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PHYLOGENETIC ANALYSIS OF THE AFRICAN GENUS *GILBERTIODENDRON* J. LÉONARD AND RELATED GENERA (LEGUMINOSAE-CAESALPINIOIDEAE-DETARIEAE)

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Premise of research. *Gilbertiodendron* is a genus endemic to Africa with ~30 species made up of trees of primary dry-land, riverine, and gallery forests. Recently, the west and central African monotypic genus *Pellegriniodendron* was merged into *Gilbertiodendron*. *Gilbertiodendron* is one of 17 genera that form the exclusively African Berlinia clade, and this study presents the findings of a phylogenetic analysis designed to evaluate the generic limits of *Gilbertiodendron* and its relationships within the Berlinia clade.

Methodology. To test the monophyly of *Gilbertiodendron* and its relationships with other genera, we analyzed nucleotide sequence data from the nuclear ribosomal internal transcribed spacer and the plastid *trnL* intron and *trnL*-F intergenic spacer, using parsimony and Bayesian analyses.

Pivotal results. *Gilbertiodendron* is recovered as monophyletic, including all the samples previously recognized as *Pellegriniodendron diphyllosum*.

Conclusions. The placement of *Pellegriniodendron* in synonymy with *Gilbertiodendron* is supported by our results. Our analyses suggest that *G. diphyllosum* is the same taxon on both sides of the Dahomey Gap. The *G. ogoouense* complex is a monophyletic group of species that needs a new taxonomic framework and within which several new species will be described. The phylogenetic framework presented here and the ongoing taxonomic revision should provide the baseline data required for adequate assessment of this group of tree species, of which only eight have been assessed under the International Union for Conservation of Nature's Red List criteria.

Keywords: *Didelotia*, Fabaceae, ITS, *Librevillea*, *Plagiosiphon*, *Pellegriniodendron*, phylogenetic analyses, tropical Africa, *trnL-trnF*.

Introduction

Leguminosae is the third-largest flowering plant family, made up ~19,500 species in ~751 genera, occurring in a great variety of habitats from rain forests and mangrove swamps to deserts and temperate zones (Lewis et al. 2005; LPWG 2013a). The family is traditionally divided into 3 subfamilies—Papilionoideae, Mimosoideae, and Caesalpinioideae—but proposals for a new classification currently being discussed will increase that number to 6, 10–12, or even 15 (LPWG 2013b). The caesalpinioid legumes form the smallest of the three traditionally recognized subfamilies and includes ~2,250 species assigned to 171 genera and four tribes (Lewis et al. 2005; LPWG 2013a). In terms of species richness, Leguminosae is the most important angiosperm family in tropical Africa (Lebrun and Stork 1998). The dominance of Caesalpinioideae species in tropical Africa was recognized by Letouzey (1968), who named a specific forest type, the *forêt biafréenne*. Caesalpinioideae species can form large expanses of forests, some

dominated by a single tree species (e.g., *Gilbertiodendron dewevrei* monodominant forest; Corlett and Primack 2011). About half of all caesalpinioid genera (82 of 171) belong to the monophyletic tribe Detarieae (Bruneau et al. 2008), which is pantropical in distribution, but the majority of the genera are confined to Africa and Madagascar (Mackinder 2005). One consistently reported group within the Detarieae is the African Berlinia clade (Bruneau et al. 2008), which is made up of 17 genera of medium to large trees. This clade includes a weakly supported group made up of *Didelotia*, *Plagiosiphon*, *Librevillea*, and *Gilbertiodendron*, sister to a large clade that includes *Anthonotha*, *Englerodendron*, *Oddonioidendron* (recently reviewed by Breteler 2006, 2008, 2010, 2011), *Berlinia*, *Isobertlinia*, *Microberlinia* (Mackinder and Pennington 2011), and the “Babijt” group; the latter, as delimited by Wieringa and Gervais (2003), includes *Brachystegia*, *Aphanocalyx*, *Bikinia*, *Julbernardia*, *Icuria*, and *Tetraberlinia*. Many of the tree species in the African forests belong to the Berlinia clade, and this is the most important group of trees in the lowland evergreen rainforest (Wieringa 1999) and a dominant component of African tropical forests (White 1983).

Gilbertiodendron is an endemic African genus with ~30 species and a dominant component in many African forests. All

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species of *Gilbertiodendron* are trees of primary rain forest and gallery forest on well-drained or periodically inundated soil (Léonard 1957; Mackinder 2005; Estrella et al. 2012a). The origin of the genus dates from J. Léonard's work in the 1950s. Léonard transferred all African species previously described within *Macrobium* to four separate genera, three of which he described as new—*Gilbertiodendron* (Léonard 1952), *Paramacrobium* (Léonard 1954), and *Pellegriniodendron* (Léonard 1955); the fourth was made up of the reinstated genus *Anthonotha* P. Beauv. (Léonard 1955). *Pellegriniodendron* (one species) was transferred to *Gilbertiodendron* following recently published phylogenetic analyses (Bruneau et al. 2008) and a morphological reevaluation of its status (Estrella et al. 2012a). A complete taxonomic history of the genus can be found in Estrella and Devesa (2014). Species belonging to *Gilbertiodendron* have leaflets with marginal or submarginal glands, a pair of bracteoles that encloses the flower bud, five sepals, and five petals. The lateral and abaxial petal pairs are reduced, alternate to the sepals and similar to them. There is a single well-developed adaxial petal, with an unguiculate base and bilobed apex. The androecium is usually formed of six staminodes and three stamens fused at the base on a short, fleshy tube, but there are a few exceptions, such as *G. splendidum*, which has nine well-developed stamens. The ovary, located on a short stipe, develops into a pod with 1–4 longitudinal nerves, the valves twisting when mature to disperse the seeds explosively (Cowan and Polhill 1981; Estrella et al. 2012a), similar to the ovary described for *Tetraberlinia* (van der Burgt 1997).

Gilbertiodendron is a genus that is important both economically (Burkill 1995), with species used for timber or traditional medicine, and ecologically, with species growing gregariously, forming large forest stands (Poorter et al. 2004; Estrella et al. 2012c; van der Burgt et al. 2012; Estrella and Devesa 2014). The highest concentration in both number of species and morphological variation is found in the Guineo-Congolian region, particularly in Gabon, which is a high-diversity area for legumes (Sosef et al. 2006; Estrella et al. 2012b). At least 18 species of *Gilbertiodendron* have been reported in this area, with 10 species considered endemic or near endemic (i.e., species for which it was estimated that 85% of the distribution area falls within Gabon; Sosef et al. 2006). Several additional species new to science are to be described during the ongoing taxonomic revision of the genus (van der Burgt et al., forthcoming).

Although *Gilbertiodendron* plays a central role in many African forest areas, as noted by Léonard (1957), the advancement of taxonomic knowledge and species delimitation within the genus has been hampered by a lack of adequate material for study, especially fruiting specimens. Recently, new collections have become available, resulting in, for example, the recognition of many new species (Estrella et al. 2012c; van der Burgt et al. 2012, forthcoming; Estrella and Devesa 2014), but even now there is a lack of good material for many taxa, preventing adequate descriptions (Estrella and Devesa 2014). With the addition of recent collections, the objectives of the current study are to complement and pursue the ongoing effort of the past few years on the taxonomy of this genus (Estrella et al. 2012a, 2012c; van der Burgt et al. 2012; Estrella and Devesa 2014) with a densely sampled species-level phyloge-

netic analysis in order to identify species groups within *Gilbertiodendron* and to resolve relationships among genera considered closely related to *Gilbertiodendron* (Bruneau et al. 2008). More specifically, the aims of the present study are (1) to test the monophyly of *Gilbertiodendron* as currently circumscribed, in particular to determine whether the inclusion of *Pellegriniodendron* within *Gilbertiodendron* based on morphological characters is supported by the phylogenetic results, and (2) to assess the phylogenetic relationships of *Gilbertiodendron* with the other genera of the Berlinia clade. To address these issues, we sequenced and analyzed the plastid *trnL* intron and *trnL-F* spacer and the nuclear ribosomal internal transcribed spacers (ITS) in *Gilbertiodendron* and related genera.

Material and Methods

Taxon Sampling

A total of 85 accessions, representing 28 species of *Gilbertiodendron* (including 10 accessions of *G. diphylum*), 9 accessions representing 4 of the 5 recognized *Plagiosiphon* species, 7 accessions representing 4 of 11 *Didelotia* species, and 1 accession of *Librevillea klainei* (monotypic genus) were sampled. This is the widest sampling of *Gilbertiodendron*, *Plagiosiphon*, and *Didelotia* assembled to date for phylogenetic analysis (the appendix provides voucher information). To test the monophyly of *Gilbertiodendron* and examine relationships among Detarieae genera, particularly in the Berlinia clade, we sampled 10 other Berlinia clade genera and 5 representative genera of the Brownea clade (sensu Bruneau et al. 2008), including *Macrobium*, which in the past has been considered a close relative of *Gilbertiodendron* (see appendix for references). *Barnebydendron riedelii* (Tul.) J. H. Kirkbr. and *Schotia latifolia* Jacq. were included as outgroup taxa to root the trees (Bruneau et al. 2008). Samples collected in the field were preserved in silica gel, and other samples were obtained from dried herbarium specimens.

Molecular Methods

DNA extraction of herbarium and silica gel-dried material was done using a modified protocol from Ky et al. (2000) rescaled for a total 3 mL of nucleic extraction buffer (15 mM Tris, 2 mM EDTA, 80 mM KCl, 20 mM NaCl, 2% β -mercaptoethanol, PPVP 2%, 0.5% Trixon-X100), and the pellet was re-covered in 2 mL of lysis buffer pH 8 (0.1 M Tris, 0.02 M EDTA, 1.25 M NaCl, MATAB 4%).

The polymerase chain reaction (PCR) amplification mix in reaction volumes of 50 μ L contained four units of Taq DNA polymerase, 1 \times Taq DNA polymerase buffer with 1.5 mmol $MgCl_2$ (New England Biolabs, Pickering, Ontario, Canada), 200 μ mol/L of each dNTP (Fermentas, Burlington, Ontario, Canada), 3 μ mol/L of each primer, and 50–100 ng of genomic DNA. For samples that were difficult to amplify, BSA (0.1 μ g/ μ L; New England BioLabs, Ipswich, MA), Tween 20 (0.03%; J.-T. Baker, Phillipsburg, NJ), and pure DMSO (4%; Fisher Scientific, Ottawa, Ontario, Canada) were also added to the mix.

To maximize the yield of PCR products for the *trnL* and *trnL-F* regions, a nested PCR method was used, as described by Sinou et al. (2009), with the primer pairs “c”–“f,” and then

Table 1

Sequence Characteristics, Parsimony and Bayesian Analysis Statistics, and Choice of Evolutionary Model for Each of the Matrices and Subsets of the Matrices Analyzed in the Phylogenetic Analysis of the Genus *Gilbertiodendron* and Related Genera

| | ITS | <i>trnL</i> intron | <i>trnL-F</i> spacer | Combined |
|--------------------------------------|-----------------|--------------------|----------------------|-----------------|
| No. sequences | 127 | 110 | 92 | 127 |
| Aligned length (bp) | 1084 | 966 | 629 | 2679 |
| Indels | 286 | 68 | 72 | 426 |
| Excluded characters (%) | 71 (6.6) | 106 (11) | 0 | 177 (6.6) |
| Variable characters (%) | 825 (63.5) | 242 (26.7) | 217 (31) | 1284 (44.2) |
| Parsimony informative characters (%) | 450 (34.6) | 99 (10.9) | 76 (10.8) | 619 (21.3) |
| CI, RI, length | .50, .75, 2227 | .82, .90, 326 | .78, .85, 303 | .55, .75 2955 |
| % GC content | 44.5 | 20.9 | 27.2 | 31.9 |
| Evolutionary model, AIC | GTR+I+ Γ | GTR+I+ Γ | GTR+I+ Γ | GTR+I+ Γ |

Note. CI = confidence index, RI = retention index, AIC = Akaike Information Criterion.

the plastid *trnL* (UAA) intron and the spacer between *trnL* (UAA) and *trnF* (GAA) were, respectively, amplified and sequenced with the primer pairs “c”-“d” and “e”-“f,” as described in Taberlet et al. (1991). For the ITS region, amplifications were performed with the “AB101” and “AB102” primers (Sun et al. 1994; Douzery et al. 1999). Conditions for the amplification of the *trnL-F* region were as follows: 5 min of initial denaturation at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at 50°C, and 90 s at 72°C, with a final step of 7 min at 72°C. For the ITS region, PCR amplification conditions were 4 min of initial denaturation at 94°C, followed by 32 cycles of 30 s at 94°C, 45 s at 53°C, and 90 s at 72°C, with a final step of 7 min at 72°C.

Sequencing was performed with Big Dye Terminator 3.1 chemistry on an ABI 3730xl DNA Analyzer (Applied Biosystems, Carlsbad, CA) at the Genome Quebec facilities (Montreal, Canada). Sequences were assembled and edited with Geneious 4.8.5 (Biomatters, <http://www.geneious.com>).

Phylogenetic Analyses

Sequence alignment was performed with Geneious 4.8.5 (Biomatters, <http://www.geneious.com>) using the default parameters. Alignments were then verified and modified manually where inconsistencies were found. Nonautapomorphic simple gaps (indels) were scored as separate presence/absence characters, following Simmons and Ochoterena (2000), as implemented in SeqState 1.4.1 (Müller 2005).

Individual matrices (plastid and nuclear markers) were analyzed separately for exploratory purposes, and a concatenated data matrix (cp+nuc) was analyzed to generate the phylogenetic tree. The *trnL* intron matrix included sequences from 110 accessions (19 genera), the *trnL-F* intergenic spacer matrix included data from 92 accessions (6 genera), and the ITS matrix included 127 accessions (21 genera).

Parsimony analyses were performed with PAUP*, version 4.0b10 (Swofford 2003). A first heuristic search was performed with 1000 replicates of random addition sequence, tree bisection-reconnection (TBR) branch-swapping, retaining only five most parsimonious trees at each replicate. Starting with the trees kept in memory from this initial analysis, a second heuristic search was performed with TBR and a limit of 100,000 trees saved. Because this second analysis uses the topologies obtained initially, it permits the investigation of more

optimal topologies than a “one-step” analysis (e.g., Davis et al. 2004). Branch support was estimated from 5000 bootstrap replicates under a heuristic strategy with one random addition-sequence replicate, TBR branch-swapping, and a maximum number of trees set at 100.

The Bayesian analyses were implemented in a parallel version of Mr. Bayes (ver. 3.2.1; Ronquist and Huelsenbeck 2003). Computations were made on the supercomputer Cottos from the Université de Montréal, managed by Calcul Québec and Compute Canada. The GTR+I+ Γ nucleotide substitution model was selected using the Akaike Information Criterion (Akaike 1974) as implemented in ModelTest (ver. 3.7; Posada and Crandall 1998) for all DNA nucleotide partitions. The model for the gap partition was set to “restriction data” (F81-like model), as suggested by Ronquist and Huelsenbeck (2003). The Bayesian estimation consisted of two independent runs, each for 50×10^6 generations, sampling trees and parameters every thousandth generation. Each run consisted of four simultaneous Monte Carlo Markov chains and four swaps per generation. All sample points prior to reaching stationarity of the chains were discarded (equivalent to discarding the first 5×10^6 generations as burn-in). Convergence was assessed by comparing majority-rule consensus trees from the two analyses and using Tracer (ver. 1.5; Rambaut and Drummond 2007) to compare density plots of the estimated parameters and of the likelihoods from the two analyses. The posterior probabilities for individual clades were compared for congruence and summarized on a majority-rule consensus.

Results

Sequence Characteristics

Length, number of indels, number of variable characters, and statistics for the phylogenetic analyses are given in table 1 for each of the regions studied (individual and combined matrices). The *trnL* intron was 470–700 bp in length, the *trnL-trnF* spacer was ~450–600 bp, and the ITS region was generally 780–930 bp in length. A total of 619 (21.3%) parsimony informative characters were obtained from the combined analysis (table 1), with a considerably higher proportion from the ITS region (34.6%) than from the *trnL* intron (10.9%) or the *trnL-trnF* spacer (10.8%).

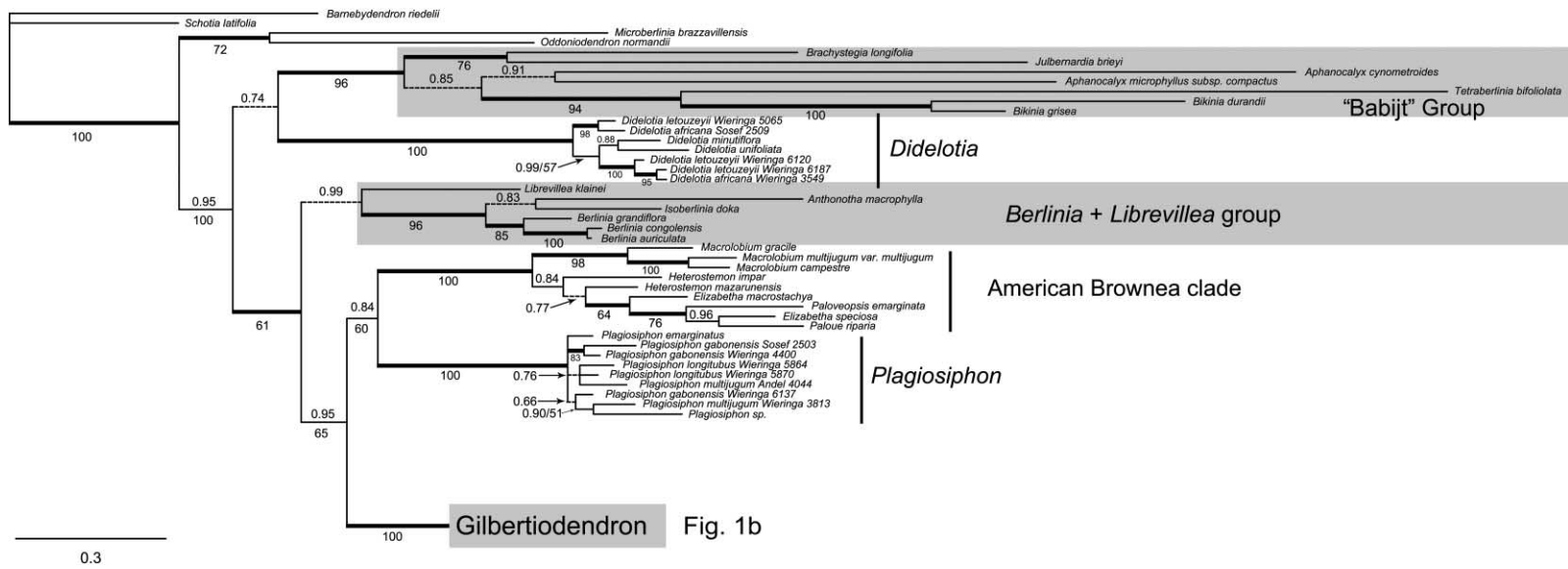
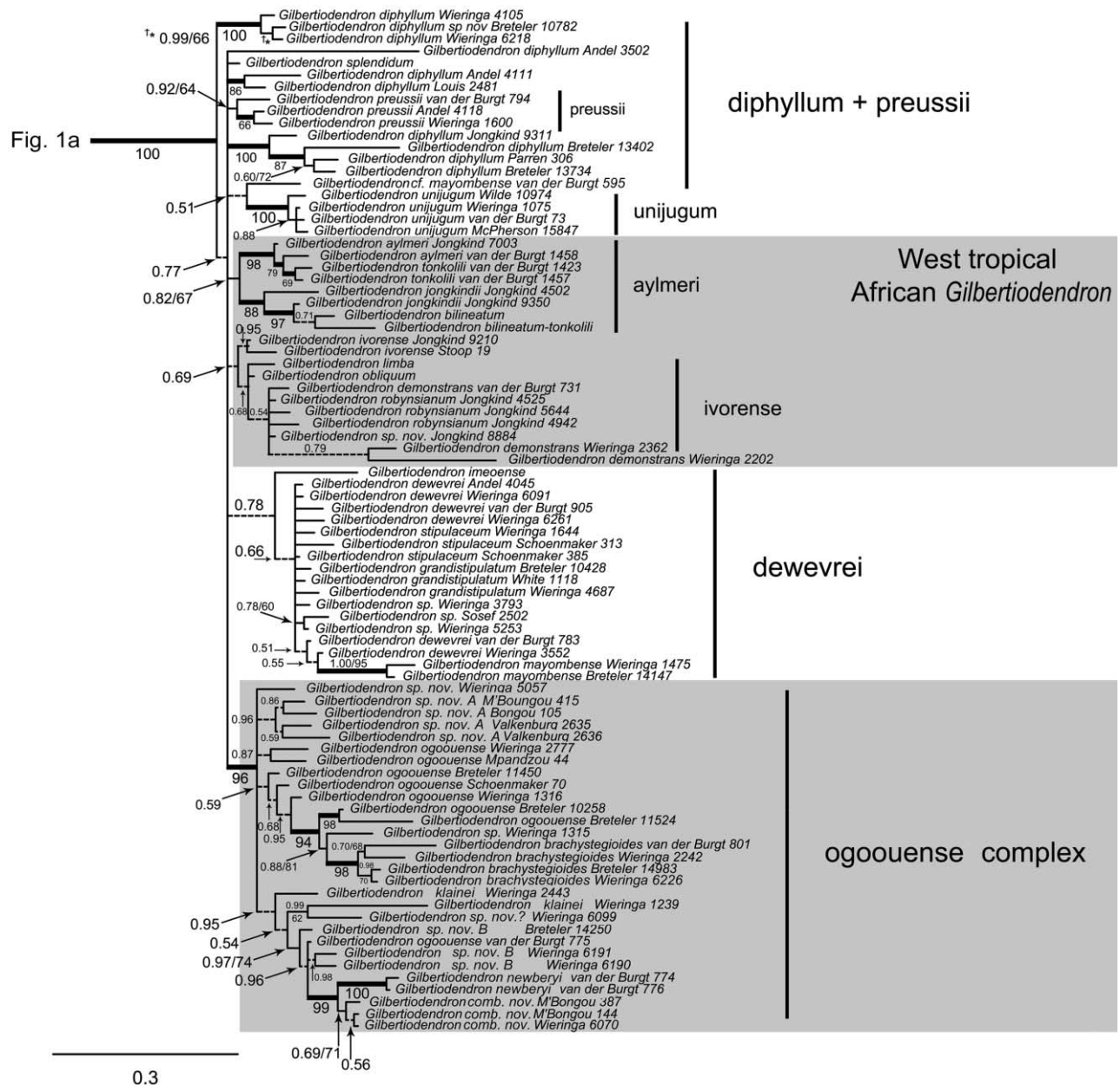


Fig. 1 Phylogenetic analysis of the chloroplast *trnL* intron and *trnL-trnF* intergenic spacer and the nuclear internal transcribed spacers for *Gilbertiodendron* and related caesalpinoid genera. Bayesian majority-rule consensus derived from 45,000 trees kept after reaching stationarity in two independent analyses. Posterior probabilities (mostly above branches) and bootstrap support values from the parsimony analysis (mostly below branches) are noted. Branches in bold are those supported by a 1.0 posterior probability; clades that are unresolved in the parsimony analysis are indicated by broken lines. For species represented by two specimens, the collector name and the collection number are indicated after the species name.



(Fig. 1, continued)

Phylogenetic Analysis

In both the parsimony and Bayesian analyses, the consensus trees resulting from the analysis of the ITS data alone (not shown) were better resolved than the consensus trees from the *trnL* and *trnL-trnF* analyses alone (not shown), but the three analyses yielded topologies with equivalent relationships among groups. The parsimony analysis of the combined data set reached the maximum number of trees retained in memory (length = 2955, confidence index = 0.55, retention index = 0.75) and yielded a poorly resolved strict consensus tree (see

fig. 1). The Bayesian majority-rule consensus tree of the combined matrix yielded a topology among groups similar to that obtained from the parsimony analysis of the combined data but with better resolution (fig. 1, with posterior probabilities indicated above the branches).

Our analysis places the genera *Microberlinia* and *Oddoniodendron* (fig. 1a, bootstrap support [BS] = 72%, posterior probability [PP] = 1.00) as a sister clade to the other *Berlinia* clade genera. The monophyletic Babijt group defined by Wieringa and Gervais (2003) is weakly supported as sister to the

Didelotia species included in our analyses (BS < 50%, PP = 0.74). A clade including all accessions of *Berlinia*, *Isoberlinia*, and *Anthonotha* is placed as sister to *Librevillea klainei* (BS < 50%, PP = 0.99), and this entire clade is moderately supported (BS = 61%, PP = 1) as sister to a group that includes the American Brownea clade sister to the *Plagiosiphon* species sampled (BS = 60%, PP = 0.84) and a clade that constitutes all the *Gilbertiodendron* species.

In our analysis, *Gilbertiodendron* is strongly supported as monophyletic (fig. 1b; BS = 100%, PP = 1.00) including all accessions of *G. diphyllum* previously recognized as *Pellegriniodendron diphyllum*. Within *Gilbertiodendron* (fig. 1b), several groups are resolved, but many with low support. The west tropical African *Gilbertiodendron* species are resolved into two clades, the “aylmeri” clade (BS = 67%, PP = 0.82) and the “ivorense” clade (<50%, 0.69), both made up of species that grow in the tropical forest from Guinea to east Ghana (with the exception of *G. demonstrans*, which occurs in central Africa). The “dewevrei” clade (BS < 50%, PP = 0.78), with species from central Africa, includes *G. dewevrei*, *G. imen-oense*, *G. stipulaceum*, *G. grandistipulatum*, and *G. mayombense*, as well as unidentified *Gilbertiodendron* samples. The strongly supported “ogooouense complex” (BS = 96%, PP = 1.00) includes a group of species identified as *G. klainei*, *G. ogoouense*, and *G. brachystegioides*, along with several new species to be described. All three samples of *G. preussii* group in a moderately supported clade, but those of its close relative, *G. diphyllum*, are not monophyletic (fig. 1b) in the combined plastid and ITS analyses. However, in the ITS analyses alone (results not shown), *G. diphyllum* samples form a single clade.

Discussion

Generic Relationships of *Gilbertiodendron*

In our analyses, *Gilbertiodendron* is resolved as a monophyletic genus moderately supported as sister to a clade constituted of *Plagiosiphon* and the American Brownea clade. Previous phylogenetic studies by Bruneau et al. (2008) placed *Gilbertiodendron* in a poorly supported clade with three genera—*Didelotia*, *Librevillea*, and *Plagiosiphon*—but with no resolution among the four genera. Although we include these 3 genera, as well as 10 other *Berlinia* clade genera, our analyses do not support a monophyletic *Berlinia* clade because of the nested position of the Brownea clade. However, as in all other phylogenetic analyses of Detarieae, generic-level relationships in the Amherstieae clade are poorly resolved (LPWG 2013a). Nevertheless, *Plagiosiphon*, a genus of five species of trees and shrubs that grow gregariously in lowland forests and along rivers (Mackinder 2005), is here supported as monophyletic with four of the five species sampled (fig. 1a). *Didelotia* is also supported as monophyletic (fig. 1a), but it occurs, albeit with little support, as sister to the Babijt clade (fig. 1a) of Wieringa and Gervais (2003). The monospecific genus *Librevillea* is here resolved as sister to representatives of three other genera of the *Berlinia* group (fig. 1a), a relationship that is supported only in the Bayesian analysis. Thus, despite better species-level sampling with more samples per species, relationships of *Gilbertiodendron* within the Amherstieae clade remain unclear.

Gilbertiodendron was included within tribe Amherstieae by

Léonard (1957) based on the position of the bracteoles at the upper part of the pedicel. Cowan and Polhill (1981) maintained *Gilbertiodendron* within that tribe, but they questioned whether Amherstieae was monophyletic. Breteler (1995) proposed a modified classification that recognized *Gilbertiodendron* (including *Pellegriniodendron* and 21 more genera) within the newly delimited tribe Macrolobieae. Phylogenetic analysis of *trnL* data confirmed the position of *Gilbertiodendron* in a Macrolobieae clade (Bruneau et al. 2001), which largely but not completely corresponded to Breteler’s (1995) delimitation of tribe Macrolobieae. Subsequently, this lineage made up of the majority of the genera (but not *Macrolobium*) was renamed the *Berlinia* clade (Bruneau et al. 2008). Mackinder (2005), in a synopsis of all previous morphological and molecular studies, considered both Detarieae and Amherstieae (including the *Berlinia* clade) within the tribe Detarieae sensu lato.

Species Relationships within *Gilbertiodendron*

Our analyses support *Gilbertiodendron* as monophyletic, including all accessions of *G. diphyllum* previously recognized as *Pellegriniodendron*. Several of the characters used by Léonard (1957) and Cowan and Raven (1981) to distinguish between *Pellegriniodendron* and *Gilbertiodendron* were found to be of weak or no value (Estrella et al. 2012a). Léonard (1957) and Cowan and Polhill (1981) characterized *Pellegriniodendron diphyllum* (*G. diphyllum*) by the presence of stipels on leaflets, but Estrella et al. (2012a) found several mature specimens of *G. unijugum* with “stipels” present at the base of the petiolules and in seedlings of other species. *Gilbertiodendron diphyllum* is an easily identifiable species with a single normal pair of leaflets and a basal pair that is reduced to small structures, sometimes referred to as stipels. The presence of submarginal crateriform glands on the leaflets blades of *G. diphyllum* is the only character that can be used to consider *Pellegriniodendron* as a segregate genus. Extrafloral nectaries are common in other genera of the Detarieae, and in some groups, this feature is an informative taxonomic character for species-level delimitations (e.g., in *Daniellia*; Estrella et al. 2010). In the Detarieae, these crateriform glands are usually present on the leaflet blades, as reported in the Babijt clade (fig. 1b) in *Aphanocalyx* (Wieringa 1999), but in *Gilbertiodendron*, crateriform glands are found only in *G. diphyllum*, which is sister to the *Gilbertiodendron* clade (fig. 1b). The remaining *Gilbertiodendron* species have a different and possibly autapomorphic type of gland, located on the leaflet margins and not found in any other Detarieae genera. These marginal glands have been used as a key character for the easy identification of the genus (e.g., Aubréville 1968). Although the function and nature of the leaflet margin glands is not clear (E. Smets, personal communication), their presence may be related to ants, which have been reported as living in association with *Gilbertiodendron* species (e.g., fig. 1 from Estrella and Devesa 2014).

The species of *Gilbertiodendron* endemic to west tropical Africa (Upper Guinea: Senegal to Togo) form two clades, but the relationship between these two clades is not resolved (fig. 1b). Clade aylmeri includes the recently published species *G. tonkolili*, which cannot be differentiated vegetatively from *G. bilineatum* but is considered distinct from this species based on

floral and fruiting characters (Estrella et al. 2012c). Our analysis resolves the two confirmed accessions of *G. tonkolili* as most closely related to *G. aylmeri*, with strong support. In the sister clade, *G. bilineatum* occurs with a recently described species, *G. jongkindii* (Estrella and Devesa 2014), which is morphologically similar to *G. obliquum*, a species that is resolved as part of the ivorensis clade, the second clade of west tropical African species (fig. 1b). *Gilbertiodendron jongkindii* has a symmetric leaflet base and sepals that are densely hairy at the margins, two characters that differentiate it from *G. obliquum*. In addition to *G. obliquum*, the ivorensis clade includes four other west tropical African species—*G. ivorensis*, *G. limba*, *G. robynsianum*, and *G. sp. nov. Jongkind 8884*—and the central African *G. demonstrans*, but with a broad distribution that extends to Nigeria. These phylogenetic results support the taxonomic framework proposed for the western African species proposed by Estrella and Devesa (2014), who recognize *G. tonkolili* and *G. jongkindii* as different species (fig. 1b).

All four accessions of *G. unijugum* form a strongly supported monophyletic group, weakly resolved as sister to one of the three specimens of *G. mayombense* sampled (fig. 1b). This *G. mayombense* specimen from the Korup National Park (Cameroon) may represent a segregate species distinct from the central African *G. mayombense* (Angola, Gabon, and D. R. Congo), which group with the dewevrei clade. A detailed comparative study of these samples will be done during the ongoing taxonomic revision.

Of the ~30 species within *Gilbertiodendron*, only *G. diphyllum* and *G. preussii* have a distribution that reaches both margins of the Dahomey Gap (fig. 2). The Dahomey Gap is a woodland and wooded grassland region from east Ghana to

Benin (Booth 1958) that results from a climatic anomaly associated with low precipitation (Salzmann and Hoelzmann 2005). Although the Dahomey Gap area is presently covered with agricultural land, savannah, and deteriorated dry forest, it has been suggested to be a barrier to rain forest species dispersal (Hawthorne and Jongkind 2006), and within it, remnant forest patches are considered biodiversity refugia that should be conserved (Backéus 1992; Chair et al. 2011). This distribution on both sides of the Dahomey Gap is uncommon for a Detarieae species (found in only 33 out of the 295 indigenous Detarieae species from west, west-central, and central Africa; Lock 1989), probably as a consequence of the relatively short and limited maximum dispersal distance of the ballistic seeds (van der Burgt et al. 2012). Despite this unusual distribution, no significant morphological differences were observed between specimens from west tropical Africa (Senegal to Ghana) and those from west-central Africa (Cameroon, Equatorial Guinea, and Gabon; Estrella et al. 2012a; Estrella and Devesa 2014). Both *G. diphyllum* and *G. preussii* have been reported from secondary forests, swampy areas, and near mangroves, possibly explaining the wide distribution of these two species beyond the Dahomey Gap, since small pockets of forests along swamps and rivers have been far more widespread during periods of forest contractions than the paper refuges of dry-land rain forest. However, all four specimens of *G. diphyllum* from west tropical Africa group into a single clade, distinct from the specimens from west-central Africa (fig. 1b), indicating the likelihood of a genetic isolation between the two areas of the distribution of this species.

The dewevrei clade (fig. 1b) is a polytomy that includes accessions representing at least five species from west-central

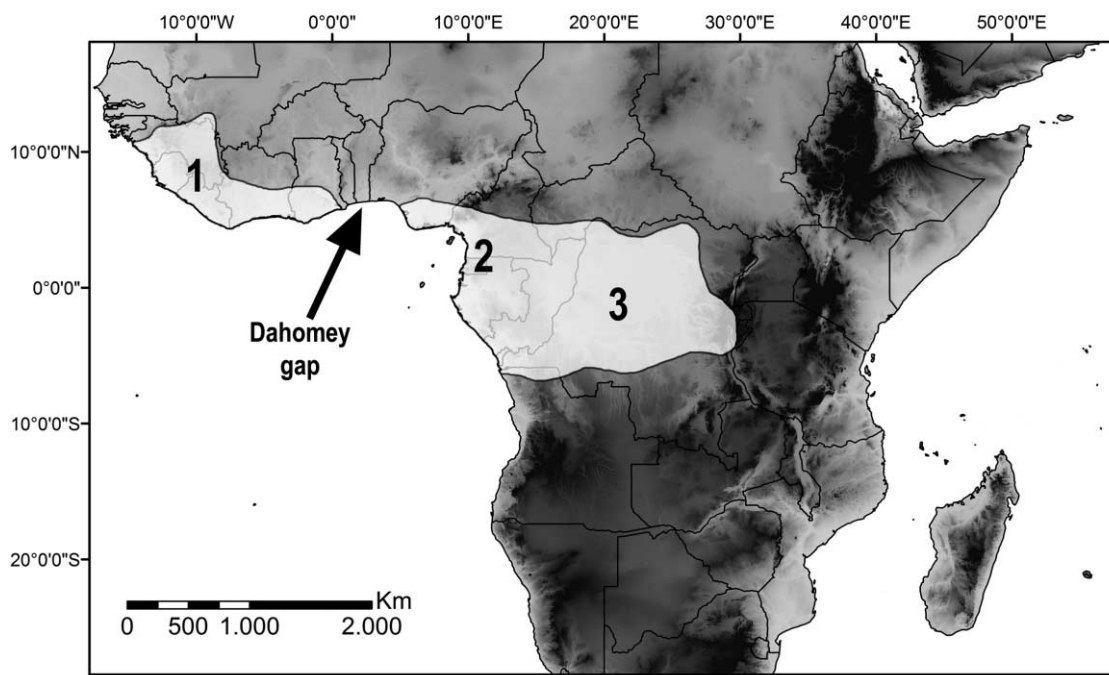


Fig. 2 Distribution of the genus *Gilbertiodendron* in Africa: 1 = west tropical Africa, 2 = west-central Africa, 3 = central Africa. The arrow indicates the location of the Dahomey Gap biogeographic area.

and central Africa, with a broad morphological variability. The dewevrei clade includes *G. grandistipulatum*, *G. mayombense*, *G. imenoense*, *G. stipulaceum*, and *G. dewevrei*. *Gilbertiodendron dewevrei* forms extensive patches of monodominant forest (Aubréville 1970; van Valkenburg et al. 1998; Peh et al. 2011) of great ecological and conservation importance (Peh et al. 2011). In studies on monodominant forests of *G. dewevrei* in central Africa, Peh et al. (2011) showed that dominance is not related to soil type but rather to seed-dispersal capacity or to the ectomycorrhizal status of the species. Other species of *Gilbertiodendron* form codominant patches, where they grow mixed with other species of Caesalpinoideae, as well as with trees from other families. Van der Burgt et al. (2012) hypothesized that the codominant patches are the consequence of the relatively short and strictly limited maximum dispersal distance of the ballistic seeds typical of most Detarieae, including all *Gilbertiodendron* species.

In the ogoouense complex clade, the unclear position of samples previously identified either as *G. ogoouense*, *G. brachystegioides*, *G. klainei*, or *G. newberyi* is in accordance with the result of the morphological study of the group suggesting the existence of several new taxa. In ongoing taxonomic revisions (van der Burgt et al., forthcoming), taxa of the ogoouense complex will be divided into several new species (fig. 1b). A common characteristic of taxa in the complex is the presence of small flowers and congested inflorescences. The specimens within this group share the absence of a gland at the apex of the bracteoles, in contrast to other *Gilbertiodendron* species where the apical glands within bracteoles can be more than 3 mm in length (Estrella and Devesa 2014).

Ongoing agriculture and clear-cutting of forest leading to severe habitat loss and degradation have been identified as well-known threats to *Gilbertiodendron* populations, and these continue to contribute to their decline. In order to be considered for a conservation rank, a species should ideally be described to reflect evolutionary entities, and the species-level phylogeny presented here provides some of the background information needed to establish the adequate assessments. Of the ~30 species within the genus, only 8 have been assessed so far under the International Union for Conservation of Nature (IUCN) Red List criteria. From west tropical Africa (fig. 2), five species are assessed: *G. bilineatum*, *G. robynianum*, and *G. splendidum* are classified as vulnerable (VU); *G. limba* is considered near threatened; and the recently pub-

lished species *G. tonkolili* is assessed as critically endangered (Estrella et al. 2012c; IUCN 2013). This would suggest that the aylmeri and ivorense clades include the most endangered groups within the genus, but this may reflect the bias in the sampling assessment with a greater focus on the west tropical African flora. For west-central Africa and central Africa (fig. 2), three species were assessed: *G. klainei* (VU), *G. newberyi* (endangered), and *G. pachyanthum* (VU; van der Burgt et al. 2012; IUCN 2013). None of the other species of *Gilbertiodendron* have been assessed, and of the eight existing assessments, five were made in 1998 and need reevaluation in light of new data. The new data derived from this phylogenetic study and the ongoing taxonomic treatment of the genus will help establish the adequate assessments for the genus, which includes many timber species currently overexploited in tropical Africa.

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Appendix

Specimens Studied, Voucher Information, and Genbank Accession Numbers for the Phylogenetic Analysis of the Genus *Gilbertiodendron* and Related Genera

All vouchers are deposited at the indicated herbaria. Sequences are compiled from previous works, indicated by superscript lowercase letters: (a) Bruneau et al. (2001), (b) Gervais and Bruneau (2002), (c) Fougère-Danezan et al. (2007), (d) Redden et al. (2010), and (e) Mackinder and Pennington (2011). Data are ordered by ITS, *trnL* intron, and *trnL-F* spacer. Missing data are denoted with a dash.

Outgroup

Barnebydendron riedelii (Tul.) J.H. Kirkbr., *Brammall* s. n., no. 1953-35501(K), AY955777^c, AF365209^a, AY958491^c; *Schotia latifolia* Jacq., Bruneau s.n., no 1948-52201 (K), AY955775^c, AF365124^a, AY958528^c. **Related genera:** *Anthonotha macrophylla* P. Beauv., *Wieringa* 2996 (WAG), AF513653^b, AF365234^a, —; *Aphanocalyx cynometroides* Oliver, *Wieringa* 2355 (WAG),

AF513654^b, AF365244^a, —; *Aphanocalyx microphyllus* subsp. *compactus* (Hutchinson ex Lane-Poole) Wieringa, *Breteler* 13356 (WAG), AF513662^b, AF365246^a, —; *Berlinia auriculata* Benth., *Wieringa* 5283 (WAG), HM041834^a, —, —; *Berlinia congolensis* (Baker f.) Keay, *Harris* 8585 (E), HM041826^c, —, —; *Berlinia grandiflora* (Vahl) Hutch. & Dalziel, *Harris* 2895 (E), HM041821^c, —, —; *Bikinia durandii* (F. Hallé & Normand) Wieringa, *Wieringa* 3021 (WAG), AF513676^b, AY116896^b, —; *Bikinia grisea* Wieringa, *Breteler* 13334 (WAG), AF513678^b, AY116897^b, —; *Brachystegia longifolia* Benth., *Herendeen* 21-xii-97-2 (US), AF513687^b, —, —; *Elizabetha macrostachya* Benth., *Redden* 3714 (US), FJ817516^d, FJ817559^d, —; *Elizabetha speciosa* Ducke, *Rodrigues & Coelho* 4850 (US), FJ817521^d, —, —; *Heterostemon impar* Spruce ex. Benth., *Amaral et al.* 229 (K), FJ817524^d, FJ817562^d, —; *Heterostemon mazarunensis* Sandwith, *Redden* 3203 (US), FJ817526^d, —, —; *Isoberlinia doka* Craib & Stapf, *Jongkind* 2552a (WAG), AF513691^b, AF365220^a, —; *Julbernardia briei* (De Wild.) Troupin, *Wieringa* 3348 (WAG), AF513692^b, AF365264^a, —; *Macrolobium campestre* Huber, *Redden* 3649 (US), FJ817499^d, FJ817551^d, —; *Macrolobium gracile* Spruce ex. Benth., *Redden* 3687 (US), FJ817500^d, FJ817552^d, —; *Macrolobium multijugum* var. *multijugum* (DC.) Benth., *Redden* 3700 (US), FJ817502^d, FJ817554^d, —; *Microberlinia brazzavillensis* A. Chev., *Wieringa* 2516 (WAG), AF513697^b, AF365222^a, —; *Oddoniodendron normandii* Aubrev., *Breteler* 12608 (WAG), AF513698^b, AF365224^a, —; *Paloue riparia* Pulle, *Redden* 1161A (US), FJ817546^d, EU361826^d, —; *Paloveopsis emarginata* R. S. Cowan, *Cid Ferreira et al.* 916 (NY), FJ817533^d, FJ817571^d, —; *Tetraberlinia bifoliolata* (Harms) Hauman, *Wieringa* 3304 (WAG), AF513700^b, AF365227^a, —. **Study group:** *Didelotia africana* Baill., *Sosef* 2509 (WAG), KJ777183, KJ777375, KJ777285; *Didelotia africana* Baill., *Wieringa* 3549 (WAG), KJ777184, KJ777376, KJ777286; *Didelotia letouzeyii* Pellegr., *Wieringa* 5065 (WAG), KJ777185, KJ777377, KJ777287; *Didelotia letouzeyii* Pellegr., *Wieringa* 6120 (WAG), KJ777186, KJ777378, KJ777288; *Didelotia letouzeyii* Pellegr., *Wieringa* 6187 (WAG), KJ777187, KJ777379, KJ777289; *Didelotia minutiflora* (A. Chev.) J. Léonard, *Wieringa* 4819 (WAG), KJ777188, KJ777380, KJ777290; *Didelotia unifoliolata* J. Léonard, *Sosef* 2517 (WAG), KJ777189, KJ777381, KJ777291; *Gilbertiodendron aylmeri* (Hutch. & Dalziel) J. Léonard, *Jongkind* 7003 (WAG), KJ777191, KJ777383, KJ777293; *Gilbertiodendron aylmeri* (Hutch. & Dalziel) J. Léonard, *van der Burgt* 1458 (WAG), KJ777190, KJ777382, KJ777292; *Gilbertiodendron bilineatum* (Hutch. & Dalziel) J. Léonard, *Jongkind* 5437 (WAG), KJ777193, —, —; *Gilbertiodendron bilineatum-tonkolili*, *Jongkind* 8775 (WAG), KJ777192, KJ777384, KJ777294; *Gilbertiodendron brachystegioides* (Harms) J. Léonard, *Breteler* 14983 (WAG), KJ777194, KJ777385, —; *Gilbertiodendron brachystegioides* (Harms) J. Léonard, *van der Burgt* 801 (WAG), KJ777195, KJ777386, KJ777295; *Gilbertiodendron brachystegioides* (Harms) J. Léonard, *Wieringa* 2242 (WAG), KJ777196, KJ777387, KJ777296; *Gilbertiodendron brachystegioides* (Harms) J. Léonard, *Wieringa* 6226 (WAG), KJ777197, KJ777388, KJ777297; *Gilbertiodendron* comb. nov., *M'Boungou* 144 (WAG), KJ777199, KJ777390, KJ777299; *Gilbertiodendron* comb. nov., *M'Boungou* 387 (WAG), KJ777200, KJ777391, —; *Gilbertiodendron* comb. nov., *Wieringa* 6070 (WAG), KJ777201, —, —; *Gilbertiodendron demonstrans* (Baill.) J. Léonard, *van der Burgt* 731 (WAG), KJ777202, KJ777392, KJ777300; *Gilbertiodendron demonstrans* (Baill.) J. Léonard, *Wieringa* 2202 (WAG), KJ777203, —, KJ777301; *Gilbertiodendron demonstrans* (Baill.) J. Léonard, *Wieringa* 2362 (WAG), KJ777204, —, —; *Gilbertiodendron dewevrei* (De Wild.) J. Léonard, *Andel* 4045 (WAG), KJ777205, KJ777393, KJ777302; *Gilbertiodendron dewevrei* (De Wild.) J. Léonard, *van der Burgt* 783 (WAG), KJ777206, KJ777394, KJ777303; *Gilbertiodendron dewevrei* (De Wild.) J. Léonard, *van der Burgt* 905 (WAG), KJ777207, KJ777395, KJ777304; *Gilbertiodendron dewevrei* (De Wild.) J. Léonard, *Wieringa* 3552 (WAG), KJ777208, KJ777396, KJ777305; *Gilbertiodendron dewevrei* (De Wild.) J. Léonard, *Wieringa* 6091 (WAG), KJ777209, KJ777397, KJ777306; *Gilbertiodendron dewevrei* (De Wild.) J. Léonard, *Wieringa* 6261 (WAG), KJ777210, KJ777398, KJ777307; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Andel* 3502 (WAG), KJ777211, KJ777399, KJ777308; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Andel* 4111 (WAG), KJ777212, KJ777400, KJ777309; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Breteler* 13402 (WAG), KJ777213, KJ777401, KJ777310; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Breteler* 13734 (WAG), KJ777214, KJ777402, KJ777311; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Jongkind* 9311 (WAG), KJ777215, KJ777403, KJ777312; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Louis* 2481 (WAG), KJ777216, KJ777404, KJ777313; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Parren* 306 (WAG), KJ777217, —, KJ777314; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Wieringa* 4105 (WAG), KJ777219, KJ777406, KJ777316; *Gilbertiodendron diphyllum* (Harms) Estrella & Devesa, *Wieringa* 6218 (WAG), KJ777220, KJ777407, KJ777317; *Gilbertiodendron diphyllum* sp. nov., *Breteler* 10782 (WAG), KJ777218, KJ777405, KJ777315; *Gilbertiodendron grandistipulatum* (De Wild.) J. Léonard, *Breteler* 10428 (WAG), KJ777221, KJ777408, KJ777318; *Gilbertiodendron grandistipulatum* (De Wild.) J. Léonard, *White* 1118 (WAG), KJ777222, KJ777409, KJ777319; *Gilbertiodendron grandistipulatum* (De Wild.) J. Léonard, *Wieringa* 4687 (WAG), KJ777223, KJ777410, KJ777320; *Gilbertiodendron imenoense* (Pellegr.) J. Léonard, *Breteler* 9974 (WAG), KJ777224, KJ777411, KJ777321; *Gilbertiodendron ivorense* (A. Chev.) J. Léonard, *Jongkind* 9210 (WAG), KJ777225, KJ777412, KJ777322; *Gilbertiodendron ivorense* (A. Chev.) J. Léonard, *Stoop* 19 (WAG), KJ777226, KJ777413, KJ777323; *Gilbertiodendron jongkindii* Estrella & Devesa, *Jongkind* 4502 (WAG), KJ777227, KJ777414, KJ777324; *Gilbertiodendron jongkindii* Estrella & Devesa, *Jongkind* 9350 (WAG), KJ777228, KJ777415, KJ777325; *Gilbertiodendron klainei* (Pierre ex Pellegr.) J. Léonard, *Wieringa* 1239 (WAG), KJ777229, KJ777416, KJ777326; *Gilbertiodendron klainei* (Pierre ex Pellegr.) J. Léonard, *Wieringa* 2443 (WAG), KJ777230, KJ777417, KJ777327; *Gilbertiodendron limba* (Scott-Elliot) J. Léonard, *Jongkind* 1438 (WAG), KJ777231, KJ777418, KJ777328; *Gilbertiodendron mayombense* (Pellegr.) J. Léonard, *Breteler* 14147 (WAG), KJ777232, KJ777419, KJ777329; *Gilbertiodendron mayombense* (Pellegr.) J. Léonard, *Wieringa* 1475 (WAG), KJ777233, —, —; *Gilbertiodendron* cf. *mayombense*, *van der Burgt* 595 (WAG), KJ777198, KJ777389, KJ777298; *Gilbertiodendron newberyi* Burgt, *van der Burgt* 774 (WAG), KJ777234, KJ777420, KJ777330; *Gilbertiodendron newberyi* Burgt, *van der Burgt* 776 (WAG), KJ777235, KJ777421, KJ777331; *Gilbertiodendron obliquum* (Stapf) J. Léonard, *Jongkind* 9972 (WAG), KJ777236, KJ777422, KJ777332; *Gilbertiodendron ogoouense* (Pellegr.)

J. Léonard, *Breteler 10258* (WAG), KJ777237, KJ777423, KJ777333; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *Breteler 11450* (WAG), KJ777238, —, —; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *Breteler 11524* (WAG), KJ777239, KJ777424, KJ777334; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *Schoenmaker 70* (WAG), KJ777242, KJ777427, KJ777336; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *van der Burgt 775* (WAG), KJ777240, KJ777425, KJ777335; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *Wieringa 1316* (WAG), KJ777243, —, —; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *Wieringa 2777* (WAG), KJ777244, KJ777428, KJ777337; *Gilbertiodendron ogoouense* (Pellegr.) J. Léonard, *Mpondzou 44* (WAG), KJ777241, KJ777426, —; *Gilbertiodendron preussii* (Harms) J. Léonard, *Andel 4118* (WAG), KJ777245, KJ777429, KJ777338; *Gilbertiodendron preussii* (Harms) J. Léonard, *van der Burgt 794* (WAG), KJ777246, KJ777430, KJ777339; *Gilbertiodendron preussii* (Harms) J. Léonard, *Wieringa 1600* (WAG), KJ777247, KJ777431, KJ777340; *Gilbertiodendron robynsianum* Aubrév. & Pellegr., *Jongkind 4525* (WAG), KJ777248, KJ777432, KJ777341; *Gilbertiodendron robynsianum* Aubrév. & Pellegr., *Jongkind 4942* (WAG), KJ777249, KJ777433, KJ777342; *Gilbertiodendron robynsianum* Aubrév. & Pellegr., *Jongkind 5644* (WAG), KJ777250, KJ777434, KJ777343; *Gilbertiodendron* sp., *Sosef 2502* (WAG), KJ777261, KJ777445, KJ777353; *Gilbertiodendron* sp., *Wieringa 1315* (WAG), KJ777262, —, —; *Gilbertiodendron* sp., *Wieringa 3793* (WAG), KJ777263, KJ777446, KJ777354; *Gilbertiodendron* sp., *Wieringa 5253* (WAG), KJ777264, KJ777447, KJ777355; *Gilbertiodendron* sp. nov., *Jongkind 8884* (WAG (Liberia), KJ777251, KJ777435, KJ777344; *Gilbertiodendron* sp. nov. A, *Bongou 105* (WAG), KJ777254, KJ777438, —; *Gilbertiodendron* sp. nov. A, *Boungou 415* (WAG), KJ777255, KJ777439, KJ777347; *Gilbertiodendron* sp. nov. A, *Valkenburg 2635* (WAG), KJ777256, KJ777440, KJ777348; *Gilbertiodendron* sp. nov. A, *Valkenburg 2636* (WAG), KJ777257, KJ777441, KJ777349; *Gilbertiodendron* sp. nov. B, *Breteler 14250* (WAG), KJ777258, KJ777442, KJ777350; *Gilbertiodendron* sp. nov. B, *Wieringa 6190* (WAG), KJ777259, KJ777443, KJ777351; *Gilbertiodendron* sp. nov. B, *Wieringa 6191* (WAG), KJ777260, KJ777444, KJ777352; *Gilbertiodendron* sp. nov.?, *Wieringa 6099* (WAG), KJ777253, KJ777437, KJ777346; *Gilbertiodendron* sp. nov., *Wieringa 5057* (WAG), KJ777252, KJ777436, KJ777345; *Gilbertiodendron splendidum* (A. Chev. ex Hutch. & Dalziel) J. Léonard, *Jongkind 8782* (WAG), KJ777265, KJ777448, KJ777356; *Gilbertiodendron stipulaceum* (Benth.) J. Léonard, *Schoenmaker 313* (WAG), KJ777266, KJ777449, KJ777357; *Gilbertiodendron stipulaceum* (Benth.) J. Léonard, *Schoenmaker 385* (WAG), KJ777267, —, —; *Gilbertiodendron stipulaceum* (Benth.) J. Léonard, *Wieringa 1644* (WAG), KJ777268, KJ777450, KJ777358; *Gilbertiodendron tonkolili* Burgt & Estrella, *van der Burgt 1423* (WAG), KJ777269, KJ777451, KJ777359; *Gilbertiodendron tonkolili* Burgt & Estrella, *van der Burgt 1457* (WAG), KJ777270, KJ777452, KJ777360; *Gilbertiodendron unijugum* (Pellegr.) J. Léonard, *McPherson 15847* (WAG), KJ777272, KJ777454, KJ777362; *Gilbertiodendron unijugum* (Pellegr.) J. Léonard, *van der Burgt 73* (WAG), KJ777271, KJ777453, KJ777361; *Gilbertiodendron unijugum* (Pellegr.) J. Léonard, *Wieringa 1075* (WAG), KJ777273, KJ777455, KJ777363; *Gilbertiodendron unijugum* (Pellegr.) J. Léonard, *Wilde 10974* (WAG), KJ777274, KJ777456, KJ777364; *Librevillea klainei* (Pierre ex Harms) Hoyle, *Sosef 2505* (WAG), KJ777275, KJ777457, KJ777365; *Plagiosiphon emarginatus* (Hutch. & Dalziel) J. Léonard, *Wieringa 6067* (WAG), KJ777276, KJ777458, KJ777366; *Plagiosiphon gabonensis* J. Léonard, *Sosef 2503* (WAG), KJ777277, KJ777459, KJ777367; *Plagiosiphon gabonensis* J. Léonard, *Wieringa 4400* (WAG), KJ777278, KJ777460, KJ777368; *Plagiosiphon gabonensis* J. Léonard, *Wieringa 6137* (WAG), KJ777279, KJ777461, KJ777369; *Plagiosiphon longitubus* J. Léonard, *Wieringa 5864* (WAG), KJ777280, —, KJ777370; *Plagiosiphon longitubus* J. Léonard, *Wieringa 5870* (WAG), KJ777281, KJ777462, KJ777371; *Plagiosiphon multijugus* J. Léonard, *Andel 4044* (WAG), KJ777282, KJ777463, KJ777372; *Plagiosiphon multijugus* J. Léonard, *Wieringa 3813* (WAG), KJ777283, KJ777464, KJ777373; *Plagiosiphon* sp., *Wieringa 4039* (WAG), KJ777284, KJ777465, KJ777374.

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