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# *Pleurostylia serrulata* and Two Allied New Species from Africa are Actually Members of the New World *Crossopetalum* (Celastraceae)

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***Abstract***—Based on phylogenetic analyses using rDNA and plastid sequence data, and the examination of morphological characters, we infer that *Pleurostylia*, as currently delimited, is a polyphyletic group. *Pleurostylia serrulata* and two newly described species from Africa are part of the New World *Crossopetalum* lineage. By contrast, *Pleurostylia* s. s. consists of all remaining species, which form a clade nested within a primarily Malagasy lineage. We present preliminary evidence that *Pleurostylia opposita*, the most geographically widespread species in the entire Celastraceae family, may include cryptic species. Although molecular evidence supports the monophyly of *Crossopetalum*, this genus does not appear to be well defined by a single morphological synapomorphy, rather the following combination of character states is diagnostic: 4-merous flowers; stamens inserted in the sinuses of a ± 4-lobed intrastaminal floral disk; pistil (2- or) 4-locular with one atropous ovule per locule; stigma conspicuously (2-) 4-branched; drupe usually single seeded and asymmetric with an excentric style remnant; and seeds exarillate with ramified postchalazal bundles visible on the surface and with endosperm present. A taxonomic treatment of *Crossopetalum* in Africa is presented, in which the new combination ***Crossopetalum serrulatum*** is proposed and two new species, ***C. bokdamii*** and ***C. mossambicense***, are described and their conservation status assessed.

***Keywords***—Cameroon, Caribbean, Congo, Mozambique, phylogeny.

*Pleurostylia* Wight & Arn. is a genus of seven currently recognized species within Celastraceae that is distributed in Africa, Madagascar and surrounding islands, India, Sri Lanka, SE Asia, Malesia, Queensland (Australia), and New Caledonia (Loesener 1942; Simmons 2004a). Like many other genera within Celastraceae, *Pleurostylia* consists of trees and shrubs with opposite leaves, axillary cymose inflorescences, bisexual flowers, and indehiscent fruits with albuminous seeds. The morphological synapomorphy that has been used to distinguish *Pleurostylia* is that their unilocular ovaries with a single fertile carpel (Matthews and Endress 2005) develop asymmetrically such that the style is positioned laterally on the nut. This synapomorphy is the basis for the generic name (Loesener 1942).

The most widely distributed species, by far, is *Pleurostylia opposita* (Wall. ex Carey) Alson, which is recorded from southern India to Australia and New Caledonia, with a single record from coastal Mozambique (Robson 1966; Robson and Sousa 1969). In contrast, *P. africana* Loes., *P. capensis* Oliv., and *P. serrulata* Loes. are endemic to Africa; *P. pachyphloea* Tul. is endemic to Mauritius and Reunion Island (Baker 1877; Perrier de la Bâthie 1946); *P. putamen* Marais is endemic to Rodrigues Island (Marais 1981); and *P. cochinchinensis* Pierre is endemic to southeast Asia, having been described from Vietnam (Pierre 1894). Within Africa, *P. africana* has been recorded from Kenya and Uganda south to Mozambique, Zimbabwe, and with a disjunct record from Huíla in southern Angola (Robson and Sousa 1969; Robson et al. 1994). The other two African endemic species are much more narrowly distributed. *Pleurostylia capensis* is endemic to South Africa (Davison 1927; Palmer and Pitman 1972) while *P. serrulata* was previously considered to be endemic to Cameroon (Villiers 1975), but there is a single depauperate specimen from Congo (Brazzaville) in the Paris herbarium (P) that the current authors believe to represent this species. *Pleurostylia* has not been reported from Ghana (Irvine 1961), Nigeria (Keay 1989), or otherwise west of Cameroon (Blakelock 1958; Geerling 1987 Hawthorne and Jongkind 2006), and *P. serrulata* has not been reported from Gabon (Villiers 1973) or the Democratic Republic of the Congo (Wilczek 1960). Other binomials have been synonymized with these seven species (or *Microtropis* Wall ex Meisn.) as follows: *P. heynei* Wight & Arn. = *P. opposita* (Ding Hou 1962; Wadhwa 1996), *P. leucocarpa* Baker = *P. pachyphloea* (Loesener 1942), *P. pallens* Pierre = *Microtropis pallens* Pierre (Merrill and Freeman 1940), *Celastrus wightianus* Wall. (and hence *P. wightianae* Tul.) = *P. wightii* (Wight and Walker-Arnott 1834), and *P. wightii* Wight & Arn. = *P. opposita* (Ding Hou 1962; Wadhwa 1996; Pullaiah and Rao 2002).

Among the seven currently recognized species in *Pleurostylia*, *P. serrulata* is unique in the following four traits. First, the young branches and leaves are pubescent rather than glabrous. Second, it has crenulate-serrulate rather than entire or undulate leaves. Third, it lacks peltate stigmas (Villiers 1975). Fourth, it has four-locular rather than unilocular ovaries. Loesener (1942, p. 180) listed the species as questionably assigned to the genus (“*P.* (?) *serrulata* Loes. in Kamerun.”), but Villiers (1975) more confidently placed the species in *Pleurostylia* after examining flowering specimens that were not available to Loesener (1908, 1942). Nonetheless, given the species’ unique traits within *Pleurostylia*, its weakly (rather than strongly) offset style (Villiers 1975), its apparently disjunct distribution relative to the other six *Pleurostylia* species, and that it has never (to our knowledge) been sampled in a published phylogenetic study, this species has been a clear priority to include in a phylogenetic analysis to determine whether or not it is a distinct genus from *Pleurostylia*. Furthermore, two previously unnamed African species have recently come to light that are morphologically close to *P. serrulata*. The first is a newly discovered species from the coastal dry forests of northeast Mozambique, first collected in 2008. The second is known from three historical collections from the Democratic Republic of the Congo. These finds have further increased the need to confirm the generic placement of *P. serrulata* and its allies.

Only a single specimen of each of three *Pleurostylia* species have been sampled in published phylogenetic analyses to date: *P. capensis* (Islam et al. 2006; Simmons et al. 2008, 2012a, 2012b; Coughenour et al. 2010, 2011), *P. opposita* (Simmons et al. 2001a, 2001c, 2008, 2012a, 2012b; Islam et al. 2006; Coughenour et al. 2010, 2011), and *P. pachyphloea* (as *P. leucocarpa*; Simmons et al. 2001c, 2008, 2012a, 2012b; Islam et al. 2006; Coughenour et al. 2010, 2011). In all of the 2001–2011 studies, *Pleurostylia* was resolved as a clade (when two or three species were sampled) that is most closely related to *Elaeodendron* Jacq. (and, when sampled, the Malagasy genus *Pseudocatha* ined.). Both of the 2012 studies included additional Malagasy genera that were found to be still more closely related to *Pleurostylia* than *Elaeodendron* or *Pseudocatha*. Simmons et al. (2012a) included *Astrocassine* ined., which was supported as sister to *Pleurostylia*. Simmons et al. (2012b) also added *Hartogiopsis trilobocarpa* (Baker) H. Perrier, which was identified as more closely related to *Pleurostylia* than *Astrocassine*. Given that *Elaeodendron* is native to Madagascar and that *Astrocassine*, *Hartogiopsis*, and *Pseudocatha* are endemic to Madagascar, we hypothesize that Madagascar is the center of origin for *Pleurostylia*, though no extant species of this genus are known from there.

In this study we expanded upon the previous phylogenetic sampling of *Pleurostylia* by sampling eight additional specimens, including three specimens from the two new species that are morphologically similar to *P. serrulata*. The two new species and *P. serrulata* are actually found to be distantly related to *Pleurostylia* s. s. These three species, together with *Wilczekra congolensis* (R. Wilczek) M. P. Simmons, are the mainland African representatives of an early derived lineage of Celastraceae that includes 18 genera with a primarily Gondwanan distribution (Bacon et al. 2016). Curiously, the closest known relatives of the new species and *P. serrulata* are in *Crossopetalum* P. Browne, which is otherwise endemic to the Caribbean and adjoining areas of North, Central, and South America. The two new species are therefore formally described in *Crossopetalum* and the new combination and an updated description are provided for *P. serrulata* (= *Crossopetalum serrulatum* (Loes.) I. Darbysh.).

## Materials and Methods

***Taxon Sampling***—Eight new terminals were sampled for morphological and molecular characters: two specimens of *Pleurostylia africana*, two African specimens of *P. opposita*, one specimen each of *P. pachyphloea* and *P. putamen*, and two specimens of the new species from Mozambique (Appendix 1). We repeatedly attempted to extract DNA from a leaf of the *P. serrulata* specimen *Letouzey 10693* (BR), which was collected in 1971, but were unsuccessful in doing so. We were unable to locate any specimens of *P. cochinchinensis* that appeared promising for DNA isolation. In addition, a DNA sample of the new species from the Democratic Republic of the Congo (*Bokdam 3263*) was extracted separately at the Jodrell Laboratory, Kew. Due to the high level of DNA degradation of this latter specimen we were unable to amplify most of the DNA regions and could not fully incorporate it into the phylogenetic analyses (see Molecular Methods below).

Preliminary phylogenetic analyses using existing Celastraceae s. s. (i.e. not including members of the former Hippocrateaceae) taxon sampling from Simmons et al. (2012a, 2012b) and Bacon et al. (2016) were conducted using 26S rDNA and *matK* independently of each other. In both cases the newly sampled specimens of *Pleurostylia* were resolved in a clade with the three previously sampled specimens of *Pleurostylia*. In contrast, the new species was resolved in a clade with *Crossopetalum* and *Xenodrys* ined. in both gene trees.

Simmons et al. (2012b) resolved *Pleurostylia* as sister to *Hartogiopsis trilobocarpa* with 94% parsimony jackknife support (Kluge and Farris 1969; Farris et al. 1996) and 97% likelihood bootstrap support (Felsenstein 1973, 1978). The next two successive sister groups of *Pleurostylia* + *Hartogiopsis* H. Perrier are *Astrocassine* ined. (99% jackknife / 100% bootstrap) and then *Elaeodendron* Jacq. + *Pseudocatha* ined. (100% jackknife / 100% bootstrap). Therefore, a compartmentalized phylogenetic analysis (Maddison et al. 1984) was performed to infer the phylogeny of *Pleurostylia* that included *Astrocassine*, *Elaeodendron*, *Hartogiopsis*, and *Pseudocatha* as outgroups. These compartmentalized analyses of *Pleurostylia* and its relatives are hereafter referred to as the *Pleurostylia* analyses.

Simmons et al. (2012b) resolved *Crossopetalum* in a clade with *Peripterygia marginata* (Baill.) Loes., *Siphonodon* Griff., and the unambiguously supported (100% jackknife / 100% bootstrap) Austral-Pacific clade (with 13 genera including *Apatophyllum* McGill., *Denhamia* Meisn., *Hexaspora* C. T. White and *Hypsophila* F. Muell.) with 96% parsimony jackknife support and 99% likelihood bootstrap support. To this clade of 16 genera Simmons and Cappa (2013) added *Wilczekra congolensis* (R. Wilczek) M. P. Simmons, and Bacon et al. (2016) added *Xenodrys micranthum* ined. *Xenodrys* was inferred to be sister to *Crossopetalum* with 100% parsimony jackknife support and 100% likelihood bootstrap support. Therefore, a compartmentalized phylogenetic analysis was performed to infer the phylogenetic relationships of the new species relative to *Crossopetalum* and *Xenodrys* by using *Apatophyllum*, *Denhamia*, *Hexaspora*, *Hypsophila*, *Peripterygia*, *Siphonodon*, and *Wilczekra* as outgroups. A still more distantly related outgroup (*Monimopetalum chinense* Rehder) was used to root the trees. These compartmentalized analyses of the new species and its relatives are hereafter referred to as the *Crossopetalum* analyses.

***Morphological Characters***—Morphological characters were derived from matrices originally published by Simmons and Hedin (1999) and most recently updated by Simmons et al. (2012a, 2012b) and Bacon et al. (2016). The morphological characters represent variation in vegetative, floral, and pollen morphology; leaf, seed, and stem anatomy; and chromosome numbers. To the degree possible, characters were scored using reductive coding rather than composite coding (Wilkinson 1995; Simmons and Freudenstein 2002). The characters and character states are listed in Simmons et al.'s (2012b) appendix 2 and the bases for most characters are described in detail by Simmons and Hedin (1999, pp. 746–751). *Pleurostylia africana*, *P. pachyphloea*, and *P. putamen* were newly scored from Loesener (1908, 1942), Marais (1981), and Robson et al. (1994). The new species from Mozambique was scored using the species description in the taxonomic treatment below. Only the parsimony-informative morphological characters were included in the simultaneous-analysis data matrices (six for the *Pleurostylia* analysis, 16 for the *Crossopetalum* analysis).

***Molecular Methods***—Total genomic DNA was extracted from herbarium specimens and silica gel-preserved (Chase and Hills 1991) leaves using the protocol described by Alexander et al. (2006). New sequences for two loci from the plastid genome (*matK* and *trnL-F*) and two gene regions from the nuclear genome (the internal transcribed spacers of nuclear ribosomal DNA [ITS] and 26S rDNA) were generated for this project. Primers, PCR, and sequencing protocols all followed those described by Simmons et al. (2012a, 2012b). All new sequences generated in this study have been deposited in GenBank under accession numbers KT862167 to KT862197 (Appendix 1).

The DNA of the new species from the Democratic Republic of the Congo (*Bokdam 3263*) was extracted at the Jodrell Laboratory, Kew (U. K.) using a modified CTAB protocol (see Buerki et al. 2009). The DNA was highly degraded and we only successfully sequenced two short plastid regions that are used as barcodes: *trnL* intron and *trnH-psbA*. Primers, PCR and sequencing protocols are available in Buerki et al. (2009) for *trnL* intron and Parmentier et al. (2013), for *trnH-psbA*.

***Alignment and Gap Coding***—Preliminary nucleotide alignments were obtained independently for each gene region using MAFFT ver. 7 (Katoh and Toh 2008a). Q-INS-i, which considers inferred secondary structure of rDNA (Katoh and Toh 2008b), was used for alignments of ITS and 26S rDNA. G-INS-i, the most accurate MAFFT algorithm for aligning loci other than rDNA, was used for all other loci. The 1PAM matrix was applied to all gene regions along with the default gap opening penalty (1.53) and the gap offset value set to 0.1.

Manual adjustments to the MAFFT alignments were performed in MacClade ver. 4.08 (Maddison and Maddison 2001) using the procedure outlined by Simmons (2004b), following Zurawski and Clegg (1987). We observed some ambiguously aligned regions where one or more sequences had a duplicate insertion (or the others had a deletion of one of two repeats) and the character-state distribution among the characters in the ambiguously-aligned region was identical for those sequences that have both repeats, such that the character-state distribution among the positions in question would be identical for either of the alternative alignments. In these cases, the ambiguously-aligned regions were kept in the analysis following Davis et al. (1998).

Two inversions were inferred in the *Crossopetalum* analyses, an autapomorphic 6-bp inversion at positions 159–164 in *matK* and a synapomorphic 3-bp inversion at positions 164–166 in the *trnL-F* intergenic spacer. We would normally score the 3-bp inversion synapomorphy as a separate character (sensu uninode coding; Simmons et al. 2000), but did not do so here because it is immediately flanked by an inferred indel and both the inversion and indel have the same character-state distribution, which indicates that they may not be independent events (Wilkinson, 1995).

A total of 155 ambiguously-aligned positions were excluded from the *Crossopetalum* analyses (26S rDNA: 23 positions from one region; ITS: 63 positions from three regions; *matK*: 0; *trnL-F*: 69 positions from three regions), and 26 ambiguously-aligned positions were excluded from the *Pleurostylia* analyses (ITS: 26 positions from two regions). Ambiguously-aligned nucleotides of individual sequences in regions that could not be unambiguously aligned with the remaining sequences were scored as ambiguous (“?”).

Gap characters, whose inclusion often affect the inferred tree topology and increase branch support values (Simmons et al. 2001b), were manually scored using modified complex indel coding (Simmons and Ochoterena 2000; Müller 2006). Fourteen parsimony-informative gap characters were scored from unambiguously aligned regions for the *Crossopetalum* analyses (26S rDNA: 2; ITS: 8; *matK*: 0; *trnL-F*: 4), and 25 were scored for the *Pleurostylia* analyses (26S rDNA: 3; ITS: 15; *matK*: 0; *trnL-F*: 7).

***Phylogenetic Analyses***—As a means of data exploration, several alternative potential process partitions (Bull et al. 1993) of the characters were analyzed. Each of the four gene regions was analyzed independently of one another to resolve their respective gene trees. Putative coalescent genes (Hudson 1990; Doyle 1995) were then analyzed and their trees compared to check for well-supported, contradictory signals that may have been caused by lineage sorting, introgression, and/or unrecognized paralogy (Doyle 1992). As such, gene trees for the adjacent rDNA gene regions and the plastid loci were analyzed independently of each other to check for potential introgression of the plastid genome or rDNA (Doyle 1992; Wendel et al. 1995) or unrecognized paralogy problems with rDNA (Álvarez and Wendel 2003; Bailey et al. 2003). An analysis of all molecular characters was then performed, followed by a simultaneous analysis of all morphological and molecular characters, which was conducted as the primary basis for phylogenetic inference (Kluge 1989; Nixon and Carpenter 1996). The simultaneous-analysis data matrices are posted as supplemental online data.

Equally weighted parsimony tree searches were conducted on each data matrix using PAUP* 4.0b10 (Swofford 2001). Branches with a minimum possible optimized length of zero were collapsed to improve efficiency of tree searches and help minimize artifacts caused by missing data (Kitching et al. 1998; Davis et al. 2005). Up to 50 trees were held within each of 2,000 random-addition-sequence (RAS) tree-bisection-reconnection (TBR) searches. Jackknife analyses were conducted with the removal probability set to approximately $e^{-1}$ (0.37). One-thousand jackknife replicates were performed with 100 RAS TBR searches (each with a maximum of 50 trees held) per replicate. Jackknife percentages were plotted onto the strict consensus (Schuh and Polhemus 1980) using TreeGraph ver. 2.0.45 (Stöver and Müller 2010) to help minimize frequency-within-replicates resampling artifacts (Davis et al. 1998; Simmons and Freudenstein 2011).

*Pleurostylia opposita* 12872 and *P. pachyphloea* 3194 were only sampled for morphological characters and 26S rDNA. Given that these two terminals were under-sampled relative to the other terminals sampled in the *Pleurostylia* simultaneous analysis, the simultaneous analysis was re-run after excluding these two terminals in an attempt to increase resolution and branch support for inferred relationships among the remaining terminals.

***Taxonomic Treatment***—The species descriptions presented are based on the study of herbarium material from K, P, WAG and YA; the description of *Crossopetalum mossambicense* was supplemented by reference to photographs of the plant in the field. Measurements are based on dry material except for the flowers of *C. mossambicense* and *C. serrulatum*, which were soaked in Aerosol OT 5% solution prior to study, and the fruits, which were heated in water for 3–6 minutes prior to dissection.

***Species Conservation Status***—The species conservation assessments follow the categories and criteria of IUCN (2012); extent of occurrence (EOO) and area of occupancy (AOO) for *Crossopetalum mossambicense* were calculated by using the Geocat tool (www.geocat.kew.org; Bachman et al. 2011). For AOO, a grid cell size of 4 $km^2$ was applied, this being recommended as an appropriate scale by the IUCN Standards and Petitions Subcommittee (2014).

## RESULTS

The simultaneous-analysis trees for the *Pleurostylia* analyses with and without *P. opposita* 12872 and *P. pachyphloea* 3194 are presented in Figs. 1A and 1B, respectively. The simultaneous-analysis tree for the *Crossopetalum* analysis is presented in Fig. 2. Strict consensus trees with jackknife values ≥ 50% plotted on them for the other 17 analyses are presented as supplementary data (Figs. S1–S17) available from the Dryad Digital Repository: doi: 10.5061/dryad.98d51 (Darbyshire et al. 2016). Data-matrix and tree statistics for all 17 analyses are presented in Table 1, which is also available via Dryad (Darbyshire et al. 2016).

***Pleurostylia***—*Pleurostylia* was resolved as a clade distinct from the other genera sampled in all nine phylogenetic analyses (Figs. 1, S1–S7) except for the *trnL-F* gene tree, wherein it was located as part of a polytomy with *Astrocassine* and *Hartogiopsis* (Fig. S5). No cases of mutually well supported (≥ 70% jackknife support) topological incongruence within *Pleurostylia* were identified among the nine analyses. On the

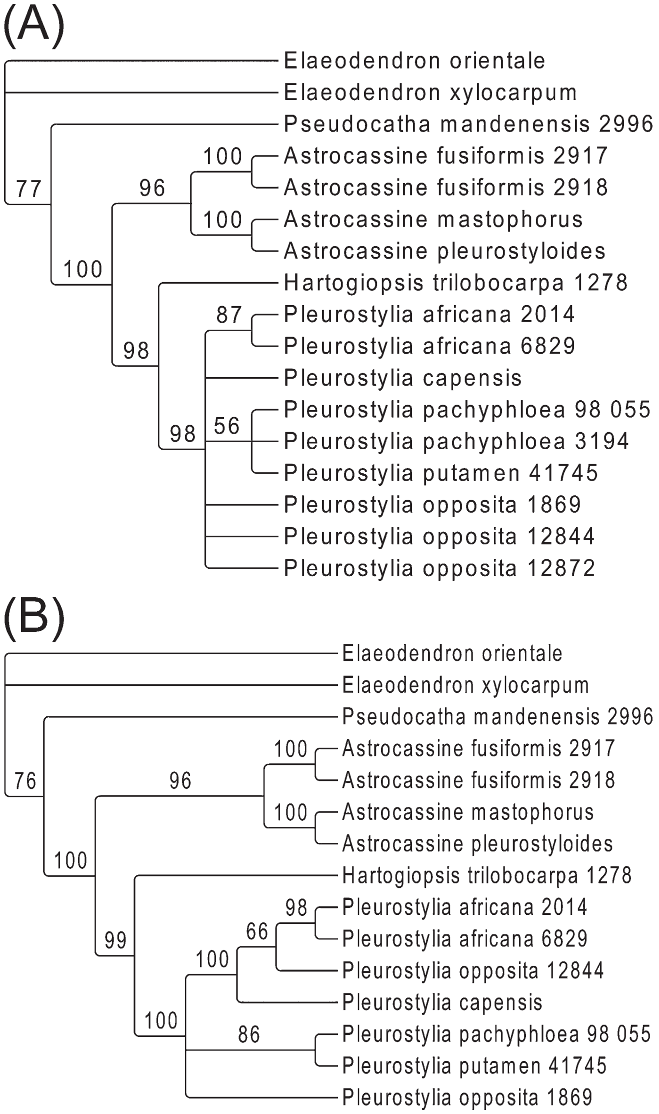

FIG. 1. Parsimony-based strict consensus of the simultaneous-analysis data matrix for the *Pleurostylia* analyses with all terminals (A), and excluding two terminals with high percentages of missing data (B). Jackknife values ≥ 50% are shown above each branch.

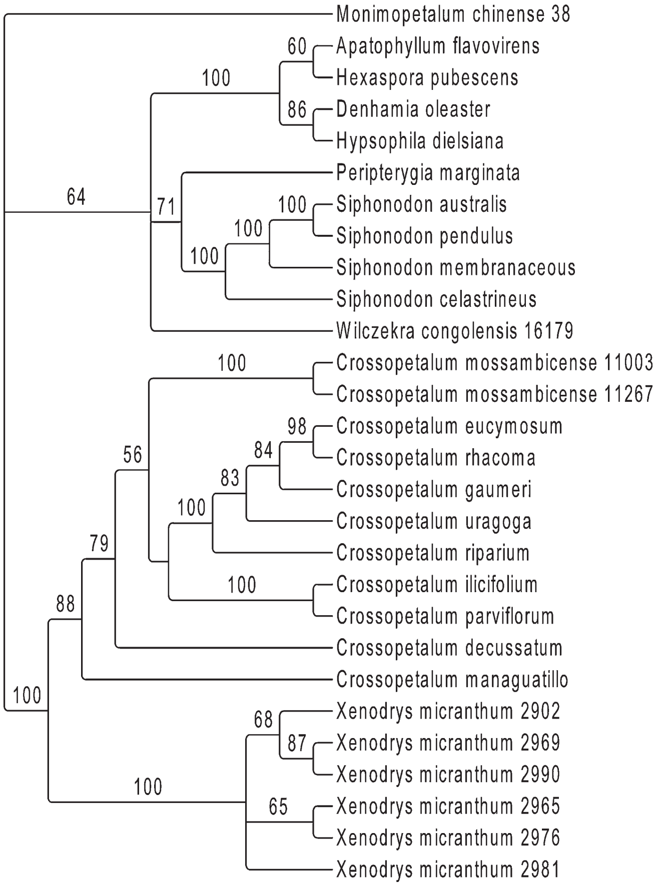

FIG. 2. Parsimony-based strict consensus of the simultaneous-analysis data matrix for the *Crossopetalum* analysis. Jackknife values ≥ 50% are shown above each branch.

simultaneous-analysis tree after excluding two terminals with high percentages of missing data, the two specimens of *P. africana* are resolved as an exclusive lineage whereas the two specimens of *P. opposita* are resolved as a polyphyletic group, with 100% and 66% jackknife support on the two clades that separate the specimens (Fig. 1B). *Pleurostylia opposita* 12844 is from Mozambique whereas *P. opposita* 1869 is from New Caledonia (Appendix 1).

***Crossopetalum***—The new species that we describe as *Crossopetalum mossambicense* I. Darbysh. below, was resolved in a clade together with *Crossopetalum* and *Xenodrys* in all eight phylogenetic analyses (Figs. 2, S8–S14). Mutually well-supported topological incongruences within this clade among the eight analyses were limited to intraspecific relationships within *Xenodrys micranthum*, and the 26S rDNA gene tree (*Crossopetalum decussatum* (Baill.) Lourteig and *C. managuatillo* (Loes.) Lundell as sister taxa with 75% jackknife support; Fig. S8) vs. the molecular and simultaneous analyses (the two species resolved as a paraphyletic group with 77% and 79% jackknife support for the separating branch). In all but the *matK* gene tree, *Xenodrys* was resolved as a clade distinct from *Crossopetalum* and the new species. In contrast, *Crossopetalum* and the new species were generally resolved as part of a polytomy and *Crossopetalum* was only resolved as a clade in the ITS gene tree, which did not include *C. decussatum* or *C. managuatillo*. *Crossopetalum*, including *C. mossambicense*, was resolved as a clade sister to *Xenodrys* in the ITS, plastid, molecular, and simultaneous analyses; in the remaining analyses they were resolved as part of a polytomy that includes *Xenodrys* (Figs. 2, S8–S14).

Among the *Crossopetalum* species, *C. mossambicense* was variously resolved as part of a basal polytomy (26S, rDNA, *matK*, *trnL-F*, plastid), sister to the other species (ITS, albeit neither *C. decussatum* nor *C. managuatillo* were sampled for this gene region) or nested within the New World species (molecular, simultaneous; Figs. 2, S8–S14). In the simultaneous analysis *C. mossambicense* was moderately well supported as nested within the New World *Crossopetalum* on two branches with 79% and 55% jackknife support, respectively. The lower branch with 79% jackknife support was supported by eight unambiguously optimized nucleotide synapomorphies (as identified using MacClade) from the 3′ region of *matK*. We were unable to sequence this region

TABLE 1. Data-matrix and tree statistics for each of the phylogenetic analyses. "CI" = ensemble consistency index (Kluge and Farris 1969) on the most parsimonious tree(s) for the parsimony-informative characters. "RI" = ensemble retention index (Farris 1989).

| Matrix | # terminals | # characters analyzed | # of parsimony informative characters | % missing / inapplicable | Most parsimonious tree length | # of most parsimonious trees | # of jackknife clades ≥ 50% | Average jackknife support (%) | CI | RI |
|---|---|---|---|---|---|---|---|---|---|---|
| *Pleurostylia* analyses: | | | | | | | | | | |
| 26S rDNA | 17 | 950 | 34 | 4.9 | 82 | 3 | 7 | 76 | 0.69 | 0.82 |
| ITS rDNA | 15 | 754 | 116 | 16.8 | 260 | 1 | 11 | 82 | 0.74 | 0.81 |
| rDNA (ITS, 26S) | 17 | 1704 | 150 | 14.5 | 345 | 5 | 8 | 85 | 0.72 | 0.81 |
| *matK* | 15 | 1348 | 19 | 13.7 | 59 | 1 | 7 | 76 | 0.96 | 0.97 |
| *trnL-F* | 14 | 1079 | 18 | 18.5 | 56 | 101 | 2 | 80 | 0.89 | 0.93 |
| plastid (*matK*, *trnL-F*) | 15 | 2427 | 37 | 18.3 | 115 | 5 | 7 | 88 | 0.92 | 0.95 |
| all molecular | 17 | 4131 | 187 | 22.4 | 468 | 10 | 8 | 88 | 0.74 | 0.80 |
| simult. Complete | 17 | 4137 | 193 | 22.3 | 477 | 10 | 9 | 90 | 0.74 | 0.82 |
| simult. Reduced | 15 | 4137 | 193 | 14.8 | 476 | 2 | 11 | 93 | 0.74 | 0.82 |
| *Crossopetalum* analyses: | | | | | | | | | | |
| 26S rDNA | 24 | 968 | 79 | 9.3 | 237 | 6 | 15 | 82 | 0.59 | 0.77 |
| ITS rDNA | 22 | 763 | 220 | 16.0 | 722 | 2 | 15 | 84 | 0.63 | 0.77 |
| rDNA (ITS, 26S) | 28 | 1731 | 299 | 27.5 | 962 | 36 | 20 | 80 | 0.62 | 0.77 |
| *matK* | 26 | 1316 | 103 | 7.3 | 411 | 12 | 14 | 85 | 0.64 | 0.80 |
| *trnL-F* | 25 | 1195 | 61 | 27.4 | 232 | 174 | 13 | 75 | 0.75 | 0.87 |
| plastid (*matK*, *trnL-F*) | 28 | 2511 | 164 | 24.1 | 649 | 90 | 19 | 78 | 0.66 | 0.82 |
| all molecular | 28 | 4242 | 463 | 25.4 | 1624 | 2 | 23 | 83 | 0.62 | 0.78 |
| simultaneous | 28 | 4258 | 479 | 25.4 | 1666 | 4 | 22 | 86 | 0.62 | 0.77 |

from the two *C. mossambicense* specimens, which were scored as having missing data for this region. The upper branch with 55% jackknife support was supported by two unambiguously optimized but homoplasious nucleotide synapomorphies from 26S rDNA, as well as three ambiguously optimized synapomorphies (one 26S rDNA nucleotide, a *trnL* indel at positions 322–327, and a *matK* nucleotide); one or both *C. mossambicense* accessions were scored for all five of these characters. We did not identify any morphological synapomorphies for either of these two branches. There are numerous other potential molecular synapomorphies for the clade of *Crossopetalum* and/or nesting of *C. mossambicense* within the genus, but these synapomorphies are ambiguously optimized because of missing data in *C. decussatum* and/or *C. managautillo* (with 49.3% and 65.6% missing or inapplicable data for the 479 parsimony informative characters).

Given that we only have comparable sequence data for the new species from the Democratic Republic of the Congo, described as *Crossopetalum bokdamii* Breteler & Buerki below, for one gene (*trnL*), we ran separate analyses (*trnL-F*: Fig. S15; plastid: Fig. S16; molecular: Fig. S17) including this sequence. Given the high amount of missing data for *C. bokdamii* in these matrices the resolution and branch support within *Crossopetalum* are low, but in all three cases *C. bokdamii* is located at the same polytomy as *C. mossambicense*.

## Discussion

***Pleurostylia***—Our reduced-taxon-sampling simultaneous analysis provides strong evidence for *Pleurostylia opposita* being a polyphyletic assemblage (Fig. 1B). The African *P. opposita* specimen (12844) is strongly supported as a clade with the other mainland African species (*P. africana* and *P. capensis*). *Pleurostylia opposita* is the most widespread species in the genus ranging from sand dunes on the Mozambique coast (Robson 1966; Robson and Sousa 1969; R. H. Archer, pers. comm. 2013) eastward to New Caledonia. We hypothesize that further sampling of *P. opposita* from across its geographic range for inclusion in phylogeographic analyses, together with morphological investigations, will reveal that *P. opposita* consists of two or more cryptic species. Robson (1966, p. 385) noted of the Mozambican material of *P. opposita*, "[*Torre 4656*]. . .agrees very well with the material from Ceylon and S. India apart from having more flowers on an average in each cyme."

The ancestral *Pleurostylia* lineage dispersed from Madagascar within the last ~11 million years (Bacon et al. 2016). Although there is a polytomy at the base of *Pleurostylia* in Fig. 1B, the topology is consistent with there being both eastward and westward dispersals from Madagascar and/or its adjacent islands.

The morphological evidence strongly supports the molecular data in showing a distant relationship between *Pleurostylia* and the three African species here assigned to *Crossopetalum* including *Pleurostylia serrulata* (= *Crossopetalum serrulatum* (Loes.) I. Darbysh., described below). These three species have pubescent young branches (albeit very sparse in *C. mossambicense*), crenulate-serrulate leaves, only slightly excentric style and branched stigmas, as opposed to the glabrous branches, entire leaves, strongly offset style and peltate stigmas in *Pleurostylia*. In addition, they differ in having a 4-locular ovary with 1 ovule per locule (observed in *C. serrulatum*), and 4-merous flowers, as opposed to ovary essentially 1-locular with 2–8 ovules and flowers usually 5-merous in *Pleurostylia*.

***Crossopetalum***—*Crossopetalum mossambicense* is well supported as part of the *Crossopetalum* lineage in our simultaneous analysis based on three branches with 88%, 79%, and 56% jackknife support (Fig. 2). But a more difficult problem is determining whether *C. mossambicense* is nested within the New World *Crossopetalum* or sister to it. Our simultaneous analysis supports *C. mossambicense* as nested within *Crossopetalum* based on two branches with 79% and 56% jackknife support, respectively, but because of the distribution of missing data in *C. decussatum*, *C. managuatillo*, and *C. mossambicense* it is difficult to identify unambiguously optimized synapomorphies for this resolution. Hence we cannot confidently conclude whether or not the African *C. mossambicense* lineage represents a dispersal event from the New World. Increased character and taxon sampling within *Crossopetalum*, including *C. serrulatum* and *C. bokdamii*, are necessary to resolve this issue.

The morphological case for inclusion of the new species and *Pleurostylia serrulata* within *Crossopetalum* is, nevertheless, strong. *Crossopetalum* does not appear to be well defined by a single morphological synapomorphy, rather a suite of character states help to delimit it from its closest allies. The following character states are of prime importance (Brizicky 1964; Adams 1972; Edwin & Hou 1975; Bornstein 1989; Mory 2001, 2010): 4-merous, bisexual flowers; stamens inserted in the sinuses of a ± 4-lobed intrastaminal floral disk (Fig. 3 A, B); pistil either 4-locular (*Crossopetalum* s. s.) or 2-locular (species previously placed in the segregate genus *Myginda* Jacq.) with corresponding 2–4 stigma/style branches; one ovule per locule, the ovules attached at the base and ascending (atropous); drupe usually single seeded (rarely more), often asymmetric with an excentric style remnant; and seeds exarillate with ramified postchalazal bundles visible on the surface and with endosperm present (Fig. 4). The African species agree with all but one of these character states, the difference being that, based upon the limited flowering material available to date (one flowering specimen of *C. mossambicense*, three each of *C. bokdamii* and *C. serrulatum*), the African species appear to be dioecious with functionally unisexual flowers. However, this is complicated by the fact that both sexual organs are present in the flowers. The functionally male flowers have a fairly well developed pistillode but in *C. serrulatum*, at least, this is markedly different from the pistil of the functionally female flowers, which is larger with a clearly differentiated style and with conspicuous, exserted branched stigmas. The

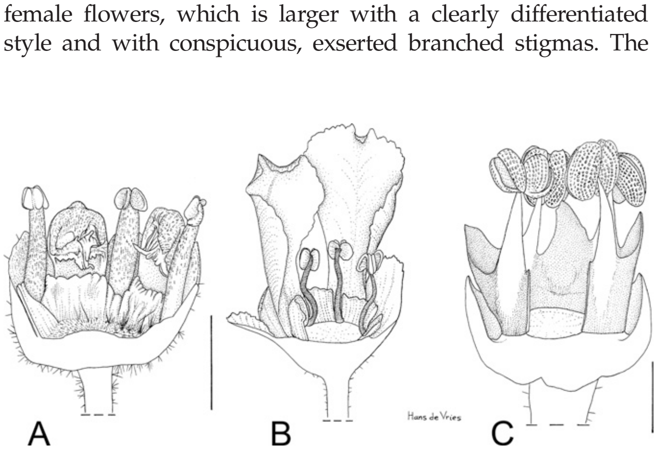

Fig. 3. Comparison of floral morphologies. A. *Crossopetalum bokdamii* Breteler & Buerki (from *Bokdam 3263*, D.R. Congo). B. *Crossopetalum parviflorum* (Hemsl.) Lundel (from *Herrera & Chacon 2361*, Costa Rica). C. *Wilczekra congolensis* (R. Wilczek) M. P. Simmons (from *Dumont 224*, D.R. Congo). Scale bars = 1 mm. Drawn by H. de Vries.

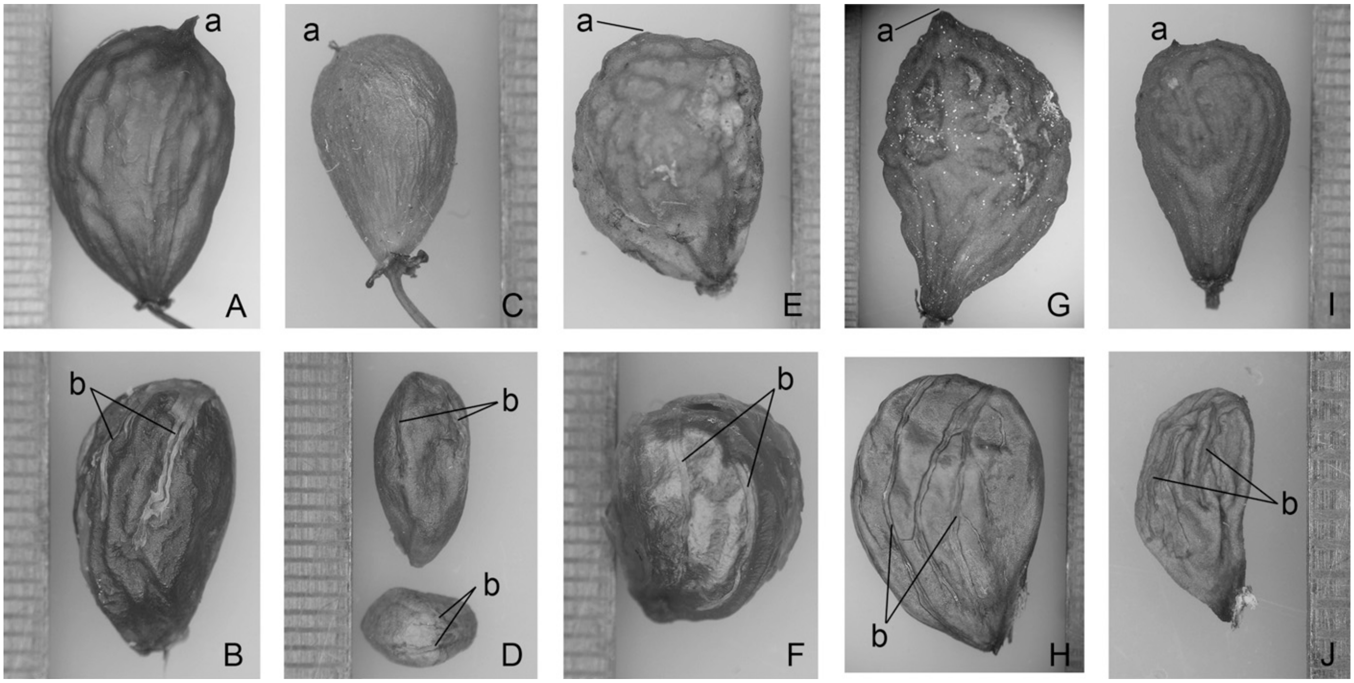

FIG. 4. Drupes (above) and seeds (below) of *Crossopetalum* species. A–B. *C. serrulatum* (A from *Letouzey 10693*, B from *Letouzey 7692*, Cameroon). C–D. *C. mossambicense* (C from *Müller 4023*, D from *Burrows 11267* [face view] and *Müller 4023* [apical view], Mozambique). E–F. *C. gomezii* (*Valverde 306*, Costa Rica). G–H. *C. parviflorum* (G from *Fernández 97*, H from *Estrada 377*, Costa Rica). I–J. *C. scoparium* (*Palmer 177*, Mexico – seed immature). a = style remnants; b= postchalazal bundles. Scale is in 0.5 mm intervals.

female flowers in *C. serrulatum* have well developed stamens but they are markedly shorter than in the functionally male flowers; from the material currently available, it is not clear as to whether these stamens are truly sterile. This is at odds with *Crossopetalum* s. s. in the New World, which is described as having bisexual (Brizicky 1964; Adams 1972; Edwin and Hou 1975; Bornstein 1989; Mory 2010) or androgynous (Mory 2001) flowers. More flowering material from Africa is required to confirm the consistency of functional dioecy in these species, and a more thorough review of New World *Crossopetalum* may also reveal instances of functional dioecy that have so far been overlooked. It is notable that in the allied Malagasy genus *Xenodrys* both bisexual and unisexual flowers have been reported (Bacon et al. 2016). *Xenodrys* is morphologically close to *Crossopetalum* but differs in having 2-locular drupes with 2 ovules per locule, and in sometimes having 5-merous flowers.

A further character that was initially thought to separate the African species of *Crossopetalum* from the New World species is the presence of a stellate indumentum. Both *C. bokdamii* and *C. serrulatum* have numerous stellate hairs on the young branches and inflorescences. Whilst this is absent in *C. mossambicense*, the drupes (and presumably also the ovaries) of that species have very fine, easily dislodged hairs, some of which appear to be stellately branched. No instances of stellate hairs have, to date, been reported in New World *Crossopetalum*. However, on close inspection of material of the widespread *C. rhacoma* Crantz for the current study, we found a more or less dense indumentum of short pale hairs, some of which are found to be bunched (e.g. on *Eggers 3803*, K). Although much less conspicuous, due to the short hair length and the density of the indumentum, these hairs are considered to be analogous to the stellate hairs of *C. bokdamii* and *C. serrulatum.* This provides further morphological evidence for a link between the New World and African taxa here assigned to *Crossopetalum*.

The only genus within continental African Celastraceae subfamilies Cassinoideae and Tripterygioideae (sensu Simmons et al. 2012b) with which *Crossopetalum* is likely to be confused is *Wilczekra* M. P. Simmons from Congo and Gabon, which was recently segregated from *Euonymus* (Simmons and Cappa 2013). However, in *Wilczekra* the flowers are 5-merous and bisexual, the petals are inserted on the receptacle, the five stamens are inserted on the disc (Fig. 3C), and the fruit is a capsule.

## Taxonomic Treatment: the Genus *Crossopetalum* in Africa

1. **Crossopetalum bokdamii** Breteler & Buerki, sp. nov.—TYPE: DEMOCRATIC REPUBLIC OF THE CONGO. Orientale, Kisangani, W bank of Lindi River, upstream of the ferry, riparian forest, 0°29′N 25°7′E, 21 Sep 1971 (fl.), *J. Bokdam & Hendrik D.C. de Wit 3263* (holotype: WAG [WAG0360832]!; isotypes: BM, BR, HUJ, MO, PE, TI, US, WAG [WAG0360833]!).

*Crossopetalum bokdamii* resembles *C. serrulatum* (see 3, below) but differs in (1) the petals having infolded lateral flaps, versus petals with a raised area centrally but lacking lateral flaps, and (2) the young stems being subterete, versus young stems flattened to triangular and with ± conspicuous ridges.

Shrub 3 m tall, putatively dioecious (only male flowers have been observed); branchlets subterete, mainly stellate-hairy. Stipules minute, narrowly triangular to subulate, sometimes bi-lobed to tri-lobed or divided to the base, with simple and stellate hairs, glabrous at the apex. Leaves opposite or subopposite; petiole semi-terete to slightly canaliculate above, 2–5 mm long, ± glabrous to sparsely stellate-hairy; lamina obovate-elliptic, 2–3 times as long as wide, 4–7 × 1.5–3 cm, base cuneate, margin crenate with small, deciduous, glandular teeth, apex acuminate for 0.5–1 cm; when young with a few simple or stellate hairs on both surfaces, soon glabrescent; midrib ± prominent on both surfaces, the (3–)4–5(–6) pairs of lateral nerves ± indistinct above, distinct beneath. Inflorescences axillary, cymose, 3–7-flowered, mainly stellate-hairy; primary peduncle 4–9 mm long; bracts and bracteoles minute, elliptic, ≤ 0.5 mm long; pedicels thread-like, articulated near base, 3–5 mm long, the lower part ≤ 0.5 mm long. Flowers minute, 2 mm in diameter, 4-merous. Functionally male flowers reddish; calyx ± urceolate, ca. 1.5 mm long, glabrous internally, almost completely united or shallowly lobed, lobes rounded, imbricate in bud; petals free, imbricate in bud, inserted on calyx tube, alternating with sepal lobes, glabrous, slightly longer than the calyx, elliptic in outline, 0.7–0.8 mm wide with reflexed apex and with infolded, lobed, 0.4–0.5 wide lateral flaps; stamens 4, opposite the sepal lobes, united with calyx tube at base for 0.5 mm, 2 mm long, glabrous; anthers exserted, ca. 0.2 mm long, dehiscence longitudinal; floral disc intra-staminal, irregularly lobulate, ca. 0.5 mm long, glabrous; pistillode narrowly oblongoid, tapering towards the apex, ca. 1 mm long, glabrous. Functionally female (or bisexual) flowers and fruits unknown. Figure 5.

*Additional Material Examined*—DEMOCRATIC REPUBLIC OF THE CONGO. La Kulu, 3°28′N 23°46′E, 19 Feb. 1931 (fl.), *J. van de Brande 383* (BR!); Lebo (Uélé-Itimbiri) 4°27′N 23°57′E, Mar. 1931 (fl.), *J. Lebrun 2404* (BR!).

***Distribution and Habitat***—This species is endemic to the Democratic Republic of the Congo, known from the northeast of the country, from near Kisangani north towards the border with the Central African Republic. It has been collected in riverine forest, forest margins, and thickets.

***Conservation Status***—The conservation status of *Crossopetalum bokdamii* cannot be calculated with any confidence because of insufficient data, so it is considered to be data deficient (DD). However, the region of the type locality has been impacted by population growth, although the specific habitat for this species provides some protection, as these forests are often periodically inundated and therefore not suitable for habitation.

***Phenology***—From the three known specimens, this species has been recorded in flower in February, March, and September.

***Etymology***—The species epithet *bokdamii* honours Dr Jan Bokdam (1946–), collector of the type material. After graduating from the Wageningen University, Jan Bokdam was affiliated from 1970 until 1974 with the Natural Science Faculty of the Université National du Zaire (now Democratic Republic of the Congo) at Kisangani. In addition to lecturing in plant taxonomy, plant ecology, and silviculture, he collected more than 2,000 herbarium specimens in the rich equatorial rainforests of the NE Province and N. Kivu. This work has provided a strong foundation for our understanding of the unique diversity of this region.

***Taxonomic Comments***—Forty-four years after Jan Bokdam collected this specimen in the Democratic Republic of the Congo, we are finally in position to assign it to the family Celastraceae and to place it in the genus *Crossopetalum*. It was found by one of the authors (F. J. B.) stored at the Herbarium Vadense (WAG) as a *Memecylon* L. species (Melastomataceae). Indeed, the shrub-like habit, the opposite leaves, and the small flowers in a cymose inflorescence are reminiscent of *Memecylon.* However, its finely crenate leaves raised some doubt and so did the unisexual flowers. Floral analysis revealed that the petals possess appendages, which, together with the stellate indumentum, indicated Sapindaceae, but no genus of this family was found with this suite of characters, certainly not in Africa. It was this puzzle that stimulated molecular analyses on the Bokdam specimen and revealed its relationship to *Crossopetalum* and its alliance to the two African species described below. The two earlier specimens of this species held at BR were found subsequently. *Crossopetalum bokdamii* is most similar to *C. serrulatum*, but clearly differs in the lobed petals and subterete stems as noted above. Future collection of this species, including female flowers and fruits, may enable identification of further diagnostic character states.

2. **Crossopetalum mossambicense** I. Darbysh., sp. nov.—TYPE: MOZAMBIQUE. Cabo Delgado: Palma Dist., 1.8 km along track to Nhica do Rovuma, from the Palma-Pundanhar road, 10°49′50″S 40°11′40″E, 6 Dec 2008 (fl.), *J. E. Burrows & S. M. Burrows 11003* (holotype: K!; isotypes: BNRH, LMA).

*Pleurostylia* ?sp. nov. aff. *serrulata* Loes. sensu Timberlake et al. Pl. Ecol. Evol. 144: 131. 2011.

*Crossopetalum mossambicense* resembles *C. serrulatum* (see 3, below) but differs in (1) being largely glabrous, lacking the conspicuous stellate and simple hairs on the young stems, petioles and peduncles of *C. serrulatum*; (2) having shorter stamens in the functionally male flowers, the filaments 0.3–0.45 mm long and the anthers positioned at the same height as the petals, rather than 0.75–1 mm long and the anthers clearly exserted beyond the petals in *C. serrulatum*; and (3) in having smaller mature drupes, 6.5–7.5 mm long, lacking a rostrum at the base of the style remnant and having only a faintly reticulate endocarp surface, rather than drupes 9.5–11 mm long, with a short prominent rostrum at the base of the style remnant and with a prominently rugose endocarp surface in *C. serrulatum*. The two species also differ in habitat; *C. mossambicense* is a species of dry coastal forests whereas *C. serrulatum* is recorded from wetter riverine and swamp forests.

Shrub 1–2.5 m tall, putatively dioecious (only male flowers and fruits seen); much branched with divergent subopposite branches; young stems green, somewhat flattened, with 2 ± prominent ridges on each flattened side, glabrous, older leafy stems with thin red-brown bark, mature stems with grey bark. Stipules minute, red- or purple-brown, narrowly triangular or sometimes divided into 2–3 linear-triangular portions, 0.3–0.5 mm long. Bud scales persistent in intrapetiolar position and somewhat accrescent, green turning red-brown or purple, triangular, 0.6–1.2 mm long. Leaves sub-opposite; petiole 1–2.5 mm long, glabrous; lamina thinly papery, (ovate-) elliptic or distal-most leaves somewhat obovate, 2.7–7 × 1–3 cm, base cuneate to obtuse, can be somewhat asymmetric, apex bluntly acuminate, margin crenulate-serrulate, teeth recurved and sometimes overlapping on adaxial leaf surface, with a caducous glandular tip, teeth can be inconspicuous on young leaves; surfaces glabrous or with very few minute, pale hairs along margin and towards base, these easily dislodged; lateral veins 3–5 pairs, anastomosing, tertiary venation reticulate, midrib pale and prominent beneath. Inflorescences of axillary cymes, solitary in each axil, up to ca. 18-flowered but usually much

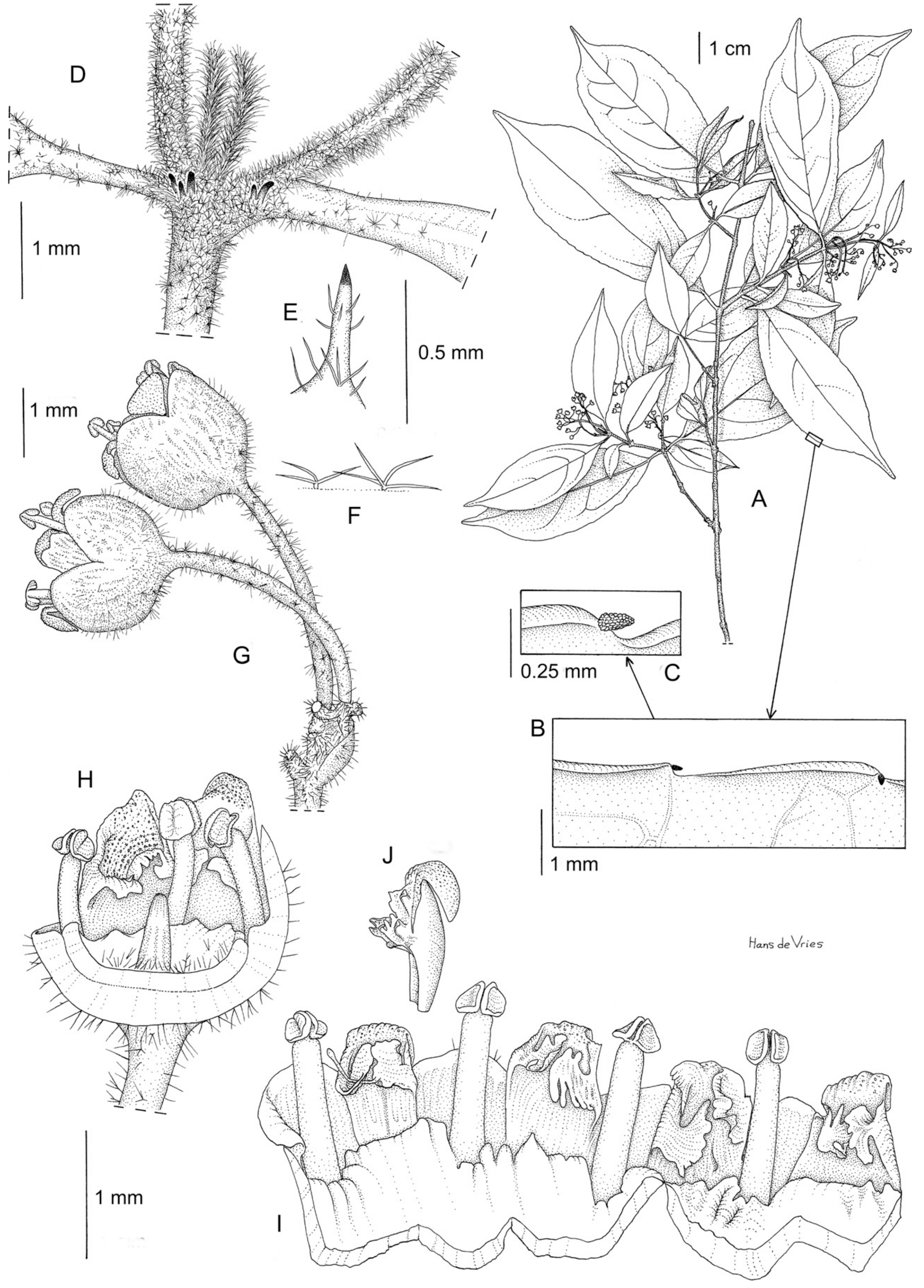

Fig. 5. *Crossopetalum bokdamii*. A. Flowering branchlet. B–C. Details of leaf margin showing glandular teeth. D. Node showing stipules and stem indumentum. E. Stipule. F. Stellate hairs. G. Male flowers. H. Male flower cut lengthwise. I. Dissected male flower rolled open. J. Petal, lateral view. (Drawn by H. de Vries from *Bokdam 3263*).

fewer; peduncles wiry, green, glabrous or with very sparse minute pale patent hairs, primary peduncle 11–24 mm long, often curved; bracts minute, green, triangular, 0.2–0.4 mm long; pedicels 1–2.5 mm long or up to 3.5 mm long in fruit, articulated in proximal half. Flowers minute, urceolate, 1.1–1.4 mm in diameter, 4-merous. Functionally male flowers: calyx green, cupular, 0.5–0.6 mm long when flattened, with 4 shallow rounded lobes, glabrous; petals free, imbricate in bud, red-pink centrally with paler greenish-white margins, ovate, 0.6–0.7 × 0.55–0.65 mm, distal portion recurved beyond calyx rim, margins sub-entire, internal surface with prominent curved raised area centrally; stamens inserted at outer margin of floral disk; filaments pale-hyaline, flattened, 0.3–0.55 × 0.15–0.2 mm, apices truncate; anthers yellow, 0.16–0.2 mm long, dehiscence longitudinal; floral disk green, concave, shallowly 4-lobed with sinuses at point of stamen attachment, lobes truncate or shallowly emarginate, ca. 1 mm wide at widest point when flattened; pistillode green, conical, 0.4–0.45 mm long, apex minutely 4-lobed or lobes barely developed. Functionally female (or bisexual) flowers not seen. Drupe 1-seeded or more rarely 2-seeded, obovoid, 6.5–7.5 × 4–6 mm, with excentric style remnant, stigma remnants sometimes persisting, (3–)4-branched with branches linear, reflexed; flesh thin, green turning yellow, with minute pale hairs easily dislodged, some hairs apparently stellate, glabrescent; endocarp pale brown, ca. 0.3 mm thick, external surface with faint raised reticulation; seeds brown, flattened-ellipsoid, 5–5.8 × 3–3.5 mm, surface smooth, with short branched postchalazal bundles, endosperm present. Figures 6A–M, 7.

***Additional Material Examined***—MOZAMBIQUE. Cabo Delgado Prov.: Palma Dist., area S of Palma, 11°06′47″S 40°20′34″E, 4 May 2008 (fr.), *T. Müller 4023* (K!, LMA); track to Pundanhar?, just N of Quelimane Village, from Mocimboa da Praia to Palma, 11°11′58″S 40°15′46″E, 21 Mar 2009 (fr.) *J. E. & S. M. Burrows 11267* (BNRH, K!, LMA).

***Distribution and Habitat***—*Crossopetalum mossambicense* is restricted to the coastal region of northern Cabo Delgado Province in northeast Mozambique. It has never been collected in neighbouring southeast Tanzania. It occurs in and along the margins of patches of coastal dry evergreen forest, including secondary regrowth, and thicket within a dry forest / miombo (*Brachystegia-Uapaca-Parinari*) woodland mosaic, occurring on sandy soils, at 60–130 m alt.

***Conservation Status***—*Crossopetalum mossambicense* is a highly localised species, restricted to the small extant patches of coastal dry forest in extreme northeast Mozambique, with an extent of occurrence (EOO) of 215 $km^2$ and an area of occupancy (AOO) of 12 $km^2$ based on our current knowledge. Estimates suggest that the extent of "dense vegetation cover" (including dry forest) in the coastal lowlands of Cabo Delgado has declined from ca. 6087 $km^2$ historically to only 1182 $km^2$ today, a loss of ca. 80% (Timberlake et al. 2011). This area has been settled for hundreds of years and there was extensive exploitation of timber during the Portuguese colonial period. Following a period of depopulation during the post-independence civil war (1960s–1991), the area is now being rapidly repopulated, aided by improved transport links and by the construction of cut-lines within the forested and wooded areas during oil prospecting in the late 2000s. The increasing human populations are resulting in widespread forest and woodland clearance for short-term subsistence agriculture. Other immediate threats include logging and uncontrolled burning (Timberlake et al. 2011). An emerging threat to the whole region is planned offshore gas extraction which, if and when it goes ahead, will result in significant migration into the region, as well as the development of onshore infrastructure and transport networks.

Whilst the miombo woodland can regenerate fairly rapidly, the dry forest is easily degraded and does not readily regenerate (J. Timberlake, pers. comm.). Of the three known locations for this species, only that at Nhica do Rovuma is considered to be secure from such activity at present, but this could change as development expands in the region in view of its proximity to the Palma-Pundanhar road. It is quite possible that the location south of Palma (*T. Müller 4023*) has already been lost due to habitat clearance but this requires confirmation (J. Timberlake, pers. comm.). The third location north of Quelimane village is close to the main Mocimboa road and is likely to have been degraded. It is likely that this species occurs in and around other coastal forest patches in the Cabo Delgado region, which remains botanically under-collected. However, with such severe threats and with the currently known range, this species is assessed as endangered (EN B1ab(iii)+2ab(iii)) under the categories and criteria of IUCN (2012).

***Phenology***—Based on the limited information available to date, this species flowers at the onset of the rainy season in December, with fruits maturing towards the end of and after the rainy season in March to May.

***Taxonomic Comments***—Despite their wide geographic and ecological isolation, *Crossopetalum serrulatum* and *C. mossambicense* are morphologically similar, particularly with respect to their flowers. However, the difference in indumentum is striking and *C. serrulatum* also has markedly larger fruits with a more conspicuously sculptured endocarp and the functionally male flowers have longer stamens that are held erect and clearly exserted beyond the petals (Fig. 6N), whereas in *C. mossambicense* the stamens converge and are positioned at the same height as the petals. These diagnostic characters, together with the differing ecology and geographic disjunction, indicate that they are best recognized as separate species.

**3. Crossopetalum serrulatum** (Loes.) I. Darbysh., comb. nov. *Pleurostylia serrulata* Loes., Bot. Jahrb. Syst. 41: 311. 1908. TYPE: CAMEROON. "ufer des Sanaga bei Sanaga Na Tinati" [fide Loesener; extant specimens labelled "Sanaga"], Jun 1897, (fr.), *G. Zenker 1465* (holotype: B, presumed †; isotypes: HBG!, K!, M!, P!, W!).

Shrub 2–5 m tall, apparently dioecious; young stems flattened with 2 ± prominent ridges on each flattened side or triangular with 3 ridges, pale-pubescent with mixed simple and stellate hairs, older leafy stems with brown or purplish-brown bark, subterete. Stipules minute, red, linear or sometimes divided with one longer linear portion, 0.3–0.45 mm long, often obscured by the stellate indumentum. Bud scales persistent in intrapetiolar position, red-brown, triangular, 0.8–1.7 mm long. Leaves sub-opposite; petiole 3–6 mm long, stellate-pubescent mainly above; lamina thinly papery, elliptic or ovate, 5.5–15 × 2.5–6.6 cm, base obtuse or rounded to cuneate, apex acuminate or smaller leaves attenuate, margin crenulate-serrulate, teeth recurved and sometimes overlapping on adaxial leaf surface, with a caducous glandular tip; surfaces glabrous except for sparse stellate hairs along midrib, these sometimes more widespread abaxially on young leaves; lateral veins 4–6 pairs, anastomosing, tertiary venation reticulate, midrib prominent beneath. Inflorescences of axillary dichasial cymes, solitary in each axil, but sometimes compounded into a paniculate thyrse on leafless lateral

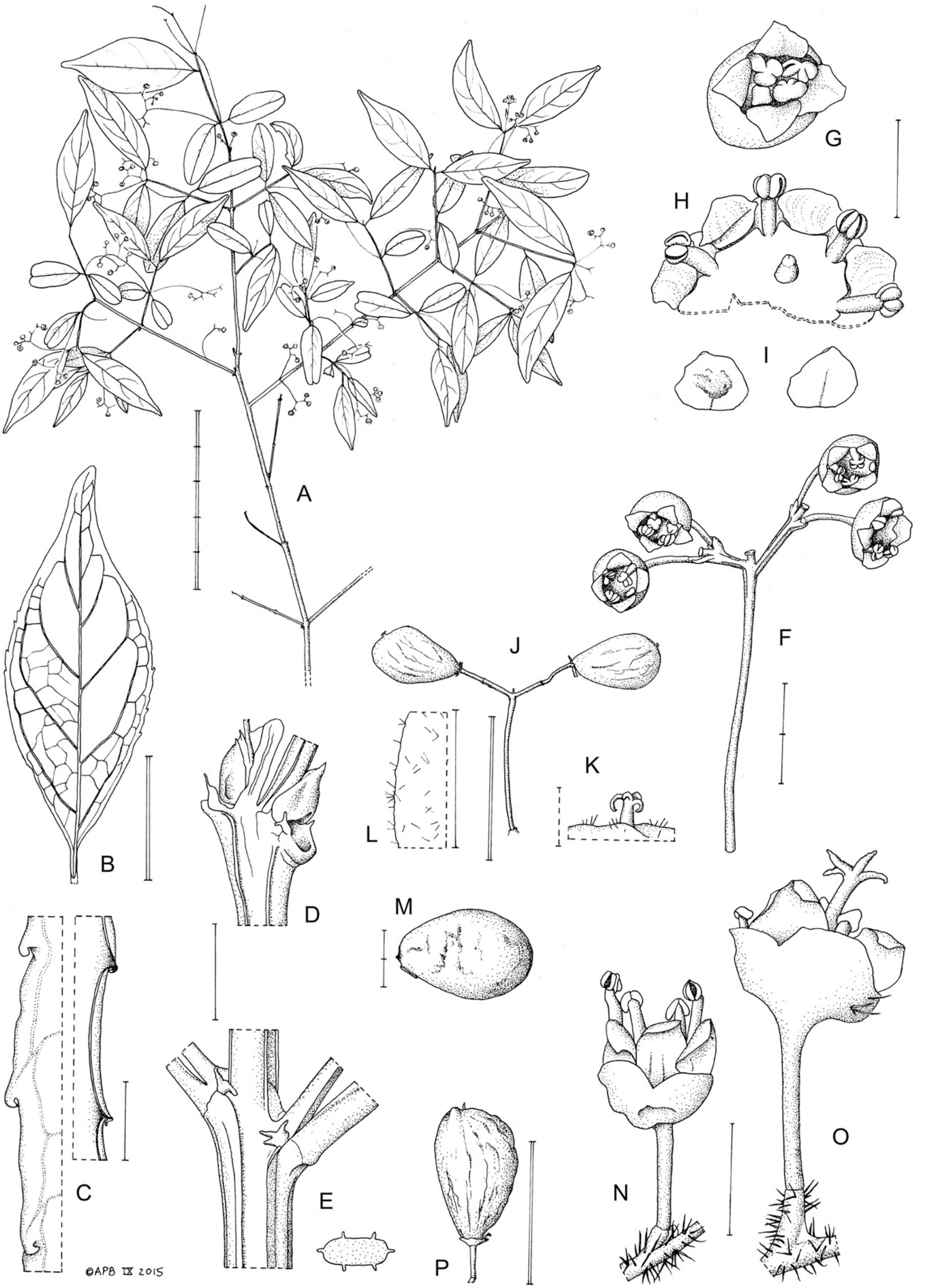

Fig. 6. *Crossopetalum mossambicense*. A. Habit. B. Leaf, adaxial surface. C. Leaf margin, adaxial (left) and abaxial (right). D. Node showing stipules and two bud scales. E. Node showing stipules, and stem in cross-section. F. Inflorescence, male flowers. G. Male flower, face view. H. Dissected male flower. I. Petal, inner face (left) and outer face (right). J. Fruits. K. Stylar remnants on fruit. L. Indumentum on fruit. M. Seed. *Crossopetalum serrulatum*. N. Male flower. O. Female (or bisexual) flower. P. Fruit. (Drawn by Andrew Brown, all from *Burrows & Burrows 11003* except J from *Müller 4023*, N from *Raynal 10722*, O from *Ngameni-Kamga 155*, and P from *Letouzey 7692*). Scale bars: dashed bar = 500 µm, single bar = 1 mm; graduated single bar = 2 mm; double bar = 1 cm; graduated double bar = 5 cm.

FIG. 7. *Crossopetalum mossambicense* in the field, Cabo Delgado Prov., Mozambique. A. In flower, 6 Dec 2008 (collected as *J. E. & S. M. Burrows 11003*). B. In fruit, 21 Mar 2009 (collected as *J. E. & S. M. Burrows 11267*). Photographs taken by J. E. Burrows.

branches, each cyme typically 3–9-flowered; peduncles wiry, green, with ± numerous pale mixed stellate and simple hairs, primary peduncle 13–33 mm long, often curved; bracts minute, green, triangular to linear, 0.2–0.5 mm long; pedicels 1–1.7 mm long or up to 2.5 mm long in fruit, articulated near base, glabrous. Flowers minute, cupular, 0.9–1.5 mm in diameter, 4-merous. Functionally male flowers: calyx green, cupular, 0.5–0.7 mm long when flattened, with shallow rounded lobes, glabrous or often with scattered stellate hairs, calyx persistent in fruit; petals yellowish, ovate, 0.7–1 × 0.5–0.7 mm, distal portion somewhat recurved beyond the calyx rim, margin entire, internal surface with prominent raised area centrally; stamens inserted at outer margin of floral disk, filaments 0.75–1 × 0.12–0.15 mm, anthers clearly exserted beyond the petals, 0.2–0.25 mm long; floral disk brown, concave, 4-lobed with sinuses at point of stamen attachment; pistillode green, conical, 0.5 mm long, apex minutely 4-lobed. Functionally female (or possibly bisexual — see note) flowers similar to male flowers except petals with less prominent central raised area; floral disk more deeply 4-lobed, stamens with filaments 0.35–0.5 mm long, anthers not exserted beyond petals, up to 0.2 mm long; pistil well-developed, ovary rounded, partially embedded within disk, c. 0.5 mm long, 4-locular, 1 ovule per locule, style clearly differentiated, 0.45–0.5 mm long; stigma exserted beyond petals, conspicuously (3-) 4-lobed, lobes spreading or somewhat reflexed, linear, 0.25–0.3 mm long. Drupe 1-seeded, obovoid-ellipsoid, 9.5–11 × 5.5–6.3 mm, with excentric style remnant broadened towards base to form a short rostrum, stigma remnant sometimes persisting; flesh thin, green turning red-orange, glabrous; endocarp pale brown, ca. 0.3–0.4 mm thick, external surface rugose; seed brown, flattened-ellipsoid, 8 × 5 mm, surface smooth, with conspicuous branched postchalazal bundles, endosperm present. Figure 5N–P.

***Additional Material Examined***—CAMEROON. Massif de Ngolè, vallée du Koboko, au NW de Banda, 4 Apr 1963 (fl.), *J. & A. Raynal 10722* (P!, YA!); km 110 route Yaoundé–Bafia, 25 Aug 1966 (fr.), *R. Letouzey 7692* (P!, YA!); près Yangba (40 k SO de Linté), 20 Sep 1966 (fr.), *R. Letouzey 7928* (P!); 18 km à l'ouest de Yenga Port Gentil, village situé à 35 km au NNE de Moloundou, 20 Apr 1971 (fr.), *R. Letouzey 10693* (BR, K!, P!, YA); près de Yaoundé, route Kribi - km 9 vers Eloumien, 22 Aug 1974 (fr.), *P. Mezili 209* (P!); sommet du Mt Tonton Leteu, massif Ngoro, 38 km N de Bafia, 28 Apr 1978 (fl.), *B. Ngameni-Kamga 70* (P!); au pied du Mt Ngolep, massif de Ngoro, 38 km au N de Bafia, 2 May 1978 (fl.), *B. Ngameni-Kamga 155* (P!, YA!).

CONGO (BRAZZAVILLE). Tronçon Bolozo–Boumdel, 22–26 June 1972 (fr.), *P. Sita 3402* (P!) — see Taxonomic Comments.

***Distribution and Habitat***—*Crossopetalum serrulatum* is restricted to central and southern Cameroon and with a single, somewhat doubtful record from northern Congo (Brazzaville). It is recorded from forest understorey including riverine forest, and from marshy clearings in semi-deciduous (Sterculiaceae-*Celtis*) forest, and can also persist in secondary bushland; it occurs at 700–900 m alt.

***Conservation Status***—This species was assessed (as *Pleurostylia serrulata*) as endangered (EN B2ab(iii)) by Cheek (2011), who recorded an extent of occurrence (EOO) of 15,718 km$^2$, an area of occupancy (AOO) of 16 km$^2$ and recognised four threat-defined locations. He considered slash and burn agriculture to be the main threat, resulting in a decline in the extent and quality of suitable habitat for this species. It is not known from any protected areas. Cheek did not include the Congolese locality within his assessment but this would only increase the AOO to 20 km$^2$ and the number of locations to five which is still within the "Endangered" thresholds under IUCN criterion B2, so the assessment of EN B2ab(iii) is accepted here.

***Taxonomic Comments***—This species was described in *Pleurostylia* based on a single fruiting specimen only, and Loesener (1908) correctly noted in the protologue that the drupes on *Zenker 1465* may be malformed and so he could not be sure of the generic or even the family placement of this species. Indeed, an undated pencil note by Norman Robson on the K isotype states "not a Celastraceae." However, having seen more material including flowering and mature fruiting specimens, Villiers (1975) noted that it agreed well with *Pleurostylia* in the appearance of the vegetative parts, inflorescences, flowers, and fruit form. However, he noted that it differed from other members in that genus in the stigma not being peltate. At that time, Villiers had seen functionally male flowers only (which he interpreted as being bisexual) so he did not note that the stigmas are in fact markedly (3-) 4-lobed or that the ovary is 4-locular with one ovule per locule as in *Crossopetalum*. This species is well illustrated in Villiers (1975: pl. 1).

The single collection seen from Congo (Brazzaville) is depauperate, with only a single fruit on the sheet and with poorly pressed foliage. More material, including flowers, is required to confirm the presence of this species in Congo.

## Comparison of the African Species of *Crossopetalum* to the New World Species

Of the New World taxa of *Crossopetalum*, the three African species appear to be morphologically closest to the Central American forest species *C. enervium* Hammel, *C. riparium* (Lundell) Lundell, and *C. standleyi* (Lundell) Lundell. Indeed, the similarity of the African species to the first of these three is striking at least on a superficial level. That said, *C. enervium* differs from all three African species in having leaves with more markedly acute or acute-acuminate apices and sharper marginal teeth, in having flowers with the petals (1–1.5 mm long) considerably longer than the stamens (filaments 0.1–0.2 mm long), that are borne just inside the margin of the floral disc, and in the petals having crenate margins (Hammel 1997). *Crossopetalum enervium* is usually glabrous, although Hammel (1997) also noted a single specimen with a puberulent inflorescence. In Africa, it would therefore most easily be confused with *C. mossambicense*, but it additionally differs from that species in having larger fruits (7–)9.5–14 × 6–7 mm, containing seeds with a more rugose endocarp similar to that in *C. serrulatum*. *Crossopetalum standleyi* and *C. riparium* are similar to *C. enervium* but the former has shorter inflorescences without a conspicuous primary peduncle, whilst the latter has subsessile anthers (Hammel 1997). Both of these characters clearly differ from the three African species.

## Key to African Species of *Crossopetalum*

1. Young stems and peduncles glabrous or largely so; filaments in functionally male flowers up to 0.55 mm long; plants of coastal dry forests and thickets, Mozambique . . . . . . . . 2. *C. mossambicense*
1. Young stems and peduncles with conspicuous pubescence including stellate hairs; filaments in functionally male flowers 0.75–2 mm long; plants of riverine, swamp, and semi-deciduous forests, Cameroon, Congo (Brazzaville) and Democratic Republic of the Congo . . . . . . . . 2
    2. Petals with infolded, lobed lateral flaps; young stems subterete . . . . . . . . 1. *C. bokdamii*
    2. Petals without lateral flaps; young stems flattened . . . . . . . . 3. *C. serrulatum*

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## Literature Cited

Adams, C. D. 1972. *Flowering plants of Jamaica*. Mona, Jamaica: University of the West Indies.

Alexander, P. J., G. Rajanikanth, C. D. Bacon, and C. D. Bailey. 2006. Recovery of plant DNA using a reciprocating saw and silica-based columns. *Molecular Ecology Notes* 7: 5–9.

Álvarez, I. and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.

Bachman, S., J. Moat, A. W. Hill, J. de la Torre, and B. Scott. 2011. Supporting red list threat assessments with GeoCAT: Geospatial conservation assessment tool. In: e-Infrastructures for data publishing in biodiversity science, eds. V. Smith and L. Penev. *ZooKeys* 150: 117–126.

Bacon, C. D., M. P. Simmons, R. H. Archer, L.-C. Zhao, and J. Andriantiana. 2016. Biogeography of the Malagasy Celastraceae: Multiple independent origins followed by widespread dispersal of genera from Madagascar. *Molecular Phylogenetics and Evolution* 94: 365–382.

Bailey, C. D., T. G. Carr, S. A. Harris, and C. E. Hughes. 2003. Characterization of angiosperm nrDNA polymorphism, paralogy, and pseudogenes. *Molecular Phylogenetics and Evolution* 29: 435–455.

Baker, J. G. 1877. *Flora of Mauritius and the Seychelles: A description of the flowering plants and ferns of those islands*. London: L. Reeve & Co.

Blakelock, R. A. 1958. 100. Celastraceae. Pp. 623–634 in *Flora of west tropical Africa* vol. 1, eds. J. Hutchinson, J. M. Dalziel, and R. W. J. Keay. London: Crown Agents for Oversea Governments.

Bornstein, A. J. 1989. Celastraceae. Pp. 113–125 in *Flora of the Lesser Antilles: Leeward and windward islands* vol. 5, ed. R. A. Howard. Jamaica Plain, Massachusetts: Arnold Arboretum.

Brizicky, G. K. 1964. The genera of Celastrales in the southeastern United States. *Journal of the Arnold Arboretum* 45: 206–234.

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

Bull, J. J., J. P. Huelsenbeck, C. W. Cunningham, D. L. Swofford, and P. J. Waddell. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384–397.

Chase, M. W. and H. H. Hills. 1991. Silica gel: An ideal material for field preservation of leaf samples for DNA studies. *Taxon* 40: 215–220.

Cheek, M. 2011. Celastraceae. Pp. 112–119 in *Red data book of the flowering plants of Cameroon: IUCN global assessments*, eds. J.-M. Onana and M. Cheek. Kew: Royal Botanic Gardens.

Coughenour, J. M., M. P. Simmons, J. A. Lombardi, and J. J. Cappa. 2010. Phylogeny of Celastraceae subfamily Salacioideae and tribe Lophopetaleae inferred from morphological characters and nuclear and plastid genes. *Systematic Botany* 35: 358–366.

Coughenour, J. M., M. P. Simmons, J. A. Lombardi, K. Yakobson, and R. H. Archer. 2011. Phylogeny of Celastraceae subfamily Hippocrateoideae inferred from morphological characters and nuclear and plastid genes. *Molecular Phylogenetics and Evolution* 59: 320–330.

Darbyshire, I., M. P. Simmons, J. J. Cappa, F. J. Breteler, and S. Buerki. 2016. Data from: *Pleurostylia serrulata* and two allied new species from Africa are actually members of the New World *Crossopetalum* (Celastraceae). Dryad Digital Repository. http://dx.doi: 10.5061/dryad.98d51.

Davis, J. I., K. C. Nixon, and D. P. Little. 2005. The limits of conventional cladistic analysis. Pp. 119–147 in *Parsimony, phylogeny, and genomics*, ed. V. A. Albert. Oxford: Oxford University Press.

Davis, J. I., M. P. Simmons, D. W. Stevenson, and J. F. Wendel. 1998. Data decisiveness, data quality, and incongruence in phylogenetic analysis: an example from the monocotyledons using mitochondrial *atpA* sequences. *Systematic Biology* 47: 282–310.

Davison, J. D. 1927. Celastraceae, *R. Br. Bothalia* 2: 289–346.

Hou, D. 1962. Celastraceae – I. Pp. 227–291 in *Flora Malesiana* vol. 6, ed. C. G. G. J. van Steenis. Leyden: Flora Malesiana Foundation.

Doyle, J. J. 1992. Gene trees and species trees: Molecular systematics as one-character taxonomy. *Systematic Botany* 17: 144–163.

Doyle, J. J. 1995. The irrelevance of allele tree topologies for species delimitation, and a non-topological alternative. *Systematic Botany* 20: 574–588.

Edwin, G. and D. Hou. 1975. Celastraceae. In *Flora of Panama* 4, eds. Woodson, E. Jr. and R. W. Schery. *Annals of the Missouri Botanical Garden* 62: 45–56.

Farris, J. S. 1989. The retention index and the rescaled consistency index. *Cladistics* 5: 417–419.

Farris, J. S., V. A. Albert, M. Källersjö, D. Lipscomb, and A. G. Kluge. 1996. Parsimony jackknifing outperforms neighbor-joining. *Cladistics* 12: 99–124.

Felsenstein, J. 1973. Maximum likelihood and minimum-steps methods for estimating evolutionary trees from data on discrete characters. *Systematic Zoology* 22: 240–249.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. *Systematic Zoology* 27: 401–410.

Geerling, C. 1987. Guide de terrain des ligneux Saheliens et soudano Guineens. *Agricultural University Wageningen Papers* 87: 1–340.

Hammel, B. E. 1997. Three new species of Celastraceae from Costa Rica, one disjunct from Mexico. *Novon* 7: 147–155.

Hawthorne, W. and C. Jongkind. 2006. *Woody plants of western African forests. A guide to the forest trees, shrubs and lianes from Senegal to Ghana.* Kew: Royal Botanic Gardens.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology* 7: 1–44.

Irvine, F. R. 1961. *Woody plants of Ghana: With special reference to their uses.* London: Oxford University Press.

Islam, M. B., M. P. Simmons, and R. H. Archer. 2006. Phylogeny of the *Elaeodendron* group (Celastraceae) inferred from morphological characters and nuclear and plastid genes. *Systematic Botany* 31: 512–524.

IUCN. 2012. IUCN Red list categories and criteria. Version 3.1, second edition. Gland: IUCN Species Survival Commission, Switzerland.

IUCN Standards and Petitions Subcommittee. 2014. Guidelines for using the IUCN red list categories and criteria. Version 11. http://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Katoh, K. and H. Toh. 2008a. Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* 9: 286–298.

Katoh, K. and H. Toh. 2008b. Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. *BMC Bioinformatics* 9: 212.

Keay, R. W. J. 1989. *Trees of Nigeria*. Oxford: Clarendon Press.

Kitching, I. J., P. L. Forey, C. J. Humphries, and D. M. Williams. 1998. *Cladistics: The theory and practice of parsimony analysis*. Oxford: Oxford University Press.

Kluge, A. G. 1989. A concern for evidence and a phylogenetic hypothesis for relationships among *Epicrates* (Boidae, Serpentes). *Systematic Zoology* 38: 7–25.

Kluge, A. G. and J. S. Farris. 1969. Quantitative phyletics and the evolution of Anurans. *Systematic Zoology* 18: 1–32.

Loesener, T. 1908. Celastraceae africanae. IV. *Botanische Jahrbücher* 41: 298–312.

Loesener, T. 1942. Celastraceae. Pp. 87–197 in *Die Natürlichen Pflanzenfamilien* vol. 20b, eds. A. Engler, H. Harms, and J. Mattfeld. Berlin: Duncker and Humblot.

Maddison, D. R. and W. P. Maddison. 2001. MacClade: Analysis of phylogeny and character evolution version 4.03. Sunderland: Sinauer Associates.

Maddison, W. P., M. J. Donoghue, and D. R. Maddison. 1984. Outgroup analysis and parsimony. *Systematic Zoology* 33: 83–103.

Marais, W. 1981. A new species of *Pleurostylia* (Celastraceae) from Rodrigues. *Kew Bulletin* 36: 229–230.

Matthews, M. L. and P. K. Endress. 2005. Comparative floral structure and systematics of Celastrales (Celastraceae, Parnassiaceae, Lepidobotryaceae). *Botanical Journal of the Linnean Society* 149: 129–194.

Merrill, E. D. and F. L. Freeman. 1940. The old world species of the Celastraceous genus *Microtropis* Wallich. *Proceedings of the American Academy of Arts and Sciences* 73: 271–310.

Mory, B. 2001. Notes on *Crossopetalum, Myginda* and *Gyminda* (Celastraceae) from Cuba. *Willdenowia* 31: 129–135.

Mory, B. 2010. Celastraceae. *Flora de la República de Cuba*. Fascículo 16(1). Liechtenstein: A. R. Gartner Verlag KG.

Müller, K. 2006. Incorporating information from length-mutational events into phylogenetic analysis. *Molecular Phylogenetics and Evolution* 38: 667–676.

Nixon, K. C. and J. M. Carpenter. 1996. On simultaneous analysis. *Cladistics* 12: 221–242.

Palmer, E. and N. Pitman. 1972. *Trees of southern Africa*. Cape Town: A. A. Balkema.

Parmentier, I., J. Duminil, M. Kuzmina, M. Philippe, D. W. Thomas, D. Kenfack, G. B. Chuyong, C. Cruaud, and O. J. Hardy. 2013. How effective are DNA barcodes in the identification of African rainforest trees? *PLoS One* 8: e54921.

Perrier de la Bâthie, H. 1946. Célastracées: (Celastraceae). Pp. 1–76 in *Flore de Madagascar et des Comores: (Plantes Vasculaires)* vol. 116, ed. H. Humbert. Paris: Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie.

Pierre, L. 1894. *Flore forestière de la Cochinchine par L. Pierre Directeur du Jardin Botanique de Saïgon*. Paris: O. Doin.

Pullaiah, T. and D. M. Rao. 2002. *Flora of eastern Ghats: Hill ranges of south east India*. New Delhi: Regency Publications.

Robson, N. K. B. 1966. Celastraceae (incl. Hippocrateaceae). Pp. 355–418 in *Flora Zambesiaca* vol. 2, pt. 2, eds. Exell, A. W., A. Fernandes, and H. Wild. London: Crown Agents for Oversea Governments and Administrations.

Robson, N. K. B. and E. P. Sousa. 1969. Celastraceae. Pp. 1–66 in *Flora de Moçambique* vol. 48, ed. A. Fernandes. Lisbon: Centro de Botânica.

Robson, N. K. B., N. Hallé, B. Mathew, and R. Blakelock. 1994. Celastraceae. Pp. 1–78 in *Flora of tropical East Africa* vol. 108, ed. R. M. Polhill. Rotterdam: A. A. Balkema.

Schuh, R. T. and J. T. Polhemus. 1980. Analysis of taxonomic congruence among morphological, ecological, and biogeographic data sets for the Leptopodomorpha (Hemiptera). *Systematic Zoology* 29: 1–26.

Simmons, M. P. 2004a. Celastraceae. Pp. 29–64 in *The families and genera of flowering plants. VI. Flowering plants. Dicotyledons. Celastrales, Oxalidales, Rosales, Cornales, Ericales*, ed. K. Kubitzki. Berlin: Springer.

Simmons, M. P. 2004b. Independence of alignment and tree search. *Molecular Phylogenetics and Evolution* 31: 874–879.

Simmons, M. P. and J. J. Cappa. 2013. *Wilczekra*, a new genus of Celastraceae for a disjunct lineage of *Euonymus. Systematic Botany* 28: 148–153.

Simmons, M. P. and J. V. Freudenstein. 2002. Artifacts of coding amino acids and other composite characters for phylogenetic analysis. *Cladistics* 18: 354–365.

Simmons, M. P. and J. V. Freudenstein. 2011. Spurious 99% bootstrap and jackknife support for unsupported clades. *Molecular Phylogenetics and Evolution* 61: 177–191.

Simmons, M. P. and J. P. Hedin. 1999. Relationships and morphological character change among genera of Celastraceae s. l. (including Hippocrateaceae). *Annals of the Missouri Botanical Garden* 86: 723–757.

Simmons, M. P. and H. Ochoterena. 2000. Gaps as characters in sequence-based phylogenetic analyses. *Systematic Biology* 49: 369–381.

Simmons, M. P., C. D. Bacon, J. J. Cappa, and M. J. McKenna. 2012b. Phylogeny of Celastraceae subfamilies Cassinoideae and Tripterygioideae inferred from morphological characters and nuclear and plastid loci. *Systematic Botany* 37: 456–467.

Simmons, M. P., C. D. Bailey, and K. C. Nixon. 2000. Phylogeny reconstruction using duplicate genes. *Molecular Biology and Evolution* 17: 469–473.

Simmons, M. P., J. J. Cappa, R. H. Archer, A. J. Ford, D. Eichstedt, and C. C. Clevinger. 2008. Phylogeny of the Celastreae (Celastraceae) and the relationships of *Catha edulis* (qat) inferred from morphological characters and nuclear and plastid genes. *Molecular Phylogenetics and Evolution* 48: 745–757.

Simmons, M. P., C. C. Clevinger, V. Savolainen, R. H. Archer, S. Mathews, and J. J. Doyle. 2001a. Phylogeny of the Celastraceae inferred from phytochrome B and morphology. *American Journal of Botany* 88: 313–325.

Simmons, M. P., M. J. McKenna, C. D. Bacon, K. Yakobson, J. J. Cappa, R. H. Archer, and A. J. Ford. 2012a. Phylogeny of Celastraceae tribe Euonymeae inferred from morphological characters and nuclear and plastid genes. *Molecular Phylogenetics and Evolution* 62: 9–20.

Simmons, M. P., H. Ochoterena, and T. G. Carr. 2001b. Incorporation, relative homoplasy, and effect of gap characters in sequence-based phylogenetic analyses. *Systematic Biology* 50: 454–462.

Simmons, M. P., V. Savolainen, C. C. Clevinger, R. H. Archer, and J. I. Davis. 2001c. Phylogeny of the Celastraceae inferred from 26S nrDNA, phytochrome B, *atpB, rbcL,* and morphology. *Molecular Phylogenetics and Evolution* 19: 353–366.

Stöver, B. C. and K. F. Müller. 2010. TreeGraph 2: Combining and visualizing evidence from different phylogenetic analyses. *BMC Bioinformatics* 11: 7.

Swofford, D. L. 2001. PAUP*: Phylogenetic analysis using parsimony (*and other methods). Sunderland: Sinauer Associates.

Timberlake, J., D. Goyder, F. Crawford, J. Burrows, G. P. Clarke, Q. Luke, H. Matimele, T. Müller, O. Pascal, C. de Sousa, and T. Alves. 2011. Coastal dry forests in northern Mozambique. *Plant Ecology and Evolution* 144: 126–137.

Villiers, J.-F. 1973. Célastracées, Pandacées, Bombacacées, Cannabacées, Bixacées, Avicenniacées. Pp. 3–71 in *Flore du Gabon* vol. 22, eds. A. Aubréville and J.-F. LeRoy. Paris: Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie.

Villiers, J.-F. 1975. Célastracées, Aquifoliacées, Salvadoracées, Pandacées, Avicenniacées, Bixacées, Cannabacées, Bombacacées. Pp. 3–32 in *Flore du Cameroun* vol. 19, eds. A. Aubréville and J.-F. Leroy. Paris: Muséum National d'Histoire Naturelle, Laboratoire de Phanérogamie.

Wadhwa, B. M. 1996. Celastraceae. Pp. 75–106 in *A revised handbook to the flora of Ceylon,* ed. M. D. Dassanayake. Lebanon: Science Publishers, Inc.

Wendel, J. F., A. Schnabel, and T. Seelanan. 1995. Bidirectional interlocus concerted evolution following allopolyploid speciation in cotton (*Gossypium*). *Proceedings of the National Academy of Sciences USA* 92: 280–284.

Wight, R. and G. A. Walker-Arnott. 1834. *Prodromus Floræ Peninsulæ Indiæ Orientalis*. London: Parbury, Allen, & Co.

Wilczek, R. 1960. 75. Celastraceae. Pp. 113–132 in *Flore du Congo Belge et du Ruanda-Urundi* vol. 9, ed. Comité exécutif de la Flore du Congo belge et le Jardin Botanique de l'État. Bruxelles: L'Institut National pour L'Étude Agronomique du Congo Belge.

Wilkinson, M. 1995. A comparison of two methods of character construction. *Cladistics* 11: 297–308.

Zurawski, G. and M. T. Clegg. 1987. Evolution of higher-plant chloroplast DNA-encoded genes: Implications for structure-function and phylogenetic studies. *Annual Review of Plant Physiology* 38: 391–418.

Appendix 1. List of new specimens sampled with taxonomic authorities, voucher information, and GenBank accession numbers (26S rDNA, ITS rDNA, *matK*, *trnL* intron, *trnL-F* spacer, respectively, unless otherwise noted) for new sequences generated for this study. Also listed for *Crossopetalum bokdamii* are GenBank accession numbers for *rpoC1* and *trnH-psbA*.

***Crossopetalum bokdamii*** Breteler & Buerki—*J. Bokdam 3263*, Democratic Republic of the Congo (WAG); —, —, —, KT862191, —. *rpoC1*: KT862196, *trnH-psbA*: KT862197. ***Crossopetalum mossambicense*** I. Darbysh.—*J. E. Burrows 11003*, Mozambique (BNRH); KT862172, KT862183, KT862177, —, KT862195. ***Crossopetalum mossambicense*** I. Darbysh.—*J. E. Burrows 11267*, Mozambique (BNRH); —, KT862185, KT862179, KT862189, —. ***Pleurostylia africana*** Loes.—*S. Bidgood et al. 2014*, Tanzania (K); KT862167, KT862180, KT862174, KT862186, KT862192. ***Pleurostylia africana*** Loes.—*S. A. Robertson & M. Ngonyo 6829*, Kenya (K); KT862168, KT862181, KT862175, KT862187, KT862193. ***Pleurostylia opposita*** (Wall. ex Carey) Alston—*J. E. Burrows 12844*, Mozambique (BNRH); KT862169, KT862182, KT862176, KT862188, KT862194. ***Pleurostylia opposita*** (Wall. ex Carey) Alston—*J. E. Burrows 12872*, Mozambique (BNRH); KT862170, —, —, —, —. ***Pleurostylia pachyphloea*** Tul.—*F. Friedmann 3194*, Reunion (K); KT862171, —, —, —, —. ***Pleurostylia putamen*** Marais—*F. Friedman 2936*, Rodrigues Islands (K); KT862173, KT862184, KT862178, KT862190 (*trnL-F*).