

Evolution of *Polyscias* sect. *Tieghemopanax* (Araliaceae) based on nuclear and chloroplast DNA sequence data

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ABSTRACT

Polyscias sect. *Tieghemopanax* comprises approximately 26 species almost entirely endemic to New Caledonia. Three independent molecular markers were used to infer phylogenetic relationships and evolutionary patterns within the *Tieghemopanax* group, and to evaluate the status of a single highly variable species, *P. dioica*. Sequence data from nuclear ribosomal ITS and 5S spacers along with intron and spacer sequences from the plastid *trnL-trnF* region were analyzed separately and in each possible combination of the three data sets. Combined analyses provided topologies largely congruent with each of the separate analyses but with increased resolution, higher bootstrap support, and decreased computational time. The resulting cladograms confirm the monophyly of section *Tieghemopanax* and suggest an allopatric or adaptive divergence model of speciation in response to geography, elevation, and/or substrate type for most species. In contrast, *P. dioica* may represent an assemblage of once distinct but closely related species whose boundaries have recently broken down through hybridization and introgression. The results suggest that the *Tieghemopanax* group originated and diversified on New Caledonia (23 spp.); subsequent long-distance dispersal to other archipelagos resulted in the evolution of three local endemics (1 sp. each in Vanuatu, Fiji, and Australasia) and the widespread distribution of *P. cissodendron* in the SW Pacific.

KEY WORDS

speciation,
Araliaceae,
biogeography,
New Caledonia,
Polyscias,
Tieghemopanax.

RÉSUMÉ

Evolution de Polyscias sect. Tieghemopanax (Araliaceae) à partir de l'analyse de l'ADN nucléaire et chloroplastique.

Polyscias sect. *Tieghemopanax* renferme environ 26 espèces presque toutes endémiques de Nouvelle-Calédonie. Trois marqueurs moléculaires indépendants ont été utilisés pour déduire les affinités et les modèles d'évolution au sein du groupe *Tieghemopanax*, et pour évaluer le statut d'une espèce particulièrement variable, *P. dioica*. Les séquences ITS et espaceur 5S de l'ADN ribosomique nucléaire ainsi que les séquences de l'intron et de l'espaceur de la région *trnL-trnF* de l'ADN chloroplastique ont été analysées séparément et dans chacune des combinaisons possibles de ces trois ensembles de données. Les topologies obtenues à partir des analyses combinées s'avèrent largement congruentes avec celles de chacune des analyses individuelles mais avec une meilleure résolution, des valeurs de bootstrap plus élevées et un temps de calcul moindre. Les cladogrammes qui en résultent confirment la monophylie de la section *Tieghemopanax* et suggèrent un modèle de spéciation allopatrique ou d'adaptation divergente, en liaison avec la géographie, l'altitude, et/ou le type de substrat, pour la plupart des espèces. Au contraire, *P. dioica* représenterait un ensemble d'espèces proches autrefois distinctes entre lesquelles les barrières ont récemment disparu par hybridation et introgression. Les résultats obtenus suggèrent que le groupe *Tieghemopanax* est originaire de Nouvelle-Calédonie où il s'est diversifié (23 spp.) et qu'une dispersion ultérieure à longue distance vers d'autres archipels a permis l'évolution de trois endémiques locaux (une espèce au Vanuatu, une à Fiji et une en Australasie) et d'une autre espèce (*P. cissodendron*) largement répandue dans le SW Pacifique.

MOTS CLÉS

spéciation,
Araliaceae,
biogéographie,
Nouvelle-Calédonie,
Polyscias,
Tieghemopanax.

INTRODUCTION

Araliaceae (the ginseng family) comprise about 50 genera and approximately 1,200-1,400 species of vascular plants (MABBERLEY 1997; PLUNKETT et al. 2001). Within this family, *Polyscias* J.R. Forst. & G. Forst. (c. 130 spp.) and *Schefflera* J.R. Forst. & G. Forst. (about 650 spp.) are the two largest genera. In contrast to the pantropical distribution of *Schefflera*, *Polyscias* (as currently circumscribed) is restricted to the Old World tropics, from Africa across the Indian Ocean to Malesia and Australia, and thence across the islands of the south Pacific as far east as Tahiti (BERNARDI 1971; PHILIPSON 1979; LOWRY 1989). BERNARDI (1979) hypothesized that Malesia may have been the cradle of araliad origin, and noted that New Caledonia has more species per unit area than any other region on earth — over 90 species in eight genera (LOWRY unpubl.). *Polyscias* is well represented on

this southwest Pacific island, with 23 indigenous species (all but one endemic) and four cultivated species of Indo-Malesian origin (LOWRY in prep.). The native species form a morphologically coherent group (BERNARDI 1979), including trees or shrubs with odd-pinnate leaves that lack sheathing petioles, and leaflets that are opposite, sessile to subsessile, entire or toothed, and pinnately veined. Their flowers have (4-)5 petals, (4-)5 stamens, (4-)5 anthers, 2-locular ovaries, 2 styles (free or partially connate), and 2 carpels; they are usually arranged in panicles of umbellules, racemules or heads, or in compound dichasia. These species have mostly an andromonoecious (or less commonly dioecious) mating system (SCHLESSMAN et al. 1990, 2001) and their fruits are fleshy drupes that are strongly compressed laterally (HUTCHINSON 1967; LOWRY 1989). The uniformity of the indigenous New Caledonian species of *Polyscias* led some authors to recognize

them as a distinct genus, *Tieghemopanax* R. Vig. (VIGUIER 1905; GUILLAUMIN 1948), which also includes several members in Fiji (SMITH & STONE 1968; SMITH 1985), as well as Vanuatu (formerly the New Hebrides) and a number of other south Pacific islands (LOWRY et al. 1986; LOWRY 1989). More recently, however, *Tieghemopanax* has been treated as an informal group or section within a more broadly defined *Polyscias* (SMITH & STONE 1968; PHILIPSON 1978, 1979; BERNARDI 1979; SMITH 1985; LOWRY 1989).

As originally defined, *Polyscias* included taxa with pinnately compound leaves, 4-5 styles and 4-5 carpels (FORSTER & FORSTER 1776), with *P. pinnata* J.R. Forst. & G. Forst. [= *P. scutellaria* (Burm. f.) Fosb.] as the type species (see SMITH & STONE 1968; LOWRY 1989). The original circumscription of *Tieghemopanax* and another genus, *Nothopanax* Miq., included pinnate leaved species having 2-3 carpellate ovaries (MIQUEL 1855, 1856; VIGUIER 1905). However, STONE (1965a,b) placed *Nothopanax* in synonymy under *Polyscias* and transferred several species of *Tieghemopanax* from Vanuatu to *Polyscias*, suggesting that the same might also be warranted for the New Caledonian members of the genus. More recently, species previously assigned to *Tieghemopanax* have likewise been treated in a broadly defined *Polyscias* following PHILIPSON'S (1978, 1979) expanded circumscription (e.g., LOWRY et al. 1986; LOWRY 1989). A recent phylogenetic study by PLUNKETT et al. (2001) suggested that this broadly defined *Polyscias* is paraphyletic. The monophyly of species traditionally assigned to *Tieghemopanax*, however, was affirmed. In light of this finding, it now seems opportune to explore in more detail questions regarding the evolution and diversification of species belonging to the *Tieghemopanax* group. Given its center of distribution in New Caledonia, where most species are endemic, *Tieghemopanax* represents a model for the study of diversification on an island with a particularly intriguing geological and botanical history.

The New Caledonian flora, with c. 3,250 native species of seed plants, exhibits remarkably high levels of endemism both at the level of species (c. 79%) and genus (~14% of the nearly

770 genera recorded) in an area of only about 17,000 km² (MORAT 1993; LOWRY 1998; MORAT et al. 2001). These include many relictual taxa representing ancient Australasian lineages present at the time New Caledonia separated from Australia c. 74 MY ago (KROENKE 1996), and others derived from more recent colonizations by long-distance dispersal. Evolution and speciation have been driven in part by the presence of highly selective ultramafic substrates covering nearly 1/3 of the island (JAFFRÉ 1976; MORAT et al. 1986; JAFFRÉ et al. 1987) and a remarkable diversity of bioclimatic and ecological conditions resulting in a wide range of vegetation formations (MORAT 1993; LOWRY 1998), including low elevation to montane rainforest (MORAT et al. 1984), dry sclerophyllous forests on calcareous substrates (JAFFRÉ et al. 1993; MORAT et al. 2001), and maquis vegetation (MORAT et al. 1986), a characteristic low, heath-like, sclerophyllous formation largely restricted to ultramafics. This unique combination of geological, climatological, and ecological features makes New Caledonia a "hotspot" for both paleo- and neoendemics, and presents an ideal context for studying evolutionary processes.

Based on evidence from molecular data (nuclear Internal Transcribed Spacer-ITS) in conjunction with present-day geographical distributions, PLUNKETT et al. (2001) speculated that an early member of section *Tieghemopanax* may have arrived in New Caledonia by long-distance dispersal from Australasia, and subsequently undergone broad radiation across the island. In addition to confirming the monophyly of those species of *Polyscias* traditionally assigned to *Tieghemopanax*, the ITS study suggested that the Australian endemic *P. sambucifolia* also belongs within the *Tieghemopanax* clade and that a second Australasian species, *P. elegans*, the sole member of sect. *Gelibia* (Hutch.) Philipson, may be sister to *Tieghemopanax*. These results pose interesting questions regarding the origin, dispersal, and radiation of section *Tieghemopanax* within New Caledonia. For example, did the two Australian species descend from the presumed Australasian progenitor species of *Tieghemopanax*, or do they represent secondary dispersals

back to Australia from New Caledonia? Similarly, are the New Caledonian species descendents of a single dispersal event from Australasia or multiple dispersals? And how might the biophysical features of New Caledonia (e.g., vegetation, climate, elevation, edaphic conditions) have contributed to speciation within the *Tieghemopanax* group after the initial dispersal(s)? Unfortunately, ITS data alone were not sufficient to resolve relationships within the section. Given these limitations, the present study has employed additional non-coding sequences from the intron and intergenic spacer of the plastid *trnL-trnF* region, and from the non-transcribed spacer of nuclear 5S rDNA. These markers were chosen because they accumulate mutations at a similar or more rapid rate than ITS. Altogether, three data sets (with comparable sampling) were assembled and analyzed, representing three independent (presumably unlinked) markers, three types of non-coding sequences (an intron, two transcribed spacers, and two non-transcribed spacers), from two inheritance units (nuclear and plastid DNA). The data sets were analyzed, both separately and in combination, in an effort to examine phylogenetic relationships within the *Tieghemopanax* group.

Circumscriptions at and below the genus level in most plant groups have in general been based on traditional taxonomic methods, relying largely on the comparison of morphological characters. These methods have proven useful in delimiting species within section *Tieghemopanax*, where numerous straightforward morphological, edaphic and/or geographical features are available to distinguish the 26 currently recognized taxa (some of which remain to be described; LOWRY 1989 and unpubl.). In applying this traditional concept to *Tieghemopanax*, however, it has been necessary to recognize one very broadly defined species, *P. dioica*, which exhibits an unusually high level of morphological variability and is distributed very widely within New Caledonia. As currently circumscribed, *P. dioica* includes several previously recognized species [e.g., *P. pulchella* (Baill.) Harms, *P. schlechteri* Harms, *P. simabae-folia* (R. Vig.) Harms, *P. suborbicularis* (Baill.) Harms, *Tieghemopanax nigrescens* Pancher ex R. Vig., and *T. viguieri* Däniker], but the corresponding morphological types often appear to

grade into one another and frequently co-occur at a give locality (LOWRY unpubl.). While the plants now placed in *P. dioica* share several features that readily allow them to be distinguished from other members of the *Tieghemopanax* group, it is nevertheless unclear whether they represent a single species with significant phenotypic variation or perhaps several distinct taxa. Resolution of this dilemma may be difficult in the absence of an explicit phylogenetic framework to assess relationships within the *Tieghemopanax* clade and the populations comprising *P. dioica*.

Apart from this single, highly variable species, the other members of section *Tieghemopanax* are morphologically coherent and apparently comprise a series of discrete, well-defined species pairs (or trios). Therefore, in using traditional taxonomic approaches we must apply two very different species definitions in section *Tieghemopanax*. This may belie two distinct modes of evolution and speciation in a single group of closely related taxa. In such cases, a robust phylogenetic hypothesis (especially one based on data independent of the morphological treatments, such as molecular data) can provide the needed framework for evaluating evolutionary patterns.

The distribution of section *Tieghemopanax*, centered on New Caledonia, provides an ideal opportunity to examine evolution on this intriguing island system, characterized by such extraordinary levels of both paleo- and neoendemism. To date, however, no detailed studies of this kind have been completed. Thus, we hope that the present paper will provide not merely a test of speciation patterns in *Polyscias*, but perhaps also a first step in evaluating evolutionary modes across many of the endemic plant groups of New Caledonia. The study reported here represents a preliminary analysis to explore further the evolutionary patterns found in New Caledonian *Polyscias*. Specifically, we have used molecular data to: (1) confirm the monophyly of section *Tieghemopanax*; (2) elucidate phylogenetic relationships among the species within the section; (3) infer evolutionary patterns (e.g., patterns of speciation, biogeography, and morphological character evolution) among these species; (4) assess the phylogenetic status of plants now assigned to the highly variable species *P. dioica*;

and (5) consider additional taxa that may provide further tests of evolutionary mechanisms in the New Caledonian flora.

MATERIALS AND METHODS

Newly derived data sets were collected from one nuclear marker (the 5S rDNA spacer) and one chloroplast marker (*trnL-trnF*). In addition, the previously published ITS data set of PLUNKETT et al. (2001) was expanded by the addition of seven new sequences. Leaf tissue was collected from 29 accessions (representing 19 of the 26 species of *Tieghemopanax*) and dried in silica gel or preserved in a CTAB/salt solution (see Table 1). Total DNAs were extracted using the CTAB method of DOYLE & DOYLE (1987) as modified by SOLTIS et al. (1991) or the DNeasy Plant Mini kit (QIAGEN Inc.). Oligonucleotide primers were obtained on the basis of previously published studies for ITS (WHITE et al. 1990; DOWNIE & KATZ-DOWNIE 1996; WEN & ZIMMER 1996), the 5S region (UDOVICIC et al. 1995) and *trnL-trnF* (TABERLET et al. 1991, although their primer "f" was modified as follows: 5'—AAC TGG TGA CAC GAG GAT TTT CAG—3').

For the *trnL-trnF* and ITS regions, each PCR reaction included 0.4 μ L of unquantified template DNA, 0.2 μ L *Taq* DNA polymerase (QIAGEN), 5 μ L 10X *Taq* buffer (supplied with enzyme), 2 μ L of 25 mM MgCl₂, 2 μ L DMSO, 1 μ L of a 5 μ M solution of each primer, 4 μ L of 10 mM dNTPs and ultrapurified water to a final volume of 50 μ L. Temperature-cycler parameters for the *trnL-trnF* regions were set at 94°C (1 min.), 49°C (1 min.), and 72°C (2 min.) for denaturing, annealing, and extension steps, respectively (35 cycles). For ITS, 40 cycles were used, each cycle including 94°C (30 sec.), 48°C (1 min.), and 68°C (1 min.). In amplifications of the 5S spacer, an identical PCR recipe was used except that 8 μ L of 10X buffer was added (rather than 5 μ L). In addition, temperature cycler parameters for the 5S amplifications were set at 93°C (10 sec.), 60°C (10 sec.), and 72°C (12 sec.) for denaturing, annealing, and extension steps, respectively (31 cycles). These modifications of

PCR parameters for 5S preferentially amplified a single repeat unit (rather than 2 or more units of this tandemly repeated gene and its spacer), thus eliminating the multiple-banding patterns observed in early PCR trials (see also UDOVICIC et al. 1995; HENEGARIU et al. 1997). PCR amplicons were purified using the QIAquick PCR cleanup kit or the agarose gel extraction kit (both QIAGEN Inc.). For the 5S spacer, sequences were obtained from complimentary strands using just two sequence reactions. For the *trnL-trnF* region, internal primers (*trnL-trnF* "d" and "e") were also necessary in most taxa to acquire complete sequences. Internal primers (ITS 3 and ITS 5.8S) were also necessary to obtain complete ITS sequences. Modified dideoxy-chain termination sequences (SANGER 1977) were performed using the BigDye Terminator cycle sequencing kit (PE Applied Biosystems) according to the manufacturer's instructions, followed by DyeEx Spin Kit (QIAGEN) purification and electrophoresis on an ABI Prism 377 automated sequencer. DNA sequence data were assembled and edited using the Sequencher (version 3.1) computer program (GeneCodes Corp.).

Despite efforts to eliminate multiple bands in 5S rDNA amplicons, several accessions of *Polyscias dioica* (e.g., Lowry 4771, 4800, 4804) and the single accessions of *P. elegans* (Plunkett 1495), *P. cissodendron* (Lowry 4749), and *P. pancheri* (Lowry 4793) yielded two or more amplification products. These bands did not express the typical multiple repeat pattern observed prior to optimization of PCR for 5S, suggesting the presence of two or more 5S spacer size variants in these samples. PCR products from two of these accessions, *P. dioica* (Lowry 4804) and *P. elegans* (Plunkett 1495), were separated in 2% TBE agarose, excised, and gel-cleaned. Each band was then cloned into the pCR2.1-TOPO vector using the TOPO TA Cloning kit (Invitrogen Corp.) and sequenced independently to determine sequence homology with 5S spacers from other species. The remaining accessions in the study, including all other *P. dioica* samples (Lowry 4647, 4712, 4713, 4781, and 5099), yielded single-banded amplicons in PCR reactions targeting the 5S spacer region, allowing for direct sequencing of the PCR products.

Table 1. — Species names for the DNA accessions used in the present study, with native range, source, and voucher information (for sequences derived from previously published studies, literature citations are provided). Names and combinations in quotation marks indicated as “ined.” will be published elsewhere. Herbarium acronyms follow Holmgren et al. (1990).

Taxon	Native range	Source and voucher/accession no.	GenBank accession
<i>Polyscias</i> sect. <i>Teghemopanax</i>			
<i>Polyscias balansae</i> (Baill.) Harms	New Caledonia	Mé Ori, New Caledonia (Lowry 4801, MO)	ITS: AF229689 5S IGS: AY035560 <i>trnL-trnF</i> : AF382165
<i>Polyscias</i> “bracteata” (R. Vlg.) Lowry, ined.	New Caledonia	Mt. Dzumac, New Caledonia (Lowry 4663, MO)	ITS: AF229692 5S IGS: AY035561 <i>trnL-trnF</i> : AF382166
<i>Polyscias</i> “calophylla” Lowry, ined.	New Caledonia	Tiéta, New Caledonia (Munzinger 341, P)	ITS: AF382939 <i>trnL-trnF</i> : AF382167
<i>Polyscias</i> “calophylla” Lowry, ined.		Plateau de Tiéa, New Caledonia (Lowry 5124, MO)	5S IGS: AY035562
<i>Polyscias cissodendron</i> (C. Moore & F. Muell.) Harms	New Caledonia, Santa Cruz, Vanuatu, Lord Howe Isl.	Mandjélla, New Caledonia (Lowry 4749, MO)	ITS: AF229693 5S IGS: AY035563 <i>trnL-trnF</i> : AF382168
<i>Polyscias</i> “crenata” (Pancher & Sebert) Lowry, ined.	New Caledonia	Baie de Tina, New Caledonia (Lowry 4664, MO)	ITS: AF229694 5S IGS: AY035564 <i>trnL-trnF</i> : AF382169
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms	New Caledonia	Mt. Dzumac, New Caledonia (Lowry 4647, MO)	ITS: AF229696 5S IGS: AY035567
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms		Mt. Mou, New Caledonia (Lowry 4712, MO)	ITS: AF382940 5S IGS: AY035568 <i>trnL-trnF</i> : AF382173
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms		Mt. Mou, New Caledonia (Lowry 4713, MO)	ITS: AF382941 5S IGS: AY035566 <i>trnL-trnF</i> : AF382170
<i>Polyscias</i> cf. <i>dioica</i> (Vieill. ex Pancher & Sebert) Harms		Roches Ouatième, New Caledonia (Lowry 4771, MO)	ITS: AF229709 5S IGS (PCR product only)
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms		Roches Ouatième, New Caledonia (Lowry 4781, MO)	ITS: AF382942 5S IGS: AY035569 <i>trnL-trnF</i> : AF382171
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms		Mé Ori, New Caledonia (Lowry 4800, MO)	5S IGS (PCR product only)
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms		Mé Ori, New Caledonia (Lowry 4804, MO)	<i>trnL-trnF</i> : AF382172
<i>Polyscias dioica</i> (Vieill. ex Pancher & Sebert) Harms		Plateau de Tiéa, New Caledonia (Lowry 5099, MO)	ITS: AF382943 5S IGS: AY035565

<i>Polyscias</i> "dzumacensis" Lowry, ined.	New Caledonia	Mt. Dzumac, New Caledonia (Lowry 4650, MO)	ITS: AF229697 5S IGS: AY035570 trnL-trnF: AF382174
<i>Polyscias</i> "jaffrei" Lowry, ined.	New Caledonia	Kopéto Massif, New Caledonia (Lowry 4740, MO)	ITS: AF229700 5S IGS: AY035571 trnL-trnF: AF382176
<i>Polyscias joskei</i> Gibbs	Fiji	Ovalau, Fiji (A.C. Smith 7584, US)	ITS: AF382944 5S IGS: AY035572 trnL-trnF: AF382177
<i>Polyscias</i> "lecardii" (R. Vlg.) Lowry, ined.	New Caledonia	Mandjéla, New Caledonia (Lowry 4754, MO)	ITS: AF229701 5S IGS: AY035573 trnL-trnF: AF382178
<i>Polyscias microbotrys</i> (Baill.) Harms	New Caledonia	Mé Ori, New Caledonia (Lowry 4802, MO)	ITS: AF229704 5S IGS: AY035574 trnL-trnF: AF382181
<i>Polyscias</i> "nitida" Lowry, ined.	New Caledonia	Sarraméa, New Caledonia (Lowry 4717A, MO)	ITS: AF382945 5S IGS: AY035575 trnL-trnF: AF382179
<i>Polyscias</i> "nothisii" Lowry, ined.	New Caledonia	Near Pouembout, New Caledonia (Lowry 4739, MO)	ITS: AF229707 5S IGS: AY035576 trnL-trnF: AF382180
<i>Polyscias pancheri</i> (Baill.) Harms	New Caledonia	Plaine des Lacs, New Caledonia (Lowry 4693, MO)	ITS: AF229710 5S IGS: AY035577 trnL-trnF: AF382182
<i>Polyscias</i> "regalis" Bernardi ex Lowry, ined.	New Caledonia	Plateau de Dogny, New Caledonia (Lowry 4720, MO)	ITS: AF229712 5S IGS: AY035578 trnL-trnF: AF382183
<i>Polyscias sambucifolia</i> (Sieb. ex DC.) Harms	Australia	Cultivated, Australian Natl. Bot. Garden (accession no.8900624, voucher Davies 1272, CBG)	ITS: AF229713 5S IGS: AY035579 trnL-trnF: AF382184
<i>Polyscias</i> "scopoliae" (Baill.) Lowry, ined.	New Caledonia	Mt. Dzumac, New Caledonia (Lowry 4638, MO)	ITS: AF229715 5S IGS: AY035580 trnL-trnF: AF382185
<i>Polyscias</i> "subincisa" (R. Vlg.) Lowry, ined.	New Caledonia	Kourmac to Tiébagni, New Caledonia (Munzinger 361, P)	5S IGS: AY035581 trnL-trnF: AF382186
<i>Polyscias</i> "subincisa" (R. Vlg.) Lowry, ined.	New Caledonia	Mandjéla, New Caledonia (Lowry 4750, MO)	ITS: AF229718
<i>Polyscias</i> "veillonii" Lowry, ined.	New Caledonia	Mt. Ouin, New Caledonia (Lowry 4658, MO)	ITS: AF229721 5S IGS: AY035582 trnL-trnF: AF382187

Table 1. (contd).

Other Polyscias					
<i>Polyscias</i> "abrahamiana" Lowry, ined.	Madagascar	Andasibe, Madagascar (Labat 3064, P)	ITS: AF229686		
<i>Polyscias</i> "abrahamiana" Lowry, ined.		Andasibe, Madagascar (Labat 3065, P)	5S IGS: AY035557 trnL-trnF: AF382148		
<i>Polyscias albersiana</i> Harms	Tanzania	W Usambara Mts., Tanzania (Lowry 5153, MO)	ITS: AF229687		
<i>Polyscias australiana</i> (F. Muell) Philipson	Australia (Queensland), New Guinea	Near Bellenden Ker Park, northern Queensland, Australia (Plunkett 1500, MO)	ITS: AF229688		
<i>Polyscias baretiana</i> Bernardi	Madagascar	Masoala Peninsula, Madagascar (Aridy 281, MO)	ITS: AF229690 5S IGS: AY035558 trnL-trnF: AF382149		
<i>Polyscias bellendenkerensis</i> (F.M. Bailey) Philipson	Australia (Queensland)	Mt. Bartle Frere, northern Queensland, Australia (Plunkett 1538, MO)	ITS: AF229691		
<i>Polyscias cumingiana</i> (C. Presl.) Fern.-Vill.	Malesia; widely cult.	Po River, Between Ponérihouen and Houaïlou, New Caledonia (Lowry 4788, MO)	ITS: AF229695		
<i>Polyscias elegans</i> (C. Moore & F. Muell.) Harms	Australia (Queensland), New Guinea	South Mission Beach, northern Queensland, Australia (Plunkett 1495, MO)	ITS: AF229698 trnL-trnF: AF382175		
<i>Polyscias fulva</i> (Hieron.) Harms	Tropical Africa	Bvumba Mts., Zimbabwe (Lowry 4806, MO)	ITS: AF229699 5S IGS: AY035559 trnL-trnF: AF382150		
<i>Polyscias fruticosa</i> (L.) Harms	Unknown, likely Melanesia; widely cult.	Mitchell & Wagstaff (1997)	ITS: U63191		
<i>Polyscias</i> cf. <i>guilfoylei</i> (W. Bull) L.H. Bailey	Unknown, likely Melanesia; widely cult.	Cultivated, Honolulu Botanical Garden (Plunkett 1357, WS)	ITS: AF229685		
<i>Polyscias macgillivrayi</i> (Seem.) Harms	Australia (Queensland), E Malesia, Solomon Isl., Micronesia	Cape Kimberly, northern Queensland, Australia (Plunkett 1536, MO)	ITS: AF229702		
<i>Polyscias mayottensis</i> Lowry, O. Pascal & Labat	Comoro Isl.	Saziley, Mayotte, Comoros Isl. (Labat 2935, MO)	ITS: AF229703		
<i>Polyscias mollis</i> (Benth.) Harms	Australia (Queensland)	Bellenden Ker Park, northern Queensland, Australia (Plunkett 1507, MO)	ITS: AF229705		
<i>Polyscias murrayi</i> (F. Muell.) Harms	Australia (Queensland)	Bellenden Ker Park, northern Queensland, Australia (Plunkett 1505, MO)	ITS: AF229706		
<i>Polyscias</i> "orientalis" Lowry, ined.	Madagascar	Ankiriandro, Madagascar (Schatz 3925, MO)	ITS: AF229708		
<i>Polyscias purpurea</i> C.T. White	Australia (Queensland)	Licuala State Forest Park, northern Queensland, Australia (Plunkett 1496, MO)	ITS: AF229711		
<i>Polyscias</i> "schatzi" Lowry, ined.	Madagascar	Ankiriandro, Madagascar (Schatz 3898, MO)	ITS: AF229714		
<i>Polyscias scutellaria</i> (Burm. f.) Fosberg	Unknown, likely Melanesia; widely cult.	Cultivated, Flecker Botanical Garden, Cairnes, Queensland, Australia (Plunkett 1491, MO)	ITS: AF229716		

<i>Polyscias sessiliflora</i> Marais	Réunion	Col de Bébour, Réunion (Lowry 4981, MO)	ITS: AF229717
<i>Polyscias tahitiensis</i> (Nadaud) Harms	Tahiti	Tahiti (Florence 12911, MO)	ITS: AF229719
<i>Polyscias tennantii</i> Bernardi	Madagascar	Andasibe, Madagascar (Labat 3074, P)	ITS: AF229720
Other Araliaceae			
<i>Apiopetalum velutinum</i> Baill.	New Caledonia	Mt. Mou, New Caledonia (Lowry 4700, MO)	ITS: AF229742
<i>Aralia nudicaulis</i> L.	North America	Wen & Zirmer (1996)	ITS: U41674 trnL-trnF: AF382157
<i>Arthrophyllum</i> "mackeei" Lowry, ined.	New Caledonia	Haute Yaté, New Caledonia (Lowry 4670, MO)	ITS: AF229736 trnL-trnF: AF382158
<i>Delarbrea paradoxo</i> Vieill. ssp. <i>paradoxa</i>	Malesia, Solomon Isl., Vanuatu, New Caledonia, Norfolk Isl.	Ponandou R., New Caledonia (Lowry 4766, MO)	ITS: AF229750
<i>Delarbrea paradoxo</i> Vieill. ssp. <i>paradoxa</i>	Réunion	Katrikoin, New Caledonia (Lowry 4791, MO)	trnL-trnF: AF382152
<i>Gastonia cutispunga</i> Lam.	Réunion	Cult., Univ. de la Réunion, Réunion (Lowry 4976, MO)	ITS: AF229722 trnL-trnF: AF382161
<i>Gastonia duplicata</i> Thouars ex Baill.	Madagascar	Masoala Peninsula, Madagascar (Aridy 299, MO)	trnL-trnF: AF382163
<i>Gastonia rodriguesiana</i> Marais	Rodrigues Isl.	Cultivated, Royal Botanic Garden Kew (acc. 662-86.06150)	ITS: AF229723 trnL-trnF: AF382162
<i>Gastonia spectabilis</i> (Harms) Philipson	New Guinea, Solomon Isl., Australia	Mt. Isley, Edmonton, Queensland, Australia (Plunkett 1537, MO)	trnL-trnF: AF382164
<i>Mackinlaya macrosciadea</i> (F. Muell.) F. Muell.	Australia (Queensland)	Cultivated, Huntington Botanical Garden San Marino, California (Plunkett 1365, WS)	ITS: AF229744
<i>Merysta balansae</i> Baill.	New Caledonia	Plateau de Dogny, New Caledonia (Lowry 4733, MO)	trnL-trnF: AF382160
<i>Merysta denhamii</i> Seem.	New Caledonia	Mé Ori, New Caledonia (Lowry 4793, MO)	ITS: AF229725
<i>Merysta "lecardii"</i> (R. Vig.) Lowry, ined.	New Caledonia	Rivière Bleue Park, New Caledonia (Lowry 4678, MO)	ITS: AF229724
<i>Merysta "pedunculata"</i> Lowry, ined.	New Caledonia	Rivière Bleue Park, New Caledonia (Lowry 4756, MO)	trnL-trnF: AF382159
<i>Merysta sinclairii</i> (Hook. f.) Seem.	New Zealand	Mitchell & Wagstaff (1997)	ITS: U63194
<i>Myodocarpus crassifolius</i> Dubard & R. Vig.	New Caledonia	Mt. Dzumac, New Caledonia (Lowry 4641, MO)	ITS: AF229751
<i>Myodocarpus crassifolius</i> Dubard & R. Vig.	North America	Mt. Mou, New Caledonia (Lowry 4704, MO)	trnL-trnF: AF382151
<i>Oplopanax horridus</i> (J. Smith) Miq.	Mesoamerica	Pacific Northwest, North America (Soule 3821, WS)	trnL-trnF: AF382155
<i>Oreopanax sanderianus</i> Hemsl.	Mesoamerica	Cultivated, Missouri Botanical Garden, no. 873066 (Plunkett 1343, WS)	trnL-trnF: AF382156
<i>Pseudosciadium balansae</i> Baill.	New Caledonia	Mt. Mou, New Caledonia (Lowry 4714, MO)	ITS: AF229760
<i>Schefflera actinophylla</i> (Endl.) Harms	Australasia	Cultivated, New York Botanical Garden (Plunkett 1316, WS)	trnL-trnF: AF382153
<i>Schefflera trevesioides</i> Harms	S. China, Viet Nam	Fan Si Pan Mtn., Vietnam (Lowry 4920, MO)	ITS: AF229732 trnL-trnF: AF382154

Sequence alignments were derived manually. Pairwise distances were calculated for each data set with PAUP* (vers. 4; D. SWOFFORD, Smithsonian Inst.) using the TAJIMA-NEI DNA distance algorithm (TAJIMA & NEI 1984). Treating gaps as missing data, the data sets were analyzed using PAUP* by maximum parsimony (MP) to infer phylogenetic relationships. Because our sampling for each marker did not completely overlap, separate analyses were first performed for each of the three data sets. For the ITS data, we sampled widely throughout Araliaceae, following the findings of PLUNKETT et al. (2001) and WEN et al. (2001) in choosing outgroups. An initial search using 100 replicates (saving no more than 100 trees per replicate) was conducted because preliminary searches yielded many tens of thousands of most parsimonious trees. The strict consensus tree based on these 100 replicates was used as a topological constraint (saving only those trees not agreeing with the constraint) for an additional 1,000 replicates, each of which was aborted if more than 1,000 trees were found. After finding no additional topologies of equal or shorter length, the shortest trees resulting from the first search were loaded as starting trees and swapped to completion saving no more than 10,000 trees. For both the *trnL-trnF* and 5S data sets, heuristic searches (each with 100 random addition replicates) were performed. Because the number of most parsimonious trees recovered was relatively low, it was unnecessary to limit the number of trees saved. Outgroup selection for these two data sets was based on results of the ITS analyses (herein, PLUNKETT et al. 2001, and WEN et al. 2001).

Previous studies (e.g., OLMSTEAD & SWEERE 1994; SOLTIS et al. 1998) have found that analyses of combined data sets can provide increased phylogenetic signal, resulting in both higher internal support (e.g., bootstrap percentages) and decreased computer run times. However, treatment of multiple data sets remains controversial (see DE QUEIROZ et al. 1995; CANNATELLA et al. 1998). Biological sources of incongruence (e.g., non-dichotomous patterns of diversification, lineage sorting of ancestral polymorphisms, and rate heterogeneity in sequence evolution) are among the potential problems that may produce mis-

leading results in phylogenetic analyses based on combined matrices. Conversely, such incongruence may also result from sampling error, either among taxa or among characters. There is little agreement in the literature regarding the best methods of testing for congruence (reviewed in JOHNSON & SOLTIS 1998), or even the basic criterion that should be tested, although most workers agree that a null hypothesis of congruence or data homogeneity must be explicitly rejected (e.g., BULL et al. 1993; MASON-GAMER & KELLOGG 1996; JOHNSON & SOLTIS 1998). To assess congruence, we constructed separate data sets for each of the three markers using an identical set of 24 taxa (to eliminate taxon sampling error). Each of these was analyzed separately and in a single combined analysis. Congruence was estimated using the incongruence test (I_{MF}) of MICKEVITCH & FARRIS (1981), calculated manually, and the partition homogeneity test of FARRIS et al. (1995) as implemented in PAUP*. Results from these tests (see below) suggest that the data sets are combinable. Therefore, all pairwise combinations were assembled, maximizing taxon sampling in each case. Combined data sets were analyzed in the same manner as the separate analyses of *trnL-trnF* and 5S.

Confidence in individual clades for all trees was estimated using bootstrap analyses (100 replicates, saving no more than 1,000 trees per replicate) (FELSENSTEIN 1985). Fitness indices and nucleotide compositions for each data set were assessed using MacClade (MADDISON & MADDISON 1992) and/or PAUP*. In addition, distance analyses based on TAJIMA-NEI estimations were used to determine relative levels of variation among a representative sample of sequences from the ITS, *trnL-trnF*, and 5S matrices.

RESULTS

Matrix comparisons

ITS sequence data represented the entire ITS1 and ITS2 non-coding regions and the intervening 5.8S coding region from 58 taxa. This matrix required approximately 41 alignment gaps and comprised an aligned length of 666 bp, 363 of which were constant, 109 variable in only one

Table 2. — Comparisons among the data sets and most parsimonious (MP) trees presented in this study and corresponding to Figs. 2-6. CI = consistency index, RI = retention index (CI calculated excluding uninformative characters).

	ITS + <i>trnL-trnF</i> + 5S spacer	ITS	<i>trnL-trnF</i>	5S spacer	ITS + <i>trnL-trnF</i>	ITS + 5S spacer	<i>trnL-trnF</i> + 5S spacer
Figure no.	2	3	4	5	6a	6b	6c
Number of taxa	24	58	40	26	33	26	24
Number of characters							
Total (incl. gaps)	1919	666	952	301	1618	967	1253
Constant	1666	363	840	199	1290	741	1123
Variable in only one taxon	149	109	56	51	149	133	68
Potentially informative	104	194	56	51	179	93	62
Nucleotide composition	n/a	22/32/29/18	34/19/18/30	26/23/30/21	n/a	n/a	n/a
Cladogram measures							
Number MP trees	1	>10,000	156	156	6	3	36
Length of MP trees	349	725	122	147	507	323	175
CI	0.666	0.518	0.938	0.744	0.647	0.682	0.765
RI	0.770	0.734	0.972	0.849	0.731	0.786	0.855

taxon, and 194 potentially informative (Table 2). The *trnL-trnF* data matrix (40 taxa) represented the entire length of both the *trnL* intron and the *trnL-trnF* intergenic spacer (plus flanking sequences from the adjacent coding regions). These sequences required approximately 27 alignment gaps, resulting in an aligned length of 952 bp, 840 of which were constant, 56 variable in only one taxon, and 56 potentially informative. Representing the entire non-transcribed spacer along with short flanking regions of the gene, the 5S rDNA matrix required approximately 17 gaps to align the matrix of 26 sequences. After alignment, these sequences were 301 bp long, containing 199 constant characters, 51 characters variable in only one taxon, and 51 characters potentially informative for parsimony. Table 2 also provides other metrics pertaining to the data sets and parsimony trees (i.e., lengths of the shortest trees, values of the consistency and retention indices, and nucleotide composition).

Rates of sequence evolution, based on TAJIMA-NEI pairwise distances, were greatest in the ITS data set, which contained the broadest sampling of araliad taxa outside of the *Tieghemopanax* group. Overall, the highest level of variation was between sequences of the outgroup taxa *Mackinlaya macrosciadea* and *Myodocarpus crassifolius* (38.2%). In the same data set, the greatest variation among species currently assigned to the

genus *Polyscias* was 10.4% (between *P. fruticosa* and *P. fulva*). Among the species of section *Tieghemopanax* this value was 6.3% (between *P. sambucifolia* and *P. joskei*). The lowest level of sequence variation among all taxa in the ITS data set was identity, found in two pairs of *P. dioica* samples. In the *trnL-trnF* data set, the greatest level of variation was 7.4% between sequences of *Myodocarpus crassifolius* and *Gastonia rodrigueziana*. Across *Polyscias*, the greatest variation was 1.4% between *P. fulva* and *P. joskei*; within section *Tieghemopanax*, this value was 1.3%, between *P. sambucifolia* and *P. joskei*. Several pairs of taxa both within and outside of section *Tieghemopanax* shared values of identity. In the 5S spacer data set, the greatest overall levels of variation were between *P. fulva* and *P. "bracteata"*¹ (19.9%), and within section *Tieghemopanax* between *P. sambucifolia* and *P. "bracteata"* (13.7%). Four species pairs shared values of identity. Comparing only taxa found across all three data sets (the same 24 taxa used for the congruence tests), ITS sequences and 5S spacer sequences have evolved on average at rates 6.2 and 16.4 times greater (respectively) than *trnL-trnF*. Although *trnL-trnF* provides few variable characters, they are highly consistent. For example, in

1. Names in quotation marks and indicated as "ined." in Table 1 will be published elsewhere.

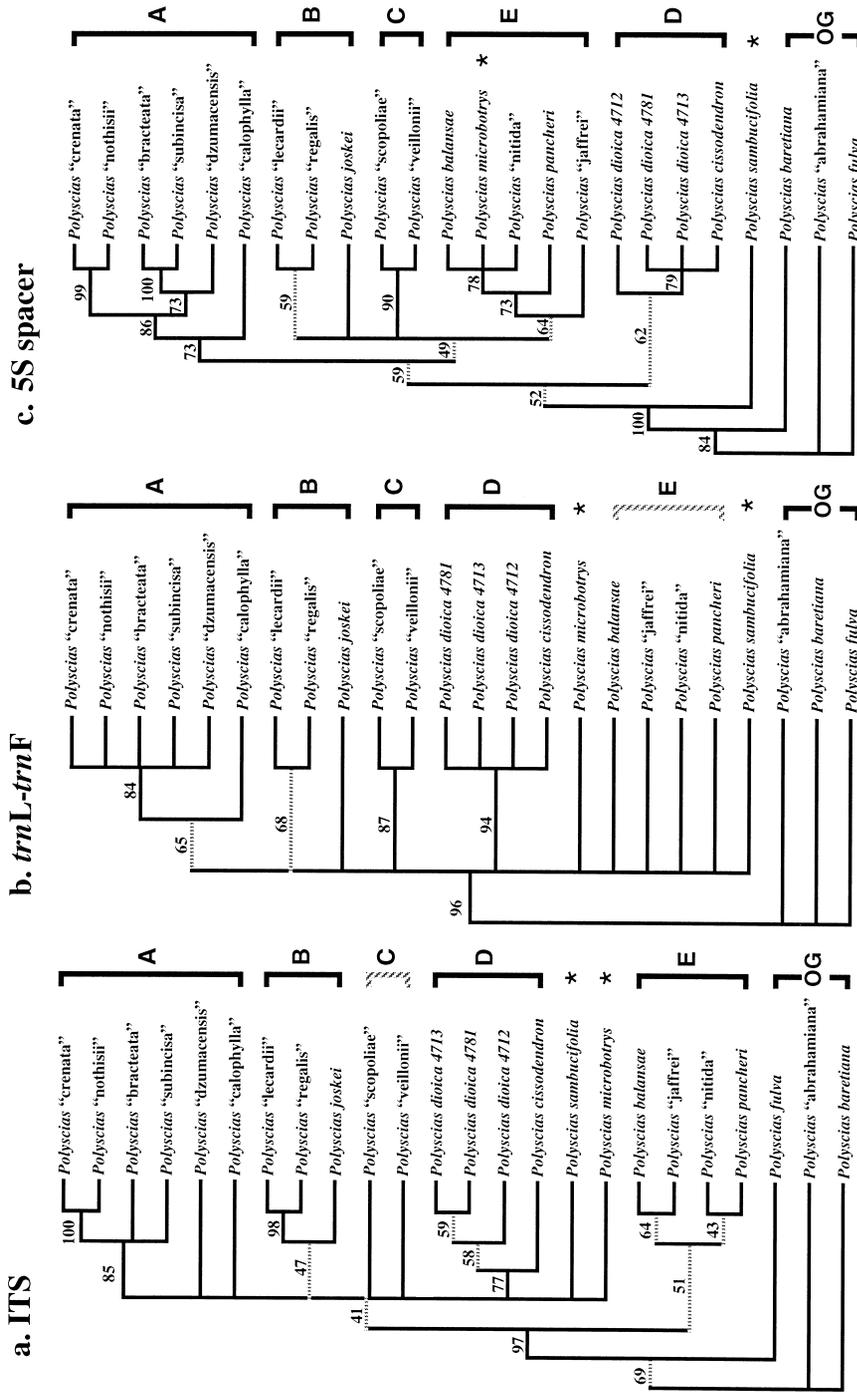


Fig. 1(a-c). — Strict consensus trees resulting from separate analyses of ITS, *trnL-trnF*, and 5S spacer data, each with an identical 24-taxon sampling. Values along nodes are bootstrap percentages; nodes with dashed branches have bootstraps below 70%. Clades consistently recovered in these trees (and in Fig. 2) are labeled A-E, although clade C in the ITS tree and clade E in the *trnL-trnF* were left unresolved (dashed brackets). Placement of *Polyscias sambucifolia* and *P. microbotrys* (discussed in text) are denoted by an asterisk. Outgroups indicated by “OG.” — (a) ITS analysis: strict consensus of 94 most parsimonious (MP) trees; tree length = 166 steps; consistency index = 0.600; retention index = 0.714. — (b) *trnL-trnF* analysis: strict consensus of 3 MP trees; tree length = 29 steps; consistency index = 0.923; retention index = 0.968. — (c) 5S-spacer analysis: strict consensus of 54 MP trees; tree length = 146 steps; consistency index = 0.556; retention index = 0.664.

the 24-taxon data set, there were only 12 parsimony informative *trnL-trnF* characters, but only one of these was homoplasious, yielding very high consistency values for the resulting trees (CI = 0.923, RI = 0.968). Thus, while at the lower limit of the phylogenetic utility of *trnL-trnF*, these data appear to make a significant contribution to the present study.

Congruence, resolution, and bootstrap support

Tests for congruence among the three 24-taxon data sets suggest that the data are homogeneous and therefore combinable. For the three-matrix comparison, the partition homogeneity test (FARRIS et al. 1995) yielded a result of $p = 0.22$ (only values of $p \leq 0.05$ indicate significant heterogeneity). In pairwise comparisons of *trnL-trnF* vs. ITS and *trnL-trnF* vs. 5S spacers, $p = 1$ (no heterogeneity). However, in the comparison of the ITS versus 5S spacer data, results suggested significant heterogeneity ($p = 0.04$). The incongruence test of MICKEVITCH & FARRIS (1981) also suggested no incongruence in pairwise comparisons of either nuclear set to the plastid data set ($I_{MF} = 0$ in both cases). Some incongruence was found between the two nuclear sets, but this was rather low ($I_{MF} = 12.7\%$).

Although evidence for incongruence is equivocal, we followed the protocol of BULL et al. (1993) and SEELANAN et al. (1997) in an attempt to investigate the sources of the possible incongruence between the two nuclear data sets. The most common biological source of incongruence in plant groups is reticulate evolution involving hybridization and/or polyploid speciation, resulting in the lateral transfer of genes or genomes. Our results do suggest that reticulation may explain at least in part the evolutionary origin of *Polyscias dioica*, a finding supported by the unusually broad morphological variability found in this species. Examination of all cladograms, however, indicates that possible reticulation involving *P. dioica* is not the source of incongruence in our data. In fact, all three separate analyses (Fig. 1) agree in resolving a single clade comprising all samples of *P. dioica* and the single sample of *P. cissodendron*. Apart from *P. dioica* (and *P. cissodendron*), evidence for reticulation in section *Tieghemopanax* is likewise scant.

We visually inspected the cladograms (Fig. 1) to identify other potentially problematic taxa in section *Tieghemopanax*. Only the placements of *P. microbotrys* and *P. sambucifolia* differed markedly from one analysis to another, but removal of these taxa did not improve the partition homogeneity score. Although combining the data sets based on the two nuclear markers may seem imprudent, results from this analysis indicate that incongruence between ITS and 5S spacers is likely due to sampling error rather than an underlying biological cause. Apart from poor resolution, examination of well-supported clades (e.g., bootstrap = 70%) reveals no disagreement among the separate trees (Fig. 1). When these data sets are combined, a single most parsimonious tree is recovered (identical in topology to Fig. 2) in which all of the well-supported clades are again resolved (with a single exception: a minor re-arrangement of terminal taxa in the clade labeled "A" in the 5S spacer tree; cf. Figs. 1c, 2). In all but three cases, bootstrap support for these same clades is higher in the combined tree than in the separate trees. Congruence between ITS and 5S spacer matrices is further supported by increases in two standard measures of homoplasy, the consistency (CI) and retention (RI) indices. For the separate (24-taxon) data sets, the CI and RI were (respectively) 0.6 and 0.714 for ITS, and 0.556 and 0.664 for the 5S spacers, but the combined (ITS + 5S spacer) analysis yielded higher values (CI = 0.644, RI = 0.745), indicating lower levels of homoplasy. Overall, these results suggest that the phylogenetic signal present in each of the separate data sets is additive when combined, effectively reducing levels of random homoplasy or "background noise" (see CHIPPENDALE & WIENS 1994; OLMSTEAD & SWEERE 1994; MASON-GAMER & KELLOGG 1996). Therefore, we present both separate and combined trees based on all available data for a total evidence approach to assessing relationships (KLUGE & WOLFE 1993).

Phylogenetic relationships

The 24-taxon data matrices (and their trees; Fig. 1) were designed to test congruence among the three separate data sets. Since these matrices represented reductions of the full data sets in each

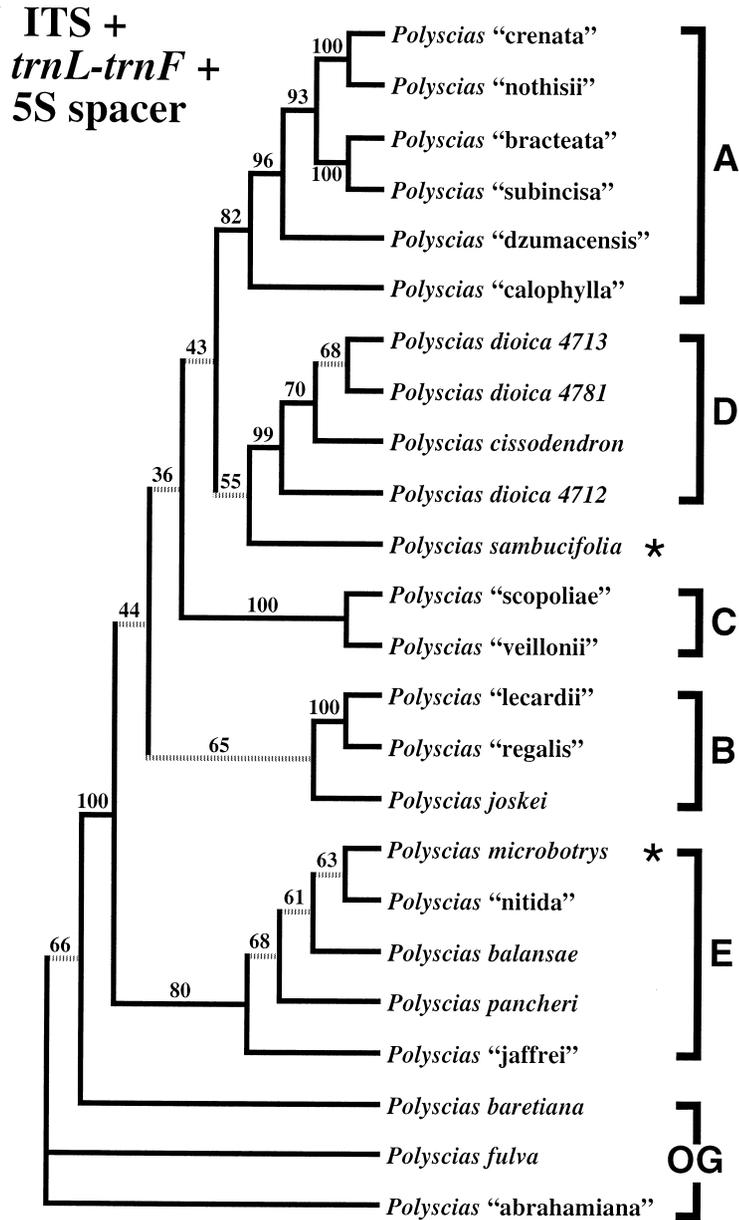


Fig. 2. — The single most parsimonious tree resulting from the combined analysis of ITS + *trnL-trnF* + 5S spacer sequences, based on the identical 24-taxon sampling as used in Fig. 1; tree length = 349 steps; consistency index = 0.663; retention index = 0.770. Values along nodes are bootstrap percentages; nodes with dashed branches have bootstraps below 70%. Identical or near identical clades (labeled A-E) from Fig. 1 are also labeled, as is the placement of *Polyscias sambucifolia* and *P. microbotrys* (asterisks), as discussed in text. Outgroups indicated by "OG."

case, we shall hereafter discuss only those results that are based on the full data sets (Figs. 3-5) for each separate analysis. In the analysis of ITS data,

the maximum limit of 10,000 trees was met, but no topologies of equal or shorter length could be found that did not agree with the strict consensus

tree, suggesting that this strict tree is a reasonable estimation of all shortest length trees. For the remaining analyses, fewer than 10,000 trees were found, allowing all analyses to be completed without tree limits. Analyses of the *trnL-trnF* and 5S matrices each yielded 156 most parsimonious trees. The combined analyses of ITS + *trnL-trnF*, ITS + 5S, and *trnL-trnF* + 5S yielded 6, 3, and 36 most parsimonious trees, respectively. In the combined analyses of all three data sets, a single, fully-resolved tree was found.

The strict consensus resulting from analysis of ITS data (Fig. 3) includes a broad sampling of species from throughout Araliaceae. This tree depicts the same major clades recovered by PLUNKETT et al. (2001), including the *Tieghemopanax*, *P. tennantii*, *Gastonia*, *P. fulva*, *Arthrophyllum*, *Meryta*, and *Polyscias* section *Polyscias* groups, which together comprise the “*Polyscias sensu lato*” clade. The recovery of most of these same clades in the parsimony analysis of the separate *trnL-trnF* data set helps to demonstrate the congruence of ITS and *trnL-trnF* topologies (see Figs. 3, 4). Congruence between topologies based on all three separate analyses (ITS, *trnL-trnF*, 5S) is evident in the recovery of many identical or near identical clades, such as the sister relationship between section *Tieghemopanax* and *Polyscias baretiana* and between the *P. “lecardii”/P. “regalis”* species pair, among many others (Figs. 3-5). Other relationships demonstrate the congruence (or at least an absence of conflict) between these topologies when polytomies are taken into consideration. For example, the *P. “scopoliae”/P. “veillonii”* species pair is well resolved in trees based on separate *trnL-trnF* and 5S data (Figs. 4, 5); because this relationship is left unresolved in the trees based on ITS sequences (Fig. 3), the ITS data set neither confirms nor refutes it.

Topologies resulting from the combined data set (ITS + *trnL-trnF*; Fig. 6a) confirm the monophyly of section *Tieghemopanax*, supported by a bootstrap value of 63%. However, outgroup sampling in this pairwise combined tree was limited, and bootstrap values supporting the monophyly of section *Tieghemopanax* in the separate trees are either lower (50% in the ITS tree; Fig. 3) or the clade fails to resolve (*trnL-trnF*

tree, although the clade comprising *Tieghemopanax* species + *P. elegans* is supported by a bootstrap of 89%; Fig. 4). These trees also help to confirm the paraphyly of *Polyscias sensu lato* (PLUNKETT et al. 2001), with taxa from several other genera appearing within the broad *Polyscias* clade (Figs. 3, 4, 6a).

Banding patterns and sequence length variation in 5S spacer sequences of *Tieghemopanax* accessions

Multiple-banding patterns of 5S PCR amplicons were observed in several accessions of taxa in the *Tieghemopanax* group, principally in some (but not most) accessions of *P. dioica* (Lowry 4771, 4800, 4804) and a single accession each of *P. elegans* (Plunkett 1495), *P. cissodendron* (Lowry 4749), and *P. pancheri* (Lowry 4793). The remaining *Tieghemopanax* accessions (including all other *P. dioica* samples) yielded only a single 5S PCR product. Individual bands from *P. dioica* (Lowry 4804) and *P. elegans* (Plunkett 1495) were gel-purified and cloned, but attempts to sequence these cloned products failed to produce clean sequence data. Therefore, these samples were not included in any of the phylogenetic analyses. Nevertheless, the resulting “dirty” sequences were compared to all sequences deposited in GenBank to assess their homology to the 5S rDNA region. The highest scores returned were in all cases for matches with angiosperm 5S sequences (from the flanking coding regions).

In general, length variability of 5S spacer products was very high throughout *Tieghemopanax*, ranging from ~220 bp to ~360 bp. Thus, despite the difficulties in obtaining usable sequence data from samples yielding multiple PCR bands, a visual inspection and comparison of these sizes may provide some insights. For example, two of the bands in the triple-banded products resulting from three *P. dioica* samples (accessions Lowry 4771, 4800, and 4804) are visually similar in size to the single-banded amplicons resulting from other accessions of *P. dioica* and several other species. The largest of these bands (~350 bp) is similar in size to the single band resulting from two *P. dioica* accessions (Lowry 4712 and 4781) and from

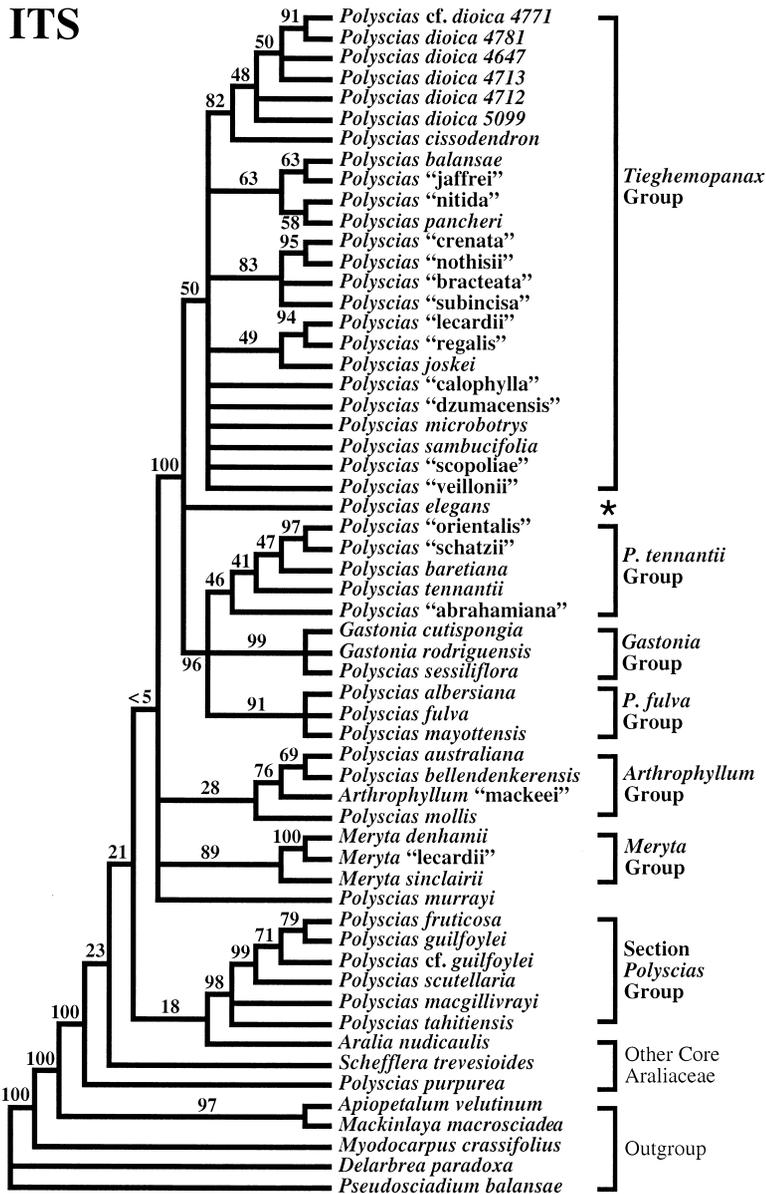


Fig. 3. — Strict consensus of 10,000 most parsimonious trees resulting from the analysis of 58 ITS sequences; tree length = 725 steps; consistency index = 0.518; retention index = 0.734. Clades denoted by brackets are those referred to in text. Values along branches are bootstrap percentages. Placement of *Polyscias elegans* (discussed in text) is denoted by an asterisk. Labels for the *Tieghepananax* group and other clades in "*Polyscias sensu lato*" follow PLUNKETT et al. (2001).

P. "jaffrei", *P. microbotrys*, and *P. "calophylla"* (*inter alia*). Similarly, the mid-sized band (~300 bp) in the same triple-banded accessions of *P. dioica* is comparable in size to the single-banded products of two different *P. dioica* accessions

(Lowry 4713 and 4647) and those of several other species (*P. scopoliae*, *P. "veillonii"*, *P. "dzumacensis"*, *P. "subincisa"*, *P. balansae*, and *P. "bracteata"*). This pattern may be explained by several alternative mechanisms, but a scenario

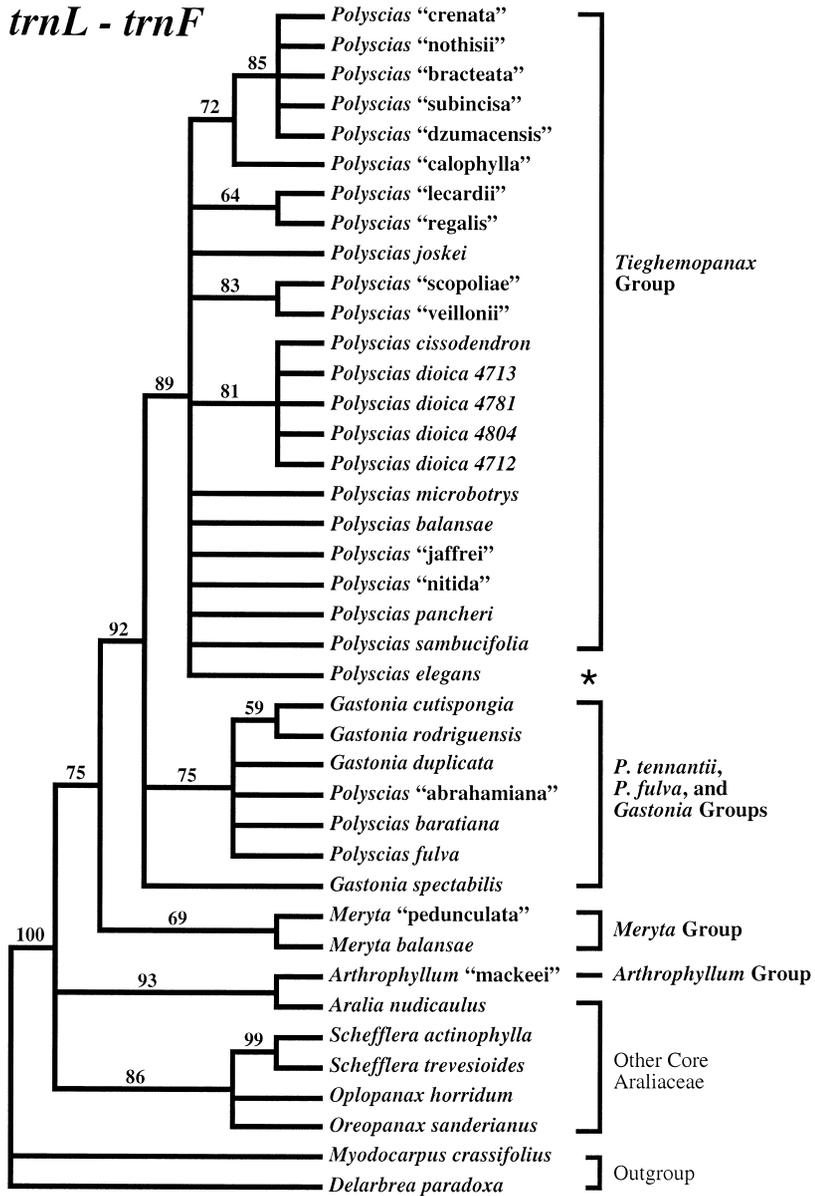


Fig. 4. — Strict consensus of 156 most parsimonious trees resulting from the analysis of 40 *trnL-trnF* sequences; tree length = 122 steps; consistency index = 0.938; retention index = 0.972. Values along branches are bootstrap percentages. Placement of *Polyscias elegans* (discussed in text) is denoted by an asterisk. Labels for the *Tieghemopanax* group and other clades in "*Polyscias sensu lato*" follow PLUNKETT et al. (2001).

involving hybridization either among variable *P. dioica* individuals or between *P. dioica* and other members of section *Tieghemopanax* could produce these results. Future work is needed to address the issue more fully.

DISCUSSION

The monophyly of *Polyscias* sect. *Tieghemopanax* was confirmed by the combined analysis of ITS + *trnL-trnF* data (Fig. 6a; bootstrap 63%) and in

the ITS tree (Fig. 3; bootstrap 50%). In the *trnL-trnF* tree (Fig. 4), the lineages of section *Tieghemopanax* form a large polytomy with *P. elegans*, supported by a bootstrap of 89%. Thus, despite the paraphyly evident in *Polyscias* as a whole, section *Tieghemopanax* appears to form a monophyletic group that is both morphologically and geographically coherent. The relationship between these New Caledonian species and two Australian taxa examined, *P. elegans* and *P. sambucifolia*, are poorly resolved in the separate analyses of ITS and *trnL-trnF* (Figs. 3, 4), but the combined analysis of these two data sets (Fig. 6a) provides a clearer picture of relationships and affords important insights into the biogeographic history of *Tieghemopanax*. Specifically, the Australian *P. elegans* is sister to the entire *Tieghemopanax* group, a result supported by a bootstrap of 86%. This finding is mirrored in many other major clades of *Polyscias* sensu lato (e.g., the section *Polyscias* and *Arthrophyllum* groups of PLUNKETT et al. 2001), and is consistent with the idea that the entire genus *Polyscias* originated in Australasia. Specifically, it seems likely that the common ancestor of *Tieghemopanax* arrived in New Caledonia through a single long-distance dispersal event after the island's separation from Australia. However, because we have no reliable estimate for the age of these lineages, we cannot rule out a more ancient vicariance event. Multiple dispersals from Australia are also conceivable, although this scenario would be less parsimonious than either a single dispersal or an ancient vicariance.

Within *Tieghemopanax*, only four taxa occur outside New Caledonia. Three species (*P. joskei*, *P. schmidii*, and *P. sambucifolia*) are found exclusively in other nearby archipelagos, and the fourth taxon, *P. cissodendron*, is native to New Caledonia but is also found on islands both to the north and south. Relationships among these species and the New Caledonian endemics provide preliminary insights into the biogeographic relationships within section *Tieghemopanax*. *Polyscias sambucifolia* is endemic (but widely ranging) in eastern Australia. Its placement in the molecular trees is somewhat unstable (cf. Figs. 1-6), and even in those cladograms where its position is resolved, bootstrap support is very weak. Two

placements, however, recur in several trees: as sister to all remaining members of section *Tieghemopanax* (Figs. 1c, 5, 6c) or as sister to the *P. dioica* + *P. cissodendron* clade (Figs. 1a, 2, 6a-b). Two alternative scenarios can thus be invoked for *P. sambucifolia*: either it represents an early branching lineage of the *Tieghemopanax* clade that remained in Australia (as did *P. elegans*), or it arrived there through secondary long-distance dispersal, most likely from New Caledonia. Without a broader consensus, we are unable to distinguish between these two hypotheses. For the remaining species, however, our results provide a clearer picture. For example, *P. cissodendron*, the most widespread member of the group, ranges from the Santa Cruz islands (southern Solomon Islands) in the north through several islands of Vanuatu (Vanua-Lava, Espiritu Santo, Erromanga, Tanna, and Aneityum) across New Caledonia, and thence farther south to Lord Howe Island (LOWRY 1989). Apart from New Caledonia, which is continental in origin, all of these are relatively young volcanic islands (less than 30 MY old, and most younger than 7 MY old; see MUELLER-DOMBOIS & FOSBERG 1998 and references therein). The age of these islands, coupled with the position of *P. cissodendron* in the molecular trees as sister to or nested within a clade of the New Caledonian endemic *P. dioica*, suggests a relatively recent series of dispersals both north and south from New Caledonia to the other islands.

The origin of the Fijian endemic *P. joskei* is likewise probably the result of long-distance dispersal. Although the major islands of Viti Levu and Vanua Levu, where this *P. joskei* occurs, have been emergent for perhaps 30 MY, they have never been in close proximity to New Caledonia. The placement of *P. joskei* as sister to the New Caledonian endemic clade of *P. "lecardii"* + *P. "regalis"* (in all trees resolving the placement of *P. joskei*; Figs. 1a, 2, 3, 6a-b), nested well within the *Tieghemopanax* group, can be explained only by dispersal. Morphologically *P. joskei* closely resembles *P. "lecardii"* and *P. "regalis"*, with which it shares a number of distinctive characters. For the last species, *P. schmidii*, we have no molecular data. On the basis of its morphology, however, it appears that this Vanuatu endemic is most closely

related to *P.* “nitida” or *P.* “nothisii”, both endemic to New Caledonia (LOWRY 1989), suggesting a third independent dispersal from New Caledonia to nearby island systems. Perhaps what is most surprising is the apparent rarity of successful dispersal events from New Caledonia to other islands involving members of the *Tieghemopanax* group. It appears that *P. cissodendron* has experienced several independent dispersals recently enough that there is little if any morphological differentiation among the widely distributed populations (LOWRY 1989). Yet, this species has no apparent traits that might suggest an increased capacity for dispersal as compared to other members of section *Tieghemopanax*. In-depth studies of both the phylogeny and natural history (e.g., identification of animal dispersers and their migration patterns) will be needed to address this issue.

In addition to broad-scale implications, the molecular analyses also provide a unified picture of relationships within section *Tieghemopanax*. In particular, nearly every tree resolves a series of identical species pairs, each of which is also supported by several morphological characters. Examination of each species pair offers useful insights into the morphological characters that unite and distinguish these species and the possible modes through which speciation may have occurred.

The *Polyscias* “lecardii”/*Polyscias* “regalis” species pair

This species pair is characterized by inflorescence features (flowers arranged in racemules) and a very similar leaf morphology (ovate to suborbicular, subcoriaceous leaflets having thickened margins). Leaflets of both species typically turn a distinctive orange-brown color when dried. *Polyscias* “lecardii”, however, has glabrous ovaries, calyces that form a low crown or rim, and flowers with distinct short pedicels, whereas *P.* “regalis” has densely puberulent ovaries, calyces with five irregular, scarios, lacinate teeth, and sessile flowers. The sister-group relationship between *P.* “lecardii” and *P.* “regalis” is well resolved in each of the seven analyses, with bootstrap support ranging from 63-100% (Figs. 2-6). Bootstrap values from 94-100% were found in the separate

ITS analysis and in three of the combined analyses (Figs. 2, 3, 6a-b). These two species occur in geographically disjunct regions (separated by c. 80-100 km). Populations of *P.* “lecardii” grow on non-ultramafic substrates in several parts of New Caledonia at middle to high elevations (600-1000 m). Conversely, *P.* “regalis” populations are known from only two locations very near one another (i.e., Plateau de Dogny and Koindé, both in south central New Caledonia), also occurring on non-ultramafic soils. In analyses including ITS data (Figs. 1a, 2, 3, 6a-b), *P. joskei* from Fiji is sister to the *P.* “lecardii”/*P.* “regalis” species pair. Although bootstrap support for this relationship is generally low (65% or lower), *P. joskei* shares most of the distinctive features mentioned above. It closely resembles *P.* “lecardii”, but can be distinguished by its thinner, membranaceous leaflets with more distinct venation, stouter secondary inflorescence axes, and styles that are longer in fruit.

The *Polyscias* “scopoliae”/*Polyscias* “veillonii” species pair

These species share several features, including a dioecious mating system, young inflorescences surrounded by large, caducous, cataphyll-like bracts, leaflets that are nearly succulent (obscuring venation on the adaxial surface), sparse branching, and similar stature (up to 4 m high). *Polyscias* “scopoliae”, however, has unifoliolate leaves, whereas those of *P.* “veillonii” are three to seven foliolate. Bootstrap values ranging from 83-100% (Figs. 2, 4, 5, 6a-c) offer significant support for the sister relationship between *P.* “scopoliae” and *P.* “veillonii”. The species in this pair are distinctly isolated for each other, with *P.* “scopoliae” restricted to dry, low elevation maquis vegetation on ultramafic substrates of the lower Tontouta and Dumbéa river valleys and on Mt. Dzumac (elevations up to c. 350 m). *Polyscias* “veillonii” also occurs on ultramafics, but ranges from 500-1,300 m elevation in more mesic conditions on several mountains (e.g., Mt. Humboldt, Mt. Dzumac, Montagne des Sources, Mt. Mou, and Prokoméo), with one population at 250 m in the upper Yaté area. The valleys where *P.* “scopoliae” occurs are situated just below the mountain slopes on which populations of *P.* “veillonii” are

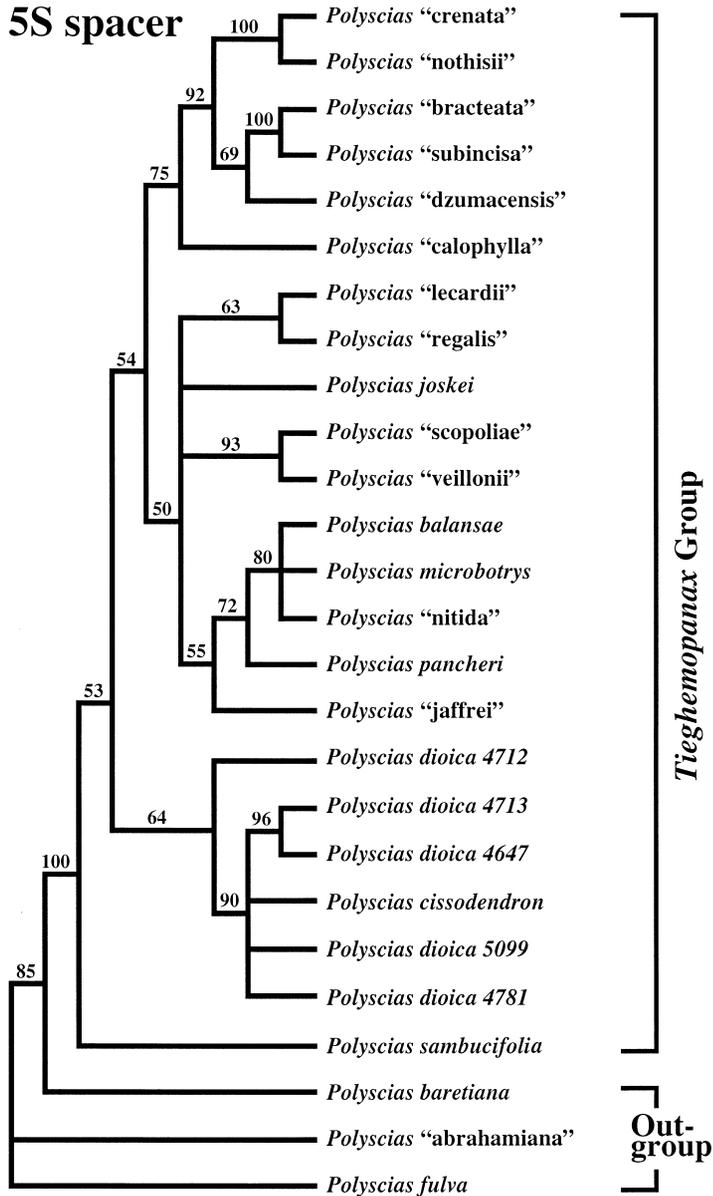


Fig. 5. — Strict consensus of 156 most parsimonious trees resulting from the analysis of 26 5S spacer sequences; tree length = 147 steps; consistency index = 0.744; retention index = 0.849. Values along branches are bootstrap percentages.

found, separated in some cases by just a few kilometers. Despite their geographical proximity, these two species are clearly distinguished by elevation (low vs. high), ecology (dry vs. more mesic conditions) and morphology (unifoliolate vs. 5- to 7-foliolate leaves).

The *Polyscias* “bracteata”/*Polyscias* “subincisa” species pair

Both of these taxa are characterized by flowers borne in heads and inflorescences with small, persistent bracts. *Polyscias* “bracteata” can be distinguished by its hermaphroditic flowers, dense,

spherical heads with (15-)20-30 flowers, stout peduncles, and large leaves with only two leaflets per node along the rachis, whereas *P.* “subincisa” has a dioecious mating system, open, hemispherical heads with 5-10(-12) flowers, slender peduncles, and smaller leaves that often have reduced secondary leaflets at each rachis node. The sister-group relationship of these species is resolved only in trees based on the separate 5S analysis and three of the four combined analyses (ITS + 5S, *trnL-trnF* + 5S, and ITS + *trnL-trnF* + 5S), but bootstrap support in these trees was 100% in all cases (Figs. 2, 5, 6b-c). *Polyscias* “bracteata” is widespread in maquis vegetation and disturbed forest and forest edges, occurring mostly on ultramafic substrates, although it is also known to occur on various other substrates in the northeastern part of New Caledonia and in the adjacent Loyalty Islands from low to fairly high elevations (up to 1000 m). Also found in maquis, *P.* “subincisa” grows mostly on ultramafic substrates but is concentrated in the northwestern region of the island. Like *P.* “bracteata,” *P.* “subincisa” can also occur on other substrates, but is limited to elevations below 200 m. These species share little or no range overlap.

The *Polyscias* “crenata”/*Polyscias* “nothisii” species pair

Both species in this pair are dioecious and share several morphological characters such as densely puberulent inflorescences and large leaves with numerous thin leaflets. In addition, individuals of these species are branched trees (5-8 m tall) with similar overall habits. *Polyscias* “crenata” differs in having puberulent petioles, sessile female flowers, and sub-ovoid fruits that are elliptical in cross-section, whereas *P.* “nothisii” has glabrous petioles, female flowers that are distinctly pedicellate, and fruits that are strongly flattened laterally. The sister relationship between *P.* “crenata” and *P.* “nothisii” is well supported in all strict trees (Figs. 2, 3, 5, 6) except that of the *trnL-trnF* data (Fig. 4). Bootstrap values ranged from 95-100%. *Polyscias* “crenata” is known to occur in sclerophyllous and transition forests in western New Caledonia on calcareous substrates at elevations ranging from sea level to 500 m. *Polyscias* “nothisii” has a similar ecological range,

but it is known from only 3 localities at low elevation (less than 50 m) in western New Caledonia.

The *Polyscias balansae*/*Polyscias* “jaffrei” species pair

Shared features between these species include their habit (monocaulous to sparsely branched treelets 1-2 m tall), a dioecious mating system, and similar leaf morphology (subcoriaceous leaflets that are ovate to sub-orbicular in shape). *Polyscias balansae* differs in having hermaphroditic flowers in umbellules and male flowers that are 5-merous, whereas *P.* “jaffrei” has hermaphroditic flowers in racemules and male flowers that are 4-merous. The sister-group relationship between these species is well resolved in only two strict trees (Figs. 3, 6a) with only moderate bootstrap support (63% in both cases). In other trees, these species come out together but in larger clades of five taxa. These species are geographically disjunct, with *P. balansae* occurring just to the south of the southern limit of *P.* “jaffrei”.

The *Polyscias dioica*/*Polyscias cissodendron* species pair

These two species share a dioecious mating system, flowers in umbellules, fruits that are 2-5 mm long, slender pedicels 0.4-0.7 mm long, calyces that form a low crown or rim, and inflorescence bracts 1-4 mm long. *Polyscias dioica* differs in having coriaceous to subcoriaceous leaves, entire to coarsely dentate and often minutely revolute leaflet margins, persistent inflorescence bracts, fruits (3.5-)4-5 mm long, and styles of female flowers and fruits united for less than half their length, whereas *P. cissodendron* has papyraceous leaves, coarsely crenulate leaflet margins, caducous inflorescence bracts, fruits 2-3(-3.3) mm long, and styles of female flowers and fruits united for more than half of their length. The relationship between *P. dioica* and *P. cissodendron* is supported by all strict trees, with bootstrap values between 64% and 99% (Figs. 2-6). Some trees show a sister-group relationship between the two species (Figs. 3, 6a) whereas others nest *P. cissodendron* within the *P. dioica* clade (Figs. 2, 5, 6b-c). These species are again largely separated geographically, but this separation is due primar-

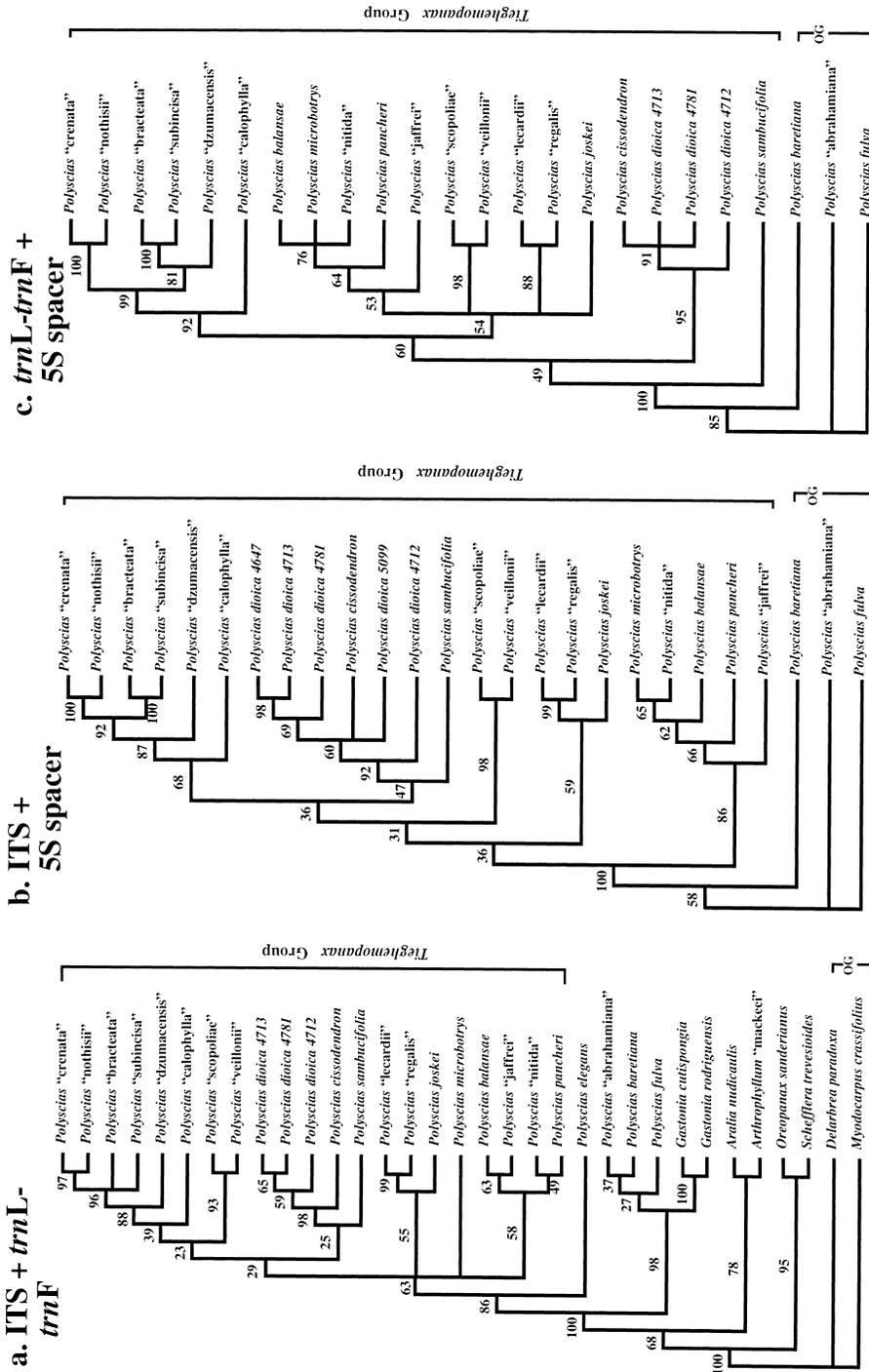


Fig. 6 (a-c). — Strict consensus trees resulting from pairwise combination of the three data sets in which maximum overlap of taxon sampling was employed; values along branches are bootstrap percentages. — (a) Strict consensus of 6 most parsimonious (MP) trees resulting from analysis of 33 ITS + trnL-trnF sequences; tree length = 507 steps; consistency index = 0.647; retention index = 0.731. — (b) Strict consensus of 3 MP trees resulting from analysis of 26 ITS + 5S spacer sequences; tree length = 323 steps; consistency index = 0.682; retention index = 0.786. — (c) Strict consensus of 36 MP trees resulting from analysis of 24 trnL-trnF + 5S spacer sequences; tree length = 175 steps; consistency index = 0.765; retention index = 0.855.

ily to edaphic constraints. *Polyscias dioica* is widespread across the island (except in the northeastern regions) but occurs almost exclusively on ultramafic substrates at low to high elevation. In contrast, *P. cissodendron* is restricted to non-ultramafic substrates in the northeastern and north-central regions of New Caledonia at elevations ranging from sea level to 750 m. As noted above, *P. cissodendron* is the only New Caledonian member of the *Tieghemopanax* group that is not endemic, with populations occurring in the southern Solomon Islands and Vanuatu to the north, and on Lord Howe Island to the south (LOWRY et al. 1986; LOWRY 1989).

Each of the species pairs described above exhibits one or more characteristics pointing either to an allopatric (MAYR 1963) or adaptive divergence (TEMPLETON 1981) model of speciation. In each pair, both molecular and morphological features help to confirm a common ancestry. Several different forms of geographic isolation, however, are evident in these pairs. For example, *P. "lecardii"* and *P. "regalis"* are separated both geographically and edaphically. *Polyscias dioica* and *P. cissodendron* are geographically sympatric (or at least parapatric) in north-central New Caledonia, but are separated primarily on the basis of edaphic conditions. Both *P. "scopoliae"* and *P. "veillonii"* have nearly identical geographic distributions (the southern region of New Caledonia) and soil requirements, but occur at different elevations with little or no overlap. Three other species pairs, *P. "bracteata"/P. "subincisa"*, *P. "crenata"/P. "nothisii"*, and *P. balansae/P. "jaffrei"*, are geographically disjunct. In general, therefore, a paradigm involving isolation and divergence appears to be the predominant mode of speciation within section *Tieghemopanax*, and this pattern may reflect a more generalized syndrome for many taxa in the New Caledonian flora. In contrast, the striking morphological variability and widespread distribution of *P. dioica*, in conjunction with the multiple banding patterns and length variation observed in the 5S PCR amplicons, seem to suggest an alternative mode of speciation for this problematic taxon.

Possible evolutionary scenarios in *Polyscias dioica*

The multiple banding patterns evident in the PCR products of the 5S region from several accessions of *P. dioica* suggest that this morphologically variable species also exhibits considerable heterogeneity in the length and copy number of the 5S repeat unit. Simple assessment of the sizes of 5S spacer PCR products from 10 accessions of *P. dioica* and from other species illustrates the great length variability of this sequence across the species of section *Tieghemopanax* (see Results). Among the samples of *P. dioica*, both single and triple banded PCR products were found; a similar result (data not discussed here) was observed in amplifications of the *trnL-trnF* marker in *P. dioica*, where both single- and double-banded amplicons were seen among different accessions. In both cases, multiple bands may have resulted from non-specific amplification of an independent sequence. In at least one case (*P. dioica*, Lowry 4804), however, homology of each of the three bands has been established with 5S coding-region sequences from other angiosperm taxa. This suggests that multiple (possibly unlinked) copies of the 5S region may be present in some (but not all) individuals of *P. dioica*.

This result may be explained by several alternative hypotheses, including gene duplication, heterogeneity among repeat units at the same locus, or hybridization among different genotypes of *P. dioica* (or alternatively between *P. dioica* and other species). Given the information presented herein (and also in EIBL 2000), we speculate that hybridization is the most likely explanation. First, two of the three size variants found among some accessions of *P. dioica* are of similar size to those found in single banded accessions (of both *P. dioica* and other species). It is relatively easy to envision an additive pattern resulting from such a hybridization (although this does not explain the smallest product of ~200 bp, which was found in none the single-banded accessions). Secondly, *P. dioica* exhibits levels of morphological variation and a geographic range far exceeding those of other species in section *Tieghemopanax*, a result confirmed by recent morphometric analysis of the group (EIBL 2000; EIBL et al. subm.). *Polyscias*

dioica may thus represent the remnants of a complex of closely related species that were once morphologically and perhaps geographically and ecologically distinct, but which have relatively recently experienced a breakdown in reproductive barriers leading to hybridization and the resulting pattern of morphological variability seen today. A thorough population-level sampling of genetic markers capable of discriminating among these alternative explanations is required to test this scenario. It is clear, however, that the general mode of divergent speciation so evident among other members of section *Tieghemopanax* is not at work in *P. dioica*. Because of its geographic distribution and the variety of its diversification patterns, *Tieghemopanax* appears to represent a model system for evaluating modes of speciation in New Caledonia. Future studies are currently underway to take advantage of these opportunities and to examine several potentially parallel cases in genera of other families.

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REFERENCES

- BERNARDI L. 1971. — Araliacearum Madagascariae et Comores propositum. 2. Revisio et taxa nova Polysciadum. *Candollea* 26: 13-89.
- BERNARDI L. 1979. — The New Caledonian genera of Araliaceae and their relationships with those of Oceania and Indonesia: 315-325, in LARSEN K. & HOLM-NIELSEN L.B. (eds.), *Tropical Botany*. Academic Press, London, New York & San Francisco.
- BULL J.J., HUELSENBECK J.P., CUNNINGHAM C.W., SWOFFORD D.L. & WADDELL P.J. 1993. — Partitioning and combining data in phylogenetic analysis. *Syst. Biol.* 42: 384-397.
- CANNATELLA D.C., HILLIS D.M., CHIPPINDALE P.T., WEIGT L., RAND A.S. & RYAN M.J. 1998. — Phylogeny of frogs of the *Physalaemus pustulosus* species group, with an examination of data incongruence. *Syst. Biol.* 47: 311-355.
- DE QUEIROZ A., DONOGHUE M.J. & JUNHYONG K. 1995. — Separate versus combined analysis of phylogenetic evidence. *Ann. Rev. Ecol. Syst.* 26: 657-681.
- DOWNIE S.R. & KATZ-DOWNIE D.S. 1996. — A molecular phylogeny of Apiaceae subfamily Apioideae: evidence from nuclear ribosomal DNA internal transcribed spacer sequences. *Amer. J. Bot.* 83: 234-251.
- DOYLE J.J. & DOYLE J.L. 1987. — A rapid DNA isolation procedure for small quantities of fresh tissue. *Phytochem. Bull.* 19: 11-15.
- EIBL J.M. 2000. — *Molecular and morphological systematics of Polyscias section Tieghemopanax (Araliaceae)*. Master's thesis, Virginia Commonwealth University, Richmond, Virginia.
- FARRIS J.S., KÄLLERSJÖ M., KLUGE A.G. & BULT C. 1995. — Testing significance of incongruence. *Cladistics* 10: 315-319.
- FELSENSTEIN J. 1985. — Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39: 783-791.
- FORSTER J.R. & FORSTER G. 1776. — *Characteres Generum Plantarum*. London.
- GUILLAUMIN A. — 1948. *Flore analytique et synoptique de la Nouvelle-Calédonie. Phanérogames*. Office de la Recherche Scientifique Coloniale, Paris.
- HARMS H. 1894-97. — Araliaceae: 1-62, in ENGLER A. and PRANTL K. (eds.), *Die Natürlichen Pflanzenfamilien* III, Vol. 8. W. Engelmann, Leipzig.
- HENEGARIU O., HEEREMA N.A., DLOUHY S.R., VANCE G.H. & VOGT P.H. 1997. — Multiple PCR: critical parameters and step-by-step protocol. *BioTechniques* 23: 504-511.
- HOLMGREN P.K., HOLMGREN N.H. & BARNETT L.C. 1990. — *Index herbariorum*. New York Botanical Garden, Bronx, New York.
- HUTCHINSON J. 1967. — *The genera of flowering plants*. Vol. 2. Oxford University Press, London.
- JAFFRÉ T. 1976. — Composition chimique et conditions d'alimentation minérale des plantes sur roches ultrabasiques (Nouvelle-Calédonie). *Cah. ORSTOM, Sér. Biol.* 9: 53-63.
- JAFFRÉ T., MORAT Ph., VEILLON J.-M. & MACKEE H.S. 1987. — Changements dans la végétation de la Nouvelle-Calédonie au cours du Tertiaire: la végétation et la flore des roches ultrabasiques. *Bull. Mus. Natl. Hist. Nat., B, Adansonia* 9: 365-391.

- JAFFRÉ T., MORAT Ph. & VEILLON J.-M. 1993. — Etude floristique et phytogéographique de la forêt sclérophylle de Nouvelle-Calédonie. *Bull. Mus. Natl. Hist. Nat., B, Adansonia* 15: 107-146.
- JOHNSON L.A. & SOLTIS D.E. 1998. — Assessing congruence: empirical examples from molecular data: 297-348, in SOLTIS D.E., SOLTIS P.S. & DOYLE J.J. (eds.), *Molecular Systematics of Plants II: DNA Sequencing*. Kluwer Publishers, Boston, Massachusetts.
- KLUGE A.G. & WOLF A.J. 1993. — Cladistics: What's in a Word? *Cladistics* 9: 183-199.
- KRONKE L.W. 1996. — Plate tectonic development of the western and southwestern Pacific: mesozoic to the present: 19-34 in KEATS A. & MILLER S.E. (eds.), *The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia, patterns and processes*. SPB Academic Publ., Amsterdam.
- LOWRY II P.P. 1989. — A revision of Araliaceae from Vanuatu. *Bull. Mus. Natl. Hist. Nat., B, Adansonia* 11: 117-155.
- LOWRY II P.P. 1998. — Diversity, endemism, and extinction in the flora of New Caledonia: a review: 181-206, in PENG C.-I & LOWRY II P.P. (eds.), *Proceedings of the International Symposium on Rare, Threatened, and Endangered Floras of Asia and the Pacific*. Academia Sinica, Taipei.
- LOWRY II P.P., DAVIES F.G. & GREEN P.S. 1986. — The distribution and synonymy of *Polyscias cissodendron* (Araliaceae). *Kew Bull.* 41: 804.
- MABBERLEY D.J. 1997. — *The Plant-Book: a portable dictionary of the higher plants*. Cambridge University Press, New York.
- MADDISON W.P. & MADDISON D.R. 1992. — *MacClade: analysis of phylogeny and character evolution*, version 3.01. Sinauer Associates Inc., Sunderland, Massachusetts.
- MAYR E. 1963. — *Animal species and evolution*. Harvard University Press, Cambridge, Massachusetts.
- MASON-GAMER R.J. & KELLOGG E.A. 1996. — Testing for phylogenetic conflict among molecular data sets in the tribe Triticeae (Gramineae). *Syst. Biol.* 45: 524-545.
- MICKEVICH M.F. & FARRIS J. S. 1981. — The implications of congruence in *Menidia*. *Syst. Zool.* 30: 351-370.
- MIQUEL F.A.G. 1855. — *Plantae Junghuhnianae*. Vol. 4. A.W. Sythoff, Leiden.
- MIQUEL F.A.G. 1856. — Araliacearum indicarum genera et species aliquot novae. *Bonplandia* 4: 137-139.
- MORAT Ph. 1993. — Our knowledge of the flora of New Caledonia: endemism and diversity in relation to vegetation type and substrates. *Biodiv. Letters* 1: 72-81.
- MORAT Ph., VEILLON J.-M & MACKEE H.S. 1984. — Floristic relationships of New Caledonian rain forest phanerogams: 71-128, in RADOVSKY F.J., RAVEN P.H. & SOHMER S.H. (eds.), *Biogeography of the Tropical Pacific*. Assoc. Syst. Collections, Lawrence, Kansas and Bernice P. Bishop Museum, Honolulu, Hawaii.
- MORAT Ph., JAFFRÉ T., VEILLON J.-M. & MACKEE H.S. 1986. — Affinités floristiques et considérations sur l'origine des maquis miniers de la Nouvelle-Calédonie. *Bull. Mus. Natl. Hist. Nat., B, Adansonia* 8: 133-182.
- MORAT Ph., JAFFRÉ T. & VEILLON J.-M. 2001. — The flora of New Caledonia's calcareous substrates. *Adansonia*, sér. 3, 23: 109-127.
- MUELLER-DOMBOIS D. & FOSBERG F.R. 1998. — *Vegetation of the tropical Pacific islands*. Sprenger-Verlag, New York.
- OLMSTEAD R.G. & SWEERE J.A. 1994. — Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the Solanaceae. *Syst. Biol.* 43: 467-481.
- PHILIPSON W.R. 1978. — A synopsis of the Malesian species of *Polyscias* (Araliaceae). *Blumea* 24: 169-172.
- PHILIPSON W.R. 1979. — Araliaceae: 1-105. *Flora Malesiana*, ser. 1, 9.
- PLUNKETT G.M., LOWRY II P.P. & BURKE M.K. 2001. — The phylogenetic status of *Polyscias* (Araliaceae) based on nuclear ITS sequence data. *Ann. Missouri Bot. Gard.* 88: 213-230.
- SANGER F., NICKLEN S. & COULSON A.R. 1977. — DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci., USA* 74: 5463-5467.
- SCHLESSMAN M.A., LLOYD D.G. & LOWRY II P.P. 1990. — Evolution of sexual systems in New Caledonian Araliaceae. *Mem. New York Bot. Gard.* 55: 105-117.
- SCHLESSMAN M.A., PLUNKETT G.M., LOWRY II P.P. & LLOYD D.G. 2001. — Sexual systems in New Caledonian Araliaceae: a preliminary phylogenetic reappraisal. *Edinburgh J. Bot.* 58: 221-228.
- SEELANAN T., SCHNABEL A. & WENDEL J.F. 1997. — Congruence and consensus in the cotton tribe (Malvaceae). *Syst. Bot.*, 22: 259-290.
- SMITH A.C. 1985. — *Flora Vitiensis Nova*. Vol. 3. Pacific Tropical Bot. Garden, Lawai, Hawaii.
- SMITH A.C. & STONE B.C. 1968. — Studies of Pacific Island plants. XIX. The Araliaceae of the New Hebrides, Fiji, Samoa, and Tonga. *J. Arnold Arbor.* 49: 431-493.
- SOLTIS D.E. & SOLTIS P.S. 1998. — Choosing an approach and an appropriate gene for phylogenetic analysis: 1-42, in SOLTIS D.E., SOLTIS P.S. & DOYLE J.J. (eds.), *Molecular Systematics of Plants II: DNA Sequencing*. Kluwer Publishers, Boston, Massachusetts.
- SOLTIS D.E., SOLTIS P.S., COLLIER T.G. & EDGERTON M.L. 1991. — The *Heuchera* group (Saxifragaceae): evidence for chloroplast transfer and paraphyly. *Amer. J. Bot.* 78: 1091-1112.
- SOLTIS D.E., SOLTIS P.S., MORT M.E., CHASE M.W., SAVOLAINEN V., HOOT S.B. & MORTON C.M.

1998. — Inferring complex phylogenies using parsimony: an empirical approach using three large DNA data sets for angiosperms. *Syst. Biol.* 47: 32-42.
- STONE B.C. 1965a. — Notes on the type species of *Polyscias* J.R. & G. Forst. (Araliaceae). *Taxon* 14: 281-285.
- STONE B.C. 1965b. — Notes on *Polyscias* (Araliaceae) from Micronesia. *Micronesica* 2: 51-59.
- TABERLET P., GIELLY L., PAUTOU G. & BOUVET J. 1991. — Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17: 1105-1109.
- TAJIMA F. & NEI M. 1984. — Estimation of evolutionary distance between nucleotide sequences. *Mol. Biol. Evol.* 1: 269-285.
- TEMPLETON A.R. 1981. — Mechanisms of speciation — a population genetic approach. *Ann. Rev. Ecol. Syst.* 12: 23-48.
- UDOVICIC F., MCFADDEN G.I. & LADIGES P.Y. 1995. — Phylogeny of *Eucalyptus* and *Angophora* based on 5S rDNA spacer sequence data. *Mol. Phylogen. Evol.* 4: 247-256.
- VIGUIER R. (1910-13) 1925. — Contribution à l'étude de la flore de la Nouvelle-Calédonie: Araliacées. *J. Bot. (Morot) II*, 3: 38-101 (dated 1910-13 but not distributed until 1925; cf. GREEN M.L., *Bull. Misc. Inform.* 1928: 155-156, 1928).
- VIGUIER R. 1905. — Sur les Araliacées du groupe des *Polyscias*. *Bull. Soc. Bot. France* 52: 285-315.
- WEN J. & ZIMMER E.A. 1996. — Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Mol. Phylogen. Evol.* 6: 167-177.
- WHITE T.J., BRUNS T., LEE S. & TAYLOR J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics: 315-322, in INNIS M.A. (ed.), *PCR Protocols: A guide to methods and applications*. Academic Press, San Diego, California.

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