a; Santamaría-Aguilar, Chaves-Fallas & Aguilar, 2017), deficiencies in the diagnostic delimitation are of great concern and there is a scientific desire to reach nomenclatural stability.

Pennington's (1990, 1991) infrageneric classification of *Chrysophyllum* and *Pouteria* recognized six sections in *Chrysophyllum* (*Aneuchrysophyllum* Engl., *Chrysophyllum*, *Donella* Pierre ex Baill. *Prieurella* Pierre, *Ragala* Pierre and *Villocuspis* (A. DC.) Aubrév. & Pellegr.) and nine in *Pouteria* (*Aneulucuma*, *Antholucuma*, *Franchetella*, *Gayella*, *Oligotheca*, *Oxythece*, *Pierrisideroxylon*, *Pouteria* and *Rivicoa*). Five sections of *Chrysophyllum* and eight of *Pouteria* are present in the Neotropics. Most of them stem from taxa established in the late 1800s by Pierre (1890, 1891) and Baillon (1891), taxa that Aubréville (1964a) still recognized as distinct genera, but were reduced to sections by Pennington (Table 1). The objectives of this study are to investigate the phylogenetic relationships of the Neotropical Chrysophylloideae based

Table 1. Overview of Pennington's classification of Neotropical Chrysophylloideae (Sapotaceae) with satellite genera including year of publication, type species and equivalent name in current classification

Genus or section, Publication year and segregate genus		Type species	Pennington's classification
Chromolucuma Ducke (2: 2)		*Chromolucuma rubriflora* Ducke	***Chromolucuma rubriflora*** Ducke
Chrysophyllum section *Aneuchrysophyllum* (11: 3)			
1891	*Chloroluma* Baill.	*Chloroluma gonocarpa* (Mart. & Eichler) Baill. ex Aubrév.	***Chrysophyllum gonocarpum*** (Mart. & Eichler) Engl.
1891	*Cornuella* Pierre	*Cornuella venezuelanensis* Pierre	***Chrysophyllum venezuelanense*** (Pierre) T.D.Penn.
1891	*Martiusella* Pierre	*Martiusella imperialis* (K.Koch & Fintelm.) Pierre	***Chrysophyllum imperiale*** (K.Koch & Fintelm.) Benth. & Hook.f.
1936	*Achrouteria* Eyma	*Achrouteria pomifera* Eyma	***Chrysophyllum pomiferum*** (Eyma) T.D.Penn.
Chrysophyllum section *Chrysophyllum* (17: 2)			
1753	*Chrysophyllum* L.	*Chrysophyllum cainito* L.	***Chrysophyllum cainito*** L.
1794	*Nycterisition* Ruiz & Pavon	*Nycterisition ferrugineum* Ruiz & Pav.	*Chrysophyllum argenteum* subsp. *ferrugineum* (Ruiz & Pav.) T.D.Penn.
1838	*Guersentia* Raf.	*Guersentia oliviformis* (L.) Raf.	***Chrysophyllum oliviforme*** L.
Chrysophyllum section *Prieurella* (5: 5)			
1891	*Prieurella* Pierre	*Prieurella cuneifolia* (Rudge) Aubrév.	*Chrysophyllum cuneifolium* (Rudge) A.DC.
Chrysophyllum section *Ragala* (4: 3)			
1891	*Ragala* Pierre	*Ragala sanguinolenta* Pierre	***Chrysophyllum sanguinolentum*** (Pierre) Baehni
Chrysophyllum section *Villocuspis* (6: 1)			
1961	*Villocuspis* (A.DC.) Aubrév. & Pellegr.	*Villocuspis flexuosa* (Mart.) Aubrév. & Pellegr.	*Chrysophyllum flexuosum* Mart.
Diploon Cronquist (1: 1)		*Diploon cuspidatum* (Hoehne) Cronquist	***Diploon cuspidatum*** (Hoehne) Cronquist
Ecclinusa Mart. (11: 3)		*Ecclinusa ramiflora* Mart.	***Ecclinusa ramiflora*** Mart.
Elaeoluma Baill. (4: 3)		*Elaeoluma schomburgkiana* (Miq.) Baill.	***Elaeoluma schomburgkiana*** (Miq.) Baill.
*Micropholis** (Griseb.) Pierre (38: 6)		*Micropholis rugosa* (Sw.) Pierre	*Micropholis rugosa* (Sw.) Pierre
1890	*Crepinodendron* Pierre	*Crepinodendron crotonoides* Pierre	*Micropholis crotonoides* (Pierre) Pierre
1891	*Meioluma* Baill.	*Meioluma guianensis* Baill.	***Micropholis venulosa*** (Mart. & Eichler ex Miq.) Pierre
1891	*Platyluma* Baill.	*Platyluma calophylloides* (Pierre) Baill.	***Micropholis venulosa*** (Mart. & Eichler ex Miq.) Pierre
1962	*Paramicropholis* Aubrév. & Pellegr.	*Paramicropholis acutangula* (Ducke) Aubrév- & Pellegr.	*Micropholis acutangula* (Ducke) Eyma
Pouteria section *Aneulucuma* (21: 0)			
1890	*Urbanella* Pierre	*Urbanella procera* (Mart.) Pierre	*Pouteria procera* (Mart.) K.Hammer
Pouteria section *Antholucuma* (13: 4)			
1890	*Radlkoferella* Pierre	*Radlkoferella multiflora* (A.DC.) Pierre	***Pouteria multiflora*** (A.DC.) Eyma

Table 1. *Continued*

Genus or section, Publication year and segregate genus		Type species	Pennington's classification
Pouteria section *Franchetella* (62: 29)			
1788	*Labatia* Sw.	*Labatia sessiliflora* Sw.	*Pouteria sessiliflora* (Sw.) Poir.
1890	*Franchetella* Pierre	*Franchetella tarapotensis* Pierre	*Pouteria tarapotensis* (Eichler ex Pierre) Baehni
1890	*Paralabatia* Pierre	*Paralabatia dictyoneura* (Griseb.) Aubrév.	*Pouteria dictyoneura* (Griseb.) Radlk.
1891	*Discoluma* Baill.	*Discoluma gardneri* (Mart. & Eichler) Baill.	***Pouteria gardneri*** (Mart. & Eichler) Baehni
1891	*Eremoluma* Baill.	*Eremoluma sagotiana* Baill.	*Pouteria sagotiana* (Baill.) Eyma
1891	*Microluma* Baill.	*Microluma parviflora* (Benth. ex Miq.) Baill.	***Pouteria ramiflora*** (Mart.) Radlk.
1891	*Nemaluma* Baill.	*Nemaluma engleri* (Eyma) Aubrév. & Pellegr.	***Pouteria engleri*** Eyma
1891	*Podoluma* Baill.	*Podoluma catocladantha* (Eichler) Aubrév.	***Pouteria gardneri*** (Mart. & Eichler) Baehni
1891	*Pseudocladia* Pierre	*Pseudocladia lateriflora* (Benth. ex Miq.) Pierre	***Pouteria ramiflora*** (Mart.) Radlk.
1962	*Sandwithiodoxa* Aubrév. & Pellegr.	*Sandwithiodoxa egregia* (Sandwith) Aubrév. & Pellegr.	*Pouteria egregia* Sandwith
1976	*Peteniodendron* Lundell	*Peteniodendron belizense* Lundell	***Pouteria durlandii*** (Standl.) Baehni
1983	*Piresodendron* Aubrév. & Pellegr.	*Piresodendron ucuqui* (Pires & R.E.Schult.) Le Thomas	*Pouteria ucuqui* Pires & R.E.Schult.
Pouteria section *Gayella* (8: 1)			
1890	*Gayella* Pierre	*Gayella valparadisaea* (Molina) Pierre	*Pouteria splendens* (A.DC.) Kuntze
1891	*Myrtiluma* Baill.	*Myrtiluma eugeniifolia* (Pierre) Aubrév.	***Pouteria eugeniifolia*** (Pierre) Baehni
1925	*Barylucuma* Ducke	*Barylucuma decussata* Ducke	*Pouteria decussata* (Ducke) Baehni
Pouteria section *Oligotheca* (8: 3)			
1925	*Syzygiopsis* Ducke	*Syzygiopsis oppositifolia* Ducke	*Pouteria oppositifolia* (Ducke) Baehni
Pouteria section *Oxythece* (11: 4)			
1961	*Caramuri* Aubrév. & Pellegr.	*Caramuri opposita* (Ducke) Aubrév. & Pellegr.	***Pouteria opposita*** (Ducke) T.D.Penn.
1961	*Neoxythece* Aubrév. & Pellegr.	*Oxythece leptocarpa* Miq.	*Pouteria elegans* (A.DC.) Baehni
1972	*Pseudoxythece* Aubrév.	*Pseudoxythece ambelaniifolia* (Sandwith) Aubrév.	***Pouteria ambelaniifolia*** (Sandwith) T.D.Penn.
Pouteria section *Pouteria* (48: 19)			
1775	*Pouteria* Aubl.	*Pouteria guianensis* Aubl.	***Pouteria guianensis*** Aubl.
1812	*Guapeba* Gomes	*Guapeba laurifolia* Gomes	***Pouteria caimito*** (Ruiz & Pav.) Radlk.
1891	*Gomphiluma* Baill.	*Gomphiluma martiana* Baill.	*Pouteria gomphiifolia* (Mart. ex Miq.) Radlk.
1891	*Krugella* Pierre	*Krugella hartii* Pierre	***Pouteria guianensis*** Aubl.
1891	*Leioluma* Baill.	*Leioluma lucens* (Mart. & Miq.) Baill.	*Pouteria lucens* (Mart. & Miq.) Radlk.
1962	*Eglerodendron* Aubrév. & Pellegr.	*Eglerodendron pariry* (Ducke) Aubrév. & Pellegr.	*Pouteria pariry* (Ducke) Baehni

Table 1. *Continued*

Genus or section, Publication year and segregate genus		Type species	Pennington's classification
1962	*Pseudolabatia* Aubrév. & Pellegr.	*Pseudolabatia psammophila* (Mart.) Aubrév.	*Pouteria psammophila* (Mart.) Radlk.
Pouteria section *Rivicoa* (10: 5)			
1782	*Lucuma* Molina	*Lucuma bifera* Molina	*Pouteria lucuma* (Ruiz & Pav.) Kuntze
1890	*Richardella* Pierre	*Richardella macrophylla* (Lam.) Aubrév.	***Pouteria macrophylla*** (Lam.) Eyma
1891	*Englerella* Pierre	*Englerella macrocarpa* Pierre	*Pouteria speciosa* (Ducke) Baehni
Pradosia Liais (25: 5)		*Pradosia glycyphloea* (Casar.) Liais	*Pradosia lactescens* (Vell.) Radlk.
Sarcaulus Radlk. (5: 1)		*Sarcaulus brasiliensis* (A.DC.) Eyma	***Sarcaulus brasiliensis*** (A.DC.) Eyma

Numbers in parentheses refer to accepted species in the Flora Neotropica Monograph (Pennington, 1990), followed by the number of sampled accessions. Asterisks (*) indicate conserved names. Species sampled in this study appear in bold type in the right column.

on nuclear ribosomal [external and internal transcribed spacer (ETS, ITS)] and nuclear (*RPB2*) DNA sequence data and a set of morphological characters using Bayesian inference and parsimony jackknifing. Our primary goals are to test (1) the monophyly of the Neotropical genera in Chrysophylloideae; (2) whether Pennington's (1990, 1991) sections in *Chrysophyllum* and *Pouteria* are natural groups; (3) whether they correspond to groups recognized by Aubréville (1964a); and (4) whether it is possible to establish diagnostic morphological character sets for the recovered clades.

MATERIAL AND METHODS

Nomenclature and taxon sampling

Pennington's (1990, 1991) classification of Neotropical Chrysophylloideae was used for the taxa we sampled. Accepted names are available from the family checklist (Govaerts *et al.*, 2001) and online at the World *Checklist of Selected Plant Families*, Royal Botanic Gardens, Kew (http://apps.kew.org/wcsp). The number of samples in this study has been expanded from 22 (Swenson *et al.*, 2008a) to 101. Terminal taxa, voucher information and GenBank accession numbers are reported in the Appendix. In addition to *Chrysophyllum* and *Pouteria*, our sample includes members of all presently accepted genera in the New World, i.e. *Chromolucuma*, *Diploon*, *Ecclinusa*, *Elaeoluma*, *Micropholis*, *Pradosia* and *Sarcaulus*. Phylogenetic estimates suggest that most form a group confined to the New World (Swenson *et al.*, 2008a; Bartish *et al.*, 2011). There are also indications that *Micropholis* is sister to all other sampled taxa (Bartish *et al.*, 2011), making it the most appropriate outgroup for the study of Neotropical Chrysophylloideae. The sample includes the type species of all genera (except *Pradosia*), three out of eight sections of *Pouteria* and three out of five sections of *Chrysophyllum* present in the Neotropics (Pennington, 1991).

Molecular data

Broad molecular phylogenetic studies in Sapotaceae have revealed plastid DNA regions to contain low numbers of informative sites (1–3%) for phylogenetic estimates (Swenson *et al.*, 2013). In contrast, molecular sequences of nuclear ribosomal DNA (rDNA) ITS1 and ITS2 (including complete 5.8S and parts of 18S and 26S), the ETS and the low copy nuclear gene *RPB2* (Oxelman & Bremer, 2000) have proved useful for phylogenetic inference in the family (Bartish *et al.*, 2005; Swenson *et al.*, 2007a, 2008a, 2013). We have here focused on these molecular markers and publish for the first time 80 ITS sequences, 96 ETS sequences and 100 *RPB2* sequences.

Total DNA was extracted from leaves dried in silica gel. Extraction, amplification and primers for ETS, ITS and *RPB2* followed the protocol described by Swenson *et al.* (2013), but also those of Bartish *et al.* (2005) for ITS and Swenson *et al.* (2008b) for ETS. Purified products of rDNA were sequenced with an ABI3130xl Automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

One drawback of ITS and ETS is that multiple copies of them occur in a typical plant genome and, depending on which copy is amplified and sequenced, inaccurate phylogeny could be reconstructed (Álvarez

& Wendel, 2003; Poczai & Hyvönen, 2010; Naciri & Linder, 2015). To identify samples with multiple copies, we carefully checked for double peaks in the proofreading procedure, with PCR products being subsequently cloned, using the TOPO-TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA, USA) and following the manufacturer's instructions. DNA from clones were amplified by PCR using the specific plasmid M13F and M13R primers following the TOPO-TA Cloning Kit manual, with subsequently purified products sequenced using the M13F primer in an ABI313xl Automated DNA Sequencer, yielding between four and ten slightly different repeats in 13 species.

Data matrix construction

Sequences of ETS, ITS and *RPB2* were partitioned into separate matrices and aligned in MAFFT v.6.818b (Katoh, Asimenos & Toh, 2009) using the *l-insi* predefined parameter setting. Resulting alignments were checked for similarity (Simmons, 2004), with subsequent minor manual adjustments to reduce potentially false homologies. Inferred gaps were coded as additional binary characters following the guidelines of Simmons & Ochoterena (2000). Gaps were assigned a simple substitution model allowing unconstrained reversible gains/losses of characters.

The sequence data include introns (ETS, ITS1, ITS2) and exons (5.8S, 18S, 26S, *RPB2*) and it is possible that jModelTest (Posada, 2008) could identify less parameter-rich substitution models for phylogenetic inference. We therefore constructed a second matrix in which introns and exons, respectively, were combined in two partitions. The original matrices (ITS, ETS, *RPB2*) with gap coding are available as Supplementary data on the journal website.

Accessions of 13 species with variable repeats (copies) of ITS or ETS were handled in two ways. The first matrix (called incomplete) was aligned with all variable repeats, but only the first repeat of each taxon was concatenated with the other unique marker, with all other copies concatenated with question marks. The second matrix (called complete) was a duplicate of the first, but the question marks were substituted with the available sequences of ITS or ETS. For example, for *Chromolucuma cespedisiiformis* J.F.Morales, four ETS copies and only one ITS sequence are available. Therefore, in the incomplete matrix it had four ETS entries, combined with one ITS sequence, and three entries with question marks. In the complete matrix, it was represented by four different ETS entries and four identical ITS sequences. Such matrices of 101 samples yielded 189 terminals, distributed in four types of matrices: (1) incomplete based on markers; (2) complete based on markers; (3) incomplete based on introns/exons; and (4) complete based on introns/exons.

Phylogenetic analyses

Phylogenetic relationships were estimated with Bayesian inference (Rannala & Yang, 1996; Yang & Rannala, 1997) and parsimony jackknifing (Farris *et al.*, 1996). We used MrBayes v3.2.1 (Ronquist & Huelsenbeck, 2003) and the BEAST 1.7.5 package (Drummond *et al.*, 2012) for phylogenetic reconstruction. To identify the best performing model for each separate partition we examined the relative fit of various nucleotide substitution models for ETS, ITS and *RPB2* and those for introns and exons. Our selection was based on the Akaike information criterion (AIC; Akaike, 1974), implemented in jModelTest (Posada, 2008). Also, since ETS and ITS are part of the same transcription unit and not considered independent datasets (Baldwin & Markos, 1998), we concatenated these and ran them against *RPB2* to reveal plausible supported incongruence.

Phylogenetic inference in MrBayes was run for ten million generations with Markov chain Monte Carlo (MCMC), starting from random trees and flat priors (default). We used three heated chains and a single cold chain, with trees sampled every 5000th generation, producing 2001 trees per run. Markov chains were assumed stationary when the log-likelihood values reached a stable equilibrium (Huelsenbeck & Ronquist, 2001), with standard deviation decreased to < 0.05 and parameters gained effective sample size (ESS) values > 200. Majority-rule consensus and posterior probabilities (PP) for nodes were assembled from all post burn-in sampled trees. Phylogenetic reconstructions for each dataset were estimated using three independent runs to confirm that they converged on similar stationary parameter estimates.

Aligned partitions of full-length sequences, respectively exons and introns, were prepared with BEAUti (part of the BEAST package) for an output xml-file for phylogenetic inference in BEAST. Substitution models (Posada, 2008) were set by manual modification of the rate parameters in the xml-file. The BEAST package was used primarily to derive a tree topology and not for divergence time estimates under a molecular clock assumption. The molecular clock was therefore unconstrained and the root was fixed by using a normal prior with an arbitrary mean (100) and a narrow standard deviation (0.1). The tree prior was set to a birth–death process (Gernhard, 2008). To ensure independent convergence on all parameters (ESS > 200), MCMCs were set to run five times, each for 30 million generations and sampling trees every 15 000 generations. Convergence and chain mixing were checked

using Tracer v.1.5 (Rambaut & Drummond, 2009). A proportion of samples in each run were discarded as burn-in, with the posterior set of trees summarized in TreeAnnotator (part of the BEAST package). The resulting maximum clade credibility (MCC) tree was then visualized with FigTree v1.3.1 (Rambaut, 2009).

To obtain jackknife values (JK) for clades, parsimony jackknifing, as implemented in PAUP* 4.0 (Swofford, 2002), was performed using the following settings: 1000 jackknife replicates with a single random addition sequence; TBR branch swapping, saving a maximum of ten trees; collapsing branches if minimum length was zero; and steepest descent not in effect. The fraction of excluded characters per replicate was set to 37%.

Support values for nodes are given as posterior probability (PP) and parsimony JK values. PP values of 0.80–0.94 were considered to be weak to moderate and those of 0.95–1.00 to be strong indicators of node support. JK values of 50–74% were considered weak, 75–89% moderate and 90–100% strong. Nodes with support < PP 0.8 and JK 50% were considered too weak and collapsed in the phylogenetic tree.

Morphological data

Selecting useful diagnostic characters of morphology represents a major challenge, since high levels of morphological homoplasy in Sapotaceae have been revealed in earlier studies (Swenson & Anderberg, 2005; Swenson *et al.*, 2007a, 2008a, b, 2013, 2015). Observed homoplasy may stem from poorly understood morphology, delimitation of character and character states, inconsistent terminology, inadequately known or cryptic species or a combination of these reasons. From nearly 50 surveyed characters, we arrived at 17 that (1) were frequently used to delimit Neotropical genera and sections of *Chrysophyllum* and *Pouteria*, (2) were the least ambiguous and (3) showed the least homoplasy across the molecular tree (Supporting Information Table S1). The data were collected from the literature (Pennington, 1990, 1991, 2006; Ribeiro *et al.*, 1999; Roosmalen & Garcia, 2000; Morales, 2012) and checked against available live and/or herbarium material deposited at FUEL, HPL, IAC, INPA, S, SPF and UEC, abbreviations according to Index Herbariorum (Thiers, continuously updated). The morphological terminology follows that of Harris & Harris (1997).

Character 1. Stipules are scattered across Sapotaceae, but unusual in subfamily Chrysophylloideae. Large, persistent stipules are present in *Chromolucuma*, *Ecclinusa* and in some *Pouteria* spp.

Character 2. Leaf venation was used by Pennington (1990) as a rich source of characters in his classification. The most common patterns are eucamptodromous and brochidodromous. However, combinations of them are frequently observed, when the lower half of the blade is eucamptodromous and a portion of the upper half is brochidodromous. Such mixed venation patterns, described by Ellis *et al.* (2009), are here called eucampto-brochidodromous.

Character 3. Tertiary veins generally form five patterns (reticulate, oblique, admedial, striate and horizontal; Pennington, 1990). Reticulate tertiaries form an irregular pattern; oblique tertiaries usually cross between and anastomose with the secondaries at an angle of *c*. 90°; admedial tertiaries descend from the leaf margin and usually do not reach the midrib; striate tertiaries are formed by several closely spaced veins parallel to the secondaries; and horizontal tertiaries are perpendicular to the midrib and fuse with it.

Character 4. Areolate venation is sometimes present, usually in the form of small round areas between the tertiaries (Munzinger & Swenson, 2009). However, in some cases the tertiaries are nearly impossible to distinguish and instead an areolate venation is directly formed as a higher order of venation.

Character 5. White latex is one of the important diagnostic features for Sapotaceae, but in rare cases it can be cream or yellowish (Pennington, 1990; Ribeiro *et al.*, 1999) or even bluish, as in some *Pycnandra* spp. (Swenson & Munzinger, 2016). As far as is known, latex colour does not change due to oxidation.

Characters 6 and 7. Flowers of Neotropical Chrysophylloideae are actinomorphic and usually isomerous. The calyx is uniseriate, with four, five or six sepals (rarely more), whereas the corolla has four, five, six or seven lobes. These characters were important in Aubréville's (1964a) classification, but since the flower parts can vary in a single species, they were down-weighted by Pennington (1990). Given that the number of sepals and corolla lobes is inconsistent with natural groups in New Caledonia (Swenson *et al.*, 2008b), we explore the possibility that this ratio shows another pattern in South American Chrysophylloideae. Four general flower types may be distinguished: (1) tetramerous; (2) pentamerous; (3) four sepals with six petals; and (5) five sepals that are outnumbered by the petals.

Character 8. Accrescent calyx refers to the continued growth of the calyx post-anthesis, resulting in a fruit with a persistent large calyx. This is a rare feature in Chrysophylloideae, found only in some species.

Character 9. The corolla of Sapotaceae is sympetalous, with a tube and free corolla lobes. Corolla form played an important role in Pennington's

(1990) circumscription of different taxonomic groups. Flower characterization is difficult and subjective, but, in this study, we use the types proposed by Swenson *et al.* (2013), with some modifications. Depending on the length of sepals, corolla tube and lobes and the general shape of the corolla, flowers may be rotate, dome- or cup-shaped, tubular, urn-shaped or campanulate. Rotate flowers have a short calyx and corolla tube, with the corolla lobes spread to form almost flat flowers (Fig. 1A). Rotate flowers should not be difficult to separate from dome- and cup-shaped ones, which have slightly longer

Figure 1. Flowers and corolla types of Neotropical Chrysophylloideae (Sapotaceae). A, *Pradosia cochlearia*, a large tree from the Amazonian with rotate flowers. B, *Pouteria minima*, a species with green flowers and dome-shaped corolla. C, *Pouteria reticulata*, one of a few species with white, dome-shaped flowers. D, *Pouteria campanulata* with cup-shaped flowers. E, *Pouteria caimito*, a widely distributed species in South America with tubular flowers. F, *Micropholis caudata* with urn-shaped flowers. G, *Chrysophyllum oliviforme* with campanulate flowers. Photographs: A–F by Aparecida de Faria, G by Hugh Nicholson.

calyces usually reaching the tube orifice and the lobes forming a kind of dome (Fig. 1B, C), or somewhat spreading (Fig. 1D), usually hiding the pistil. Tubular flowers have a cylindrical corolla tube that is longer than the sepals and usually have erect corolla lobes (Fig. 1E). Urn-shaped flowers (not present in the sampled taxa) are similar to tubular flowers, differing from them in having rather short calyx lobes, a prominent corolla tube that is broadest at the mid-section and has a contracted mouth and short recurved lobes (Fig. 1F). Campanulate flowers are similar to tubular and/or urn-shaped flowers, but their corolla tube is broadest at the tube orifice, forming a small bell (Fig. 1G). Some taxa with unisexual flowers are difficult to code since the pistillate flowers (female) are tubular and the staminate flowers (male) are cup-shaped, as in *Chrysophyllum sparsiflorum* Klotzsch ex Miq.

Character 10. Corolla margin in most species is glabrous, but is ciliate (and resembling trichomes) in some taxa and papillate in others.

Character 11. Anther filaments are inserted at various levels in the corolla tube. They can be inserted in the tube orifice, just below the tube orifice, in the mid-section of the tube or near the base. Pennington (1990, 1991) considered this character of low taxonomic value due to its wide homoplasticity in his recognized groups. In contrast, Swenson *et al.* (2007a, 2013), using molecular data, have demonstrated it to be quite consistent in the clades recovered in their analyses and it is therefore explored here.

Character 12. Pollen morphology of Sapotaceae has been surveyed for 398 species and classified into 12 pollen types (Harley, 1991). These types were used by Pennington, but numbered differently in his *Flora Neotropica* (1990) and *The Genera of Sapotaceae* (1991). Pollen types vary in the genera *sensu* Pennington (1991), with *Chrysophyllum*, for example, represented by four and *Pouteria* by eight pollen types. Pollen types in Chrysophylloideae, in a phylogenetic context, were discussed for the first time by Swenson *et al.* (2008a), who found some patterns consistent with clades. We repeat that exercise here, following Harley's (1991) classification, even though data are missing for 48% of the sampled taxa.

Character 13. Pennington (1990, 1991) used absence of staminodes as a diagnostic character to separate *Chrysophyllum* from *Pouteria*. However, Swenson *et al.* (2008a) demonstrated that staminodes had been lost multiple times across the subfamily and therefore they represent a character that is not as useful as commonly believed. Nevertheless, with an expanded sampling of Neotropical species, their distribution (presence, vestigial, absence) could still be diagnostic. Staminodes are inserted in the corolla lobe sinuses and are scored as vestigial, as long as one (or more) is missing (or reduced).

Character 14. Fruits of Sapotaceae are usually classified as berries with a leathery or somewhat woody outer pericarp and an inner fleshy endocarp that serves as the pulp (Pennington, 1991). However, fruits in *Pradosia* have been called drupes, but drupaceous is a better term since the endocarp differentiates and the inner layer becomes jelly-like, partially transparent, but never hard as in a true drupe (Terra-Araujo *et al.*, 2013). Fruits are distinguished here as berries and drupaceous.

Character 15–17. Three seed characters have been frequently used in the systematics of Sapotaceae: distinction between foliaceous or plano-convex cotyledons; radicle included in the cotyledons or exserted below the commissure; and presence or absence of an endosperm (Pennington, 1990, 1991). Phylogenetic analyses have confirmed that these characters covary in the phylogeny of Sapotaceae (Swenson et al., 2007a, 2008b, 2013; Gautier *et al.*, 2013) and result in three types: foliaceous cotyledons, with the radicle extending below the commissure, and the presence of endosperm (Type 1); plano-convex cotyledons, with the radical not exserted, and no endosperm (Type 2); and plano-convex cotyledons, with radicle exserted, and no endosperm (Type 3).

These morphological characters were added to the molecular dataset and mapped as discrete units as implemented in BEAST (Lemey *et al.*, 2009). However, this approach was not feasible (see Results) and morphological characters were mapped on the molecular MCC tree obtained from the BEAST analyses using MacClade 4.0 (Maddison & Maddison, 2005).

RESULTS

Partitions and multiple copies

The number of aligned characters is 2664, including 424 base pairs of ETS, 918 of ITS, 1230 of *RPB2* and gaps. If the sequences are partitioned as exons and introns, there are 938 and 1634 nucleotides, respectively. The relative fit of various models of nucleotide substitution and aligned partitions are reported in Table 2.

Multiple copies of ETS and ITS were never found in the same species. Multiple copies of ETS were found and cloned in ten species: *Chromolucuma cespedesiiformis* (four copies), *Pouteria campanulata* (Kunth) Baehni (seven copies), *P. cuspidata* (A.DC.) Baehni subsp. *cuspidata* (seven copies), *P. eugeniifolia* (Pierre) Baehni (six copies), *P. freitasii* T.D.Penn. (five copies), *P. minima* T.D.Penn. (ten copies), *P. pallens* T.D.Penn.

Table 2. Characteristics of nuclear sequences for each data partition and substitution model based on the Akaike information criterion (Akaike, 1974) in this phylogenetic analysis of Neotropical Chrysophylloideae (Sapotaceae)

Data	Aligned length (bp)	Number of characters			Gaps	Model
		Constant	Uninformative	Potentially informative		
ETS	424	75	45	304 (71.7%)	25	TIM3+G
ITS	918	293	195	430 (46.8%)	51	TPM1uf+G
RPB2	1230	619	332	279 (22.7%)	16	TPM2uf+G
Exons	938	550	242	146 (15.6%)	1	TVM+G
Introns	1634	394	335	905 (55.4%)	91	TVM+G

(six copies), *Pradosia decipiens* Ducke (eight copies), *P. schomburgkiana* (A.DC.) Cronquist (three copies) and *Sarcaulus brasiliensis* (A.DC.) Eyma (nine copies). Similarly, four species contained multiple copies of ITS and were cloned: *Chrysophyllum prieurii* A.DC. (eight copies), *Pouteria ambelaniifolia* (Sandwith) T.D.Penn. (nine copies), *P. fulva* T.D.Penn. (ten copies) and *Pouteria* sp. from Tree183 in Picinguaba (Ubatuba, SP, Brazil) (ten copies) (Appendix).

Separate analyses of the ETS+ITS and *RPB2* recovered weak support for the backbone of the phylogenetic trees, with no supported (PP > 0.8) incongruence between major clades, except for two minor cases. Analyses of the ETS+ITS recovered *Pouteria anomala* T.D.Penn. and *P. engleri* Eyma, apart from *Micropholis*, as sister to all other taxa, whereas analysis of the *RPB2* recovered them in a moderately (PP 0.93) supported polytomy similar to what is reported in Figure 2. The other case is represented by a single species, *P. ramiflora* (Mart.) Radlk., which falls as sister to clade N, when ETS+ITS is used (PP 1.0), but inside clade P (PP 0.93) when *RPB2* is used. As will be shown below, neither of these cases has an impact on the bigger picture reported in this paper and therefore we kept the entire sample and concatenated the ETS+ITS and *RPB2* in other analyses.

Tree topology

All analyses (MrBayes, BEAST, Jackknife) using nuclear data partitioned either by loci or exons/introns identified the same backbone phylogenetic relationships with similar support values, but with slightly lower support and less resolved topologies when incomplete matrices were used. *Pouteria laevigata* (Mart.) Radlk. and *P. maxima* T.D.Penn. clearly belong to *Micropholis* and had to be moved to the outgroup in order to recover a monophyletic ingroup.

Analyses of the incomplete matrices rendered most species monophyletic or as members of clades with no hard incongruence. Only *Pouteria ambelaniifolia*, *P. eugeniifolia* and two *Pradosia* spp. proved to be exceptions to this pattern. We therefore analysed the data using the complete matrices, with question marks substituted by the corresponding ETS or ITS sequences. Once more, all species except *P. ambelaniifolia* and the two *Pradosia* spp. were recovered as monophyletic, but with an improved overall resolution and support (Fig. 2).

Bayesian inference of molecular sequence data and morphological data mapped as discrete units in BEAST failed to reach ESS values > 200 for several critical parameters. We therefore reran the analyses excluding all but four fruit characters, with the same result for ESS values. This outcome is interpreted as evidence for the morphological model failing to provide an unambiguous signal in informing the compound likelihood of the analysis, which translates to extensive morphological homoplasy (Swenson & Anderberg, 2005; Swenson *et al.*, 2007a, 2008a, b).

Overall tree resolution may be envisaged to include 17 supported clades (A–Q), plus a few species of uncertain affinity. Our analysis recovered *Chrysophyllum* and *Pouteria*, with most sections of the latter, as poly- or paraphyletic in their current circumscriptions. *Chrysophyllum sensu* Pennington (1990) is divided into five sections, of which sections *Ragala* (clade B), *Prieurella* (clade F) and *Chrysophyllum* (clade H) are mutually monophyletic, but not closest relatives to each other. Members of *C.* sections *Aneuchrysophyllum* and *Diploon* are indicated as affiliated, but only with weak branch support (clade G).

Species currently classified in *Pouteria* are scattered throughout the phylogenetic trees, from the outgroup (clade A) to *Pouteria* section *Oxythece* (clade Q). *Pouteria* sections *Oxythece* and *Rivicoa* are the only monophyletic groups that correspond to Pennington's classification. Sections *Antholucuma*, *Franchetella* and *Pouteria* are all polyphyletic.

The small genera *Elaeoluma* (clade C), *Ecclinusa* (clade E) and *Pradosia* (clade K) are all monophyletic and recovered with strong support. Nevertheless, it must be noted that different copies of *Pradosia decipiens* and *P. schomburgkiana* from the same

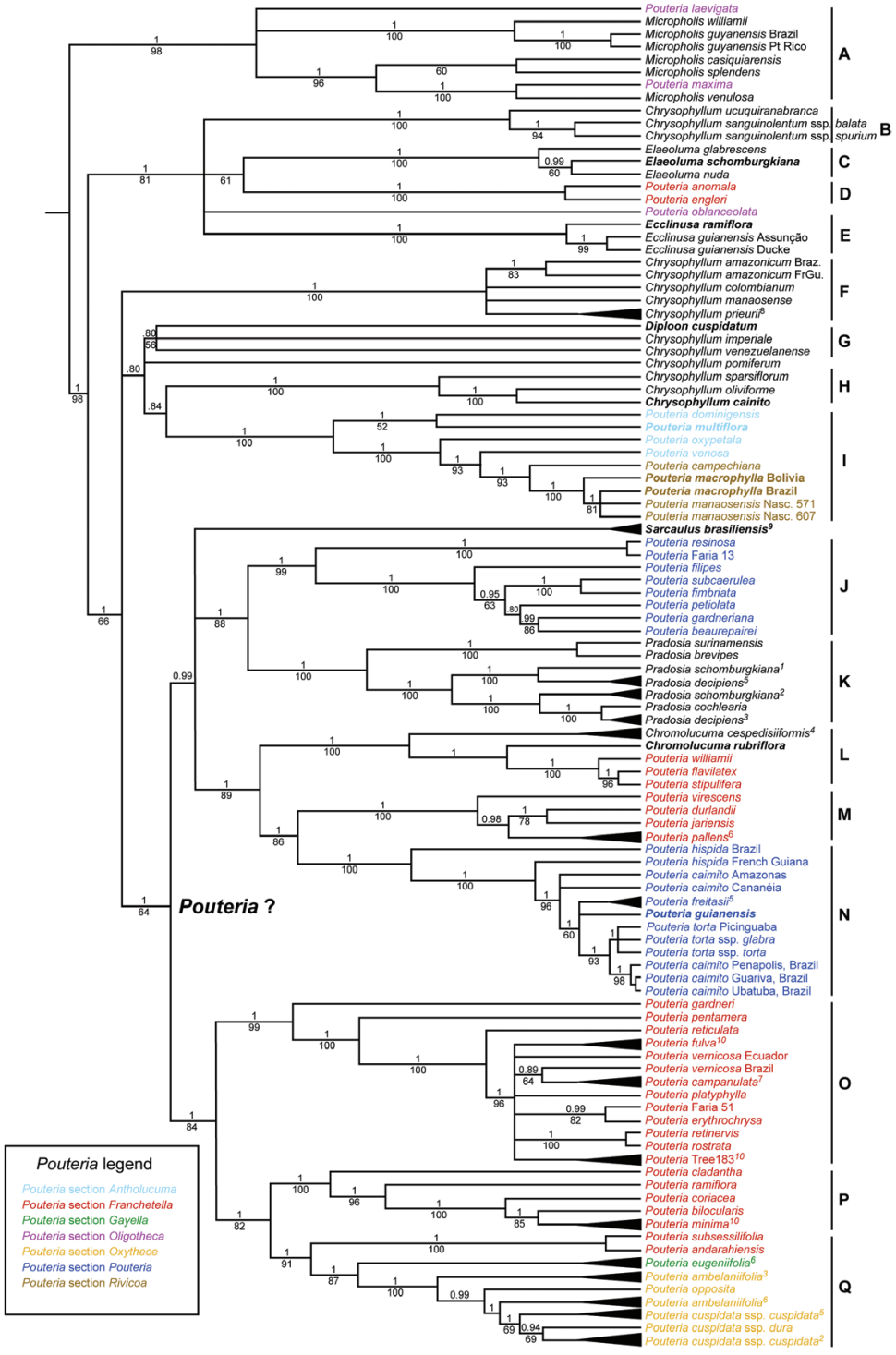

Figure 2. Phylogenetic tree obtained from Bayesian inference and parsimony analyses of nuclear sequences of Neotropical Chrysophylloideae (Sapotaceae). Sections of *Pouteria* are colour-coded according to the legend. Species containing several ETS or ITS copies are illustrated with a black triangle, followed by the name and the number of obtained copies. Posterior probabilities (above) and parsimony jackknifing (below) are indicated along the branches. The type species of genera recognized by Pennington (1990) are indicated in bold. Clades A–Q are discussed in the text.

source forced accessions to group in two clades. This suggests hybridization or incomplete lineage sorting. Evolutionary history is investigated elsewhere (Terra-Araujo *et al.*, 2015).

Multiple accessions of *Pouteria caimito* (Ruiz. & Pav.) Radlk, *P. macrophylla* (Lam.) Eyma, *P. manaosensis* (Aubrév. & Pellegr.) T.D.Penn. and *Ecclinusa guianensis* Eyma. were included. All five accessions of *P. caimito* fell in clade N, but three accessions from the Atlantic forest grouped together with strong support, whereas one specimen from the same biome was recovered in close proximity to a specimen from the Amazon forest. All accessions of *P. macrophylla* and *P. manaosensis* grouped in clade I, but not as distinct species. The two accessions of *E. guianensis* grouped together (clade E). Phylogenetic relationships of *Diploon cuspidatum* (Hoehne) Cronquist, *Chrysophyllum imperiale* (Linden ex K.Koch & Fintelm.) Benth. & Hook.f., *C. pomiferum* (Eyma) T.D.Penn., *C. venezuelanense* (Pierre) T.D.Penn., *Pouteria oblanceolata* and *Sarcaulus brasiliensis* remained unresolved.

Optimization of morphology

To identify diagnostic character combinations (Figs 3, 4), 17 morphological characters were mapped on the molecular MCC tree obtained from the BEAST analysis. The majority of features were highly variable, concurring with previous studies (Swenson & Anderberg, 2005; Swenson *et al.*, 2007a, 2008a, b). However, several characters were congruent with more inclusive clades. The number of sepals and corolla lobes and the three seed characters are presented in Figures 3F and 4F, respectively. Character distribution is discussed below.

DISCUSSION

Our phylogenetic analyses of 101 terminal taxa of Neotropical Chrysophylloideae unambiguously demonstrate that Pennington's (1990, 1991) classification of *Chrysophyllum* and *Pouteria* is untenable (Fig. 2). Depending on clade circumscription, five to 17 clades (clades A–Q) are recovered with various success of morphological coherence (Figs 3, 4). Terminals of *Chrysophyllum* are distributed in four to five groups, with *C.* section *Ragala* corresponding to the genus *Ragala* (clade B), section *Prieurella* corresponding to the genus *Prieurella* (clade F) and section *Chrysophyllum* possibly corresponding to a narrow circumscription of *Chrysophyllum* (clade H). Petersen, Parker & Potter (2012) used ITS sequences to demonstrate that five to six species of *C.* section *Chrysophyllum*, including the resurrected *C. bicolor* Poir, are close relatives of the generic type *C. cainito* L. In that study, other species of the genus seemed to be distantly related to *C.* section *Chrysophyllum*, but their conclusions were limited by the inclusion of only two *Pouteria* spp. apart from those of *Chrysophyllum*.

In our analyses, and depending on delimitation, accessions of *Pouteria* are recovered in some ten clades, with a topology that does not concur with Aubréville's or Pennington's classifications (cf. Appendix), but strongly supports recent conclusions based on plastid DNA, nrDNA and morphology (Swenson & Anderberg, 2005; Swenson *et al.*, 2008a; Bartish *et al.*, 2011) that both *Chrysophyllum* and *Pouteria* remain 'catch-all' baskets, despite recent resurrections of several Australasian genera (Swenson *et al.*, 2007a, 2013).

Multiple copies of ETS or ITS were found in 13 species, all but three species being monophyletic. The three non-monophyletic species are *Pouteria ambelaniifolia*, *Pradosia decipiens* and *Pradosia schomburgkiana*, but accessions of each one are recovered near each other in three different subclades and do not challenge the overall topology, i.e. the taxa with multiple copies of ETS or ITS represent local problems, not hindering rebuilding of a natural classification of Neotropical Chrysophylloideae.

Utility of mapped morphological characters

Morphological characters in Sapotaceae have been demonstrated to be homoplastic and their use in phylogenetic analysis, until their homology is better understood, is bound to introduce noise (Swenson & Anderberg, 2005; Swenson *et al.*, 2007a, 2008a, b, 2013, 2015). The present study supports that notion. Seventeen characters have been selected for evaluation of their usefulness for taxon characterization. Since the current generic classification to a large extent does not agree with the proposed tree topology, in the following discussion we therefore refer to clades A–Q instead of generic names.

Stipules

Small caducous stipules are scattered across Sapotaceae, e.g. being diagnostic for the Malagasy tribe Tseboneae (Sapotoideae; Gautier *et al.*, 2013). They are less common in Chrysophylloideae and are present only in the recovered clades E and L, except for *Pouteria williamii* (Aubrév. & Pellegr.) T.D.Penn. of the latter clade. Our clade E corresponds to Pennington's *Ecclinusa*, whereas clade L would accord with *Chromolucuma*, if three *Pouteria* spp. were transferred to it (Fig. 3A). In fact, Alves-Araújo & Alves (2012a) transferred *Pouteria congestifolia* to *Chromolucuma* precisely based on presence of stipules and yellow latex.

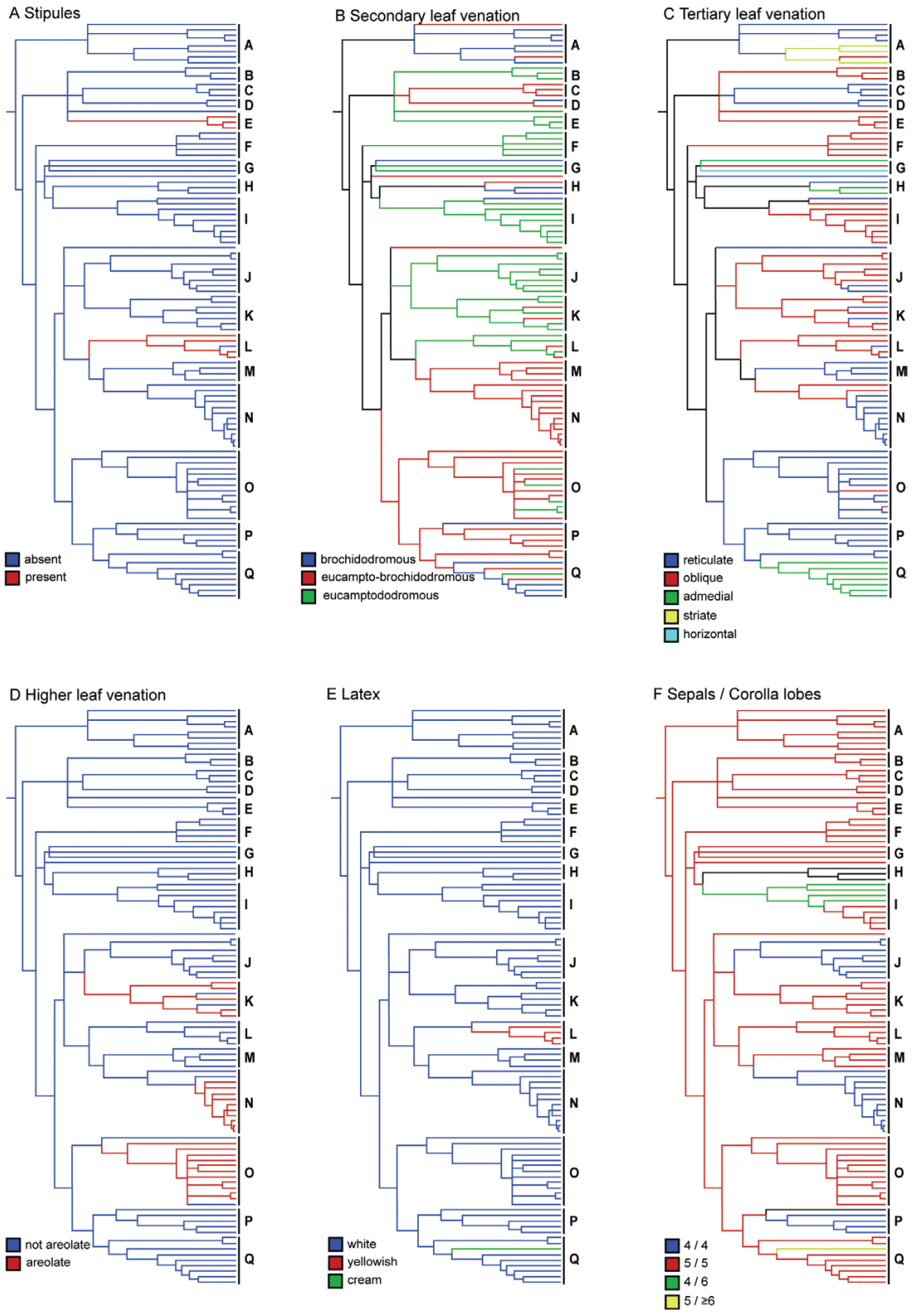

Figure 3. Seven morphological characters mapped on the phylogenetic tree obtained from Bayesian and parsimony analyses of Neotropical Chrysophylloideae (Sapotaceae). Clades A–Q are discussed in the text. Black branches represent ambiguous character states.

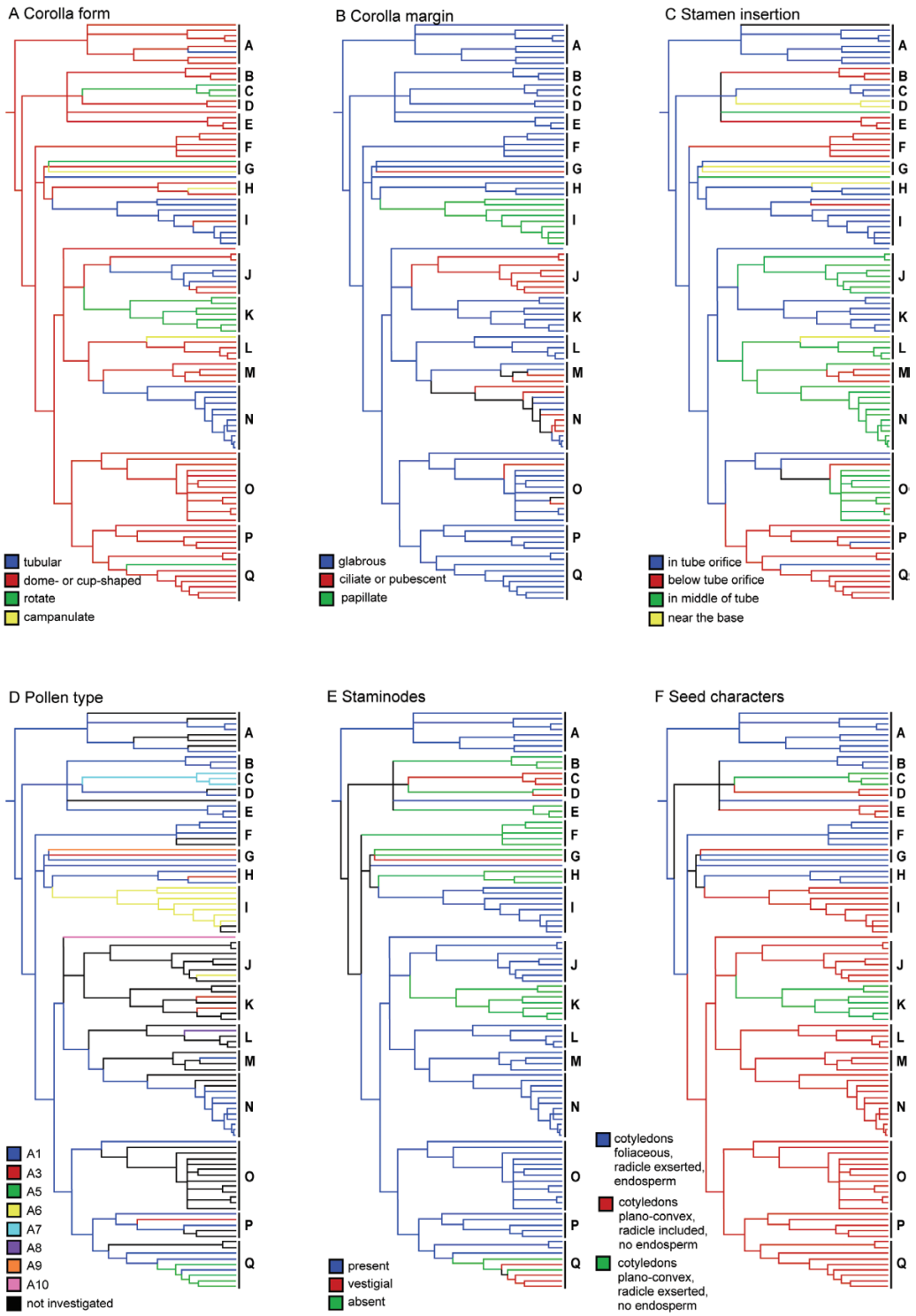

Figure 4. Eight morphological characters mapped on the phylogenetic tree obtained from Bayesian and parsimony analyses of Neotropical Chrysophylloideae (Sapotaceae). Note that three characters are amalgamated in F. Clades A–Q are discussed in the text.

Secondary venation

Leaf venation is a useful field character, but has been found to be fairly homoplasious (Swenson & Anderberg, 2005; Swenson *et al.*, 2008a). Eucamptodromous and the mixed eucampto-brochidodromous venation types are the two most common patterns of secondary venation, although with variable coherence with the recovered clades (Fig. 3B). All members of clades B, E, F and J have eucamptodromous venation, with only clades M and N having the exclusively mixed eucampto-brochidodromous type.

Tertiary venation

Tertiary venation has been found to covary and be diagnostic for groups of Australasian Sapotaceae (Swenson *et al.*, 2013). Five patterns of tertiary venation (reticulate, oblique, admedial, striate and horizontal) were distinguished and found to fit the phylogenetic tree slightly better than those of the secondary venation. Reticulate tertiaries are consistent with clades C, D, M and P and nearly so with clade N (Fig. 3C). Oblique tertiaries are found throughout clades B, E and F. Admedial tertiaries are present in clades H and Q, whereas striate tertiaries are restricted to some taxa of clade A (*Micropholis*). Higher-order leaf venation may be characterized by areolate patterns that are mostly diagnostic for clades K, N and O, although always with some exceptions (Fig. 3D).

Latex

Latex in Sapotaceae is usually white, but one clade of Neotropical species has yellow or yellowish latex (Fig. 3E), diagnostic of *Chromolucuma* (Pennington, 1991; Alves-Araújo & Alves, 2012a). A few species may also have cream latex, one being *P. eugeniifolia*, occurring in clade Q.

Sepals and corolla lobes

Pennington (1991) stressed that American *Pouteria* spp. fall into several well-defined groups based on the number of flower parts. Isomerous flowers with the same number of sepals and corolla lobes (four or five), often with some variation, are the dominating types (Fig. 3F). The pentamerous flower corresponds to the symplesiomorphic state, reduced three times, in clades J, N and P, to a tetramerous flower through evolution. Four species of *Pouteria* section *Antholucuma* (clade I) have four sepals and six corolla lobes. These form a well-supported grade to the members of *P.* section *Rivicoa*, which all have pentamerous flowers. Thus, the number of flower parts agrees well with our tree and, in combination with others, should be considered a useful character, as suggested by Pennington (1991).

Accrescent calyx

An accrescent calyx is rare in Chrysophylloideae, but present in all species of *Chrysophyllum* section *Ragala* (clade B). In fact, for Pierre (1891) it was the key character when he described the genus *Ragala*. *Chrysophyllum eximium* Ducke (not included) has this feature too, but was placed in *C.* section *Aneuchrysophyllum* on the basis of leaf venation with a note that it may belong in section *Ragala* (Pennington, 1990).

Corolla form

Classification of corolla forms, guided by the above definitions, is admittedly subjective and difficult. The cup-shaped corolla with spreading lobes is the most common type in Neotropical Chrysophylloideae (Fig. 4A). It is sometimes hard to separate from the slightly rounder dome-shaped corolla, with corolla lobes turned inward, not opening up as much as the former. These two types combine well with our clades and only a few taxa embedded among them have clearly different corolla types. The two exceptions are *Chromolucuma cespedisiiformis*, with campanulate corollas, embedded in clade L, and *Pouteria eugeniifolia*, with rotate corollas, embedded in clade Q. A tubular corolla is nearly consistent in clades I, J and N, but, as usual, with occasional exceptions. The rotate corolla is characteristic of *Elaeoluma* (clade C) and *Pradosia* (clade K), but also found in *Diploon* (part of clade G). It should also be kept in mind that corolla form variation may be due to sexual dimorphism. For example, *Chrysophyllum venezuelanense* (clade G) has cup-shaped pistillate flowers and campanulate staminate flowers, whereas *C. sparsiflorum* (in clade H) has tubular pistillate flowers and cup-shaped staminate flowers.

Corolla margin

Corolla margins in Chrysophylloideae are usually glabrous, without trichomes or papillae (Fig. 4B). However, all members of clade I have papillate corolla margins. Short cilia or trichomes are present in *Chrysophyllum imperiale* (of clade G), clade J and several taxa of clades M, N and O.

Stamen insertion

Stamens in Sapotaceae are opposite the corolla lobes and the filaments are inserted to the corolla tube at different levels. In addition, at the point of insertion, the filament can be completely fused with the corolla tube tissue or run above the corolla tube like a keel. The view of these features has varied, being considered taxonomically important (Aubréville, 1964a) or of

little value (Pennington, 1991). In a phylogenetic context, the level of insertion is diagnostic for groups in Australasia (Swenson *et al.*, 2007a). We believe that the level of insertion is quite consistent and useful across the South American clades (Fig. 4C). Stamens inserted in the tube orifice seem to be diagnostic for *Micropholis* (clade A), *Elaeoluma* (clade C), *Chrysophyllum* section *Chrysophyllum* (part of clade H), *Diploon* (part of clade G), clade I [except *Pouteria multiflora* (A.DC.) Eyma], *Pradosia* (clade K) and *Sarcaulus* and for some scattered species in clades O–Q. Stamens inserted just below the tube orifice are found in ten clades and consistent for *Chrysophyllum* section *Ragala* (clade B), *Ecclinusa* (clade E), *C.* section *Prieurella* (clade F) and, to a large extent, for clades P–Q. Stamens inserted in the mid-portion of the corolla tube are consistent only for clade N, J and, with some exceptions, *Chromolucuma* (clade L) and clade O. Taxa with stamens inserted near the corolla tube base are found in clades D, G, H and L, usually with some exceptions. In some rare cases scoring of this character is difficult; for example, in *Pouteria laevigata*, with a corolla tube only 0.5–1.5 mm long, stamen insertion could be interpreted either as just below the tube orifice or near the base of the corolla tube.

Pollen types

Harley (1991) explored pollen types in Neotropical Sapotaceae, but many species remain to be investigated. Eight pollen types were represented in this study and Harley's numbering system is followed here, with Pennington's (1991) numbering cited in parentheses. Pollen morphology seems to convey a phylogenetic clue (Fig. 4D). Thus, all taxa in clades A, B, D, E, F, M and N have (or may have) pollen type A1 (7). *Elaeoluma* (clade C) is the only lineage with pollen type A7 (10). The monotypic genus *Diploon* (clade G) is the only taxon in the family with the spiny pollen type A9 (12). Pollen type A3 (3) was found in clades G, H, K and P, such as in *Chrysophyllum imperiale* (clade G) and *Pradosia* (clade K), a genus also with other pollen types (Harley, 1991). Pollen type A6 (5) is rare but distinctive for clade I, and may be also indicative for clade J. *Chromolucuma* and *Sarcaulus* have the pollen types A8 and A10, respectively (combined by Pennington into his type 11), and are not known elsewhere in the family, except for three anomalous collections of *Pouteria*. Pollen type A5 (9), usually with entire and striate surface, is also rare and found exclusively in clade Q. Overall, pollen type A1 (7) is the most widespread in the subfamily and appears to be symplesiomorphic. If the so-far uninvestigated taxa in our sample were surveyed for pollen types, we predict that they would be largely compatible with the clades recovered in this study.

Staminodes

Loss of staminodes was shown to have occurred several times in the subfamily (Swenson *et al.*, 2008a) and therefore their absence in *Chrysophyllum* or presence in *Pouteria* cannot be used as a synapomorphy. Our study, although supporting the above conclusions, also indicates that their loss is an ongoing process, with vestigial staminodes present, for instance, in *Elaeoluma* (clade C), *Pouteria engleri* (clade D) and *C. venezuelanense* (clade G) (Fig. 4E). Furthermore, in unisexual flowers of clade Q (*Pouteria* section *Oxythece*), they are absent in male (staminate) flowers but present in female (pistillate) flowers. We therefore consider their presence, reduction and absence as a useful morphological character.

Seed characters

Distribution of the three seed character state types shows strong congruence with the recovered tree topology (Fig. 4F). Foliaceous cotyledons, radicle exserted below the cotyledon commissure and presence of endosperm, suggested to be a symplesiomorphic character state combination (Swenson *et al.*, 2008a), was found in clades A, B, F, G and H. The second type, with plano-convex cotyledons, included radicle and absent endosperm, concur with being an advanced and widely distributed character state combination, consistent for clades D, E, I, J and L–Q. The third type is similar to the second, but with the radicle exserted (vs. included) below the cotyledon commissure, a combination of character states diagnostic for *Elaeoluma* (clade C) and *Pradosia* (clade K). This latter type is rare in the subfamily, found only in the New Guinean genus *Magodendron* Vink and in some taxa of the poorly known assemblage of *Synsepalum* (A.DC.) Daniell in Africa (Swenson *et al.*, 2013). Pennington (1991) had assumed *Chromolucuma* (clade L) to be of the latter type, but he knew fruits of only one species, with a slightly exserted radicle. Our re-examination of *Chromolucuma* seeds could not confirm presence of an exserted radicle.

In summary, several characters including floral merosity, corolla form, corolla margin, level of stamen insertion and seed features show acceptable to good congruence with the recovered clades. We hope that combinations of these features will be useful in future endeavours of circumscribing natural groups in Neotropical Chrysophylloideae.

Towards a revised generic classification

Our phylogenetic study of Chrysophylloideae in South America recovered an overall well-resolved topology with high posterior probabilities (PP ≥ 0.95) and usually moderate (JK 75–89%) to strong (JK 90–100%)

jackknife support. Diagnostic character combinations are required to describe genera in Sapotaceae (Swenson *et al.*, 2007a, 2008a, 2013; Gautier *et al.*, 2013) and we believe we have identified some of the most useful characters in this study. Broad concepts of *Chrysophyllum* and *Pouteria* (Pennington 1991) have been found untenable and it is therefore better to consider clades. In the discussion below, we have opted for clades that covary with morphology and often refer to clades A–Q.

Micropholis

Micropholis, a genus of *c*. 40 species (Govaerts *et al.*, 2001), with ephemeral flowers (Terra-Araujo *et al.*, 2012b), was instated in 1890 by Pierre and recognized by Aubréville (1964a) and Pennington (1990, 1991), with the latter dividing it into two sections. The generic type, *M. rugosa* (Sw.) Pierre (*M.* section *Micropholis*), unfortunately remained unavailable for this study, but its overall morphology suggests that it should be recovered in clade A. *Micropholis splendens* Gilly ex Aubrév., the type of section Micropholis sect. *Exsertistamen*, T.D. Penn. was found embedded in clade A with strong support (Fig. 2). If *Pouteria laevigata* and *P. maxima* were transferred to *Micropholis*, the genus would be rendered monophyletic. Pennington (1990, 2006) placed *P. laevigata* and *P. maxima* in *P.* section *Oligotheca* on the basis of a pentamerous flower with staminodes and seeds with foliaceous cotyledons, exserted radicle and endosperm (Pennington 1990, 2006). However, *P.* section *Oligotheca* is without doubt an unnatural assemblage, as shown in several previous studies (Bartish *et al.*, 2005; Swenson & Anderberg, 2005; Swenson *et al.*, 2007a, 2008a), with its Australasian and Pacific members distributed among *Planchonella*, *Pleioluma* and *Sersalisia* (Swenson *et al.* 2013). The seed characters of *P. laevigata* and *P. maxima* concur with those of *Micropholis* and differ from the seed characters of other sections of *Pouteria*, which have plano-convex cotyledons, included radicle and no endosperm. *Pouteria maxima* is furthermore characterized by alternate distichous leaves, a feature of *Micropholis sensu* Pennington (1990, 1991). A more thorough phylogenetic analysis of *Micropholis* is therefore needed to test its monophyly and find all members of the group.

Ecclinusa, Elaeoluma, Clade B and Clade D

Clades B–E

Our phylogenetic analyses show clades B–E, recovered with maximum Bayesian and moderate jackknife support, to be sister to the remaining taxa (clade F–Q). Clades B–E include five strongly supported lineages in a polytomy. No particular morphological character or character combination is diagnostic for the larger clade. However, each subclade is possible to characterize.

Clade B circumscribes three accessions of *Chrysophyllum*, corresponding to the genus *Ragala sensu* Aubréville (1964a), or *C.* section *Ragala sensu* Pennington (1990), with *C. sanguinolentum* (Pierre) Baehni as the type species. Pierre (1891) originally established this genus on the basis of its leaf and stomata anatomy and affiliated it with *Ecclinusa* (clade E). *Ecclinusa*, established by Martius (1839), includes 11 species of trees and shrubs (Govaerts *et al.*, 2001). The two lineages share several morphological characters, including pollen type (A1), but differ in others. If *Ragala* were to be reinstated, it would be defined by the absence of stipules, eucamptodromous venation, oblique tertiaries, stamen filaments inserted just below the tube orifice, absence of staminodes, seeds with foliaceous cotyledons, exserted radicle and the presence of the endosperm. One additional important character, rarely reported elsewhere for Neotropical Chrysophylloideae, is an accrescent calyx, subtending the fruit. *Ecclinusa* differs from the above character combination by the presence of stipules, absence of an accrescent calyx and seeds with plano-convex cotyledons, included radicle and the absence of the endosperm (Figs 3, 4). In addition, flowers of *Ecclinusa* are sessile, a character rare for the subfamily in South America and known only in a few species of *Micropholis* (two species) and *Pouteria* (eight species) (Pennington, 1990).

Elaeoluma (clade C), established by Baillon (1891), includes four species distributed from Panama to central Brazil (Aubréville, 1964a; Pennington, 1990). Its monophyly has previously been suggested (Swenson & Anderberg, 2005; Swenson *et al.*, 2008a) and in our analyses it was recovered with maximum support. The genus is readily recognized by the densely and minutely punctuated lower leaf surfaces, pale green colour and reticulate tertiaries. Apart from its exclusive A7 pollen type, it differs from the rest of the clade B–E lineages by the presence of vestigial staminodes in the pistillate flowers and an exserted radicle, notwithstanding the plano-convex cotyledons and the absence of endosperm.

Pouteria anomala and *P. engleri* (clade D), two species recovered with maximum clade support, are closely related to their congener *P. oblanceolata*, but not to the majority of *Pouteria* spp. or even the type species (*Sarcaulus*–clade Q). Lacking staminodes, *P. anomala* has been associated with *Chrysophyllum*, whereas *P. engleri* is the generic type of *Nemaluma* (Pennington, 1991), a monotypic genus established by Baillon (1891) and recognized only once since then (Aubréville, 1961a). Aubréville characterized the genus by a poorly developed tertiary venation, pentamerous flower, stamens fused to the base of the corolla tube

and vestigial staminodes. Our observations agree with those of Aubréville, except that the tertiaries have been found to be reticulate and the staminodes either absent or vestigial.

Pouteria oblanceolata is one of *c.* 160 species of *P.* section *Oligotheca sensu* Pennington (1990, 1991), with about 150 of them distributed in the natural genera *Planchonella*, *Pleioluma*, *Sersalisia* and *Vanroyena* of Oceania and Southeast Asia (Swenson *et al.*, 2013). *Pouteria* section *Oligotheca* is represented in the Neotropics by ten species, three of them sampled in this study. Two of these (*P. laevigata and P. maxima*) are best transferred to *Micropholis* (clade A), but *P. oblanceolata* occurs as a sole member in the polytomy B–E. It has pentamerous, cup-shaped, unisexual flowers, and shares characters with clades B (Figs 3B, C, 4F) and E (Fig. 3B, C). Filaments are inserted in the mid-section of the corolla tube (Fig. 4C) and well-developed staminodes in both pistillate (*c.* 0.5 mm long) and staminate (1.7–2.5 mm long) flowers (Fig. 4E) are the only odd characters for this clade. If its ITS sequence is blasted on the European Nucleotide Archive web search, the sequence is most similar to the Australasian genus *Pleioluma*, with which it shares several features, including the presence of staminodes, filaments inserted in the mid-section of the corolla tube and seed characters. However, it lacks areolate venation and campanulate flowers diagnostic for *Pleioluma* (Swenson *et al.*, 2013). It thus represents an odd member of the subfamily of uncertain sister relationships and with a unique combination of characters; it may deserve generic recognition.

To summarize, clades B–E, although with strong molecular support, have practically no clear subclade relationships. Since amalgamating two or more lineages in any combination would create heterogeneous genera, we suggest that *Ragala* (clade B), *Elaeoluma* (clade C), *Nemaluma* (clade D) and *Ecclinusa* (clade E) are recognized as separate genera and that *Pouteria oblanceolata* is included in a broader analysis in order to search for possible closer relatives.

Resurrection of *Prieurella*

Clade F

Five samples of *Chrysophyllum* are grouped in clade F with maximum support and are only distantly related to the generic type *C. cainito*. Clade F corresponds to the genus *Prieurella* established by Pierre (1891), recognized with five species by Aubréville (1964a), but reduced to a section of *Chrysophyllum* by Pennington (1990). Aubréville (1964b) distinguished *Prieurella* based on eucamptodromous leaf venation, oblique tertiaries, pentamerous and globose (dome-shaped) flowers, stamen filaments inserted just below the tube orifice, absence of staminodes, seeds with foliaceous cotyledons, exserted radicle and endosperm. This character combination fully agrees with our observations of the included species and with that of the generic type *P. cuneifolia* (not validly published), which was not included here due to its presumed hybrid origin (Swenson *et al.*, 2008a). Bark and crushed leaves of plants of this group are also usually characterized by the odour of bitter almonds (hydrocyanic acid), a useful field character. Since these taxa are clearly not close relatives to *Chrysophyllum s.s.* and the clade is rather easy to recognize, *Prieurella* deserves to be resurrected.

Clade G and *Chrysophyllum pomiferum*

Clade G is a group of three species (*Diploon cuspidatum, Chrysophyllum imperiale* and *C. venezuelanense*) with a relationship support of only PP 0.80 and JK 56. These three taxa have been historically treated as separate genera or associated with *Chrysophyllum*. The monotypic *Diploon*, established by Cronquist (1946), was based on *C. cuspidatum*. Because of an odd character combination (unilocular ovary, basi-lateral seed scar and the absence of endosperm), Cronquist was unsure if it belonged in Sapotaceae, but eventually decided to keep it in the family. Aubréville (1964a) and Pennington (1991) accepted *Diploon*, but its phylogenetic affiliations remain uncertain (Swenson & Anderberg, 2005; Swenson *et al.*, 2008a). The brochidodromous leaf venation, admedial tertiaries, rotate flowers, absence of staminodes, plano-convex cotyledons and the spiny type (A9) of the pollen grains (Fig. 4D), known only in one other species in the family, *Micropholis retusa* (Spruce ex Miq.) Eyma (not sampled in this study), readily distinguish *Diploon* from other genera in Sapotaceae.

Chrysophyllum imperiale from the Atlantic forest in Brazil and *C. venezuelanense* from Central America and the north-western corner of South America are two large tree species placed by Pennington (1990) in *C.* section *Aneuchrysophyllum*. Molecular and morphological phylogenetic analyses, both in this study and elsewhere (Swenson *et al.*, 2008a; Bartish *et al.*, 2011), have shown *C.* section *Aneuchrysophyllum* to be an unnatural assemblage of African and American lineages, with the American members related to each other but of uncertain affinity in the subfamily. *Chrysophyllum imperiale* and *C. subspinosum* Monach. (not sampled in this study) are the only family members with a spinous-serrate leaf margin, a feature that led to the initial placement of the former in Theophrastaceae (= Primulaceae *sensu* APG IV, 2016), with an eventual transfer to Sapotaceae and *Chrysophyllum* by Bentham & Hooker (1876).

Pierre (1891) used these two species to establish two closely associated genera, *Martiusella* Pierre and *Cornuella* Pierre, respectively, but neither was recognized by Aubréville (1964a) or Pennington (1991). If accepted as genera, both are readily distinguished from other members of the subfamily by a combination of eucamptodromous leaf venation, pentamerous flowers and stamen filaments inserted near the base of the corolla tube. In combination with oblique tertiaries, bisexual flowers and absence of staminodes, the spinous-serrate leaf margin is a distinguishing character of *Martiusella*. *Cornuella* differs from *Martiusella* in the entire leaves, more or less horizontal tertiaries, unisexual flowers and vestigial staminodes.

Chrysophyllum pomiferum was originally described as the monotypic genus *Achrouteria* Eyma, a name referring to the endospermous seeds of *Achras* (*Manilkara*), or *Chrysophyllum*, and staminodes of *Pouteria*, with no known intermediates at the time (Eyma, 1936). Pennington (1990) transferred it to *Chrysophyllum*, despite the presence of well-developed staminodes, placing it in section *Aneuchrysophyllum*, with *C. gonocarpum* (Mart. & Eichler) Engl., *C. lucentifolium* Cronquist and *C. venezuelanense*. Our study indicates a weak relationship between *C. pomiferum* and clade G. Apart from the common pollen type (A1) and pentamerous flowers, *C. pomiferum* shows a combination of characters typical of several different clades, such as reticulate tertiaries (a widespread feature), the presence of staminodes (clades I, J and L–P) and endospermous seeds (clades A, B, F and H). As far as we know, only one other species, *C. durifructum* (W.A.Rodrigues) T.D.Penn. (not sampled in this study), has the same combination of characters.

In summary, the three species of clade G and *C. pomiferum* are impossible to maintain in *Chrysophyllum*. If they were to be united in a single genus, based on a certain phylogenetic affinity, a heteromorphic group would be created (Figs 3, 4). On the other hand, by retaining *Diploon*, reinstatements of *Achrouteria*, *Cornuella* and *Martiusella* would be justified, with *Achrouteria* and possibly *Martiusella* containing two species each and *Cornuella* and *Diploon* being monotypic. However, *Chrysophyllum gonocarpum*, the type species of *Chloroluma* (Table 1), remains to be analysed in a phylogenetic context. It is a species with an unusual character combination and may be a candidate for generic recognition. If future phylogenetic analyses group *C. gonocarpum* with *C. pomiferum*, *Chloroluma* would have priority over *Achrouteria* (McNeill *et al.*, 2012), if a narrow generic concept were used. For the meantime, we recommend that existing names of *Achrouteria*, *Cornuella* and *Martiusella* be used, simply because that is a better solution than forcing them into an unnatural assemblage of *Chrysophyllum*.

Chrysophyllum s.s. and *Villocuspis*

Clade H receives maximum support (PP 1.0, JK 100%) and groups two sections of *Chrysophyllum* as sisters, *C.* sections *Chrysophyllum* (*C. cainito*, *C. oliviforme* L.) and *Villocuspis* (*C. sparsiflorum*), sections that, respectively, include 17 and six species (Pennington, 1990, 1991). Phylogenetic analysis groups these two sections with some *Pouteria* spp. instead of members of *Chrysophyllum*. In any event, Aubréville and Pellegrin (in Aubréville, 1961a) recognized *Villocuspis* at the generic level using the pubescent anthers as a cardinal character, but Pennington emphasized the homogeneity of floral and endospermous seeds and returned to a sectional concept in *Chrysophyllum*. Our phylogenetic analyses and examination of morphology of terminals in clade H show that flowers have variable numbers of sepals and corolla lobes, usually a sericeous corolla on the outer surface, absence of staminodes and endospermous seeds, but other characters are different. For example, *C.* section *Chrysophyllum* has admedial tertiaries, stamens inserted in the tube orifice, and glabrous anthers, contrasting with *C.* section *Villocuspis* with laxly reticulate tertiaries, stamens inserted near the corolla base and pubescent anthers (cf. Pennington, 1990). Despite this study including only three species of the two sections, we suspect that an expanded phylogenetic analysis of these two groups may support recognition of two clades corresponding to *Chrysophyllum s.s.* and *Villocuspis*.

Resurrection of *Lucuma*?

Clade I, recovered with maximum support (PP 1.0, JK 100%), includes all of the sampled species of *Pouteria* section *Antholucuma* and of section *Rivicoa sensu* Pennington (1990) (Fig. 2). The type species of these two sections, *P. multiflora* and *P. macrophylla*, respectively, were recognized by Pierre (1890) as the genera *Radlkoferella* Pierre and *Richardella* Pierre, the former on the basis of flowers with four sepals and usually six petals and the latter with pentamerous flowers (Fig. 3F). However, members of section *Antholucuma* form a well-supported grade to section *Rivicoa* in our analysis, making recognition of two sections or two genera untenable. Another option would be to unite all members of clade I in a single genus, recognized on two synapomorphies, the papillate corolla margin (Fig. 4B) and the A6 pollen type with unusually thick walls in proportion to the size of the grain (Fig. 4D; Harley, 1991). In addition, this clade would further be recognized based on a series of rather constant characters: oblique tertiaries [except in *P. dominigensis* (C.F.Gaertn.) Baehni]; bisexual flowers; filaments inserted in the corolla tube orifice (except in *P. multiflora*); the presence of staminodes; and non-endospermous seeds with plano-convex cotyledons and an

included radicle. The oldest name in section *Rivicoa* is *Lucuma* Molina (Table 1), based on *Lucuma bifera* Molina [= *P. lucuma* (Ruiz & Pav.) Kuntze]. Although *Pouteria lucuma* was not sampled in this study, its morphology fully agrees with the above combination of characters (pollen type unknown), making *Lucuma* a good candidate to represent a monophyletic group, with its circumscription remaining to be further investigated.

A NEW CIRCUMSCRIPTION OF *POUTERIA*?

Our phylogenetic analyses, based on ribosomal and nuclear sequence data, group *Sarcaulus*–clade Q with maximum Bayesian (PP 1.0) and weak jackknife (JK 64%) support in one large clade. It includes all species of *Pouteria* not discussed above, plus three widely accepted genera, *Chromolucuma*, *Pradosia* and *Sarcaulus* (Aubréville, 1964a; Pennington, 1990, 1991, 2006, 2007; Swenson & Anderberg, 2005; Alves-Araújo & Alves, 2012a; Terra-Araujo *et al.*, 2013, 2015). Different character combinations readily distinguish these three genera, but in our analyses, they are embedded among the clades of *Pouteria*. Terminals of clades J and N are usually placed in *Pouteria* section *Pouteria*, have nearly identical character distributions and are expected to form *Pouteria s.s.* with the type species *P. guianensis*. However, *Chromolucuma*, *Pradosia* and part of *P.* section *Franchetella* render the two clades of section *Pouteria* polyphyletic with strong branch support. We foresee two possible solutions: (1) circumscribing *Sarcaulus*–clade Q as *Pouteria*, with several recognized subgenera; or (2) assigning generic rank to each clade. Neither of these alternatives is unproblematic, but most of the taxa in the clade *Sarcaulus*–clade Q are still identified by a simple character combination of a non-papillate corolla margin, presence of staminodes and seeds with plano-convex cotyledons, included radicle and absence of endosperm. However, clade K (*Pradosia*) is still an exception with similar seed characters, but with an exserted radicle and absence of staminodes.

Sarcaulus includes five species with white to yellowish, dome-shaped, carnose flowers that have a strong jasmine-like scent. The stamens are borne on short filaments inserted in the tube orifice and the fruits have plano-convex cotyledons with an included radicle and no endosperm. Its pollen is of type A10, not found elsewhere in the family, a type that has provided evidence to uphold *Sarcaulus* as a distinct genus (Harley, 1991). Monophyly of *Sarcaulus* has not been tested.

Pradosia (clade K) includes 23 species of trees or shrubs and has been united with *Pouteria* (Eyma, 1936) and *Chrysophyllum* (Baehni, 1965), but for some time it has been recognized at the generic level (Aubréville, 1964a; Pennington, 1991; Swenson & Anderberg, 2005; Terra-Araujo *et al.*, 2012a, 2015; Terra-Araujo, Faria & Swenson, 2016). Monophyly of the group receives maximum branch support. *Pradosia* is possibly the easiest group to identify of all Chrysophylloideae in the Neotropics on the basis of the drupaceous fruit, a synapomorphy for the genus, with a jelly-like (cartilaginous) endocarp that surrounds the single seed with plano-convex cotyledons and an exserted radicle (Terra-Araujo *et al.*, 2013, 2015, 2016).

Chromolucuma is paraphyletic in its present circumscription because it forms a grade with three species of *Pouteria* section *Franchetella* (clade L). Alves-Araújo & Alves (2012a) expanded *Chromolucuma* with two species on the basis of presence of stipules, so the group would now circumscribe eight species occurring in the Amazon, the Atlantic forest and north to Costa Rica. The clade is readily distinguished by persistent stipules (absent or small in *Pouteria williamii*) (Fig. 3A), yellow latex (Fig. 3E) and pollen type A8, if it is unique to the group (Fig. 4D). The group needs to be rendered monophyletic either by transferring three *Chromolucuma* spp. to *Pouteria* or another three *Pouteria* spp. to *Chromolucuma*.

Clades J, M–N and O–Q include members of sections *Franchetella*, *Gayella*, *Oxythece* and *Pouteria*, supported with high-to-maximum Bayesian and parsimony values. None of these can be satisfactorily distinguished by a combination of characters analysed in this study. For example, tetramerous flowers correlate well with clades J, N and P, but seem to have evolved in *Pouteria* at least three times (Fig. 3F). Other floral characters, such as the corolla form (Fig. 4A) and stamen insertion in the corolla tube (Fig. 4C), are homoplastic, providing no consistent synapomorphies. There is some indication of correlation in the secondary leaf venation (Fig. 3B), with clade J differing from clades M–N and O–Q, but the evidence is not strong enough to merit a generic recognition for any of the clades. Other reviewed characters, proven useful for the Australasian taxa (Swenson *et al.*, 2013), such as pubescence of the inner surface of sepals, or trichomes on the corolla, have shown no significantly useful pattern.

CONCLUSIONS AND PERSPECTIVES

Searching for a natural classification of Neotropical Chrysophylloideae proved to be more complicated than first expected. In retrospect, we realize that our sampling is not sufficiently exhaustive for overall conclusions, but our subfamilial phylogenetic assessment provides new insights for a multitude of conclusions and suggests hypotheses to be tested in the future. The monophyly of Neotropical Chrysophylloideae with one or two establishments in the region should be tested. Broad generic concepts of *Chrysophyllum* and *Pouteria*

are untenable and unpractical and their lineages must be explored, delimited, recognizable and named. Early diverging lineages, often with aberrant morphological character distributions, are easier to distinguish than more recent and diverse radiations. We conclude that the usually recognized genera *Diploon*, *Ecclinusa* and *Elaeoluma* should be maintained and that the satellite genera (or sections) *Ragala* (clade B), *Nemaluma* (clade D), *Prieurella* (clade F), *Achrouteria*, *Cornuella* and *Martiusella* (clade G) merit resurrection. The presumed intercontinental hybrid origin of *Chrysophyllum cuneifolium* and its status as the generic type of *Prieurella* must be clarified. Monophyly of *Micropholis*, here used to orientate the tree, needs to be tested in a broader analysis. Likewise, the true members of *Chrysophyllum*, i.e. sections *Chrysophyllum* and *Villocuspis*, must be explored with a larger taxon sampling. Phylogenetic relationships of *Chrysophyllum durifructum*, *C. eximium*, *C. flexuosum*, *C. gonocarpum*, *C. subspinosum*, *Pouteria lucuma*, *P. oblanceolata* and the remaining seven species of section *Oligotheca* are all of particular interest, not the least because some are types. For example, the relationship of *P. lucuma* is important for a possible resurrection of *Lucuma*. It is recommended that multiple accessions are included of species with disjunct distributions or that are divided into several subspecies. Then there is the challenge to circumscribe the assemblage of *Pouteria*, including the relationships and monophyly of *Sarcaulus*. An evaluation of the alternatives mentioned above must include the type species (Table 1), a more complete taxon sampling and a further pursuit of useful morphological characters. Micromorphological leaf characters were recently successfully used to separate morphologically similar *Pouteria* spp. (Popovkin, Faria & Swenson, 2016) and here might provide useful features that concur with recovered clades. Making a qualified prediction, we would be surprised if yet-to-be-analysed species of *Pouteria* sections *Franchetella*, *Gayella*, *Oxythece* and *Pouteria* and members of the currently accepted genera *Chromolucuma*, *Pradosia* and *Sarcaulus* would fall outside of clade *Sarcaulus*–Q.

ACKNOWLEDGMENTS

Igor Bartish, Laurent Gautier, one anonymous reviewer and, especially, the Associate Editor Lars Chatrou are thanked for many constructive comments on the manuscript. We thank the herbarium curators Carlos Henrique Franciscon (INPA), Roseli Torres (IAC) and Ana Odete Santos Vieira (FUEL) for making specimens available for this study. Hugh Nicholson is thanked for his contribution of field images. ADF is grateful to the National Institute for Amazonian Research (INPA) by supporting the work in the Amazon, with special thanks to the Postgraduate Course in Plant Biology and the Biological Dynamics of Forest Fragments (PDBFF). Cynthia de Oliveira Ferreira, Laboratory of Molecular Biology at the Hospital for Tropical Biology, State of Amazonas, and Mattias Myrenås and Bodil Cronholm, Swedish Museum of Natural History, are acknowledged for their invaluable laboratory assistance. José Ribamar Mesquita Ferreira and Everaldo da Costa Pereira provided invaluable field assistance by sampling most of the Amazonian specimens. For productive collaboration in the field, we thank the team of Flora do Uatuma Project in the State of Amazon, Lidyanne Aona in the State of Bahia and João Carlos Galvão, André Rochelle and Catia Urbanetz in the State of São Paulo. This work was supported by grants to ADF and JRP from the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP – 06/55507-9 and 06/55508-5) and to US from the Swedish Research Council (VR 621-2007-5845). JRP is also supported by a CNPq grant.

REFERENCES

Akaike H. 1974. A new look at the statistical model identification. *IEEE Transactions on Automatic Control* **19:** 716–723.

Álvarez I, Wendel JF. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* **29:** 417–434.

Alves-Araújo A, Alves M. 2011. Two new species of *Pouteria* (Sapotaceae) from the Atlantic Forest in Brazil. *Systematic Botany*. **36:** 1004–1007.

Alves-Araújo A, Alves M. 2012a. Two new species and a new combination of Neotropical Sapotaceae. *Brittonia* **64:** 23–29.

Alves-Araújo A, Alves M. 2012b. *Pouteria ciliata*, *P. confusa*, *P. nordestinensis* and *P. velutinicarpa* spp. nov. (Sapotaceae) from Brazil. *Nordic Jounal of Botany* **30:** 399–406.

APG IV. 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Botanical Journal of the Linnean Society* **181:** 1–20.

Aubréville A. 1961a. Notes sur les Sapotacées Africaines et Sud-Américaines. *Adansonia*, n.s. **1:** 6–38.

Aubréville A. 1961b. Notes sur des Poutériées Américaines. *Adansonia*, n.s. **1:** 150–191.

Aubréville A. 1964a. Les Sapotacées: taxonomie et phytogéographie. *Adansonia, série 2, Mémoire* **1:** 1–157.

Aubréville A. 1964b. Notes sur des Sapotacées. III. *Adansonia*, n.s. **4:** 367–391.

Aubréville A. 1967. *Flore de la Nouvelle Calédonie et dépendances, Vol. 1, Sapotacées.* Paris: Muséum National d'Histoire Naturelle.

Baehni C. 1942. Mémoires sur les Sapotacées II. Le genre *Pouteria*. *Candollea* **9:** 147–476.

Baehni C. 1965. Mémoires sur les Sapotacées III. Inventaire des genres. *Boissiera* **11:** 1–262.

Baillon H. 1891. *Histoire des plantes, Vol. 11*. Paris: Librairie Hachette.

Baldwin BG, Markos S. 1998. Phylogenetic utility of the external transcribed spacer (ETS) of 18S–26S rDNA: congruence of ETS and ITS trees of *Calycadenia* (Compositae). *Molecular Phylogenetics and Evolution* **10:** 449–463.

Bartish IV, Swenson U, Munzinger J, Anderberg AA. 2005. Phylogenetic relationships among New Caledonian Sapotaceae (Ericales): molecular evidence for generic polyphyly and repeated dispersal. *American Journal of Botany* **92:** 667–673.

Bartish IV, Antonelli A, Richardson JE, Swenson U. 2011. Vicariance or long-distance dispersal: historical biogeography of the pantropical subfamily Chrysophylloideae (Sapotaceae). *Journal of Biogeography* **38:** 177–190.

Bentham G, Hooker JD. 1876. *Genera plantarum 2*. London: Reeve & Co.

Cronquist A. 1946. Studies in the Sapotaceae — VI: miscellaneous notes. *Bulletin of the Torrey Botanical Club* **73:** 465–471.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29:** 1969–1973.

Ellis B, Daly DC, Hickey LJ, Johnson KR, Mitchell JD, Wilf P, Wing SL. 2009. *Manual of leaf architecture.* Ithaca: The New York Botanical Garden Press and Comstock Publishing Associates.

Eyma PJ. 1936. Notes on Guiana Sapotaceae. *Recueil des Travaux Botaniques Néerlandais* **33:** 156–210.

Farris JS, Albert VA, Källersjö M, Lipscomb D, Kluge AG. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* **12:** 99–124.

Gautier L, Naciri Y, Anderberg AA, Smedmark JEE, Randrianaivo R, Swenson U. 2013. A new species, genus and tribe of Sapotaceae, endemic to Madagascar. *Taxon* **62:** 972–983.

Gautier L, Lachenaud O, van der Burgt X, Kenfack D. 2016. Five new species of *Englerophytum* K. Krause (Sapotaceae) from central Africa. *Candollea* **71:** 287–305.

Gernhard T. 2008. The conditioned reconstructed process. *Journal of Theoretical Biology* **253:** 769–778.

Govaerts R, Frodin DG, Pennington TD. 2001. *World checklist and bibliography of Sapotaceae.* Kew: Royal Botanic Gardens.

Harley MM. 1991. The pollen morphology of the Sapotaceae. *Kew Bulletin* **46:** 379–491.

Harris GJ, Harris MW. 1997. *Plant identification terminology: an illustrated glossary, 1st edn*. 5th printing. Springer Lake: Springer Lake Publishing.

Huelsenbeck JP, Ronquist F. 2001. MrBayes: Bayesian inference of phylogenetic trees. *Bioinformatics* **17:** 754–755.

Katoh K, Asimenos G, Toh H. 2009. *Multiple alignment of DNA sequences with MAFFT.* In: Posada D, ed. *Bioinformatics for DNA sequence analysis.* Totowa: Human Press, 39–64.

Lemey P, Rambaut A, Drummond AJ, Suchard MA. 2009. Bayesian phylogeography finds its roots. *PLoS Computational Biology* **5:** e1000520.

Mackinder B, Harris DJ, Gautier L. 2016. A reinstatement, recircumscription and revision of the genus *Donella* (Sapotaceae). *Edinburgh Journal of Botany* **73:** 297–339.

Maddison DR, Maddison WP. 2005. *MacClade 4: analysis of phylogeny and character evolution, version 4.0*. Sunderland: Sinauer.

Martius CFP. 1839. (19.) *Ecclinusa ramiflora* Mart. *Flora* **22** (Beiblatt 1): 2.

McNeill J, Barrie FR, Buck WR, Demoulin V, Greuter W, Hawksworth DL, Herendeen PS, Knapp S, Marhold K, Prado J, Prud'homme van Reine WF, Smith GF, Wiersema JH, Turland NJ, eds. 2012. *International Code of Botanical Nomenclature for algae, fungi, and plants (Melbourne Code): adopted by the Eighteenth International Botanical Congress Melbourne, Australia, July 2011.* Regnum Vegetabile 154. Königstein: Koeltz.

Morales F. 2012. Nuevas especies de Sapotaceae para Cosa Rica. *Darwiniana* **50:** 107–113.

Munzinger J, Swenson U. 2009. Three new species of *Planchonella* Pierre (Sapotaceae) with a dichotomous and online key to the genus in New Caledonia. *Adansonia*, séries 3, **31:** 175–189.

Naciri Y, Linder HP. 2015. Species delimitation and relationships: the dance of the seven veils. *Taxon* **64:** 3–16.

Oxelman B, Bremer B. 2000. Discovery of paralogous nuclear gene sequences coding for the second-largest subunit of RNA polymerase II (*RPB2*) and their phylogenetic utility in gentianales of the asterids. *Molecular Biology and Evolution* **17:** 1131–1145.

Pennington TD. 1990. *Flora Neotropica Monograph 52: Sapotaceae.* New York: New York Botanical Garden.

Pennington TD. 1991. *The genera of Sapotaceae.* Kew: Royal Botanic Gardens.

Pennington TD. 2006. Flora da Reserva Ducke, Amazonas, Brasil: Sapotaceae. *Rodriguésia* **57:** 251–366.

Pennington TD. 2007. *Flora of Ecuador 80. 152. Sapotaceae*. Gothenburg: Department of Plant and Environmental Sciences, Göteborg University.

Petersen JJ, Parker IM, Potter D. 2012. Origins and close relatives of a semi-domesticated neotropical fruit tree: *Chrysophyllum cainito* (Sapotaceae). *American Journal of Botany* **99:** 585–604.

Pierre L. 1890. *Notes botaniques: Sapotacées*, pp. 1–36. Paris: Librairie des sciences Paul Klincksieck.

Pierre L. 1891. *Notes botaniques: Sapotacées*, pp. 37–67. Paris: Librairie des sciences Paul Klincksieck.

Poczai P, Hyvönen J. 2010. Nuclear ribosomal spacer regions in plant phylogenetics: problems and prospects. *Molecular Biology Reports* **37:** 1897–1912.

Popovkin AV, Faria AD, Swenson U. 2016. *Pouteria synsepala* (Sapotaceae: Chrysophylloideae): a new species from the northern littoral of Bahia, Brazil. *Phytotaxa* **286:** 39–46.

Posada D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253–1256.

Rambaut A. 2009. *FigTree version 1.3.1*. Computer program available at: http://tree.bio.ed.ac.uk/software/figtree/ (last accessed November 2015).

Rambaut A, Drummond AJ. 2009. *Tracer version 1.5*. Computer program available at: http://tree.bio.ed.ac.uk/software/tracer/ (last accessed November 2015).

Rannala B, Yang Z. 1996. Probability distribution of molecular evolutionary trees: a new method of phylogenetic inference. *Journal of Molecular Evolution* **43:** 304–311.

Ribeiro JELS, Hopkins MJG, Vicentini A, Sothers CA, Costa MAS, Brito JM, Souza MA, Martins LHP, Lohmann LG, Assunção PACL, Pereira EC, Silva CF, Mesquita MR, Procópio LC. 1999. *Flora da Reserva Ducke: guia de identificação das plantas vasculares de uma floresta de terra firme na Amazônia Central.* Manaus: INPA.

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19:** 1572–1574.

Roosmalen MGM, Garcia OMCG. 2000. Fruits of the Amazonian forest. Part II: Sapotaceae. *Acta Amazonica* **30:** 187–290.

Santamaría-Aguilar D, Chaves-Fallas JM, Aguilar R. 2017. Two new species of *Chrysophyllum* (Sapotaceae) endemic to Costa Rica. *Brittonia* **69:** 1–9.

Simmons MP. 2004. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* **31:** 874–879.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* **49:** 369–381.

Smedmark JE, Anderberg AA. 2007. Boreotropical migration explains hybridization between geographically distant lineages in the pantropical clade Sideroxyleae (Sapotaceae). *American Journal of Botany* **94:** 1491–1505.

Smedmark JE, Swenson U, Anderberg AA. 2006. Accounting for variation of substitution rates through time in Bayesian phylogeny reconstruction of Sapotoideae (Sapotaceae). *Molecular Phylogenetics and Evolution* **39:** 706–721.

Swenson U, Anderberg AA. 2005. Phylogeny, character evolution, and classification of Sapotaceae (Ericales). *Cladistics* **21:** 101–130.

Swenson U, Munzinger J. 2012. Revision of *Pichonia* (Sapotaceae) in New Caledonia. *Australian Systematic Botany* **25:** 31–48.

Swenson U, Munzinger J. 2016. Five new species and a systematic synopsis of *Pycnandra* (Sapotaceae), the largest endemic genus in New Caledonia. *Australian Systematic Botany* **29:** 1–40.

Swenson U, Bartish IV, Munzinger J. 2007a. Phylogeny, diagnostic characters, and generic limitation of Australasian Chrysophylloideae (Sapotaceae, Ericales): evidence from ITS sequence data and morphology. *Cladistics* **23:** 201–228.

Swenson U, Munzinger J, Bartish IV. 2007b. Molecular phylogeny of *Planchonella* (Sapotaceae) and eight new species from New Caledonia. *Taxon* **56:** 329–354.

Swenson U, Richardson JE, Bartish IV. 2008a. Multi-gene phylogeny of the pantropical subfamily Chrysophylloideae (Sapotaceae): evidence of generic polyphyly and extensive morphological homoplasy. *Cladistics* **24:** 1006–1031.

Swenson U, Nylinder S, Munzinger J. 2013. Towards a natural classification of Sapotaceae subfamily Chrysophylloideae in Oceania and Southeast Asia based on nuclear sequence data. *Taxon* **62:** 746–770.

Swenson U, Lowry PP 2nd, Munzinger J, Rydin C, Bartish IV. 2008b. Phylogeny and generic limits in the *Niemeyera* complex of New Caledonian Sapotaceae: evidence of multiple origins of the anisomerous flower. *Molecular Phylogenetics and Evolution* **49:** 909–929.

Swenson U, Munzinger J, Lowry PP II, Cronholm B, Nylinder S. 2015. Island life – classification, speciation and cryptic species of *Pycnandra* (Sapotaceae) in New Caledonia. *Botanical Journal of the Linnean Society* **179:** 57–77.

Swofford DL. 2002. *PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sunderland: Sinauer.

Terra-Araujo MH, Faria AD, Vicentini A. 2012a. A new species of *Pradosia* (Sapotaceae) from Central Amazonia. *Brittonia* **64:** 139–142.

Terra-Araujo MH, Faria AD, Ribeiro JELS, Swenson U. 2012b. Flower biology and subspecies concepts in *Micropholis guyanensis* (Sapotaceae): evidence of ephemeral flowers in the family. *Australian Systematic Botany* **25:** 295–303.

Terra-Araujo MH, Faria AD, Alves-Araujo A, Alves M. 2013. *Pradosia restingae* sp. nov. from the Atlantic forest, Brazil. *Nordic Journal of Botany* **31:** 437–441.

Terra-Araujo MH, de Faria AD, Vicentini A, Nylinder S, Swenson U. 2015. Species tree phylogeny and biogeography of the Neotropical genus *Pradosia* (Sapotaceae, Chrysophylloideae). *Molecular Phylogenetics and Evolution* **87:** 1–13.

Terra-Araujo MH, Faria AD, Swenson U. 2016. A taxonomic update of Neotropical *Pradosia* (Sapotaceae, Chrysophylloideae). *Systematic Botany* **41:** 634–650.

Thiers B. (continuously updated). *Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium.* Available at: http://sweetgum.nybg.org/science/ih/ (last accessed March 2017).

Triono T, Brown AHD, West JG, Crisp MD. 2007. A phylogeny of *Pouteria* (Sapotaceae) from Malesia and Australasia. *Australian Systematic Botany* **20:** 107–118.

van Royen P. 1957. Revision of the Sapotaceae of the Malaysian area in a wider sense. VII. *Planchonella* Pierre. *Blumea* **8:** 235–445.

Vink W. 1958. Revision of the Sapotaceae of the Malaysian area in a wider sense XIII. *Chrysophyllum* L. *Blumea* **9:** 21–74.

Yang Z, Rannala B. 1997. Bayesian phylogenetic inference using DNA sequences: a Markov Chain Monte Carlo Method. *Molecular Biology and Evolution* **14:** 717–724.

APPENDIX

Appendix. Accessions of Neotropical Chrysophylloideae sampled in this study, following Pennington's (1990, 1991) classification compared to that of Aubréville (1961b, 1964a). Type species for genera or sections are in bold type. Sequences published for the first time are prefixed with KJ and multiple accessions with asterisks (*).

Taxon Pennington (1990,1991)	Aubréville (1961b,1964a)	Origin: Voucher	ETS	ITS	*RPB2*
Chromolucuma Ducke	*Chromolucuma*				
**Chromolucuma cespedisiiformis* J.F.Morales	unknown at the time	Costa Rica: *Arne Anderberg & al. 20* (S)	KJ453560–63*	EF558614	KJ453710
Chromolucuma rubriflora Ducke	*Chromolucuma*	Brazil: *Vicentini & al. 1229* (INPA)	NS	KJ399340	KJ453711
Chrysophyllum L.	*Chrysophyllum*				
Chrysophyllum section *Aneuchrysophyllum*	*Chloroluma* Baill.				
C. imperiale (K.Koch & Fintelm.) Benth. & Hook.f.	*Chloroluma*	Brazil: *Pennington s.n.* (S)	KJ453567	EF558615	KJ453715
C. pomiferum (Eyma) T.D.Penn.	*Chloroluma*	Brazil: *Assunção & Silva 651* (INPA)	KJ453570	KJ399345	KJ453718
C. venezuelanense (Pierre) T.D.Penn.	*Chloroluma*	Ecuador: *Ståhl & al. 5755* (S)	KJ453576	DQ246673	KJ453724
Chrysophyllum section *Chrysophyllum*	*Chrysophyllum*				
C. cainito L.	*Chrysophyllum*	Mexico: *Petersen 94* (UCD)	KJ453565	KJ399342	KJ453713
C. oliviforme L.	*Chrysophyllum*	Cuba: *Gutiérrez & Nilsson 1* (S)	KJ453569	DQ246670	KJ453717
Chrysophyllum section *Prieurella*	*Prieurella* Pierre				
C. amazonicum T.D.Penn.	*Prieurella*	Brazil: *Assunção & Pereira 207* (INPA)	KJ453564	KJ399341	KJ453712
C. amazonicum T.D.Penn.	*Prieurella*	French Guiana: *Poncy 1745* (P)	NS	DQ246690	KJ453791
C. colombianum T.D.Penn.	*Prieurella*	Brazil: *Vicentini & Pereira 913* (INPA)	KJ453566	KJ399343	KJ453714
C. manaosense (Aubrév.) T.D.Penn.	*Prieurella*	Brazil: *Ribeiro & al. 1246* (INPA)	KJ453568	KJ399344	KJ453716
**C. prieurii* A.DC.	*Prieurella*	Brazil: *Nascimento & al. 778* (INPA)	KJ453571	KJ399346–53*	KJ453719
Chrysophyllum section *Ragala*	*Ragala* Pierre				
C. sanguinolentum (Pierre) Baehni	*Ragala*				
subsp. *balata* (Ducke) T.D.Penn.	*Ragala*	Brazil: *Vicentini & Silva 379* (INPA)	KJ453572	KJ399354	KJ453720
subsp. *spurium* (Ducke) T.D.Penn.	*Ragala*	Brazil: *Nascimento & al. 549* (INPA)	KJ453573	KJ399355	KJ453721
C. ucuquiranabranca (Aubrév. & Pellegr.) T.D.Penn.	*Ragala*	Brazil: *Nascimento & al. 777* (INPA)	KJ453575	KJ399357	KJ453723
Chrysophyllum section *Villocuspis*	*Villocuspis* Aubrév. & Pellegr.				
C. sparsiflorum Klotzsch ex Miq.	*Villocuspis*	Brazil: *Sothers & Silva 927* (INPA)	KJ453574	KJ399356	KJ453722
Diploon Cronquist	*Diploon*				
Diploon cuspidatum (Hoehne) Cronq.	*Diploon*	French Guiana: *Pennington & al. 13843* (U)	KJ453577	DQ246676	KJ453725

Appendix. *Continued*

Taxon Pennington (1990,1991)	Aubréville (1961b,1964a)	Origin: Voucher	ETS	ITS	*RPB2*
Ecclinusa Mart.	*Ecclinusa*				
Ecclinusa guianensis Eyma	*Ecclinusa*	Brazil: *Assunção & al. 162* (INPA)	KJ453578	KJ399358	KJ453726
Ecclinusa guianensis Eyma	*Ecclinusa*	Brazil: *Ducke Reserve 05-906* (K)	KJ453579	DQ246677	KJ453727
Ecclinusa ramiflora Mart.	*Ecclinusa*	Suriname: *Irwing & al. 55081* (S)	KJ453580	DQ246678	KJ453728
Elaeoluma Baill.	*Elaeoluma*				
Elaeoluma glabrescens (Mart. & Eichler ex Miq.) Aubrév.	*Elaeoluma*	Costa Rica: *Anderberg & al. 33* (S)	KJ453581	EF558616	KJ453729
Elaeoluma nuda (Baehni) Aubrév.	*Elaeoluma*	Brazil: *Souza & al. 409* (INPA)	KJ453582	KJ399359	KJ453730
Elaeoluma schomburgkiana (Miq.) Baill.	*Elaeoluma*	Brazil: *Keel & Coelho 243* (S)	KJ453583	DQ246679	KJ453731
Micropholis Pierre section *Micropholis*	*Micropholis*				
Micropholis casiquiarensis Aubrév.	*Micropholis*	Brazil: *Nascimento & al. 770* (INPA)	NS	KJ399360	KJ453732
Micropholis guyanensis (A.DC.) Pierre	*Micropholis*	Brazil: *Hopkins & al. 1475* (INPA)	KJ453584	KJ399361	KJ453733
Micropholis guyanensis (A.DC.) Pierre	*Micropholis*	Puerto Rico: *Taylor 11691* (MO)	NS	DQ246682	KJ453734
Micropholis venulosa (Mart. & Eichler ex Miq.) Pierre	*Micropholis*	Brazil: *Assunção 122* (U)	KJ453586	DQ246683	KJ453736
Micropholis williamii Aubrév. & Pellegr.	*Micropholis*	Brazil: *Ribeiro & al. 1199* (INPA)	KJ453587	KJ399363	KJ453737
Micropholis section *Exsertistamen*	not defined at the time				
Micropholis splendens Gilly ex Aubrév.	*Micropholis*	Brazil: *Assunção & Pereira 13* (INPA)	KJ453585	KJ399362	KJ453735
Pouteria Aubl.	*Pouteria*				
Pouteria section *Antholucuma*	*Radlkoferella* Pierre				
P. dominigensis (C.F.Gaertn.) Baehni	*Radlkoferella*	Cuba: *Gutiérrez & Nilsson 13* (S)	KJ453615	AY552106	KJ453754
P. multiflora (A.DC.) Eyma	*Radlkoferella*	Ecuador: *Villa & Rivaz 257* (BM)	NS	DQ246693	KJ453777
P. oxypetala T.D.Penn.	unknown at the time	Brazil: *Bertoni & Geremias 293* (IAC)	KJ453658	KJ399417	KJ453780
P. venosa (Mart.) Baehni	*Radlkoferella*	Brazil: *Faria & Ribeiro 2007/55* (INPA)	KJ453682	KJ399445	KJ453800
Pouteria section *Franchetella*	*Franchetella* Pierre				
P. andarahiensis T.D.Penn.	unknown at the time	Brazil: *Faria & Ribeiro 2008/09* (SPF)	KJ453589	KJ399373	KJ453739
P. anomala (Pires) T.D.Penn.	?	Brazil: *Sothers & Pereira 1068* (INPA)	KJ453590	KJ399374	KJ453740
P. bilocularis (H.Winkl.) Baehni	*Labatia* Sw.	Brazil: *Nascimento & al. 508* (INPA)	KJ453592	KJ399376	KJ453742
**P. campanulata* Baehni	*Pouteria*	Brazil: *Assunção & al. 673* (INPA)	KJ453598–604*	KJ399382	KJ453748
P. cladantha Sandwith	*Neoxythece* Aubrév. & Pellegr.	Brazil: *Assunção & Pereira 616* (INPA)	KJ453606	KJ399383	KJ453750
P. coriacea (Pierre) Pierre	*Pouteria*	Brazil: *Faria & Ribeiro 2007/07* (INPA)	NS.	KJ399384	KJ453751
P. durlandii (Standl.) Baehni	*Paralabatia* Pierre	Brazil: *Ribeiro & al. 1904* (INPA)	KJ453616	KJ399387	KJ453755
P. engleri Eyma	*Nemaluma* Baill.	Brazil: *Faria & Ribeiro 735* (INPA)	KJ453617	KJ399388	KJ453756
P. erythrochrysa T.D.Penn.	unknown at the time	Brazil: *Ribeiro & al. 1785* (INPA)	KJ453619	KJ399390	KJ453758
P. flavilatex T.D.Penn.	unknown at the time	Brazil: *Ribeiro & al. 1906* (INPA)	KJ453628	KJ399394	KJ453762
**P. fulva* T.D.Penn.	unknown at the time	Brazil: *Faria & Ribeiro 2007/52* (INPA)	KJ453634	KJ399396–405*	KJ453764

Appendix. *Continued*

Taxon Pennington (1990,1991)	Aubréville (1961b,1964a)	Origin: Voucher	ETS	ITS	*RPB2*
P. gardneri (Mart. & Miq.) Baehni	*Podoluma* Baill.	Brazil, Picinguaba: Tree 992 PJ sub50 (INPA)	KJ453674	KJ399437	KJ453792
P. jariensis Pires & T.D.Penn.	?	Brazil: *Oliveira & al. 215* (INPA)	KJ453639	KJ399408	KJ453769
**P. minima* T.D.Penn.	unknown at the time	Brazil: *Ribeiro & Pereira 1876* (INPA)	KJ453646–55*	KJ399414	KJ453776
**P. pallens* T.D.Penn.	unknown at the time	Brazil: *Ribeiro & al. 1905* (INPA)	KJ453659–64*	KJ399418	KJ453781
P. pentamera T.D.Penn.	unknown at the time	Brazil: *Faria & Ribeiro 2007/54* (INPA)	KJ453665	KJ399419	KJ453782
P. platyphylla (A.C.Sm.) Baehni	*Pouteria*	Brazil: *Faria & Ribeiro 2007/39* (INPA)	KJ453667	KJ399421	KJ453784
P. ramiflora (Mart.) Radlk.	*Paralabatia*	Brazil: *Faria & Ribeiro 2008/5* (SPF)	KJ453669	KJ399432	KJ453786
P. reticulata (Engl.) Eyma	*Franchetella* Pierre	Brazil: *Assunção & al. 728* (INPA)	KJ453671	KJ399434	KJ453788
P. retinervis T.D.Penn.	unknown at the time	Brazil: *Ribeiro & al. 1936* (INPA)	KJ453672	KJ399435	KJ453789
P. rostrata (Huber) Baehni	*Pouteria*	Brazil: *Faria & Ribeiro 2007/40* (INPA)	KJ453673	KJ399436	KJ453790
P. stipulifera T.D.Penn.	unknown at the time	Brazil: *Ribeiro & Pereira 1956* (INPA)	KJ453677	KJ399440	KJ453795
P. subsessilifolia Cronquist	?	Brazil: *Faria & Ribeiro 2008/11* (SPF)	KJ453679	KJ399442	KJ453797
P. vernicosa T.D.Penn.	unknown at the time	Brazil: *Sothers & Pereira 380* (INPA)	KJ453683	KJ399446	KJ453801
P. vernicosa T.D.Penn.	unknown at the time	Ecuador: *Villa, Velez & Rivaz 1304* (BM)	KJ453684	DQ246694	KJ453802
P. virescens Baehni	*Pouteria*	Brazil: *Martins & al. 49* (INPA)	KJ453685	KJ399447	KJ453803
P. williamii (Aubrév. & Pellegr.) T.D.Penn.	*Eremoluma* Baill.	Brazil: *Souza & al. 477* (INPA)	KJ453686	KJ399448	KJ453804
P. Faria13	?	Brazil: *Faria & Ribeiro 2007/13* (INPA)	KJ453593	KJ399377	KJ453743
P. Faria51	?	Brazil: *Faria & Ribeiro 2007/51* (INPA)	KJ453618	KJ399389	KJ453757
**P.* Tree183	?	Brazil, Picinguaba: Tree 183 PJ sub11	KJ453668	KJ399422–31*	KJ453785
Pouteria section *Gayella*	*Gayella* Pierre				
**P. eugeniifolia* (Pierre) Baehni	*Myrtiluma* Baill.	Brazil: *Faria & Ribeiro 2007/38* (INPA)	KJ453620–25*	KJ399391	KJ453759
Pouteria section *Oligotheca*					
P. laevigata (Mart.) Radlk.	*Labatia*	Brazil: *Vicentini & al. 762* (INPA)	KJ453640	KJ399409	KJ453770
P. maxima T.D.Penn.	unknown at the time	Brazil: *Vicentini & al. 1203* (INPA)	KJ453645	KJ399413	KJ453775
P. oblanceolata Pires	?	Brazil: *Ribeiro & Silva 1373* (INPA)	KJ453656	KJ399415	KJ453778
Pouteria section *Oxythece*					
**P. ambelaniifolia* (Sandwith) T.D.Penn.	*Pseudoxythece* Aubrév.	Brazil: *Ribeiro & al. 1895* (INPA)	KJ453588	KJ399364–72*	KJ453738
P. cuspidata (A.DC.) Baehni					
*subsp. *cuspidata*	*Pouteria*	Brazil: *Faria & Ribeiro 2007/21* (INPA)	KJ453607–13*	KJ399385	KJ453752
subsp. dura (Eyma) T.D.Penn.	*Neoxythece*	Brazil: *Sothers & al. 200* (INPA)	KJ453614	KJ399386	KJ453753
P. opposita (Ducke) T.D.Penn.	*Caramuri* Aubrév. & Pellegr.	Brazil: *Martins & al. 45* (INPA)	KJ453657	KJ399416	KJ453779

Appendix. *Continued*

Taxon Pennington (1990,1991)	Aubréville (1961b,1964a)	Origin: Voucher	ETS	ITS	*RPB2*
Pouteria section *Pouteria*					
P. beaureparei (Glaz. & Raunk.) Baehni	*Pseudolabatia* Aubrév. & Pellegr.	Brazil: Tree D1906, Cananéia	KJ453591	KJ399375	KJ453741
P. caimito (Ruiz & Pav.) Radlk.	*Labatia*	Brazil: Manaus: *Assunção & Silva 649* (INPA)	KJ453594	KJ399378	KJ453744
P. caimito (Ruiz & Pav.) Radlk.	*Labatia*	Brazil, Guariva: *Bertoni & Geremias 11* (IAC)	KJ453595	KJ399379	KJ453745
P. caimito (Ruiz & Pav.) Radlk.	*Labatia*	Brazil, Penápolis: *Bertoni & Geremias 391* (IAC)	KJ453596	KJ399380	KJ453746
P. caimito (Ruiz & Pav.) Radlk.	*Labatia*	Brazil, Ubatuba: *Bertoni & Geremias 295* (IAC)	KJ453597	KJ399381	KJ453747
P. caimito (Ruiz & Pav.) Radlk.	*Labatia*	Brazil, Cananéia: Tree C1913 (IAC)	KJ453676	KJ399439	KJ453794
P. filipes Eyma	*Pseudolabatia*	Brazil: *Faria & Ribeiro 2007/43* (INPA)	KJ453626	KJ399392	KJ453760
P. fimbriata Baehni	*Pouteria*	Brazil: *Ribeiro & al. 1908* (INPA)	KJ453627	KJ399393	KJ453761
**P. freitasii* T.D.Penn.	unknown at the time	Brazil: *Assunção & Silva 770* (INPA)	KJ453629–33*	KJ399395	KJ453763
P. gardneriana (A.DC.) Radlk.	*Pouteria*	Argentina: *Schwarz 8216* (UPS)	KJ453635	DQ246689	KJ453765
P. guianensis Aubl.	*Pouteria*	Brazil: *Ribeiro & al. 1902* (INPA)	KJ453636	KJ399406	KJ453766
P. hispida Eyma	*Pouteria*	Brazil: *Assunção & Silva 197* (INPA)	KJ453637	KJ399407	KJ453767
P. hispida Eyma	*Pouteria*	French Guiana: *Mori & al. 25432* (NY)	KJ453638	DQ246691	KJ453768
P. petiolata T.D.Penn.	unknown at the time	Brazil: *Sothers 946* (INPA)	KJ453666	KJ399420	KJ453783
P. resinosa T.D.Penn.	unknown at the time	Brazil: *Faria & Ribeiro 2007/33* (INPA)	KJ453670	KJ399433	KJ453787
P. subcaerulea Pierre ex Dubard	*Pseudolabatia*	Brazil: *Faria & Ribeiro 2008/3* (SPF)	KJ453678	KJ399441	KJ453796
P. torta (Mart.) Radlk.					
subsp. *glabra* T.D.Penn.	*Pouteria*	Brazil: *Ribeiro & al. 1310* (INPA)	KJ453680	KJ399443	KJ453798
subsp. *torta*	*Pouteria*	Brazil, Picinguaba: Tree 0412 PJ sub21 (INPA)	KJ453675	KJ399438	KJ453793
subsp. *torta*	*Pouteria*	Brazil: *Faria & Ribeiro 2008/2* (SPF)	KJ453681	KJ399444	KJ453799
Pouteria section *Rivicoa*	*Richardella* Pierre				
P. campechiana (Kunth) Baehni	*Richardella*	Taiwan (cultivated): *Wang 798* (HAST)	KJ453605	DQ246688	KJ453749
P. macrophylla (Lam.) Eyma	*Richardella*	Brazil: *Vicentini & Pereira 771* (INPA)	KJ453642	KJ399410	KJ453772
P. macrophylla (Lam.) Eyma	*Richardella*	Bolivia: *Seidel & al. 5905* (K)	KJ453641	DQ246692	KJ453771
P. manaosensis (Aubrév. & Pellegr.) T.D.Penn.	*Richardella*	Brazil: *Nascimento & Pereira 571* (INPA)	KJ453644	KJ399412	KJ453774
P. manaosensis (Aubrév. & Pellegr.) T.D.Penn.	*Richardella*	Brazil: *Nascimento 607* (INPA)	KJ453643	KJ399411	KJ453773
Pradosia Liais	*Pradosia*				
Pradosia brevipes (Pierre) T.D.Penn.	*Ecclinusa*	Brazil: *Lindeman 6743* (U)	KJ453687	AY552158	NS
Pradosia cochlearia (Lecomte) T.D.Penn.	*Ecclinusa*	Brazil: *Brito & al. 29* (INPA)	KJ453688	KJ399449	KJ453805
**Pradosia decipiens* Ducke	*Pradosia*	Brazil: *Brito & al. 1950* (INPA)	KJ453689–96*	KJ399450	KJ453806

Appendix. *Continued*

Taxon Pennington (1990,1991)	Aubréville (1961b,1964a)	Origin: Voucher	ETS	ITS	*RPB2*
**Pradosia schomburgkiana* (A.DC.) Cronquist	*Pradosia*	Brazil: *Faria & Ribeiro 2007/09* (INPA)	KJ453697–99*	KJ399451	KJ453807
Pradosia surinamensis (Eyma) T.D.Penn.	*Pradosia*	Guyana: *Harris 1076* (U)	KJ453700	AY552157	KJ453808
Sarcaulus Radlk.					
****Sarcaulus brasiliensis*** (A.DC.) Eyma	*Sarcaulus*	Brazil: *Martins & al. 48* (INPA)	KJ453701–09*	KJ399452	KJ453809

NS, not sampled.
*Species with variable repeats in either ETS or ITS.

SUPPORTING INORMATION

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

Table S1. Characters sampled for this phylogenetic study of Neotropical Chrysophylloideae (Sapotaceae). An aligned data matrix.