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 Rotheca, and the unspecific 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, trnL-F, trnF- F, trnD-F, and trnS-F/M, 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 Pantropical Coastal clade. The Asian clade and African clade are sister groups and together form a monophyletic group. However, the Pantropical 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 Pantropical 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 heterostylos 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 Rotheca 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 Pantropical 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 unspecific Australian genus Huxleya Ewart, put the delimitation of Clerodendrum (sensu Steane & Mabberley, 1998) into question again. Huxleya was found nested within the Pantropical 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
representing 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, 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-Ajugoideae (Ajuga L., Teucrium L., Rotheca). 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-trnF. 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-trnF: 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.](image-url)
of the dataset. Six poly-nucleotide or microsatellite regions (a microsatellite with “AT” repeats and a poly-T region in the trnD-trnF 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 trnFM-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 (lset 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 strongly supported sister groups (100%/1.0, BB/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. quadrioculare, 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. hildegbrandtii, 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. brachiatrica clade, and the A. elata + A. martinciscis 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 Verdecourt’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 Rotheca 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 inermis). 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). Lamiaceae 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 heterostyly 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.

<table>
<thead>
<tr>
<th>Character</th>
<th>Clerodendrum s.str.</th>
<th>Volkameria</th>
<th>Ovieda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branches</td>
<td>Not tuberculate</td>
<td>Not tuberculate</td>
<td>Tuberculate</td>
</tr>
<tr>
<td>Leaf</td>
<td>Blade frequently longer than 6 cm, never spiny; venation not camptodromous</td>
<td>Blade usually shorter than 6 cm, never spiny; venation not camptodromous</td>
<td>Blade longer than 6 cm, margin usually with spiny teeth; venation camptodromous and reticulate with pronounced abaxial relief</td>
</tr>
<tr>
<td>Inflorescence</td>
<td>Usually terminal</td>
<td>Frequently axillary</td>
<td>Terminal</td>
</tr>
<tr>
<td>Fruiting calyx</td>
<td>Accrescent, larger than fruits, brightly coloured</td>
<td>Rarely accrescent, smaller than fruits, enclosing the fruit base, not brightly coloured</td>
<td>Accrescent, enclosing fruit, not brightly coloured</td>
</tr>
<tr>
<td>Fruits</td>
<td>Often fleshy, with bright colour contrast- ing with calyx</td>
<td>Usually dryish, not brightly coloured</td>
<td>Not recorded recently (Burman in Pluimer, 1760, has ‘bacca obovata’), apparently not brightly coloured</td>
</tr>
</tbody>
</table>
“Clerodendrum s.str. + Volkameria + Ovieda + Aegiphila + Amasonia + Tetraclea + Kalalaharia” 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 & Mabberley, 1998 for *Rotheca*) as a result of this work:

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

   Trees, shrubs sometimes suckerimg, lianes or subherbacose 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 tooid. 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.


   Shrubs to 1.5 m, sometimes subherbacose; branches tuberculate. Leaves simple, decussate (occasionally some in spirals), coriaceous, margin toothed, teeth usually spiny, veination cymptodromous, 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, exerted; 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.


**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* (Kest) Baill., l.c. (*C. bracteosum* Kestel. [type: Rheed, Hort. Malab. 4: t. 29. 1683] = *Rotheca serrata* (L.) Steane & Mabb. [syn. nov.]); *O. fragrans* (Wild.) Hitchcock (= *C. chinese* (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.) Baakh. [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 synon.] = *C. indicum* (L.) Kuntze).

*Additions to Index kewensis and other standard lists.


   Shrub, sometimes subherbacose, 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, exerted; ovary cylindrical; stigma shortly bifid. Fruits generally globose to obovate, 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

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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:


2. **V. aculeata** L. ≡ *C. aculeatum* (L.) Schdl. Tropical America.


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**LITERATURE CITED**


### Appendix 1

Plant material used in this study consists of: 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.


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