

Further disintegration and redefinition of *Clerodendrum* (Lamiaceae): Implications for the understanding of the evolution of an intriguing breeding strategy

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Abstract The genus *Clerodendrum* s.l. is polyphyletic. Although recent studies have resulted in *C.* subg. *Cyclonema* and *C.* sect. *Konocalyx* being removed to the resurrected genus *Rothea*, and the unispecific genus *Huxleya* being sunk into *Clerodendrum*, it has been unclear whether *Clerodendrum* as currently circumscribed is monophyletic, particularly in relation to the American genera *Aegiphila*, *Amasonia*, and *Tetraclea*. This phylogenetic study employs four relatively fast-evolving chloroplast DNA regions, *trnT-L*, *trnL-F*, *trnD-T*, and *trnS-fM*, to clarify the generic boundaries of *Clerodendrum* and its relationship to allied genera. The results corroborate previous studies that there are three well-supported clades in the currently recognized *Clerodendrum*: an Asian clade, an African clade, and a Pan-tropical Coastal clade. The Asian clade and African clade are sister groups and together form a monophyletic group. However, the Pan-tropical Coastal clade is more closely related to the three American genera than it is to the other two *Clerodendrum* clades. In addition, a Caribbean species, *C. spinosum*, is found to be more closely related to the American genera than it is to any of the three major *Clerodendrum* groups. These results indicate that *Clerodendrum* as currently circumscribed is not monophyletic. We propose to separate the Pan-tropical Coastal clade and *C. spinosum* by reviving the genera *Volkameria* (including *Huxleya*) and *Ovieda*, respectively for these, and to restrict *Clerodendrum* to the Asian and African clades. Brief descriptions of the genera to be recognized are provided. All Neotropical ‘*Clerodendrum*’ taxa are referred to other genera, necessitating six new combinations, which are also provided, where required, for two other well-studied Old World *Volkameria* species; all names ever used in *Ovieda* are given their modern placings (two placed newly in synonymy). The study also sheds light on the evolution of an intriguing breeding strategy that avoids self-pollination or/and sexual interference. This strategy involves presentation of pollen and stigma in the centre of the flower in a sequential fashion by moving the filaments and style. It appears to have evolved in the common ancestor of *Clerodendrum*, *Volkameria*, *Ovieda*, *Amasonia*, *Tetraclea*, *Aegiphila* and *Kalaharia*, and still occurs in all of these taxa except *Aegiphila*, where it has been succeeded by a heterostylous system.

Keywords *Aegiphila*; breeding strategy; chloroplast DNA; *Clerodendrum*; *Huxleya*; *Ovieda*; phylogeny; *Volkameria*

■ INTRODUCTION

The genus *Clerodendrum* L. as delimited by nineteenth-century botanists (Schauer, 1847; Briquet, 1895) is heterogeneous. However, this delimitation has been followed reasonably closely by subsequent authors, even though they recognized it to be problematic (Lam, 1919; Thomas, 1936; Moldenke, 1985; Verdcourt, 1992). Cladistic analyses of morphological data (Cantino, 1992; Rimpler & al., 1992) provided preliminary evidence that *Clerodendrum* sensu lato (s.l.) was not monophyletic. But these analyses, primarily focused at subfamily or family level, included relatively few *Clerodendrum* s.l. species and did not provide good resolution of relationships within *Clerodendrum* s.l. or between the genus and other related ones. Phylogenetic studies based on chloroplast DNA (cpDNA) restriction site data (Steane & al., 1997) and nuclear ITS sequences (Steane & al., 1999), with extensive sampling within *Clerodendrum* s.l. and related genera, strongly suggested that

Clerodendrum s.l. is polyphyletic. Subsequently, a number of species comprising the *C.* subg. *Cyclonema* (Hochst.) Gürke and *C.* sect. *Konocalyx* (Thomas) Verdc. were removed to the resurrected genus *Rothea* Raf. (Steane & Mabberley, 1998). In addition, the molecular studies divided *Clerodendrum* (sensu Steane & Mabberley, 1998) into three major clades that are in general associated with geographic distribution: an Asian clade, an African clade, and a Pan-tropical Coastal clade (Fig. 1A).

A more recent study (Steane & al., 2004) which included three New World genera, *Aegiphila* Jacq., *Amasonia* L. f., and *Tetraclea* A. Gray, and a unispecific Australian genus *Huxleya* Ewart, put the delimitation of *Clerodendrum* (sensu Steane & Mabberley, 1998) into question again. *Huxleya* was found nested within the Pan-tropical Coastal clade and therefore was sunk into the genus *Clerodendrum* (Steane & al., 2004). The African and Asian clades were still recovered as sister groups and together formed a monophyletic group (Fig. 1B). The three New World genera, *Aegiphila*, *Amasonia*, and *Tetraclea*, each

represented by a single species in that study, formed a New World clade. However, the relationships among the New World clade, the Pantropical Coastal *Clerodendrum*, and the remaining *Clerodendrum* species (Asian + African), were unresolved (Steane & al., 2004; Fig. 1B), leaving the possibility that *Clerodendrum* (sensu Steane & al., 2004) as currently circumscribed is paraphyletic in relation to the clade of New World genera. In addition, the phylogenetic framework presented in those studies (Steane & al., 1997, 1999, 2004) is mainly based on cpDNA restriction site data and nuclear ITS sequences, with only a few chloroplast *ndhF* sequences. It is difficult to add more data to a restriction site dataset, due to the nature of this type of marker. For many *Clerodendrum* species the ITS region is difficult to sequence directly without cloning, possibly because of their being polyploids, which is indicated by the high chromosome number, $2n = 46, 48, \text{ or } 52$, of most species (see the Index to Plant Chromosome Numbers Database, <http://mobot.mobot.org/W3T/Search/ipcn.html>).

The major objectives of this paper are, therefore, to: (1) test the monophyly of *Clerodendrum* as currently circumscribed; (2) present a phylogenetic framework of *Clerodendrum* and its related genera based on cpDNA sequence data, to which additional sequence data may be added easily in future studies; and (3) use this phylogenetic framework to examine the evolution of morphological characters.

■ MATERIALS AND METHODS

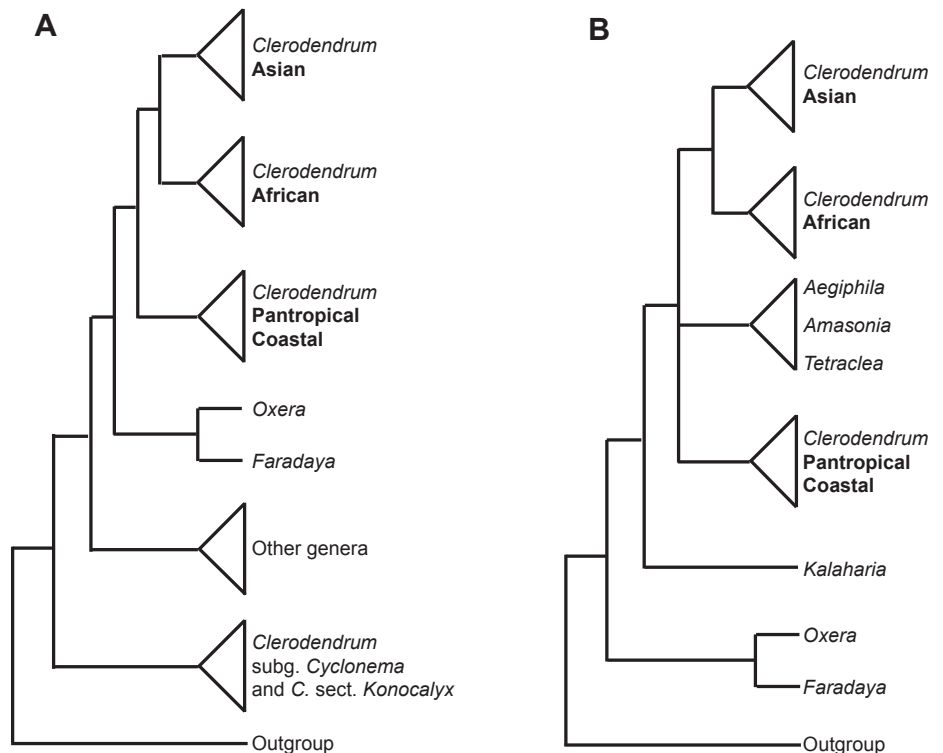
Our sampling included 40 species of *Clerodendrum* (sensu Steane & al., 2004), representing the three major clades

identified in previous studies, 13 species from six closely related genera (*Aegiphila*, *Amasonia*, *Tetraclea*, *Kalaharia* Baill., *Oxera* Labill., *Faradaya* F. Muell.), and three species from more distantly related genera in the Lamiaceae-Ajugoi-deae (*Ajuga* L., *Teucrium* L., *Rothea*). Voucher information for these 56 samples is listed in Appendix 1.

Total genomic DNA was extracted from either silica-gel dried leaf tissue or herbarium specimens using the modified CTAB method (Doyle & Doyle, 1987). Four relatively fast-evolving non-coding cpDNA regions (Shaw & al., 2005) were chosen for sequencing. These were *trnD-trnT*, *trnT-trnL*, *trnL-trnF* (*trnL* intron and *trnL-F* intergenic spacer), and *trnS-trnM*. PCR and sequencing primers with corresponding references are listed in Appendix 2. Procedures for PCR and sequencing are described in Yuan & Olmstead (2008). Sequences of the two outgroup species, *Verbena officinalis* L., *Aloysia virgata* (Ruiz & Pav.) Pers., are from Yuan & Olmstead (2008), while sequences of all other species were generated in this study and have been deposited in GenBank (*trnD-trnT*: EU160617–EU160666, FJ951910–FJ951915; *trnS-trnM*: FJ951916–FJ951970; *trnT-trnL*: FJ951971–FJ952025; *trnL-trnF*: FJ952026–FJ952081).

Sequence alignments were made manually using Se-AL v.2.0a11 (Rambaut, 1996) based on the similarity criterion (Simmons, 2004). The four cpDNA regions were combined as a single dataset for phylogenetic analyses because these regions are part of the haploid chloroplast genome and, therefore, share the same evolutionary history. Phylogenetically informative insertions/deletions (indels) were coded as binary characters using the simple gap coding method (Graham & al., 2000; Simmons & Ochoterena, 2000) and appended to the end

Fig. 1. Summarized phylogenies from previous studies. **A**, results from Steane & al. (1997, 1999), showing that *Clerodendrum* s.l. is polyphyletic; **B**, results from Steane & al. (2004), showing the unresolved relationship between Asian + African *Clerodendrum*, Pantropical Coastal *Clerodendrum*, and a clade comprising *Aegiphila*, *Amasonia*, and *Tetraclea*.



of the dataset. Six poly-nucleotide or microsatellite regions (a microsatellite with “AT” repeats and a poly-T region in the *trnD-trnT* segment, two poly-T regions in the *trnT-trnL* segment, a poly-A region in the *trnL-trnF* segment, and a poly-C/T/G region in the *trnM-trnS* segment) have been excluded from analyses due to uncertainty of homology assessment. Both parsimony and Bayesian analyses were performed on the final dataset.

Parsimony analysis was conducted using PAUP* v.4.0b10 (Swofford, 2002). Heuristic searches were performed with 1000 random stepwise addition replicates and TBR branch swapping with the MULTREES option in effect. Nodal support was determined by bootstrap analyses (Felsenstein, 1985) of 500 replicates, each with 20 random stepwise addition replicates and TBR branch swapping with MULTREES on.

Bayesian analyses were conducted using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003). A mixed-model approach (Ronquist & Huelsenbeck, 2003) was employed to integrate the phylogenetically informative gaps as binary characters with nucleotide data. The final dataset was divided into two partitions, the “nucleotide” partition and “gap” partition. We used Akaike information criterion (AIC) implemented in Modeltest v.3.7 (Posada & Crandall, 1998) to determine the model of sequence evolution that best fits the “nucleotide” partition (GTR+G). The restriction site (binary) model in MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) was used for the “gap” partition, with ascertainment bias for gap characters incorporated (Iset coding = informative). We performed two independent runs of 1,000,000 generations from a random starting tree using the default priors and four Markov chains (one cold and three heated chains), sampling one tree every 100 generations. Plots of log likelihood scores were used to determine stationarity and trees from the first 100,000 generations were discarded as burn-in.

RESULTS

The final dataset consisted of 44 scored gap characters and 4002 aligned nucleotides, of which 127 from the six poly-nucleotide or microsatellite regions were excluded due to uncertainty of homology assessment. One of the eight maximum parsimony trees resulting from parsimony analysis is shown in Fig. 2. The results are consistent with previous studies (Steane & al., 1997, 1999) in that *Clerodendrum* sensu Steane & al. (2004) is divided into three major clades: an African clade, an Asian clade, and a Pantropical Coastal clade. All three clades are supported by 99%–100% bootstrap (BS) values and posterior probabilities (PP) of 1.0. However, our results reveal that the Pantropical Coastal clade is more closely related to the New World genera (*Aegiphila*, *Amasonia*, *Tetraclea*) than it is to the Asian or African *Clerodendrum* clades (Fig. 2), a matter not revealed by earlier studies. In addition, a Caribbean species, *Clerodendrum spinosum* (L.) Spreng., is found not to belong to any of the three major clades. Together with *Aegiphila*, *Amasonia*, *Tetraclea*, it forms a well-supported (84%/1.0, BS/PP) New World clade. The African and Asian *Clerodendrum*

clades are strongly supported sister groups (100%/1.0, BS/PP), whereas the Pantropical Coastal *Clerodendrum* clade is sister to the New World clade (97%/1.0, BS/PP). Also consistent with previous studies (Steane & al., 2004), *Kalaharia*, a unispecific African genus, is recovered as sister group of the larger clade that includes all three *Clerodendrum* groups and the New World clade. The *Oxera/Faradaya* clade is sister to the even more inclusive clade including *Kalaharia* (Fig. 2).

Within the Asian clade, relationships are fairly well resolved. One strongly supported monophyletic group, in particular, is worth mentioning. It consists of species (*C. floribundum*, *C. indicum* [type of *Siphonanthus* L.], *C. minahassae*, *C. quadriloculare*, *C. tomentosum*) that are characterized by an extremely long and narrow corolla tube (99%/1.0, BS/PP; Fig. 2), probably an adaptation to a particular type of pollinator. Within the African clade, however, relationships are poorly resolved but it is noticeable that one species, *C. hildebrandtii*, is strongly supported as sister to the rest of the African group (Fig. 2). Within the Pantropical Coastal clade, branches are short, indicating little sequence diversification between species. Within the New World clade, both *Aegiphila* and *Amasonia* are strongly supported monophyletic groups (Fig. 2). *Tetraclea* is resolved to be the sister lineage of *Amasonia*, but this relationship is only weakly supported (52%/0.49, BS/PP, these values are not shown in Fig. 2). Likewise, *Clerodendrum spinosum* is recovered as sister to the *Amasonia/Tetraclea* clade, but weakly supported (52%/0.78, BS/PP).

Bayesian analyses gave very similar results. The only difference between the Bayesian majority consensus tree and parsimony tree shown in Fig. 2 is on the relationship between *Aegiphila anomala*, *A. alba*, the *A. hassleri* + *A. brachiata* clade, and the *A. elata* + *A. martinicensis* clade, but neither the relationship suggested by parsimony analyses nor that indicated by Bayesian inference is well supported (BS < 50%, PP < 0.7).

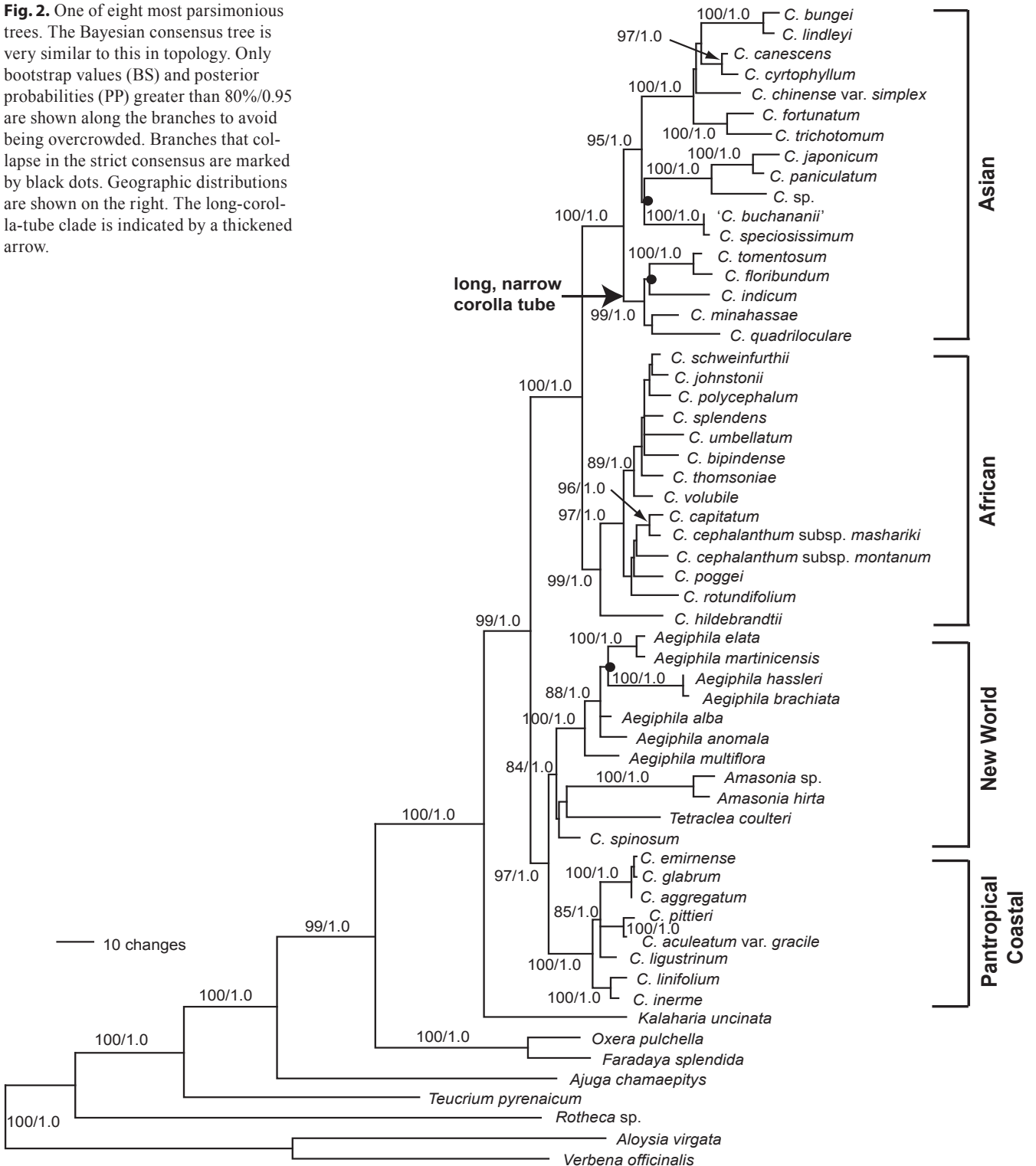
DISCUSSION

Redefinition of *Clerodendrum* and revival of *Volkameria* and *Ovieda*. — *Clerodendrum* as currently circumscribed (Steane & Mabberley, 1998; Steane & al., 2004) is certainly not monophyletic (Fig. 2). In order to delimit *Clerodendrum* as a monophyletic group, either the New World clade should be incorporated in *Clerodendrum* or the Pantropical Coastal clade should be removed from *Clerodendrum* and raised to generic level. Renaming species will therefore be inevitable. We choose the second option here for two reasons: (1) *Aegiphila*, *Amasonia*, and *Tetraclea*, have ca. 120, 8, and 2 species, respectively, whereas the Pantropical Coastal clade comprises ca. 30 species. To minimize the number of name changes, it is more sensible to separate the Pantropical Coastal clade into a distinct genus, for which the earliest name is *Volkameria* L. in which a number of the germane names have already been published. (2) Retaining the generic distinction for *Aegiphila*, *Amasonia*, and *Tetraclea* while resurrecting *Volkameria* provides increased information about evolutionary relationships

in the classification of this group. Besides separating the Pantropical Coastal clade as the revived genus *Volkameria*, *Clerodendrum spinosum* also needs to be removed from *Clerodendrum*. Its original name, *Ovieda spinosa* L., is revived for it. Therefore, the newly delimited *Clerodendrum* is restricted to the Asian and African clades. This is of no little historical

interest in that molecular work has confirmed three of the four generic concepts used by Linnaeus for this group (see Taxonomy). It is also noteworthy that the long narrow corolla tube has evolved at least twice independently in the group: once in *Ovieda* and also in the common ancestor of the Asian clade comprising *C. indicum* (which was independently described

Fig. 2. One of eight most parsimonious trees. The Bayesian consensus tree is very similar to this in topology. Only bootstrap values (BS) and posterior probabilities (PP) greater than 80%/0.95 are shown along the branches to avoid being overcrowded. Branches that collapse in the strict consensus are marked by black dots. Geographic distributions are shown on the right. The long-corolla-tube clade is indicated by a thickened arrow.



in *Ovieda* at least three times; see below), *C. quadriloculare*, and others, as mentioned above.

Although it is difficult to find unique morphological synapomorphies to separate *Clerodendrum*, *Volkameria*, and *Ovieda*, no doubt a cardinal reason why a broad view of *Clerodendrum* has prevailed for so long, a combination of several characters, as listed in Table 1, can be readily used to distinguish the three genera.

Phylogenetic position of *C. hildebrandtii*. — Within the African clade, one species, *C. hildebrandtii*, is sister to the rest of the African group. This species is distinguished from other African species by its large corolla (few flowers in each inflorescence) and large cylindrical calyx. In fact, *C. hildebrandtii* is the sole member of Verdcourt's (1992) *C. sect. Cylindrocalyx* (Thomas) Verd. in his treatment of the genus in East Africa. However, approximately 20 species of *Clerodendrum* that are restricted to Madagascar closely resemble *C. hildebrandtii* in morphology. Unfortunately, we were unable to obtain any living material of these species or herbarium specimens of sufficient quality for DNA extraction. But we predict that this particular Madagascan group, together with *C. hildebrandtii*, will form a clade that is sister to the rest of the African clade. A future study with extensive sampling of this group will shed light on the evolution of this, perhaps the most beautiful, *Clerodendrum* group.

Evolution of an intriguing breeding strategy. — An interesting breeding strategy has been reported in some species of *Clerodendrum* sensu stricto (s.str.) and *Volkameria* (Corner, 1940; Primack & al., 1981; Reddy & Reddi, 1995). The stamens and the style are curled upwards tightly inside the flower bud. When the flower opens, the filaments and style start uncoiling. While the filaments project to the centre, the style continues to bend down towards the lower side of the flower. The flower is strongly protandrous and this is the functional male phase. After pollen has been released and the anthers wither, the filaments curl back sideways and the style with its receptive stigma projects back to the centre, taking the position occupied by the stamens in the male phase (see Fig. 3; also see illustration and a detailed description in Reddy & Reddi, 1995). This strategy was first noted by Corner (1940: 700). He mentioned this as typical of species of the genus native in the Malay Peninsula except for *C. serratum*, which is indeed referable to another genus

as *Rothea serrata* (L.) Steane & Mabb., where “the stamens and style arch over the top of the flower and one of the petals is modified into a lower lip or landing platform” (Corner 1940: 700). Four decades later the same strategy was described by Primack & al. (1981) in *Volkameria inermis* (as *Clerodendrum inerme*). Then in 1995, a detailed description was made by Reddy & Reddi from their observation of *C. infortunatum*, an African species. Recently, it was observed in *C. trichotomum*, an Asian species, and *C. thomsoniae*, an African species, by the first author of this paper before he was aware of the earlier work. This presentation of pollen and stigma in the centre of the flower in a sequential fashion by moving the filaments and style is an elegant combination of dichogamy and herkogamy, that avoids self-fertilization or/and sexual interference (i.e., receiving pollen by stigmas and exporting pollen from anthers: Lloyd & Webb, 1986; Webb & Lloyd, 1986).

A subsequent search for floral images has revealed that not only *Clerodendrum* s.str. and *Volkameria*, but also *Ovieda*, *Amasonia*, *Tetraclea*, and *Kalaharia*, all display this particular floral presentation with curled stamens and style at different stages (Fig. 3). Field observations of the pollination ecology of *Oxera* and *Faradaya* by de Kok (1997) found these two genera resemble the aforementioned taxa by having protandrous flowers, but not in displaying the alternating movement between the filaments and style. In addition, floral presentation is heterogeneous in *Oxera* and *Faradaya* (de Kok, 1997), by contrast with the uniform system found in *Clerodendrum* s.str. and allies. *Clerodendrum* s.str., *Volkameria*, *Ovieda*, *Aegiphila*, *Amasonia*, *Tetraclea*, and *Kalaharia* together form a strongly supported clade (Fig. 2). Lamiaceous taxa outside this clade do not show such floral presentation. Therefore, this breeding strategy appears to have evolved only once in the common ancestor of these lineages and is a synapomorphy defining this clade. It has also been lost once and been replaced by a heterostylous system on the path leading to the extant *Aegiphila* lineage (Fig. 3G–H). This shift has interesting implications for the understanding of evolutionary pathways from homostyly to heterostyly.

Our examination of this intriguing breeding strategy in a phylogenetic context provides a striking example of how molecular phylogenetics can re-direct our effort in finding and re-interpreting overlooked morphological characters. The

Table 1. Comparison of morphological characters of *Clerodendrum* s.str., *Volkameria*, and *Ovieda*.

	<i>Clerodendrum</i>	<i>Volkameria</i>	<i>Ovieda</i>
Branches	Not tuberculate	Not tuberculate	Tuberculate
Leaf	Blade frequently longer than 6 cm, never spiny; venation not camptodromous	Blade usually shorter than 6 cm, never spiny; venation not camptodromous	Blade longer than 6 cm, margin usually with spiny teeth; venation camptodromous and reticulate with pronounced abaxial relief
Inflorescence	Usually terminal	Frequently axillary	Terminal
Fruiting calyx	Accrescent, larger than fruits, brightly coloured	Rarely accrescent, smaller than fruits, enclosing the fruit base, not brightly coloured	Accrescent, enclosing fruit, not brightly coloured
Fruits	Often fleshy, with bright colour contrasting with calyx	Usually dryish, not brightly coloured	Not recorded recently (Burman in Plumier, 1760, has ‘bacca obovata’), apparently not brightly coloured

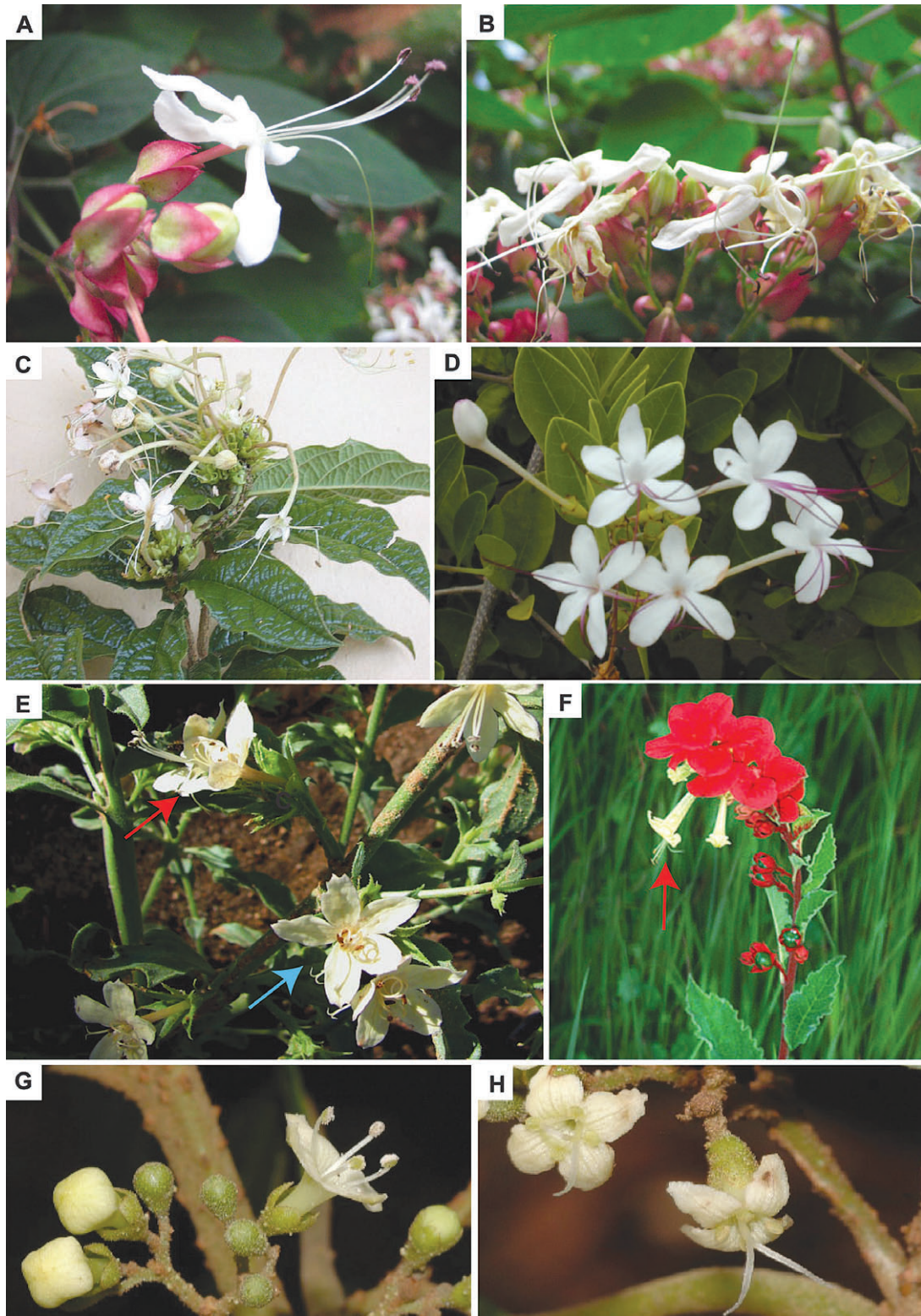


Fig. 3. Representative floral images of *Clerodendrum* s.str. and allied genera. **A–B**, *Clerodendrum trichotomum* Thunb. (photos by Y.-W. Yuan). **A**, flower at male phase. Note the stamens project to the centre, whereas the style bends down towards the lower side of the flower. **B**, flower at female phase. The filaments curl back sideways and the style with its receptive stigma projects back to the centre. **C**, *Ovidia spinosa* L. (photo courtesy of Jackeline Salazar). **D**, *Volkameria inermis* L. (photo courtesy of Forest & Kim Starr). **E**, *Tetraclea coulteri* A. Gray (photo courtesy of Burr Williams). The red and blue arrows indicate the flower at male and female phase, respectively. **F**, *Amasonia campestris* Moldenke (photo courtesy of Robin Foster). The red arrow indicates the flower at male phase. **G–H**, *Aegiphila* sp. (photos courtesy of Kevin Nixon, www.plant-systematics.org), showing the heterostylous system. **G**, thrum flower; **H**, pin flower.

“*Clerodendrum* s.str. + *Volkameria* + *Ovieda* + *Aegiphila* + *Amasonia* + *Tetraclea* + *Kalaharia*” clade recovered by molecular data has never been recognized in traditional classification schemes. But, after finding that this complex breeding strategy is shared by these taxa, it becomes obvious that they are all closely related as indicated by DNA sequences.

■ TAXONOMY

Genera recognized (see also Steane & Mabblerley, 1998 for *Rothea*) as a result of this work:

1. *Clerodendrum* L., Sp. Pl. 2: 637. 1753 – Type: *C. infortunatum* L.

= *Siphonanthus* L., Sp. Pl. 1: 109. 1753 – Type: *S. indicus* L. (*indica*) = *Clerodendrum indicum* (L.) Kuntze

= *Cryptanthus* Osbeck, Dagb. Ostind. Resa.: 215. 1757 – Type: *C. chinensis* Osbeck = *Clerodendrum chinense* (Osbeck) Mabb.

Trees, shrubs sometimes suckering, *lianes* or *subherbaceous perennials*. *Leaves* simple (sometimes lobed), decussate or (rarely) whorled, never spiny. *Inflorescences* cymose, usually terminal. *Flowers* bisexual; calyx campanulate to tubular, variously lobed, often coloured, usually accrescent; corolla red to yellow, pink or white, with narrow tube, 5-lobed, the lobes usually unequal; stamens 4 (or 5), didynamous, inserted within corolla tube, usually long-exserted; ovary imperfectly 4-locular, each locule with 1 ovule, style terminal, elongate, shortly 2-lobed. *Fruit* a drupe, often 4-sulcate or 4-lobed; endocarp tough, separating into 4 (or 2 pairs of) pyrenes (sometimes only two maturing), each with one seed.

Circa 150 species in tropical Old World with some species found as far south as Australia and as far north as China and Japan.

2. *Ovieda* L., Sp. Pl. 2: 637. 1753 – Type: *O. spinosa* L. ≡ *Clerodendrum spinosum* (L.) Spreng.

Shrub to 1.5 m, sometimes subherbaceous; *branches* tuberculate. *Leaves* simple, decussate (occasionally some in spirals), coriaceous, margin toothed, teeth usually spiny, venation camptodromous, pinnate-reticulate, with conspicuous pronounced venation in relief on abaxial surface. *Inflorescences* corymbose, terminal. *Flowers* bisexual; calyx large, campanulate with 5 acute lobes, accrescent enclosing fruit, not brightly coloured; corolla white with long narrow tube, mouth 5-lobed; stamens 4, exserted; style solitary, as long as stamens; ovary globose. *Fruit* a drupe, globose to obovate, with 2 locules, each with one seed.

One species, restricted to Hispaniola.

Ovieda spinosa L., Sp. Pl. 2: 637. 1753 – Lectotype (designated here): Burman in Plumier, Pl. Amer.: t. 256. 1760.

Notes. – Hitherto type material has not been designated (Jarvis, 2007: 716). Linnaeus cited Plumier (1703, as *Valdia*) but that shows insufficient detail to be the basis for his description. A much more detailed description, together with a plate

that shows the inflorescence and leaf features that Linnaeus described in 1753 are shown in Burman’s 1760 work. Burman’s plate is based on a tracing of Plumier’s drawing that was made by Claude Aubriet for Herman Boerhaave, and later prepared for publication by Burman. Burman sent proof copies to Linnaeus ahead of their publication, and it seems clear that Linnaeus must have received this one prior to 1753, hence its being appropriate as type material, even though published after Linnaeus’s own work (Jarvis, 2007: 151).

Note: *Ovieda* Spreng. (1824) = *Lapeirousia* Pourr. (Iridaceae). For *Clerodendrum* s.l., Baillon (1891) resurrected *Ovieda* L., which had been made a synonym of *Clerodendrum* by authors from the 1820s onwards, so his action was inadmissible, though *Ovieda* as the name for *Clerodendrum* s.l. (including *Volkameria*) gained some currency in American publications (**Ovieda aculeata* (L.) Baill., Hist. Pl. 11: 95. 1891 non *O. aculeata* Klatt (1864 = *Lapeirousia fabricii* (de la Roche) Ker, Iridaceae) = *Volkameria aculeata* L.; **O. bracteosa* (Kostel.) Baill., l.c. (*C. bracteosum* Kostel. [type (icon): Rheede, Hort. Malab. 4: t. 29. 1683] = *Rothea serrata* (L.) Steane & Mabb. [**syn. nov.**]); *O. fragrans* (Willd.) Hitchcock (= *C. chinense* (Osbeck) Mabb.); *O. inermis* Burm. f. (= *C. indicum* (L.) Kuntze); **O. infortunata* (L.) Baill., l.c. (= *C. infortunatum* L.); *O. mitis* L. (= *C. indicum* (L.) Kuntze); *O. ovalifolia* A. Juss. (*C. ovalifolia* (A. Juss.) Bakh. [type: India, Pondichéry, Commerson s.n. in Hb. Juss. (microfiche 347/19-P-JUSS)] = *V. inermis* L. [**syn. nov.**]); **O. trichotoma* (Thunb.) Baill., l.c. (= *C. trichotomum* Thunb.); *O. verticillata* Roxb. ex D. Don [*nom. in synonym.*] = *C. indicum* (L.) Kuntze).

*Additions to *Index kewensis* and other standard lists.

3. *Volkameria* L., Sp. Pl. 2: 637. 1753 – Type: *V. aculeata* L. ≡ *Clerodendrum aculeatum* (L.) Schldl.

= *Huxleya* Ewart in Proc. Roy. Soc. Victoria, ser. 2, 25: 109. 1912, **syn. nov.** – Type: *H. linifolia* Ewart & B. Rees, l.c. ≡ *C. linifolium* (Ewart & B. Rees) de Kok = *V. linifolia* (Ewart & B. Rees) Mabb. & Y.W. Yuan, **comb. nov.** – Type: Australia, Northern Territory, Darwin, 1892, *N. Holtze* 1322 (lecto MEL).

Shrubs, sometimes *subherbaceous*, *lianes*, rarely *small trees*; branches ± tetragonal, usually ash-grey, nodes swollen. *Leaves* decussate (to ternate), subglabrous, with entire margin, never spiny, venation arcuate-reticulate. *Inflorescences* axillary to supra-axillary cymes. *Flowers* usually fragrant; calyx campanulate, only rarely accrescent, margin with 5 broadly triangular small teeth; corolla hypocrateriform, white, sometimes pink or purple, lobes unequal; stamens 4 (or 5), didynamous, inserted within corolla tube, exserted; ovary cylindrical; stigma shortly bifid. *Fruits* generally globose to obovoid, becoming black or brown and separating into 4 corky pyrenes, each with 1 seed.

Approximately 25–30 species, pantropical but with apparently only one species in Asia (*V. inermis* L.)

Notes. – Pending a critical review of *Volkameria* taxa of Madagascar, where they appear to be numerous (Moldenke, 1956), the germane species are not transferred from *Clerodendrum* here, though one at least already has a name in

Volkameria: *V. heterophylla* Vent. (*C. heterophyllum* (Vent.) R. Br., Mascarenes, Madagascar), nor are those of Australia except *C. linifolium* above. However, all the five species native in Mesoamerica can now be placed as they have been monographed by Rueda (1993, see for species descriptions). To these can be safely added two widespread African species to add to those already with names in *Volkameria*, plus *C. aggregatum* used in our analysis:

1. *Volkameria acerbiana* Vis. \equiv *Clerodendrum acerbianum* (Vis.) B.D. Jacks.
Tropical Africa. See Verdcourt (1992) for a description.
2. *V. aculeata* L. \equiv *C. aculeatum* (L.) Schldl.
Tropical America.
3. *Volkameria aggregata* (Gürke) Mabb. & Y.W. Yuan, **comb. nov.** \equiv *Clerodendrum aggregatum* Gürke in Bot. Jahrb. Syst. 18: 177. 1894 – Type: Madagascar, Loko-be, *Hildebrandt 3339* (B \dagger holo; K iso, NY iso).
Tropical Africa.
4. *Volkameria costaricensis* (Standl.) Mabb. & Y.W. Yuan, **comb. nov.** \equiv *Clerodendrum costaricense* Standl. in Publ. Field Mus. Nat. Hist., Bot. Ser. 18: 1002. 1938 – Type: Costa Rica, La Pena de Zarcero, *Smith H588* (H holo; MO iso, NY fragm.).
Caribbean.
5. *Volkameria eriophylla* (Gürke) Mabb. & Y.W. Yuan, **comb. nov.** \equiv *Clerodendrum eriophyllum* Gürke in Bot. Jahrb. Syst. 18: 178. 1894 – Type: [Tanzania] *Fischer ser. I, 331* (B \dagger holo).
Tropical Africa. See Verdcourt (1992) for a description.
6. *Volkameria glabra* (E. Mey.) Mabb. & Y.W. Yuan, **comb. nov.** \equiv *Clerodendrum glabrum* E. Mey., Comm. Pl. Afr. Austr.: 273. 1837 – Type: South Africa, Cape Province, R. Basche, *Drège s.n.*, (B \dagger holo; K iso).
See Verdcourt (1992) for a description.
7. *V. inermis* L. \equiv *C. inerme* (L.) Gaertn.
Tropical Asia and Pacific. See Mabberley (2004) for a description.
8. *V. ligustrina* Jacq. \equiv *C. ligustrinum* (Jacq.) R. Br.
Tropical America.
9. *V. linifolia* (Ewart & B. Rees) Mabb. & Y.W. Yuan (see above).
Northern Australia.
10. *Volkameria mollis* (Kunth) Mabb. & Y.W. Yuan, **comb. nov.** \equiv *Clerodendrum molle* Kunth., Nov. Gen. Sp. 2, quarto ed.: 244. 1818 – Type: Ecuador, *Bonpland 3387* (P-HB 6209.46: III: 1 (microfiche)).
Tropical America.

11. *Volkameria pittieri* (Moldenke) Mabb. & Y.W. Yuan, **comb. nov.** \equiv *Clerodendrum pittieri* Moldenke in Phytologia 1: 416. 1940 – Type: Peru, *Pittier 4965* (US holo; MO iso).
Caribbean.

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Appendix 1. Plant material used in this study. Each entry consists of: taxon name, country in which it was collected, year of collection, collector with collection number, and herbarium (accession number when available) where voucher was deposited.

Aegiphila alba Moldenke, Ecuador, 2004, *J. Clark* 7960, US; *A. anomala* Pittier, Costa Rica, 1995, *J.F. Morales & V. Urena* 3760, MO (5748376); *A. brachiata* Vell., Brazil, 2003, *A. Kegl* 1581, MO (5772239); *A. elata* Sw., Panama, 1986, *G. McPherson* 8473, MO (4290179); *A. hassleri* Briq., Argentina, 2000, *De Romero & al.* 2201, MO (5830202); *A. martinicensis* Jacq., Puerto Rico, 1980, *J.C. Solomon* 5732, MO (2897682); *A. multiflora* Ruiz & Pav., Bolivia, 1983, *St. G. Beck* 8688, MO (4275754); *Ajuga chamaepitys* (L.) Schreb., *Cantino* 185, K; *Amasonia hirta* Benth., Brazil, 1982, *B.A.S. Percira* 251, MO (2994726); *Amasonia* sp., Brazil, 1997, *Giulietti & al.*, *PCD* 6176, K; *Clerodendrum aculeatum* (L.) Schldl. var. *gracile* Griseb. ex Moldenke, Fairchild Tropical Botanic Garden, 2006, *Zona* 1100 & *Gillis* 9169, FTG; *C. aggregatum* Gürke, Madagascar, 1996, *Gautier & S.T. Be*, *LG* 2873, MO (5208533); *C. bipindense* Gürke, Guinea, 1987, *Carvalho* 3033, MO (4322999); *C. buchananii* auctt., non (Roxb.) Walp., Oxford Botanic Garden, *Steane* 76, FHO; *C. bungei* Steud., Oxford Botanic Garden, *Steane* 78, FHO; *C. canescens* Wall. ex Walp., China, *S.X. Luo* 242, IBSC; *C. capitatum* (Willd.) Schumacher & Thonn., Tanzania, 1998, *O.A. Kibure* 183, MO (5310537); *C. cephalanthum* Oliv. subsp. *mashariki* Verdc., Tanzania, 1999, *M.A. Mwangoka* 974, MO (5290487); *C. cephalanthum* subsp. *montanum* (Thomas) Verdc., Tanzania, 2003, *O.A. Kibure* 982, MO (04473598); *C. chinense* (Osbeck) Mabb. var. *simplex* (Moldenke) S.L.Chen, Fairchild Tropical Botanic Garden, 2006, *Fantz* 3419, FTG; *C. cyrtophyllum* Turcz., China, *S.X. Luo* 253, IBSC; *C. emirnense* Boj. ex Hook., Madagascar, 1990, *P.B. Phillipson & al.* 3414, MO (3842769); *C. floribundum* R. Br. Australia, 1989, *C.R. Dunlop* 8055, L(0625213); *C. fortunatum* L., China, *S.X. Luo* 252, IBSC; *C. glabrum* E. Mey., Oxford Botanic Garden, *Steane* 87, FHO; *C. hildebrandtii* Vatke var. *puberula* Verdc., Tanzania, 1999, *C.J. Kayombo* 2302, MO (5291257); *C. indicum* (L.) Kuntze, Singapore, RK 5394; *C. inerme* (L.) Gaertn., Oxford Botanic Garden, *Steane* 86, FHO; *C. japonicum* (Thunb.) Sweet, China, *S.X. Luo* 254, IBSC; *C. johnstonii* Oliv., Tanzania, 1999, *P. Phillipson & J. Mwangwa* 2017, MO (5189370); *C. ligustrinum* (Jacq.) R. Br., Mexico, 1998, *R. Novelo & Ay Ramos* 2510, L (0625210); *C. lindleyi* Decne. ex Planch. China, *S.X. Luo* 251, IBSC; *C. linifolium* (Ewart & B. Rees) de Kok, Australia, 1999, *Cowie* 8213, K; *C. minahassae* Teijsm. & Binn., Fairchild Tropical Botanic Garden, 2006, *Houghton & White* 1145, FTG; *C. paniculatum* L., Palau (in cultivation), 1966, *N.H. Cheatham* 32, US(3356180); *C. pittieri* Moldenke, Peru, 1976, *D.C. Wasshausen & F. Encarnarium* 686, US (2956667); *C. poggei* Gürke, Tanzania, 1999, *G. Gobbo* 281, MO (5290499); *C. polycephalum* Bak., Ghana, 1994, *C.C.H. Jongkind & H.H. Schmidt* 1735, MO (04667183); *C. quadriloculare* Merr. Fairchild Tropical Botanic Garden, 2006, *Zona* 1104, FTG; *C. rotundifolium* Oliv., Tanzania, 2000, *S. Bidgood & al.* 4819, MO (5658503); *C. splendens* G. Don, National Botanic Garden, Zimbabwe; *C. schweinfurthii* Gürke, Oxford Botanic Garden, *Steane* 82, FHO; *C. sp.*, Indonesia, 2001, *Ramadhanil & al.* 461, L (0334140); *C. speciosissimum* Hort. Gand. ex Drapiez, Oxford Botanic Garden, *Steane* 90, FHO; *C. spinosum* (L.) Spreng. Dominican Republic, 2001, *F. Jimenez* 3332, MAPR (26103); *C. thomsoniae* Balf., UW Greenhouse, 2002, *RG0* 2002-07, WTU; *C. tomentosum* (Vent.) R. Br., Australia, 1984, *S.J. Forbes* 2510, L (0625210); *C. trichotomum* Thunb., R.G. Olmstead home garden, 2002, *RG0* 2002-134, WTU; *C. umbellatum* Poir., Tanzania, 1999, *G. Gobbo* 486, MO (5290496); *C. volubile* P. Beauv., Ghana, 1996, *M. Merello & al.* 1345, MO (05030536); *Faradaya splendida* F. Muell., *H. Rimpler* 2144, FB; *Kalaharia uncinata* (Schinz) Moldenke, Tanzania, 2002, *N.A. Mwangulango* 973, MO (5721485); *Oxera pulchella* Labill., *H. Rimpler* 1328, FB; *Rothea* sp., Madagascar, *Wen* 9487, US; *Tetraclea coulteri* A. Gray, U.S.A., *Ki-Joong Kim* 100026, TEX; *Teucrium pyrenaicum* L., Voucher unknown.

Appendix 2. Primers used for PCR and sequencing; the ones that were specifically designed for this study are marked by an asterisk (*).

trnL-F: c, d, e, f (Taberlet & al., 1991); *trnS-fM*: trnS^{UGA} & trnM^{CAU} (Shaw & al., 2005), psbZF(V) (Yuan & Olmstead, 2008), psbZR(C)* (5'-CAT-CAATCTTATTGATTAGCGTA-3'); *trnD-T*: trnD^{GUC}F & trnT^{GGU}R (Shaw & al., 2005), trnD-TE2T* (5'-AATTCGAATCCCCGCTGCCTCC-3'), trnD-TE2D* (5'-CATTCCATTATATTGACAATT-3'); *trnT-L*: a (Taberlet & al., 1991), R(Cler)* (5'-ACCTATAGGAACCCATATT-3').