CSIRO PUBLISHING
www.publish.csiro.au/journals/asb *Australian Systematic Botany* **20**, 107–118

A phylogeny of *Pouteria* (Sapotaceae) from Malesia and Australasia

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Abstract. The genus *Pouteria* Aublet is a pantropical group and many of its species produce high-quality timber and edible fruit. In 1991, on the basis of morphological characters, Pennington combined the genus *Planchonella* Pierre with *Pouteria*, expanding the latter genus to nine sections and 325 species. However, many *Planchonella* species were not included in his account and doubt remains about the generic limits of *Pouteria sensu* Pennington. This paper re-assesses the generic delimitation of *Pouteria* and its affinities with *Planchonella* from molecular data generated from the nuclear-encoded internal transcribed spacer (ITS) region. The analysis includes 22 *Planchonella* species and three *Pouteria* species *sensu* van Royen collected from Malesia and Australia, and seven additional *Planchonella* species from New Caledonia with molecular data available from GenBank. Other genera from Sapotaceae included in the analysis were *Chrysophyllum*, *Niemeyera*, *Pichonia*, *Pycnandra* and *Xantolis* (tribe Chrysophylleae) and *Mimusops*, *Palaquium* and *Manilkara* (outgroups from other tribes). The resulting ITS cladograms from both Bayesian and maximum parsimony analyses indicated that Malesian and Australasian *Pouteria* species are not monophyletic and comprise three separate lineages, therefore providing evidence against the broad circumscription of this genus by Pennington. Tertiary leaf venation type (reticulate, parallel or ramified), when mapped onto the phylogeny, correlated with these groupings, indicating that this character is taxonomically informative.

Introduction

Pouteria Aublet is the largest pantropical woody genus in family Sapotaceae, with centres of diversification in Malesia, Australia and the Pacific (~120 spp.) (Pennington 1991) and in the neotropics (~188 spp.) (Pennington 1990). The genus also occurs in Africa (~5 spp.). *Pouteria* species are characterised by their tree or shrubby habit, the absence of stipules (except in *P. congestifolia*), spirally arranged leaves (rarely opposite) and eucamptodromous or brochidodromous secondary venation, usually without submarginal veins. *Pouteria* has axillary or ramiflorous, fasciculate inflorescences and the flower includes a single whorl of sepals. The corolla lobes, stamens and staminodes are isomerous with, or similar in number to, the sepals; the corolla lobes are undivided; and the stamens are either exserted or included, with staminodes small or lacking. The genus has a one- to several-seeded berry fruit with broadly ellipsoid to plano-convex seeds. The seed has an adaxial or, less often, basiventral hilum and vertical embryo with plano-convex or thin foliaceous cotyledons, an exserted or included radicle and the endosperm may be absent (Pennington 1991).

Recognition of more than one genus in *Pouteria sensu lato* of Malesia and Australasia began with the work of Dubard (1912, 1915, cited in Pennington 1991), Lam (1925, 1927, 1939) and Lam and Varossieau (1938). The outcome of this work was recognition of *Pouteria* (= *Lucuma* Molina *sensu* Dubard) with non-endospermous seed and *Planchonella* Pierre with endospermous seed. In contrast, Baehni (1938, 1942) proposed a different concept in his worldwide taxonomic treatment for Sapotaceae, combining *Planchonella sensu* Lam and *Lucuma sensu* Dubard & Lam into a large single genus *Pouteria* (318 spp.). As van Royen (1957) noted, Baehni's reason for the union was not explicitly stated. Pennington (1991) suggested that the reason was Baehni's rejection of the reliability of certain characters such as the absence or presence of endosperm. However, in the introduction to his manuscript, Baehni wrote that though the union was not simple, it was justified in view of similarities in morphology, anatomy and the evolutionary history of the genus and its species' geographical distributions (Baehni 1942). The taxonomic importance of the presence or absence of endosperm has been debated ever since (Pennington 1991).

The present study employs molecular data in an attempt to resolve the generic and specific delimitation problems and to provide additional morphological evidence for classification of *Pouteria* in the Australo-Malesian region. Molecular data from the chloroplast (*rbcL, atpB, atp1, matR, matK, psbN, psbH, ndhF, rps12, rps16, trnL/F* and *trnS/G*) have been used for phylogenetic studies on the genera and species of Sapotaceae by several authors (Morton *et al*. 1997; Källersjö *et al*. 2000; Savolainen *et al*. 2000; Anderberg *et al*. 2001, 2002; Bremer *et al*. 2002; Anderberg and Swenson 2003; Hamilton *et al*. 2003; Swenson and Anderberg 2005). Nuclear rDNA, in contrast with chloroplast DNA (cpDNA) has been found to be informative

 10.1071/SB06011 1030-1887/07/020107

about the phylogeny of Sapotaceae at lower taxonomic levels (Bartish *et al*. 2005). Hence internal transcribed spacer region (ITS) sequence data have been employed in this investigation of relationships within Malesian and Australasian *Pouteria sensu lato*.

In the early studies by Lam (1925, 1927) and van Royen (1957), leaf venation was used to key species but its value for higher-order classification was not investigated before Pennington (1990, 1991). The phylogenetic value of this feature in *Pouteria* has not been tested previously.

The present study uses selected representatives of *Pouteria* and *Planchonella* to (1) test the monophyly and classification of *Pouteria sensu* Pennington; (2) investigate relationships between Malesian and Australian species; and (3) examine the distribution of leaf tertiary venation types compared with the molecular estimate of phylogeny.

Materials and methods

Taxon sampling

Sampling of representative taxa was based on morphological diversity and knowledge of the previous phylogenies and classifications. The ingroup was the Sapotaceae tribe Chrysophylleae (Pennington 1991), which is weakly monophyletic according to *ndhF* sequences (Anderberg and Swenson 2003). Within the tribe, 21 species that were previously classified as *Planchonella* and *Pouteria sensu lato* (Lam 1925, 1927; Herrmann-Erlee and van Royen 1957; van Royen 1957), and currently classified as *Pouteria sensu* Pennington (Pennington 1990, 1991; Govaerts *et al*. 2001; Vink 2002), were sampled for molecular and leaf tertiary venation characters. Of these taxa, 13 species have multiple accessions (Table 1). Additional ingroup samples were taken from *Niemeyera*, the putative sister lineage to *Pouteria*, and also from *Chrysophyllum*, *Pycnandra*, *Pichonia* and *Xantolis*. Outgroup samples were taken from *Palaquium* (tribe Isonandreae), *Mimusops* and *Manilkara* (tribe Mimusopeae). All leaf tissues for DNA extraction were preserved in silica gel (Chase and Hills 1991) and/or in CTAB gel (Rogstad 1992). One or more voucher specimens per species were used for morphological study. The previously published ITS sequences of eight New Caledonian *Pouteria* and other genera in Chrysophylleae: *Pycnandra*, *Niemeyera*, *Pichonia* and *Xantolis* (Bartish *et al*. 2005), were taken from GenBank (www.ncbi.nlm.nih.gov/Genbank/index.html) and added to the molecular data matrix. Overall, the sampling covered a wide geographic range within Malesia and Australasia, i.e. Malaysia, The Philippines, Indonesia, Papua New Guinea, Australia, Vanuatu, New Zealand and New Caledonia.

DNA extraction, amplification and sequencing

Total DNA was obtained from dried leaves stored in silica or CTAB gel upon collecting in the field and from dried herbarium specimens. The leaves were ground in liquid nitrogen, and the DNA was extracted following the CTAB method (Doyle and Doyle 1987) or by a DNeasy Plant Minikit following the protocol provided by the company (QIAGEN, Melbourne, Australia).

Double-stranded DNA fragments were amplified by polymerase chain reaction (PCR). The amplifications were performed in 100-μL volumes containing ~1 μg of DNA, 10 μL of PCR buffer (Life Technologies, Melbourne, Australia), 0.1 μM of each primer, 4 mM magnesium chloride, 0.05 mM dNTPs and 2.25 U of *Taq* DNA polymerase (New England BioLabs Inc., Ipswic, MA, USA). Initially the reaction mix was heated to 96°C for 3 min to denature the secondary structure of the template and assist in primer annealling (Cross *et al*. 2002). This was followed by 30 PCR cycles, each consisting of three steps: 94°C for 30 s (denaturation); 48–52°C (depending on the template) for 60 s (annealing); and 72°C for 2 min (extension). After 30 cycles of amplification, there was a final extension period of 7 min at 72°C. The PCR products were purified by QIAquick PCR purification kits following the procedures supplied by QIAGEN.

The purified DNA was used as the template for the sequencing reaction, by 'Big Dye' terminators and following the methods prescribed by PerkinElmer (Norwalk, CT, USA). The sequencing reaction was conducted in 25 cycles of denaturation at 96°C for 10 s alternating with annealing at 57°C for 5 s. Five ITS primers (Table 2) were employed for both the DNA amplification and the sequencing reaction following White *et al*. (1990) and Sun *et al*. (1994), so that both strands were sequenced.

Sequences showing ambiguities were re-sequenced where possible and only clean sequences were retained for further analysis, as recommended by Alvarez and Wendel (2003). Three species that did not produce clean sequences were excluded from the analysis. Multiple samples taken within 13 species provided a further check for the presence of paralogues and pseudogenes (Alvarez and Wendel 2003).

Sequence analysis

Sequences of the complete ITS region (ITS1, 5.8S, ITS2) were assembled with the aid of the computer alignment program Sequence Navigator (PerkinElmer). Each sequence accession was stored and aligned with ClustalW in BioEdit Sequence Alignment Editor (Hall 1999). The resulting automated alignment was edited manually in BioEdit, then exported as a Nexus file for the maximum parsimony and Bayesian analyses.

Maximum parsimony analysis of the data was conducted using PAUP* Version 4.0b10 (Swofford 2002). The initial analysis had the following settings: a heuristic search of 1000 replicates each saving 100 trees, random addition sequence, tree bisection-reconnection (TBR) branch swapping, collapsing branches if minimum length is zero and all characters equally weighted. All the trees thus saved in memory were then branch-swapped to completion. No shorter trees were found in the additional analysis and a strict consensus was constructed from the trees in memory. Clade support was estimated using bootstrap analysis (BS) in PAUP with 5000 replicates and saving only 100 trees per replicate (Mort *et al*. 2000). Additionally, jackknife (JN) analysis was performed in PAUP with settings recommended by Farris *et al*. (1996), including 'jac' emulation, 10 000 'fast' addition-sequence replicates and deleting $1/e$ characters at each iteration (see also Felsenstein 2004). For both BS and JN, nodes with values $<50\%$ were considered unsupported and nodes with values $>90\%$ were considered to have strong support.

Bayesian analyses were conducted with MrBayes 3.0 (Huelsenbeck and Ronquist 2001), with a GTR + I + G model (general time reversible + invariable sites + gamma) selected by

Table 1. List of taxa sampled (general time reversible + invariable sites + gamma) selected bywith collection details and leaf tertiary venation type [species names follow Govaerts *et al*. (2001) and Jessup (2001)]

Tertiary leaf venation scoring: 1 = reticulate, 2 = ramified, 3 = parallel, NS = not seen

Taxon name	Origin	Collector detail	GenBank accession number	Herbarium holding voucher	Leaf tertiary venation
***Pouteria* (tribe Chrysophylleae)**					
P. asterocarpon (P.Royen) Jessup	Cook District, Queensland, Australia	Telford and Butler 9444		CANB	1
P. arnhemica (F.Muell. ex Benth.) Baehni	NT, Australia	Telford 11707		CANB	1
P. arnhemica (F.Muell. ex Benth.) Baehni	Fitzgerald District, WA, Australia	Purdie 4751		CANB	1
P. australis (R.Br.) Baehni	NSW, Cult. ANBG, Australia	Beesley 1035		CANB	1
P. australis (R.Br.) Baehni	Cult. Booderee Botanic Garden, NSW, Australia	NSW 8504673		CANB	1
P. baillonii (Zahlbr.) Baehni	Noumea, New Caledonia	Hartley 14843		CANB	1
P. baillonii (Zahlbr.) Baehni	New Caledonia		AY552141		1
P. baueri (Montoruz.) Baehni	New Caledonia	Biffin and Craven 92		CANB	3
P. chartacea (F.Muell. ex Benth.) Baehni	Queensland, Australia	Telford 11339		CANB	1
P. cinerea (Pancher ex Baill.) Baehni	Noumea, New Caledonia	Schodde 5282		CANB	1
P. costata (Endl.) Baehni	Malakewu Island, Vanuatu	Curry 1565		CANB	1
P. costata (Endl.) Baehni	New Zealand		AF396230		1
P. cotinifolia (A.DC.) Baehni var. *cotinifolia* P.Royen	Queensland, Cult. ANBG, Australia	NSW 9301601		CANB	1
P. cotinifolia (A.DC.) Baehni var. *pubescens* (P.Royen) Jessup	Atherton, Queensland, Australia	Gray 08457		CANB	1
P. duclitan (Blanco) Baehni	Sulawesi, Cult. Purwodadi Botanic Garden, Indonesia	Triono 208		BO	1
P. eerwah (F.M.Bailey) Baehni	Queensland, Cult. ANBG, Australia	Triono 203		CANB	1
P. eerwah (F.M.Bailey) Baehni	NSW, Cult. Booderee Botanic Garden, Australia	Triono 202		CANB	1
P. firma (Miq.) Baehni	Cult. FRIM, Malaysia	Leng Guan FRI 42470		FRIM	3
P. firma (Miq.) Baehni	Sumatra, Cult. Bogor Botanic Garden, Indonesia	Triono 201		BO	3
P. howeana (F.Muell.) Baehni	Lord Howe Island, NSW, Australia	Telford 7043		CANB	1
P. howeana (F.Muell.) Baehni	Lord Howe Island, NSW, Australia	LHI s.n.		CANB	1
P. howeana (F.Muell.) Baehni	New Caledonia		AY552142		1
P. kaalensis Aubrev.	New Caledonia		AY552104		1
P. lauracea (Baill.) Baehni	New Caledonia		AY552145		1
P. linggensis (Burck) Baehni	Port Narvin, Vanuatu	Curry 600		BO	1
P. luzoniensis (Merr.) Baehni var. *papuana* Erlee	W. Papua, Cult. Purwodadi Botanic Garden, Indonesia	Triono 207		BO	1
P. myrsinifolia (F.Muell.) Jessup	NSW, Cult. ANBG, Australia	Triono 205		CANB	1
P. myrsinodendron (F.Muell.) Jessup	Wetar Island, Indonesia	Dunlop s.n.		BO	1
P. myrsinodendron (F.Muell.) Jessup	Cult. Mt Annan Botanic Garden, NSW, Australia	ACC 862059		CANB	1
P. obovata (R.Br.) Baehni	Palfrey Island, Queensland, Australia	Beesley 566		CANB	1
P. obovata (R.Br.) Baehni	Hinchinbrook Island, Queensland, Australia	Vink 17723		CANB	1
P. obovata (R.Br.) Baehni	Java, Cult. Bogor Botanic Garden, Indonesia	Triono 347		BO, CANB	1
P. obovata (R.Br.) Baehni	Milne Bay, Papua New Guinea	Brass 21872		CANB	1
P. obovata (R.Br.) Baehni	Bakaro, Manokwari, West Papua, Indonesia	Triono 369		CANB	1
P. pinifolia (Baill.) Baehni	New Caledonia		AY552111		1
P. pohlmaniana (F.Muell.) Baehni var. *pohlmaniana*	Cult. Adelaide Botanic Garden, SA, Australia	Triono 310		CANB	1
P. pohlmaniana (F.Muell.) Baehni var. *pohlmaniana*	Cooktown, Queensland, Australia	Triono 179		CANB	1
P. richardii (F.Muell.) Baehni	Litchfield National Park, NT, Australia	Triono 342		BO, CANB	3
P. rubicunda (Pierre ex Baill.) Baehni	New Caledonia		AY552160		3

Table 1. (*continued*)

Taxon name	Origin	Collector detail	GenBank accession number	Herbarium holding voucher	Leaf tertiary venation
P. sandwicensis (A.Gray) Baehni & O.Deg.	Honouliuli, Hawaii	Takeuchi 2417		CANB	2
P. sandwicensis (A.Gray) Baehni & O.Deg.	Kauai, Hawaii	Stone 3404		BO	2
P. sericea (Aiton) Baehni	Bamboo Range Peninsula, Queensland, Australia	Jones and Gray 418905		CANB	1
P. sericea (Aiton) Baehni	Cooktown, Queensland, Australia	Triono 209		CANB	1
P. wakere (Pancer & Sebert) Baehni	Cult. Royal Botanic Garden Sydney, NSW, Australia	Boucher 1575		SYD	1
Other genera in tribe Chrysophylleae					
Chrysophyllum bangweolense R.E.Fr.	Africa		AY552152		NS
C. cainito L.	Cult. Darwin, NT, Australia	Triono 344		BO, CANB	2
C. cainito L.	W. Indies, Cult. Bogor Botanic Garden, Indonesia	Triono 314		BO	2
Pichonia balansana Pierre	New Caledonia		AY552109		1
Pichonia calomeris (Baill. ex Guillaumin) T.D.Penn.	New Caledonia		AY 552102		1
Pichonia novocaledonica (Engl.) T.D.Penn.	New Caledonia		AY552103		NS
Pichonia cf. *sessiliflora* (C.T.White) Aubrev.	Solomon Islands	Wayne s.n.		BO	1
Niemeyera balansae (Baill.) Aubrev.	New Caledonia		AY552123		3
N. deplanchei (Baill.) T.D.Penn.	New Caledonia		AY552120		1
N. francei (Guillaumin & Dubard) T.D.Penn.	New Caledonia		AY552117		NS
N. sessilifolia (Pancher & Sebert) T.D.Penn.	New Caledonia		AY552118		NS
N. whitei (Aubrev.) L.W.Jessup	Mt Moombil, NSW, Australia	Telford 9561		CANB	1
N. chartacea (Baill.) Aubrev.	Cult. Adelaide Botanic Garden, SA, Australia	Triono 211		CANB	1
Pycnandra comptonii (S.Moore) Vink	New Caledonia		AY552131		3
Pycnandra decandra (Montrouz.) Vink	New Caledonia		AY552132		1
Pycnandra neocaledonica (S.Moore) Vink	New Caledonia		AY552129		1
Pycnandra paniensis Aubrev.	New Caledonia		AY552121		1
Xantolis cambodiana (Pierre) van Royen	Cambodia		AY552155		1
X. siamensis (Fletcher) van Royen	Thailand		AY552154		1
Outgroups					
Tribe Isonandeae					
Palaquium amboinense Burck	Cult. Herbarium Bogoriense, Indonesia	Triono 348		BO	3
Tribe Mimusopeae					
Manilkara kauki (L.) Dubard	West Bali, Indonesia	Triono 345		BO, CANB	3
Mimusops elengi L.	Broome, WA, Australia	West 5000		CANB	1

Modeltest version 3.06 (Posada and Crandall 1998) and by a Markov chain Monte Carlo (MCMC) method (Larget and Simon 1999) to search tree and parameter space. Four chains were run simultaneously for 2 million generations, sampling trees and parameters every 100 generations. After determining that a burn-in period of 500 generations sufficed, the remaining 19 501 samples were saved for further analysis. Clade support was represented by posterior probability (PP) values, with PP values between 50 and 90% indicating weakly supported nodes and PP values >90% indicating strong support.

Leaf tertiary venation

Leaf tertiary venation of the voucher specimens, and of additional herbarium material, was observed under constant

Table 2. List of the internal transcribed spacer (ITS) region primers used in polymerase chain reaction (PCR) amplification and in sequencing, following White *et al.* (1990) and Sun *et al.* (1994, marked with *)
All sequences shown 5′ to 3′

Primer name	Primer sequence	Use
17SE or ABI101*	ACGAATTCATGGTCCGGTGAAGTGTTCG	PCR
ITS 5	GGAAGTAAAAGTCGTAACAAGG	PCR/sequencing
ITS 1	TCCGTAGGTGAACCTGCGG	Sequencing
ITS 4	TCCTCCGCTTATTGATATGC	PCR/sequencing
26SE or ABI 102*	TAGAATTCCCCGGTTCGCTCGCCGTTAC	PCR

magnification (0.4 × objective lens) with a stereo dissecting microscope. Venation images were captured with a digital camera and processed with AnalySIS software from Soft Imaging System (Olympus).

For each sampled taxon in the ingroup, one to three representative specimens were scored for their leaf tertiary venation type. The scores were checked against previously published taxonomic results. Tertiary venation was classified into three character states and named following the definition provided by Hickey (1973), van Royen (1957) and Pennington (1990): reticulate (state 1), ramified (state 2) and parallel (state 3) (Fig. 1). To supplement the direct observations of herbarium specimens, taxa were also scored from published descriptions (cf. Farmer and Schilling 2002), but the results from direct observation were given priority when there was a conflict with the literature (cf. Cross *et al.* 2002). These data then were mapped onto the molecular phylogeny manually.

Results

ITS sequence characteristics

The overall length of the ITS region varied from 667 bp in *Pouteria sandwicensis* to 672 bp in *P. firma* within *Pouteria sensu lato* and from 606 bp in *Niemeyera deplanchei*, *N. francei*, *N. sessilifolia* and *N. whitei* to 626 bp in *Mimusops elengi* among the other taxa. The ITS region subunits comprised: ITS1, varying from 254 to 270 bp (G + C content 50–58%); 5.8S, invariably 164 bp (G + C content 50–55%); and ITS2, 188 to 238 bp (G + C content 54–60%). The aligned region included numerous insertions or deletions (indels) of 1 to 14 bp and contained 747 characters of which 338 were constant, 101 were variable but parsimony uninformative and 308 (41%) were parsimony informative. All ingroup and outgroup sequences could be aligned with confidence except for problematic areas in *Manilkara*, *Palaquium*, *Xantolis* and *Mimusops*. Exclusion of the problematic sites made no difference to the topology found in the analyses. Twelve potentially informative indels within ITS1 and ITS2 were scored as additional presence or absence characters following the method used by Cross *et al.* (2002) and included in the data analysis. The final data matrix is available from the first author. There was no significant base composition bias difference (non-stationarity) among taxa ($\chi^2 = 32.6042$, df = 216, $P = 1.00$). There was no evidence for multiple paralogues or pseudogenes following re-sequencing of ambiguous sequences and all species with replicated samples were monophyletic (Fig. 2).

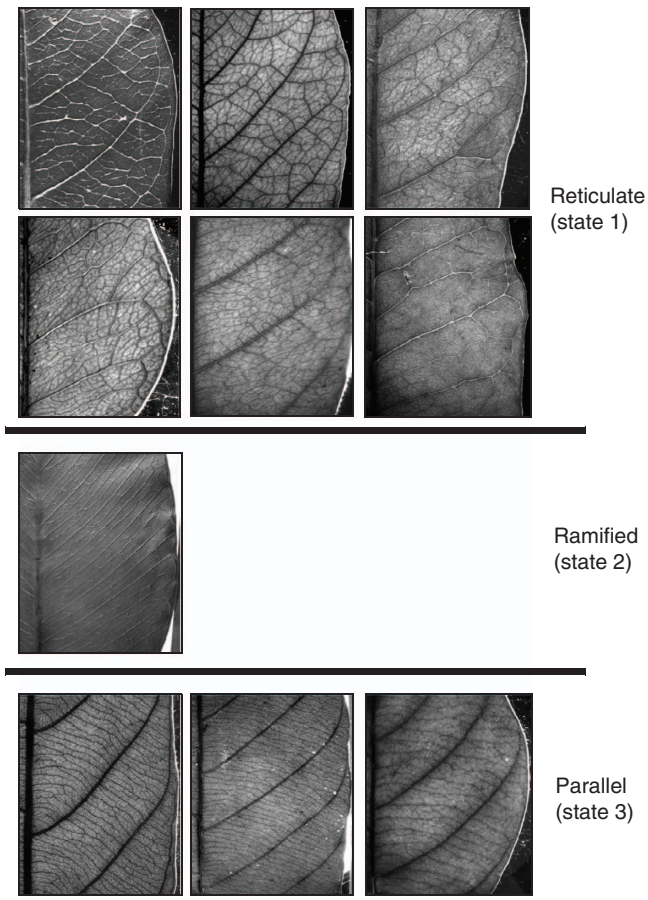

Fig. 1. Three tertiary leaf venation patterns: reticulate (state 1 in Table 1), ramified (state 2) and parallel (state 3). Terminology follows the definition of Hickey (1973), van Royen (1957) and Pennington (1990).

Phylogenetic analyses

The parsimony search found 2160 shortest trees at 875 steps with: consistency index (CI) excluding uninformative characters 0.62; homoplasy index (HI) excluding uninformative characters 0.38; retention index (RI) 0.79; and rescaled consistency index (RC) 0.49. The strict maximum parsimony consensus (not shown) scarcely differed from the Bayesian consensus and therefore we present only the latter (Figs 2, 3), with the results of the BS and JN parsimony analyses mapped onto Fig. 2.

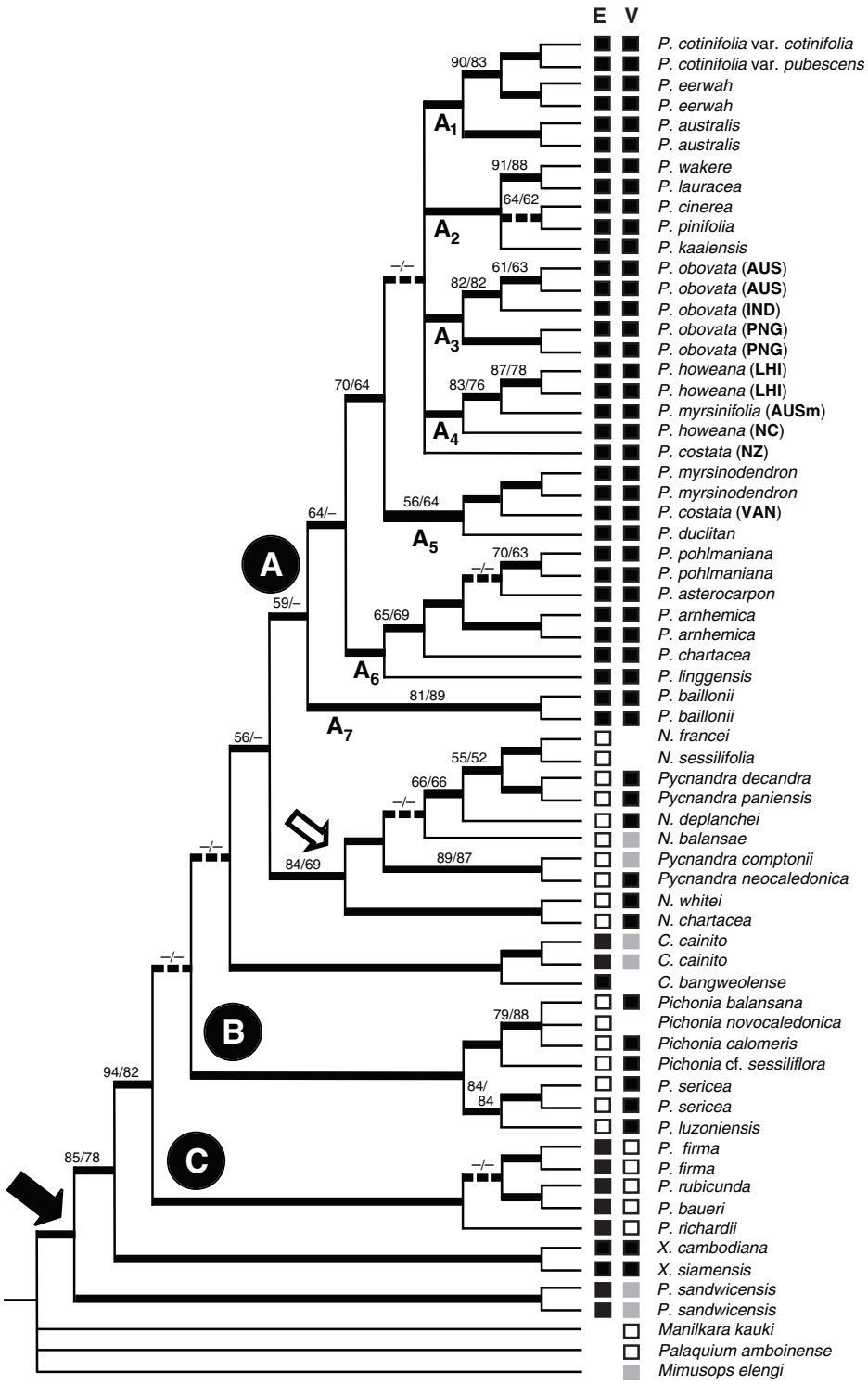

Fig. 2. A 50% majority rule Bayesian consensus tree based on the analysis of sequence data from the internal transcribed spacer region (ITS) nrDNA. Posterior probabilities above 90 are represented by a thick line and values between 50 and 90 by a broken line. Parsimony bootstrap/jackknife support values <90% are given above each line and a dash indicates values <50%. The black arrow indicates tribe Chrysophylleae *sensu* Pennington (1991) and the white arrow indicates the *Pycnandra/Niemeyera* clade (see text). Abbreviations representing geographical origins of some taxa: Australia (AUS), Australian mainland (AUSm), Lord Howe Island (LHI), Indonesia (IND), New Caledonia (NC), New Zealand (NZ), Papua New Guinea (PNG) and Vanuatu (VAN). Distribution of two morphological characters among *Pouteria* species is mapped onto the tree, represented by shaded boxes. Endosperm (column E): black = present; white = absent; no box = not scored. Tertiary venation type (column V): black = reticulate (state 1 in Table 1); grey = ramified (state 2); white = parallel (state 3); no box = tertiary venation unknown. See text for references to clades labelled A to C.

At the conclusion of the Bayesian search, a 50% majority rule consensus was constructed from the 19 501 post-burn-in samples (Fig. 2) and is shown as a phylogram in Fig. 3 with branch lengths calculated by the model and the average sampled parameter values. This tree was more resolved than the maximum parsimony tree, which differed by the collapse of some unsupported nodes, e.g. the two consecutive dotted branches near the base of the backbone, the node uniting *Pouteria pohlmaniana* and *P. asterocarpon* and sister-group relationships between *P. firma* and *P. baueri* plus *P. rubicunda* (Fig. 2). In general, results from the three measures of clade support (BS, JN and PP) are correlated, although with some minor differences, for example four nodes along the backbone scored PP >90 but BS and JN ≤70 (Fig. 2).

Generic relationships

Two clades comprising *Xantolis* (*X. cambodiana* and *X. siamensis*) and *Chrysophyllum* (*C. cainito* and *C. bangweolense*) correspond to the genera proposed by Pennington (1991). However, other genera included in Pennington's family account, such as *Niemeyera* and *Pycnandra*, do not appear to be monophyletic (clade marked with white arrow in Fig. 2). Genus *Pouteria sensu* Pennington does not form a monophyletic group and instead four distinct lineages were recovered. These were clades A and C; *P. sericea* plus *P. luzonensis* within clade B; and *P. sandwicensis* in clade D (Fig. 2). All these formed a monophyletic group with the other genera of the tribe Chrysophylleae *sensu* Pennington (marked with black arrow in Fig. 2).

Clade A (Fig. 2) received mixed support (PP >90, BS 59, JN <50). All the species in this clade were formerly classified under genus *Planchonella sensu* van Royen (1957) and Aubréville (1967); the only other species included in our sample that were previously regarded as *Planchonella* fell in clade C. Clade A also included *P. wakere*, which was previously classified under *Pouteria sensu* Hermann-Erlee and van Royen (1957).

Within clade A, three Australian species (*P. cotinifolia*, *P. eerwah* and *P. australis*) grouped together (clade A_1) with fairly strong support (PP >90, BS 90, JN 83) and the five New Caledonian species formed a strongly supported group (clade A_2, PP >90, BS 97, JN 97). The populations of the widely distributed *Pouteria obovata* from Indonesia, Australia and New Guinea grouped in clade A_3 with strong support in all indices. Within this clade, the samples from Australia (marked AUS in Fig. 2) and Indonesia (marked IND) formed a sister group to *P. obovata* from Papua New Guinea (marked PNG). Two populations of *P. myrsinodendron* (formerly *P. obovoidea*) from Malesia and Australia formed a strongly supported group within clade A_5, with *P. costata* from Vanuatu and *P. duclitan* as its successive sister species.

Clade A_4, with strong support, comprises *P. howeana* (formerly classified as *P. myrsinoides* subsp. *reticulata*) from Lord Howe Island (marked LHI in Fig. 2), *P. myrsinifolia* (formerly classified as *P. myrsinoides* subsp. *myrsinoides*) from mainland Australia (marked AUSm in Fig. 2), and a representative of *P. howeana* (*P. myrsinoides* subsp. *reticulata)* from New Caledonia (marked NC in Fig. 2). The strong support for this resolution indicates that *P. howeana* from New Caledonia (NC) may be distinct from *P. howeana* of Lord Howe Island (LHI). However, these taxa are no more divergent than the samples taken within other species (Fig. 3).

Clade A_6 is another strongly supported group, comprising Australian taxa *P. pohlmaniana* var. *pohlmaniana* and var. *vestita*, *P. asterocarpon* (formerly classified as *P. pohlmaniana* var. *asterocarpon*), *P. arnhemica*, *P. chartacea* and *P. linggensis* from Papua New Guinea. Finally, *P. baillonii* from New Caledonia constitutes the sister group (clade A_7) to the rest of clade A.

Clade B, with strong support from all indices, comprises four species of *Pichonia*, together with two non-endospermous species formerly classified under *Pouteria* (*P. sericea* and *P. luzoniensis*) (Herrmann-Erlee and van Royen 1957). Except for *P. sandwicensis*, all the remaining *Pouteria* species sampled fell into the strongly supported clade C. These are endospermous species formerly classified under *Planchonella sensu* van Royen (1957): *P. rubicunda* and *P. baueri* from New Caledonia, *P. firma* from the Malay Peninsula and Indonesia and *P. richardii* from northern Australia.

Distribution of leaf venation patterns

Three leaf tertiary venation patterns (reticulate, ramified and parallel) occur among Malesian and Australasian *Pouteria* species. Most of the *Pouteria* species with reticulate tertiary leaf venation were nested in *Pouteria* clade A (Fig. 2). However, an exception occurred in which *Pouteria sericea* and *P. luzoniensis* var. *papuana* with reticulate tertiary leaf venation nested in clade B together with the four *Pichonia* species, which possess similarly reticulate venation. Meanwhile, all sampled *Pouteria* species with parallel tertiary leaf venation grouped in clade C, consisting of *P. rubicunda*, *P. baueri*, *P. firma* and *P. richardii*. The only *Pouteria* species with ramified leaf tertiary venation, *P. sandwicensis* (clade D), was placed as sister group to the rest of the tribe Chrysophylleae (Fig. 3).

Discussion

Are the tribe Chrysophylleae and genus Pouteria monophyletic?

Monophyly of the tribe Chrysophylleae is strongly supported by both the Bayesian and maximum parsimony estimates of phylogeny from ITS, and this accords with evidence from cpDNA (Anderberg and Swenson 2003). There is moderate to strong support from ITS for *P. sandwicensis* (clade D in Figs 2, 3) being the sister group to the rest of the tribe, although this relationship is not evident in a less resolved cpDNA dataset (Anderberg and Swenson 2003).

By contrast, there is no support in the present study for the monophyly of *Pouteria* as currently circumscribed (Fig. 3) by Baehni (1942), Pennington (1991) and Vink (2002). Our analyses have defined three distinct evolutionary lineages (clades A, B and C in Fig. 2) that include the Malesian and Australasian *Pouteria* species previously classified under either *Planchonella sensu* van Royen (1957) or *Pouteria sensu* Herrmann-Erlee and van Royen (1957). These clades also include ITS sequences from New Caledonian *Pouteria* (Bartish *et al*. 2005). Although support for nodes along the backbone of the tree is generally weak to absent (Fig. 2), some *Pouteria* lineages are separated from the others by

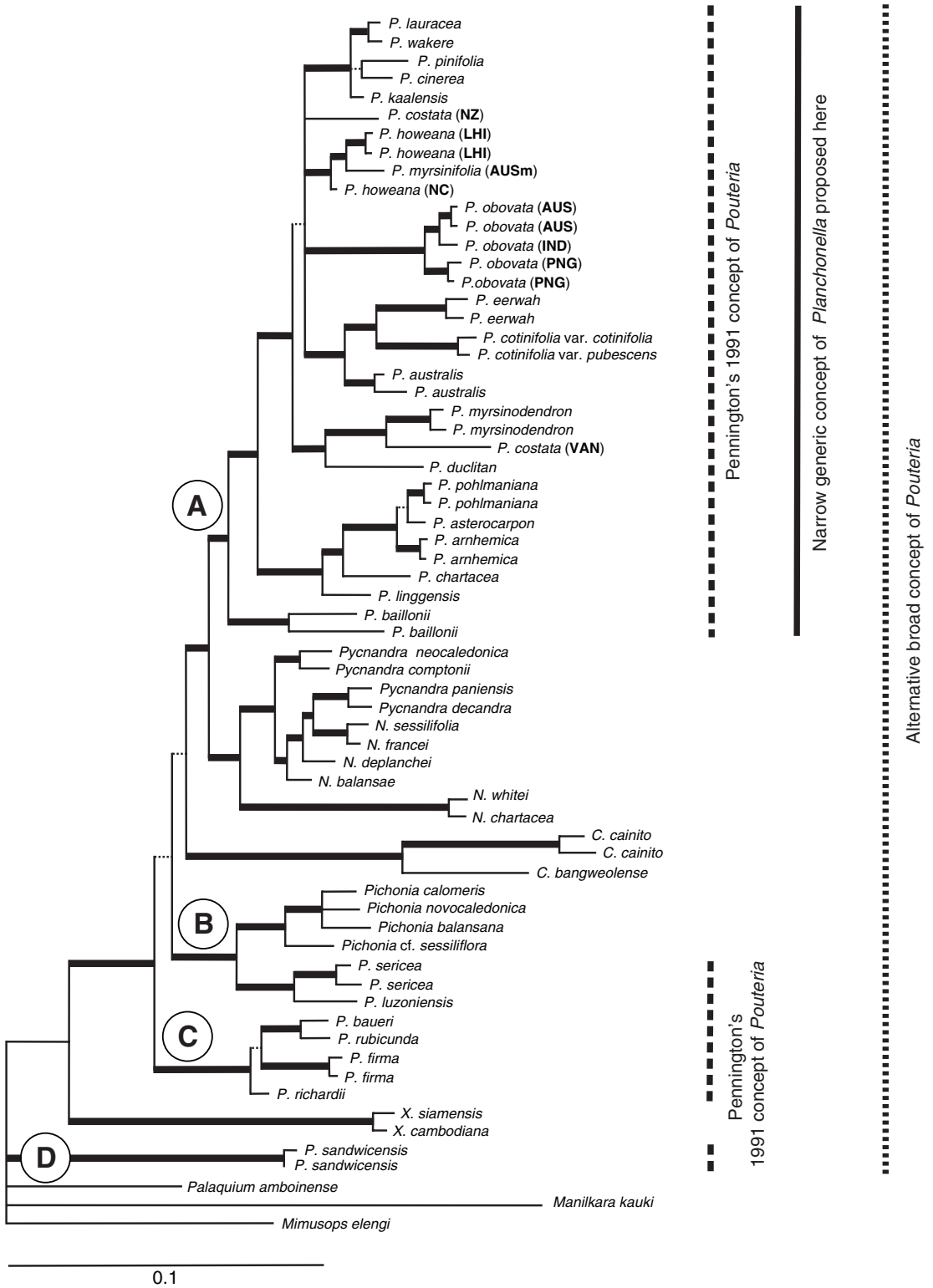

Fig. 3. A phylogram of the tree shown in Fig. 2, with line styles of branches and geographical abbreviations with the same meaning as in Fig. 2. The broken vertical line represents the generic concept of *Pouteria sensu* Pennington (1991), the continuous line represents the narrow generic concept proposed here for a reinstated *Planchonella* and the dotted line represents an alternative broader concept in which *Pouteria* is equivalent to the whole tribe Chrysophylleae. See text for references to clades labelled A to D. The scale bar represents branch length in substitutions per site.

strongly supported nodes, e.g. *P. sandwicensis*, *P. sericea* and *P. luzonensis*. This result broadly agrees with those of previous molecular phylogenetic studies on New Caledonian Sapotaceae (Bartish *et al*. 2005), and on the whole family (Anderberg and Swenson 2003; Swenson and Anderberg 2005). Therefore, it appears unlikely that *Pouteria* in the broad sense of Pennington (1991) is monophyletic and distinct from the other genera of Chrysophylleae (*Niemeyera*, *Pycnandra*, *Chrysophyllum*, *Pichonia* and *Xantolis*). Additionally, our ITS phylogeny does not support the narrow concept of *Pouteria*, resulting from the segregation of species with endosperm into the genus *Planchonella* (Lam 1927; van Royen 1957; Herrmann-Erlee and van Royen 1957; Aubréville 1964; Baehni 1965). Under this classification, neither *Planchonella* (clades A and C) nor *Pouteria* (part of clade B and *P. sandwicensis* in clade D) is monophyletic.

The distribution of leaf tertiary venation types

Van Royen (1957), Herrmann-Erlee and van Royen (1957) and Pennington (1990, 1991) established the importance of the leaf tertiary venation type in *Pouteria* taxonomy. In the present study, we re-assessed the diagnostic value of the leaf tertiary venation character by examining its distribution compared with the ITS phylogeny. All *Pouteria* species with reticulate tertiary venation are nested in clade A, with the exception of *Pouteria sericea* and *Pouteria luzoniensis* var. *papuana* in clade B. The species nested in clade A correspond to the endospermous species of the former *Planchonella sensu* van Royen (1957). However, van Royen's concept of *Planchonella* also included a group of species with parallel tertiary leaf venation pattern that, according to ITS, are excluded from group A and constitute clade C. van Royen's concept of *Planchonella* also included *P. sandwicensis* from Hawaii (clade D) with ramified tertiary leaf venation, which is a sister group to the rest of the tribe Chrysophylleae (Fig. 3). In summary, with the exception of *Pouteria sericea* and *Pouteria luzoniensis* var. *papuana*, the species of Malesian and Australasian *Pouteria* are separated into two groups on the basis of the reticulate (clade A) and parallel (clade C) tertiary leaf venation possessed by its member species. Thus, leaf tertiary venation patterns are of phylogenetic significance and should be used as a major character in the taxonomy of Malesian and Australasian *Pouteria.*

A narrowed generic delimitation

The concept of *Pouteria* could be narrowed by reinstating the genus *Planchonella* to correspond with clade A, which comprises those *Pouteria sensu* Pennington species that have reticulate tertiary leaf venation and endosperm (Figs 2, 3). Thus, the new concept of *Planchonella* would exclude species with ramified tertiary venation (*P. sandwicensis* in clade D) and species with parallel tertiary leaf venation pattern (clade C). A difficulty with this circumscription is that BS and JN support for clade A is not strong (Fig. 2) and further testing of the monophyly of this clade is needed.

Clade B is strongly supported and includes two species of *Pouteria* (*P. sericea* and *P. luzoniensis* var. *papuana*) that are sister group to four species from genus *Pichonia sensu* Pennington (1991): *Pichonia* cf. *sessiliflora* from Solomon Islands and *P. balansana, P. calomeris* and *P. novocaledonica* from New Caledonia. Pennington (1991) and Govaerts *et al*. (2001) suggested a close relationship between these two genera, and that *Pichonia* is a possible section within *Pouteria*. We suggest instead that both of these taxa, *Pouteria sericea* and *P. luzoniensis* var. *papuana*, be placed into *Pichonia* on the basis of the ITS result and shared morphologies such as: short pedicel (inflorescence almost sessile); staminodes alternating with stamens at the same level; and single-seeded fruits lacking endosperm. This combination of characters provides strong support for the recognition of *Pichonia* as a distinct genus (Bartish *et al*. 2005).

Clade C is strongly supported and comprises *P. rubicunda* and *P. baueri* from New Caledonia, *P. firma* from the Malay Peninsula and Indonesia and *P. richardii* from northern Australia. These taxa share parallel tertiary leaf venation; spathulate, obovate to oblanceolate leaves, and a higher floral number per inflorescence compared with other species within *Pouteria*. Van Royen (1957) separated these taxa as a distinct group in genus *Planchonella* but they are not monophyletic with the concept of the genus that we propose here. This clade groups with *Pichonia* in the ITS analysis of Bartish *et al*. (2005) but only weakly (JN 52). Therefore, further study, potentially using a different gene, is required to determine the relationship of this group compared with other taxa of Chrysophylleae.

Infrageneric relationships

Our result also calls into question the sectional division of *Pouteria* species in the system by Pennington, which classified all Malesian and Australasian (including New Caledonian) species into two sections, Oligotheca and Pierrisideroxylon. His section Oligotheca includes all species in clades A and C, and *P. sericea* (clade B), whereas his section Pierrisideroxylon includes *P. luzoniensis* (clade B) and nine others not sampled here. Three additional Malesian and Australasian *Pouteria* species, *P. asterocarpon*, *P. chartacea* and *P. duclitan*, which were not assigned to any of Pennington's nine *Pouteria* sections, are also in clade A. These results imply that the circumscription of sectional classification for the Malesian and Australasian *Pouteria* species needs revision, pending a new generic classification.

Some of the smaller clades in Fig. 2 show interesting geographic and/or taxonomic patterns. The endemic Australian species *P. cotinifolia*, *P. eerwah* and *P. australis* form a distinct clade (A_1). This accords with van Royen's prediction in his circular diagram (van Royen 1957, fig. 1). Within clade A_1, *P. cotinifolia* var. *pubescens* and *P. cotinifolia* var. *cotinifolia* formed a strongly supported natural group. These two varieties have overlapping geographic distributions and are morphologically distinguished by the presence or absence of indumentum on mature leaves, pedicels, sepals and ovary (van Royen 1957). More samples for sequencing of both varieties are needed to assess the differentiation of the two taxa and whether they should be treated as a single species.

The second interesting result occurs in clade A_2 (Fig. 2). The species *P. wakere* formerly classified under 'non-endospermous' *Pouteria sens*. van Royen, is nested, with strong support, with endospermous species *P. lauracea*, *P. cinerea*, *P. pinifolia* and *P. kaalensis* (Fig. 2). This result indicates that the presence of membranous endosperm of *P. wakere* (as noted by van Royen in his 1957 monograph, p. 478) should be considered an important

feature to distinguish this particular species from other 'non-endospermous' taxa. Therefore, the presence and absence of endosperm alone does not provide clear separation of the genera *Planchonella* and *Pouteria* and conflicts with the suggestion that the presence *v.* absence of endosperm and exserted *v.* included radicle, could be used to differentiate between these genera (Bartish *et al*. 2005; Swenson and Anderberg 2005).

The widely distributed *Pouteria obovata* is strongly supported as monophyletic (clade A_3), with populations from Australia (AUS) and Indonesia (IND) in one subgroup and those from Papua New Guinea (PNG) in the other. The two subgroups can be distinguished by the presence of indumentum on the leaf surface of the Australian and Indonesian samples and its absence in samples from Papua New Guinea. We propose to recognise two infraspecific taxa on the basis of this character and the geographical isolation of New Guinea from the rest of the regions.

Clade A_4 shows strong support for the monophyly of the *P. myrsinifolia* group and its internal structure raises questions about past taxonomy of the group. Green (1990) concluded that the populations from Lord Howe Island (LHI) and those from New Caledonia (NC) belong to the same subspecies, *P. myrsinoides* subsp. *reticulata*. Jessup (2001) questioned this taxonomy and proposed use of the name *P. howeana* for these populations until further studies resolved their status. Our results indicate that the populations from New Caledonia (NC) and Lord Howe Island (LHI) are paraphyletic (Fig. 2) because *P. myrsinifolia* (F.Muell.) Jessup, from the Australian mainland (marked AUSm in Fig. 2), is sister taxon to the Lord Howe Island (LHI) population. Because these three populations have previously been recognised as the same species, *Pouteria myrsinoides* (Baehni 1942, van Royen 1957), we propose to undertake further work involving morphological comparisons to resolve their relationships and status.

Another issue of species-level paraphyly involves *P. costata* from Vanuatu (marked VAN in clade A_5, Fig. 2) and *P. costata* (formerly *P. novozelandica*) from New Zealand (marked NZ in Clade A_4, Fig. 2). These populations had been recognised as separate species before several authors (van Royen 1957; Pennington 1991; Govaerts *et al*. 2001) merged them into the single species *P. costata*. Our results clearly indicate that the New Zealand (NZ) population should remain distinct from that from Vanuatu (VAN) with these populations falling into separate, well-supported clades.

Overall, interspecific relationships in clade A partly correspond to the relationships illustrated in van Royen's diagram (van Royen 1957, fig. 1): *P. cotinifolia*, *P. eerwah* and *P. australis* form a monophyletic group; *P. lauracea* and *P. cinerea* are closely related; and *P. arnhemica* is closely related to *P. pohlmaniana*, *P. chartacea* and *P. linggensis* (although according to van Royen, these taxa belong to different sections of the same group).

Sampling constraints

The sampling limitations of this study should be borne in mind. The estimate of phylogeny is based on a single locus (ITS). Other loci could produce conflicting patterns (Maddison 1997); however, our phylogeny is consistent with one derived from cpDNA (Anderberg and Swenson 2003) and is more resolved. Several problems have been found in using ITS for estimating phylogenies, such as multiple loci and/or tandem copies, lineage sorting, pseudogenes and recombination among copies (e.g. Buckler *et al*. 1997; Alvarez and Wendel 2003), but these may not differ qualitatively from other nuclear DNA regions that are not yet as well characterised, e.g. *rpb2* (Pfeil *et al*. 2004) and cinnamoyl CoA reductase (Poke *et al*. 2006). Also, we have sampled a minority of species from the Malesian and Australasian region (Table 1). Nevertheless, our sampling scheme should be representative of the study group in this region because we targeted the known infrageneric taxa and morphological variants. On a global scale, the lack of sampling from the neotropics and Africa limits the generality of the conclusions from this study. We propose to fill these sampling gaps in future work but, given these caveats, the taxonomic changes proposed here should be considered provisional.

Conclusions

The present study shows that the ITS region is informative for inferring phylogeny and specific classification of the genus *Pouteria* in Malesia and Australasia. This conclusion parallels that of Bartish *et al*. (2005) for New Caledonian Sapotaceae.

Pouteria sensu Pennington (1991) in Malesia and Australasia is not monophyletic according to our ITS phylogeny and therefore this circumscription for the genus (broken line, Fig. 3) is not supported. Instead, the species fall into three separate clades (A, B and C) in Fig. 2. One taxonomic solution would be to narrow generic concepts, treating each of these clades as a genus. Clade A would be a narrowed concept of *Planchonella*, clade B would be an expanded concept of *Pichonia* and clade C could be described as a new genus but requires additional supporting evidence. *Pouteria sandwicensis*, a Hawaiian species (in clade D), should be separated at genus level from these Malesian–Australian taxa, being distinguished by ramified venation and its placement by ITS as sister taxon to the rest of Chrysophylleae.

Alternatively, the ITS phylogeny could be viewed as supporting a broad generic circumscription of the genus *Pouteria*, by extending the concept by Pennington (1991) to include such genera as *Niemeyera*, *Pycnandra*, *Chrysophyllum*, *Pichonia* and *Xantolis* (thick line in Fig. 3). However, this would challenge long-held generic delimitations in the family and would effectively reduce the tribe Chrysophylleae to a single large genus. Also, this would be inconsistent with the classification of the sister group, which includes three tribes and several genera (Anderberg and Swenson 2003). Moreover, the type of *Pouteria* is a species from the neotropics, which was not sampled for this study. Therefore further supporting evidence, both from additional genes and from sampling of neotropical and African species, is needed before generic circumscriptions should be changed in the Chrysophylleae.

In addition to the main conclusion above, our analysis of ITS affinities (1) suggests the separation of *P. obovata* into two varieties that differ in the presence or absence of leaf indumentum; (2) is in accord with separating the paraphyletic species *Pouteria myrsinoides* subsp. *reticulata* into two subspecies *Pouteria myrsinoides* subsp. *reticulata* and *Pouteria myrsinoides* subsp. *howeana*; (3) indicates the need

for additional study of *Pouteria costata* which is shown to be paraphyletic; and (4) resolves a group of taxa within *Pouteria* with parallel tertiary venation (clade C in Fig. 2).

Finally, this study confirms that leaf tertiary venation patterns are of taxonomic importance when considered in the light of molecular phylogenies of *Pouteria sensu lato*.

Acknowledgements

This work was part of the first author's Ph.D. project and it was supported by the Ebbe Nielsen Scholarship provided by Australian Biological Resources Study (ABRS), The Australian National University International Postgraduate by Research Tuition Scholarship, and CSIRO – Plant Industry; the first author also received a research grant from the Systematics Association and the Linnaean Society, United Kingdom for the field work. We thank the following institutions and persons for their assistance in providing plant materials: The Australian National Botanic Gardens and Booderee Botanic Garden, The Adelaide Botanic Garden and Mt Lofty Botanic Garden, The Royal Botanic Garden Sydney and Mt Annan Botanic Garden, The Australian National Herbarium (CANB), Lyn Craven, Andrew Slee, and David Jones from the Centre for Plant Biodiversity Research (CPBR), CSIRO – Plant Industry; Bogor Botanic Garden, Purwodadi Botanic Garden, Herbarium Bogoriense (BO), West Bali National Park, Wana Riset Samboja (East Kalimantan), and Forestry Research Division Manokwari (West Papua), Indonesia; Dr Saw Leng Guan (FRIM, Malaysia) and Dr Edwino S. Fernando (Los Banos, Philippines). Our further thanks to Randy Bayer, Ed Biffin, Curt Brubaker, Mark Clements, Ed Cross, Marlien van der Merwe, Ish Sharma, Matt Unwin and many other colleagues from the Centre for Plant Biodiversity Research (CPBR), CSIRO – Plant Industry, and to Lyn Cook from the School of Botany and Zoology, the Australian National University for invaluable advice and support. Thanks also go to the reviewers for the helpful comments on earlier versions of this paper.

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Manuscript received 13 April 2006, accepted 20 February 2007