ORIGINAL ARTICLE

Phylogenetic placement of *Ivodea* and biogeographic affinities of Malagasy Rutaceae

Marc S. Appelhans[1,2] · Jun Wen[2]

Abstract

The genus *Ivodea* is endemic to Madagascar and the Comoros and consists of 30 species. This study is the first to include the genus in a molecular phylogenetic analysis. We sequenced the plastid *trn*L–*trn*F and the nuclear ITS regions for three *Ivodea* species and revealed that the genus is monophyletic and most closely related to the African and Malagasy *Vepris*, refuting earlier suggestions of a close relationship between *Ivodea* and the Asian, Malesian, Australasian and Pacific genera *Euodia* and *Melicope*. *Ivodea* and *Vepris* provide another example of closely related pairs of Rutaceous groups that have drupaceous and capsular/follicular fruits, respectively, thus further confirming that fruit types are not suited to delimit subfamilies in Rutaceae, as has often been done in the past. *Ivodea* was the last of the seven Malagasy genera to be included in the Rutaceae phylogeny, making it possible to conduct an assessment of biogeographic affinities of the genera that occur on the island. Our assessments based on sister-group relationships suggest that the eight lineages (representing seven genera) of Malagasy Rutaceae either have African or have Asian affinities. Two lineages have an African origin, and one lineage has an Asian origin. Taxon sampling is insufficient to interpret the directionality of dispersal events in the remaining lineages.

Keywords Biogeography · *Ivodea* · Madagascar · Rutaceae

Introduction

Rutaceae are represented in Madagascar by eight genera: *Cedrelopsis* Baill., *Chloroxylon* Scop., *Citrus* L., *Fagaropsis* Mildbr. ex Siebenl., *Ivodea* Capuron, *Melicope* J.R.Forst. & G.Forst., *Vepris* Comm. ex A.Juss., and *Zanthoxylum* L. (including *Toddalia* Juss.; Appelhans et al. 2018a). Of these, *Citrus* is not native, while *Cedrelopsis* is endemic to Madagascar and *Ivodea* is endemic to Madagascar and the Comoro Islands (Schatz 2001; Rabarimanarivo et al. 2015).

Ivodea is the only Malagasy genus that has never been included in a molecular phylogenetic study, and

✉ Marc S. Appelhans
 marc.appelhans@biologie.uni-goettingen.de

1 Department of Systematics, Biodiversity and Evolution
   of Plants, Albrecht-von-Haller Institute of Plant
   Sciences, University of Goettingen, Untere Karspuele 2,
   37073 Göttingen, Germany

2 Department of Botany, National Museum of Natural History,
   Smithsonian Institution, PO Box 37012, Washington,
   DC 20013-7012, USA

consequently, its phylogenetic position and biogeographic affinities are unknown. The genus has recently been revised, and the number of published species has risen from 17 to 30 (Rabarimanarivo et al. 2015). *Ivodea* was believed to be endemic to Madagascar until Labat et al. (2005) described a species from the Comoro Islands. Of the 30 currently accepted species, 27 are endemic to Madagascar, two are endemic to the Comoro Islands, and one species occurs on both Madagascar and the Comoro Islands. *Ivodea* is morphologically characterized by a membranaceous and brittle testa, seeds without endosperm, as well as the lack of a nectary disc (Capuron 1961; Kubitzki et al. 2011; Rabarimanarivo et al. 2015). *Euodia* J.R.Forst. & G.Forst. and *Melicope* have been hypothesized to be the closest relatives of *Ivodea* (Capuron 1961; Rabarimanarivo et al. 2015). *Euodia* resembles *Ivodea* in its thin and brittle seed coat, but *Euodia* is restricted to a relatively small area in Australasia and the West Pacific (Hartley 2001; Kubitzki et al. 2011; Appelhans et al. 2014, 2018b). A close relationship of the two genera would therefore be surprising from a biogeographic perspective, although it is not unconceivable since many examples of largely disjunct sister groups at various taxonomic levels exist (e.g. Le Roux et al. 2014). *Melicope*

has a broad distribution throughout southern, eastern and SE Asia, Malesia, Australasia and many Pacific Islands as far east as the Hawaiian and Marquesas Islands. Additionally, *Melicope* is also known from Madagascar and the Mascarene Islands (Hartley 2001). From a biogeographic point of view, a close relationship of *Ivodea* and *Melicope* would not be surprising. Yet, the membranaceous and brittle testa, and the absence of endosperm and nectary disc in *Ivodea* are not indicative of a close affinity with *Melicope* (Perrier de la Bâthie 1950; Hartley 2001).

Apart from *Euodia/Melicope*, *Ivodea* has not yet been compared with other Rutaceae genera. In order to place *Ivodea* in the Rutaceae phylogeny and to evaluate whether *Euodia* and *Melicope* are indeed among the closest relatives of *Ivodea*, we assembled a dataset that contains representatives of all major clades of Rutaceae to which we added newly generated sequence data for *Ivodea*. Taxon sampling was based on previous phylogenetic studies (Chase et al. 1999; Groppo et al. 2008, 2012; Morton and Telmer 2014) and includes all four currently recognized subfamilies (sensu Morton and Telmer 2014), 11 out of 13 informal taxonomic units ('alliances'; all except the Neotropical *Amyris* and *Polyaster* alliances that contain two and seven genera, respectively) used by Kubitzki et al. (2011), and covers the distribution of the family.

The main goals of the present study are to: (1) determine the phylogenetic position of *Ivodea* and (2) identify its biogeographic affinity. All Rutaceae genera from Madagascar except *Ivodea* have been sampled in previous phylogenetic studies. With the last previously unsampled genus included, an additional goal of this study is to (3) evaluate the biogeographic affinities of Malagasy Rutaceae as a whole.

Materials and methods

Taxon sampling

Our final dataset includes 94 samples representing 63 of the 154 genera of Rutaceae (Kubitzki et al. 2011) and contains samples of all major Rutaceae clades and all genera occurring on Madagascar (Schatz 2001; Groppo et al. 2008). Subfamily Aurantioideae, the diverse South American Galipeinae and the Southern African Diosmeae are represented only by few samples since they are not closely related to any Malagasy taxon, and initial BLAST searches and phylogenetic tree reconstructions using our newly generated data revealed no close relationship between *Ivodea* and these three groups. Five genera (out of eight) of subfamily Cneoroideae, which has been shown to be the sister group to Rutaceae s.s. (Appelhans et al. 2011), are included as outgroups.

Samples throughout the distributional range have been included for all genera occurring in Madagascar except *Chloroxylon* and *Vepris*. *Chloroxylon* has a disjunct distribution in Madagascar (two species), southern India and Sri Lanka (one species; Kubitzki et al. 2011), and only sequence data for the species from India and Sri Lanka were available. *Vepris* is represented by 13 out of about 80 species (Kubitzki et al. 2011) because BLAST searches revealed that *Vepris* species had the highest sequence similarity compared to our newly generated data for *Ivodea*. All GenBank entries for *Vepris* represent mainland African species, so we also sequenced a *Vepris* specimen from Madagascar. However, the genus also occurs on the Mascarenes, as well as in tropical Arabia and SW India, so that our sampling does not cover the whole distribution range. Several samples have been included for the species-rich and widespread genera *Melicope* and *Zanthoxylum* (Appelhans et al. 2014, 2018a).

Molecular laboratory work

DNA was extracted from herbarium samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. We initially amplified the internal transcribed spacer (ITS) region for the *Ivodea* samples using primers ITS4 and ITS5 (White et al. 1990) and performing a PCR with 35 cycles of 1 min at 95 °C, 1 min at 52 °C and 1 min at 72 °C, including initial denaturation of 7 min at 95 °C and final elongation steps of 7 min at 72 °C. PCR products were cleaned using ExoSAP-IT (affymetrix USB, Cleveland, Ohio, USA) and sequenced on an ABI 3100 capillary sequencer at Microsynth Seqlab (Göttingen, Germany). Forward and reverse sequences were combined, and the consensus sequence manually edited using the CLC Genomics Workbench 11.0.1 (QIAGEN, Aarhus, Denmark) and BLASTed against the nucleotide database at GenBank. The best matches belonged to the genus *Vepris*, and we decided to assemble a dataset consisting of ITS and *trn*L–*trn*F for our analyses since this marker set was used in the only published phylogenetic study of *Vepris* (Morton 2017). The *trn*L–*trn*F region was amplified and sequenced in two pieces using primers c, d, e and f (Taberlet et al. 1991) and applying the same PCR and sequencing protocols as outlined for ITS.

No ITS sequences could be obtained from GenBank for six genera. We had material at our disposal for three of them (*Cedrelopsis*, *Ptaeroxylon* and *Spathelia*) and complemented the data for these genera. Voucher information and GenBank numbers for all accessions are presented in Tables 1 and 2.

Table 1 GenBank accessions for all sequences used in this study

Genus	Species	ITS	*trn*L–*trn*F
Acronychia	baeuerlenii	AY588596	EU853774
Adenandra	uniflora	–	JX307298
Aegle	marmelos	FJ434169	AY295294
Afraegle	paniculata	FJ434170	AY295295
Almeidea	albiflora	KP866620 & KP866643	KP866579
Andreadoxa	flava	KP866627 & KP866649	KP866586
Atalantia	ceylanica	FJ434159	AY295288
Balfourodendron	riedelianum	KC502921	EU853779
Bergera	koenigii	FJ434147	JX144258
Boenninghausenia	albiflora	LT558105	EF489218
Boronia	heterophylla	KP867657	EU853780
Brombya	platynema	HG971315	HG971163
Casimiroa	edulis	DQ225795	EF126639
Cedrelopsis	***gracilis***	MK882476*	HM637911
Chloroxylon	faho	–	AY295276
Chorilaena	quercifolia	AY631915	EU853785
Citrus	aurantiifolia	GQ225865	EF126645
Clausena	excavata	FJ434152	EF126674
Cneoridium	dumosum	LT558106	EF489256
Cneorum	tricoccon	GU178973	GU178982
Coatesia	paniculata	HG971316	HG971164
Coleonema	pulchrum	–	EU853788
Conchocarpus	heterophyllus	KP866628 & KP866653	KP866588
Dictamnus	albus	GQ434819	EU853792
Drummondita	calida	KU861262	KU861303
Esenbeckia	febrifuga	KP866657 & KP866634	KP866594
Euodia	hylandii	DQ225814	HG971169
Fagaropsis	angolensis	KU193665	KU193633
Fagaropsis	***glabra***	FJ440571	FJ440575
Fagaropsis	spec	MH016465	MG975302
Flindersia	australis	HM116975	EF126677
Galipea	jasminiflora	KP866636	KP866595
Geleznowia	verrucosa	KU861264	KU861305
Glycosmis	pentaphylla	FJ434151	AY295279
Halfordia	kendack	DQ225785	EU853798
Haplophyllum	bastetanum	AY484576	EF489245
Harrisonia	abyssinica	GU178980	GU178986
Helietta	puberula	KC502924	EU853799
Hortia	oreadica	KP866637	EU853803
Ivodea	***decaryana***	MK882477*	MK883748*
Ivodea	***mahaboensis***	MK882478*	MK883749*
Ivodea	***sahafariensis***	MK882479*	MK883750*
Lunasia	amara	HG971328	EU853805
Melicope	ternata	DQ225804	EU853808
Melicope	***madagascariensis***	HG971387	HG971220
Melicope	obtusifolia	HG971396	HG971226
Metrodorea	nigra	KC502928	EU853809
Murraya	paniculata	FJ434153	AY295280
Neoschmidea	pallida	KU861301	KU861342
Orixa	japonica	HM851496	DQ225930 + DQ225875
Pamburus	missionis	FJ434155	AY295300

Table 1 (continued)

Genus	Species	ITS	*trn*L–*trn*F
Perryodendron	*parviflorum*	HG971443	HG971267
Phellodendron	*amurense*	JN226791	JN226785
Pilocarpus	*spicatus*	P866639	KP866597
Psilopeganum	*sinense*	LT558111	LT558094
Ptaeroxylon	*obliquum*	MK882480*	EU853812
Ptelea	*trifoliata*	DQ225790	EU853813
Rauia	*Nodosa*	KP866640 & KP866662	KP866598
Ruta	*graveolens*	EU591989	AY295275
Severinia	*buxifolia*	JX144180	EU369566
Skimmia	*anquetila*	DQ225796	EF126698
Spathelia	*sorbifolia*	MK882481*	FR747914
Tetractomia	*tetrandra*	MG595152	HG971271
Tetradium	*glabrifolium*	KM506896	DQ225902
Thamnosma	*hirschii*	FN552652 & FN552668	EF489219
Triphasia	*trifolia*	JX144220	EU853822
Vepris	*amaniensis_1*	KU193666	KU193634
Vepris	*amaniensis_2*	KU193683	KU193651
Vepris	*amaniensis_3*	KU193669	KU193637
Vepris	***elliotii***	MK882482*	MK883751*
Vepris	*eugeniifolia*	KU193684	KU193652
Vepris	*fadenii*	KU193677	KU193645
Vepris	*glomerata*	KU193686	KU193654
Vepris	*hanangensis*	KU193672	KU193640
Vepris	*heterophylla*	KU193682	KU193650
Vepris	*morogorensis*	KU193663	KU193631
Vepris	*nobilis_1*	KY508613	KY508614
Vepris	*nobilis_2*	KU193670	KU193638
Vepris	*sansibarensis*	KU193681	KU193649
Vepris	*simplicifolia_1*	KU193689	KU193657
Vepris	*simplicifolia_2*	KU193675	KU193643
Vepris	*simplicifolia_3*	KU193668	KU193636
Vepris	*stolzii_1*	KU193676	KU193644
Vepris	*stolzii_2*	KU193680	KU193648
Vepris	*trichocarpa_1*	KU193678	KU193646
Vepris	*trichocarpa_2*	KU193679	KU193647
Vepris	*trichocarpa_3*	KU193671	KU193639
Vepris	*trichocarpa_4*	KU193667	KU193635
Zanthoxylum	*ailanthoides*	JN226790	FN599489
Zanthoxylum	***asiaticum***	KM506901	FN599488
Zanthoxylum	*holtzianum*	MH016515	MG975352
Zanthoxylum	***madagascariense***	MH016522	MG975359
Zanthoxylum	*simulans*	MH016545	NC037482
Zieria	*collina*	EU281864	KP188914

Sequences newly generated for this study are marked with an asterisk, and taxa occurring in Madagascar are marked in bold. In the case of *Chloroxylon faho*, the genus occurs in Madagascar, but the sampled species (*C. faho*) does not, and therefore, only the genus name is highlighted in bold

Alignment and phylogenetic analyses

Forward and reverse sequences were merged and edited manually using the CLC Genomics Workbench. Sequence alignments were generated using MUSCLE (Katoh et al. 2002; Katoh and Toh 2008) and edited by hand in Mesquite 3.40 (Maddison and Maddison 2015). The *trn*L–*trn*F alignment had a length of 1280 bp and contained 540

Table 2 Voucher information for newly generated samples used for the molecular studies

Species	Collector	Coll. Year	Location	Herbarium
Cedrelopsis gracilis	*Randrianarivelojosia, 003*	2001	Madagascar	TAN
Ivodea decaryana	*Andriamihajarivo, 296*	2004	Madagascar. Prov. Fianarantsoa, Itremo, Ambato nandrahana	MO
Ivodea mahaboensis	*Ludovic, 720* (Paratype)	2004	Madagascar. Prov. Fianarantsoa, Atsimo Atsinana, Farafangana, Mahabo-Mananivo, Baboaka	MO
Ivodea sahafariensis	*Be et al., 85*	2004	Madagascar. Prov. Antsiranana, Ramena, Baie des Sakalava	MO
Ptaeroxylon obliquum	*Balkwill et al., 5309*	1990	South Africa	B
Spathelia sorbifolia	*Van Ee, 750*	2007	Jamaica	NY
Vepris elliotii	*Ranaivojaone, 592*	2004	Madagascar	US

Herbarium codes are according to Index Herbariorum (https://sweetgum.nybg.org/science/ih/)

variable sites (42.2%) of which 286 (22.3%) were parsimony informative. The length of the ITS alignment was 743 bp of which 511 (68.8%) were variable and 379 (51.0%) were parsimony informative. Three regions with a combined length of 31 bp in the *trn*L–*trn*F alignment and two regions with a combined length of 65 bp in the ITS alignment could not be aligned with confidence and were excluded from the analyses.

Phylogenetic analysis of the ITS and *trn*L–*trn*F datasets as well as the two datasets combined was carried out using Bayesian inference (BI) and maximum likelihood (ML). Analyses were performed on the high-performance computing (HPC) cluster of Göttingen University. MrBayes 3.2.6. (Ronquist et al. 2012) was used for BI. The best-fitting substitution models were determined using jModelTest 2.1.3 (Darriba et al. 2012) and were set to GTR + G and GTR + I + G for *trn*L–*trn*F and ITS, respectively. BI analyses consisted of two independent Markov chain Monte Carlo (MCMC) runs with four chains each, which were observed for 10 million generations, and a tree was sampled every 100th generation. All runs reached stationarity (standard deviation of split frequencies < 0.01) after the 10 million generations. After inspecting the results in Tracer 1.6.0 (Rambaut et al. 2014; all effective sampling size [ESS] values were above 200) and removing 25% of the trees as burn-in, 50% majority-rule consensus trees were calculated in MrBayes. Only branches with posterior probability (pp) values of ≥ 0.95 pp were considered as supported.

ML analyses were carried out using RAxML 8.2.4 (Stamatakis 2014). The substitution models were specified as in the BI analyses for the single-marker analyses. For the combined analyses of *trn*L–*trn*F and ITS, the dataset was partitioned and the GTR + G model was used. All ML searches consisted of 1000 rapid bootstrap analyses followed by a search for the best-scoring tree. Branches with bootstrap (bs) values of 50–69 were considered to have low support, branches with bs values of 70–89 were regarded to have moderate support, and branches with bs values ≥ 90 were regarded as strongly supported.

The alignments and all consensus trees have been deposited at TreeBASE (https://purl.org/phylo/treebase/phylows/study/TB2:S24384).

Results

The consensus trees based on *trn*L–*trn*F (Fig. 1) and ITS (Fig. 2) alone showed congruent patterns of relationships, but differed in the resolution of clades. The more conserved *trn*L–*trn*F region provided better resolution at deeper nodes in the phylogeny, while ITS had better resolution towards the tips. There were supported conflicts between the *trn*L–*trn*F and ITS datasets concerning the position of *Helietta* Tul. (sister to *Esenbeckia* Brid. in the ITS dataset, but sister to *Metrodorea* A.St.-Hil. in the *trn*L–*trn*F dataset) and of several branches within *Vepris*. The concatenation might therefore be problematic, and we show the results for both the single-marker analyses and the combined analyses in the results and discussion, consequently.

All consensus trees (*trn*L–*trn*F and ITS alone and the two makers combined; Figs. 1, 2, 3) reveal a close relationship between *Ivodea* and *Vepris*. While the two genera form a polytomy in the analyses based on the *trn*L–*trn*F dataset (1.00 pp, 100bs), the analyses based on ITS alone and *trn*L–*trn*F and ITS combined reveal a sister-group relationship of the two genera (ITS: 0.96 pp, 87bs; combined: 1.00 pp, 100bs) and support monophyly of both genera (*Ivodea*: ITS: 0.99 pp, 83bs; combined: 1.00 pp, 88bs; *Vepris*: ITS: 0.90 pp, 54bs; combined: 0.94 pp, 60bs). This study is the first to sample a Malagasy species of *Vepris* in a molecular phylogenetic context (*V. elliottii* (Radlk.) I.Verd.); it is here resolved as sister to the African representatives of *Vepris* albeit with only low support (ITS: 0.72 pp, < 50bs; combined: 0.92 pp, 55bs). *Euodia* and *Melicope*, which have been hypothesized to be the closest relatives of *Ivodea*, are only distantly related to it. Both *Euodia* and *Melicope* belong to a species-rich clade of Asian, Malesian and Australasian genera, which is sister to the *Vepris–Ivodea* clade (Figs. 1, 2, 3).
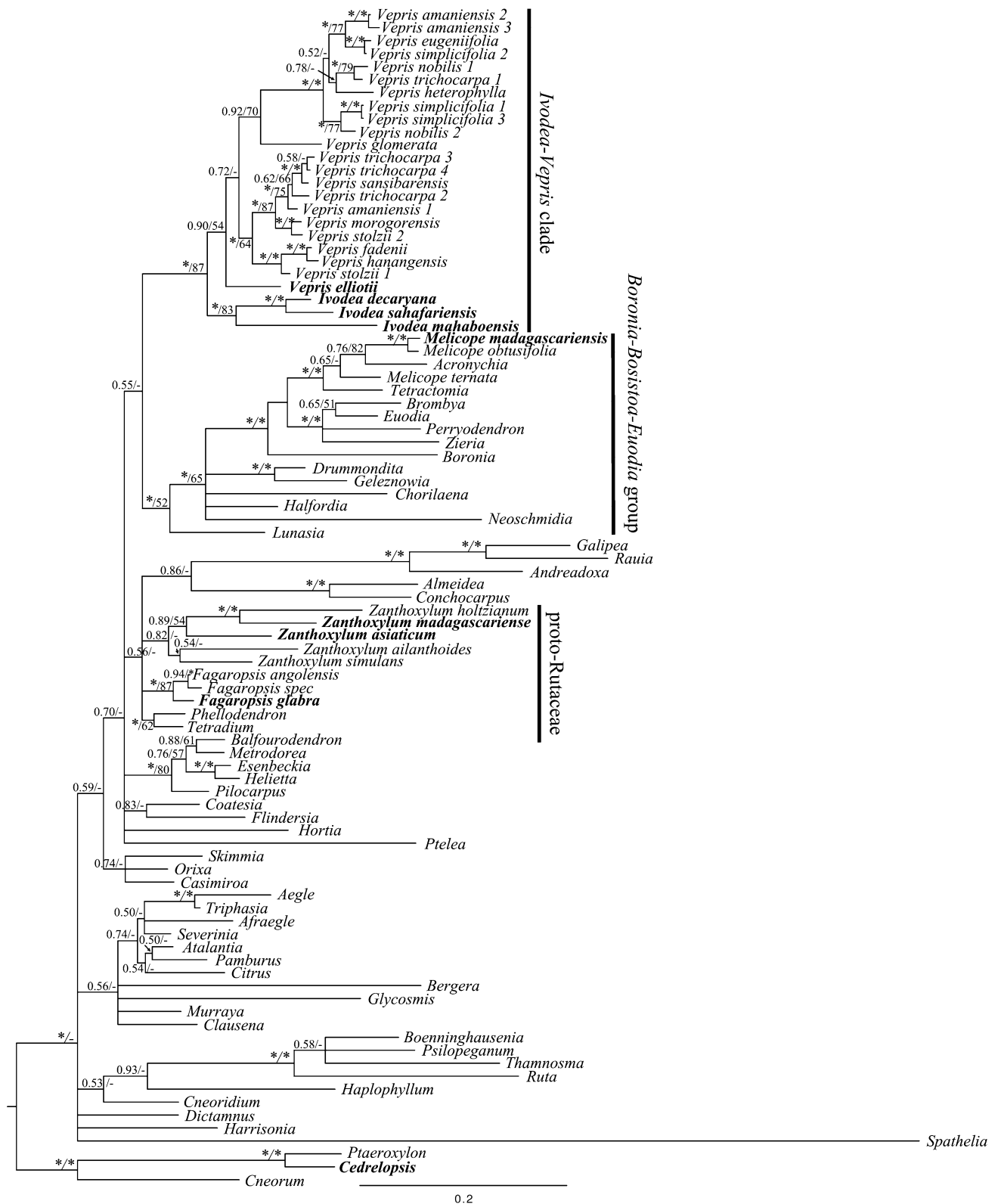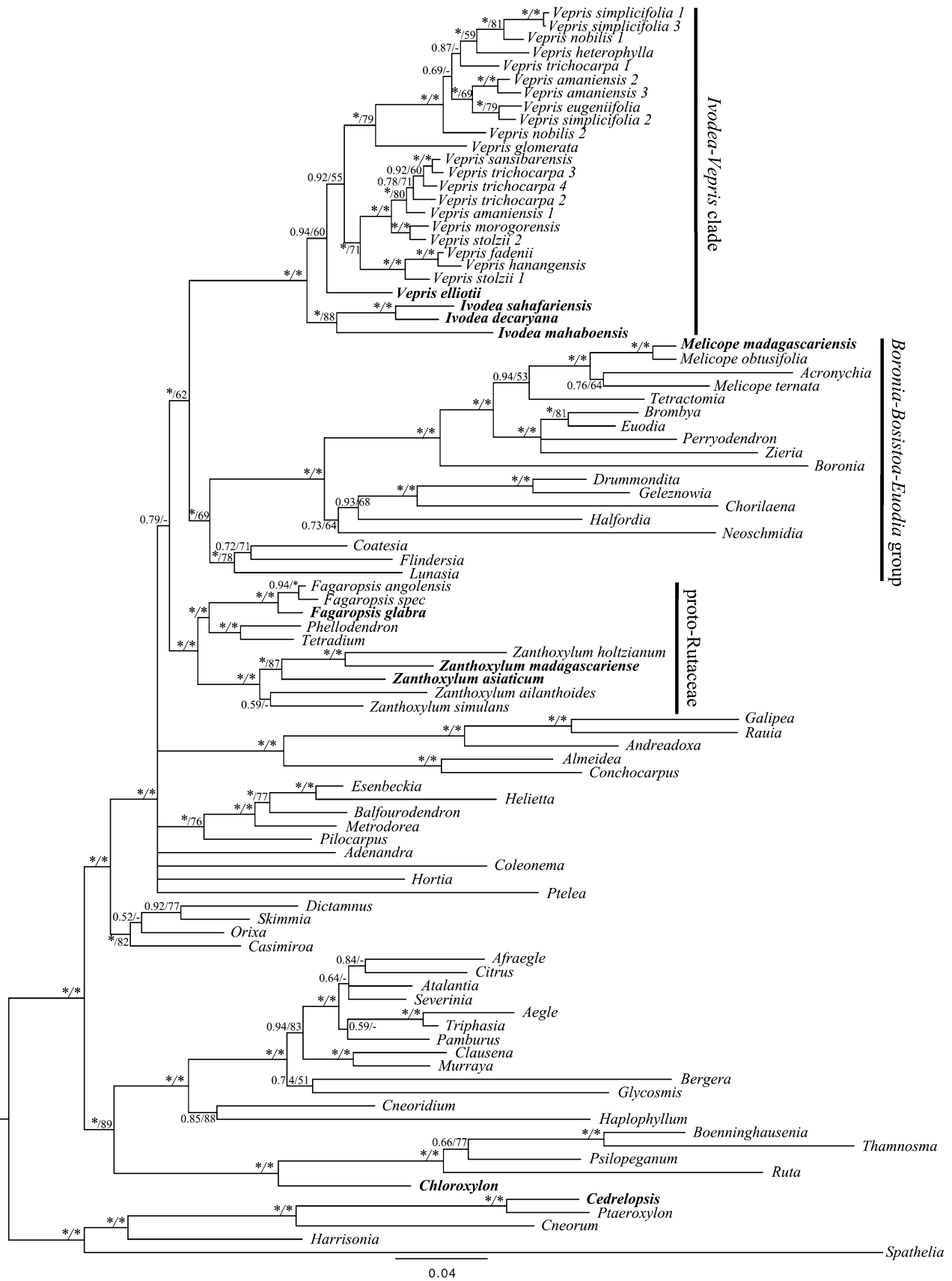
Fig. 1 Bayesian 50% majority-rule consensus tree based on the analysis of the ITS dataset. Posterior probabilities (pp; left) and the bootstrap values (bs; right) of the maximum likelihood analysis are displayed next to the branches. Asterisks indicate branches with high support ≥ 0.95 pp or ≥ 90% bs, respectively, and a hyphen represents unsupported branches in the ML analysis. Taxa native to Madagascar are highlighted in bold. Species epithets are included only for genera with several accessions, and numbers indicate several accessions of a species
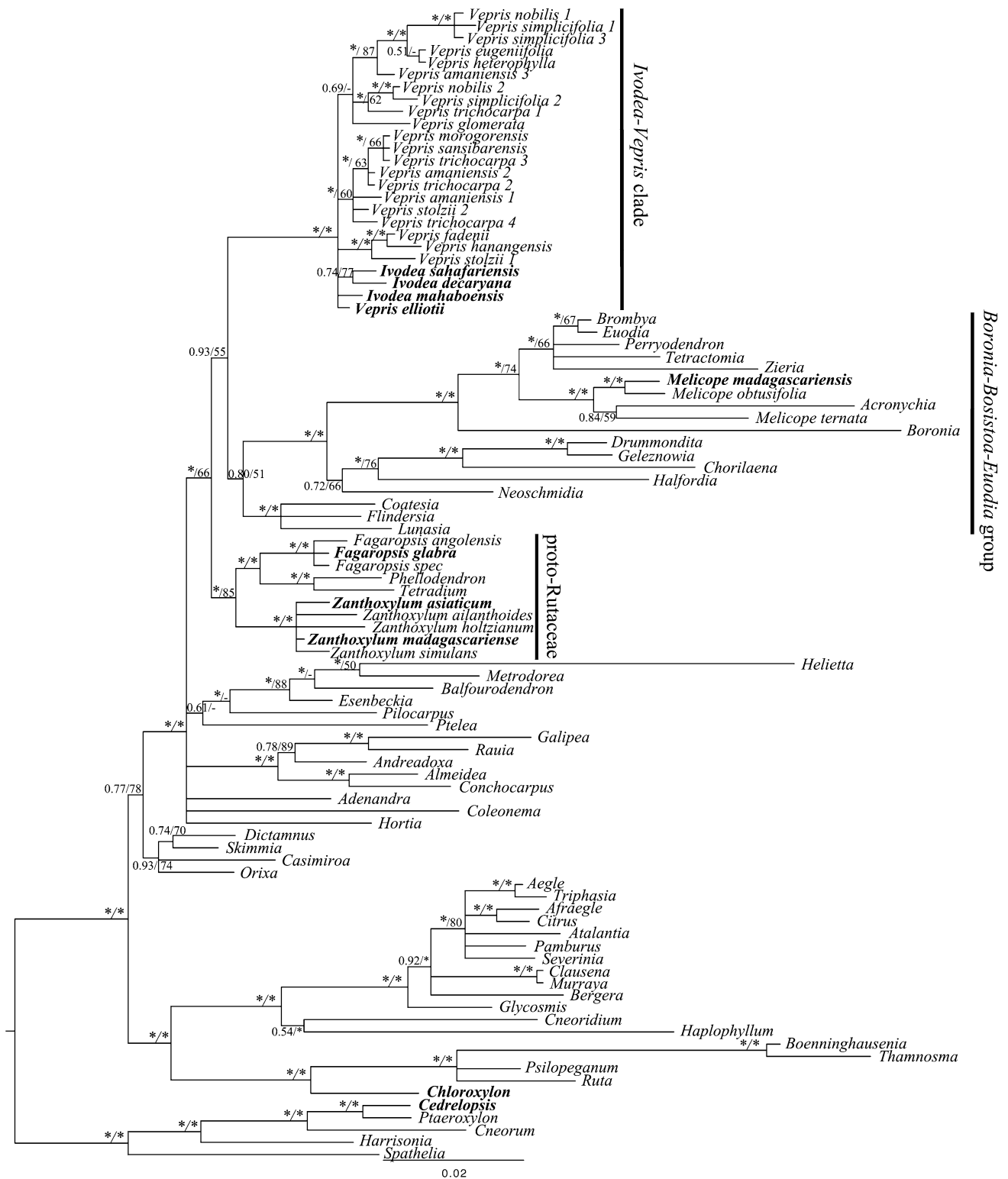
Fig. 2 Bayesian 50% majority-rule consensus tree based on the *trn*L–*trn*F dataset. Posterior probabilities (pp; left) and the bootstrap values (bs; right) of the maximum likelihood analysis are displayed next to the branches. Asterisks indicate branches with high support ≥ 0.95 pp or ≥ 90% bs, respectively, and a hyphen represents unsup-ported branches in the ML analysis. Taxa native to Madagascar are highlighted in bold. Species epithets are included only for genera with several accessions, and numbers indicate several accessions of a species

This is also the first time that more than one species of *Fagaropsis* is included in a phylogenetic analysis. Our results provide the first evidence for the monophyly of the genus (ITS: 0.99 pp, 87bs; *trn*L–*trn*F: 1.00 pp, 96bs; combined: 1.00 pp, 100 bs). *Fagaropsis* is confirmed to be part of the proto-Rutaceae clade (Appelhans et al. 2018a) and is sister to the Asian *Phellodendron* Rupr. and *Tetradium* Lour. (ITS: unresolved; *trn*L–*trn*F: 1.00 pp, 94bs; combined: 0.99 pp, 94bs). The relationships between the three *Fagaropsis* specimens are not resolved in the *trn*L–*trn*F dataset, but the ITS and the combined datasets reveal a grouping of the two sampled African specimens which are sister to the single sampled Malagasy specimen (ITS: 0.94 pp, 100bs; combined: 0.94 pp, 100bs).

Among the Rutaceae genera that contain species in Madagascar, only *Ivodea* and *Vepris* are close relatives (sister genera). All other genera with Malagasy representatives are not closely related to one another. Malagasy Rutaceae have representatives belonging to three of the four currently recognized subfamilies (all except Aurantioideae), with *Cedrelopsis* in Cneoroideae, *Chloroxylon* in Rutoideae and all others in the largest subfamily, Amyridoideae (sensu Morton and Telmer 2014).

Discussion

Phylogenetic placement of *Ivodea*

Our results reveal a sister-group relationship between the genera *Ivodea* and *Vepris*. *Ivodea* has been linked to *Euodia* and *Melicope* in previous studies (Capuron 1961; Rabarimanarivo et al. 2015). *Euodia* has been reduced from about 120 to seven species restricted to New Guinea, Australia and Pacific archipelagos as far east as Vanuatu (naturalized in other South Pacific Islands), and most of the species that were previously placed in *Euodia* have been transferred to *Melicope* and *Tetradium* (Hartley 1981, 2001). Species with a thin and brittle testa belong to *Euodia* s.s., which forms a clade with *Brombya* F.Muell., *Perryodendron* T.G.Hartley and *Pitaviaster* T.G.Hartley (Appelhans et al. 2014; Figs. 1, 2, 3). These three genera are also characterized by a thin and brittle testa, and they have overlapping distribution ranges in the Moluccas, New Guinea, tropical Australia and

the western part of the South Pacific as far east as Vanuatu (Kubitzki et al. 2011; Appelhans et al. 2014). Moreover, the brittle seed coat and the lack of a nutritious and spongy exotesta probably make *Euodia* and its allies unsuited for long-distance dispersal via endozoochory (Appelhans et al. 2014). It would have been rather surprising if *Ivodea* were closely related to *Euodia* and its relatives given the large distance to Madagascar and no apparent suitability for bird dispersal (Appelhans et al. 2014). However, there are examples of Asian–Malagasy disjunctions of plants that lack obvious adaptations for long-distance dispersal. A well-known example is *Nepenthes* (Meimberg et al. 2001; Biswal et al. 2018). Moreover, long-distance dispersal events might not have been caused by the standard dispersal vector(s), but either by unusual behaviour of the standard vector or by a different vector (Higgins et al. 2003; Nathan et al. 2008). A close relationship of *Ivodea* and *Melicope* would have been more conceivable from a biogeographic point of view. *Melicope* has a broad distribution throughout southern, eastern and SE Asia, Malesia, Australasia and many Pacific Islands as far east as the Hawaiian and Marquesas Islands. Additionally, the genus is known from Madagascar and the Mascarene Islands (Hartley 2001). *Melicope* has a broad morphological circumscription (e.g. fruits follicular or capsular, monoecious or dioecious flowers), but species in some clades within *Melicope* and especially the Malagasy species resemble *Ivodea* in most morphological characters. Yet, the two genera differ substantially in the anatomy of the testa (membranaceous and brittle in *Ivodea* vs. thick sclerotesta and nutritious sarcotesta in *Melicope*), and the absence of endosperm and a nectary disc (*Ivodea*) vs. copious endosperm and a well-developed disc (*Melicope*) (Perrier de la Bâthie 1950; Hartley 2001).

The relationship of *Ivodea* and *Vepris* was surprising at first glance because of their different fruit types. On the other hand, Rabarimanarivo et al. (2015) mentioned that some species of *Ivodea* closely resemble the unifoliolate species of *Vepris* vegetatively as well as in flowering specimens. Engler (1931) based his Rutaceae classification mainly on fruit characters and placed *Vepris* in the subfamily Toddalioideae (Toddalieae–Toddaliinae) together with genera with exclusively drupaceous fruits. Engler's treatment predated the recognition of *Ivodea*, but Capuron (1961) placed *Ivodea* in subfamily Rutoideae (Xanthoxyleae–Evodiinae), defined by the presence of dehiscent fruits. The separation into subfamilies with dehiscent and non-dehiscent fruits seems straightforward, but Engler (1931) made an exception by including the monotypic Chilean genus *Pitavia* Molina in Rutoideae despite its having drupaceous fruits. Since then, phytochemical and molecular phylogenetic studies have revealed that the two subfamilies are largely polyphyletic and that fruit characters are not good indicators of relationships at deeper phylogenetic scales in Rutaceae (Poon et al. 2007; Waterman 2007; Groppo et al. 2008). Several examples of pairs

of taxa have been identified which have dehiscent and non-dehiscent fruits, respectively, or in which a taxon with one of these fruit types is nested within a clade that otherwise contains members with the other fruit type. These examples include *Melicope* (dehiscent) and *Acronychia* J.R.Forst. & G.Forst. (indehiscent); *Sarcomelicope* Engl./*Dutaillyea* Baill./*Comptonella* Baker f./*Picrella* Baill. (all indehiscent) and *Melicope* p.p. (dehiscent); *Euodia* (dehiscent) and *Pitaviaster* (indehiscent) (Appelhans et al. 2014); *Zanthoxylum* (dehiscent) and *Toddalia* (indehiscent; now merged into *Zanthoxylum*); *Phellodendron* (indehiscent) and *Tetradium* (dehiscent) (Appelhans et al. 2018a); as well as *Acradenia* Kippist (dehiscent) and *Crossosperma* T.G.Hartley (indehiscent) (Bayly et al. 2013). *Ivodea* and *Vepris* represent one more example in this growing list.

A characteristic feature of *Ivodea* is the lack of endosperm (Capuron 1961; Rabarimanarivo et al. 2015). Seeds lacking endosperm are typical for the southern African *Diosma* L. alliance and Aurantioideae, but are rare in other Rutaceae (Kubitzki et al. 2011). Interestingly, *Vepris* is polymorphic in this respect, and species may have copious or sparse endosperm or it may be completely lacking (Kubitzki et al. 2011). Moreover, *Ivodea* and *Vepris* share a characteristic thin seed coat (Kubitzki et al. 2011). Phyllotaxis is important for recognizing genera and major clades in Rutaceae (e.g. Appelhans et al. 2014); however, both *Ivodea* and *Vepris* exhibit variation in phyllotaxis, from alternate to subopposite or opposite (*Ivodea*; Rabarimanarivo et al. 2015) and alternate to rarely subopposite (*Vepris*; Schatz 2001). For most other morphological characters, *Ivodea* represents a subset of the character states of the more broadly defined *Vepris*, e.g. dioecious (in *Ivodea*) vs. dioecious or polygamous (in *Vepris*), leaves unifoliolate versus 1-, 3–9(-12)-foliolate, flowers 4(-5)-merous versus (3-)4(-5)-merous and 2 versus 1 or 2 ovules per locule. The only entirely discriminating characters between the two genera are the lack of a nectary disc in *Ivodea* and the different fruit types (Kubitzki et al. 2011).

Biogeographic affinities of Malagasy Rutaceae

The highest proportion of Malagasy angiosperm colonists stems from Africa (Yoder and Nowak 2006). However, there is also a high proportion of Malagasy lineages with an Indian, E Asian, SE Asian and Australian origin (Yoder and Nowak 2006; Buerki et al. 2013; Janssens et al. 2016). The direction of dispersal has not always been from these areas to Madagascar, but Madagascar has also been identified as the source of dispersal events (Bacon et al. 2016). The Asian affinities of the Malagasy flora have been attributed to vicariance events resulting from the breakup of Gondwana by some authors, but most time-calibrated molecular phylogenetic studies have revealed a much more recent colonization of Madagascar (Warren et al. 2010; Buerki et al. 2013; Bacon et al. 2016). Instead of Gondwanan vicariance, long-distance dispersal and stepping-stone dispersal have been proposed as the major mechanisms of migrations from Asia to Madagascar. A prominent route for stepping-stone dispersal might have been from Southern India via several Indian Ocean archipelagos to Madagascar and the Mascarenes during times of lower sea levels (Schatz 1996; Warren et al. 2010).

Now that DNA sequence data are available from representatives of all currently recognized genera of Malagasy Rutaceae, a first comparison of origins of Malagasy Rutaceae can be made. The sister-taxon relationships of Malagasy Rutaceae suggest African and Asian affinities, although limited taxon sampling and relatively old and isolated clades prevent a conclusive inference in some cases (Table 3). This is particularly true for *Chloroxylon*, for which only one of its three species could be sampled and only part of the distribution is covered, and *Vepris*, whose immediate relatives are not known and for which only a part of the distribution likewise is covered in our sampling.

The closest relatives of *Cedrelopsis* (eight species) are the monotypic S African genus *Ptaeroxylon* Eckl. & Zeyh. and the monotypic E African genus *Bottegoa* Chiov. The former might either be sister to *Cedrelopsis* (Razafimandimbison et al. 2010) or nested within it, although this

Table 3 Distribution of genera of Rutaceae native to Madagascar and their inferred geographic origin based on sister-group relationships as indicated by the phylogenetic trees

Genus	Distribution	Possible origin of Malagasy lineage
Cedrelopsis	Madagascar	Africa
Chloroxylon	Madagascar, S India, Sri Lanka	S Asia
Fagaropsis	Africa, Madagascar	Africa
Ivodea	Madagascar, the Comoros	Africa or S Asia
Melicope	Tropical Asia, Malesia, Australasia, Pacific, Madagascar and Mascarene Islands	S Asia
Vepris	Tropical Africa, Madagascar and 1 sp in SW India	Africa or S Asia
Zanthoxylum	Pantropical, extending to temperate areas in E Asia and N America	Africa

topology has very low support (Appelhans et al. 2011). An Ancestral Area Reconstruction suggested an African origin for the three genera (Appelhans et al. 2012). Since only the sister-group relationship of *Cedrelopsis* and *Ptaeroxylon* had strong support in previous analyses, an African origin for *Cedrelopsis* is likely. If future studies provide evidence for *Ptaeroxylon* being nested within *Cedrelopsis*, a Malagasy origin of the two genera and a subsequent colonization to mainland Africa by the ancestor of *Ptaeroxylon* would be the most parsimonious scenario. The split of *Cedrelopsis*/*Ptaeroxylon* from *Bottegoa* and the initial diversification of *Cedrelopsis*/*Ptaeroxylon* have been dated to the Oligocene and Miocene (Appelhans et al. 2012), so a long-distance dispersal event from mainland Africa (or from Madagascar in case the alternative topology will be supported in future analyses) is likely.

A single exemplar of *Chloroxylon* has been included in several deeper-level phylogenetic analyses (Chase et al. 1999; Groppo et al. 2008; Morton and Telmer 2014; Appelhans et al. 2016), and it was not possible to sample additional specimens in the present study. Thus, the monophyly of this genus with a disjunct distribution in Madagascar, India and Sri Lanka remains untested using molecular methods. From a morphological perspective, the genus is characterized by some unusual features for Rutaceae (e.g. six ovules per locule, 3-valved capsule, apically winged seeds; Schatz 2001), so that morphology strongly suggests monophyly of the genus. Assuming that the genus is monophyletic, it remains to be tested whether the origin of *Chloroxylon* is in Madagascar with a dispersal to India and Sri Lanka, or vice versa. The closest relatives of *Chloroxylon* are the rare central Chinese *Psilopeganum* Hemsl., the Asian and Malesian *Boenninghausenia* Rchb., *Thamnosma* Torr. & Frém., which has a disjunct distribution in N America, Somalia and southern Africa, and the Macaronesian, Mediterranean to SW Asian *Ruta* L. (Kubitzki et al. 2011; Appelhans et al. 2016). Given the large and disjunct distribution of the clade and because monophyly of *Chloroxylon* has not yet been tested using molecular methods, the biogeographic origin of *Chloroxylon* remains unresolved.

Previous studies have only included a single species of *Fagaropsis*. The genus consists of four species, which occur in central and eastern Africa (two species) and on Madagascar (two species; Kubitzki et al. 2011). We sampled African and Malagasy species and provide the first evidence for monophyly of the genus. *Fagaropsis* belongs to a group of four genera referred to as proto-Rutaceae, which also includes the Asian *Phellodendron* and *Tetradium*, as well as the pantropical *Zanthoxylum* (Ling et al. 2009; Appelhans et al. 2018a). Despite the predominance of Asian taxa in this group, the oldest known fossils are European (Chandler 1961; Gregor 1989), so a Eurasian origin of the proto-Rutaceae has been proposed (Appelhans et al. 2018a). The

ancestor of *Fagaropsis* likely originated in Eurasia and either colonized Africa first and later dispersed to Madagascar or vice versa.

The second member of proto-Rutaceae native to Madagascar is *Zanthoxylum*, and the Malagasy members of this large, pantropical genus belong to two separate lineages within it. *Zanthoxlyum* contains about 225 species and is divided into four main clades, and all African, Malagasy and Mascarene species form a clade that is sister to the remainder of the genus (taxa from Asia, the Americas and the Pacific; Appelhans et al. 2018a). The Malagasy and Mascarene endemic species are deeply nested within the African clade of *Zanthoxylum* (Appelhans et al. 2018a); thus, the phylogenetic reconstructions provide support for an African origin of the ancestor of the Malagasy and Mascarene species. The split of the Malagasy and Mascarene species from their African ancestors has been dated from the Oligocene to the Miocene (Appelhans et al. 2018a). *Zanthoxylum asiaticum* (formerly *Toddalia asiatica*) represents the second lineage of *Zanthoxylum* that occurs in Madagascar. This species has a large distribution, ranging from E Africa, the Comoros, Madagascar and the Mascarene Islands to S and SE Asia and the Philippines (Schatz 2001; Kubitzki et al. 2011). It was resolved as sister to the African clade of *Zanthoxylum* (Appelhans et al. 2018a), but in another study it was found to be sister to a main Asian clade (M.S. Appelhans, unpublished). An increased sampling of *Z. asiaticum* specimens throughout its distribution is needed in future phylogenetic analyses to determine whether this taxon originated in Africa and migrated to Asia, or vice versa, and which area is the source for the Malagasy populations.

The Malagasy and Mascarene species of *Melicope* have been resolved as monophyletic within *Melicope* section *Lepta* (Lour.) T.G.Hartley although several internal nodes had low support. They are deeply nested within this species-rich (about 234 species) and widespread genus (Kubitzki et al. 2011) and their closest relatives are taxa from mainland S and SE Asia as well as from Borneo (Appelhans et al. 2014). The split of the Malagasy and Mascarene lineage from their closest relatives has been dated to the Pliocene (Appelhans et al. 2014, 2018b). While Appelhans et al. (2014, 2018b) did not sample a crucial species from India and taxon sampling from Sumatra was low, an Asian origin for the Malagasy and Mascarene species is evident, but further studies are needed to evaluate whether the source area was India, SE Asia or Malesia and whether a single long-distance dispersal event or a stepping-stone dispersal via Indian Ocean archipelagos led to the presence of *Melicope* in Madagascar and the Mascarene Islands.

The sister position of *Ivodea* to *Vepris*, with the Malagasy *V. elliotii* resolved as sister to the African *Vepris* species, suggests the importance of Madagascar in the origin and evolution of the *Ivodea*–*Vepris* clade. The two earliest

diverged clades within the *Ivodea–Vepris* clade from Madagascar (Figs. 1, 2, 3) suggest an origin of the group in Madagascar and one migration event to Africa, which led to the species-rich African clade of *Vepris*. A less parsimonious alternative hypothesis is an African origin of the *Ivodea–Vepris* clade followed by two independent migration events from Africa to Madagascar. A denser sampling of *Vepris* from Madagascar and the Mascarenes, and an inclusion of the two species from Arabia and India, are needed for a conclusive assessment. The African–Malagasy–Indian (S Asian) disjunct distribution has been reported in other plant lineages (Raven and Axelrod 1974), e.g. in *Cyphostemma* (Vitaceae, Hearn et al. 2018; Wen et al. 2018) and *Exacum* (Gentianaceae, Yuan et al. 2005; Pirie et al. 2015). The closest relatives of the *Ivodea–Vepris* lineage (Figs. 1, 2, 3; Groppo et al. 2008) might be a large SE Asian, Malesian and Australasian clade consisting of the *Boronia* Sm. alliance, the *Bosistoa* F.Muell. alliance and the *Euodia* alliance (sensu Kubitzki et al. 2011). Given these relationships, it is conceivable that the ancestor of *Vepris* and *Ivodea* might have dispersed from SE Asia, Malesia or Australasia to Madagascar and Africa. Alternatively, the species-rich SE Asian, Malesian and Australasian clade might have its origin in Madagascar or Africa. A well-supported backbone phylogeny is needed to elucidate the directionality of this dispersal event. The split of *Vepris* from the *Boronia–Bosistoa–Euodia* group has been dated to the Late Paleocene to Early Eocene (Appelhans et al. 2012). During these epochs, the migration of the Indian tectonic plate, and islands such as the Seychelles, which presumably existed as stepping stones (Schatz 1996; Yoder and Nowak 2006; Warren et al. 2010; Scotese 2014), might have facilitated dispersal. Further research with enhanced taxon sampling of the Malagasy *Vepris* species is needed in order to infer the directionality of the dispersal events. If our hypothesis about the SE Asian, Malesian or Australasian origin of the *Ivodea–Vepris* clade proves to be correct, the original hypothesis of a relationship of *Ivodea* with SE Asian taxa would be confirmed even though the closest relatives are not *Euodia* or *Melicope* (Capuron 1961).

Conclusions

Our analyses suggest a sister-group relationship of *Ivodea* and *Vepris*. A close relationship of *Ivodea* with the largely Asian and Pacific genera *Euodia* and *Melicope*, as was suggested in previous, morphology-based studies (Capuron 1961; Rabarimanarivo et al. 2015), is not supported in our study. Based on our sampling, the two early-diverged lineages within the *Ivodea–Vepris* clade represent Malagasy species, while the remainder of the clade consists of African species. The two sister clades of *Ivodea–Vepris* consist of

Asian taxa or taxa with an Asian origin, although the support for these relationships is moderate to low. These results suggest that the *Ivodea–Vepris* clade may have originated in Asia and initially colonized Madagascar, followed by dispersal of *Vepris* to Africa. Species of *Vepris* from Arabia and India (one species each; Kubitzki et al. 2011) could not be sampled in this study. Future studies including these species will shed more light on the origin of this clade. Of the other Malagasy genera of Rutaceae, *Cedrelopsis* and one of the two Malagasy lineages of *Zanthoxylum* are inferred to have an African origin, while *Melicope* originated in Asia. *Fagaropsis* has an African affinity, but it remains unclear whether the ancestor of the genus colonized Madagascar first (likely from Eurasia) followed by dispersal to Africa or whether Africa was colonized first. Further studies are needed to assess the monophyly and geographic origin of *Chloroxylon* and *Zanthoxylum asiaticum*. Both taxa have a large and disjunct distribution, and sampling throughout their distribution is needed for deeper insights into their evolutionary diversification.

Compliance with ethical standards

References

Appelhans MS, Smets E, Razafimandimbison SG, Haevermans T, van Marle EJ, Rabarison H, Couloux A, Randrianarivelojosia M, Keßler PJA (2011) Phylogeny, evolutionary trends, and classification of the *Spathelia/Ptaeroxylon* clade: morphological and

molecular insights. Ann Bot (Oxford) 107:1259–1277. https://doi.org/10.1093/aob/mcr076

Appelhans MS, Keßler PJA, Smets E, Razafimandimbison SG, Janssens SB (2012) Age and historical biogeography of the pantropically distributed Spathelioideae (Rutaceae, Sapindales). J Biogeogr 39:1235–1250. https://doi.org/10.1111/j.1365-2699.2012.02686.x

Appelhans MS, Wen J, Wagner WL (2014) A molecular phylogeny of *Acronychia*, *Euodia*, *Melicope* and relatives (Rutaceae) reveals polyphyletic genera and key innovations for species richness. Molec Phylogen Evol 79:54–68. https://doi.org/10.1016/j.ympev.2014.06.014

Appelhans MS, Krohm S, Manafzadeh S, Wen J (2016) Phylogenetic placement of *Psilopeganum*, a rare monotypic genus of Rutaceae (the citrus family) endemic to China. J Syst Evol 54:535–544. https://doi.org/10.1111/jse.12208

Appelhans MS, Reichelt N, Groppo M, Paetzold C, Wen J (2018a) Phylogeny and biogeography of the pantropical genus *Zanthoxylum* and its closest relatives in the proto-Rutaceae group (Rutaceae). Molec Phylogen Evol 126:31–44. https://doi.org/10.1016/j.ympev.2018.04.013

Appelhans MS, Wen J, Duretto M, Crayn D, Wagner WL (2018b) Historical biogeography of *Melicope* (Rutaceae) and its close relatives with a special emphasis on Pacific dispersals. J Syst Evol 56:576–599. https://doi.org/10.1111/jse.12299

Bacon CD, Simmons MP, Archer RH, Zhao LC, Andriantiana J (2016) Biogeography of the Malagasy Celastraceae: Multiple independent origins followed by widespread dispersal of genera from Madagascar. Molec Phylogen Evol 94:365–382

Bayly MJ, Holmes GD, Forster PI, Cantrill DJ, Ladiges PY (2013) Major clades of Australasian Rutoideae (Rutaceae) based on *rbcL* and *atpB* sequences. PLoS ONE 8:e72493

Biswal DK, Debnath M, Konhar R, Yanthan S, Tandon P (2018) Phylogeny and biogeography of carnivorous plant family Nepenthaceae with reference to the Indian pitcher plant *Nepenthes khasiana* reveals an Indian subcontinent origin of Nepenthes colonization in South East Asia during the Miocene epoch. Frontiers Ecol Evol 6:108. https://doi.org/10.3389/fevo.2018.00108

Buerki S, Devey DS, Callmander MW, Phillipson PB, Forest F (2013) Spatio-temporal history of the endemic genera of Madagascar. Bot J Linn Soc 171:304–329

Capuron R (1961) Contribution à l'étude de la ore forestière de Madagascar. III. Sur quelques plantes ayant contribué au peuplement de Madagascar. A. Rutacées nouvelles. 4. *Ivodea* R. Capuron gen. nov. (Rutoideae–Xanthoxyleae–Evodiinae). Adansonia 1:73–82

Chandler MEJ (1961) The lower Tertiary floras of Southern England, I, Paleocene Floras, London Clay Flora (supplement). British Museum, London

Chase MW, Morton CM, Kallunki JA (1999) Phylogenetic relationships of Rutaceae: a cladistics analysis of the subfamilies using evidence from rbcL and atpB sequence variation. Amer J Bot 86:1191–1999

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. Nat Meth 9:772

Engler A (1931) Rutaceae. In: Engler A, Harms H (eds) Die natürlichen Pflanzenfamilien—Band 19a. Wilhelm Engelmann, Leipzig, pp 187–359

Gregor HJ (1989) Aspects of the fossil record and phylogeny of the family Rutaceae (Zanthoxyleae, Toddalioideae). Pl Syst Evol 161:251–265

Groppo M, Pirani JR, Salatino MLF, Blanco SR, Kallunki JA (2008) Phylogeny of Rutaceae based on two non-coding regions from cpDNA. Amer J Bot 95:985–1005. https://doi.org/10.3732/ajb.2007313

Groppo M, Kallunki JA, Pirani JR, Antonelli A (2012) Chilean Pitavia more closely related to Oceania and Old World Rutaceae than to Neotropical groups: evidence from two cpDNA non-coding regions, with a new subfamilial classification of the family. Phytokeys 19:9–29

Hartley TG (1981) A revision of the genus *Tetradium* (Rutaceae). Garden's Bull Singapore 34:91–131

Hartley TG (2001) On the taxonomy and biogeography of *Euodia* and *Melicope* (Rutaceae). Allertonia 8:1–341

Hearn DJ, Evans M, Wolf B, McGinty M, Wen J (2018) Dispersal is associated with morphological innovation, but not increased diversification, in *Cyphostemma* (Vitaceae). J Syst Evol 56:340–359

Higgins SI, Nathan R, Cain ML (2003) Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? Ecology 84:1945–1956

Janssens SB, Groeninckx I, De Block PJ, Verstraete B, Smets EF (2016) Dispersing towards Madagascar: Biogeography and evolution of the Madagascan endemics of the Spermacoceae tribe (Rubiaceae). Molec Phylogen Evol 95:58–66

Katoh K, Toh H (2008) Recent developments in the MAFFT multiple sequence alignment program. Brief Bioinf 9:286–298

Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res 30:3059–3066

Kubitzki K, Kallunki JA, Duretto M, Wilson PG (2011) Rutaceae. In: Kubitzki K (ed) The families and genera of vascular plants, vol. 10. Springer, Berlin, pp 276–356

Labat JN, Pignal M, Pascal O (2005) Deux espèces nouvelles et une combinaison nouvelle chez les Rutaceae de l'Archipel des Comores. Monogr Syst Bot Missouri Bot Gard 104:362–365

Le Roux JJ, Strasberg D, Rouget M, Morden CW, Koordom M, Richardson DM (2014) Relatedness defies biogeography: the tale of two island endemics (*Acacia heterophylla* and *A. koa*). New Phytol 204:230–242

Ling KH, Wang Y, Poon WS, Shaw PC, But PPH (2009) The relationship of *Fagaropsis* and *Luvunga* in Rutaceae. Taiwania 54:338–342

Maddison WP, Maddison DR (2015) Mesquite: a modular system for evolutionary analysis, version 3.40 [online]. Available at: https://mesquiteproject.org. Accessed 15 Apr 2018

Meimberg H, Wistuba A, Dittrich P, Heubl G (2001) Molecular phylogeny of Nepenthaceae based on cladistics analysis of plastid trnK intron sequence data. Pl Biol 3:164–175

Morton CM (2017) Phylogenetic relationships of *Vepris* (Rutaceae) inferred from chloroplast, nuclear, and morphological data. PLoS ONE 12:e0172708. https://doi.org/10.1371/journal.pone.0172708

Morton CM, Telmer C (2014) New subfamily classification for the Rutaceae. Ann Missouri Bot Gard 99:620–641

Nathan R, Schurr FM, Spiegel O, Steinitz O, Trakhtenbrot A, Tsoar A (2008) Mechanisms of long-distance seed dispersal. Trends Ecol Evol 23:638–647

Perrier de la Bâthie H (1950) Rutacées. In: Humbert H (ed) Flore de Madagascar et des Comores 104e familie. Firmin-Didot et Cie, Paris, pp 1–89

Pirie MD, Litsios G, Bellstedt DU, Salamin N, Kissling J (2015) Back to Gondwanaland: can ancient vicariance explain (some) Indian Ocean disjunct plant distributions? Biol Lett 11:20150086

Poon WS, Shaw PC, Simmons MP, But PPH (2007) Congruence of molecular, morphological, and biochemical profiles in Rutaceae: a cladistic analysis of the subfamilies Rutoideae and Toddalioideae. Syst Bot 32:837–846

Rabarimanarivo M, Rakotonirina N, Phillipson PB, Lowry PP II, Labat JN, Pignal M (2015) Révision du genre *Ivodea* Capuron (Rutaceae), endémique de Madagascar et de l'archipel des Comores. Adansonia, sér 3 37:63–102

Rambaut A, Suchard MA, Xie D, Drummond AJ (2014) Tracer v1.6. Available at: https://tree.bio.ed.ac.uk/software/tracer/. Accessed 15 Apr 2018

Raven PH, Axelrod DI (1974) Angiosperm biogeography and past continental movements. Ann Missouri Bot Gard 61:539–673

Razafimandimbison SG, Appelhans MS, Rabarison H, Haevermans T, Rakotondrafara A, Rakotonandrasana SR, Ratsimbason M, Labat J-N, Keßler PJA, Smets E, Cruaud C, Couloux A, Randrianarivelojosia M (2010) Implications of a molecular phylogenetic study of the Malagasy genus *Cedrelopsis* and its relatives (Ptaeroxylaceae). Molec Phylogen Evol 57:258–265. https://doi.org/10.1016/j.ympev.2010.06.023

Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542

Schatz G (1996) Malagasy/Indo-Australo-Malesian phytogeographic connections. In: Lourenço WR (ed) Biogéographie de Madagascar. ORSTOM, Paris, pp 73–83

Schatz G (2001) Generic Tree Flora of Madagascar. Royal Botanic Gardens. Kew & Missouri Botanical Garden, St. Louis

Scotese CR (2014) Atlas of Paleogene Paleogeographic Maps (Mollweide Projection), Maps 8-15, vol. 1. The Cenozoic, PALEOMAP Atlas for ArcGIS. PALEOMAP Project, Evanston

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. Pl Molec Biol 17:1105–1109

Warren BH, Strasberg D, Bruggemann JH, Prys-Jones RP, Thébaud C (2010) Why does the biota of the Madagascar region have such a strong Asiatic flavour? Cladistics 26:526–538. https://doi.org/10.1111/j.1096-0031.2009.00300.x

Waterman PG (2007) The current status of chemical systematics. Phytochemistry 68:2896–2903

Wen J, Lu LM, Nie ZL, Liu XQ, Zhang N, Ickert-Bond SM, Gerrath J, Manchester SR, Boggan J, Chen ZD (2018) A new phylogenetic tribal classification of the grape family (Vitaceae). J Syst Evol 56:262–272

White TJ, Burns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and amplifications. Academic Press, San Diego, pp 315–322

Yoder AD, Nowak MD (2006) Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. Annual Rev Ecol Evol Syst 37:405–431

Yuan Y-M, Wohlhauser S, Moller M, Klackenberg J, Callmander M, Kupfer P (2005) Phylogeny and biogeography of *Exacum* (Gentianaceae): a disjunctive distribution in the Indian Ocean Basin resulted from long distance dispersal and extensive radiation. Syst Biol 54:21–34