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Phylogenetic position of *Meteoromyrtus* (Myrtaceae)

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Abstract

We investigated the phylogenetic affinities of the rare and threatened Indian species *Meteoromyrtus wynadensis* (Bedd.) Gamble. Sequences from the ITS and ETS regions of nrDNA and the *trnK/matK* and *psbA-trnH* regions of the plastid genome were compiled in a 45 taxon dataset augmented by sequences from Genbank. Phylogenetic analysis using maximum parsimony and Bayesian approaches showed that *Meteoromyrtus* is deeply embedded in *Eugenia* and sister to *E. reinwardtiana* in a clade with other Old World taxa. The evidence clearly indicates that the genus should be considered a synonym of *Eugenia* and that the species should henceforth be known by its original name *E. wynadensis* Bedd.

Introduction

Meteoromyrtus Gamble is a monotypic genus from India. The sole species, *M. wynadensis* (Bedd.) Gamble, was originally described as a *Eugenia* by Richard Beddome (1864). Beddome's publications in Indian botany have recently been enumerated by Turner (2012) who also clarified the dates of publication of his major works, *Flora Sylvatica for Southern India* and *Icones plantarum Indiae orientalis*. In the *Forester's Manual of Botany*, included in the former publication, Beddome (1872) stated that his species was intermediate between *Eugenia* L. and *Pimenta* Lindl. "having the pendulous ovules of the former and the habit and inflorescence of the latter." Beddome retained his species in *Eugenia* with a question mark but Gamble (1918) transferred it to a new genus on the basis of the distinctive pendulous ovules. Note that Gamble changed the spelling of Beddome's epithet to 'wynaadensis' but that change is not justified (see Byng et al. 2015).

Meteoromyrtus wynadensis is confined to the southern part of the Western Ghats, near the conjunction of the states of Karnataka, Kerala and Tamil Nadu. The species was last assessed by the IUCN in 1998 for the Red List and is categorised as Critically Endangered B1+2c (version 2.3) (WCMC 1998, Irwin & Narasimhan 2011). According to the IUCN assessment, "the only remaining population apparently occurs at Theerthundamala in Cannanore District" (Tamil Nadu), and "the habitat for only remaining population is steadily declining. The populations in Wynnad [sic] and Nilgiri Hills in Tamil Nadu are thought to have been destroyed." However, there appears to have been no more recent assessment of the records of the species from Kerala (Pandurangan et al. 1984) or Karnataka (Rao and Yoganarasimhan 1986).

Recent phylogenetic analyses, using both plastid and nuclear ribosomal DNA sequences, have established the major clades in Myrtaceae (Sytsma et al. 2004; Wilson et al. 2005; Biffin et al. 2007; Thornhill et al. 2015) and a new classification, based on a *matK/trnK* sequence analysis, has been published (Wilson et al. 2005). Other workers have published more in-depth analyses of some of these clades. Amongst these, there have been several

investigations of the tribe Myrteae, which includes both *Eugenia* and *Pimenta*, as well as *Myrtus* L. and other genera, like *Psidium* L. The tribe Myrteae is diverse and geographically widespread. It is defined by possession of opposite leaves, stems bearing simple (occasionally 2-armed) hairs, and fleshy fruits, often containing a number of seeds (Wilson et al. 2005; Wilson 2011). The most comprehensive phylogeny of this tribe was published by Lucas et al. (2007) who sampled approximately 60% of its genera. These authors combined data from ITS, ETS, *psbA-trnH* and part of *matK* in an analysis that recovered seven clades, with only a few taxa that were not strongly associated with any particular clade. The tribe itself was very strongly supported with the genus *Myrtus* sister to all other taxa. Other studies have focussed on individual clades or genera within the tribe: the *Eugenia* group (Van der Merwe et al. 2005, Cruz et al. 2013, Mazine et al. 2014), the *Myrcia* group (Lucas et al. 2011, Staggemeier et al. 2015), *Myrceugenia* O.Berg. (Murillo et al. 2012, 2013), and *Rhodomyrtus* (DC.) Rchb. and its allies (Snow et al. 2011). As a result of these studies, changes have been proposed that affect the circumscription of several genera. For example, *Eugenia* is now considered to include both *Hexachlamys* O.Berg. (Cruz et al. 2013, Mazine et al. 2014) and *Monimiastrum* J.Guého & A.J.Scott (Van der Merwe et al. 2005, Snow 2008).

The phylogenetic study of *Eugenia* by Van der Merwe et al. (2005) focused primarily on taxa from southern Africa and Mauritius and confirmed the monophyly of the African species groups designated X and Y (Van Wyk et al. 1980, 1982, Van Wyk and Botha 1984) supported by morphological and anatomical characters. The phylogeny of Van der Merwe et al. (2005) also gave support to the hypothesis, suggested by Van Wyk and co-workers, that group X was more closely related to New World species and that group Y was more closely related to Old World species, including those from Mauritius. It should also be noted that both of these groups fall into the poorly supported clade 9 of Mazine et al. (2014).

The aim of the present study is to determine the phylogenetic position of *Meteoromyrtus*, querying its status as a distinct genus and testing the conjecture that its affinities lie with either *Eugenia* or *Pimenta*.

Materials and Methods

Sampling: We compiled a 45-taxon dataset, 43 taxa sampled from Myrteae, which largely represented previously published data. Five accessions were new in the dataset and additional sequence data for at least one locus was added for nine further taxa. Ingroup sampling included *Meteoromyrtus*, representatives of *Eugenia* from six of the eight previously identified subclades (Mazine et al. 2014), as well as the sister genus *Myrcianthes* O.Berg. Outgroups were chosen on the basis of the previous molecular study of the tribe by Lucas et al. (2007). That study identified the *Myrteola* O.Berg. and *Pimenta* clades as closely related to *Eugenia* and forming a well-supported clade, which was recently dubbed the MEP clade by Vasconcelos et al. (2015). Therefore, we used samples from the *Pimenta* and *Myrteola* groups of genera and added *Myrtus communis* L. and *Calycolpus moritzianus* (O.Berg) Burret as further outgroups within Myrteae. We rooted the trees on *Lindsayomyrtus* B.Hyland & Steenis and *Tristaniopsis* Brongn. & Gris representing tribes outside Myrteae. Details of all taxa studied and associated GenBank numbers are listed in Table 1.

Table 1. Voucher and accession numbers for taxa included in this study. Herbarium abbreviation codes follow Index Herbariorum (RBGS = Royal Botanic Gardens, Sydney for cultivated plants). Bold text indicates some or all data for a taxon are new to this study.

Taxon	Voucher	Source	ITS	ETS	<i>psbA</i>	<i>matK</i>
<i>Calycolpus moritzianus</i> (O.Berg) Burret	COL; C. Parra-O 480	Colombia: Santander, El Mortiño	KU945986	KU945977	KU945999	KU945991
<i>Campomanesia guazumifolia</i> (Cambess.) O.Berg	UNSW; P.G. Wilson s.n.	cult. RBGS (Wild source unknown)	AM234076	KU945978	AM489821	AY521532.2
<i>Eugenia albanensis</i> Sond.	PRU; A.E. van Wyk 12723	South Africa	AY487286	AY454129	—	—
<i>Eugenia arenosa</i> Mattos	K; Mazine 1021	Brazil	KJ187605	KJ187658	KJ469654	—
<i>Eugenia axillaris</i> (Sw.) Willd.	K; Hamilton 553	Turks and Caicos	KJ187607	KJ187660	KJ469656	—
<i>Eugenia ternatifolia</i> Cambess. (<i>Eugenia beaurepairiana</i>)	K; Mazine 1008	Brazil	KJ187609	KJ187662	KJ469658	—
<i>Eugenia brasiliensis</i> Lam.	K; Lucas 126	Brazil	KJ187613	KJ187666	KJ469662	—

Taxon	Voucher	Source	ITS	ETS	<i>psbA</i>	<i>matK</i>
<i>Eugenia brongniartiana</i> Guillaumin	NOU; Pillon 176	New Caledonia	KJ187615	KJ187668	KJ469664	—
<i>Eugenia buxifolia</i> Lam.	NSW; K.Wilson 10740	France: Reunion Is. (cult. Conservatoire Botanique National de Mascarin, Les Colimaçons)	KU945987	KU945979	KU946000	KU945992
<i>Eugenia capensis</i> (Eckl. & Zeyh.) Harv.	PRU; A.E. van Wyk 12694	South Africa	AY487292	AY454134	—	—
<i>Eugenia cerasiflora</i> Miq.	PRU; A.J. Urban s.n.	Brazil	AY487296	AY454137	—	—
<i>Eugenia crassipetala</i> J.Guého & A.J.Scott	PRU; D. Florens s.n.	Mauritius	AY487288	AY454131	—	—
<i>Eugenia erythrophylla</i> Strey	PRU; M.M. van der Merwe 259	cult. South Africa	AY463139	AY454145	—	—
<i>Eugenia florida</i> DC.	K; Lucas 106	French Guiana	AM234090	AM489912	AM489830	—
<i>Eugenia foetida</i> Pers.	ASU; A. Salywon 1208	USA, Florida	AY487298	AY454139	—	—
<i>Eugenia gregii</i> (Sw.) Poir.	NSW; P.G.Wilson 1534	cult. RBGS (Wild source unknown)	AY487285	AY454128	KU946001	KU945993
<i>Eugenia involucrata</i> DC.	PRU; A.J. Urban s.n.	Brazil	AY487294	AY454135	—	—
<i>Eugenia kanakana</i> N.Snow. (<i>Monimiastrum globosum</i>)	PRU; D. Florens s.n.	Mauritius	AY487297	AY454138	—	—
<i>Eugenia langsdorffii</i> O.Berg	K; Da Silva & Farias 4528	Brazil	AM234092	AM489914	—	—
<i>Eugenia latifolia</i> Aubl.	K; M. Prevost 4707	French Guiana	AM234091	AM489913	AM489831	—
<i>Eugenia lucida</i> Lam.	PRU; D. Florens s.n.	cult. Mauritius	AY487289	AY454132	—	—
<i>Eugenia mespiloides</i> Lam.	NSW; K.Wilson 10741	France: Reunion Is. (cult. Conservatoire Botanique National de Mascarin, Les Colimaçons)	KU945988	KU945980	KU946002	KU945994
<i>Eugenia myrcianthes</i> Nied. (<i>Hexachlamys edulis</i>)	LPAG; L. Katinas 201	South America (Wild source unknown)	—	KU945982	—	AY525131.2
	K; Mazine 1091	Brazil	KJ187652	—	KJ469702	—
<i>Eugenia natalitia</i> Sond.	PRU; A.J. Urban s.n.	cult. South Africa	AY463135	AY454141	—	—
	Maurin 1796	South Africa	—	—	—	JF270773
<i>Eugenia orbiculata</i> Lam.	PRU; D. Florens s.n.	Mauritius	AY487290	AY454133	KJ469679	—
<i>Eugenia picardiae</i> Krug & Urb.	PRU; A.J. Urban s.n.	Brazil	AY487295	AY454136	—	—
<i>Eugenia puniceifolia</i> (Kunth) DC.	K; M. Prevost 4724	French Guiana	AM234087	AM489909	AM489827	—
<i>Eugenia reinwardtiana</i> (Blume) DC.	NSW; P.G. Wilson s.n.	cult. RBGS (Wild source unknown)	KU945989	KU945981	KU946003	KU945995
<i>Eugenia simii</i> Dümmer	PRU; A.E. van Wyk 12704	South Africa	AY463134	AY454140	—	—
<i>Eugenia stictosepala</i> DC.	K; Zappi 406	Brazil	AM234086	AM489908	—	—
<i>Eugenia sulcata</i> Spring ex Mart.	K; Lucas 68	Brazil	AM234089	AM489911	AM489829	AM489987
<i>Eugenia tinifolia</i> Lam.	PRU; D. Florens s.n.	Mauritius	AY487287	AY454130	—	—

Taxon	Voucher	Source	ITS	ETS	<i>psbA</i>	<i>matK</i>
<i>Eugenia tropophylla</i> H.Perrier	PRU; T.K. Lowrey 2134	Madagascar	AY487303	AY454150	—	—
<i>Eugenia uniflora</i> L.	ASU; D. Damrel 2304 (ITS, ETS), FLAS; J.R. Abbott 23855 (<i>psbA</i>)	USA.; Florida	AY487284	AY454127	GU135338.2	—
	NSW; P.G. Wilson 1335	cult. RBGS (Wild source unknown)	—	—	—	AF368207.3
<i>Eugenia verdoorniae</i> A.E.van Wyk	PRU; A.E. van Wyk 12697	cult. South Africa	AY463137	AY454143	—	—
<i>Eugenia woodii</i> Dummer	PRU; A.E. van Wyk 12695	cult. South Africa	AY463138	AY454144	—	—
<i>Eugenia zeyheri</i> (Harv.) Harv.	PRU; A.E. van Wyk 12696	cult. South Africa	AY463136	AY454142	—	—
<i>Lindsayomyrtus racemoides</i> (Greves) Craven	NSW; K.D. Hill 2039	cult. RBGS (Wild source: Australia: Queensland, Daintree NP, Noah Creek)	HM160111/ HM160112	KU945983	—	AF184706.3
<i>Lophomyrtus bullata</i> Burret	OTA; Belsham M31	cult. New Zealand	AM234145	AM489923	AM489841	—
	Shane Wright s.n.	New Zealand (Wild source unknown)	—	—	—	KU945996
<i>Meteoromyrtus wynadensis</i> (Bedd.) Gamble	UGoa; Rajkumar 310	India: Kerala, Wayanad Forest	KU945990	KU945984	KU946004	KU945997
<i>Myrcianthes cisplatensis</i> (Cambess.) O.Berg	ASU; Landrum 11233	Uruguay	JN660914	—	JQ033349	JN661013
<i>Myrcianthes pseudomato</i> (D.Legrand) McVaugh	K; Beck 9667	Bolivia	AM234100	AM489951	AM489868	—
<i>Myrcianthes pungens</i> (O.Berg) D.Legrand	ESA; Forster 1013	Brazil	KJ187656	KJ187709	KJ469706	—
<i>Myrtus communis</i> L.	K; Lucas 211	cult. RBG Kew	AM234149	AM489955	AM489872	—
	WIS; Sytsma 7205	cult.	—	—	—	AY525136.2
<i>Neomyrtus pedunculata</i> (Hook.f.) Allan	OTA; Belsham M42	cult. New Zealand	AM234144	AM489956	AM490637	—
	CHR; D. Glenny 8174	New Zealand: Westland, Kelly's Creek	—	—	—	KU945998
<i>Pimenta racemosa</i> (Mill.) J.W.Moore	K; B. Holst 8866	cult.	AM234082	AM489959	AM489875	AY521545
<i>Tristaniopsis laurina</i> (Sm.) Peter G.Wilson & J.T.Waterh.	UNSW22390	cult. RBGS (Wild source: Australia: unknown)	EF041514	KU945985	—	AF184710.3

Molecular data: New extractions of total genomic DNA were made from silica-dried, herbarium or fresh plant material. Tissue was disrupted dry with tungsten beads using the Qiagen Tissue Lyser and extractions used the DNeasy Plant DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

Sequences were compiled for 6 regions: 2 nuclear regions, the nuclear encoded internal transcribed spacer (ITS) and external transcribed spacer (ETS) of the ribosomal RNA gene; and 4 plastid regions were amplified for a subset of available taxa, a portion of the chloroplast *trnK* intron inclusive of the *matK* gene and its 5' and 3' spacers and the *psbA-trnH* intergenic spacer. Data for all loci was supplemented with published sequences from GenBank. Primers used for PCR amplification and sequencing as follows: ETS, *myrtF* (Lucas

et al. 2007) and ETS–18S (Wright et al. 2001); ITS, s3 (Kass and Wink 1997) and 26se (Sun et al. 1994), 5' *matK* spacer, 909 (Lam et al. 2002) and 2518 (Gadek et al. 1996); *matK*, 2516 and 2519 (Gadek et al. 1996); 3' *matK* spacer, *matK5* and *trnK2R* (Steele and Vilgalys 1994); *psbA-trnH*, *psbA* (Sang et al. 2007) and *trnH2* (Tate and Simpson 2003). In addition to the above, internal primers 756R (O'Brien et al. 2000) and 2521 (Gadek et al. 1996) were also used to sequence *matK*

All PCR reactions were carried out in 25 µL volumes containing 200 µM of each primer, 200 µM of each dNTP, 0.004% BSA, 2–2.5 mmol MgCl₂ and 1U Immolase™ DNA polymerase (Bioline, Luckenwalde, Germany) with a hot start PCR cycle. After an initial 10 min hot start at 95°C PCR reactions were subjected to 40 cycles as follows: denaturation for 30 s at 94°C; annealing for 30 s at 50–53°C; and extension for 1 min at 72°C, with a final extension for 4 min at 72°C. The annealing temperature for ETS, ITS and *psbA-trnH* was 53°C, and *matK* 50°C. Double-stranded PCR templates were purified with SureClean (Bioline (Aust) Pty. Ltd, Australia) and direct sequencing was processed at the DNA Analysis Facility of The Ramaciotti Centre for Gene Function Analysis (University of New South Wales, Sydney, Australia).

Table 2. Sequence statistics for all loci in the combined dataset. Aligned sequence lengths, variable characters, number of scored potentially parsimony informative indels and nucleotide substitution models applied to each partition for Bayesian analyses are presented.

Locus	ITS	ETS	5' <i>matK</i> spacer	<i>matK</i>	3' <i>matK</i> spacer	<i>psbA</i>
Taxa with data (of 45 total taxa)	45	44	9	18	12	24
Aligned length (bp)	847	541	801	1528	277	640
Parsimony informative characters (bp)	151	154	15	48	6	43
Parsimony uninformative characters (bp)	95	104	43	162	28	92
Indels	10	28	3	1	2	4
AIC model	GTR+I+G	GTR+G	GTR	GTR+G	HKY	GTR+I+G
Inverted sequence (bp)	-	-	-	-	8	21

Sequence alignment: Sequence chromatograms were edited in ABI software Sequence Navigator 1.0.1 and consensus sequences generated which were then aligned manually in PAUP* version 4.0b10 (Swofford 2002). In aligning sequences, gaps were positioned to maximize conformity to known indel types such as simple and inverted duplications of adjacent sequences (Golenberg et al., 1993; Levinson and Gutman 1987). Overlapping indels of different length, and insertions of the same length but bearing different relationships to surrounding sequence, were treated as having independent origins, while indels of the same length and position and showing minor differences in nucleotide sequence were scored as the same state (Simmons and Ochoterena 2000). Potentially informative indels were scored as additional presence/absence characters and appended to the sequence matrix. Gaps were treated as missing data in the phylogenetic analyses. Coding sequences of the *matK* gene were translated in MacClade (version 4.08a; Maddison and Maddison 2000) to check for internal stop codons.

Heuristic searches were conducted in PAUP* using tree bisection reconnection branch-swapping on best trees to recover the most-parsimonious (MP) trees. 1000 replicates of random taxon addition searching were conducted in which multistate characters were treated as polymorphisms, in order to detect multiple islands of trees. Relative support for the clades identified by parsimony analysis was estimated using the jackknife rather than bootstrap resampling in PAUP*, following the recommendations of Simmons and Freudenstein (2011). For jackknife analyses, 10000 replicates of faststep searching were conducted in which each replicate used random-taxon addition, no branch swapping, and the percentage of characters deleted was set at 33%. Jackknife (JK) values 50–74% were interpreted as weak support for clades; 75–89% moderate support; 90–99% strong support and 100% jackknife was considered robust. Sequence statistics for each locus are presented in Table 2.

The MP phylogenies generated were compared to those obtained using the Markov Chain Monte Carlo (MCMC) method implemented in MrBayes 3.2.2 (Ronquist et al. 2012). The most appropriate nucleotide substitution models to apply in likelihood-based analyses were determined using the Akaike's information criterion in MrModeltest 2.3 (Nylander 2004), with data partitioned into the 6 regions indicated above and excluding the appended scored indels, with each partition assigned a unique substitution model (Table 2). Bayesian analyses also included indels from all regions combined as an extra partition, with the indels binary encoded, and applying a default two-state Markov model with gamma distribution of rates and coding set to variable (as there were no invariant sites). Statefreqpr was set to fixed (empirical) for this partition to reflect only having two states.

Bayesian posterior probabilities (PP) were estimated using three independent runs of four million generations using four chains with tree sampling every 1000 generations. All parameters were set to be unlinked and with rates variable between partitions, with all other priors for the analysis set flat (i.e. as Dirichlet priors). Runs were assessed as sufficient when displaying convergence of effective sampling size (ESS) for all statistics in Tracer 1.6 (Rambaut et al. 2014), the standard deviation of split frequencies was clearly <0.01 and the potential scale reduction factor (PSRF) for all parameters was 1.000. Trees generated prior to the four Markov chains reaching stationarity (the burn-in $\sim 25\%$) were discarded. The remaining trees were used to construct a 50% majority rule consensus tree, with nodes assigned posterior probabilities (PP) of 0.95–1.00 considered as supported.

Results

The nuclear dataset was almost complete: all taxa had ITS sequence, all but one had ETS sequence (Table 1). There was substantial missing data for all plastid loci however: only nine taxa had *matK* 5' spacer sequences, 18 had at least partial *matK* gene sequences and 12 taxa had sequence for the 3' *matK* spacer, 24 taxa had *psbA-trnH* sequence, but analyses excluding those loci were not inconsistent in structure and showed only minimally different support for clades, with all loci included in the final analyses.

Aligned sequence lengths, variable characters, number of scored informative indels and models applied to each partition for Bayesian analyses are presented in Table 2. Sequences for most of the *matK* gene and its 5' spacer could not be generated for *Meteoromyrtus*, reflecting the degraded nature of the sourced herbarium material.

Inclusion of indels in both Bayesian and parsimony analyses resulted in moderate improvements in branch supports, and for the Bayesian analysis produced the only fully resolved tree, though many branches lacked support. Therefore indels were included in all the analyses presented here. Regions containing inversions in the 3' *matK* spacer (8bp) and the *psbA-trnH* intergenic spacer (21bp), were scored as a single presence/absence event with the indel partition and the actual bases excluded from analyses.

As separate analyses of nuclear and chloroplast loci indicated no conflict in phylogenetic signal all analyses presented are for the combined dataset. Heuristic searching of the combined dataset inclusive of scored indels yielded 12 equally most parsimonious (MP) trees of 1787 steps in a single island. For the most part the MP strict consensus tree was well resolved although supports between clades were generally weak (figure not shown). Jackknife support $>50\%$ are indicated on the Bayesian majority-rule consensus tree (Fig. 1).

Calycolpus and *Myrtus* are strongly supported as sister taxa in these analyses (PP = 1.00, JK = 99%) and form the earliest diverging lineage within Myrteae, although this position is poorly supported (PP = 0.87, JK = 57%). As with earlier studies these data retrieve a strongly supported clade including *Eugenia*, *Hexachlamys* and *Monimiastrum* and which also includes *Meteoromyrtus* (PP = 1.00, JK = 97%).

Although the MEP clade received strong Bayesian support in the study by Lucas et al. (2007) and the two unpublished phylogenies shown in Vasconcelos et al. (2015), it is poorly supported by our data (PP = 0.87, JK = 57%). While the *Myrteola* and *Pimenta* groups are strongly supported (PP = 1.00, JK = $\geq 95\%$), the *Eugenia* group itself (*Eugenia* + *Myrcianthes*) has weak jackknife support even though the posterior probability is strong (PP = 1.00, JK = 70%).

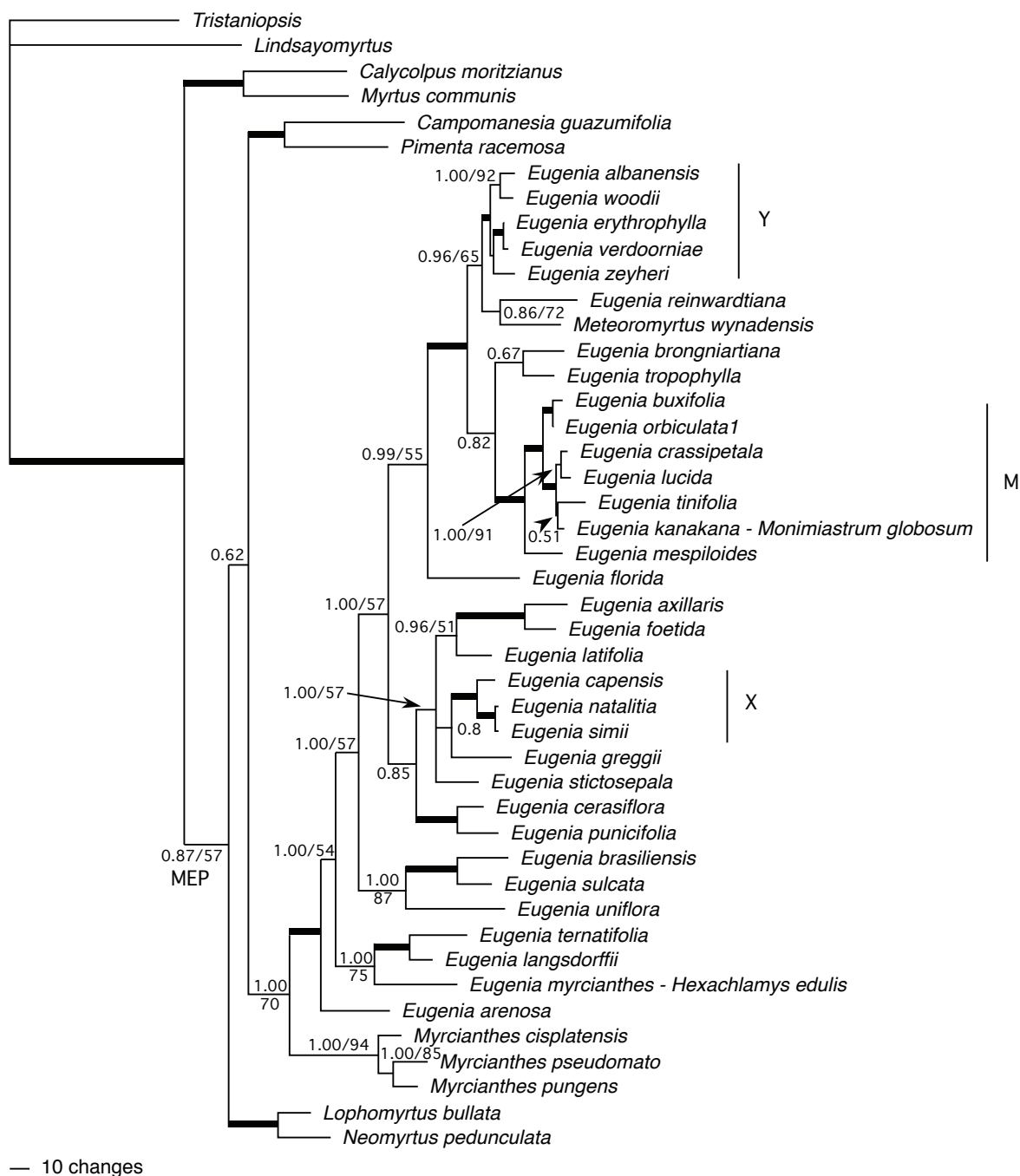


Fig. 1. Bayesian 50% majority rule consensus tree. Thick lines received support PP = >0.95 and JK = ≥95%. Other values shown on tree indicate clade support from Bayesian posterior probabilities (PP, left) and jackknife values from maximum parsimony analysis of >50% (JK, right).

Discussion

Morphological characters: The molecular phylogenetic results confirm that *Meteoromyrtus* was correctly placed by Beddome (1864) in *Eugenia* and that there is no support for a relationship with *Pimenta*. The suggested relationship with *Pimenta* was based on the pendulous ovules, which is a feature of most species in that genus. Placentation in *Eugenia* is variable and the arrangement of ovules has generally been described as follows: “ovules 2–numerous per locule, usually on a pad-like placenta arising from the medial septum” (Landrum and Kawasaki 1997). Placentation and ovule number and position are rarely recorded in Floras and other publications that describe species of *Eugenia*. When descriptions of these features in non-South American

taxa can be found, they always conform to the general description given above. This includes *E. reinwardtiana* (Blume) DC., which appears as sister to *Meteoromyrtus* in our analysis. So the distinctly pendulous ovules, which inspired the generic name, are quite unusual in the genus but must be interpreted as a likely autapomorphy.

One feature of *Meteoromyrtus* that does not seem to have been noted previously is the nature of the hairs. In the protologue, the young growth and inflorescences are described as being ‘densely fulvo-tomentose’ but the hairs themselves are not described. Examination of leaves revealed that the hairs are unequally 2-armed with one short and one very long arm. Landrum and Kawasaki (1997) record 2-armed, or dibrachiate, hairs in Brazilian Myrtaceae as “common in *Myrceugenia*, *Calyptanthus*, *Marlierea*, and some species of *Myrcia* and *Eugenia*.” Schmid (1972, p. 427) records and illustrates the range of variation of trichomes in *Eugenia*, including some that are asymmetrically dibrachiate. Snow (2011) also illustrates asymmetrically dibrachiate hairs and notes that they are a distinguishing feature of the Malagasy species *Eugenia ardyceae* N. Snow. Asymmetrically dibrachiate hairs have also been recorded for *Blepharocalyx cruckshanksii* (Hook. & Arn.) Nied. by Landrum (1986) and *Ugni candollei* O.Berg. by Landrum (1988) but *Eugenia* is the only genus of Myrteae occurring in the Old World where this type of hair has been recorded.

Phylogenetic position: *Calycolpus* is clearly closely allied to *Myrtus*. Landrum (2010) suggested that if this relationship, mooted by unpublished data, was shown to be well supported, it would “indicate that *Calycolpus* is an independent lineage, separate from most other American genera of Myrtaceae.” The isolated position of the sister species, *Myrtus communis*, in the broader Lucas et al. (2007) analysis, is further evidence that this could be the case.

The *Eugenia* clade is strongly supported as monophyletic (PP = 1.00, JK = 97%) and, although sampling is limited, most of the species groupings agree with those identified by Mazine et al. (2014). The only variation is that *E. florida* DC., which was a member of clade 8 in that study, is here included amongst taxa of their clade 9. However, this is probably not significant since their clade 9 was a grouping of convenience and much of the backbone of their analysis lacked Bayesian or bootstrap support.

Meteoromyrtus is found to be sister to *E. reinwardtiana* in all analyses, with weak jackknife support (JK = 72%) but no Bayesian support (PP = 0.86). This species pair occurs in a well-supported clade (PP = 1.00, JK = 100%) consisting of some African species (group Y), Mascarene species (including those formerly assigned to the genus *Monimiastrum*), a Madagascan species, *E. tropophylla* H.Perrier and *E. brongniartiana* Guillaumin, the lone New Caledonian species that appears to be closer to the Mascarene species than it is to the geographically closer Australasian species, *E. reinwardtiana*. The lack of strong support for the relationship between *E. reinwardtiana* and *Meteoromyrtus* is not surprising given that, apart from taxa from the African and Indian Ocean areas, few species from the Asia-Pacific area have been sequenced.

In India, the south-western Ghats are an area of high endemism; apart from *Meteoromyrtus* seven of the recorded species of *Eugenia* are endemic, three of which are listed as rare and threatened by Ahmedullah and Nayar (1986). If future studies could access material from Indian species like *E. argentea* Bedd., *E. rottleriana* Wight & Arn., and *E. roxburghii* DC., as well as other taxa from Malesia and the Pacific, this would allow a better understanding of patterns of diversity and dispersal. Such an understanding will be of great importance since it is clear that all Old World taxa have their origins in the New World but a wider analysis has the potential to clarify the history of the independent origins of the two African groups of species, X and Y.

All *Eugenia* species found in the Mascarenes are endemic to single islands (Baider and Florens 2013): 17 Mauritius, 3 Réunion, 1 Rodrigues. We newly sampled two Réunion species and their placement lies within the robust exclusively Mascarene clade (PP = 1.00, JK = 100%; clade M, Van der Merwe et al. 2005), not as sister taxa but both as early-diverging lineages (Fig. 1). This may suggest several dispersal scenarios with either multiple dispersals to Réunion and Mauritius of ancestral species, or an initial dispersal to Réunion from outside with all the Mauritian taxa arising after subsequent dispersals from Réunion. *Eugenia mespiloides* Lam. which is IUCN listed as vulnerable (UICN et al. 2013) occurs as the earliest lineage amongst the mostly Mauritian taxa included here, while *E. buxifolia* Lam. is sister to *E. orbiculata* Lam. (PP = 1.00, JK = 98%). Inclusion of more taxa from this island group within an expanded dataset may clarify the position of *E. mespiloides*.

It is interesting to note that all non-American species of *Eugenia* sampled in the present study are found in the large group 9 of Mazine et al. (2014), hinting that every dispersal event outside South America within *Eugenia* was from this group. We agree with the assertion by Mazine et al. (2014) that, to confirm this, a more extensive sampling of *Eugenia* species from Africa and Asia is required.

Conclusion

Our results show that *Meteoromyrtus* is a *Eugenia* despite the unique morphological characters that led to its recognition as a separate genus, a finding foreshadowed by Byng et al. (2015). Based on this result we propose that the correct name should now be *Eugenia wynadensis* Bedd.

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