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TWO NEW SPECIES OF METROSIDEROS (MYRTACEAE) FROM NEW CALEDONIA: DUAL CHARACTERISATION WITH MORPHOLOGY AND nrDNA SEQUENCE VARIATION

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SUMMARY

We have identified two new species of *Metrosideros* subg. *Mearnsia* (Myrtaceae) growing on, and apparently endemic to, the massif known as the Roches d'Ouaïème in New Caledonia. Of these two species, we include *M. whitakeri* in sect. *Mearnsia* and *M. rotundifolia* in sect. *Calyptropetala*. Both morphological assessments and phylogenetic analyses based on nrDNA sequence variation produced congruent interpretations of the novelty of the new species and of their classification.

Key words: ITS, *Metrosideros, Mearnsia*, Myrtaceae, nuclear rDNA.

INTRODUCTION

Metrosideros Banks ex Gaertn. with about 50 species (Dawson, 1976), is the only widespread genus of the capsular Myrtaceae in the Pacific basin (Wilson, 1996). The genus comprises two subgenera, subg. *Metrosideros* (c. 26 spp.) and subg. *Mearnsia* (c. 24 spp.) (Dawson, 1970). It has diversity centres in temperate New Zealand (12 spp.) and adjacent subtropical New Caledonia (16 spp.).

Here we identify two new species, *Metrosideros whitakeri* and *M. rotundifolia*, of *Metrosideros* subg. *Mearnsia* on the dual bases of morphological description and DNA sequence variation analyses. Both species were collected on the massif known as the Roches d'Ouaïème, 10 km north-west of Hienghène in north-eastern Grand Terre, New Caledonia, and are apparently endemic to that landform (and the closely contiguous massif Ton-Non) (Map 1).

Nuclear rDNA – ITS1-5.8S-ITS2 – was analysed to indicate the phylogenetic relationships of the two new species to eight other subg. *Mearnsia* species in New Caledonia, to basal *M. perforata* (J.R. & G. Forst.) A. Rich. of New Zealand, and to the outgroup species, *Cloezia floribunda* Brongn. & Gris. Together with first morphological descriptions this provides a combined view of the relationships of these previously undescribed taxa.

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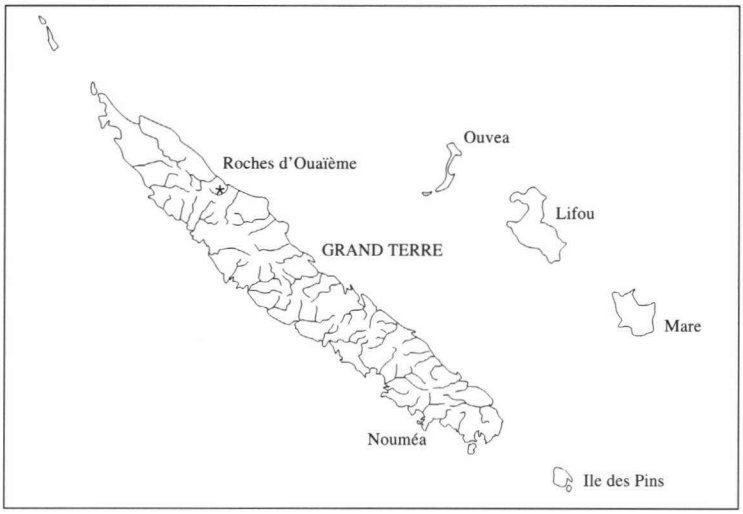

Map 1. Distribution of *Metrosideros whitakeri* J.W. Dawson and *M. rotundifolia* J.W. Dawson in New Caledonia.

MATERIALS AND METHODS – DNA PHYLOGENY

The 11 subg. *Mearnsia* species and the single outgroup species were sampled from naturally occurring populations in New Caledonia and New Zealand and were transported as live material with subsequent storage at -80 °C. Vouchers for these samples are held at: Auckland Museum Herbarium, Auckland, New Zealand (AK) and Victoria University Herbarium, Wellington, New Zealand (WELTU) (Table 1). Collection localities were mostly identified by GPS or by grid reference. The outgroup taxon is *Cloezia floribunda*, a species endemic to New Caledonia and classified within the *Metrosideros* alliance of the Myrtaceae (Briggs & Johnson, 1979).

Genomic DNA was prepared from frozen leaf material (0.5–2 g) using a modified Doyle & Doyle (1987) procedure. The ITS region of rDNA was amplified using the primers ITSa and ITSb (Wendel et al., 1995) in the following conditions: 50 pmol primers, 0.2 mM each dNTP, 1–100 ng genomic DNA, 1.5–2 mM $MgCl_2$, 1 U AmpliTaq Gold (Perkin Elmer) in a reaction volume of 100 µL. PCR products were purified using the High Pure PCR kit (Boehringer Mannheim) and sequenced using an ABI 373A or 377 sequencer. Sequences were edited visually using the ABI Auto Assembler program and initially aligned using the clustal option of the ABI Sequence Navigator, with final alignment done manually.

Phylogenies were generated using PAUP 4b2 provided by D.L. Swofford of the Smithsonian Institution. Parsimony analysis for all 12 species was applied using the heuristic search option with tree-bisection-reconnection branch swapping, random addition sequence (10 replicates) and gaps treated as 'missing'. Bootstrapping (Felsenstein, 1985) was applied under parsimony using the heuristic option with 1000 replicates. Trees were also constructed using neighbour-joining with a variety of substitution models; the derived topologies from these were all identical and confirmed the relationships shown in the parsimony analysis.

Table 1. Location and Herbarium details for samples used in DNA sequence analyses.

Taxon Name	Collection Site / Date	Global Position or Grid Reference	Herbarium Accession
C. floribunda Brongn. & Gris	Marais Kiki, New Caledonia 5/97		*WELTU 19376* Det. J.W. Dawson
M. brevistylis J.W. Dawson	Mt Aoupinie, New Caledonia 5/97	21° 10' 49 S 165° 17' 55 E	*WELTU 19353* Det. J.W. Dawson
M. cacuminum J.W. Dawson	Mt Mandjelia, New Caledonia 5/97	20° 23' 48 S 164° 31' 55 E	*WELTU 19358* Det. J.W. Dawson
M. dolichandra Schltr. ex Guillaumin	Mt Dzumac, New Caledonia 5/97	22° 01' 53 S 166° 28' 02 E	*WELTU 19359* Det. J.W. Dawson
M. longipetiolata J.W. Dawson	Roches d'Ouaïème, New Caledonia 10/98	940 m cliff edge	*AK 236304* Det. A.H. Whitaker
M. operculata Labill.	Mt Dzumac, New Caledonia 5/97	22° 01' 58 S 166° 28' 37 E	*WELTU 19382* Det. J.W. Dawson
M. paniensis J.W. Dawson	Mt Panie, New Caledonia 5/97	summit	*WELTU 19397* Det. J.W. Dawson
M. patens J.W. Dawson	Yate Heights, New Caledonia 5/97	22° 10' 02 S 166° 53' 50 E	*WELTU 19352* Det. J.W. Dawson
M. perforata (J.R. & G. Forst.) A. Rich.	Waitakere Ranges, New Zealand 4/97	36° 56' 08 S 174° 38' 12 E	*AK 232563* Det. S.D. Wright
M. porphyrea Schltr.	Mt Humboldt, New Caledonia 5/97	21° 52' 46 S 166° 25' 21 E	*WELTU 19380* Det. J.W. Dawson
M. rotundifolia J.W. Dawson	Roches d'Ouaïème, New Caledonia 5/97	20° 38' 19 S 164° 51' 44 E	*WELTU 19350* Det. J.W. Dawson
M. whitakeri J.W. Dawson	Roches d'Ouaïème, New Caledonia 10/98	04835 77169	*AK 236307* Det. A.H. Whitaker

MORPHOLOGICAL AND LOCATION DESCRIPTIONS

1. **Metrosideros whitakeri** J.W. Dawson, *spec. nov.* — Fig. 2, Map 1

Frutex humilis, effusus. Virgae valde elatae. Partes juvenes glabrae. Folia crassa, late ovata vel orbiculata, 17–23 mm longa, 18–26 mm lata. Inflorescentia in axillis foliorum multiflora. Hypanthium floris 2.5–4 mm longum, 2–3 mm latum, sepalis rotundatis 1–2 mm longis, 1.5–2 mm latis, petalis coccineus orbicularibus 1.5–2 mm longis, 2 mm latis. Stamina 12–15 mm longa. Stylus 15–20 mm longus. Fructus valde nervatus, 4.5–5 mm longus, 3–3.5 mm latus. — Typus: *MacKee 34106* (holo P), Massif Ton-Non, 650 m, 20.10.1977.

A low, patch-forming shrub, from a few cm to 0.5 m in height, with many erect stems. Young parts glabrous. Twigs strongly 4-winged and moderately flattened, the wings forming rounded lobes adjacent to the nodes. *Leaves* nearly sessile or with short petioles up to 2 by 2 mm; lamina thick, broadly ovate to almost orbicular, 17–23 by 18–26 mm, tip rounded to obtusely angled, base rounded to cordate; oil glands evident to

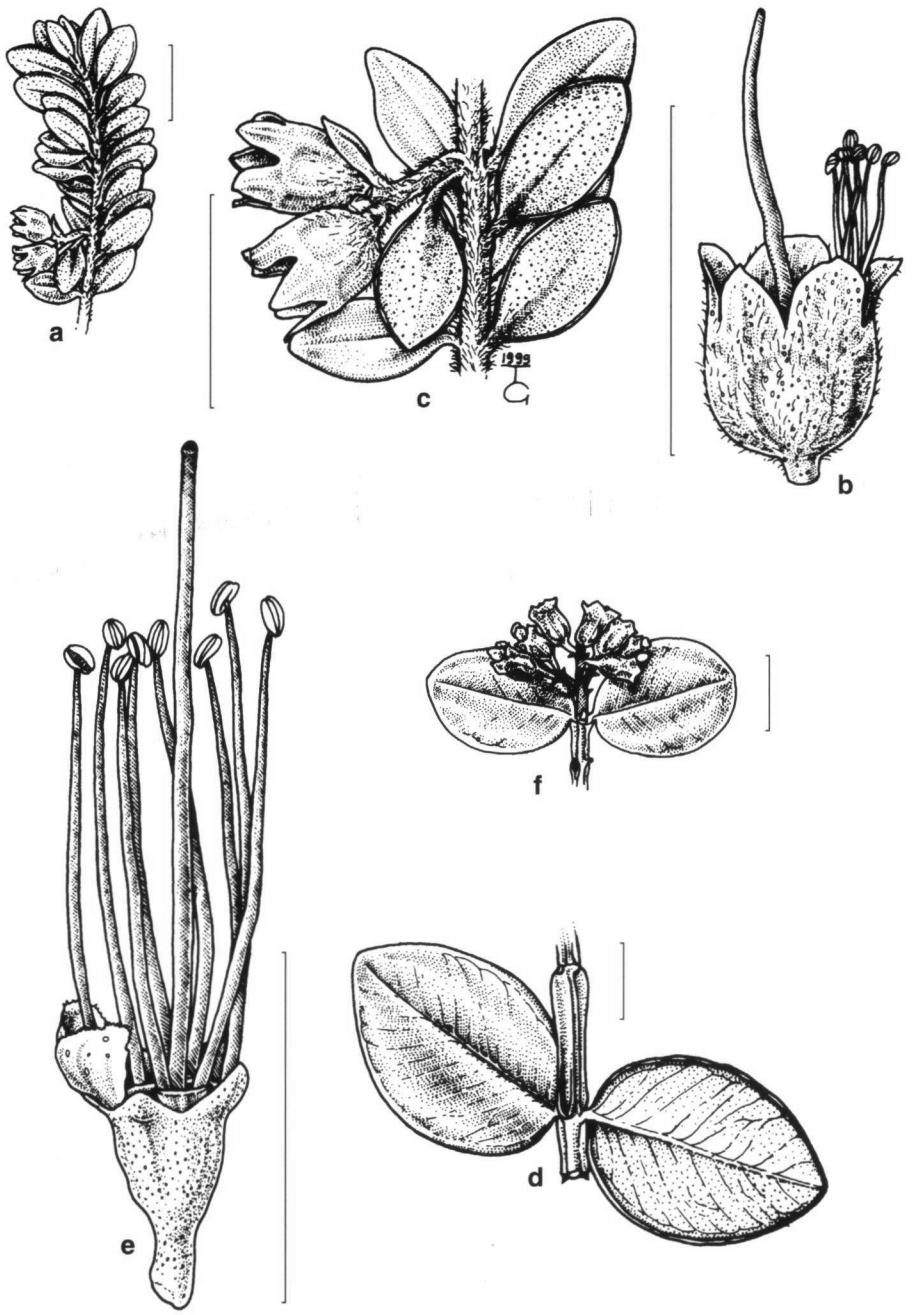

Fig. 2. — a–c: *Metrosideros rotundifolia* J.W. Dawson. a. Habit of twig; b. old flower with a few persistent stamens; c. leaves and a pair of capsules (a, c: *WELTU 19350*; b: *MacKee 19132*). — d–f: *Metrosideros whitakeri* J.W. Dawson. d. Habit of leaves and stems; e. flower; f. habit of capsules (d: *WELTU 19527*; e: *Mackee 34106*; f: *WELTU 19526*). — Scale bar = 1 cm.

obscure. *Inflorescences* mostly paired, each in the axil of a leaf adjacent to the aborted apex of a twig; mostly about as wide as long, 15–20 by 15–25 mm, with 2–4 pairs of secondary axes each bearing 2–5 flowers. Pedicels 1.5–2 by 0.5 mm. Hypanthium cupulate, 2.5–4 mm by 2–3 mm. Sepals rounded, 1–2 by 1.5–2 mm. Petals orbicular, crimson, 1.5–2 by 2 mm. Stamens 12–15 mm long, anther c. 0.8–1 by 0.4 mm. Style 15–20 mm long. *Fruit* an urceolate capsule with strongly developed veins, 4.5–5 by 3–3.5 mm; included in and much shorter than the hypanthium.

Distribution — New Caledonia, NE Grand Terre, on the crest of the Roches d'Ouaïème, Massif Ton-Non.

Habitat & Ecology — Exposed rock on the cliff edge and face, also in low open forest some metres away from the cliff. On schists. Altitude 640–970 m. Flowering and fruiting in October.

Notes — On the basis of our morphological assessment, the nearest relative of *Metrosideros whitakeri* appears to be *M. porphyrea* Schltr. of sect. *Mearnsia* of subg. *Mearnsia*. Both species have orbicular leaves of similar size with short petioles. *Metrosideros whitakeri* differs most notably from *M. porphyrea* in its strongly winged twigs and low, patch-forming growth habit. The latter is not just a result of stunting with exposure on the cliff edge as the habit is still evident in plants growing in shelter and shade some metres back from the edge. Other differences are the thicker leaves of *M. whitakeri*, the lack of hairs even on young parts, and the smaller inflorescences, although the latter is judged on limited material of the species. The growth habit and lack of pubescence also sets *M. whitakeri* apart from the other New Caledonian species in the section.

Specimens:

MacKee 34106, leg. Cherrier, Massif Ton-Non, crete E, 650 m, 20.10.1977 (fl., young fr.); *Whitaker WELTU 19526*, Massif Ton-Non, 940 m, 22.10.1998 (fl., fr.); *19527*, Massif Ton-Non, 970 m, 10.12.1998.

2. Metrosideros rotundifolia J.W. Dawson, *spec. nov.* — Fig. 2, Map 1

Frutex usque ad 3 m alta. Virgae rotundatae. Partes juvenes pubescentes. Folia ovalia vel ovata, 5–10 mm longa, 4–6.5 mm lata. Inflorescentia in axillis foliorum 2- vel 3-flora. Hypanthium floris c. 3 mm longum, 3 mm lata, sepalis rotundatis 2 mm longis, 2 mm latis, petalis non visis probabaliter caducis. Stamina alba vel subrosea, 3.5–5 mm longa. Stylus 6–10 mm longus. Fructus 3–4 mm longus, 3.5–4.5 mm latus. — Typus: *MacKee 19132* (holo P), summit, Roches d'Ouaïème (Massif Ton-Non), 900 m, 13.7.1968.

A much-branched rounded shrub up to 3 m high. Young parts with dense, spreading greyish hairs, gradually becoming glabrous. Twigs rounded. *Leaves* with slender petioles 1–2 by 0.3–0.5 mm; lamina oval to ovate, 5–10 by 4–6.5 mm, tip rounded, base rounded; oil glands evident below. Young leaves fawn to tan coloured, the colouring persisting below. *Inflorescences* 2- or 3-flowered in leaf axils. Flowers with short pedicels, c. 1 by 1 mm. Hypanthium pubescent, cupulate, c. 3 by 3 mm. Sepals rounded, c. 2 by 2 mm. Petals not seen. Stamens white to pinkish, 3.5–5 mm long, anther 0.5–0.8 by 0.3–0.5 mm. Style 6–10 mm long. *Fruits* cupulate, 3–4 by 3.5–4.5 mm, tip of capsule level with the rim of the hypanthium.

Distribution — New Caledonia, NE Grand Terre, on the crest of the Roches d'Ouaïème, Massif Ton-Non.

Habitat & Ecology — Crest and upper back slope of the cliff of the Roches d'Ouaïème, Massif Ton-Non. On schists. Altitude 700–900 m (low shrubbery at 700 m and in low, open forest at 900 m). Flowering and fruiting in May.

Notes — On the basis of our morphological assessment, *Metrosideros rotundifolia* appears to belong to sect. *Calyptropetala* of subg. *Mearnsia*. Specimens lacking flowers were tentatively included in *M. paniensis* J.W. Dawson by Dawson (1992), but *M. rotundifolia* differs from that species and from *M. cacuminum* J.W. Dawson in its type of pubescence (which is spreading rather than appressed); its small, broad, orbicular leaves; white to pinkish flowers; and short stamens and styles. The similar leaf size and the geographic proximity (*M. cacuminum* occurs on the Roches d'Ouaïème and *M. paniensis* on adjacent Mt Panie) of these three species is suggestive of their close relationships. However, with the exception of *M. patens* J.W. Dawson, *M. rotundifolia* differs from all species of the section in its small leaves and short stamens. *Metrosideros patens* is unusually reduced in all its parts, having the smallest leaves and shortest stamens of any species in the section. By contrast, *M. operculata* Labill. has substantially larger leaves than any of the other species within the section.

Specimens:

Dawson WELTU 12740, Massif Ton-Non, 700 m, 12.12.1977 (fr.); *19350*, Massif Ton-Non, 700 m, 29.5.1997 (fl., fr.); *MacKee 19132*, summit, Roches d'Ouaïème (Massif Ton-Non), 900 m, 13.7.1968 (fl., young fr.).

RESULTS AND DISCUSSION – DNA PHYLOGENY

PCR products from the 12 taxa collected in New Caledonia and New Zealand were sequenced for ITS1-5.8S-ITS2. The 12 sequences were aligned and subjected to parsimony and distance-based analyses (see Methods). Under parsimony, a single most parsimonious tree was retained (76 steps, Fig. 2). Of 633 total characters, 562 were constant, 57 variable characters were uninformative and 14 were informative. Under equal character weighting, the Consistency Index value is 0.974 for informative characters (Homoplasy Index value is 0.026), and the Retention Index value is 0.947. Neighbour-joining applying a wide variety of substitution models uniformly produces the topology presented as Fig. 3.

The existing taxonomy divides the New Caledonian component of subg. *Mearnsia* into two major sections; one encompassing the *M. operculata* group (sect. *Calyptropetala*) and the other the *M. brevistylis* group (sect. *Mearnsia*) (Dawson, 1992). Analyses of DNA variation reveals that in their higher order structures both the parsimony and neighbour-joining topologies show two clades, with five species apiece, which correspond precisely to these two sections in their composition (as seen in Fig. 2 & 3). Bootstrap support under parsimony for each clade is strong (86% – sect. *Mearnsia* and 96% – sect. *Calyptropetala*, Fig. 2). In both analyses, *M. rotundifolia* is located within sect. *Calyptropetala* and *M. whitakeri* within sect. *Mearnsia*. This is consistent with our interpretation on morphological grounds of the relationships of the two new species (see above).

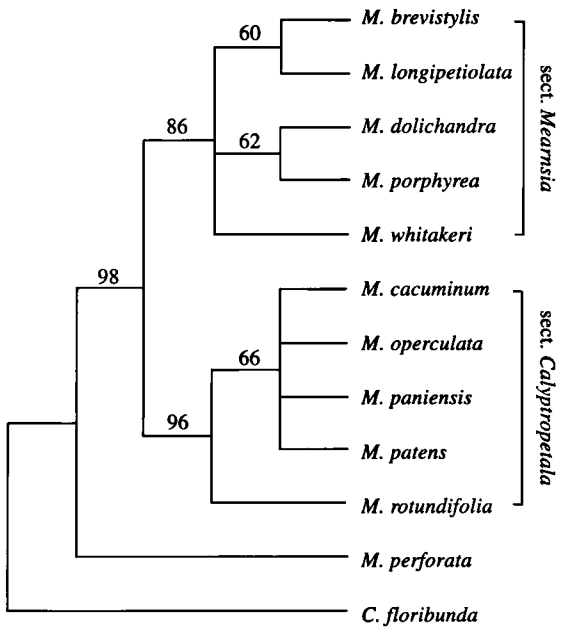

Fig. 2. Strict consensus of parsimony analysis for 12 taxa. Bootstrap values >50% shown. The clades are identified by existing taxonomic distinctions.

Interestingly, in the parsimony and neighbour-joining topologies *M. rotundifolia* is basal to the other species in its clade (Fig. 2 & 3), with weak to moderate bootstrap support (66%) for that position. This suggests that it may represent a plesiomorphic form in sect. *Calyptropetala* within New Caledonia. For the parsimony analysis, *M. whitakeri* also appears as an isolate within its clade, although it is not in a basal position (the other four species occur as two pairs with weak bootstrap support for each grouping) (Fig. 2). In the neighbour-joining analysis this species is more closely affiliated with *M. porphyrea* and *M. dolichandra* Schltr. ex Guillaumin than with the other two in the clade and appears to be basal within that subclade (Fig. 3). The association of *M. whitakeri* with *M. porphyrea* under neighbour-joining provides some affirmation of our morphological interpretation of the likely closest relative for that species.

Metrosideros perforata (monotypic in sect. *Calyptropetala* subsect. *Exertis*) of New Zealand is located basal to both the New Caledonian clades in the two topologies (Fig. 2 & 3), with very strong bootstrap support (98%) for that position. This accords with a suggested (Wilson, 1996) basal relationship within *Metrosideros* for that species on morphological grounds. *Metrosideros tetrasticha* Guillaumin, presently classified within subg. *Mearnsia* in the monotypic sect. *Neocaledonica* (Dawson, 1992), is not included here since other work done by us with ITS strongly suggests that the species should be reclassified within subg. *Metrosideros*.

Within subg. *Mearnsia* there are nine previously recognised species endemic to New Caledonia (Dawson, 1992) so that the two new species described here represent a substantial addition to the diversity of this group.

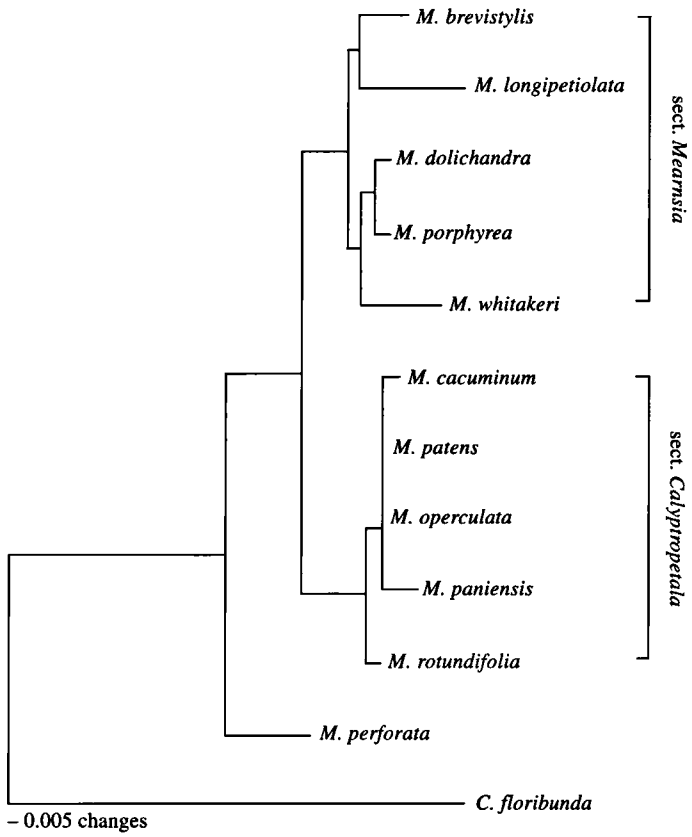

Fig. 3. Neighbour-joining analysis for 12 taxa. This topology remains unchanged regardless of the substitution model applied. The clades are identified by existing taxonomic distinctions.

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REFERENCES

Briggs, B.G. & L.A.S. Johnson. 1979. Evolution in the Myrtaceae – evidence from inflorescence structure. Proc. Linn. Soc. NSW 102: 157–256.

Dawson, J.W. 1970. Pacific capsular Myrtaceae 2. The Metrosideros complex: M. collina group. Blumea 18: 441–445.

Dawson, J.W. 1976. Pacific capsular Myrtaceae XI. Redefinition of Metrosideros Banks ex Gaertn. and definition of infrageneric categories. Blumea 23: 7–11.

Dawson, J.W. 1992. Flore de la Nouvelle-Calédonie et Dependances 18. Myrtacées Leptospermoidées. Paris, Museum National d'Histoire Naturelle.

Doyle, J.J. & J.L. Doyle. 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bull. 19: 11–15.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using bootstrap. Evolution 39: 783–791.

Wendel, J.F., A. Schnabel & T. Seelanan. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (Gossipium). Proc. Natn. Acad. Sci., USA 92: 280–284.

Wilson, P.G. 1996. Myrtaceae in the Pacific, with special reference to Metrosideros. In: A. Keast & S.E. Miller (eds.), The origin and evolution of Pacific island biotas, New Guinea to eastern Polynesia: patterns and processes: 233–246. Academic Publishing, Amsterdam.