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PHYLOGENY OF *TRICALYSIA*  
(RUBIACEAE) AND ITS  
RELATIONSHIPS WITH ALLIED  
GENERA BASED ON PLASTID  
DNA DATA: RESURRECTION OF  
THE GENUS *EMPOGONA*<sup>1</sup>

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ABSTRACT

Recent studies on the circumscription of the tribe Coffeae (Rubiaceae) revealed a weakly supported clade containing *Tricalysia* A. Rich. and the allied genera *Argocoffeopsis* Lebrun, *Calycosiphonia* Pierre ex Robbr., *Belonophora* Hook. f., *Diplospora* DC., *Discospermum* Dalzell, *Nostolachma* T. Durand, and *Xantonnea* Pierre ex Pit. The phylogenetic relationships of *Tricalysia* and these allied taxa are investigated further using sequence data from four plastid regions (*trnL-F* intron and intergenic spacer, *rpl16* intron, *accD-psaI* intergenic spacer, and *PetD*). Our results demonstrate that *Tricalysia* sensu Robbrecht is not monophyletic. The genus name *Tricalysia* should be restricted to taxa from subgenus *Tricalysia*; subgenus *Empogona* (Hook. f.) Robbr. is sister to the genus *Diplospora* and is recognized at the generic level. The 34 necessary new combinations for *Empogona* Hook. f. are provided: *E. acidophylla* (Robbr.) J. Tosh & Robbr., *E. aequatoria* (Robbr.) J. Tosh & Robbr., *E. africana* (Sim) J. Tosh & Robbr., *E. aulacosperma* (Robbr.) J. Tosh & Robbr., *E. bequaertii* (De Wild.) J. Tosh & Robbr., *E. bracteata* (Hiern) J. Tosh & Robbr., *E. breterlei* (Robbr.) J. Tosh & Robbr., *E. buxifolia* (Hiern) J. Tosh & Robbr. subsp. *buxifolia*, *E. buxifolia* subsp. *australis* (Robbr.) J. Tosh & Robbr., *E. cacondensis* (Hiern) J. Tosh & Robbr., *E. concolor* (N. Hallé) J. Tosh & Robbr., *E. coriacea* (Sond.) J. Tosh & Robbr., *E. crepiniana* (De Wild. & T. Durand) J. Tosh & Robbr., *E. deightonii* (Brenan) J. Tosh & Robbr., *E. discolor* (Brenan) J. Tosh & Robbr., *E. filiformistipulata* (De Wild.) Bremek. subsp. *filiformistipulata*, *E. filiformistipulata* subsp. *epipsila* (Robbr.) J. Tosh & Robbr., *E. glabra* (K. Schum.) J. Tosh & Robbr., *E. gossweileri* (S. Moore) J. Tosh & Robbr., *E. kirkii* Hook. f. subsp. *junodii* (Schinz) J. Tosh & Robbr., *E. lanceolata* (Sond.) J. Tosh & Robbr., *E. macrophylla* (K. Schum.) J. Tosh & Robbr., *E. maputenis* (Bridson & A. E. van Wyk) J. Tosh & Robbr., *E. ngalaensis* (Robbr.) J. Tosh & Robbr., *E. nogueirae* (Robbr.) J. Tosh & Robbr., *E. ovalifolia* (Hiern) J. Tosh & Robbr. var. *ovalifolia*, *E. ovalifolia* var. *glabrata* (Oliv.) J. Tosh & Robbr., *E. ovalifolia* var. *taylorii* (S. Moore) J. Tosh & Robbr., *E. reflexa* (Hutch.) J. Tosh & Robbr. var. *reflexa*, *E. reflexa* var. *ivorensis* (Robbr.) J. Tosh & Robbr., *E. ruandensis* (Bremek.) J. Tosh & Robbr., *E. somaliensis* (Robbr.) J. Tosh & Robbr., *E. talbotii* (Wernham) J. Tosh & Robbr., and *E. welwitschii* (K. Schum.) J. Tosh & Robbr.

*Key words:* *accD-psaI*, *Coffea*, coffee, Coffeae, *Empogona*, molecular systematics, *petD*, *rpl16*, Rubiaceae, *Tricalysia*, *trnL-F*.

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The genus *Tricalysia* A. Rich. is one of the largest genera of Rubiaceae in Africa and occurs in continental Africa (ca. 95 species), Madagascar (12 species), and the Comoros (one species). The genus typically possesses the distinguishing characteristics of the tribe Coffeae (Bridson & Verdcourt, 2003; Davis et al., 2007). These include axillary inflorescences paired at the nodes with obvious calyculi, flowers with left contorted corolla aestivation and a distinctly 2-lobed style, and relatively small and few-seeded fleshy fruits. Most *Tricalysia* species can be

separated readily from other Coffeae by the presence of stipules with needlelike awns, truncate to distinctly lobed calyces, and seeds with a shallow hilum. Identification of *Tricalysia* at the species level is notoriously difficult, as the genus contains a large number of species across a broad geographic and ecologic range, often separated by minor and continuous characters.

In a series of papers, Robbrecht (1978, 1979, 1982, 1983, 1987) conducted a taxonomic revision of *Tricalysia*, with later contributions by Ali and

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Robbrecht (1991) and Ranarivelo-Randriambovonjy et al. (2007). Robbrecht (1979, 1982, 1983, 1987) recognized and revised two subgenera: subgenera *Tricalysia* A. Rich with five sections (*Probletostemon* (K. Schum.) Robbr., *Tricalysia*, *Rosea* (Klotzsch) Robbr., *Ephedranthera* Robbr., and an unnamed Madagascan section) and subgenus *Empogona* (Hook. f.) Robbr. with two sections (*Empogona* Hook. f. and *Kraussiopsis* Robbr.). Separation of the two subgenera in *Tricalysia* was supported by differences in calyx lobe morphology, corolla throat pubescence, fruit color, and the presence/absence of a sterile appendage on the anther connective.

*Empogona* Hook. f. was originally recognized at the generic level by Hooker (1873) based on a single Zambezi species, *E. kirkii* Hook. f. Brenan (1947) reduced the genus *Empogona* to a section of *Tricalysia*, containing six mainly eastern and southern African species. During his revision of *Tricalysia* and, in particular, his treatment of subgenus *Empogona*, Robbrecht (1979) showed that ca. 20 other tropical African species, many of them with Guineo-Congolian distribution, also belonged to this subgenus.

Robbrecht (1978) also investigated the closely related genus *Neorosea* N. Hallé, consisting of 17 species, many of which were formerly included in *Tricalysia*. Two of these 17 species, including the type species *N. jasminiflora* (Klotzsch) N. Hallé, proved to be genuine *Tricalysia* species; a new genus, *Sericanthe* Robbr., was described to accommodate the remaining species (Robbrecht, 1978).

The close association between *Diplospora* DC. and *Discospermum* Dalzell with *Tricalysia* has long been recognized, with some authors (e.g., Schumann, 1891) considering *Diplospora* and *Tricalysia* to be synonymous. Ali and Robbrecht (1991) broadly surveyed *Diplospora* and *Discospermum* and enumerated a whole suite of characters that could be used to distinguish these Asian taxa from the closely related African *Tricalysia* species. They also justified *Diplospora* and *Discospermum* as separate genera on the basis of fruit morphology.

The most recent taxonomic work on *Tricalysia*, by Ranarivelo-Randriambovonjy et al. (2007), focused on the unnamed Madagascan section that was alluded to, but not treated by, Robbrecht (1987). Of the 12 species of *Tricalysia* occurring in Madagascar, only one species belongs to subgenus *Empogona* (*T. ovalifolia* Hiern). The other 11 species, characterized by the presence of unisexual flowers, belong to subgenus *Tricalysia*. Ranarivelo-Randriambovonjy et al. (2007) observed that the Madagascan taxa could be accommodated within section *Tricalysia* were it not for the presence of unisexual flowers. As a result, they

formally placed these 11 taxa in *Androgyne* Robbr., a new section within subgenus *Tricalysia*.

Recent phylogenetic investigations incorporating morphological and molecular data sets have enabled us to improve our understanding of the systematic position of *Tricalysia* and its relationships with associated genera (Andreasen & Bremer, 2000; Persson, 2000; Bridson & Verdcourt, 2003; Robbrecht & Manen, 2006; Davis et al., 2007). Andreasen and Bremer (2000) assessed tribal and generic delimitation in subfamily Ixoroideae using morphology, plastid and nuclear ribosomal DNA sequences, and restriction site (restriction fragment length polymorphism) data. Their results highlighted the close affinity between *Coffea* L. and *Psilanthus* Hook. f. (Coffeae s. str.) and several members of the Gardenieae subtribe Diplosporinae (*Diplospora* and *Tricalysia*), resulting in an expanded circumscription of the tribe Coffeae to include *Tricalysia*, *Diplospora*, *Discospermum*, *Sericanthe*, *Coffea*, *Psilanthus*, and *Bertiera* Aubl. Bridson and Verdcourt (2003) further enlarged and modified the concept of Coffeae on the basis of morphology and provisional plastid data (provided by A. P. Davis, unpublished). In contrast to the studies of Andreasen and Bremer (2000) and a broader study of the Rubiaceae (Robbrecht & Manen, 2006), the genus *Bertiera* was excluded from Coffeae and placed in its own tribe, Bertiereae.

Davis et al. (2007) reexamined the circumscription and phylogeny of Coffeae and *Bertiera* using sequence data from three plastid regions (*trnL-F* intron and intergenic spacer, *accD-psaI*, and *rpl16*) in combination with morphological data. Their study confirmed the placement of *Tricalysia* and related taxa (*Sericanthe*, *Diplospora*, and *Discospermum*) with *Coffea* and *Psilanthus*, and expanded Coffeae to include six other genera (*Argocoffeopsis* Lebrun, *Belonophora* Hook. f., *Nostolachma* T. Durand, *Calycosiphonia* Pierre ex Robbr., and *Xantonnea* Pierre ex Pit.). However, this study only surveyed a limited number of *Tricalysia* species, all of which belong to subgenus *Tricalysia*. *Bertiera* was excluded from Coffeae and retained in Bertiereae, in agreement with Bridson and Verdcourt (2003), and Gardenieae subtribe Diplosporinae was placed in synonymy with Coffeae.

The current investigation uses DNA sequence data to test the monophyly of *Tricalysia* as currently circumscribed and to assess the accuracy of the subgeneric classification for the genus (Robbrecht, 1979, 1982, 1983, 1987). This is the first molecular study to include widespread and representative sampling of *Tricalysia*. In addition, we reassess the phylogenetic relationships within the broadly circumscribed Coffeae, with an expanded sampling from both subgenera of *Tricalysia*. Given the wealth of *trnL-F*, *rpl16*, and *accD-psaI* sequence data already

Table 1. Summary of species from *Tricalysia* subgen. *Empogona* and subgen. *Tricalysia* sampled in this study (following classification of Robbrecht, 1979, 1982, 1983, 1987).

| A) <i>Tricalysia</i> subgen. <i>Empogona</i> (ca. 27 spp., Robbrecht, 1979)      |   |  |
|--|---|--|
| Section  | Species group   | Species                                |
| <i>Tricalysia</i> sect. <i>Empogona</i> Hook. f. 12 spp. sensu Robbrecht, 1979   | <i>T. discolor</i> group                                | <i>T. acidophylla</i> Robbr.           |
|  | <i>T. junodii</i> group                                 | <i>T. junodii</i> (Schinz) Brenan      |
|  |   | <i>T. ngalaensis</i> Robbr.            |
|  |   | <i>T. ovalifolia</i> Hiern             |
|  | No known group affiliation within sect. <i>Empogona</i> | <i>T. concolor</i> N. Hallé            |
|  |   | <i>T. gossweileri</i> S. Moore         |
| <i>Tricalysia</i> sect. <i>Kraussiopsis</i> Robbr. 15 spp. sensu Robbrecht, 1979 | <i>T. crepiniana</i> group                              | <i>T. bequaertii</i> De Wild.          |
|  |   | <i>T. talbotii</i> (Wernham) Keay      |
|  | <i>T. ruandensis</i> group                              | <i>T. cacondensis</i> Hiern            |
|  |   | <i>T. lanceolata</i> (Sond.) Burt Davy |
|  |   | <i>T. ruandensis</i> Bremek.           |

B) *Tricalysia* subgen. *Tricalysia* (ca. 75 spp., Robbrecht, 1982, 1983, 1987)

| Section  | Species group                          | Species  |
|--|--|--|
| <i>Tricalysia</i> sect. <i>Probletostemon</i> (K. Schum.) Robbr. 4 spp. sensu Robbrecht, 1983          |  | <i>T. anomala</i> E. A. Bruce                                |
|  |  | <i>T. elliotii</i> (K. Schum.) Hutch. & Dalziel              |
| <i>Tricalysia</i> sect. <i>Ephedranthera</i> Robbr. 9 spp. sensu Robbrecht, 1982                       |  | <i>T. aciculiflora</i> Robbr.                                |
|  |  | <i>T. acocantheroides</i> K. Schum.                          |
|  |  | <i>T. bridsoniana</i> Robbr.                                 |
| <i>Tricalysia</i> sect. <i>Tricalysia</i> 40 spp. sensu Robbrecht, 1987                                | <i>T. angolensis</i> group             | <i>T. griseiflora</i> K. Schum.                              |
|  | Core group for sect. <i>Tricalysia</i> | <i>T. bagshawei</i> S. Moore                                 |
|  |  | <i>T. coriacea</i> (Benth.) Hiern                            |
|  |  | <i>T. microphylla</i> Hiern                                  |
|  |  | <i>T. okelensis</i> Hiern                                    |
|  |  | <i>T. pallens</i> Hiern                                      |
| <i>Tricalysia</i> sect. <i>Rosea</i> (Klotzsch) Robbr. 9 spp. sensu Robbrecht, 1987                    |  | <i>T. jasminiflora</i> (Klotzsch) Benth. & Hook. f. ex Hiern |
|  |  | <i>T. schliebenii</i> Robbr.                                 |
| <i>Tricalysia</i> sect. <i>Androgyne</i> Robbr. 11 spp. sensu Ranarivelo-Randriamboavonjy et al., 2007 |  | <i>T. ambrensis</i> Randriamb. & De Block                    |
|  |  | <i>T. analamazaotrensis</i> Homolle ex Randriamb. & De Block |
|  |  | <i>T. cryptocalyx</i> Baker                                  |
|  |  | <i>T. dauphinensis</i> Randriamb. & De Block                 |
|  |  | <i>T. leucocarpa</i> (Baill.) Randriamb. & De Block          |
|  |  | <i>T. perrieri</i> Homolle ex Randriamb. & De Block          |

Table 2. Amplification primers used in this study.

| Region           | Primer           | Primer sequence (5'-3')           | Reference             |
|------------------|------------------|-----------------------------------|-----------------------|
| <i>trnL-F</i>    | Forward (c)      | CGA AAT CGG TAG ACG CTA CG        | Taberlet et al., 1991 |
|                  | Reverse (f)      | AAT TGA ACT GGT GAC ACG AG        |                       |
| <i>rpl16</i>     | Forward (71f)    | GCT ATG CTT AGT GTG TGA CTC GTT G | Jordan et al., 1996   |
|                  | Reverse (1661r)  | CGT ACC CAT ATT TTT CCA CCA CGA C |                       |
|                  | Reverse (1516r)  | CCC TTC ATT CTT CCT CTA TGT TG    | Shaw et al., 2005     |
|                  | Internal forward | GTA AGA AGT GAT GGG AAC GA        | Davis et al., 2007    |
|                  | Internal reverse | TGC TTC CCA TCA CTT CTT AC        |                       |
| <i>accD-psaI</i> | Forward (769 F)  | GGA AGT TTG AGC TTT ATG CAA ATG   | Mendenhall, 1994      |
|                  | Reverse (75 R)   | AGA AGC CAT TGC AAT TGC CGG AAA   |                       |
| <i>petD</i>      | Forward (1365)   | TTG ACY CGT TTT TAT AGT TTA C     | Löhne & Borsch, 2004  |
|                  | Reverse (738)    | AAT TTA GCY CTT AAT ACA GG        |                       |

available for Coffeae (Davis et al., 2007), we have focused on these three plastid regions in the current investigation and included further sequence data from the plastid region *petD*.

#### MATERIALS AND METHODS

##### TAXON SAMPLING

An expanded sampling of *Tricalysia*, *Diplospora*, *Discospermum*, *Sericanthe*, and *Bertiera* was combined with sequence data generated by Davis et al. (2007). *Tricalysia* samples representing both subgenera and all of the seven sections of the genus (Robbrecht, 1979, 1982, 1983, 1987) were included in the analyses (Table 1). Representative taxa from Ixoreae, Octotropideae, and Gardenieae were selected as the outgroup. A list of the 80 accessions used in the study is given in Appendix 1.

##### DNA EXTRACTION, POLYMERASE CHAIN REACTION AMPLIFICATION, AND SEQUENCING

Most DNA samples were obtained from silica gel collections or, alternatively, from seed, flower, or leaf samples taken from herbarium specimens (BR, K, MO, WAG). A small number of DNA samples were obtained from fresh leaf material collected from the living collections of the National Botanic Garden of Belgium.

For silica gel samples, DNA was isolated using a modified DNA Mini Extraction Protocol (Royal Botanic Gardens, Kew [K] protocol). DNA samples were obtained from herbarium material using the 2× CTAB protocol of Doyle and Doyle (1987), with the DNA subsequently purified using cesium chloride/ethidium bromide gradients and concentrated by dialysis before inclusion in the DNA Bank at K. All DNA samples were purified using a NucleoSpin purification column (Macherey-Nagel, Bethlehem,

Pennsylvania, U.S.A.) according to the manufacturer's instructions in order to remove any potential polymerase chain reaction (PCR) inhibitors.

Amplification of the *trnL-F*, *rpl16*, *petD*, and *accD-psaI* plastid regions was carried out using the primers listed in Table 2. Amplification of the *rpl16* region was primarily carried out using the forward primer 71f and the reverse primers 1661r (Jordan et al., 1996) and 1516R (Shaw et al., 2005), although Coffeae specific internal primers designed by K were also required for certain taxa (Davis et al., 2007).

All PCR and sequencing reactions were performed using a Perkin Elmer (Waltham, Massachusetts, U.S.A) GeneAmp 9700 Thermal Cycler machine. Amplification of *trnL-F* was carried out using the following profile: 94°C for 3 min.; 32 cycles of 94°C for 1 min., 51°C for 1 min., 72°C for 2 min.; and a final extension of 72°C for 7 min. *accD-psaI* and *rpl16* were amplified as follows: 94°C for 3 min.; 32 cycles of 94°C for 1 min., 52°C for 1 min., 72°C for 1 min. 30 sec.; and a final extension of 72°C for 7 min. Amplification of *petD* was carried out as follows: 96°C for 2 min.; 34 cycles of 94°C for 1 min., 50°C for 1 min., 72°C for 1 min. 30 sec.; and a final extension of 72°C for 10 min.

For the *trnL-F*, *petD*, and *rpl16* regions, 25 µl PCR reactions were made using a commercial PCR master mix (2.5 mM MgCl<sub>2</sub> ReddyMix; ABgene; Epsom, Surrey, U.K.). *accD-psaI* did not amplify successfully with the commercial master mix, so 25 µl PCR master mixes were prepared using Biotaq DNA polymerase (Bioline, London, U.K.), 2.5 µl of 10× NH<sub>4</sub> reaction buffer (Bioline), 1.5 µl of 50 mM MgCl<sub>2</sub>, and 2.5 µl of dNTPs (Promega, Madison, Wisconsin, U.S.A.). All amplified PCR products were purified using NucleoSpin purification columns following the manufacturer's protocol.

Cycle sequencing reactions were carried out using BigDye Terminator Mix version 3.1 (Applied Biosystems, Inc., Warrington, Cheshire, U.K.). The cycle

sequence reaction consisted of 26 cycles of 10 sec. at 96°C, 5 sec. at 50°C, and 4 min. at 60°C. Cycle sequencing products were cleaned with the MagneSil Clean-Up System (Promega) using an automated robot (Biomek NX S8; Beckman Coulter, High Wycombe, Buckinghamshire, U.K.). Analysis of cycle sequencing products was performed using an AB 3730 DNA Analyzer (Applied Biosystems). In addition, a number of the *trnL-F* and *petD* samples were sent to Macrogen (Seoul, South Korea) for sequencing.

#### ALIGNMENT AND GAP CODING

Sequences were assembled and edited using the Staden software package (Staden et al., 1998). All sequences were aligned manually in MacClade (version 4.04, Maddison & Maddison, 2002). Low levels of sequence variation enabled sequences to be aligned without difficulty. Regions of ambiguous alignment, such as the beginning and end of sequences, were removed. The edited sequences were analyzed with gaps treated as missing data and phylogenetically informative indels (insertions and/or deletions) coded according to the “simple indel coding” method of Simmons and Ochoterena (2000).

#### PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed on the four separate plastid data sets in addition to the combined four-region plastid matrix.

*Maximum parsimony.* Heuristic tree searches were carried out in PAUP\* version 4.0b10 (Swofford, 2003) using 10,000 replicates of random taxon sequence addition, holding 10 trees at each step, with tree bisection-reconnection (TBR) branch swapping, delayed transformation (DELTRAN) optimization, and MULTREES in effect, and saving no more than 10 trees per replicate. Support values for clades recovered in the analyses were estimated using bootstrap analysis (Felsenstein, 1985). One thousand replicates of simple sequence addition, TBR swapping, and saving 10 trees per replicate were performed in PAUP\*. We interpreted bootstrap values greater than 85% as being well supported, 75%–84% as being moderately supported, and 50%–74% as having low support.

*Bayesian inference.* Evolutionary models for each plastid region were selected using Modeltest v3.06 (Posada & Crandall, 1998) under the Akaike information criterion. In the case of *accD-psaI*, *petD*, and *rpl16*, the nucleotide substitution model that best fits the data was HKY + I + G. The HKY + I model

was selected for the *trnL-F* sequence matrix. The combined data set was partitioned into five discrete units. In addition to the four plastid regions, there was a fifth partition for the phylogenetically informative indels. The restriction site (binary) model of evolution was implemented for the indel data, following the recommendation of Ronquist et al. (2005). Four independent Bayesian searches, each consisting of two simultaneous parallel analyses, were carried out using MrBayes 3.1 (Huelsenbeck & Ronquist, 2001). In each Bayesian analysis, four Markov chains (three heated, one cold) were run simultaneously for 2,000,000 generations, sampling trees every 100 generations. The initial 25% of trees were discarded as a conservative burn-in. After confirming by eye that trees generated from separate analyses had consistent topologies, the “post-burn-in” trees from each analysis were pooled together, imported into PAUP\* version 4.0b10 (Swofford, 2003), and summarized by majority rule consensus, with values on the tree equating to posterior probabilities (PP).

#### RESULTS

This study generated 229 sequences, which were combined with the 75 sequences obtained by Davis et al. (2007). In total, this study included 79 *accD-psaI* sequences (53 newly generated), 80 *trnL-F* sequences (54 newly generated), 78 *rpl16* sequences (55 newly generated), and 67 *petD* sequences (all newly generated). The *rpl16* region proved to be the most problematic region to amplify, due in part to two poly-A stretches (one 373 bp from the 5' end, the other 466 bp from the 3' end). As a result, it was often difficult to obtain sufficient overlap during sequence assembly. Internal primers, designed specifically for Coffeae taxa (Davis et al., 2007), were used to obtain a complete sequence for *rpl16* in problematic taxa.

In general, the amount of genetic variability in all plastid regions was low (Table 3). A large proportion of the total genetic variation occurred between the ingroup (Coffeae) taxa and outgroup (other Ixoroideae). We observed considerable length variability in the *accD-psaI* region. As with all the plastid regions investigated, *accD-psaI* is particularly AT-rich and subject to several repeat units, giving rise to a number of potentially phylogenetically informative indels. In the case of *Tricalysia* subgen. *Empogona*, all taxa included in the study share a 250 bp deletion in the *accD-psaI* region. Less length variation was observed in *petD*, *rpl16*, and *trnL-F*. The gross tree topologies of all four individual analyses were examined by eye and found to be topologically consistent, and the four data sets were subsequently combined in all further analyses.

Table 3. Characteristics of *accD-psaI*, *rpl16*, *petD*, *trnL-F*, and combined data sets and tree statistics.

|   | <i>accD-psaI</i> | <i>rpl16</i> | <i>petD</i> | <i>trnL-F</i> | Combined plastid |
|---|------------------|--------------|-------------|---------------|------------------|
| No. of taxa   | 79               | 78           | 67          | 80            | 80               |
| Total length (base pairs)                             | 1255             | 1207         | 1064        | 889           | 4415             |
| Sequence length variation                             | 737–1061         | 995–1068     | 937–966     | 772–822       | —                |
| No. of constant characters                            | 1075             | 982          | 974         | 765           | 3796             |
| No. of phylogenetically informative indels            | 22               | 11           | 8           | 9             | 50               |
| No. of variable PI characters (% of total characters) | 117 (9.3)        | 116 (9.6)    | 45 (4.2)    | 74 (8.3)      | 352 (7.9)        |
| Tree length   | 283              | 339          | 123         | 174           | 929              |
| Consistency index                                     | 0.827            | 0.814        | 0.854       | 0.822         | 0.816            |
| Retention index                                       | 0.923            | 0.890        | 0.937       | 0.916         | 0.908            |
| No. of trees saved                                    | 9920             | 1056         | 1392        | 9990          | 8853             |

The aligned combined matrix had a total length of 4465 bp. There were 669 variable characters and, of these, 352 characters were parsimony informative (7.9% of total number of characters). In total, the matrix contained 50 parsimony informative indels, consisting of repeat sequences in addition to insertion/deletion events. Exclusion of outgroup taxa (Ixoreae, Gardenieae, Octotropideae, and Bertierieae) revealed 211 parsimony informative characters within Coffeae.

PHYLOGENETIC RESULTS

The heuristic maximum parsimony (MP) analysis of the combined plastid data matrix generated 8853 most parsimonious trees with a length of 929 steps, a consistency index (CI) of 0.816, and a retention index (RI) of 0.908. Table 3 summarizes the tree statistics for the individual and combined analyses.

The topologies of the MP strict consensus tree and the Bayesian majority rule tree (Fig. 1) were consistent with each other. Figure 2 displays one of the most parsimonious trees and indicates both bootstrap support (BS) and branch length. Both MP and Bayesian analyses reconfirm the monophyly of the ingroup (BS 99%, PP 1.00). *Bertiera*, here represented by its two subgenera, is monophyletic (BS 100%, PP 1.00) and is sister to the ingroup (BS 79%, PP 1.00).

The clade of *Coffea* and *Psilanthus* is well supported (BS 100%, PP 1.00) and is sister to the remaining ingroup taxa (BS 93%, PP 1.00). There is also strong support for the clade of *Argocoffeopsis* and *Calycosiphonia* (BS 99%, PP 1.00). The sister relationship of *Calycosiphonia* and *Argocoffeopsis* to the rest of the ingroup receives weak bootstrap support (BS 50%), but is supported by a PP of 0.98.

Both MP and Bayesian analyses recovered a clade including *Sericanthe*, *Diplospora*, *Discospermum*, and *Tricalysia* subgen. *Empogona*. Although there is no bootstrap support for this clade (BS < 50%), the clade does receive support in the Bayesian analyses (PP 0.98). Within this clade, there is strong support for the monophyly of *Sericanthe* (BS 99%, PP 1.00), *Discospermum* (BS 100%, PP 1.00), and the group of *Diplospora* and *Tricalysia* subgen. *Empogona* (BS 99%, PP 1.00). The monophyly of both *Diplospora* (BS 90%, PP 1.00) and *Tricalysia* subgen. *Empogona* (BS 98%, PP 1.00) is confirmed. Within *Tricalysia* subgen. *Empogona*, two groups receive high levels of support: the group of *T. cacondensis* Hiern, *T. lanceolata* (Sond.) Burt Davy, and *T. ruandensis* Bremek. (BS 85%, PP 1.00); and the group of *T. junodii* (Schinz) Brenan, *T. ovalifolia*, and *T. acidophylla* Robbr. (BS 98%, PP 1.00).

The clade of *Belonophora* and *Tricalysia* subgen. *Tricalysia* is present in both the MP strict consensus

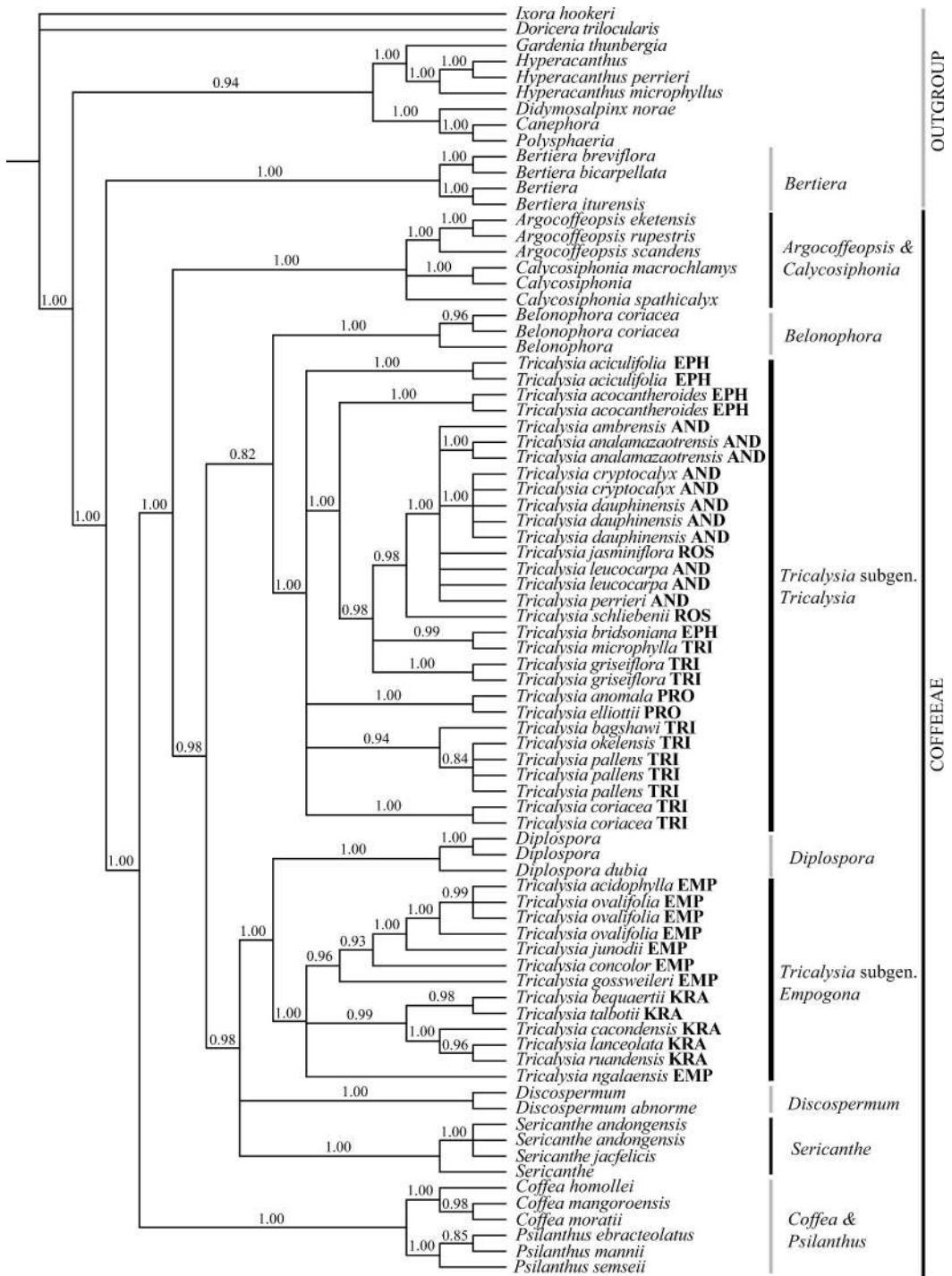


Figure 1. Maximum parsimony strict consensus/Bayesian majority rule consensus tree. Bayesian posterior probabilities are indicated above branches. Sectional groupings are annotated after species names: AND, *Tricalysia* sect. *Androgyne*; EMP, *Tricalysia* sect. *Empogona*; EPH, *Tricalysia* sect. *Ephedranthera*; KRA, *Tricalysia* sect. *Kraussiopsis*; PRO, *Tricalysia* sect. *Proletostemon*; ROS, *Tricalysia* sect. *Rosea*; TRI, *Tricalysia* sect. *Tricalysia*. See Table 1 for species authorities and provenance.



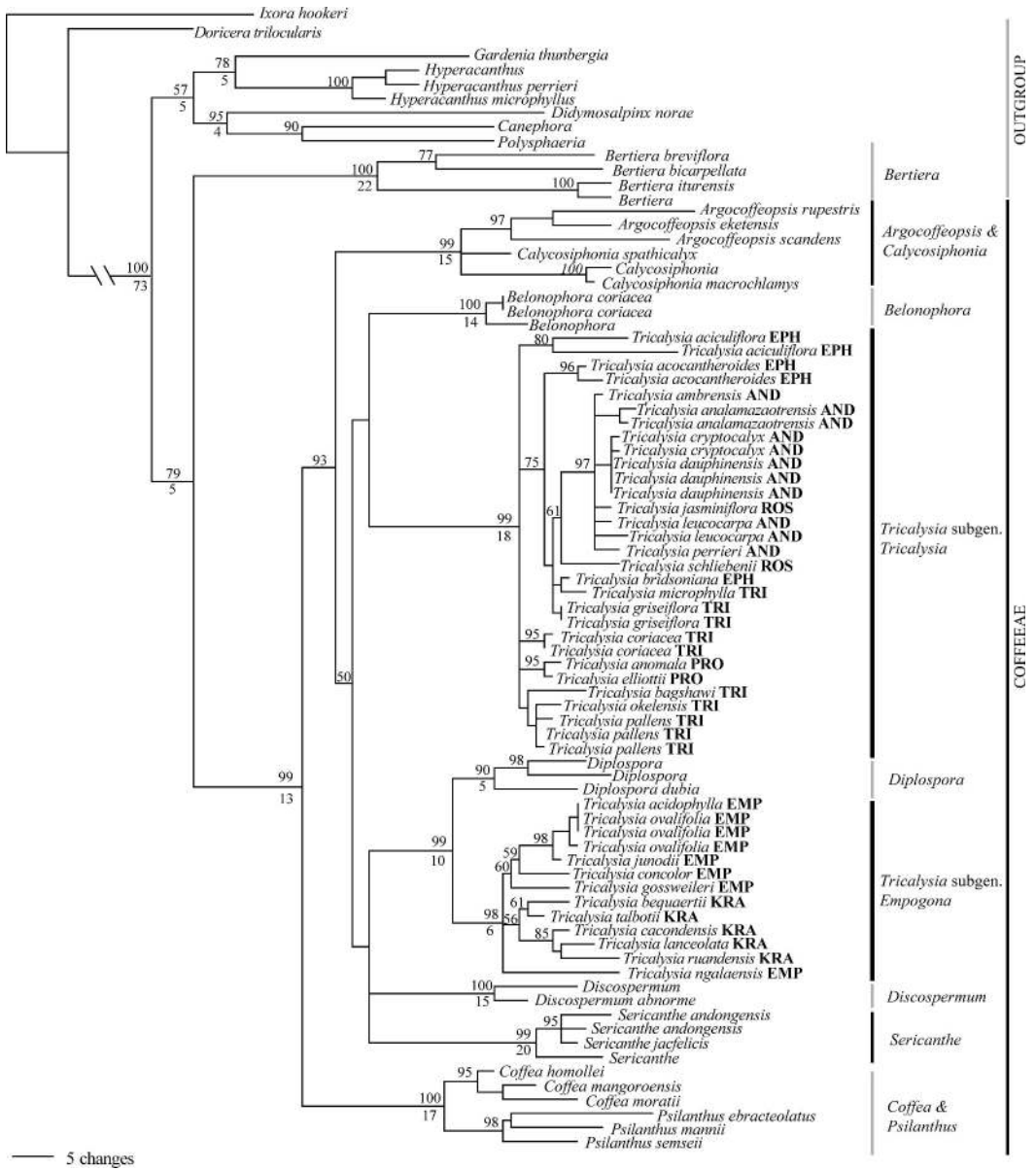


Figure 2. One of the 8853 most parsimonious trees generated in the maximum parsimony analysis. Bootstrap values > 50% are indicated above branches, and selected branch lengths are indicated below branches. Sectional groupings are annotated after species names: AND, *Tricalysia* sect. *Androgyne*; EMP, *Tricalysia* sect. *Empogona*; EPH, *Tricalysia* sect. *Ephedranthera*; KRA, *Tricalysia* sect. *Kraussiopsis*; ROS, *Tricalysia* sect. *Rosea*; PRO, *Tricalysia* sect. *Probletostemon*; TRI, *Tricalysia* sect. *Tricalysia*. See Table 1 for species authorities and provenance.

tree and the Bayesian majority rule tree, although there is negligible support for this clade (BS < 50%, PP 0.82). However, the monophyly of *Belonophora* (BS 100%, PP 1.00) and *Tricalysia* subgen. *Tricalysia* (BS 99%, PP 1.00) is strongly supported. Within *Tricalysia* subgen. *Tricalysia*, several groups receive strong support: the group of *T. elliotii* (K. Schum.) Hutch. &

Dalziel and *T. anomala* E. A. Bruce (BS 95%, PP 1.00), and a group of predominantly Madagascan taxa with the inclusion of *T. jasminiflora* (Klotzsch) Benth. & Hook. f. ex Hiern (BS 97%, PP 1.00). There is also moderate bootstrap (BS 75%) and high PP (PP 1.00) for the clade of *T. acoantheroides* K. Schum., *T. griseiflora* K. Schum., *T. bridsoniana* Robbr., *T.*

*microphylla* Hiern, *T. schliebenii* Robbr., and the aforementioned Madagascan group together with *T. jasminiflora*.

#### DISCUSSION

Previous taxonomic work on *Tricalysia* has focused on the use of traditional morphological and anatomical characters to infer relationships within the genus. In the most recent classification of the genus, Robbrecht (1979, 1982, 1983, 1987) subdivided it into two subgenera and seven sections. Here, for the first time, we have addressed relationships in this group using molecular data.

In the current investigation, we obtained sequence data from four plastid regions for both subgenera and all seven sections of *Tricalysia* and generated estimates of phylogeny using both MP and Bayesian inference methods. The consensus tree topologies of both analyses (strict consensus for MP, majority rule consensus for Bayesian) were consistent. As is often observed, Bayesian PP were higher than bootstrap support values for any given node (Huelsenbeck et al., 2002; Erixon et al., 2003; Randle et al., 2005).

#### TESTING THE MONOPHYLY OF THE GENUS *TRICALYSIA*

Our phylogenetic analyses indicate that *Tricalysia*, as currently circumscribed, is not monophyletic. The monophyly of subgenera *Tricalysia* and *Empogona* is confirmed, but they are not sister to each other. This represents a new, though perhaps unsurprising, observation, which has implications for the taxonomy of the group (see below).

Davis et al. (2007) included five species of *Tricalysia* in their molecular and morphological reassessment of the circumscription and phylogeny of Coffeae. All five species were representatives of subgenus *Tricalysia*. In both their combined molecular and combined morphological-molecular phylogenies, *Tricalysia* (subgen. *Tricalysia*) was placed in a poorly supported and unresolved clade containing *Sericanthe*, *Belonophora*, and an Asian clade (including *Diplospora* and *Discospermum*). The study of Davis et al. (2007) incorporated molecular data from three plastid regions (*trnL-F*, *accD-psaI*, and *rpl16*). In our investigation, we included an additional plastid region, the group II intron *petD*. The extra characters provided by this fourth plastid marker were still not sufficient to fully elucidate systematic relationships within the clade containing *Tricalysia* subgen. *Tricalysia*, *Sericanthe*, *Belonophora*, *Diplospora*, and *Discospermum*.

The inclusion of taxa from *Tricalysia* subgen. *Empogona* led to results conflicting with the study

of Davis et al. (2007). First, we did not recover an Asian clade. Instead, *Diplospora* formed a well-supported monophyletic group with *Tricalysia* subgen. *Empogona* (BS 99%, PP 1.00). Second, both the MP strict consensus tree and the Bayesian majority rule consensus tree indicated sister relationships between *Tricalysia* subgen. *Tricalysia* and *Belonophora*, and recovered a clade containing *Sericanthe*, *Discospermum*, *Diplospora*, and *Tricalysia* subgen. *Empogona*. The clade of *Belonophora* and subgenus *Tricalysia* received poor internal support (BS < 50%, PP 0.82), but there was support for the second clade in the Bayesian analysis (BS < 50%, PP 0.98).

#### TAXONOMIC IMPLICATIONS FOR GENERIC CONCEPTS

The revelation that *Tricalysia* sensu Robbrecht is not monophyletic calls for a reconsideration of the taxonomic delimitation of *Tricalysia* and closely related taxa. One taxonomic option would be to widen the genus *Tricalysia* to include *Belonophora*, *Diplospora*, *Discospermum*, and *Sericanthe*. However, these genera are easily identified (e.g., by the use of a key) and are so diverse morphologically and anatomically that consolidating them into one genus does not seem justified (Table 4). A more logical option would be to separate these taxa into groups at the generic level, based on morphological and molecular synapomorphies.

Robbrecht (1979) enumerated four potential field characters that distinguish the subgenera *Empogona* and *Tricalysia*. Taxa of subgenus *Empogona* are identified by the presence of distinctly lobed calyces (vs. short and truncate in subgenus *Tricalysia*), densely pubescent corolla throats (vs. glabrous to sparsely hairy), the presence of a large flattened sterile appendage protruding from the anther connective (vs. blunt anthers, occasionally forming a short triangular appendage), and fruits that turn black at maturity (vs. red fruits). Robbrecht (1979) considered recognizing *Empogona* at the generic rank, but opted to incorporate it as a subgenus of *Tricalysia*, given the similarity in a number of other key characters (placentation, pollen morphology, fruit and seed morphology, and seed coat anatomy). This decision was also pragmatic in terms of taxonomic stability, as it required the fewest nomenclatural changes (Robbrecht, 1979).

The revision of *Sericanthe* (Robbrecht, 1978) and the survey of the Asian relatives of *Tricalysia* (Ali & Robbrecht, 1991) provided ample morphological and anatomical evidence to justify the exclusion of these genera from *Tricalysia*. The genus *Sericanthe* is distinguished from *Tricalysia* by the presence of bacterial leaf galls (rare in Rubiaceae), wing-shaped

colleters, and pollen with a verrucate sexine (in contrast to the reticulate sexine occurring in all other members of Coffeaeae). Davis et al. (2007) also presented the following synapomorphic characters for the genus: 7- to 9-merous flowers, distinctly basifixed anthers, and horizontal micropyle orientation.

*Diplospora* and *Discospermum* consistently have tetramerous flowers, which occur only rarely in African *Tricalysia*, and the flowers of Asian taxa are smaller than their African counterparts (Ali & Robbrecht, 1991). In addition, there is a strong tendency toward unisexual flowers in Asian taxa, a trait that is absent in all but a few representatives of *Tricalysia* confined to Madagascar (Ranarivelo-Randriamboavonjy et al., 2007). Ali and Robbrecht (1991) also justified maintaining *Diplospora* and *Discospermum* as separate genera on the basis of rather divergent fruit types (small, fleshy, and red fruits in *Diplospora* and large, leathery, and purplish black fruits in *Discospermum*). The decision to maintain *Diplospora* and *Discospermum* as separate genera is also supported by our molecular analyses.

The tribal position of *Belonophora* has been fairly unstable since its initial description by Hooker (1873), partly due to the erroneous observation by Hooker that *Belonophora* possesses a solitary, pendulous ovule in each of the two locules. Keay (1958) observed that *Belonophora* species actually possess two collateral ovules per locule, on the inner surface of a pendulous placenta, but he felt it premature to assign the genus to a new tribe until a more satisfactory tribal classification within Rubiaceae had been proposed. Robbrecht and Puff (1986) tentatively placed *Belonophora* in the tribe Aulacocalyceae, although the axillary inflorescences of *Belonophora* contrasted with the terminal or subterminal inflorescences possessed by other members of the tribe. The placement of *Belonophora* in the tribe Coffeaeae was first proposed by Bridson and Verdcourt (2003) and later supported by the study of Davis et al. (2007). The imbricate calyx lobes of *Belonophora* were synapomorphic for the genus in the study of Davis et al. (2007), and the genus is also distinguished from other members of Coffeaeae by the presence of a superior embryo radicle (Cheek & Dawson, 2000).

In light of evidence from our own molecular investigation, and in combination with morphological and anatomical observations reported elsewhere, we believe it is appropriate and fully justified to recognize *Empogona* (sensu Robbrecht, 1979) at generic rank. The necessary taxonomic changes for the inclusion of many former *Tricalysia* species in the genus *Empogona* are provided at the end of the Discussion.

#### RECOGNITION OF INTRAGENERIC GROUPS IN *TRICALYSIA*

In addition to testing the monophyly of *Tricalysia* sensu Robbrecht, we were able to assess the levels of support for his sectional groups within the genus. All seven sections were sampled in our analysis, although some were better represented. Low levels of genetic variation between species limited the amount of resolution between taxa, but there are some provisional findings from this study.

*Tricalysia* subgen. *Tricalysia* was subdivided into five sections by Robbrecht (1982, 1983, 1987). *Tricalysia* sect. *Probletostemon*, here represented in our molecular study by *T. elliotii* and *T. anomala* (Table 1), was thought to possess many morphological and anatomical features regarded as primitive for the group. These included free bracteoles, standard colleters (Robbrecht, 1988), large pleiomorous flowers with many ovules per placenta, and large fruits (Robbrecht, 1983). Our study confirms the monophyly of section *Probletostemon* (BS 95%, PP 1.00), but it remains unresolved in a basal polytomy.

*Tricalysia* sect. *Ephedranthera*, here represented by three species, is characterized by the presence of anthers that are sessile in the corolla throat and partly included within the corolla tube (Robbrecht, 1982). The monophyly of this section is not supported in our investigation. *Tricalysia aciculiflora* Robbr. falls within the basal polytomy, whereas *T. acantheroides* and *T. bridsoniana* are situated within the moderately to well-supported clade (BS 75%, PP 1.00) containing all the remaining taxa of subgenus *Tricalysia*.

The other three sections (*Tricalysia*, *Rosea*, and *Androgyne*) are very similar morphologically. Most species in subgenus *Tricalysia* belong to section *Tricalysia*, which Robbrecht (1987) further subdivided into four informal groups. Only two of these informal groups are included in this investigation. The core group of taxa within section *Tricalysia*, here represented by *T. coriacea* (Benth.) Hiern and the weakly supported clade of *T. pallens* Hiern, *T. okelensis* Hiern, and *T. bagshawei* S. Moore, is unresolved in the basal polytomy. The group of *T. angolensis* A. Rich. ex DC., represented by *T. griseiflora* K. Schum., falls within the clade containing *T. bridsoniana*, *T. microphylla*, and representatives from sections *Rosea* and *Androgyne*.

In section *Rosea*, species differ conspicuously from those in section *Tricalysia* due to the presence of a spathaceous calyx (Robbrecht, 1987). In section *Androgyne*, which comprises the Madagascan representatives of subgenus *Tricalysia*, species are characterized by the presence of unisexual flowers. There is weak bootstrap and significant Bayesian support

Table 4. Salient morphological characters of *Tricalysia* and close relatives. Characters in boldface represent unique features for *Empogona*. Figures in single parentheses = rarely; figures in double parentheses = very rarely.

|                          | <i>Tricalysia</i> , excluding <i>Tricalysia</i> sect.   |   |  |  | <i>Sericanthus</i><br>(ca. 20 spp.)   |
|--------------------------|---|---|--|--|---|
|                          | <i>Diplospora</i><br>(ca. 10 spp.)  | <i>Empogona</i><br>(29 spp.)  | sect. <i>Androgyne</i><br>(ca. 80 spp.)  | <i>Belonophora</i><br>(5 spp.)   |   |
| Bracts and<br>bracteoles | free or fused into<br>calyculi  | free alternate, sect.<br><i>Empogona</i> ; fused into<br>calyculi, sect.<br><i>Kraussiopsis</i>                     | fused into calyculi;<br>free alternate in<br>sect.<br><i>Probletostemon</i>                                      | fused into<br>calyculi<br>free, opposite   | fused into calyculi   |
| Corolla length<br>(mm)   | 8–15  | 5–10  | 8–50   | 5–10   | (8–)12–25   |
| Flower<br>organization   | hermaphroditic or unisexual   | hermaphroditic  | hermaphroditic <sup>a</sup>  | unisexual  | hermaphroditic  |
| Merosity                 | 4   | 4<br>4(–(–5))   | ((4–)5(–6))  | 4–7  | (5–)7–8(–9)   |
| Calyx                    | tube short, rounded lobes<br>present or absent  | tube short, lobes<br>mostly triangular  | tube short, lobes well-<br><b>developed and</b><br><b>often overlapping</b>                                      | tube well-<br>developed,<br>with minute<br>teeth   | tube short, lobes well-<br>developed  |
| Corolla throat           | glabrous to bearded   | glabrous or hairy   | glabrous to hairy  | hairy  | glabrous or hairy   |
| Anthers                  | medifixed; on short<br>filaments in throat,<br>exserted   | medifixed; on short<br>filaments in throat,<br>exserted   | medifixed; on long<br>filaments in<br>throat, exserted   | medifixed; on<br>short<br>filaments in<br>throat or<br>sessile,<br>exserted  | basifixed; on short<br>filaments in<br>throat, exserted   |
| Anther<br>connective     | sometimes protruding in very<br>short triangle  | sometimes protruding<br>in very short<br>triangle   | mostly protruding in<br>short triangle   | short apical<br>appendage  | strongly flattened;<br>no appendage   |
| Placentation             | (3–)5–15 ovules on a hemi-<br>circular to ± hemi-ellipsoid<br>placenta; attached to the<br>middle of the septum | 1–3(–6) ovules on a<br>hemi-circular to<br>± hemi-ellipsoid<br>placenta; attached<br>to the middle of<br>the septum | 1–12 ovules on a<br>hemi-circular to<br>± hemi-ellipsoid<br>placenta; attached<br>to the middle of<br>the septum | 2–8 ovules on a<br>hemi-circular<br>to ± hemi-<br>ellipsoid<br>placenta;<br>attached to<br>the middle of<br>the septum | (1–)2(–5) ovules on<br>a hemi-circular<br>± hemi-<br>ellipsoid<br>placenta;<br>attached to the<br>apex of the<br>septum |
| Fruit (mm)               | 20–30   | 5–7   | 5–20   | 5–9  | 10–30   |

Table 4. Continued.

|                        | <i>Discospermium</i><br>(ca. 7 spp.) | <i>Diplospora</i><br>(ca. 10 spp.)       | <i>Empogona</i><br>(29 spp.)                       | <i>Tricalysia</i> , excluding<br>sect. <i>Androgyne</i><br>(ca. 80 spp.) | <i>Tricalysia</i> sect.<br><i>Androgyne</i><br>(11 spp.) | <i>Belonophora</i><br>(5 spp.) | <i>Sericanthe</i><br>(ca. 20 spp.)      |
|------------------------|--------------------------------------|--|--|--|--|--------------------------------|---|
| Fruit color            | purplish black                       | turning from yellow<br>and orange to red | <b>first white, turning<br/>purple, then black</b> | red, rarely orange   | red  | yellow                         | orange                                  |
| Pericarp               | sclerified or leathery               | fleshy                                   | fleshy   | fleshy; rarely<br>sclerotic, sect.<br><i>Probletostemon</i>              | fleshy   | fleshy                         | fleshy                                  |
| Placental<br>outgrowth | massive, mostly surrounding<br>seeds | mostly none                              | none, with weak<br>outgrowths in some<br>spp.      | mostly none  | none   | massive, surrounding<br>seeds  | mostly none;<br>massive in some<br>spp. |
| Endosperm              | entire, ruminant in some spp.        | entire or ruminant                       | entire, ruminant in some<br>spp.                   | entire   | entire   | entire                         | entire                                  |
| Embryo radicle         | away from septum                     | inferior                                 | inferior   | inferior   | inferior   | superior                       | lateral                                 |

<sup>a</sup> Heterostyly in section *Ephedranthera*.

<sup>b</sup> Glabrous in a few species, e.g., *Empogona concolor*.

<sup>c</sup> Some species with an inconspicuous appendix, e.g., *Empogona uelutitschii*.

(BS 61%, PP 0.98) for a clade containing these two sections. *Tricalysia schliebenii* (section *Rosea*) is sister to a strongly supported clade (BS 97%, PP 1.00) containing members of section *Androgyne* and *T. jasminiflora* of section *Rosea*.

Robbrecht (1979) recognized two sections within subgenus *Empogona*: section *Empogona* is characterized by free bracteoles and distinct non-overlapping calyx lobes; in contrast, the bracteoles in section *Kraussiopsis* are fused to form calyculi, and the calyx lobes either touch or overlap each other (with the exception of *Tricalysia bequaertii* De Wild., where the calyx lobes are not touching). *Tricalysia ngalaensis* Robbr., previously thought to be closely related to *T. junodii* (Schinz) Brenan (Robbrecht, 1979), is in an unresolved position (Figs. 1, 2). There is weak bootstrap but significant Bayesian support for the monophyly of section *Kraussiopsis* (BS 56%, PP 0.99), and the informal group of *T. ruandensis* is also well supported (BS 85%, PP 100). The remaining taxa of section *Empogona* are weakly supported (BS 60%, PP 0.96), although the clade of *T. junodii*, *T. ovalifolia*, and *T. acidophylla* is well supported (BS 98%, PP 1.00).

OTHER RELATIONSHIPS WITHIN COFFEEAE AND THE  
RELATIONSHIP TO *BERTIERA*

The sister relationship of *Bertiera* and Coffeeae is recovered with moderate bootstrap and significant Bayesian support (BS 79%, PP 100), although our outgroup sampling is not complete. In order to confirm this result, more extensive sampling of representative groups within subfamily Ixoroideae is needed. Robbrecht and Manen (2006) opted to place *Bertiera* in subtribe Bertierinae, sister to Coffeinae, as the characteristic features of *Bertiera* differ from those of Coffeeae. Davis et al. (2007) found only weak bootstrap support for the sister relationship between *Bertiera* and Coffeeae (BS < 40%) based on molecular data alone, and the sister relationship was not recovered following the addition of morphological characters in their combined molecular-morphological analysis. Based on the decision of Bridson and Verdcourt (2003), they opted to place *Bertiera* in the monogeneric tribe Bertiereae. Whether *Bertiera* is recognized at the tribal or subtribal level is still open to debate, but we agree with Davis et al. (2007: 321) that “Coffeeae, with the addition of new genera and the removal of *Bertiera*, is both scientifically coherent and practical.”

In the three-region plastid analysis of Davis et al. (2007), *Coffea* and *Psilanthus* form a well-supported monophyletic clade supported by a bootstrap of 87%, and are placed sister to the rest of Coffeeae. This relationship is recovered in our four-region analysis,

with increased support values (BS 93%, PP 1.00). There was also strong support for the sister relationship between the well-supported *Argocoffeopsis* and *Calycosiphonia* clade and the remaining ingroup taxa in our Bayesian analysis (PP 0.98), but weak support for this relationship in the MP analysis (BS 50%). This relationship was also recovered in the strict consensus tree of Davis et al. (2007).

TAXONOMIC NOVELTIES RESULTING FROM THE GENERIC  
RESURRECTION OF *EMPOGONA*

An outline of an emended infrageneric classification for *Empogona* is provided below. It contains a formal new combination for one of the two sections recognized. The outline is followed by a checklist of species, providing all necessary new combinations at the species level and below.

OUTLINE OF AN EMENDED CLASSIFICATION FOR *EMPOGONA*

**Empogona** Hook. f., Hooker's Icon. Pl. 11: 72, t. 1091. 1871. TYPE: *Empogona kirkii* Hook. f.

*Tricalysia* subgen. *Empogona* (Hook. f.) Robbr., Bull. Jard. Bot. Natl. Belg. 49: 259. 1979.

The further synonymy of subgenus *Empogona* (Robbrecht, 1979: 259) remains applicable to the genus *Empogona*.

**Empogona** Hook. f. sect. **Empogona**. *Tricalysia* subgen. *Empogona* (Hook. f.) Robbr. sect. *Empogona* (Hook. f.) Brenan.

*EMPOGONA KIRKII* SPECIES GROUP

This corresponds to the group of *Tricalysia junodii* (Robbrecht, 1979: 269). The group comprises the species numbered 11, 18, 22, and 24 in the checklist below. The position of *Empogona ngalaensis* (species 22 below) was not confirmed by our molecular analysis.

*EMPOGONA DISCOLOR* SPECIES GROUP

This corresponds to the group of *Tricalysia discolor* (Robbrecht, 1979: 292). The group comprises the species numbered 1, 4, 6, and 14 in the checklist below. The group is only represented by *Empogona acidophylla* in the analysis, which falls in a clade corresponding to the previous species group.

Section *Empogona* further comprises the three species numbered 10, 17, and 25 in the checklist below. They were considered to be of isolated position (Robbrecht, 1979: 300). Two of these species (10. *E. concolor* and 17. *E. gossweileri*) are included in the

analysis. They have a basal position in the clade corresponding to section *Empogona*.

**Empogona** sect. **Kraussiopsis** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia* subgen. *Empogona* sect. *Kraussiopsis* Robbr., Bull. Jard. Bot. Natl. Belg. 49: 309. 1979. TYPE: *Empogona crepiniana* (De Wild. & T. Durand) J. Tosh & Robbr.

*EMPOGONA RUANDENSIS* SPECIES GROUP

This corresponds to the group of *Tricalysia ruandensis* (Robbrecht, 1979: 310). The group comprises the species numbered 8, 9, 19, 26, and 27 in the checklist below.

*EMPOGONA GLABRA* SPECIES GROUP

This corresponds to the group of *Tricalysia glabra* (Robbrecht, 1979: 292). This small group comprises only two species, numbers 16 and 23 in the checklist below.

*EMPOGONA CREPINIANA* SPECIES GROUP

This corresponds to the group of *Tricalysia crepiniana* (Robbrecht, 1979: 329). It is the most speciose group comprising 11 species, numbered 2, 3, 5, 7, 12, 13, 15, 20, 21, 28, and 29 of the checklist below.

CHECKLIST OF SPECIES AND INFRASPECIFIC TAXA,  
INCLUDING TAXONOMIC NOVELTIES

The list below, ordered alphabetically, enumerates all known taxa of *Empogona*, including the four species (species numbered 3, 7, 21, and 27 below) treated or described after Robbrecht's (1979) revision. The infrageneric assignment of the species is given in the preceding section of the present paper. Taxa preceded by an asterisk (\*) were included in the molecular analysis (see Table 1).

The checklist includes taxonomic novelties for all species, i.e., 34 new combinations and three modifications of infraspecific status. In his revision, Robbrecht (1979) used varietal status for all infraspecific taxa recognized. Here we reconsider the appropriateness of that treatment in applying du Rietz's criteria (as cited in Stace, 1991) for distinguishing subspecies and varieties. Therefore, when infraspecific taxa are allopatric and differing in several features, we propose subspecific rather than varietal status.

(\*) 1. **Empogona acidophylla** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia acid-*

- ophylla* Robbr., Bull. Jard. Bot. Natl. Belg. 49: 292. 1979. TYPE: Tanzania. Eastern Usambaras, 2 mi. E of Sigi railway station, 27 July 1953, *R. B. Drummond & J. H. Hemsley 3490* (holotype, K!; isotypes, B!, BR!, LISU!, S!).
- 2. *Empogona aequatoria*** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia aequatoria* Robbr., Bull. Jard. Bot. Natl. Belg. 48: 465. 1978. TYPE: [Democratic Republic of the Congo.] Congo belge. Yangambi, 4 Dec. 1937, *J. Louis 6887* (holotype, BR!; isotypes, B!, BR!, C!, COI!, EA!, HBG!, K!, MO!, P!, PRE!, UPS!, WAG!).
- 3. *Empogona africana*** (Sim) J. Tosh & Robbr., comb. nov. Basionym: *Diplospora africana* Sim, Forest Fl. Cape, 238. 1907. *Tricalysia africana* (Sim) Robbr., S. African J. Bot. 51: 331. 1985. TYPE: South Africa. E Pondoland, Egossa Forest, Aug. 1899, *T. R. Sim 2386* (holotype, NU!).
- 4. *Empogona aulacosperma*** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia aulacosperma* Robbr., Bull. Jard. Bot. Natl. Belg. 49: 296. 1979. TYPE: [Democratic Republic of the Congo.] Congo belge. Musenge, 20 Dec. 1958, *A. Léonard 2088* (holotype, BR!; isotypes, EA!, K!, MO!, WAG!).
- (\* **5. *Empogona bequaertii*** (De Wild.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia bequaertii* De Wild., Pl. Bequaert. 3: 157. 1925. TYPE: [Democratic Republic of the Congo.] Congo belge. [Kisangani] Stanleyville, Tshopo River, 25 Feb. 1915, *J. Bequaert 6969* (holotype, BR!).
- 6. *Empogona bracteata*** (Hiern) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia bracteata* Hiern, Fl. Trop. Afr. [Oliver et al.] 3: 120. 1877. TYPE: [Guinea.] Senegambia. Karkandy, s.d., *Heudelot 855* (holotype, K!).
- 7. *Empogona breteleri*** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia breteleri* Robbr., Bull. Jard. Bot. Natl. Belg. 51: 166. 1981. TYPE: Gabon. Moanda–Franceville Km 23, 12 Sep. 1970, *F. J. Breteler 6431* (holotype, WAG!; isotypes, BR!, P!).
- 8. *Empogona buxifolia*** (Hiern) J. Tosh & Robbr.
- 8a. *Empogona buxifolia*** (Hiern) J. Tosh & Robbr. subsp. *buxifolia*, comb. nov. Basionym: *Tricalysia buxifolia* Hiern, Fl. Trop. Afr. [Oliver et al.] 3: 119. 1877. TYPE: Angola. Ambriz, Nov. 1872, *J. Monteiro s.n.* (holotype, K!; isotype, W!).
- 8b. *Empogona buxifolia* subsp. *australis*** (Robbr.) J. Tosh & Robbr., comb. et stat. nov. Basionym: *Tricalysia buxifolia* var. *australis* Robbr., Bull. Jard. Bot. Natl. Belg. 48: 465. 1978. TYPE: Angola. Tchivinguiro, 13 Dec. 1961, *G. Barbosa 9650* (holotype, LISC!; isotypes, COI!, K!, LUAI!).
- (\* **9. *Empogona cacondensis*** (Hiern) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia cacondensis* Hiern, Cat. Afr. Pl. (Hiern) 1(2): 467. 1898. TYPE: Angola. Rd. from Quipaca to fortress near Ferão, Oct. 1859, *F. Welwitsch 3112* (lectotype, designated by Robbrecht [1979: 320], LISU!; duplicates, BM!, COI!, K!).
- (\* **10. *Empogona concolor*** (N. Hallé) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia concolor* N. Hallé, Fl. Gabon 17: 283. 1970. TYPE: Gabon. Bélinga, mine de fer, 21 July 1966, *N. Hallé & A. Le Thomas 119* (holotype, P!; isotypes, K!, P!).
- 11. *Empogona coriacea*** (Sond.) J. Tosh & Robbr., comb. nov. Basionym: *Kraussia coriacea* Sond., Linnaea 23: 54. 1850. *Tricalysia sonderiana* Hiern, Fl. Trop. Afr. [Oliver et al.] 3: 119. 1877, replacement for *Kraussia coriacea* Sond., non *Randia coriacea* Benth., Niger Fl. [W. J. Hooker] 387. 1849 [= *Tricalysia coriacea* (Benth.) Hiern]. TYPE: [South Africa. KwaZulu-Natal:] Natal: Durban, s.d., *W. Guenzius 100* (holotype, W!; isotypes, BM!, C!, K!, PRE!, S!).
- 12. *Empogona crepiniana*** (De Wild. & T. Durand) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia crepiniana* De Wild. & T. Durand, Ann. Mus. Congo Belg., Bot. ser. 3, 1: 120. 1901. TYPE: [Democratic Republic of the Congo.] Wangata, 17 Feb. 1896, *A. Dewèvre 740* (holotype, BR!; isotype, COI!).
- 13. *Empogona deightonii*** (Brenan) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia deightonii* Brenan, Kew Bull. 8: 112. 1953. TYPE: Sierra Leone. Jama, 10 Mar. 1948, *F. C. Deighton 4723* (holotype, K!; isotype, P!).
- 14. *Empogona discolor*** (Brenan) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia discolor* Brenan, Kew Bull. 2: 72. 1947. TYPE: [Ghana.] Gold Coast. Mampong Scarp, Feb. 1933, *C. Vigne 2748* (holotype, K!; isotype, MO!).
- 15. *Empogona filiformistipulata*** (De Wild.) Bremek.
- 15a. *Empogona filiformistipulata*** (De Wild.) Bremek. subsp. *filiformistipulata*, Bot. Jahrb. 71:

201, 222. 1940. Basionym: *Urophyllum filiformi-stipulatum* De Wild., Pl. Bequaert. 3: 211. 1925. *Tricalysia filiformi-stipulata* (De Wild.) Brenan, Kew Bull. 8: 112. 1953. TYPE: [Democratic Republic of the Congo.] Congo belge. Kisangani, Tshopo River, 12 Jan. 1915, *J. Bequaert 6580* (holotype, BR!; isotype, K not seen).

**15b. *Empogona filiformistipulata* subsp. *epipsila***

(Robbr.) J. Tosh & Robbr., comb. et stat. nov. Basionym: *Tricalysia filiformistipulata* (De Wild.) Brenan var. *epipsila* Robbr., Bull. Jard. Bot. Natl. Belg. 48: 465. 1978. TYPE: [Democratic Republic of the Congo.] Congo belge. Yangambi, Feb. 1933, *J. Louis 14233* (holotype, BR!; isotypes, COI!, K!, MO!, P!, WAG!).

**16. *Empogona glabra* (K. Schum.) J. Tosh & Robbr., comb. nov.**

Basionym: *Tricalysia glabra* K. Schum., Bot. Jahrb. Syst. 23: 445. 1896. TYPE: Angola. Catete, Nov. 1856, *F. Welwitsch 3117* (holotype, LISU!; isotypes, BM!, C!, COI!, K!, P!).

(\* **17. *Empogona gossweileri* (S. Moore) J. Tosh & Robbr., comb. nov.**

Basionym: *Tricalysia gossweileri* S. Moore, J. Linn. Soc. Bot. 37: 305. 1906. TYPE: Angola. Cuanza Norte, Cazengo, 1903, *J. Gossweiler 688* (holotype, BM!; isotypes, K!, P!).

**18. *Empogona kirkii* Hook. f.**

**18a. *Empogona kirkii* Hook. f. subsp. *kirkii*,**

Hooker's Icon. Pl. 11: 72, t. 1091. 1871. *Tricalysia junodii* (Schinz) Brenan var. *kirkii* (Hook. f.) Robbr., Bull. Jard. Bot. Natl. Belg. 49: 271. 1979. TYPE: Malawi. Cape Maclear, Oct. 1861, *J. Kirk s.n.* (holotype, K!).

*Empogona allenii* Stapf is the only species validly published in the genus *Empogona* not taken up as a result of the present study. It is a synonym of the present taxon (Robbrecht, 1979: 272).

(\* **18b. *Empogona kirkii* subsp. *junodii* (Schinz)**

J. Tosh & Robbr., comb. et stat. nov. Basionym: *Empogona junodii* Schinz, Mém. Herb. Boiss. 10: 67. 1900. *Tricalysia junodii* (Schinz) Brenan, Kew Bull. 2: 60. 1947. TYPE: Mozambique. Baia de Laurenço Marques (Delagoa Bay), s.d., *H. Junod 311* (holotype, Z!).

(\* **19. *Empogona lanceolata* (Sond.) J. Tosh & Robbr., comb. nov.**

Basionym: *Kraussia lanceolata* Sond., Linnaea 23: 53. 1850. *Tricalysia lanceolata* (Sond.) Burt Davy, Ann. Transvaal

Mus. 3: 122. 1912. TYPE: [South Africa. KwaZulu-Natal:] Natal: Durban, *W. Gueinzus 68* (lectotype, designated by Robbrecht [1979: 313], W!; duplicates, P!, S!).

**20. *Empogona macrophylla* (K. Schum.) J. Tosh & Robbr., comb. nov.**

Basionym: *Tricalysia macrophylla* K. Schum., Bot. Jahrb. Syst. 28: 66. 1899. TYPE: Cameroon. Bipinde, *Zenker 1569* (lectotype, designated by Robbrecht [1979: 339], COI!; duplicates, BM!, BR!, COI!, E!, G!, GOET!, HBG!, L!, M!, MO!, P!, S!, W!, WAG!).

**21. *Empogona maputenis* (Bridson & A. E. van Wyk) J. Tosh & Robbr., comb. nov.**

Basionym: *Tricalysia maputensis* Bridson & A. E. van Wyk, Fl. Zambes. 5(3): 475. 2003. TYPE: Mozambique. Matutuine, 8 Aug. 1957, *L. A. G. Barbosa & F. L. de Lemos 7807* (holotype, LISC not seen).

(\* **22. *Empogona ngalaensis* (Robbr.) J. Tosh & Robbr., comb. nov.**

Basionym: *Tricalysia ngalaensis* Robbr., Bull. Jard. Bot. Natl. Belg. 49: 277. 1979. TYPE: Malawi. North Ngala, 20 mi. N of Chilumba, 17 Dec. 1969, *J. Pawek 3095* (holotype, K!).

**23. *Empogona nogueirae* (Robbr.) J. Tosh & Robbr., comb. nov.**

Basionym: *Tricalysia nogueirae* Robbr., Bull. Jard. Bot. Natl. Belg. 48: 466. 1978. TYPE: Angola. Musenge, 14 Oct. 1966, *J. B. Teixeira 10701* (holotype, LISC!; isotype, COI!).

**24. *Empogona ovalifolia* (Hiern) J. Tosh & Robbr.**

(\* **24a. *Empogona ovalifolia* (Hiern) J. Tosh & Robbr. var. *ovalifolia*, comb. nov.**

Basionym: *Tricalysia ovalifolia* Hiern, Fl. Trop. Afr. [Oliver et al.] 3: 119. 1877. TYPE: [Tanzania.] Zanzibar: s. loc., s.d. [acc. K Sep. 1868], *J. Kirk s.n.* (lectotype, designated by Robbrecht [1979: 339], K!).

**24b. *Empogona ovalifolia* var. *glabrata* (Oliv.) J. Tosh & Robbr., comb. nov.**

Basionym: *Empogona kirkii* Hook. f. var. *glabrata* Oliv., Trans. Linn. Soc., Bot., 2: 336. 1887. *Tricalysia ovalifolia* Hiern var. *glabrata* (Oliv.) Brenan, Kew Bull. 2: 58. 1947. TYPE: Kenya or Tanzania. 40–60 mi. from coast, [1884], *H. H. Johnston s.n.* [*Kilimanjaro Exp.*] (holotype, K!).

**24c. *Empogona ovalifolia* var. *taylorii* (S. Moore)**

J. Tosh & Robbr., comb. nov. Basionym: *Empogona taylorii* S. Moore, J. Bot. 63: 145. 1925. *Tricalysia ovalifolia* Hiern var. *taylorii* (S.



Moore) Brenan, Kew Bull. 2: 59. 1947. TYPE: Kenya. Giriama, Oct. 1887, *W. E. Taylor s.n.* (holotype, BM!).

**25. *Empogona reflexa*** (Hutch.) J. Tosh & Robbr.

**25a. *Empogona reflexa*** (Hutch.) J. Tosh & Robbr. var. **reflexa**, comb. nov. Basionym: *Tricalysia reflexa* Hutch., Kew Bull. 1915: 44. 1915. TYPE: Sierra Leone. Kessewe, 17 Apr. 1913, *C. E. Lane-Poole 131* (holotype, K!).

**25b. *Empogona reflexa*** var. **ivorensis** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia reflexa* var. *ivorensis* Robbr., Bull. Jard. Bot. Natl. Belg. 48: 466. 1978. TYPE: Ivory Coast. W of Niapidou, 20 Jan. 1959, *A. J. M. Leeuwenberg 2500* (holotype, WAG!; isotypes, BR!, K!).

(\* **26. *Empogona ruandensis*** (Bremek.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia ruandensis* Bremek., Bull. Jard. Bot. État Bruxelles 26: 253. 1956. TYPE: [Rwanda.] Mayaga, Mutema, 19 May 1954, *L. Liben 1416* (holotype, U!; isotypes, BR!, WAG!).

**27. *Empogona somaliensis*** (Robbr.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia somaliensis* Robbr., Bull. Jard. Bot. Natl. Belg. 56: 149. 1986. TYPE: Somalia. 17 km W of Badade, 30 June 1983, *J. B. Gillett, C. F. Hemming, R. M. Watson & H. Julin 25153* (holotype, K!).

(\* **28. *Empogona talbotii*** (Wernham) J. Tosh & Robbr., comb. nov. Basionym: *Cremaspora talbotii* Wernham, Cat. Pl. Oban 49. 1913. *Tricalysia talbotii* (Wernham) Keay, Bull. Jard. Bot. État Bruxelles 28: 291. 1958. TYPE: Nigeria. Southern Nigeria, Oban, 1911, *P. A. Talbot 287* (holotype, BM!; isotype, K!).

**29. *Empogona welwitschii*** (K. Schum.) J. Tosh & Robbr., comb. nov. Basionym: *Tricalysia welwitschii* K. Schum., Bot. Jahrb. Syst. 23: 449. 1897. TYPE: Angola. Near Ponte do Felix Simões, Apr. 1855, *F. Welwitsch 3106* (holotype, LISU!; duplicates, BM not seen, COI!, K!, P!).

CONCLUSIONS AND FUTURE DIRECTIONS

We have been able to demonstrate that the two subgenera comprising the large Afro-Malagasy genus *Tricalysia* do not form a monophyletic group and should be treated as separate genera. *Empogona* has been previously recognized at generic rank, and

subsequent authors have considered reviving its generic status. On the basis of our molecular evidence, it is now fully justified to revive *Empogona* at the generic rank. The Asian genus *Diplospora* is sister to *Empogona*, with both genera forming a strongly supported monophyletic group. As a consequence, the weakly supported Asian clade reported by Davis et al. (2007) is not recovered in this investigation. Further data are still required to fully elucidate the phylogenetic relationships between *Belonophora*, *Diplospora*, *Discospermum*, *Empogona*, *Sericanthe*, and *Tricalysia*. There is increased support for the placement of a *Coffea* and *Psilanthus* clade as sister to the rest of Coffeaceae.

Future work requires the inclusion of nuclear ribosomal and low copy nuclear DNA sequence data, as well as expanded taxon sampling, in an effort to improve resolution between terminal taxa within the genera *Tricalysia* and *Empogona*. It seems prudent to defer detailed discussion on the biogeography of *Tricalysia* and *Empogona* until we have a broader sampling and a more resolved phylogenetic hypothesis of both genera.

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Appendix 1. Taxon voucher and accession data.

| Taxon  | Voucher                                    | <i>accD-psaI</i> | <i>petD</i> | <i>rpl16</i> | <i>trnL-F</i> |
|--|--|------------------|-------------|--------------|---------------|
| <i>Argocoffeopsis eketensis</i> (Wernham) Robbr.                                     | Davis 3031 (K), Cameroon                   | DQ180497         | AM999399    | DQ180531     | DQ180566      |
| <i>Argocoffeopsis rupestris</i> (Hiem) Robbr. subsp. <i>thomneri</i> (Lebrun) Robbr. | Harris 8168 (K), Central African Republic  | DQ180496         | NA          | DQ180532     | DQ180567      |
| <i>Argocoffeopsis scandens</i> (K. Schum.) Lebrun                                    | Davis 3016 (K), Cameroon                   | DQ180498         | AM999400    | DQ180533     | DQ180568      |
| <i>Belonophora coriacea</i> Hoyle  | Maurin 5 (K), Cameroon                     | DQ180499         | AM999401    | DQ180534     | DQ180569      |
| <i>Belonophora coriacea</i> Hoyle  | Maurin 19 (K), Cameroon                    | DQ180500         | AM999402    | DQ180535     | DQ180570      |
| <i>Belonophora</i> sp. indet.  | Tadjoueu 480 (K), Cameroon                 | DQ180501         | AM999403    | DQ180536     | DQ180571      |
| <i>Bertiera bicarpellata</i> (K. Schum.) N. Hallé                                    | Davis 3051 (K), Cameroon                   | DQ180502         | AM999396    | DQ180537     | DQ180572      |
| <i>Bertiera breiflora</i> Hiem   | Van Caekenberghe 41 (BR), Gabon*           | NA               | AM999397    | AM999524     | AM999466      |
| <i>Bertiera iturensis</i> K. Krause  | Van Caekenberghe 40 (BR), Gabon*           | FMI160622        | AM999398    | AM999525     | AM999467      |
| <i>Bertiera</i> sp. indet.   | Davis 3017 (K), Cameroon                   | DQ180504         | NA          | DQ180539     | DQ180574      |
| <i>Calycosiphonia macrochlamys</i> (K. Schum.) Robbr.                                | Davis 3044 (K), Cameroon                   | DQ180507         | NA          | DQ180542     | DQ180576      |
| <i>Calycosiphonia macrochlamys</i> (K. Schum.) Robbr.                                | Davis 3036 (K), Cameroon                   | DQ180506         | AM999404    | DQ180541     | DQ180575      |
| <i>Calycosiphonia spathicalyx</i> (K. Schum.) Robbr.                                 | Davis 2925 (K), Tanzania                   | DQ180509         | AM999405    | DQ180544     | DQ180578      |
| <i>Canephora</i> sp. indet.  | Davis 2727 (K), Madagascar                 | DQ180510         | NA          | AM999523     | DQ180579      |
| <i>Coffea homollet</i> J.-F. Leroy   | Davis 2305 (K), Madagascar                 | DQ153402         | NA          | DQ153651     | DQ153769      |
| <i>Coffea mangorenensis</i> Portères   | Rakotonasolo 41 (K), Madagascar            | DQ153503         | AM999406    | DQ153752     | DQ153870      |
| <i>Coffea moratii</i> J.-F. Leroy ex A. P. Davis & Rakotonas.                        | Davis 2326 (K), Madagascar                 | DQ153502         | AM999407    | DQ153751     | DQ153869      |
| <i>Didymosalphix norae</i> (Swynn.) Keay   | Van Caekenberghe 62 (BR), Zimbabwe*        | FMI160621        | AM999395    | AM999522     | AM999465      |
| <i>Diplospora dubia</i> (Lindl.) Masam.  | Van Caekenberghe 49 (BR) <sup>n</sup>      | AM999388         | AM999408    | AM999526     | AM999468      |
| <i>Diplospora</i> sp. indet.   | Bremer 15238 (K), Borneo (Brunei)          | DQ180511         | NA          | DQ180546     | DQ180580      |
| <i>Diplospora</i> sp. indet.   | Nangkai 15238 (K), Borneo (Brunei)         | AM999389         | AM999409    | AM999527     | AM999510      |
| <i>Disospernum abnorme</i> (Korth.) S. J. Ali & Robbr.                               | Sidiyasa 2148 (K), Borneo (Kalimantan)     | AM999380         | AM999410    | AM999528     | AM999469      |
| <i>Disospernum</i> sp. indet.  | Ismail 16846 (K), Borneo (Brunei)          | AM999390         | AM999411    | AM999529     | AM999470      |
| <i>Doricera trilocularis</i> (Balf. f.) Verdc.                                       | Friedmann 2939 (K), Mascarenes (Rodrigues) | DQ180513         | NA          | DQ180548     | DQ180582      |
| <i>Gardenia thunbergia</i> L. f.   | Davis et al. 1961-29703 (K), SE Africa     | DQ180514         | NA          | DQ180549     | DQ180583      |
| <i>Hyperacanthus microphyllus</i> (K. Schum.) Bridson                                | Goyder 5024 (K), Madagascar                | AM999387         | NA          | AM999520     | AM999464      |
| <i>Hyperacanthus perrieri</i> (Drake) Rakotonas. & A. P. Davis                       | Davis 2584 (K), Madagascar                 | FMI160619        | NA          | AM999519     | AM999462      |
| <i>Hyperacanthus</i> sp. indet.  | Davis 2586 (K), Madagascar                 | FMI160620        | NA          | AM999521     | AM999463      |
| <i>Ixora guillottii</i> Hochr.   | Tosh et al. 4088 (BR), Madagascar          | FMI160624        | AM999394    | AM999518     | AM999461      |
| <i>Psilanthus ebracteolatus</i> Hiem   | Billiet 53054 (BR), Ivory Coast*           | AM999392         | AM999412    | AM999530     | AM999471      |
| <i>Psilanthus manni</i> Hook. f.   | Van Caekenberghe 78 (BR), Ghana*           | FMI160623        | AM999413    | AM999531     | AM999472      |
| <i>Psilanthus sensei</i> Bridson   | Kisera 1473 (K), Tanzania                  | DQ153395         | AM999414    | DQ153644     | DQ153762      |
| <i>Polysphaeria</i> sp. indet.   | Mnungi 15 (K), Tanzania                    | DQ180517         | NA          | DQ180552     | DQ180586      |
| <i>Sericanthe andogenensis</i> (Hiem) Robbr.   | Bidgood 3490 (K), Tanzania                 | DQ180522         | AM999416    | DQ180557     | DQ180591      |
| <i>Sericanthe andogenensis</i> (Hiem) Robbr.   | Dessein 1097 (BR), Zambia                  | FMI77157         | AM999415    | AM999532     | AM999473      |

## Appendix 1. Continued.

| Taxon  | Voucher   | <i>accD-psaI</i> | <i>petD</i> | <i>rpl16</i> | <i>trnL-F</i> |
|--|---|------------------|-------------|--------------|---------------|
| <i>Sericanthe jacqelicis</i> (N. Hallé) Robbr.                       | <i>Carnalho 4169</i> (K), Gulf of Guinea Islands (Bioko)  | DQ180523         | NA          | NA           | DQ180592      |
| <i>Sericanthe</i> sp. indet.   | <i>Valkenberg 3160</i> (WAG), Gabon                       | AM999391         | AM999417    | AM999533     | AM999511      |
| <i>Tricalysia acutiliflora</i> Robbr.                                | <i>Manktelow 91215</i> (K), Tanzania                      | AM999345         | AM999419    | AM999535     | AM999475      |
| <i>Tricalysia acutiliflora</i> Robbr.                                | <i>Luke 7071</i> (K), Tanzania                            | AM999344         | AM999418    | AM999534     | AM999474      |
| <i>Tricalysia acidophylla</i> Robbr.                                 | <i>Kindekat 122</i> (BR), Tanzania                        | AM999346         | AM999420    | AM999536     | AM999512      |
| <i>Tricalysia aocantheroïdes</i> K. Schum.                           | <i>Dessein 1212</i> (BR), Zambia                          | AM999347         | AM999421    | AM999537     | AM999476      |
| <i>Tricalysia aocantheroïdes</i> K. Schum.                           | <i>Brummit 320</i> (K), Malawi                            | AM999348         | AM999422    | FMI60581     | AM999513      |
| <i>Tricalysia ambrensis</i> Randriamb. & De Block                    | <i>De Block 1313</i> (BR), Madagascar                     | AM999349         | AM999423    | FMI60582     | AM999477      |
| <i>Tricalysia analamazoatrensis</i> Homolle ex Randriamb. & De Block | <i>Tosh et al. 11</i> (BR), Madagascar                    | AM999350         | AM999424    | FMI60583     | AM999478      |
| <i>Tricalysia analamazoatrensis</i> Homolle ex Randriamb. & De Block | <i>De Block et al. 1874</i> (BR), Madagascar              | AM999351         | AM999425    | FMI60584     | AM999514      |
| <i>Tricalysia anomala</i> E. A. Bruce var. <i>guineensis</i> Robbr.  | <i>Danis 3045</i> (K), Cameroon                           | DQ180526         | AM999426    | DQ180560     | DQ180595      |
| <i>Tricalysia bagshawei</i> S. Moore                                 | <i>Malasse 2052</i> (K), Democratic Republic of the Congo | AM999352         | AM999427    | FMI60585     | AM999479      |
| <i>Tricalysia bequaertii</i> De Wild.                                | <i>Walters 942</i> (MO), Gabon                            | AM999353         | AM999428    | FMI60586     | AM999480      |
| <i>Tricalysia bridsomiana</i> Robbr.                                 | <i>De Block 389</i> (BR), Kenya                           | AM999354         | AM999429    | FMI60587     | AM999481      |
| <i>Tricalysia caudensis</i> Hiern                                    | <i>Dessein 1031</i> (BR), Zambia                          | AM999355         | AM999430    | FMI60588     | AM999482      |
| <i>Tricalysia concolor</i> N. Hallé                                  | <i>Degreef 95</i> (BR), Gabon                             | AM999356         | AM999431    | FMI60589     | AM999483      |
| <i>Tricalysia coriacea</i> (Benth.) Hiern                            | <i>Dessein 1283</i> (BR), Zambia                          | AM999358         | AM999433    | FMI60591     | AM999485      |
| <i>Tricalysia coriacea</i> (Benth.) Hiern                            | <i>Dessein 1359</i> (BR), Zambia                          | AM999357         | AM999432    | FMI60590     | AM999484      |
| <i>Tricalysia cryptocalyx</i> Baker                                  | <i>De Block 527</i> (BR), Madagascar                      | AM999359         | AM999434    | FMI60592     | AM999486      |
| <i>Tricalysia cryptocalyx</i> Baker                                  | <i>Tosh et al. 322</i> (BR), Madagascar                   | AM999360         | AM999435    | FMI60593     | AM999487      |
| <i>Tricalysia dauphinensis</i> Randriamb. & De Block                 | <i>De Block 694</i> (BR), Madagascar                      | AM999361         | AM999436    | FMI60594     | AM999488      |
| <i>Tricalysia dauphinensis</i> Randriamb. & De Block                 | <i>Tosh et al. 349</i> (BR), Madagascar                   | AM999362         | AM999436    | FMI60595     | AM999489      |
| <i>Tricalysia dauphinensis</i> Randriamb. & De Block                 | <i>Rabenhaitra 2115</i> (K), Madagascar                   | AM999363         | AM999438    | FMI60596     | AM999490      |
| <i>Tricalysia elliptica</i> (K. Schum.) Hutch. & Dalziel             | <i>Jonghind 1806</i> (K), Ghana                           | AM999364         | AM999439    | FMI60597     | AM999491      |
| <i>Tricalysia gosswileri</i> S. Moore                                | <i>Senterre 4041</i> , Equatorial Guinea                  | AM999365         | AM999440    | FMI60598     | AM999492      |
| <i>Tricalysia griseiflora</i> K. Schum.                              | <i>Dessein 1044</i> (BR), Zambia                          | AM999367         | AM999442    | FMI60600     | AM999494      |
| <i>Tricalysia griseiflora</i> K. Schum.                              | <i>Dessein 305</i> (BR), Zambia                           | AM999366         | AM999441    | FMI60599     | AM999493      |
| <i>Tricalysia jasmiflora</i> (Klotzsch) Benth. & Hook. f. ex Hiern   | <i>Ayami 42</i> (K), Malawi                               | AM999368         | AM999443    | FMI60601     | AM999495      |
| <i>Tricalysia junodii</i> (Schinz) Brenan                            | <i>Van Coelenberghe 79</i> (BR), Zimbabwe*                | AM999369         | AM999444    | FMI60602     | AM999496      |
| <i>Tricalysia lanceolata</i> (Sond.) Burtt Davy                      | <i>Bagliss 1519</i> (K), South Africa                     | AM999370         | AM999445    | FMI60603     | AM999497      |
| <i>Tricalysia leucocarpa</i> (Baill.) Randriamb. & De Block          | <i>Gautier 2442</i> (K), Madagascar                       | AM999371         | AM999446    | FMI60604     | AM999498      |
| <i>Tricalysia leucocarpa</i> (Baill.) Randriamb. & De Block          | <i>Tosh et al. 398</i> (BR), Madagascar                   | AM999372         | AM999447    | FMI60605     | AM999499      |
| <i>Tricalysia microphylla</i> Hiern                                  | <i>De Block 405</i> (BR), Kenya                           | AM999373         | AM999448    | FMI60606     | AM999500      |
| <i>Tricalysia ngalaensis</i> Robbr.                                  | <i>Bidgood 2966</i> (K), Tanzania                         | AM999374         | AM999449    | FMI60607     | AM999501      |
| <i>Tricalysia okelensis</i> Hiern                                    | <i>Schmidt 2139</i> (K), Ghana                            | AM999375         | AM999450    | FMI60608     | AM999505      |

Appendix 1. Continued.

| Taxon   | Voucher                               | <i>accD-psaI</i> | <i>petD</i> | <i>rpl116</i> | <i>trnL-F</i> |
|---|---------------------------------------|------------------|-------------|---------------|---------------|
| <i>Tricalysia ovalifolia</i> Hiern                          | De Block et al. 1072 (BR), Madagascar | AM999378         | AM999453    | FMI60611      | AM999504      |
| <i>Tricalysia ovalifolia</i> Hiern                          | De Block et al. 1090 (BR), Madagascar | AM999376         | AM999452    | FMI60609      | AM999503      |
| <i>Tricalysia ovalifolia</i> Hiern                          | Butly 309 (K), Tanzania               | AM999377         | AM999451    | FMI60610      | AM999502      |
| <i>Tricalysia pallens</i> Hiern                             | Dessein 1266 (BR), Zambia             | AM999381         | AM999455    | FMI60613      | AM999515      |
| <i>Tricalysia pallens</i> Hiern                             | Dessein 953 (BR), Zambia              | AM999382         | AM999456    | FMI60614      | AM999516      |
| <i>Tricalysia pallens</i> Hiern                             | Adams 831 (K), Liberia                | AM999379         | AM999454    | FMI60612      | AM999506      |
| <i>Tricalysia perrieri</i> Homolle ex Randriamb. & De Block | De Block 766 (BR), Madagascar         | AM999383         | AM999457    | FMI60615      | AM999507      |
| <i>Tricalysia ruandensis</i> Bremek.                        | Kuchar 22323 (BR), Tanzania           | AM999384         | AM999458    | FMI60616      | AM999517      |
| <i>Tricalysia schliebenii</i> Robbr.                        | Bidgood 1913 (K), Tanzania            | AM999385         | AM999459    | FMI60617      | AM999508      |
| <i>Tricalysia talbotii</i> (Wernham) Keay                   | Latilo 67674 (K), Nigeria             | AM999386         | AM999460    | FMI60618      | AM999509      |

\* Leaf material and vouchers collected from the living collections of National Botanic Garden of Belgium. Country of origin given in the table.

<sup>a</sup> Origin unknown. Living material given to National Botanic Garden of Belgium by Hong Kong Herbarium.