Phylogeny of the Turneraceae clade (Passifloraceae s.l.): Trans-Atlantic disjunctions and two new genera in Africa

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Abstract Turneraceae, with just over 200 species in 10 genera, is today often included in a widely circumscribed Passifloraceae. The vast majority of the species are found in the New World, whereas generic diversity is largest in the Old World. According to current circumscriptions, three of the genera show trans-Atlantic disjunctions: *Turnera* with over 135 species in America and two species in Africa (one in the south-western and one in the north-eastern part), *Piriqueta* with 44 species in America and one in southern Africa, and *Erblichia* with one species in Central America and four in Madagascar. The phylogeny of Turneraceae is reconstructed based on DNA sequences from plastid *trnL-F* and nuclear ITS and sampling for all genera, including both New and Old World species for the trans-Atlantic groups to test their monophyly. The genera of Turneraceae form a strongly supported monophyletic group, the Turneraceae clade, within Passifloraceae s.l. The phylogeny is geographically structured, with one clade comprising American species only, except for the two African species of *Turnera*, and another clade with all other African species plus the Central American *Erblichia odorata*. *Turnera* is retrieved as monophyletic with the two African species appearing as close relatives of *T. ulmifolia*, the type of *Turnera*. The existence of a trans-Atlantic disjunction in *Turnera* is therefore supported. It is most likely caused by long-distance dispersal and estimated to be not older than late Miocene. In *Piriqueta* only the American species are supported as a monophyletic group, whereas the single African species is resolved as a member of the African clade. The trans-Atlantic disjunction in *Piriqueta* is therefore not supported and the African species is proposed to be placed in a genus of its own, *Afroqueta* gen. nov., as *Afroqueta capensis* comb. nov. *Erblichia* on Madagascar is supported as sister to *Mathurina*, a genus endemic to Rodrigues Island in the Mascarenes, and does not group with *E. odorata* in Central America, the type of *Erblichia*. The trans-Atlantic disjunction in *Erblichia* is therefore not supported either and the genus *Arboa* gen. nov. is proposed to accommodate the four Malagasy species, *Arboa integrifolia* comb. nov., *Arboa berneriana* comb. nov., *Arboa madagascariensis* comb. nov., and *Arboa antsingyae* comb. nov.

Keywords *Afroqueta*; *Arboa*; *Erblichia*; molecular phylogenetics; Passifloraceae; *Piriqueta*; taxonomy; trans-Atlantic disjunctions; *Turnera*; Turneraceae; typification

Supplementary Material Figures S1 and S2 (in the Electronic Supplement) and the alignment files are available in the Supplementary Data section of the online version of this article (http://www.ingentaconnect.com/content/iapt/tax).

■ INTRODUCTION

Turneraceae in its traditional sense comprises just over 200 species in 10 genera (Arbo, 2007; Arbo & Espert, 2009): *Adenoa* Arbo (one species, south-eastern Cuba), *Erblichia* Seem. (five species, Central America and Madagascar), *Hyalocalyx* Rolfe (one species, south-east tropical Africa and Madagascar), *Loewia* Urb. (one species, north-east tropical Africa), *Mathurina* Balf. f. (one species, Rodrigues Island in the Mascarenes), *Piriqueta* Aubl. (45 species, tropical America and southern Africa), *Stapfiella* Gilg (six species, tropical Africa), *Streptopetalum* Hochst. (six species, tropical Africa), *Tricliceras* Thonn. ex DC. (16 species, tropical Africa) and *Turnera* L. (ca. 140 species, tropical America and Africa). The vast majority of the species in the group are found in the New World, whereas generic diversity is largest in the Old World.

A remarkable fact is that, according to current circumscriptions, three of the genera show trans-Atlantic disjunctions: *Turnera* with over 135 species in America and two species in Africa (Fig. 1A), *Piriqueta* with 44 species in America and one in southern Africa (Fig. 1B), and *Erblichia* with one species in Central America and four in Madagascar (Fig. 1C). None of these disjunct distributions has previously been tested in molecular-based phylogenetic studies.

Since its publication by Candolle (1828), Turneraceae has generally been recognized as a family in the order Violales (or superorder Violanae), closely related to Passifloraceae and Malesherbiaceae (e.g., Melchior, 1964; Dahlgren, 1980; Cronquist, 1988; Takhtajan, 1997). However, Hutchinson (1973) placed it along with Loasaceae in Loasales. APG (1998) included Violales in Malpighiales, but proposed no other changes for this group of families. Soltis & al. (2000) used sequence
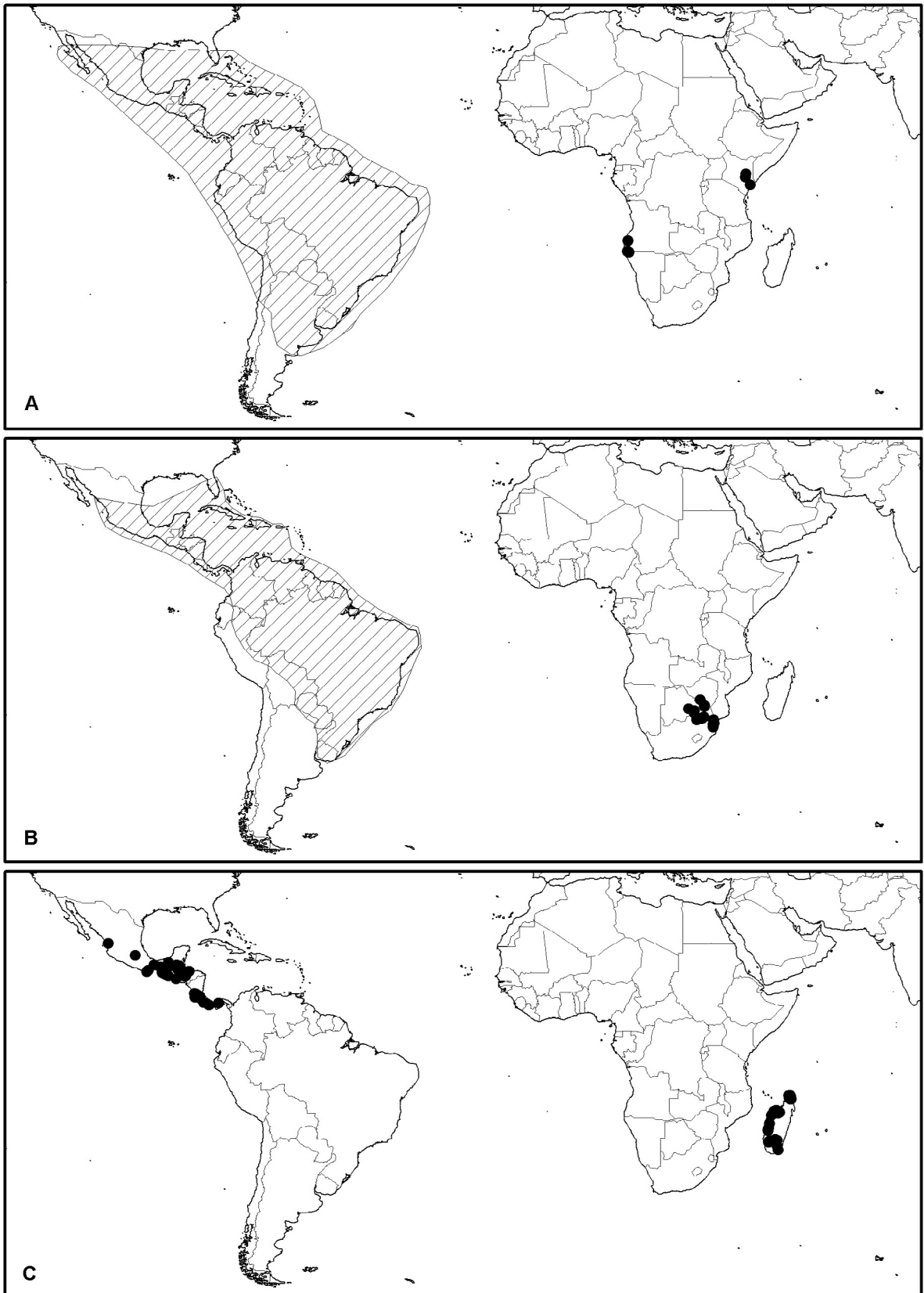
Fig. 1. Trans-Atlantic disjunctions in Turneraceae. **A,** Distribution of *Turnera* with over 135 species in America (hatched) and two in Africa; **B,** distribution of *Piriqueta* with 44 species in America (hatched) and one in Africa; **C,** distribution of *Erblichia* with one species in Central America and four in Madagascar.

data from *Turnera ulmifolia* (type of *Turnera* and hence of Turneraceae) in large-scale analyses of angiosperm phylogeny, which confirmed the close relationship between Turneraceae, Malesherbiaceae and Passifloraceae.

Chase & al. (2002), in a molecular study based on *rbcL* sequences and focusing on Malpighiales, again included *Turnera ulmifolia*. *Turnera* was found to be sister to *Malesherbia* Ruíz & Pávon and these two genera together were sister to a clade with representatives of three genera of Passifloraceae, all five genera together forming the "Passifloraceae clade". Based on this result, APG II (APG, 2003) proposed a wider circumscription of Passifloraceae, with optional recognition of Turneraceae and Malesherbiaceae.

Davis & Chase (2004), in a study of Malpighiales based on both plastid and nuclear genes, again with *Turnera ulmifolia* representing Turneraceae, found *Malesherbia* to be sister to *Turnera* plus *Passiflora* L. The same topology was retrieved in the study by Korotkova & al. (2009) based on sequences of the *petD* group II intron, but with Turneraceae represented by *Turnera grandidentata* (Urb.) Arbo. Wurdack & Davis (2009) included *Turnera ulmifolia* and *Tricliceras longipedunculatum* from Turneraceae in a study of Malpighiales based on sequence data from 13 gene regions, again retrieving *Malesherbia* as sister to a clade with *Passiflora* and *Paropsia* Noronha ex Thouars in Passifloraceae as sister to *Turnera* plus *Tricliceras*. APG III (APG, 2009) retained a broadly circumscribed Passifloraceae, but now without optional recognition of Turneraceae and Malesherbiaceae.

Davis & al. (2005), in a broad study of Malpighiales focusing on the origin of modern tropical rain forests, also included members of the Passifloraceae clade. They estimated the age of the Passifloraceae crown clade to more or less coincide with the Cretaceous-Tertiary boundary.

In this paper we present for the first time a molecular phylogeny of Turneraceae, with sampling from all genera, seven of which have previously not been included in any molecular study, and including representatives from both the New and the Old World for the trans-Atlantic groups. The main questions we ask are: (1) do the members of Turneraceae in its traditional sense form a monophyletic group and how are they related to each other, and (2) are the genera with trans-Atlantic disjunctions monophyletic? If the answer to (2) is yes, the existence of the trans-Atlantic disjunctions would be supported. If the answer is no, the trans-Atlantic disjunctions would be just artefacts based on a misleading taxonomy and changes in generic circumscriptions would be needed.

■ MATERIALS AND METHODS

Taxon sampling. — We sampled from all 10 currently recognized genera of Turneraceae in its traditional sense (members of *Adenoa*, *Erblichia*, *Hyalocalyx*, *Loewia*, *Mathurina*, *Stapfiella* and *Streptopetalum* were sequenced for the first time). In genera with trans-Atlantic disjunctions (*Erblichia*, *Piriqueta*, *Turnera*), we included representatives from both the New and Old World. A total of 28 species of Turneraceae

was included in the analyses and a data matrix with plastid *trnL-F* and nuclear ribosomal ITS sequences was assembled.

Outside Turneraceae species of *Malesherbia* (formerly Malesherbiaceae) and *Basananthe* Peyr., *Paropsia* and *Passiflora* from Passifloraceae s.str. were sampled. *Humiria* Aubl. (Humiriaceae) and *Populus* L. (Salicaceae) were outgroups from Malpighiales based on Chase & al. (2002). Accession numbers and vouchers for all sequences used are summarized in the Appendix.

DNA extraction, amplification and sequencing. — Total DNA from silica-gel-dried and herbarium material was extracted using a slightly modified version of the 2× CTAB method of Doyle & Doyle (1987) and purified using the QIAquick purification kit (Qiagen, Sollentuna, Sweden) following the manufacturer's protocol. Two markers were used for this study, the nuclear ribosomal internal transcribed spacer (ITS) and the plastid *trnL-F*, which both have been shown to be useful for inferring phylogenetic relationships within several angiosperm families. The entire ITS region (including the 5.8S gene) of all newly studied species was amplified and sequenced using the protocols outlined in Alejandro & al. (2005). For amplification and sequencing of the *trnL-F* region the protocols are described in Razafimandimbison & Bremer (2002). In all PCRs, one reaction was run with water only as a negative control. PCR products were purified using the Multi-Screen PCR-plates (Millipore, Solna, Sweden) according to the manufacturer's protocol. Cycle sequencing was done using the Dye Terminator Cycle Sequencing Kit v.1.0 or with the DYEnamic ET terminator Cycle Sequencing Kit (GE Healthcare, Uppsala, Sweden). The sequencing reactions were subsequently run on a MegaBACE 1000 capillary machine.

Phylogenetic analyses. — Sequence fragments were assembled and edited using the Sequencher v.3.1.1 (Gene Codes Corp., Ann Arbor, Michigan, U.S.A.) software package. For each species all new sequences and the published ones from GenBank were aligned using the computer program CLUSTAL X (Thompson & al., 1997) to produce an initial alignment that was manually adjusted using the software Se-Al v.2.0 (Rambaut, 1996). Gaps were treated as missing data in all alignments. The aligned matrices are available as supplementary data to the online version of this article.

To investigate the monophyly of the traditional Turneraceae within Passifloraceae s.l., the 28 sequences of members of Turneraceae were analysed with parsimony bootstrapping, implemented in PAUP* v.4.0b10 (Swofford, 2002), along with sequences of *Malesherbia*, *Basananthe*, *Paropsia* and *Passiflora*, using *Humiria* and *Populus* as outgroups. Due to alignment problems of the ITS sequences of *Humiria* and *Populus* with those of the ingroup, only *trnL-F* sequences could be used for this analysis. The following settings were used: 1000 replicates, MULTREES option on, tree bisection-reconnection (TBR) branch swapping, and five random addition sequences.

To investigate the phylogeny within Turneraceae in more detail, *trnL-F* and ITS sequences were analysed separately and in combination, using parsimony (PAUP*) for the separate analyses and both parsimony and Bayesian inference (MrBayes v.3.1, Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck,

2003) for the combined analysis. One species each of *Malesherbia*, *Paropsia* and *Passiflora* were used as outgroups in these analyses.

A combined parsimony analysis of the 32 terminals was subsequently performed using the following settings: heuristic search option, 5000 random sequence additions, TBR branch swapping, and MULTREES option on. The consistency index (CI) and retention index (RI) were calculated (all uninformative characters excluded) to estimate homoplasy. Bootstrap (BS) values were computed using heuristic searches, with MULTREES on, TBR, five random additions, and 1000 replicates to assess relative support of retained clades. A strict consensus tree was produced from the resulting most parsimonious trees (MPT) saved from the combined analysis.

A combined Bayesian analysis of sequence data from the ITS and *trnL-F* regions of the 32 terminals was performed using MrBayes. For each single gene dataset, the best performing nucleotide substitution model was selected using the computer program MrModeltest v.2.0 (Nylander, 2004). In the latter, the best performing evolutionary model was estimated under three different model selection criteria: Akaike information criterion (AIC) (Akaike, 1973), AICc (a second order AIC, necessary for small samples) and the Bayesian information criterion (BIC; Schwartz, 1978). The combined Bayesian analysis was conducted with four independent Markov chains run for 10×10^6 Metropolis-coupled Markov chain Monte Carlo (MCMC) generations, with tree sampling every 1000th generations. The temperature coefficient of the chain-heating scheme was set to 0.15. We partitioned the combined datasets into two partitions, each with the GTR+G+I model, suggested as best fit to the data under all three criteria (AIC, AICc, BIC). Partitions were unlinked so that each dataset was allowed to have its own parameter values. Flat prior probabilities were specified according to suggestions produced by the software MrModeltest v.2.0 (Nylander, 2004). Stationarity and convergence of runs, as well as the correlation of split frequencies between the runs were checked using the program AWTY (Nylander & al., 2008). We checked the effective sample size (ESS) of parameters using the program Tracer v.1.4 (Rambaut & Drummond, 2007). The combined Bayesian analyses were run twice using different random starting trees to evaluate the convergence of the likelihood values and posterior probabilities. Trees sampled from the first 5×10^6 generations were discarded, because they represented the burn-in stage of the analysis. All saved trees (after excluding the burn-in) from the two independent runs were pooled for a consensus tree.

Divergence time estimation. — Divergence times were estimated using BEAST v.1.6.1 (Drummond & Rambaut, 2007) on the combined partitioned dataset with a Yule speciation tree prior, the GTR+G+I model, and an uncorrelated relaxed lognormal clock. BEAST was set to run an MCMC for 10 million generations, with tree sampling every 1000th generation. The trace files were analyzed in Tracer v.1.4 (Rambaut & Drummond, 2007) to check for convergence and to determine the 95% highest posterior density (HPD) interval for the estimated divergence dates. The first 1000 trees were discarded as burn-in. A separate analysis in which BEAST only sampled from the

prior distributions was run to check if the data was informative (Drummond & al., 2006). The tree was calibrated using a normally distributed age prior of 66 million years (Ma) (with a standard deviation of 2.5) assigned to the Passifloraceae s.l. crown clade, based on the age estimate by Davis & al. (2005).

■ RESULTS

Details of the datasets and resulting trees for the parsimony analyses are presented in Table 1. A total of 62 sequences were used, of which 57 were newly generated for this project and are published here (see Appendix).

trnL-F **dataset. —** The *trnL-F* dataset yielded 1933 positions, of which 355 were parsimony-informative (Table 1). A bootstrap tree from the analysis of the *trnL-F* data is shown in Fig. 2. Passifloraceae s.l. is retrieved with strong support (100% BS), with *Malesherbia* as weakly supported sister to the rest of the family (52% BS). Within Passifloraceae there is strong support for a Turneraceae clade (98% BS), comprising representatives from all genera previously referred to Turneraceae.

Combined dataset. — Separate parsimony bootstrap analyses of the *trnL-F* and the ITS data produced trees with a similar topology (Figs. S1–S2). Visual inspection of the trees showed no well-supported conflict between them and, accordingly, we merged the *trnL-F* and ITS data for combined analyses.

The combined dataset yielded 2471 positions, of which 551 were parsimony-informative (Table 1). A parsimony analysis of the combined data produced 24 most parsimonious trees. The topology of the strict consensus tree was also entirely congruent with that of the Bayesian majority-rule consensus tree shown in Fig. 3 from a Bayesian analysis of the same combined dataset. Therefore, both bootstrap support from the parsimony analysis and Bayesian posterior probabilities are shown on the tree in Fig. 3. The Turneraceae clade comprises two main clades, one (the American clade) moderately supported (0.98 PP, 70% BS) with *Adenoa*, all sampled members of *Turnera*, and all sequenced American members of *Piriqueta*, and the other (the African clade) strongly supported (1.00 PP, 99% BS) with *Erblichia odorata* and all remaining African groups.

In the American clade *Turnera* is weakly supported as monophyletic (0.91 PP, 57% BS) with the two African species,

Table 1. Comparison of *trnL-F* and combined ITS/*trnL-F* datasets and resulting trees for parsimony analyses of the Turneraceae clade.

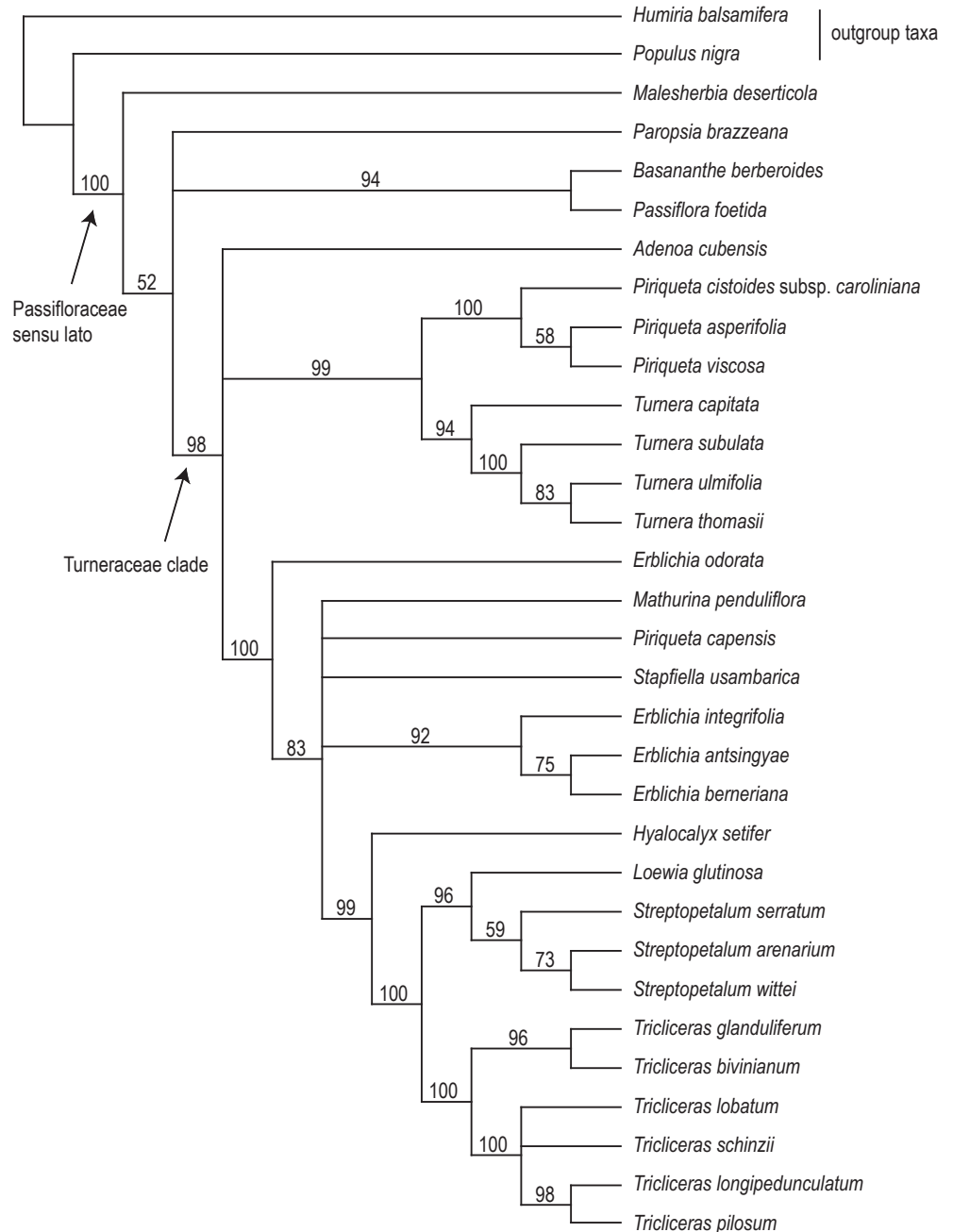|	*trnL-F*	Combined ITS/*trnL-F*
Number of sequences	32	60
Lengths of aligned matrices	1933	2471
Parsimony-informative characters	355	551
Lengths of most parsimonious trees	778	1703
Consistency index	0.665	0.543
Retention index	0.799	0.721

T. oculata and *T. thomasii*, with strong support (1.00 PP, 100% BS) in a clade also comprising *T. ulmifolia* and *T. subulata*. The clade with the American members of *Piriqueta* is strongly supported (1.00 PP, 100% BS) as is the clade with *Turnera* and *Piriqueta* together.

In the African clade the Central American *Erblichia odorata* is retrieved with weak support (0.99 PP, 53% BS) as sister to a clade with all sequenced African members of Turneraceae except *Turnera oculata* and *T. thomasii*. However, the sampled Malagasy species of *Erblichia* form a strongly supported clade (1.00 PP, 100% BS), which is resolved as sister to the Rodriguan *Mathurina* (1.00 PP, 96% BS). Within Malagasy *Erblichia*,

E. integrifolia is sister to *E. antsingyae* plus *E. berneriana*. The single African species of *Piriqueta*, *P. capensis*, is strongly supported (1.00 PP, 99% BS) as member of a clade with the African genera *Stapfiella*, *Hyalocalyx*, *Loewia*, *Streptopetalum* and *Tricliceras*. *Streptopetalum* and *Tricliceras* are retrieved as monophyletic with strong support (respectively, 1.00 PP, 95% BS and 1.00 PP, 100% BS), and *Loewia* and *Streptopetalum* together form a strongly supported clade (1.00 PP, 90% BS).

Divergence time estimates. — The divergence time estimates resulted in an age of (39.8–)51.4(–63.0) Ma for the Turneraceae clade. The American clade was estimated to be (26.8–)37.6 (–49.3) Ma and the African clade (22.6–)32.3(–42.5) Ma. The

Fig. 2. Maximum parsimony bootstrap consensus tree from the *trnL-F* analysis. Bootstrap support is shown above branches.

crown clade with *Mathurina* and Malagasy *Erblichia* was dated at (7.0–)14.6(–23.3) Ma and diverged from its potential sister group, *E. odorata*, (12.7–)22.5(–33.4) Ma. The unresolved Afro-American *Turnera* clade with *T. oculata*, *T. thomasii*, *T. subulata* and *T. ulmifolia* was estimated to be (2.8–)5.6(–9.6) Ma.

■ DISCUSSION

According to the results of the analysis of the *trnL-F* sequence data (Fig. 2), the genera of Turneraceae form a strongly supported monophyletic group, the Turneraceae clade, within Passifloraceae s.l. This group has no commonly recognized formal name in the current classification of Passifloraceae, although both Turneroideae and Turnereae are available. There seem to be no clear morphological synapomorphies for the Turneraceae clade, but heterostyly (Shore & al., 2006) is common (not present in other Passifloraceae) and the stigmas are more or less distinctly penicillate. The position of *Malesherbia* as sister to the rest of Passifloraceae s.l. is in agreement with the results of the studies by Davis & Chase (2004), Korotkova & al. (2009) and Wurdack & Davis (2009). According to the divergence time estimates, the Turneraceae clade dates back at least to the Eocene (mean age estimate ≈51.4 Ma)
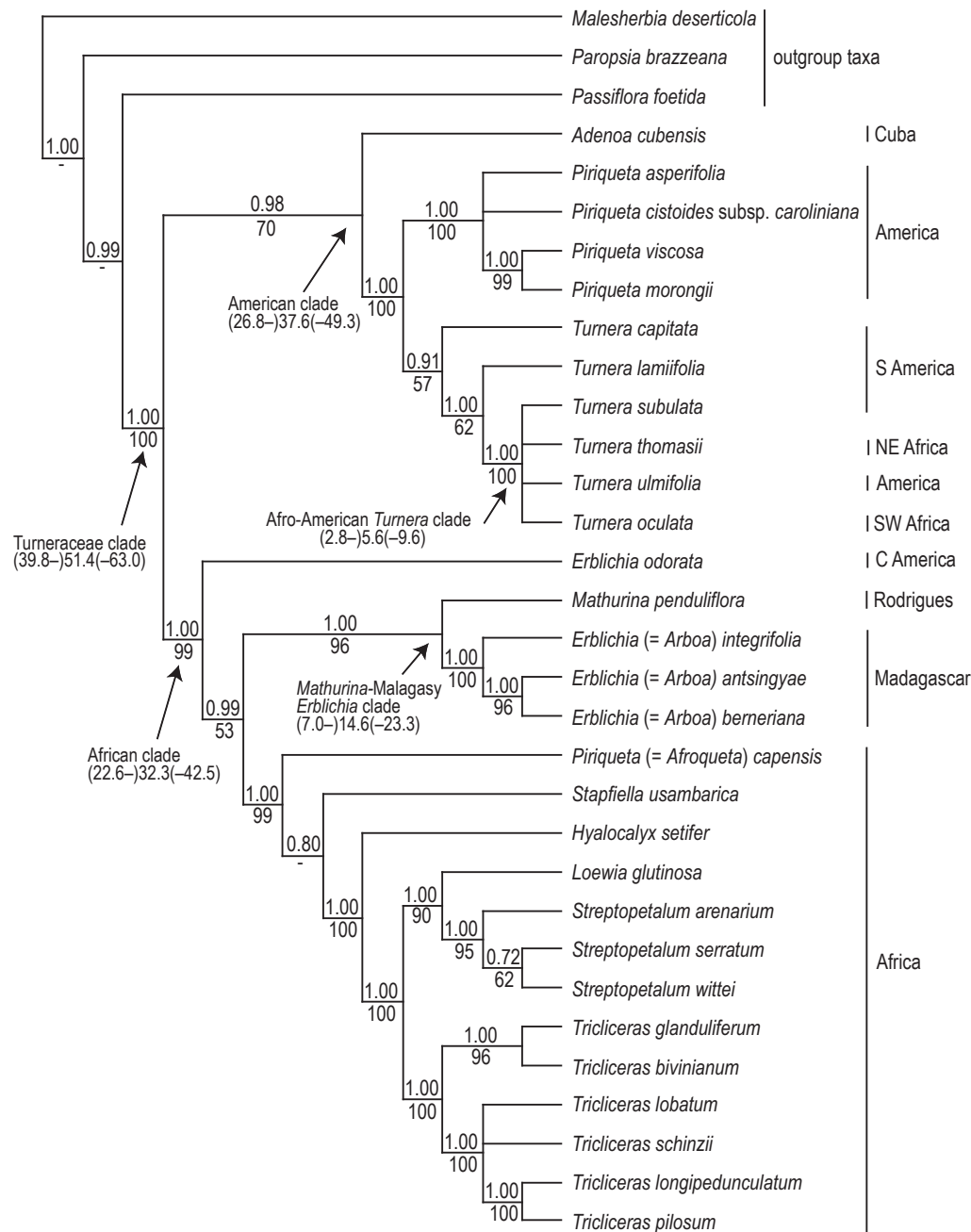

Fig. 3. Bayesian majority-rule consensus tree from the combined *trnL-F*/ITS analysis with Bayesian posterior probabilities shown above and bootstrap support below branches. For each named clade the mean age estimate (in Ma) with 95% HPD interval is given.

The phylogeny of the Turneraceae clade as retrieved in the analyses of the combined dataset (Fig. 3) is strongly geographically structured. The species-rich American clade (mean age estimate ≈37.6 Ma) with *Adenoa*, *Piriqueta* and *Turnera* is morphologically supported by having flowers with a 10-nerved calyx tube (Arbo, 1995, 2007). The African clade (mean age estimate ≈32.3 Ma), with the African/Malagasy/Mascarenian members of Turneraceae (except the two African species of *Turnera*) plus the Central American *Erblichia odorata* has, in contrast, either calyx tubes with more than 10 nerves, or flowers with practically free sepals.

Probably the Turneraceae clade has an American origin. *Malesherbia* is entirely American, Passifloraceae in general is most diverse in America, and within the Turneraceae clade itself, the *Adenoa-Piriqueta-Turnera* clade is almost entirely American, and *Erblichia odorata* is restricted to Central America.

Turnera is weakly supported as monophyletic (Fig. 3), with *T. capitata* as sister to the rest of the genus. This agrees with the topology retrieved in the study by Truyens & al. (2005), where ITS sequences of 37 species or infraspecific taxa of *Turnera* were analysed.

The African species *T. oculata* and *T. thomasii* are found in a strongly supported clade (mean age estimate ≈5.6 Ma) with the tropical American species *T. subulata* and *T. ulmifolia* (type of *Turnera*). The existence of a trans-Atlantic disjunction in *Turnera* (Fig. 1A) is therefore supported, which is in agreement with the results of the phylogenetic analysis based on morphological characters by Arbo & Espert (2009), although the allopolyploid *T. ulmifolia* was excluded in that study. The two African species are closely related, although not retrieved as sisters (Fig. 3), and a single introduction to Africa may have given rise to the two species that are found there today. According to the age estimate the trans-Atlantic disjunction in *Turnera* would date back to the Pliocene or near the Miocene-Pliocene boundary, and we regard long-distance dispersal as the most likely explanation. The similar trans-Atlantic disjunction in *Thamnosma* Torr. & Frém. (Rutaceae) studied by Thiv & al. (2011) was dated to the Miocene and also believed to be caused by long-distance dispersal.

The African species of *Turnera* are confined to arid habitats in two widely separated areas, *T. oculata* in south-western Angola and north-western Namibia and *T. thomasii* in eastern Kenya. This disjunction between south-western and north-eastern Africa indicates that the two species are most likely relics of a so called "arid corridor" distribution (Thulin & al., 2012). An arid corridor linking south-western and north-eastern Africa has been postulated for the Miocene (Caujapé-Castells & al., 2001), as well as for the repeated dry glacial periods of the Pleistocene (Bellstedt & al., 2008). The trans-Atlantic *Thamnosma*, mentioned above, also shows an arid corridor disjunction in Africa (Thiv & al., 2011), where the diversification between the southern African and north-east African members was dated to the Miocene. The age of the African disjunction in *Turnera* is apparently not older than late Miocene.

As for *Piriqueta*, the American species only (including the type, *P. cistoides*) are supported as a monophyletic group

(Fig. 3). The single African species, *P. capensis* in southern Africa, is instead firmly placed as a member of the African clade. The trans-Atlantic disjunction of *Piriqueta* (Fig. 1B) is therefore an artefact of an incorrect generic delimitation and a new taxonomic position for *P. capensis* is needed.

Already Urban (1883), when transferring *Turnera capensis* to *Piriqueta*, indicated its difference from the American species of this genus by placing it in his *P.* sect. *Erblichia* (Seem.) Urb., along with the Malagasy species *P. berneriana* (Tul.) Urb. (= *Erblichia berneriana* (Tul.) Arbo) and *P. madagascariensis* (O. Hoffm.) Urb. (= *E. madagascariensis* O. Hoffm.), and the Central American *P. odorata* (Seem.) Urb. (= *E. odorata* Seem.). These species were differentiated from other members of *Piriqueta* primarily by having practically free sepals, but Urban also noted their indumentum of simple hairs compared to the variable indumentum including also stellate hairs in the other species.

Arbo (1995) in her revision of *Piriqueta* placed *P. capensis* as the sole member of *P.* sect. *Africana* Arbo, whereas all American species were placed in *P.* sect. *Piriqueta*. Arbo pointed again to the practically free sepals and indumentum of simple hairs of *P. capensis*, and added the presence of foliar nectaries in *P. capensis* as a further difference from the American species. The deviating indumentum of *P. capensis* compared to that of the other species of *Piriqueta* was stressed also by Gonzalez & Arbo (2004).

In our analyses *P. capensis* never groups with the American species of *Piriqueta*, but instead always appears with strong support as a member of the African clade. In the combined analyses (Fig. 3) it is weakly supported as sister to a clade with *Stapfiella*, *Hyalocalyx*, *Loewia*, *Streptopetalum* and *Tricliceras*. Morphologically it differs markedly from all these genera by having a corona (petals ligulate in *Tricliceras*), albeit very reduced, and by its almost free sepals. Its isolated position in the phylogeny and unique morphology support its placement in a genus of its own (see below).

Stapfiella, *Loewia*, *Streptopetalum* and *Tricliceras* are all confined to the African mainland, but the single species of *Hyalocalyx* is distributed in both south-east tropical Africa and Madagascar. It obviously represents an introduction to Madagascar from mainland Africa.

Within the African clade there is strong support for *Loewia* as sister to *Streptopetalum*. *Loewia* was considered monospecific by Thulin (1993), the north-east Somali *L. microphylla* (Chiov.) Roti Mich. being just a depauperate form of *L. glutinosa*, and *L. tanaensis* Urb. in eastern Kenya being probably conspecific as well. A sister-group relationship between *Loewia* and *Streptopetalum* has not previously been suggested, but we think the two genera should be retained also in the future. *Loewia* differs markedly from *Streptopetalum*, for example, by being distinctly woody shrubs (not herbs or subshrubs) with an indumentum of mostly stellate (not simple and glandular) hairs.

The trans-Atlantic disjunction in *Erblichia* (Fig. 1C) is not supported either. The Malagasy species are strongly supported (Fig. 3) as sister to *Mathurina* on the geographically neighbouring Rodrigues Island (situated ca. 1500 km E of

Madagascar), whereas *Erblichia odorata* (type of *Erblichia*) in Central America is weakly supported as sister to all other members of the African clade. The mean age estimate for the crown clade with *Mathurina* and Malagasy *Erblichia* is ≈14.6 Ma (95% HPD 7.0–23.3 Ma). In an alternative topology retrieved with weak support by BEAST (and also weakly supported in the analysis of the ITS data only; Fig. S1), *Erblichia odorata* is instead sister to *Mathurina* plus the Malagasy species of *Erblichia*, the clade having a mean age estimate of ≈22.6 Ma.

The strong biogeographical link between Rodrigues and Madagascar detected here seems to be unparalleled in other plant groups. Rodrigues is a volcanic island with an age of ≈1.5 Ma, whereas the submarine Rodrigues Ridge is dated to 7.5–11 Ma (Duncan, 2009). Therefore, *Mathurina* apparently represents an example of an island lineage that is considerably older than the island on which it occurs. Other examples of this phenomenon in the Mascarenes are *Monimia* Thouars (Monimiaceae) on Mauritius and Réunion (Renner & al., 2010) and perhaps *Roussea* Sm. (Rousseaceae) on Mauritius (Lundberg, 2001).

As *Erblichia* as currently circumscribed is retrieved as polyphyletic, taxonomic change is needed. According to the topology in Fig. 3, the Malagasy species could either be placed in a genus of their own or be included in *Mathurina* that resembles *Erblichia* by having free or almost free sepals (Arbo, 2007) and homostylous flowers (Shore & al., 2006). However, the Malagasy species of *Erblichia* differ from *Mathurina* by having, for example, homophyllous leaves, erect flowers and fruits, flowers with corona, and seeds with a lobed or undivided aril. *Mathurina*, in contrast, has heterophyllous leaves, pendulous flowers and fruits, no corona, and numerous minute seeds with the aril divided into thin threads. The pendulous capsules of *Mathurina* open in dry weather (M. Thulin, pers. obs.) to release the seeds that are dispersed by wind, aided by the long threads of the aril. Wind dispersal is unique for *Mathurina* in the Turneraceae clade. In the Malagasy species of *Erblichia*, as in most other members of the Turneraceae clade, the seeds apparently drop to the ground and are dispersed by ants that are attracted by the aril. Because of the profound morphological differences between the Malagasy species of *Erblichia* and *Mathurina*, we see no point in expanding the latter, but instead propose a taxonomic change, where the Malagasy species of *Erblichia* are accommodated in a genus of their own.

Erblichia odorata differs from the Malagasy species previously accommodated in *Erblichia* by having petals with a basal ligule only, but without an annular corona. It is pollinated by hummingbirds (Arbo, 2007), and is apparently unique in the Turneraceae clade by having seeds retained in the woody capsules and dispersed by seed-eating birds attracted by the orange arils (M. Thulin, pers. obs.).

The alternative topology retrieved by BEAST and the ITS data (see above), where *Erblichia odorata* is sister to *Mathurina* plus the Malagasy species of *Erblichia*, would imply that *Erblichia* could be circumscribed widely to include all these taxa. However, the support for this alternative is weak, and even

if it had been strongly supported we would, for morphological reasons, have preferred to place the Malagasy species of *Erblichia* in a genus of their own.

The weakly supported phylogenetic position of *E. odorata* in Central America as sister to the rest of the African clade (or alternatively as sister to *Mathurina* plus the Malagasy species of *Erblichia*) indicates a not previously detected trans-Atlantic disjunction. It has an age of ≈32.3 Ma (or ≈22.5 Ma with the alternative topology), dating back at least to the Oligocene (or the Miocene). In either case, it would be older than the trans-Atlantic disjunction seen in *Turnera*, and would reflect an older dispersal event from America to Africa.

■ TAXONOMIC IMPLICATIONS

Afroqueta Thulin & Razafim., **gen. nov.** – Type: *A. capensis* (Harv.) Thulin & Razafim.

Differs from *Piriqueta* by its indumentum of simple unicellular hairs (without stellate or glandular hairs), leaves with submarginal glands near the base of the lamina beneath, and calyx of almost free sepals.

Perennial herbs or subshrubs, with indumentum of simple unicellular hairs. Leaves alternate, simple, sessile or shortly petiolate, pinnately veined, with submarginal glands near base of lamina beneath; stipules reduced to minute subulate glands. Flowers axillary, solitary, usually heterostylous; pedicel articulated, with 2 bracteoles. Calyx of 5 almost free sepals, with quincuncial aestivation. Petals 5, yellow, unguiculate, contorted in bud; corona present, reduced to tufts of curly hairs at base of sepals and petals. Stamens 5, antesepalous, inserted at the base of the sepals; filaments free; anthers dorsifixed, with longitudinal dehiscence. Ovary superior, 3-carpellate, 1-locular, with several ovules on parietal placentae; styles 3, connivent at the base; stigmas shortly penicillate. Fruit a subglobose capsule, 3-valved, with loculicidal dehiscence from the apex to the base. Seeds slightly curved, reticulate-areolate; aril membranous, unilateral, with subentire to crenulate margin, whitish.

A genus of a single species in south-central and southern Africa. The name is derived from *Piriqueta*, the American genus to which the single species of *Afroqueta* was previously referred. *Piriqueta* is of uncertain etymology, and the meaning of the name was not explained by Aublet (1775) in the protologue.

In *Piriqueta* the corona is annular with a membranous base and a fimbriate upper margin (Arbo, 1995). However, in *Afroqueta* no membranous part can be detected, but tufts of curly hairs (Fig. 4E) are present at the base of sepals and petals that we interpret as a reduced corona.

Afroqueta capensis (Harv.) Thulin & Razafim., **comb. nov.** ≡ *Turnera capensis* Harv., Fl. Cap. 2: 599. 1862 ≡ *Piriqueta capensis* (Harv.) Urb. in Jahrb. Königl. Bot. Gart. Berlin 2: 78. 1883 – Type: South Africa, Transvaal, Apjes River, *Burke 152* (TCD, lectotype, TCD0017316, digital image!; K!, PRE, SAM, isolectotypes).
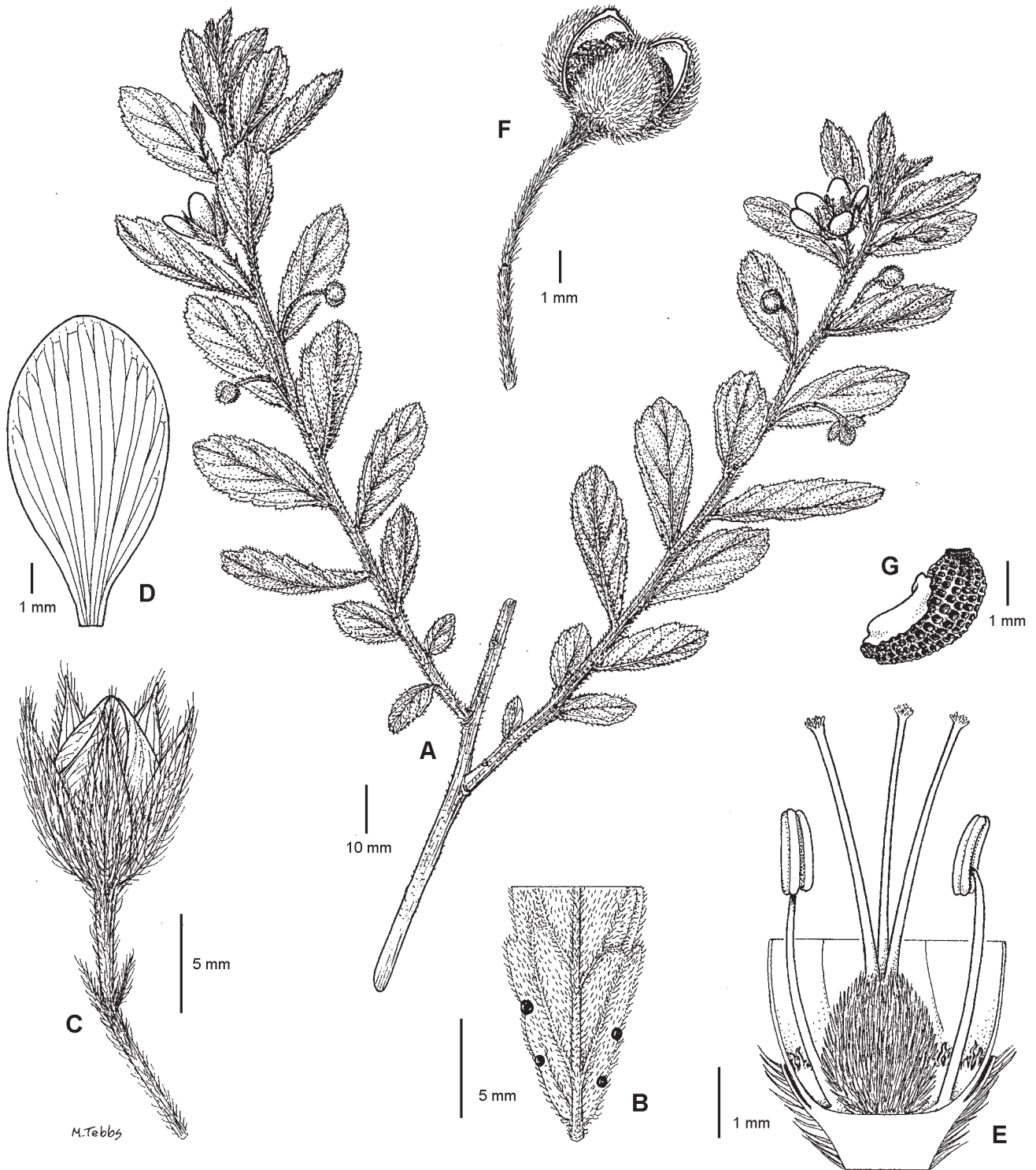
Fig. 4. *Afroqueta capensis*. **A,** Flowering and fruiting branch; **B,** base of leaf, lower surface, showing glands; **C,** flower bud; **D,** petal; **E,** portion of flower, showing corona at base of sepals and petals, two of the stamens and the pistil; **F,** capsule; **G,** seed. — A and B from *Marques 2348* (K), C–E from *Balsinhas 157* (K), F and G from *Drummond 5927* (K). Drawn by Margaret Tebbs.

In the protologue of *Turnera capensis*, Harvey (1862) cited "*Burke & Zeyher*" from "Aapje's river" in "Herb. Hook. D." as type. "Herb. Hook." here refers to the Hooker Herbarium and "D." to the Dublin Herbarium (now TCD), and "*Burke & Zeyher*" in TCD was cited as a holotype by Obermeyer (1976). The label information on the single sheet present in TCD is "*Burke 152* Ex Herb. Hooker. Apjes Rivier S. Africa". As *Burke 152* is also present in Herbarium Hookerianum at K, Obermeyer's action is here regarded as a lectotypification according to the *ICBN*, Art. 9.8 (McNeill & al., 2006).

Perennial herb or subshrub, up to 1 m tall, but mostly less than 30 cm; stems pilose with ± spreading hairs. Leaves elliptic to oblong or narrowly obovate, 1–5 × 0.5–1.5(–2) cm, densely pubescent on both sides, acute to obtuse at the apex, cuneate at the base and sometimes shortly petiolate, margin serrate, with 1–3(–4) pairs of sessile subcircular glands 0.5–1 mm in diameter near the base. Flowers on 4–30 mm long pubescent pedicels; bracteoles subulate, 1–2 mm long. Sepals lanceolate-oblong, 7–13 × 2–3 mm, apiculate, pubescent, 3-nerved; calyx-tube less than 1 mm long. Petals 6–12 × 3–6 mm, narrowly obovate, obtuse. Corona of ca. 0.5 mm long hairs. Stamens 3–4.5 mm long in long-styled flowers, 6–8 mm long in short-styled flowers; filaments glabrous, very narrowly triangular; anthers 0.75–1.5 mm long. Ovary ca. 2 mm long, pubescent; styles 3.5–6 mm long in long-styled flowers, ca. 2 mm long in short-styled flowers. Capsule 4–7 mm long, often pendulous on ± curved pedicels, pubescent. Seeds 3–3.5 mm long; aril ca. 2/3 the length of the seed. — Figure 4.

Distribution and habitat. – *Afroqueta capensis* is known from southern Zimbabwe, southern Mozambique, Botswana, Swaziland, and from Transvaal and northern Natal in South Africa (Fig. 5). It occurs in open woodland and bushland, often in stony places, at 300–1100 m above sea level.

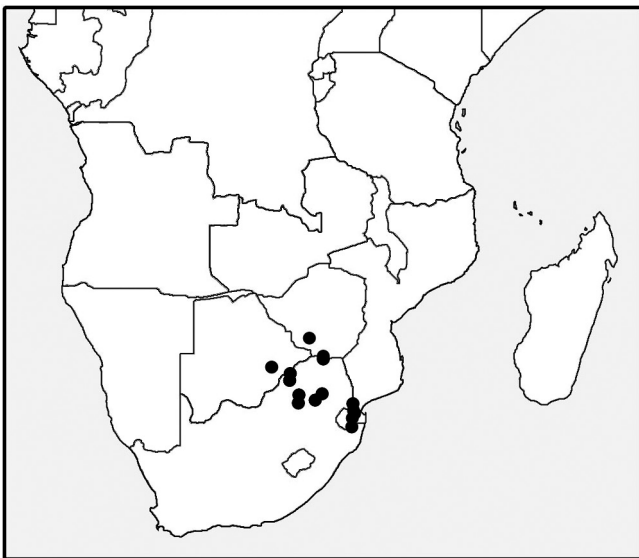*Representative collections examined.* – BOTSWANA: ca. 3 km N of Mosolotshane along road to Serowe, 25 Jan. 1983,

Fig. 5. Distribution of *Afroqueta capensis*.

Blomberg & al. BMP 113 (UPS). MOZAMBIQUE: Lourenço Marques, Namaacha, Fonte de Goba, 11 Nov. 1971, *Marques 2348* (BM, K). SOUTH AFRICA: Transvaal, Loskop Dam on road to "The Hell", 28 Nov. 1963, *Codd & Verdoorn 10361* (K). SWAZILAND: Stegi Distr., Palata, 11 Jan. 1962, *Compton 31209* (K). ZIMBABWE: Beitbridge Distr., ca. 8 km N of Shashi-Limpopo confluence, 22 Mar. 1959, *Drummond 5927* (K, P).

Taxonomic remarks. – According to Fernandes (1978) the plants of *Afroqueta capensis* (as "*Piriqueta capensis*") in Mozambique differ from the ones in Zimbabwe by having an indumentum with longer and more appressed hairs, and sessile leaves with larger more acute and closely spaced teeth and glands nearer the leaf margin. However, the variation is continuous and no taxonomical subdivision of the species seems warranted. Fernandes (1978) further noted that among 10 studied collections from Mozambique, six comprised only long-styled plants, two were mixtures of short-styled and long-styled plants, one included only short-styled plants, whereas one collection was homostylous.

Arboa Thulin & Razafim., **gen. nov.** – Type: *A. integrifolia* (Claverie) Thulin & Razafim.

Differs from *Erblichia* by having an annular corona (not just ligulate petals), seeds only loosely attached (not retained in capsule) and with whitish (not orange) aril, and from *Mathurina* by its homophyllous (not heterophyllous) leaves, erect (not pendulous) flowers and fruits, corolla with corona, and seeds with lobed or undivided aril (aril not divided into thin threads).

Shrubs or trees, with indumentum of simple unicellular hairs or glabrous, simple multicellular hairs present in leaf axils. Leaves alternate, simple, shortly petiolate or with a petiole-like base, pinnately veined, with glands on the margin; stipules small, ± caducous. Flowers axillary, solitary, erect, homostylous; pedicel articulated, with 2 bracteoles. Calyx of 5 almost free sepals, with quincuncial aestivation. Petals 5, yellow to orange-red or red, free, unguiculate, contorted in bud; corona present, annular, ± distinctly lobed, with fimbriate margin. Stamens 5, antesepalous; filaments free; anthers subbasifixed, with longitudinal dehiscence. Ovary superior, 3-carpellate, 1-locular, with numerous ovules on parietal placentae; styles 3, ± divergent at the base; stigmas shortly fimbriate to penicillate. Fruit an erect capsule, granulate outside, 3-valved, with loculicidal dehiscence from the apex. Seeds only loosely attached (not retained in capsule), slightly curved, striate-scrobiculate; aril membranous, ± enveloping the seed, lobed or undivided with ± lacerate margin, whitish.

A genus of four species, restricted to Madagascar. The name is given in honour of Maria Mercedes Arbo, since long a prominent student of Turneraceae, who in 1979 revised the species now included in *Arboa*.

Arbo (2007: 459) stated that a free corona is present in *Mathurina* and *Erblichia*, and presence of a corona in *Mathurina* is also implied in her key to the genera of Turneraceae (Arbo, 2007: 462). However, this is a mistake, there is no corona in *Mathurina* and in *Erblichia* a free corona is only found in *E. integrifolia*.

Key to the species of *Arboa*

1. Flower buds and sepals obtuse; anthers 1.5–2 mm long, obtuse; ovary shortly stipitate; corona free; scars of floral parts forming a 2–3 mm long stipe-like part below the fruit .... 1. *A. integrifolia*
1. Flower buds and sepals acute or acuminate; anthers longer, apiculate at the apex; ovary sessile; corona inserted at the base of petals and sepals; scars of floral parts forming a narrow band below the fruit 2
2. Leaves 13–20 cm long, elliptic, narrowing below into a petiole-like base with a pair of projecting ca. 3 mm wide cup-shaped glands in upper part .... 4. *A. antsingyae*
2. Leaves up to 11 cm long, mostly obovate, shortly petiolate, without or with much smaller projecting glands near the base 3
3. Leaves up to 5 cm long; sepals 8–15 mm long; anthers 2.5–5.5 mm long.......................... 2. *A. berneriana*
3. Leaves, at least most of them, more than 5 cm long; sepals 18–28 mm long; anthers 5–10 mm long .... 3. *A. madagascariensis*

1. ***Arboa integrifolia*** (Claverie) Thulin & Razafim., **comb. nov.** ≡ *Paropsia integrifolia* Claverie in Ann. Mus. Colon. Marseille, sér. 2, 7: 66. 1909 ≡ *Piriqueta integrifolia* (Claverie) Capuron in Adansonia, sér. 2, 3: 135. 1963 ≡ *Erblichia integrifolia* (Claverie) Arbo in Adansonia, sér. 2, 18: 467. 1979 – Type: Madagascar, "bois sablonneaux des environs de Majunga", Nov. 1903, *Perrier de la Bâthie 1629* (P!, lectotype, P00077562, designated here; K!, P!, isolectotypes).
= *Piriqueta mandrarensis* Humbert in Notul. Syst. (Paris) 12: 125. 1946 – Type: Madagascar, "basin de reception de la Mananara, affluent de Mandrare, pentes occidentales des montagnes entre l'Andohahelo et l'Elakelaka entre Ampahiso et Mahamavo", Jan. 1934, *Humbert 13790* (P!, lectotype, P00077564, designated here; K!, P!, isolectotypes).

Shrub or small tree; young branchlets ± densely pubescent with ± yellowish hairs. Leaves narrowly elliptic to lanceolate or obovate, 2.5–11 × 1–5.5 cm, ± densely pubescent on both surfaces, paler beneath, obtuse to acuminate at the apex, narrowing below into a short petiole, margin shallowly crenulate to serrulate, the teeth gland-tipped, sometimes with a pair of larger glands, ca. 0.7 mm in diam., at base of lamina; lateral veins 4–11 pairs. Floral buds obtuse; pedicel 5–20 mm long, articulated in lower part, pubescent; bracteoles 1–7 mm long, pubescent. Sepals 5.5–10 × 2.5–4 mm, pubescent, obtuse; corona 1–1.5 mm high, free, ± distinctly 10-lobed, inserted at base of ovary. Stamens inserted at base of ovary; filaments 6.5–14 mm long; anthers 1.5–2 mm long, obtuse. Ovary 3–4 mm long, with up to 0.5 mm long stipe at the base; styles 2–12 mm long, slightly diverging at the base, shortly fimbriate at the tips. Capsule subglobose, 6–12 mm long, sometimes slightly umbonate at the apex, with scars of floral parts forming a 2–3 mm long stipe-like part below the fruit. Seeds 2.5–4 mm long; aril lobed, almost as long as the seed. — Figure 6.

Distribution and habitat. – *Arboa integrifolia* is widespread in Madagascar with scattered occurrences in the northern, western and southern parts of the island (Fig. 7A). It occurs in areas of deciduous, seasonally dry forest or bushland, often on sandy or rocky ground, at elevations from near sea level up to about 1200 m.

Representative collections examined. – MADAGASCAR: Antsiranana, Daraina, forêt de Binara, 13°14′ N, 49°37′ E, 2 Dec. 2005, *Nusbaumer & Ranarison LN1716* (G, K, MO, P). Mahajanga, Mampikony, Bongolava, Betaramahamay, forêt sèche sur sable d'Ambohimanga, 15°57′ S, 47°26′ E, 6 Dec. 2004, *Razakamalala & al. 1840* (MO); Tsaramandroso, Ankarafantsika, 17 Oct. 1950, *Ramamonjisoa 2010-RN* (P, TAN). Toliara, Morondava, 11 km N of Morondava on the road to Belo-Tsiribihina, 18 Jan. 1998, *Luckow 4284* (K, MO); vallée de la Menarahaka, à l'Est d'Ihosy, 19 Dec. 1968, *Capuron 20572-SF* (MO, P); forêt de Zombitse, à l'Est de Sakaraha, 27–28 Dec. 1961, *Capuron 20589-SF* (K, MO, P); Mt Vohipolaka au N de Betroka, Nov. 1933, *Humbert 11689* (P).

Taxonomic remarks. – According to the phylogeny in Fig. 3, *Arboa integrifolia* (= *Erblichia integrifolia*) is sister to *A. antsingyae* (= *E. antsingyae*) plus *A. berneriana* (= *E. berneriana*) with strong support. It differs markedly from the other three species of *Arboa* by its more or less densely pubescent stems and leaves, obtuse floral buds and sepals (Fig. 6C), short and obtuse anthers (Fig. 6E), shortly stipitate ovary, free corona (Fig. 6E), and by the scars of the floral parts forming a 2–3 mm long stipe-like part below the fruit (Fig. 6F).

Over its fairly wide range *Arboa integrifolia* is quite variable in, for example, leaf size, density of indumentum, size of sepals, petals and fruits, and in the degree of development of the umbo that is sometimes found at the tip of the fruit (Fig. 6F–G).

2. ***Arboa berneriana*** (Tul.) Thulin & Razafim., **comb. nov.** ≡ *Turnera berneriana* Tul. in Ann. Sci. Nat., Bot., sér. 5, 9: 322. 1868 ≡ *Piriqueta berneriana* (Tul.) Urb. in Jahrb. Königl. Bot. Gart. Berlin 2: 78. 1883 ≡ *Erblichia berneriana* (Tul.) Arbo in Adansonia, sér. 2, 18: 469. 1979 – Type: Madagascar, Lingvatou, 1835, *Bernier (2me envoi) 268* (P!, lectotype, P00077559, designated here; P!, isolectotypes).

The epithet "*berneriana*" has been corrected to "*bernieriana*" by, e.g., Perrier de la Bâthie (1950), as the name of the collector of the type is J. Bernier. However, Tulasne (1868) in the protologue deliberately latinized the name as Bernerius, which is why his spelling is to be preserved (*ICBN*, Art. 60.7, McNeill & al., 2006).

There are three duplicates of *Bernier 268* in P, but only one of them has information on locality and other details of the plant. This sheet, P00077559, is here selected as lectotype. The two other sheets, P00077558 and P00077560, are treated as isolectotypes. The sheet P00077561, communicated as *Boivin 2560 bis* and cited as such by Tulasne (1868) is, in effect, also a duplicate of *Bernier 268* and is to be regarded as an isolectotype as well.

Shrub, 0.5–5 m tall; branchlets glabrous. Leaves oblanceolate to obovate or elliptic, 1–5 × 0.5–2.5 cm, glabrous or sparsely pubescent, obtuse to retuse or sometimes subacute at the apex, narrowing below into a short petiole, margin subentire to denticulate-crenulate, sometimes ± revolute, the
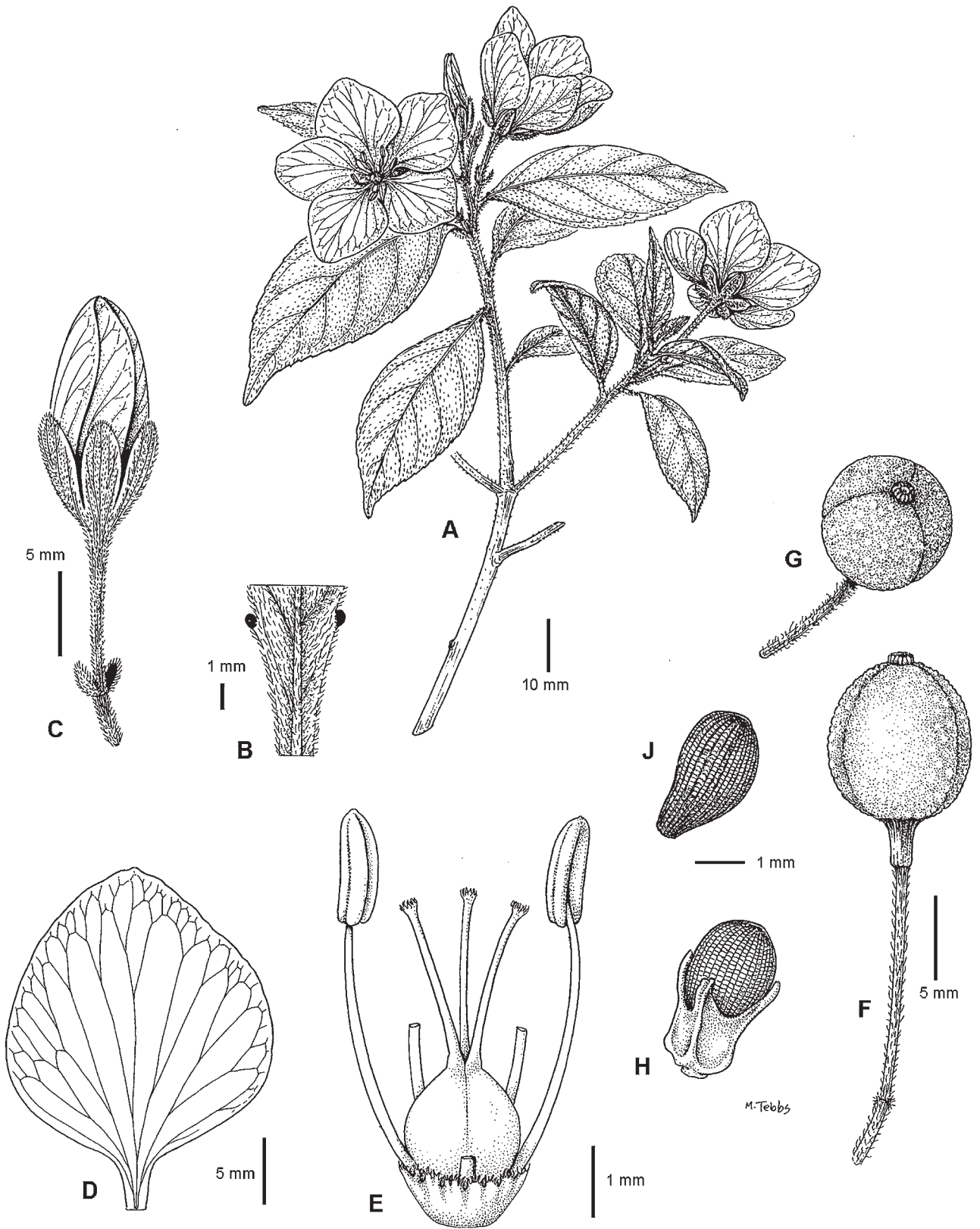
Fig. 6. *Arboa integrifolia*. **A,** flowering branch; **B,** base of leaf, lower surface, showing glands; **C,** flower bud; **D,** petal; **E,** portion of flower showing corona, two of the stamens and the pistil; **F–G,** capsule, side view and top view; **H,** seed; **J,** seed with aril removed. — A–E from *Luckow 4284* (K) and photograph, F–I from *Nusbaumer 923* (K). Drawn by Margaret Tebbs.

teeth ± distinctly gland-tipped; lateral veins 3–6 pairs. Flower buds acute; pedicel 8–45 mm long, articulated in lower part; bracteoles 0.2–1 mm long. Sepals 8–15 × ≈3 mm, glabrous or sparsely appressed pubescent, acute, the outer ones usually provided with 2–3 pairs of minute acute glands near margin. Petals 12–20 × 8–16 mm, yellow, orange-red or red, obtuse; corona 1.7–2.5 mm high, ± distinctly 10-lobed, inserted at the base of the petals and sepals. Stamens inserted at base of sepals; filaments 3–9 mm long; anthers 2.5–5.5 mm long, curved, apiculate. Ovary ca. 2 mm long, sessile; styles 7–11 mm long, widely diverging at the base, penicillate at the tips. Capsule sub-globose, 6–8 mm long, scars of floral parts forming a narrow band below the fruit. Seeds 3–3.5 mm long, very finely pitted only; aril unilateral, up to about as long as the seed.

Distribution and habitat. – *Arboa berneriana* is restricted to the northern part of Madagascar (Fig. 7B), where it grows in deciduous, seasonally dry forest on sand or limestone at 5–380 m above sea level.

Representative collections examined. – MADAGASCAR: Antsiranana, Andrafiabe, Ambalobozokely, Baie de Rigny, Nosy Voanio, 12°26′S, 49°33′E, 23 Mar. 2006, *Ratovoson & al. 1120* (MO, P, TAN, UPS); banks of Ambatabe (= Antafiamantsina) River, 17 Mar. 1994, *Du Puy & al. M725* (K, MO, P, TAN); Daraina, forêt de Bekaraoka, 13°06′S, 49°40′E, 19 Feb. 2004, *Nusbaumer & Ranirison 1198* (G, K, MO, P); entre Ambilobe et Anivorano, 4–9 Mar. 1951, *Humbert & Capuron 25542* (P).

Taxonomic remarks. – *Arboa berneriana* differs from *A. madagascariensis* and *A. antsingyae* by its smaller leaves and shorter sepals and anthers. Also, the marginal glands usually present on the outer sepals of *A. berneriana* seem to be unique to this species. Still, there is a considerable, although continuous, variation in leaf and flower size within *A. berneriana*. The flower colour is recorded as orange-red or red in all recent material and old reports of yellow flowers need confirmation.

3. ***Arboa madagascariensis*** (O. Hoffm.) Thulin & Razafim., **comb. nov.** ≡ *Erblichia madagascariensis* O. Hoffm., Sert. Pl. Madagasc.: 19. 1881 ≡ *Piriqueta madagascariensis* (O. Hoffm.) Urb. in Jahrb. Königl. Bot. Gart. Berlin 2: 79. 1883 ≡ *Turnera madagascariensis* (O. Hoffm.) Baill. in Bull. Mens. Soc. Linn. Paris 1: 582. 1886 – Type: Madagascar, Mts Ambóhitsi (Ambre), Mar. 1880, *Hildebrandt 3376* (B, holotype, destroyed; K!, lectotype, designated by Arbo, 1979; BM!, G!, M, P!, W, WU, isolectotypes).

Shrub or small tree; branchlets glabrous. Leaves obovate to elliptic, 5–11 × 2–5.5 cm, glabrous or sparsely pubescent, obtuse to shortly acuminate at the apex, narrowing below into a short petiole; margin subentire to denticulate-crenulate, the teeth obtuse and ± distinctly gland-tipped, generally with a pair of larger glands, up to 1.5 mm in diam., near the base; lateral veins 5–8 pairs. Flower buds acute; pedicel 25–40 mm long, articulated in lower part; bracteoles ca. 1.5 mm long. Sepals 18–20 × ≈3 mm, sparsely appressed pubescent, acute. Petals 20–35 × 12–20 mm, orange-red, shortly acuminate; corona 2–3 mm high, ± distinctly 10-lobed, inserted at the base of petals and sepals. Stamens inserted at base of sepals; filaments 9–14 mm long; anthers 5–10 mm long, curved, apiculate. Ovary 3–4 mm long, sessile; styles 12–20 mm long, widely divergent at the base, penicillate at the tip. Capsule subglobose, 9–10 mm in diam., scars of floral parts forming a narrow band below fruit. Seeds 3–4.5 mm long; aril shorter than seed.

Distribution and habitat. – *Arboa madagascariensis* is known from two collections only, both from deciduous forest. According to Perrier de la Bâthie (1950) the type locality is at the base of Montagne d'Ambre at 300–400 m above sea level. The second collection, *Perrier de la Bâthie 1227*, is from limestone rocks near sea level at Amboanio near Mahajanga. These two localities (Fig. 7C) are about 460 km apart and the respective collections were made in 1880 and 1901. From this it seems that there is a considerable risk that the species has gone extinct.
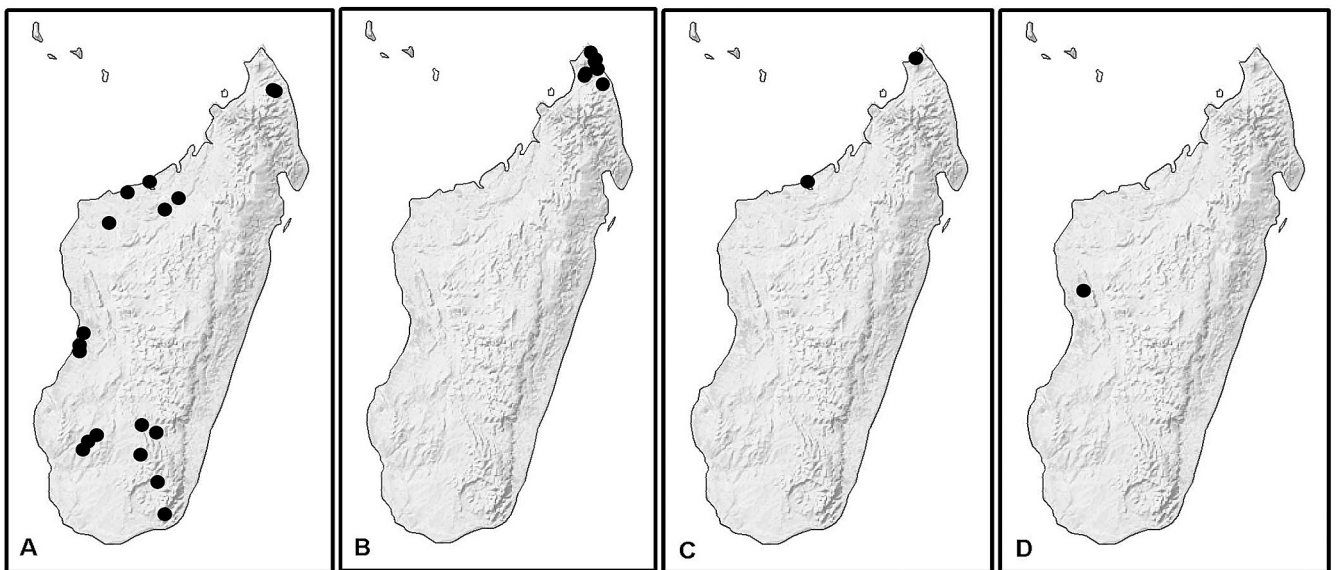

Fig. 7. Distributions of **A,** *Arboa integrifolia*; **B,** *A. berneriana*; **C,** *A. madagascariensis*; **D,** *A. antsingyae*.

Additional collection studied. – MADAGASCAR: Mahajanga, Amboanio near Mahajanga, Feb. 1901, *Perrier de la Bâthie 1227* (K, P).

Taxonomic remarks. – *Arboa madagascariensis* is close to *A. berneriana*, but seems distinctive by its larger leaves, usually well over 5 cm long, larger sepals (18–28 versus 8–15 mm long) without marginal glands, and larger anthers (5–10 mm versus 2.5–5 mm long).

4. **Arboa antsingyae** (Arbo) Thulin & Razafim., **comb. nov.** ≡
Erblichia antsingyae Arbo in Adansonia, sér. 2, 18: 476. 1979 – Type: Madagascar, "forêt de l'Antsingy, près de la clairière d'Ambodiriana (piste Antsalova–Tsiandro), 15 Dec. 1952, *Capuron 6832-SF* (P!, lectotype, P00077555, designated here; K!, P!, S!, TEF, isolectotypes).

Piriqueta antsingyae Capuron in Adansonia, sér. 2, 3: 133 (1963) was not validly published as three collections with different collectors and numbers were cited (*Leandri 2173*, *Capuron 6832-SF*, *Razafindrakoto 4703-RN*). The three collections were made at the same occasion and Capuron therefore regarded them all as parts of one single collection. However, this view is not in accordance with the *ICBN*, Art. 8 (McNeill & al., 2006). Furthermore, only two of the collections were made 15 Dec. 1952, *Razafindrakoto 4703-RN* being dated 14 Dec. 1952.

Arbo (1979) treated this species as *Erblichia antsingyae*, cited a single collection (*Capuron 6832-SF*) as (lecto)type, and at the same time provided a full and direct reference to the Latin diagnosis and description of *Piriqueta antsingyae*. Her name therefore fulfills the requirements for valid publication of the *ICBN*, Art. 45.1 (McNeill & al., 2006), although she intended it as a new combination.

The collection *Capuron 6832-SF* in P consists of four duplicates. The best sheet, P00077555, is here designated as lectotype, whereas P00077556, P00077557, plus one unnumbered sheet, are treated as isolectotypes.

Tree, 8–10 m tall; branchlets glabrous. Leaves elliptic, 13–20 × 5–10 cm, glabrous, shortly acuminate or rarely obtuse at the apex, narrowing below into a 10–20 mm long petiole-like base with a pair of projecting ca. 3 mm wide cup-shaped glands in upper part, margin subentire to denticulate-crenulate, the teeth obtuse and minutely gland-tipped; lateral veins 13–20 pairs. Flower buds acute; pedicel 60–105 mm long, glabrous, articulated 15–28 mm from the tip; bracteoles ca. 1 mm long. Sepals 30 × 7–8 mm, glabrous, acute. Petals 35–45 × 20–30 mm, orange-red, acute to shortly acuminate; corona ca. 3 mm high, inserted at base of petals and sepals, with 5 ± marked lobes opposite the petals. Stamens inserted at base of sepals; filaments 17–20 mm long; anthers ca. 13 mm long, curved, apiculate. Ovary 4–5 mm long, sessile; styles 20–25 mm long, widely divergent at the base, penicillate at the tips. Capsule ovoid, 25–30 × 20 mm, scars of floral parts forming a narrow band below the fruit. Seeds (unripe) 4–5 mm long, very finely pitted only; aril unilateral, lacerate, up to about as long as the seed.

Distribution and habitat. – *Arboa antsingyae* is known only from the type locality in the Bemaraha Massif in western Madagascar (Fig. 7D), where it grows in dry deciduous forest on limestone at 100–150 m above sea level. All three collections were made on the same occasion, in July 1952, and perhaps even from the same tree. The Bemaraha Massif ("Tsingy de Bemaraha") is still botanically little known and further localities for this remarkable species may be anticipated.

Additional collection studied. – MADAGASCAR: Antsingy, near Ambodiriana (Antsalova –Tsiandro road), 15 Jul. 1952, *Leandri 2173* (B, G, P) & *Razafindrakoto 4703-RN* (P, TAN).

Taxonomic remarks. – *Arboa antsingyae* is the only species in the genus that grows to a medium-sized tree. It also differs markedly from all the other species by its large leaves (13–20 cm long) with a pair of projecting and about 3 mm wide, cup-shaped glands in the upper part of the petiole-like leaf base. Also, the flowers in *A. antsingyae* are larger than in any of the other species.

■ LITERATURE CITED

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. Pp. 267–281 in: Petrov, B.N. & Csaki, F. (eds.), *Second International Symposium of Information Theory.* Budapest: Akademiai Kiado.

Alejandro, G.D., Razafimandimbison, S.G. & Liede-Schumann, S. 2005. Polyphyly of *Mussaenda* inferred from ITS and *trnT-F* data and its implication for generic limits in Mussaendeae (Rubiaceae). *Amer. J. Bot.* 92: 544–557.

APG. 1998. An ordinal classification for the families of flowering plants. *Ann. Missouri Bot. Gard.* 85: 531–553.

APG. 2003. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG II. *Bot. J. Linn. Soc.* 141: 339–436.

APG. 2009. An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG III. *Bot. J. Linn. Soc.* 161: 105–121.

Arbo, M.M. 1979. Revisión del género *Erblichia* (Turneraceae). *Adansonia,* sér. 2, 18: 459–482.

Arbo, M.M. 1995. *Turneraceae–parte 1. Piriqueta.* Flora Neotropica Monograph 67. Bronx, New York: The New York Botanical Garden.

Arbo, M.M. 2007. Turneraceae. Pp. 458–466 in: Kubitzki, K. (ed.), *The families and genera of vascular plants,* vol. 9. Berlin, Heidelberg: Springer.

Arbo, M.M. & Espert, S.M. 2009. Morphology, phylogeny and biogeography of *Turnera* L. (Turneraceae). *Taxon* 58: 457–467.

Aublet, J.B.C.F. 1775. *Histoire des plantes de la Guiane françoise*, vol. 1. London, Paris: Pierre-Francois Didot jeune.

Bellstedt, D.U., Van Zyl, L., Marais, E.M., Bytebier, B., Villiers, C.A. de, Makwarela, A.M. & Dreyer, L.L. 2008. Phylogenetic relationships, character evolution and biogeography of southern African members of *Zygophyllum* (Zygophyllaceae) based on three plastid regions. *Molec. Phylogenet. Evol.* 47: 932–949.

Candolle, A.P. de. 1828. *Prodromus systematis naturalis regni vegetabilis*, vol. 3. Paris: Treuttel et Würtz.

Caujapé-Castells, J., Jansen, R.K., Membrives, N., Pedrola-Monfort, J., Montserrat, J.M. & Ardanuy, A. 2001. Historical biogeography of *Androcymbium* Willd. (Colchicaceae) in Africa: Evidence from cpDNA RFLPs. *Bot. J. Linn. Soc.* 136: 379–392.

Chase, M.W., Zmarzty, S., Lledó, M.D., Wurdack, K.J., Swensen, S.M. & Fay, M.F. 2002. When in doubt, put it in Flacourtiaceae: A molecular phylogenetic analysis based on plastid *rbcL* DNA sequences. *Kew Bull.* 57: 141–181.

Cronquist, A. 1988. *The evolution and classification of flowering plants*, ed. 2. Bronx, New York: The New York Botanical Garden.

Dahlgren, R.M.T. 1980. A revised system of classification of the angiosperms. *Bot. J. Linn. Soc.* 80: 91–124.

Davis, C.C. & Chase, M.W. 2004. Elatinaceae are sister to Malpighiaceae; Peridiscaceae belong to Saxifragales. *Amer. J. Bot.* 91: 262–273.

Davis, C.C., Webb, C.O., Wurdack, K.J., Jaramillo, C.A. & Donoghue, M.J. 2005. Explosive radiation of Malpighiales supports a mid-Cretaceous origin of modern tropical rain forests. *Amer. Naturalist* 165: E36–E65.

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

Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *B. M. C. Evol. Biol.* 7: 214, doi:10.1186/1471-2148-7-214.

Drummond, A.J., Ho, S.Y.W., Phillips, M.J. & Rambaut, A. 2006. Relaxed phylogenetics and dating with confidence. *PLoS Biol.* 4(5): e88, doi:10.1371/journal.pbio.0040088.

Duncan, R.A. 2009. Mascarene Islands, geology. Pp. 620–621 in Gillespie, R.G. & Clague, D.A. (eds.), *Encyclopedia of islands.* Encyclopedias of the Natural World 2. Berkeley: University of California Press.

Fernandes, R. 1978. Turneraceae. Pp. 348–368 in: Launert, E. (ed.), *Flora Zambesiaca*, vol. 4. Glasgow: Robert MacLehose & Co.

Gonzalez, A.M. & Arbo, M.M. 2004. Trichome complement of *Turnera* and *Piriqueta. Bot. J. Linn. Soc.* 144: 85–97.

Harvey, W.H. 1862. Turneraceae. P. 599 in: Harvey, W.H. & Sonder, O.W. (eds.), *Flora capensis*, vol. 2. Dublin: Hodges, Smith, and Co.

Huelsenbeck, J.P. & Ronquist, F. 2001. MrBayes: Bayesian inference of phylogeny. *Bioinformatics* 17: 754–755.

Hutchinson, J. 1973. *The families of flowering plants*, ed. 3. Oxford: Clarendon Press.

Korotkova, J., Schneider, J.V., Quandt, D., Worberg, A., Zizka, G. & Borsch, T. 2009. Phylogeny of the eudicot order Malpighiales: Analysis of a recalcitrant clade with sequences of the *petD* group II intron. *Pl. Syst. Evol.* 282: 201–228.

Lundberg, J. 2001. The asteralean affinity of the Mauritian *Roussea* (Rousseaceae). *Bot. J. Linn. Soc.* 137: 267–276.

McNeill, J., Barrie, F.R., Burdet, H.M., Demoulin, V., Hawksworth, D.L., Marhold, K., Nicolson, D.H., Prado, J., Silva, P.C., Skog, J.E., Wiersema, J.H. & Turland, N.J. (eds.) 2006. *International Code of Botanical Nomenclature (Vienna Code): Adopted by the Seventeenth International Botanical Congress Vienna, Austria, July 2005.* Regnum Vegetabile 146. Rugell: Gantner.

Melchior, H. 1964. *Engler's Syllabus der Pflanzenfamilien*, ed. 12, vol. 2. Berlin: Borntraeger.

Nylander, J.A.A. 2004. MrModeltest, version 2. Program distributed by the author, Evolutionary Biology Centre, Uppsala University, Uppsala. http://www.abc.se/~nylander/.

Nylander, J.A.A., Wilgenbusch, J.C., Warren, D.L. & Swofford, D.L. 2008. AWTY (Are We There Yet?): A system for graphical exploration of MCMC convergence in Bayesian phylogenetics. *Bioinformatics* 24: 581–583.

Obermeyer, A.A. 1976. Turneraceae. Pp. 93–103 in: Ross, J.H. (ed.), *Flora of southern Africa*, vol. 22. Pretoria: Botanical Research Institute.

Perrier de la Bâthie, H. 1950. *Turnéracées.* In: Humbert, H. (ed.), *Flore de Madagascar et des Comores*, fam. 142. Paris: Firmin-Didot et Cie.

Rambaut, A. 1996. Se-Al: Sequence alignment editor. http://evolve.zoo.ox.ac.uk.

Rambaut, A. & Drummond, A.J. 2007. Tracer, version 1.4. http://beast.bio.ed.ac.uk/Tracer.

Razafimandimbison, S.G. & Bremer, B. 2002. Phylogeny and classification of Naucleae s.l. (Rubiaceae) inferred from molecular (ITS, *rbcL*, and *trnT-F*) and morphological data. *Amer. J. Bot.* 89: 1027–1041.

Renner, S.S., Strijk, J.S., Strasberg, D. & Thébaud, C. 2010. Biogeography of the Monimiaceae (Laurales): A role for East Gondwana and long-distance dispersal, but not West Gondwana. *J. Biogeogr.* 37: 1227–1238.

Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Schwartz, G. 1978. Estimating the dimensions of a model. *Ann. Statist.* 6: 461–464.

Shore, J.S., Arbo, M.M. & Fernández, A. 2006. Breeding system variation, genetics and evolution in the Turneraceae. *New Phytol.* 171: 539–551.

Soltis, D.E., Soltis, P.S., Chase, M.W., Mort, M.E., Albach, D.C., Zanis, M., Savolainen, V., Hahn, W.H., Hoot, S.B., Fay, M.F., Axtell, M., Swensen, S.M., Nixon, K.C. & Farris, J.S. 2000. Angiosperm phylogeny inferred from a combined data set of 18S rDNA, *rbcL*, and *atpB* sequences. *Bot. J. Linn. Soc.* 133: 381–461.

Swofford, D.L. 2002. PAUP*: Phylogenetic analysis using parsimony (*and other methods), version 4.0b10. Sunderland, Massachusetts: Sinauer.

Takhtajan, A. 1997. *Diversity and classification of flowering plants.* New York: Columbia University Press.

Thiv, M., Van der Niet, T., Rutschmann, F., Thulin, M., Brune, T. & Linder, H.P. 2011. Old–New World and trans-African disjunctions of *Thamnosma* (Rutaceae): Intercontinental long-distance dispersal and local differentiation in the succulent biome. *Amer. J. Bot.* 98: 76–87.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The CLUSTAL X windows interface: Flexible strategies for multiple alignment aided by quality analysis tools. *Nucl. Acids Res.* 22: 4673–4680.

Thulin, M. 1993. Turneraceae. Pp. 34–36 in: Thulin, M. (ed.), *Flora of Somalia*, vol. 1. Kew: Royal Botanic Gardens.

Thulin, M., Thiede, J. & Liede-Schumann, S. 2012. Phylogeny and taxonomy of *Tribulocarpus* (Aizoaceae): A paraphyletic species and an adaptive shift from zoochorous trample burrs to anemochorous nuts. *Taxon* 61: 55–66.

Truyens, S., Arbo, M.M. & Shore, J.S. 2005. Phylogenetic relationships, chromosome and breeding system evolution in *Turnera* (Turneraceae): Inferences from ITS sequence data. *Amer. J. Bot.* 92: 1749–1758.

Tulasne, L.-R. 1868. Flora madagascariensis, fragmentum tertium. *Ann. Sci. Nat., Bot.*, sér. 5, 9: 298–344.

Urban, I. 1883. Monographie der Familie der Turneraceen. *Jahrb. Königl. Bot. Gart. Berlin* 2: 1–152.

Wurdack, K.J. & Davis, C.C. 2009. Malpighiales phylogenetics: Gaining ground on one of the most recalcitrant clades in the angiosperm tree of life. *Amer. J. Bot.* 96: 1551–1570.

Appendix. Species sampled with voucher information and GenBank accession numbers. Sequences (ITS, *trnL-F*) generated for this study are marked with an asterisk, other sequences are from Truyens & al. (2005).

Adenoa cubensis (Britton & P. Wilson) Arbo, Cuba, *Zuloaga & al. 9600* (SI), JQ723349*, JQ723376*. ***Basananthe berberoides*** (Chiov.) de Wilde, Yemen, *Thulin & al. 8010* (UPS), –, JQ723377*. ***Erblichia antsingyae*** Arbo (= ***Arboa antsingyae*** (Arbo) Thulin & Razafim.), Madagascar, *Capuron 6832-SF* (S), JQ723351*, JQ723378*. ***Erblichia berneriana*** (Tul.) Arbo (= ***Arboa berneriana*** (Tul.) Thulin & Razafim., Madagascar, *Allorge, Brulfert & Bardot-Vaucoulon 2712* (P), JQ723352*, JQ723379*. ***Erblichia integrifolia*** (Claverie) Arbo (= ***Arboa integrifolia*** (Claverie) Thulin & Razafim.), Madagascar, *Thulin & Razafindraibe 11906* (UPS), JQ723353*, JQ723380*. ***Erblichia odorata*** Seem., Costa Rica, *Thulin & Poveda 11697* (UPS), JQ723350*, JQ723381*. ***Humiria balsamifera*** Aubl., *Anderson 13654* (MICH), –, AF350941. ***Hyalocalyx setifer*** Rolfe, Madagascar, *Thulin & al. 10398* (UPS), JQ723354*, JQ723382*. ***Loewia glutinosa*** Urb., Somalia, *Thulin & al. 6436* (UPS), JQ723355*, JQ723383*. ***Malesherbia deserticola*** Phil., Chile, *Werdermann 1049* (S), JQ723356*, JQ723384*. ***Mathurina penduliflora*** Balf. f., Rodrigues Island, *DFHJJ2* (C), JQ723357*, JQ723385*. ***Paropsia brazzeana*** Baill., Zambia, *Luwiika & al. 116* (UPS), JQ723358*, JQ723386*. ***Passiflora foetida*** L., Ecuador, *Madsen & Chiombo 7897* (UPS), JQ723359*, JQ723387*. ***Piriqueta asperifolia*** Arbo, Brazil, *Franca & al. 3135* (CTES), AY973394, JQ723388*. ***Piriqueta capensis*** (Harv.) Urb. (= ***Afroqueta capensis*** (Harv.) Thulin & Razafim.), Botswana, *Blomberg & al. BMP 113* (UPS), JQ723360*, JQ723389*. ***Piriqueta cistoides*** (L.) Griseb. subsp. ***caroliniana*** (Walter) Arbo, U.S.A., Florida, *Shore 311* (CTES), AY973391, JQ723390*. ***Piriqueta morongii*** Rolfe, Argentina, *Krapovickas 45204* (CTES), AY973393, –. ***Piriqueta viscosa*** Griseb., Brazil, *Groppo & al. 1050* (SPF), AY973392, JQ723391*. ***Stapfiella usambarica*** J. Lewis, Tanzania, *Borhidi & al. 85625* (UPS), JQ723361*, JQ723392*. ***Streptopetalum arenarium*** Thulin, Somalia, *Thulin 6375* (UPS), JQ723362*, JQ723393*. ***Populus nigra*** L., no voucher, –, FJ490818. ***Streptopetalum serratum*** Hochst., Somalia, *Thulin & al. 7618* (UPS), JQ723363*, JQ723394*. ***Streptopetalum wittei*** Staner, Tanzania, *Bidgood & al. 914* (UPS), JQ723364*, JQ723395*. ***Tricliceras bivinianum*** (Tul.) R. Fern., Ethiopia, *Gilbert & Ermias 8354* (UPS), JQ723365*, JQ723396*. ***Tricliceras glanduliferum*** (Klotzsch) R. Fern., Namibia, *Wanntorp 539* (S), JQ723366*, JQ723397*. ***Tricliceras lobatum*** (Urb.) R. Fern., Tanzania, *Aleljung 368* (UPS), JQ723367*, JQ723398*. ***Tricliceras longipedunculatum*** (Mast.) R. Fern., Tanzania, *Mhoro 310* (UPS), JQ723368*, JQ723399*. ***Tricliceras pilosum*** (Willd.) R. Fern., Ethiopia, *Gilbert & Thulin 357* (UPS), JQ723370*, JQ723400*. ***Tricliceras schinzii*** (Urb.) R. Fern., Botswana, *Hansen 3538* (UPS), JQ723369*, JQ723401*. ***Turnera capitata*** Camb., Brazil, *Irwin & al. 30589* (UPS), –, JQ723402*. ***Turnera lamiifolia*** Camb., Brazil, *Irwin & al. 11232* (UPS), JQ723373*, –. ***Turnera oculata*** Story, Namibia, *Giess 9377* (PRE), JQ723375*, –. ***Turnera subulata*** Sm., Costa Rica, *Thulin 11705* (UPS), JQ723371*, JQ723403*. ***Turnera thomasii*** (Urb.) Story, Kenya, *Gilbert & Thulin 1700* (UPS), JQ723372*, JQ723404*. ***Turnera ulmifolia*** L., Spain, Gran Canaria, *Thulin 11625* (UPS), JQ723374*, JQ723405*.