How to kill two genera with one tree: clarifying generic circumscriptions in an endemic Malagasy clade of Sapindaceae

SVEN BUERKI[1,2]*, PORTER P. LOWRY II[3,4], SYLVIE ANDRIAMBOLOLONERA[5], PETER B. PHILLIPSON[3,4], LAURA VARY[6] and MARTIN W. CALLMANDER[3,7]

[1]*Department of Biodiversity and Conservation, Real Jardín Botánico, CSIC, Plaza de Murillo 2, 28014 Madrid, Spain*

[2]*Molecular Systematics Section, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3DS, UK*

[3]*Missouri Botanical Garden, P.O. Box 299, St Louis, MO 63166-0299, USA*

[4]*Muséum National d'Histoire Naturelle, Case Postale 39, 57 rue Cuvier, 75231 05 CEDEX, Paris, France*

[5]*Missouri Botanical Garden, Madagascar Research and Conservation Program, B.P. 3391, Antananarivo 101, Madagascar*

[6]*Department of Ecology and Evolutionary Biology, University of California-Irvine, 321 Steinhaus Hall, Irvine, CA 92697, USA*

[7]*Conservatoire et Jardin botaniques de la Ville de Genève, ch. de l'Impératrice 1, CH-1292 Chambésy, Switzerland*

Phylogenetic relationships in a Malagasy clade of Sapindaceae, encompassing *Molinaea* (with members also in the Mascarene Islands), *Neotina*, *Tina* and *Tinopsis*, were inferred by expanding a previous nuclear and plastid DNA data set for the family. The circumscription of these morphologically similar genera has remained problematic since the first family-wide treatment. To investigate this situation, representative taxa were analysed to: (1) test the monophyly of the genera; (2) investigate their phylogenetic relationships; and (3) explore alternative circumscriptions that reflect phylogeny and yield genera that are morphologically coherent and easily characterized. Phylogenetic inferences supported the monophyly of the group and its subdivision into three clades. All species of *Molinaea* sampled belong to a clade (Clade I) that is sister to a clade comprising *Neotina*, *Tina* and *Tinopsis*, within which one clade (Clade II) encompasses *Tinopsis* and *Neotina* (with the latter nested within the former) and another (Clade III) comprises all taxa of *Tina*. These three genera can be easily distinguished from *Molinaea* by having two rather than three carpels, which represents an unambiguous synapomorphy. Given the paraphyly of *Tinopsis* with regard to *Neotina* and the strong support for the monophyly of *Tina*, two potentially viable options are available for the generic delimitation of the taxa in this clade: (1) to recognize two genera corresponding, respectively, to Clades II and III; or (2) to place all of the taxa in a single genus encompassing both clades. Based on a review of morphological evidence the second option is favoured and consequently a broad generic concept is applied. © 2011 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2011, **165**, 223–234.

ADDITIONAL KEYWORDS: Cupania group – *Molinaea* – *Neotina* – phylogenetic inference – taxonomy – *Tina* – *Tinopsis*.

*Corresponding author. E-mail: s.buerki@kew.org

INTRODUCTION

Recent phylogenetic analyses of Sapindaceae inferred from nuclear and plastid sequence data have revealed a high level of para- and polyphyly at the subfamilial, tribal and even generic levels (Harrington *et al.*, 2005; Buerki *et al.*, 2009a). The worldwide Sapindaceae, as circumscribed by the Angiosperm Phylogeny Group (APG II, 2003; APG III, 2009), include *c.* 1900 species in 142 genera and four subfamilies (Xanthoceroideae, Hippocastanoideae, Dodonaeoideae and Sapindoideae) (Buerki *et al.*, 2009a), although Buerki *et al.* (2010a) recently adopted a narrower family circumscription based on molecular, biogeographical, dating and morphological evidence. To accommodate the high level of tribal para/polyphyly, a new informal infra-familial classification was proposed by Buerki *et al.* (2009a), mainly based on molecular evidence, and additional work has been conducted to develop new generic circumscriptions (see Buerki *et al.*, 2010b). These studies have clarified relationships at the family level and made important advances towards an improved classification of Sapindaceae. They have also brought into focus the need for further investigations to identify new synapomorphies that support the groups defined in the molecular analyses and that can provide the basis for developing a formal tribal classification (see Buerki *et al.*, 2009a).

Madagascar is home to a remarkable array of morphological and genetic diversity within Sapindaceae and an exceptional level of endemism (Capuron, 1969). In total, *c.* 100 species in 27 genera are currently recognized in Madagascar, with 11 genera endemic to the island; namely, *Beguea* Capuron, *Chouxia* Capuron, *Conchopetalum* Radlk., *Gereaua* Buerki & Callm*., Neotina* Capuron, *Plagioscyphus* Radlk., *Pseudopteris* Baill., *Tina* Schult., *Tinopsis* Radlk. and *Tsingya* Capuron (Capuron, 1969; Buerki *et al.*, 2010b). In the context of preparing a treatment of the family for the Catalogue of the Vascular Plants of Madagascar (http://www.efloras.org/madagascar), the aim of which is to provide an authoritative taxonomic synthesis of the Malagasy flora, an initial set of revisions has been conducted on several Malagasy genera (e.g. Schatz, Gereau & Lowry, 1999; Buerki *et al.*, 2009b, 2010b) and others are in progress. Phylogenetic analyses have shown that most Sapindaceae present on this large Indian Ocean island (especially those in endemic genera) belong to one of two well-supported clades, referred to as the Macphersonia and Cupania groups (Buerki *et al.*, 2009a). Relationships within the first of these clades, members of which are distributed mainly in Madagascar with some taxa in eastern Africa, were recently investigated by Buerki *et al.* (2010b), whereas the second group, which has a wider distribution, with taxa occurring in Australasia,

South America and Madagascar (Buerki *et al.*, 2009a), has not yet been examined in detail. Within the Cupania group, four genera form a strongly supported clade: *Molinaea* Comm. ex Juss. (with eight species in the Malagasy region: five in Madagascar and three in the Mascarene Islands), *Neotina* (two species), *Tina* (six species) and *Tinopsis* (11 species), the latter three all endemic to Madagascar. These genera are closely related to several South American genera, notably *Cupania* and *Matayba* Aubl. (Buerki *et al.*, 2009a). Although the four Malagasy genera form a monophyletic group, their circumscription and defining characters have been problematic ever since Radlkofer (1933) published the first comprehensive classification for the family and they have posed problems for taxonomists since then (Capuron, 1969; Andriambololonera, 1999).

There has been considerable confusion regarding the definition and circumscription of these four genera (see Capuron, 1969; Acevedo-Rodríguez, 2003), but *Molinaea* can be easily distinguished morphologically from members of the other genera by its three-carpellate gynoecium (vs. two carpels in *Neotina*, *Tina* and *Tinopsis*) (Fig. 1). When Radlkofer (in Durand, 1888) described *Tinopsis*, based on *T. apiculata* Radlk., he distinguished it from *Tina* on the basis of the number of stamens (five vs. eight, respectively), which prompted Choux (1925) to transfer *Tina isoneura* Radlk. to *Tinopsis* as it also has five stamens, although it has a dehiscent fruit characteristic of *Tina* (*Tinopsis* is the only genus with indehiscent fruit; see below and Table 1). Choux (1927) and Radlkofer (1933) subsequently changed their minds and chose to recognise a single genus, *Tina*, because of the absence of strong discriminating morphological characters. In contrast, in his monograph of Malagasy Sapindaceae, Capuron (1969) resurrected *Tinopsis* (in which he described eight new species) and described *Neotina* as a new genus to accommodate *Tina isoneura* because of its unique combination of fruit morphology, number of stamens and lomatorrhizal embryo (a character shared with *Tinopsis*), whereas the embryo of *Tina* is notorrhizal (see Table 1). Although these genera are morphologically similar in many respects, Capuron (1969) assigned them to two different tribes: *Neotina* and *Tina* (and *Molinaea*) were placed in Cupanieae, characterized by a dehiscent fruit with a ceraceous (waxy), coloured (generally orange to pale red) arillode that partially surrounds the seed (in some cases the arillode is somewhat obscure), whereas *Tinopsis* was assigned to Schleichereae, members of which have an indehiscent fruit with a fleshy, translucent arillode surrounding the entire seed (similar to that of the widely cultivated *Litchi chinensis* Sonn.; see Fig. 1 for a summary of fruit morphology).

As mentioned above, the phylogenetic analyses of Buerki *et al.* (2009a, 2010a, b) were in agreement
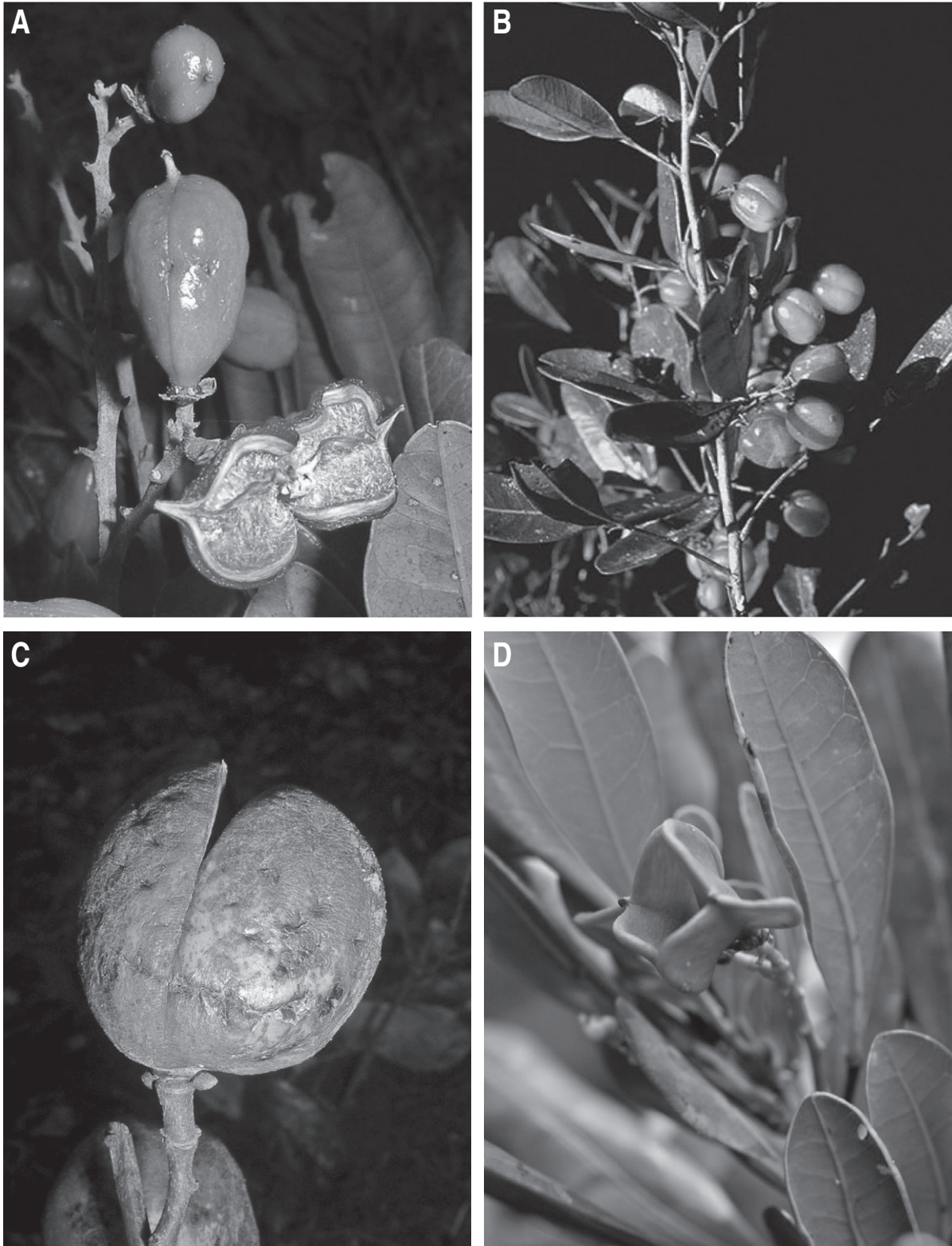
Figure 1. A survey of fruit morphology in representative members of *Molinaea*, *Neotina, Tina* and *Tinopsis*. A, *Tina striata* Radlk. ssp. *striata* (*Buerki 75*; photograph: S. Buerki); B, *Neotina coursii* Capuron (*Malcomber 1293*; photograph: G.E. Schatz); C, *Tinopsis macrocarpa* Capuron (*Buerki 134*; photograph: S. Buerki); D, *Molinaea retusa* Radlk. (*Callmander 572*; photograph: M.W. Callmander).

Table 1. Comparison of the Malagasy genera *Tina*, *Neotina* and *Tinopsis*

	Tina Roemer & Schult.	*Neotina* Capuron	*Tinopsis* Radlk.
Tribe	Cupanieae	Cupanieae	Schleichereae
Phylogenetic grouping	Cupania group	Cupania group	Cupania group
Leaflet	Denticulate (at least in part)	Entire	Entire
Petal scale	Free	Free or united	Free or united
Stamens	(5 or) 6–8 (or 9)	5 (6 or 7)	5 (6 or 7)
Anther	Subcordiform, apiculus and glandular at the apex	Oblong, emarginate and eglandular at the apex	Oblong, emarginate and eglandular at the apex
Stigmatic line	Short	Well developed along the style	Well developed along the style
Fruit	Dehiscent, splitting into two valves that become widely separated	Dehiscent, splitting into two valves that become widely separated	Indehiscent or incompletely splitting into two erect valves
Endocarp	Glabrous or pubescent	Glabrous	Glabrous
Arillode	Not surrounding the entire seed (sometimes reduced or absent), ceraceous, coloured (usually orange or pale red)	Not surrounding the entire seed (sometimes reduced or absent), ceraceous, coloured (usually orange or pale red)	Surrounding the entire seed, fleshy, translucent
Embryo type	Notorrhizal	Lomatorrhizal	Lomatorrhizal

The definition of tribes follows Radlkofer (1933) and the phylogenetic groupings are those of Buerki *et al*. (2009a). Morphological characters were adapted from Capuron (1969).

with the views of Choux (1927) and Radlkofer (1933) with regard to considering *Molinaea*, *Neotina*, *Tina* and *Tinopsis* as closely related genera. In these molecular studies, *Molinaea* was shown to be the sister lineage of the remaining genera (with *Tina* in turn being sister to *Neotina* + *Tinopsis; Buerki et al*., 2009a, 2010a, b). However, because these phylogenetic analyses were based on limited sampling (just one or two exemplars per genus), they do not provide a robust understanding of relationships within this clade. In an attempt to address this deficiency, we have expanded the data set of Buerki *et al*. (2010b) by significantly augmenting the number of taxa within this clade (hereafter referred to as the ingroup) in order to: (1) test the monophyly of the four genera as currently defined; (2) investigate phylogenetic relationships among the members of the ingroup; and (3) explore alternative circumscriptions that reflect phylogeny and yield genera that are morphologically coherent and easily characterized.

MATERIAL AND METHODS
SAMPLING, SEQUENCE DATA AND PHYLOGENETIC ANALYSES

Species names, voucher information and GenBank accession numbers for all sequences are provided in Buerki *et al*. (2010b) except for the taxa added for this study (see Appendix), which include representative species of the Malagasy genera *Molinaea*, *Neotina*, *Tina* and *Tinopsis*, all of which are members of the Cupania group (Buerki *et al*., 2009a). The outgroup sampling included one species of Anacardiaceae (species of *Sorindeia* Thou.; defined as the outgroup in all analyses; Buerki *et al*., 2009a) and one species of Simaroubaceae (*Harrisonia abyssinica* Oliv.). The DNA extraction, amplification and sequencing protocols for the nuclear and plastid regions studied are provided in Buerki *et al*. (2009a). The nuclear sequences include the entire internal transcribed spacer (ITS) region (ITS1, 5.8S and ITS2) and the plastid markers include both coding (*matK* and *rpoB*) and non-coding regions (the *trnL* intron and the intergenic spacers *trnD-trnT*, *trnK-matK*, *trnL-trnF* and *trnS-trnG*).

In earlier phylogenetic studies of Sapindaceae (Buerki *et al*., 2009a, 2010a, b, c), none of the moderately to strongly supported relationships recovered (i.e. with bootstrap support > 75%) showed incongruence between the single-gene analyses performed and a total evidence approach was therefore adopted. As the present study employs an expanded version of the same basic data set, we have again chosen to present the result of our analyses based on a combined data set, using maximum likelihood (ML) and maximum parsimony (MP) criteria, following the same procedure as in Buerki *et al*. (2009a). The parsimony ratchet (Nixon, 1999) was performed using PAUPrat (Sikes & Lewis, 2001). Ten independent searches were performed with 200 iterations and 15% of the parsimony informative characters perturbed. A strict consensus tree was constructed based on the shortest equally

parsimonious trees. To assess support at each node, non-parametric bootstrap analyses (Felsenstein, 1985) were performed using PAUP* (Swofford, 2002) following the same procedure as in Buerki *et al.* (2009a). An ML analysis was performed using RAxML version 7.0.0 (Stamatakis, 2006; Stamatakis, Hoover & Rougemont, 2008) with 1000 rapid bootstrap analyses followed by the search of the best-scoring tree in one single run based on the GTR + G + I model (see Buerki *et al.*, 2009a). These analyses were conducted using the facilities made available by the Vital-IT portal at the Swiss Institute of Bioinformatics (Lausanne, Switzerland; http://www.vital-it.ch/about/).

RESULTS

The ML and MP total evidence trees were highly congruent and revealed the same major groups of Sapindaceae as presented in Buerki *et al.* (2009a, 2010a, b). The most parsimonious tree for the combined analysis was 10 376 steps in length [consistency index (CI) = 0.497 and retention index (RI) = 0.750] and the consensus tree was based on 1269 trees. The best ML tree had a log likelihood of −73 138.44. Statistics for each marker within the ingroup are provided in Table 2 (statistics for the full data set are given in Buerki *et al.*, 2009a, 2010b). As our results are congruent with those of earlier studies of Sapindaceae, i.e. phylogenetic relationships and bootstrap support values (BS) are similar (Fig. 2A), only the ML total evidence tree is discussed below because it contains the maximum amount of phylogenetic information (Fig. 2). Both ML and MP analyses strongly support the monophyly of the ingroup and its position within the Cupania group (BS: 100; Fig. 2). Within the ingroup, three clades can be recognized: Clade I (BS: 100) is sister to Clade II (BS: 65) + Clade III (BS: 98) (Fig. 2B). Clade I exclusively comprises taxa of *Molinaea*. The composition of Clade II suggests that *Tinopsis* is paraphyletic with respect to *Neotina* (Fig. 2B), although relationships are weakly supported and might better be regarded as unresolved. All species belonging to *Tina* are placed in Clade III, within which accessions belonging to *T. striata* appear to be paraphyletic with respect to certain other members of the genus, although some nodes are weakly supported and further analyses will be needed to confirm this finding (Fig. 2B).

DISCUSSION

RELATIONSHIPS WITHIN THE INGROUP

The phylogenetic analyses conducted in this study using significantly expanded ingroup sampling (including 47 specimens representative of ingroup diversity) confirm: (1) the monophyly of the ingroup (BS: 100); (2) its placement within the Cupania group

Table 2. Characteristics of partitions used in the phylogenetic analyses

	ITS	matK	rpoB	trnD-trnT IGS	trnK-matK IGS	trnL intron	trnL-trnF IGS	trnS-trnG IGS	All eight regions
Number of sequences	36	32	43	31	32	46	46	28	47
Alignment length (including outgroup)	783	1089	357	1498	723	529	431	1374	6784
Number of constant characters (%)	653 (83.4)	1034 (94.95)	349 (97.76)	1436 (95.86)	691 (95.57)	499 (94.33)	413 (95.82)	1319 (96.0)	6394 (94.25)
Number of variable characters (%)	130 (16.6)	55 (5.05)	8 (2.24)	62 (4.14)	32 (4.43)	30 (5.67)	18 (4.18)	55 (4.0)	390 (5.75)
Number of potentially parsimony-informative characters (%)	75 (9.58)	20 (1.84)	4 (1.12)	21 (1.4)	15 (2.07)	10 (1.89)	11 (2.55)	27 (1.97)	183 (2.7)

The values reported correspond to the ingroup sampling only. See Buerki *et al.* (2009a) for values related to the entire data set. IGS, intergenic spacer; ITS, internal transcribed spacer.
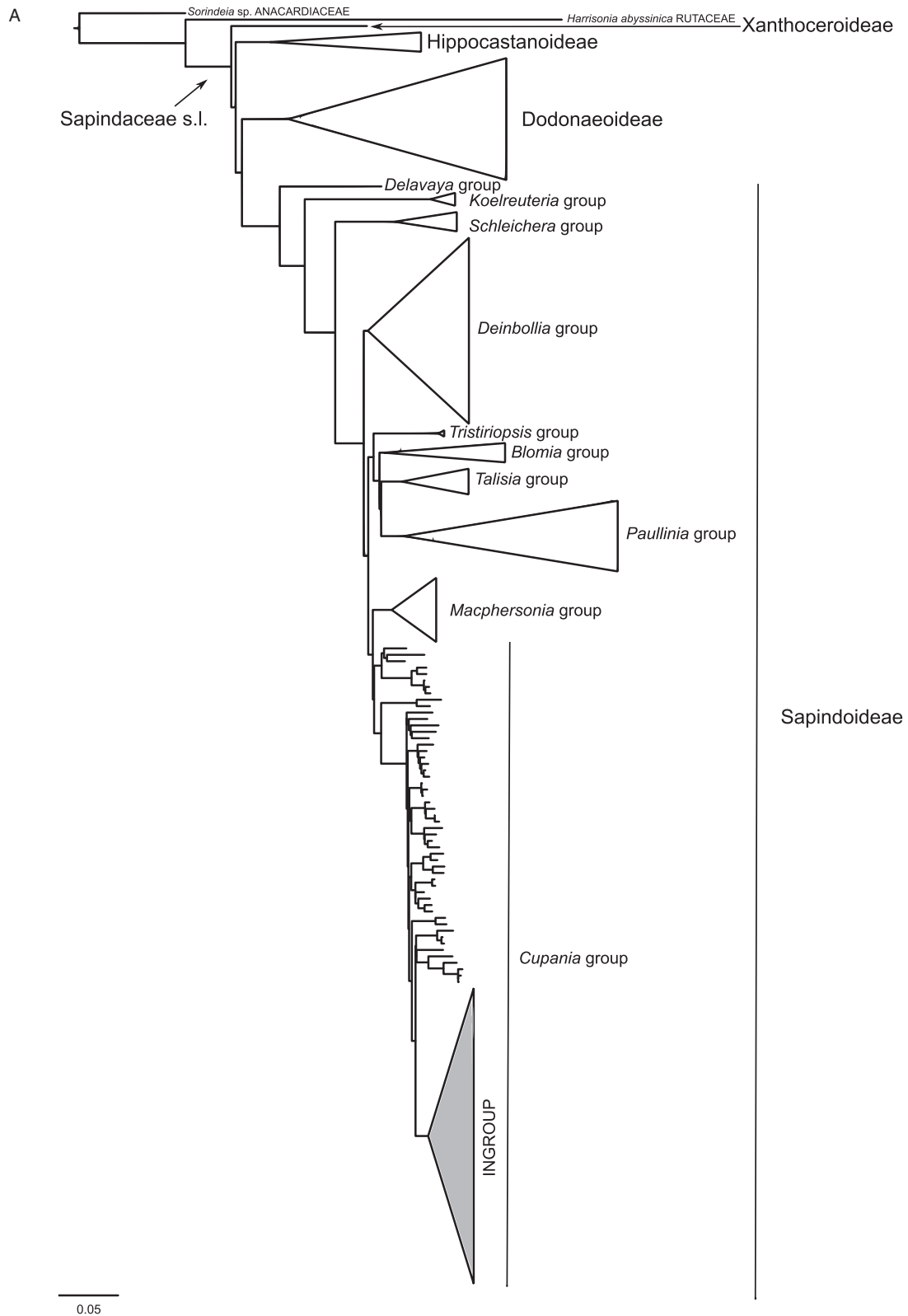
Figure 2. A, best maximum likelihood phylogenetic tree inferred from eight plastid and nuclear markers summarizing relationships within Sapindaceae.

B
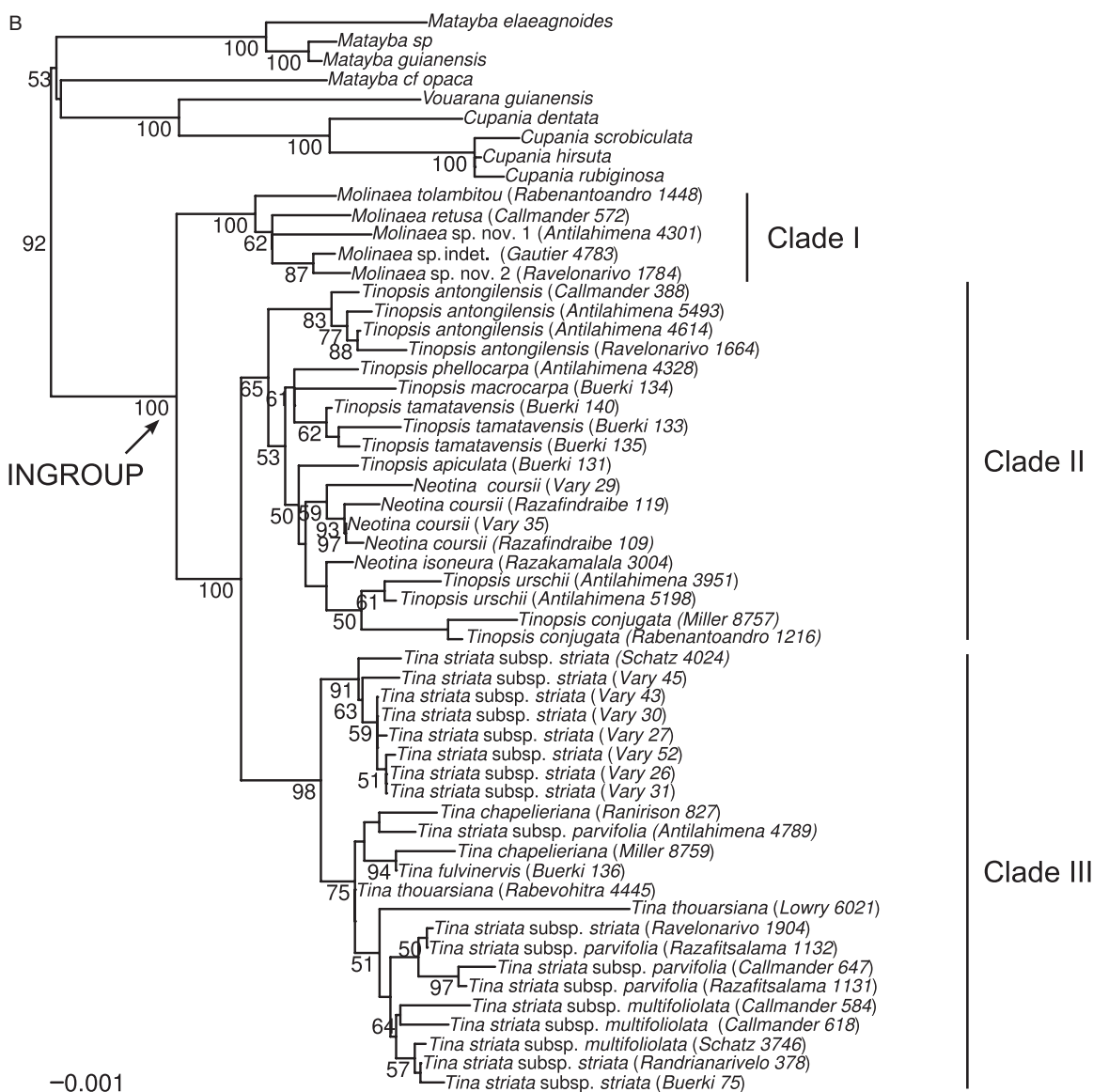
Figure 2. B, phylogenetic relationships within the Malagasy Sapindaceae clade (ingroup). The South American sister clade is also represented and used as the outgroup. Bootstrap supports are indicated above each branch. The classification follows Buerki *et al.* (2009a).

(Fig. 2A); (3) the sister position of *Molinaea* (Clade I; Fig. 2B) to the other genera within the ingroup, as suggested earlier based on much more limited sampling (Buerki *et al.*, 2009a); (4) the monophyly of *Tina* (Clade III); and (5) the close relationship between *Tinopsis* and *Neotina* (Clade II BS: 65; previously suggested by Buerki *et al.*, 2009a; Fig. 2B). Based exclusively on phylogenetic evidence, the monophyly of both *Molinaea* and *Tina* is supported, whereas the status of the two other genera remains problematic. In the following discussion, we will explore the taxonomic implications of the paraphyly of *Tinopsis* (based on morphological evidence) and will attempt to propose a coherent generic treatment.

GENERIC CIRCUMSCRIPTION: RADLKOFER VS. CAPURON

One of the goals of the present study is to improve on the current generic level taxonomy for this group of Malagasy Sapindaceae by proposing an alternative that reflects evolutionary relationships and is supported by easily discernable morphological features. Although Capuron (1969) resurrected *Tinopsis* and described *Neotina* in an attempt to characterize the diversity exhibited by members of this group, he acknowledged that no vegetative or floral features unambiguously distinguished *Tina* from *Tinopsis* and/or *Neotina*. As a consequence, hundreds of sterile

and flowering herbarium specimens remain unassigned to genus, serving as a clear indication of the inadequacies of the current generic framework and providing strong motivation for our efforts to improve on it. Using the phylogenetic hypotheses resulting from our molecular analyses, we have considered alternatives for generic circumscriptions with particular regard to potentially diagnostic morphological characters (see Table 1). Such an approach constitutes the first step towards understanding the evolution of this Malagasy clade and will provide a basis for further investigations focusing, for example, on species delimitations and patterns of morphological character evolution.

Molinaea, which forms a clade sister to all other members of the ingroup (Clade I; Fig. 2B), is characterized by an ovary with three carpels, a feature found in most Sapindaceae, including the South American genera (such as *Cupania* L.; Fig. 2B) that are sister to the clade comprising the genera being studied here (i.e. the ingroup). This suggests that a reduction in the number of carpels took place in a common ancestor of the clade comprising *Neotina*, *Tina* and *Tinopsis* and that this feature thus constitutes a synapomorphy for them (Fig. 2B). Capuron (1969) hypothesized just such a trend and he also argued that, although the Malagasy genera are morphologically similar to those from South America (especially *Cupania*), they are nevertheless sufficiently distinctive and geographically separate to be retained. He further suggested that *Tina*, *Tinopsis* and *Neotina* shared a common ancestor with *Molinaea*, a hypothesis that is strongly supported by our results (Fig. 2B). Among the four ingroup genera, *Molinaea* is also the only one to occur outside Madagascar, with three of the nine described species found in the Mascarene Islands (Capuron, 1969). A taxonomic revision of this genus will soon be completed (M. W. Callmander, P. B. Phillipson and S. Buerki, unpubl. data).

Based on the evidence presented here, *Molinaea* can be comfortably maintained as a well-supported and easily recognized genus, but the status of the three other genera is less clear. Given the paraphyly of *Tinopsis* with regard to *Neotina* in Clade II and the strong support for the monophyly of *Tina* (comprising Clade III; Fig. 2), two potentially viable alternatives are available for the generic delimitation of the taxa in this clade: (1) to recognize two genera corresponding to Clades II and III, respectively; and (2) to place all of the taxa in a single genus encompassing both clades (Fig. 2B; Table 1). Below we will consider the advantages and drawbacks of these alternative classifications.

Option 1 would result in the circumscription of two genera, *Tinopsis* (including *Neotina*) and *Tina*, only the latter of which is well supported by molecular data (BS: 98; Fig. 2B). However, adopting this generic alignment would present practical problems with regard to morphology. A broadened circumscription of *Tinopsis* would include some members with indehiscent fruits and fleshy, translucent arillodes and others (those currently assigned to *Neotina*) with dehiscent fruits and ceraceous, coloured arillodes, a combination of characters also found in *Tina*. Moreover, recent field observations made by the authors have shown that the fresh fruits of the species currently referred to as *Tinopsis macrocarpa* Capuron exhibit a well-defined line of dehiscence that is initiated early in development (Fig. 1), despite the fact that this taxon is nested well within a subclade that otherwise comprises species with indehiscent fruits (Fig. 2B). This finding of homoplasy in fruit dehiscence further calls into question the taxonomic utility of this character, which Capuron (1969) regarded as important for distinguishing genera within the group. Our results also lend support to studies that have revealed a trend of homoplasy in fruit morphology more broadly within the family (Harrington *et al*., 2005; Buerki *et al*., 2009a) and they suggest that the importance attached to fruit structure in the past (e.g. by Radlkofer, 1933; Capuron, 1969) may have been misplaced. Further field investigations may show that the true mode of dehiscence of some members of the family is not fully reflected in what can be observed on dried herbarium specimen, as in the case of *Tinopsis macrocarpa*.

If fruit characters prove to be less informative than once supposed, it will be necessary to identify other attributes that are more reliable for distinguishing major groups within the family. In the case of the Malagasy clade being examined here, several features, including details of the margin of the leaflet, and the number of stamens and stigmatic lines, appear to corroborate the close relationship between *Neotina* and *Tinopsis* revealed by molecular evidence (Table 1) and might thus lend support to option 1 mentioned above. According to Capuron (1969), species of *Tina* have denticulate leaflets, whereas those of the two other genera he recognized have entire leaflets (Table 1). Although this character appears to have diagnostic value for species, it does not correlate well with the current circumscription of genera, contrary to Capuron's assertion. For example, *Tina thouarsiana* (Cambess.) Capuron has entire leaflets, whereas those of *Tina dasycarpa* Radlk. may either be entire or have evident teeth (as observed recently by the authors). With regard to the number of stamens, *Tina* has long been seen as distinctive in having six to eight stamens vs. five in the two other genera (Table 1). However, field observations reported by Capuron (1969), and confirmed by the authors of the present study, clearly show a high level of variability in this character, especially within *Tina* (for a

detailed review see Capuron, 1969), casting doubt over its value for circumscribing genera. Alternatively, the presence and shape of a gland at the apex of the anther appears to have potential for distinguishing *Tinopsis* (including *Neotina*), which has oblong, emarginate anthers without a gland at the apex, from *Tina*, members of which have subcordiform, apiculous anthers with an apical gland (Table 1). The presence of a short stigmatic line in *Tina*, vs. a well-developed stigmatic line that extends along the style in the other genera (Table 1), also seems to support option 1. Finally, Capuron (1969), like Radlkofer (1933) before him, attributed great importance to embryo type, and on this basis he distinguished *Neotina*, with its lomatorrhizal embryo (a character shared with *Tinopsis*), from *Tina*, which has a notorrhizal embryo (Table 1). We have not found any exceptions to this pattern (based on limited observations), but embryo type is not easily observed in the field or on dried specimens, a fact that significantly limits its utility as a generic level character.

Option 2, which would involve placing all the taxa currently assigned to *Neotina*, *Tina* and *Tinopsis* in a single, significantly expanded genus, would be fully consistent with our molecular findings, which provide strong support for this clade (BS: 100). If this option were adopted, the name *Tina* would have nomenclatural priority (Acevedo-Rodríguez, 2003). Circumscribed in this manner, the genus would comprise *c*. 20 species, encompassing all members of the ingroup with a bicarpellate gynoecium, which would constitute a robust and easily observed synapomorphy, unambiguously enabling fertile material of *Tina* to be distinguished from specimens of *Molinaea*. This option thus has a clear practical advantage over the alternative outlined above by avoiding problems involving characters related to the anthers and stigmas, which may be of value for distinguishing the three traditionally recognized genera, but are often particularly difficult to observe in the small flowers produced by these plants, frequently requiring magnification to be certain of which character state is expressed.

Our observations suggest that the floral and vegetative characters mentioned above will prove to be more suitable for distinguishing species within a newly expanded *Tina*. Moreover, they may be of value for clarifying taxonomic limits within the most problematic member of the genus, *T. striata* Radlk., which exhibits a high level of morphological polymorphism (with five subspecies recognized by Capuron, 1969) and appears to be polyphyletic as currently defined (Fig. 2B). An evaluation of the potential utility of these morphological features will be facilitated by making use of the microsatellites recently developed by Vary *et al.* (2009) to investigate species limits within the *T. striata* complex.

It should be noted that the phylogenetic distances between taxa within the Cupania group are quite low, especially compared with other groups of Sapindaceae (Fig. 2A), despite the high level of generic diversity observed in the group (*c*. 32 genera; Buerki *et al.*, 2009a, 2010a, b). The significant non-monophyly found in some of the currently recognized genera (*Cupaniopsis* Radlk., *Guioa* Cav., *Matayba*, *Sarcotoechia* Radlk. and *Tinopsis*) might reflect a historical tendency toward taxonomic over-splitting. If this proves to be the case, then the option we have proposed here for expanding *Tina* to include *Neotina* and *Tinopsis* may be the first of several such modifications to the current generic level taxonomy within the group. Also, we note that further studies (along the same lines as the one presented here) will be needed to test the utility and robustness of these genera. From an evolutionary point of view, the phylogenetic information presented here is consistent with a rapid diversification within the widely distributed Cupania group, the various subgroups of which have members throughout the Tropics (with the sole exception of Africa).

Let's kill two genera with one tree: Radlkofer won!

Based on the results presented above, we recommend that an expanded circumscription of *Tina* be adopted to encompass all bicarpellate members of the Malagasy clade under consideration here (the required taxonomic and nomenclatural changes will be formally published in a forthcoming paper). This approach avoids the problems associated with homoplasy found in fruit features historically emphasized and the difficulty of accurately observing minute and often cryptic floral characters. Moreover, placing *Tinopsis* and *Neotina* in synonymy under *Tina* provides a more practical and easily applied taxonomic framework that is fully consistent with our molecular findings and more easily accommodates material the morphology of which does not conform to historical generic limits.

In view of the rapidly increasing affordability and efficiency of using molecular techniques to elucidate evolutionary relationships, generic circumscriptions and taxonomic revisions should, whenever possible, be based on a strong phylogenetic framework. At the same time, when taxa are circumscribed, they must be supported by unambiguous and easily observable morphological synapomorphies, insofar as possible. This is the approach we have used in proposing our revised circumscription of *Tina* in a way that enables it to be easily recognized and unambiguously distinguished from *Molinaea*, returning to the generic alignment adopted more than 80 years ago by Choux (1925, 1927) and Radlkofer (1933) based solely on morphological evidence.

ACKNOWLEDGEMENTS

REFERENCES

Acevedo-Rodríguez P. 2003. (1592) Proposal to conserve the name *Tina* (Sapindaceae) with a conserved type. *Taxon* **52:** 373–374.

Andriambololonera S. 1999. *Contribution à l'étude du genre endémique Tina Roem. & Schultes (Sapindaceae). Mémoires de Diplôme d'Etudes Approfondies en Sciences Biologiques Appliquées.* Université d'Antananarivo.

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II. *Botanical Journal of the Linnean Society* **141:** 399–436.

APG III. 2009. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Botanical Journal of the Linnean Society* **161:** 105–121.

Buerki S, Forest F, Acevedo-Rodríguez P, Callmander MW, Nylander JAA, Harrington M, Sanmartín I, Küpfer P, Alvarez P. 2009a. Plastid and nuclear DNA markers reveal intricate relationships at subfamilial and tribal levels in the soapberry family (Sapindaceae). *Molecular Phylogenetics and Evolution* **51:** 238–258.

Buerki S, Callmander MW, Lowry PP II, Phillipson PB. 2009b. A synoptic revision of the genus *Lepisanthes* Blume (Sapindaceae) in Madagascar. *Adansonia, Série 3* **31:** 301–309.

Buerki S, Lowry PP II, Alvarez N, Razafimandimbison SG, Küpfer P, Callmander MW. 2010a. Phylogeny and circumscription of Sapindaceae revisited: molecular sequence data, morphology and biogeography support recognition of a new family, Xanthoceraceae. *Plant Ecology and Evolution* **143:** 148–161.

Buerki S, Phillipson PB, Lowry PP II, Callmander MW. 2010b. Molecular phylogenetics and morphological evidence support recognition of *Gereaua*, a new endemic genus of Sapindaceae from Madagascar. *Systematic Botany* **35:** 172–180.

Buerki S, Forest F, Alvarez N, Nylander JAA, Arrigo N, Sanmartín I. 2010c. An evaluation of new parsimony-based versus parametric inference methods in biogeography: a case study using the globally distributed plant family Sapindaceae. *Journal of Biogeography* **38:** 531–550.

Capuron R. 1969. Révision des Sapindacées de Madagascar et des Comores. *Mémoires du Muséum National d'Histoire Naturelle, Série B Botanique* **19:** 1–189.

Choux P. 1925. Les Cupaniées malgaches. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences* **181:** 71–72.

Choux P. 1927. Les Sapindacées de Madagascar. *Mémoires de l'Académie Malgache* **4:** 1–118.

Durand TH. 1888. *Index generum phanerogamorum.* Brussels: Sumptibus Auctoris.

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

Harrington MG, Edwards KJ, Johnson SA, Chase MW, Gadek PA. 2005. Phylogenetic inference in Sapindaceae *sensu lato* using plastid *matK* and *rbcL* DNA sequences. *Systematic Botany* **30:** 366–382.

Nixon KC. 1999. The parsimony ratchet, a new method for rapid parsimony analysis. *Cladistics* **15:** 407–414.

Radlkofer L. 1933. Sapindaceae. In: Engler A, ed. *Das Pflanzenreich IV,* 165 (Heft 98a,h). Leipzig: Verlag von Wilhelm Engelmann, 1–1539.

Schatz GE, Gereau RE, Lowry PP II. 1999. A revision of the Malagasy endemic genus *Chouxia* Capuron (Sapindaceae). *Adansonia, série 3* **21:** 51–62.

Sikes DS, Lewis PO. 2001. *Pauprat.* Storrs: University of Connecticut.

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22:** 2688–2690.

Stamatakis A, Hoover P, Rougemont J. 2008. A rapid bootstrap algorithm for the RAxML web-servers. *Systematic Biology* **57:** 758–771.

Swofford DL. 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4.* Sunderland: Sinauer Associates.

Vary LB, Lance SL, Hagen C, Tsyusko O, Glenn TC, Sakai AK, Weller SG. 2009. Characterization of microsatellite loci from the Malagasy endemic, *Tina striata* Radlk. (Sapindaceae). *Conservation Genetics* **10:** 1113–1115.

APPENDIX

Ingroup voucher information and GenBank accession numbers for taxa used in the phylogenetic analysis (see Buerki *et al.*, 2010b for details on the other taxa). G, Conservatoire et Jardin Botaniques de la Ville de Genève, Switzerland; MO, Missouri Botanical Garden, USA; P, Muséum National d'Histoire Naturelle, France; IGS, intragenic spacer; ITS, internal transcribed spacer.

Taxon	Author	Voucher	Herbarium	ITS	matK	rpoB	trnD-trnT IGS	trnK-matK IGS	trnL intron	trnL-trnF IGS	trnS-trnG IGS	N markers
Molinaea retusa	Radlk.	*Callmander 572*	G	–	HQ399243	HQ399278	HQ399306	HQ399332	HQ399410	HQ399370	HQ399436	7
Molinaea sp. indet.		*Gautier 4783*	G	HQ399202	HQ399227	HQ399257	–	HQ399316	HQ399387	HQ399347	–	6
Molinaea sp. nov. 1		*Antilahimena 4301*	MO	EU720510	EU720662	EU720854	EU720983	EU721099	EU721280	EU721468	EU721578	8
Molinaea sp. nov. 2		*Ravelonarivo 1784*	MO	–	HQ399240	HQ399275	HQ399303	HQ399329	HQ399407	HQ399367	–	6
Molinaea tolambitou	(Camb.) Radlk.	*Rabenantoandro 1448*	MO	EU720554	EU720700	EU720902	EU721007	EU721138	EU721324	EU721512	–	7
Neotina coursii	Capuron	*Razafindraibe 109*	MO	–	HQ399237	HQ399272	HQ399300	HQ399326	HQ399404	HQ399364	HQ399431	7
Neotina coursii	Capuron	*Razafindraibe 119*	MO	EU720543	EU720690	EU720891	EU721002	EU721128	EU721313	EU721501	EU721594	8
Neotina coursii	Capuron	*Vary 29*	P	HQ399203	–	HQ399258	–	–	HQ399388	HQ399348	–	4
Neotina coursii	Capuron	*Vary 35*	P	–	HQ399244	HQ399279	–	HQ399333	HQ399411	HQ399371	–	5
Neotina isoneura	(Radlk.) Capuron	*Razakamalala 3004*	MO	HQ399188	–	HQ399245	HQ399282	–	HQ399374	HQ399334	HQ399414	6
Tina chapelieriana	(Camb.) Kalk.	*Miller 8759*	MO	HQ399197	HQ399224	HQ399253	HQ399289	HQ399313	HQ399383	HQ399343	HQ399420	8
Tina chapelieriana	(Camb.) Kalk.	*Ranirison 827*	MO	EU720520	EU720667	EU720864	EU720986	EU721104	EU721286	EU721474	EU721579	8
Tina fulvinervis	Radlk.	*Buerki 136*	G	–	HQ399239	HQ399274	HQ399302	HQ399328	HQ399406	HQ399366	HQ399433	7
Tina striata subsp. *multifoliata*	Capuron	*Callmander 584*	G	HQ399204	HQ399228	HQ399259	–	HQ399317	HQ399389	HQ399349	–	6
Tina striata subsp. *multifoliata*	Capuron	*Callmander 618*	MO	HQ399209	HQ399232	HQ399264	HQ399296	HQ399321	HQ399394	HQ399354	HQ399427	8
Tina striata subsp. *multifoliata*	Capuron	*Schatz 3746*	MO	HQ399207	–	HQ399262	HQ399294	–	HQ399392	HQ399352	HQ399425	6
Tina striata subsp. *parvifolia*	Capuron	*Antilahimena 4789*	MO	–	HQ399241	HQ399276	HQ399304	HQ399330	HQ399408	HQ399368	HQ399434	7
Tina striata subsp. *parvifolia*	Capuron	*Callmander 647*	MO	HQ399212	HQ399235	HQ399267	HQ399299	HQ399324	HQ399397	HQ399357	HQ399430	8
Tina striata subsp. *parvifolia*	Capuron	*Razafitsalama 1131*	MO	HQ399210	HQ399233	HQ399265	HQ399297	HQ399322	HQ399395	HQ399355	HQ399428	8
Tina striata subsp. *parvifolia*	Capuron	*Razafitsalama 1132*	MO	HQ399211	HQ399234	HQ399266	HQ399298	HQ399323	HQ399396	HQ399356	HQ399429	8
Tina striata subsp. *striata*	Radlk.	*Buerki 75*	G	–	HQ399238	HQ399273	HQ399301	HQ399327	HQ399405	HQ399365	HQ399432	7
Tina striata subsp. *striata*	Radlk.	*Randrianarivelo 378*	MO	HQ399206	HQ399230	HQ399261	HQ399293	HQ399319	HQ399391	HQ399351	HQ399424	8

APPENDIX *Continued*

Taxon	Author	Voucher	Herbarium	ITS	matK	rpoB	trnD-trnT IGS	trnK-matK IGS	trnL intron	trnL-trnF IGS	trnS-trnG IGS	N markers
Tina striata subsp. striata	Radlk.	Ravelonarivo 1904	MO	–	HQ399242	HQ399277	HQ399305	HQ399331	HQ399409	HQ399369	HQ399435	7
Tina striata Radlk. subsp. striata		Schatz 4024	MO	HQ399208	HQ399231	HQ399263	HQ399295	HQ399320	HQ399393	HQ399353	HQ399426	8
Tina striata Radlk. subsp. striata		Vary 26	P	HQ399214	–	–	–	–	HQ399399	HQ399359	–	3
Tina striata Radlk. subsp. striata		Vary 27	P	HQ399215	–	–	–	–	HQ399400	HQ399360	–	3
Tina striata Radlk. subsp. striata		Vary 30	P	HQ399213	–	HQ399268	–	–	HQ399398	HQ399358	–	4
Tina striata Radlk. subsp. striata		Vary 31	MO	HQ399216	–	HQ399269	–	–	HQ399401	HQ399361	–	4
Tina striata Radlk. subsp. striata		Vary 43	P	HQ399217	HQ399236	HQ399270	–	HQ399325	HQ399402	HQ399362	–	6
Tina striata Radlk. subsp. striata		Vary 45	P	EU720509	EU720661	EU720853	–	EU721098	EU721279	EU721467	–	6
Tina striata Radlk. subsp. striata		Vary 52	MO	HQ399218	–	HQ399271	–	–	HQ399403	HQ399363	–	4
Tina thouarsiana	(Camb.) Capuron	Lowry 6021	MO	HQ399205	HQ399229	HQ399260	–	HQ399318	HQ399390	HQ399350	–	6
Tina thouarsiana	(Camb.) Capuron	Rabevohitra 4445	MO	–	–	HQ399280	–	–	HQ399412	HQ399372	–	3
Tinopsis antongilensis	Capuron	Antilahimena 4614	MO	HQ399195	–	HQ399251	HQ399288	–	HQ399381	HQ399341	HQ399419	6
Tinopsis antongilensis	Capuron	Antilahimena 5493	MO	HQ399199	–	–	–	–	–	–	–	1
Tinopsis antongilensis	Capuron	Callmander 388	G	HQ399198	–	HQ399254	HQ399290	–	HQ399384	HQ399344	HQ399421	6
Tinopsis antongilensis	Capuron	Ravelonarivo 1664	MO	HQ399194	–	HQ399250	HQ399287	–	HQ399380	HQ399340	HQ399418	6
Tinopsis apiculata	Radlk.	Buerki 131	G	EU720422	EU720589	EU720744	EU720936	EU721034	EU721180	EU721368	EU721540	8
Tinopsis conjugata	(Radlk.) Capuron	Miller 8757	MO	HQ399196	HQ399223	HQ399252	HQ399307	HQ399312	HQ399382	HQ399342	–	6
Tinopsis conjugata	(Radlk.) Capuron	Rabenantoandro 1216	MO	–	–	HQ399281	–	–	HQ399413	HQ399373	HQ399437	5
Tinopsis macrocarpa	Capuron	Buerki 134	G	HQ399201	HQ399226	HQ399256	HQ399292	HQ399315	HQ399386	HQ399346	HQ399423	8
Tinopsis phellocarpa	Capuron	Antilahimena 4328	MO	HQ399200	HQ399225	HQ399255	HQ399291	HQ399314	HQ399385	HQ399345	HQ399422	8
Tinopsis tamatavensis	Capuron	Buerki 133	G	HQ399191	HQ399221	HQ399247	HQ399284	HQ399310	HQ399377	HQ399337	HQ399416	8
Tinopsis tamatavensis	Capuron	Buerki 135	G	HQ399192	–	HQ399248	HQ399285	–	HQ399378	HQ399338	HQ399417	6
Tinopsis tamatavensis	Capuron	Buerki 140	G	HQ399193	HQ399222	HQ399249	HQ399286	HQ399311	HQ399379	HQ399339	–	7
Tinopsis urschii	Capuron	Antilahimena 3951	MO	HQ399190	HQ399220	HQ399246	HQ399283	HQ399309	HQ399376	HQ399336	HQ399415	8
Tinopsis urschii	Capuron	Antilahimena 5198	MO	HQ399189	HQ399219	–	–	HQ399308	HQ399375	HQ399335	–	5