

PHYLOGENY OF TWO AFRICAN GENERA OF SAPOTACEAE – *ENGLEROPHYTUM* AND *SYNSEPALUM*

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Englerophytum and *Synsepalum* are two closely related genera of trees and shrubs from the African tropics. Previous molecular studies have shown that these genera collectively form a clade within the subfamily Chrysophylloideae (Sapotaceae). However, little is known about the inter-relationships of the taxa within the *Englerophytum*–*Synsepalum* clade. In this study, nuclear ribosomal DNA and plastid *trnH-psbA* sequences were used to estimate the phylogeny within the clade. Results indicate that the clade consists of six major lineages, two composed solely of taxa from the genus *Englerophytum* and four composed of taxa from the genus *Synsepalum*. Each lineage can be distinguished by suites of vegetative and floral characters. Leaf venation patterns, calyx fusion, style length and staminodal structure were among the most useful characters for distinguishing clades. Some of the subclades within the *Englerophytum*–*Synsepalum* clade were also found to closely fit descriptions of former genera, most of which were described by Aubréville, that have since been placed in synonymy with *Englerophytum* and *Synsepalum*. The clade with the type species of *Englerophytum* also contains the type species of the genera *Wildemaniodoxa* and *Zeyherella*, which are confirmed as synonyms.

Keywords. Africa, *Englerophytum*, morphology, phylogeny, Sapotaceae, *Synsepalum*.

INTRODUCTION

Englerophytum K.Krause and *Synsepalum* (A.DC.) Daniell (Chrysophylloideae, Sapotaceae) are two genera of woody trees and shrubs distributed in forest and savannah across tropical Africa (Govaerts *et al.*, 2001). The genus *Synsepalum* is estimated to contain around 35 species and *Englerophytum* has approximately 19, of which 14 are recognised in the WCSP (2013) and five were added by Gautier *et al.* (2016). Several members of these genera have edible fruits (e.g. *Englerophytum magalismontanum* (Sond.) T.D.Penn., *Synsepalum afzelii* (Engl.) T.D.Penn.) and good-quality wood (*S. brevipes* (Baker) T.D.Penn., *S. msolo* (Engl.) T.D.Penn.). Probably the best-known species is the miracle fruit, *Synsepalum dulcificum* (Schumach. & Thonn.) Daniell, acknowledged worldwide for its ability to turn sour-tasting foods sweet.

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The current generic circumscriptions of *Englerophytum* and *Synsepalum* were made by Pennington (1991) and are based solely on morphological evidence. In his account, Pennington recognises a close relationship between *Englerophytum* and *Synsepalum* and highlights their shared characteristics, the most prominent being (i) the frequent presence of stipules, (ii) the 5-merous flower structure, (iii) the irregular presence of staminodes, and (iv) similar seed and embryo. Despite these similarities, Pennington (1991) split these two genera based on two characters in leaf venation pattern and filament fusion. *Englerophytum* has striate brochidodromous leaf venation and a tendency for filament fusion. In contrast, *Synsepalum* usually exhibits eucamptodromous leaf venation patterns and has free filaments. Before Pennington (1991) there had been several classifications of Sapotaceae (Pierre, 1890; Engler, 1904; Chevalier, 1943; Meeuse, 1960; Aubréville, 1961a, 1964a; Baehni, 1965). One of the most comprehensive, which was one of the main references before Pennington's account, was Aubréville's (1964a). In Aubréville's account, *Englerophytum* and *Synsepalum* were much more narrowly circumscribed, and species that are today considered as being part of these genera were placed in other smaller genera. These other genera are presented in Table 1 with the states of a selection of characters used to separate them.

The widely divergent views of Pennington and Aubréville resulted from their different approaches towards classification. Aubréville adopted narrow morphogeographical generic concepts, whereas Pennington (1991) used wider generic concepts and more evidence to ensure that his genera were circumscribed using a suite of characters. However, we note that although Pennington (1991) did effectively synonymise all Aubréville's genera into *Englerophytum* or *Synsepalum*, he did not publish all the species transfers. In this paper from here onwards we use '*Englerophytum* s. str.' and '*Synsepalum* s. str.' to refer to Aubréville's concepts of the genera and otherwise simply '*Englerophytum*' and '*Synsepalum*' for Pennington's wider concepts.

Recent molecular studies on a few members of *Englerophytum* and *Synsepalum* (Swenson & Anderberg, 2005; Swenson *et al.*, 2008; Bartish *et al.*, 2011) have shown that these two genera form a strongly supported clade within the subfamily Chrysophylloideae, supporting the inferences of close relatedness made through morphology. However, little is known about the relationships of the members within the clade. Studies by Swenson and colleagues (Swenson & Anderberg, 2005; Swenson *et al.*, 2008) gave mixed results. Parsimony analysis of combined data from plastid DNA, nuclear DNA and morphology showed species from *Englerophytum* and *Synsepalum* segregating into monophyletic subclades, suggesting that the two genera are distinct. However, in trees obtained using either only nuclear DNA or only plastid DNA these genera were not monophyletic.

No study has focused on resolving the relationships within the *Englerophytum*–*Synsepalum* (ES) clade. All the molecular data available were obtained indirectly from studies dealing with family relationships and that used few species from the target genera. In this study the nuclear transcribed spacers, together with the 5.8S region and partial 18S and 26S regions of ribosomal DNA (internal transcribed spacer, ITS) and the

TABLE 1. The 10 genera accepted by Aubréville (1964a) and included by Pennington (1991) in *Englerophytum* and *Synsepalum*, with a selection of distinctive characters states

Aubréville (1964a)	Fusion of sepals	Corolla tube length: corolla lobe length ratio	No. of corolla lobes and stamens	Staminodes	Filament length	Filament fusion	Anther dehiscence	Seed scar	Pennington (1991)
<i>Englerophytum</i> s. str.	< 1/3	1	5–8	Absent	Short	Yes	Extrorse	Broad, ventral	<i>Englerophytum</i>
<i>Zeyherella</i>	< 1/3	1	5	Absent	Long	No	Extrorse	Narrow, ventral	<i>Englerophytum</i>
<i>Wildemaniodoxa</i>	1/2	1	10	Minute, irregular	Long	No	Extrorse	Broad, ventral	<i>Englerophytum</i>
<i>Neoboivinella</i>	< 1/3	> 1	5	Minute or absent	Short	No	Introrse	Narrow, ventral	<i>Englerophytum</i>
<i>Pseudoboivinella</i>	< 1/3	1	5	Small, subulate	Long	No	Extrorse	Narrow, ventral	<i>Englerophytum</i>
<i>Synsepalum</i> s. str.	1/2	1	5	Large, petaloid	Long	No	Extrorse	Very broad, ventral	<i>Synsepalum</i>
<i>Vincentella</i>	< 1/3	<< 1	5	Large, linear	Long or short	No	Extrorse	Narrow, ventral	<i>Synsepalum</i>
<i>Pachystela</i>	< 1/3	1	5	Present or absent	Long	No	Extrorse	Broad, ventral	<i>Synsepalum</i>
<i>Afrosersalisia</i>	1/3 to 1/2	1	5	Minute	Long	No	Extrorse	Broad, ventral	<i>Synsepalum</i>
<i>Tulestea</i>	< 1/3	≤ 1	5	Minute	Short	No	Extrorse	Unknown	<i>Synsepalum</i>

plastid *trnH-psbA* region, were sequenced to (i) verify whether the monophyly of the ES clade is supported with a larger species sample, (ii) elucidate relationships within the ES clade, (iii) determine whether molecular data are consistent with the current generic delimitations of *Englerophytum* and *Synsepalum*, and (iv) gain insight into the morphological synapomorphies shared by members of the ES clade and in particular its subclades.

MATERIALS AND METHODS

Plant material

An attempt was made to sample taxa within the ES clade from throughout their geographical range. A total of 41 accessions of either *Englerophytum* or *Synsepalum* were sampled for this study. Table 2 shows all genera included by Pennington (1991) in the complex, with their type species, their acceptance status following Aubréville (1964a), and their representation in our sampling. Of the ten genera accepted by Aubréville (1964a) in the complex, only *Tulestea* Aubrév., a small genus of three or four rare species he considered close to *Afrosersalisia* A.Chev., could not be included. Therefore, nine genera were included in our sampling, eight of which were represented by their type species. We were unfortunately not successful in obtaining DNA from our only sample of *Synsepalum revolutum* (Baker) T.D.Penn., type species of the genus *Vincentella* Pierre, but this group was represented by *Synsepalum passargei* (Engl.) T.D.Penn.

Nine outgroups were selected by reference to Swenson & Anderberg (2005), Swenson *et al.* (2008) and Bartish *et al.* (2011). Seven outgroups were chosen from the subfamily Chrysophylloideae to provide a subfamilial framework in which to verify the monophyly of the ES clade. The other two outgroups were *Xantolis* Raf., a sister genus to the Chrysophylloideae, and *Eberhardtia aurata* (Pierre ex Dubard) Lecomte, which may belong to the subfamily Sarcospermatoideae that is sister to the rest of the family. Table 3 provides information on all the published sequenced accessions, and Table 4 on all those published here.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from herbarium and silica gel-dried material by using the DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA). The ITS region was amplified using primers ITS 5p and 8p (Möller & Cronk, 1997), following the recipe and program of Armstrong (2010) but with less BioTaq (0.125 µL). Accessions whose ITS region did not amplify well in the first PCR were subjected to an additional nested PCR using the same recipe and program from Armstrong (2010) but with primers ITS1 and ITS4 (White *et al.*, 1990) and a reduced number of cycles (29). The plastid *trnH-psbA* region was amplified using the primers *trnH* and *psbA* (Hamilton, 1999). The recipe from Armstrong (2010) and the ‘rp116 program’ of Shaw *et al.* (2005) were used.

All the PCR products from both the ITS and the plastid regions were purified using the ExoSAP-IT protocol (GE Healthcare, Chicago, Illinois, USA). They were then run on an

TABLE 2. The 16 genera included by Pennington (1991) in *Englerophytum* and *Synsepalum*, with their type species, their acceptance status following Aubréville (1964a), and their representation in our sampling

Genus	Type species	Representation in the sampling	Status according to Aubréville (1964a)	Representation in the sampling	Status according to Pennington (1991)
<i>Englerophytum</i> K. Krause	<i>E. stelechanthum</i>	T	<i>Englerophytum</i> s. str.	T	<i>Englerophytum</i>
<i>Bequaertiodendron</i> De Wild.	<i>B. congolense</i> (= <i>E. congolense</i>)	NR	<i>Englerophytum</i> s. str.	T	<i>Englerophytum</i>
<i>Tisserantiodoxa</i> Aubrév. & Pellegr.	<i>T. oubanguiensis</i>	T	<i>Englerophytum</i> s. str.	T	<i>Englerophytum</i>
<i>Zeyherella</i> (Pierre ex Baill.) Aubrév. & Pellegr.	<i>Z. magalismontana</i>	T	<i>Zeyherella</i>	T	<i>Englerophytum</i>
<i>Wildemaniodoxa</i> Aubrév. & Pellegr.	<i>W. laurentii</i>	T	<i>Wildemaniodoxa</i>	T	<i>Englerophytum</i>
<i>Neoboivinella</i> Aubrév. & Pellegr.	<i>N. natalensis</i> (= <i>E. natalense</i>)	T	<i>Neoboivinella</i>	T	<i>Englerophytum</i>
<i>Pseudoboivinella</i> Aubrév. & Pellegr.	<i>P. oblanceolata</i>	T	<i>Pseudoboivinella</i>	T	<i>Englerophytum</i>
<i>Synsepalum</i> (A.DC.) Daniell	<i>S. dulcificum</i>	T	<i>Synsepalum</i> s. str.	T	<i>Synsepalum</i>
<i>Stironeurum</i> Radlk.	<i>S. stipulatum</i>	T	<i>Synsepalum</i> s. str.	T	<i>Synsepalum</i>
<i>Vincentella</i> Pierre	<i>V. densiflora</i> (= <i>S. revolutum</i>)	S	<i>Vincentella</i>	S	<i>Synsepalum</i>
<i>Bakeriella</i> Dubard	<i>B. revoluta</i>	S	<i>Vincentella</i>	S	<i>Synsepalum</i>
<i>Pachystela</i> Pierre ex Radlk.	<i>P. longistyla</i>	T	<i>Pachystela</i>	T	<i>Synsepalum</i>
<i>Pseudopachystela</i> Aubrév. & Pellegr.	<i>P. lastourvillensis</i>	T	<i>Pachystela</i>	T	<i>Synsepalum</i>
<i>Afrosersalisia</i> A. Chev.	<i>A. afzelii</i> (= <i>S. afzelii</i>)	T	<i>Afrosersalisia</i>	T	<i>Synsepalum</i>
<i>Rogeonella</i> A. Chev.	<i>R. chevalieri</i> (= <i>S. cerasiferum</i> (Welw.) T.D.Penn.)	NR	<i>Afrosersalisia</i>	T	<i>Synsepalum</i>
<i>Tulestea</i> Aubrév. & Pellegr.	<i>T. gabonensis</i>	NR	<i>Tulestea</i>	NR	<i>Synsepalum</i>

NR, not represented; S, represented but not by its type species; T, represented by its type species.

TABLE 3. Details of the outgroup sequences used in the analysis

Species	Collector and collector no. (herbarium code)	Sequence	GenBank accession no.	Reference
<i>Donella pruniformis</i> (Pierre ex Engl.) Aubrév. & Pellegr.	<i>Jongkind</i> 3762 (WAG)	ITS	DQ246671	Swenson <i>et al.</i> (2008)
		<i>trnH-psbA</i>	DQ344100	Swenson <i>et al.</i> (2008)
<i>Donella lanceolata</i> (Blume) Aubrév.	<i>Solo & Randrianasolo</i> 33 (WAG)	ITS	DQ246672	Swenson <i>et al.</i> (2008)
		<i>trnH-psbA</i>	DQ344101	Swenson <i>et al.</i> (2008)
<i>Eberhardtia aurata</i>	<i>Hao</i> 534 (S)	ITS	EF558617	Swenson <i>et al.</i> (2008)
		<i>trnH-psbA</i>	DQ344106	Swenson <i>et al.</i> (2008)
<i>Omphalocarpum pachysteloides</i> Mildbr. ex Hutch. & Dalziel	<i>Jongkind</i> 2351 (WAG)	ITS	AY552151	Bartish <i>et al.</i> (2005)
		<i>trnH-psbA</i>	DQ344122	Swenson <i>et al.</i> (2008)
<i>Omphalocarpum strombocarpum</i> Y.B.Harv. & Lovett	<i>Frimodt-Moller, Joker & Ndangalasi</i> TZ538 (C)	ITS	DQ246685	Swenson <i>et al.</i> (2008)
		<i>trnH-psbA</i>	DQ344123	Swenson <i>et al.</i> (2008)
<i>Pouteria adolfi-friedericii</i> (Engl.) A.Meeuse	<i>Friis et al.</i> 3502 (UPS)	ITS	AY552115	Bartish <i>et al.</i> (2005)
		<i>trnH-psbA</i>	DQ344127	Swenson <i>et al.</i> (2008)
<i>Pouteria macrophylla</i> (Lam.) Eyma	<i>Seidel & al.</i> 5905 (K)	ITS	DQ246692	Swenson <i>et al.</i> (2008)
		<i>trnH-psbA</i>	DQ344137	Swenson <i>et al.</i> (2008)
<i>Xantolis siamensis</i> (H.R.Fletcher) P.Royen	<i>Smitairi</i> 1 (L)	ITS	AY552154	Bartish <i>et al.</i> (2005)
		<i>trnH-psbA</i>	DQ344151	Swenson <i>et al.</i> (2008)

ITS, internal transcribed spacer.

TABLE 4. Voucher information for the sequences published in this analysis

No.	Species	Collector and collector no. (herbarium code)	Source	Country	ITS sequence obtained	<i>trnH-psbA</i> sequence obtained
1	<i>Englerophytum stelechanthum</i>	Wieringa 7702 (WAG)	S	Gabon	✓	✓
2	<i>Englerophytum</i> sp.	Jongkind 5084 (FHO)	H	Ivory Coast	✓	✓
3	<i>Englerophytum paludosum</i>	Maas 10325 (WAG)	H	Gabon	✓	✓
4	<i>Englerophytum laurentii</i> ^a	Harris 9685 (E)	H	Congo	✓	✓
5	<i>Englerophytum laurentii</i> ^a	Van der Laan 231 (WAG)	H	Cameroon	X	X
6	<i>Englerophytum letestui</i> ^b	Sosef 2025 (WAG)	S	Gabon	✓	✓
7	<i>Englerophytum magalismsontanum</i>	Balkwill et al. 11986 (E)	H	South Africa	✓	✓
8	<i>Englerophytum magalismsontanum</i>	Stronkhorst 1 (WAG)	H	Botswana	✓	✓
9	<i>Englerophytum magalismsontanum</i>	Chapman 6922 (E)	H	Malawi	✓	✓
10	<i>Englerophytum natalense</i>	Gereau et al. 6120 (E)	H	Tanzania	✓	✓
11	<i>Englerophytum natalense</i>	Chapman 6479 (E)	H	Malawi	✓	✓
12	<i>Englerophytum oblanceolatum</i>	Van der Maesen 6154 (WAG)	H	Benin	✓	✓
13	<i>Englerophytum oubanguiense</i>	Harris 8166 (E)	H	Congo	✓	✓
14	<i>Englerophytum oubanguiense</i>	Harris 4924 (E)	H	Central African Republic	✓	✓
15	<i>Englerophytum oubanguiense</i>	Jongkind 11443 (WAG)	H	Guinea	✓	✓
16	<i>Englerophytum stelechanthum</i>	Waterman & Mckey 868 (E)	H	Cameroon	✓	✓
17	<i>Synsepalum afzelii</i>	Hawthorne, Gyakari 201a 121 (FHO)	H	Ghana	✓	✓
18	<i>Synsepalum afzelii</i>	Hawthorne, Gyakari 200b 212 (FHO)	H	Ghana	✓	✓
19	<i>Synsepalum aubrevillei</i>	Hawthorne, Gyakari 200b 32 (FHO)	H	Ghana	✓	✓
20	<i>Synsepalum aubrevillei</i>	Hawthorne, Gyakari 200b 166 (FHO)	H	Ghana	✓	✓
21	<i>Synsepalum brevipes</i>	Harris 8441 (E)	S	Gabon	✓	✓

TABLE 4. (Continued)

No.	Species	Collector and collector no. (herbarium code)	Source	Country	ITS sequence obtained	<i>trnH-psbA</i> sequence obtained
22	<i>Synsepalum brevipes</i>	Hawthorne, Gyakari 200b 131 (FHO)	H	Ghana	✓	✓
23	<i>Synsepalum brevipes</i>	Hawthorne et al. AM1219 (FHO)	H	Senegal	✓	✓
24	<i>Synsepalum brevipes</i>	Moutsamboté 6093 (E)	H	Congo	✓	✓
25	<i>Synsepalum brevipes</i>	Harris 9712 (E)	H	Congo	✓	✓
26	<i>Synsepalum brevipes</i>	Sosef 2134 (WAG)	S	Gabon	X	X
27	<i>Synsepalum congolense</i>	Harris 8325 (E)	S	Gabon	✓	✓
28	<i>Synsepalum dulcificum</i>	Moutsamboté 6060 (E)	S	Congo	✓	✓
29	<i>Synsepalum dulcificum</i>	Hawthorne, Gyakari 200b 138 (FHO)	H	Ghana	✓	✓
30	<i>Synsepalum dulcificum</i>	Moutsamboté 6013 (E)	H	Congo	✓	✓
31	<i>Synsepalum dulcificum</i>	Kami 4327 (E)	H	Congo	✓	✓
32	<i>Synsepalum fleuryanum</i>	Harris 8456 (E)	S	Gabon	✓	✓
33	<i>Pseudopachystela lastoursvillensis</i>	Bissiengou 771 (WAG)	S	Gabon	✓	✓
34	<i>Synsepalum ntimii</i>	Hawthorne, Gyakari 203a 24 (FHO)	H	Ghana	✓	✓
35	<i>Synsepalum passargei</i>	Reitsma 3820 (FHO)	H	Guinea	✓	✓
36	<i>Synsepalum revolutum</i>	Harris 5735 (E)	H	Central African Republic	X	X
37	<i>Synsepalum</i> sp.	Harris 9579 (E)	S	Congo	✓	✓
38	<i>Synsepalum</i> sp.	Harris 8702 (E)	S	Gabon	✓	✓

TABLE 4. (Continued)

No.	Species	Collector and collector no. (herbarium code)	Source	Country	ITS sequence obtained	<i>trnH-psbA</i> sequence obtained
39	<i>Synsepalum</i> sp.	<i>Sosef</i> 2619 (WAG)	H	Gabon	✓	✓
40	<i>Synsepalum stipulatum</i>	<i>Harris</i> 9130 (E)	S	Congo	✓	✓
41	<i>Synsepalum stipulatum</i>	<i>Harris</i> 9014 (E)	S	Congo	✓	✓
42	<i>Synsepalum stipulatum</i>	<i>Wieringa</i> 5228 (WAG)	S	Gabon	✓	✓
43	<i>Synsepalum subcordatum</i>	<i>Harris</i> 7562 (E)	H	Central African Republic	✓	✓
44	<i>Synsepalum tsoumkpe</i>	<i>Hawthorne, Gyakari</i> H200 661 (FHO)	H	Ivory Coast	✓	✓

^a New combination, made in this paper, for *Wildemaniodoxa laurentii*.

^b New combination, made in this paper, for *Zeyherella letestui*.

X, No sequence obtained; ✓, sequence obtained; H, herbarium specimen; ITS, internal transcribed spacer; S, silica gel-dried leaf fragment.

ABI 3730 sequencer at the University of Edinburgh's GenePool facility, using the manufacturer's protocols. Sequences were then edited in Sequencher version 5.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA) and aligned using BioEdit version 7.2.0 (Hall, 1999). An automatic alignment was carried out using Clustal W (Thompson *et al.*, 1994) and the results were then refined manually. Gaps were coded as additional binary characters using the approach of Simmons & Ochoterena (2000).

Phylogenetic analysis

Heuristic parsimony searches were implemented on the ITS, *trnH-psbA* and combined matrices by using PAUP* version 4.0 (Swofford, 2003). All character states were treated as unordered and equally weighted. The heuristic search was made using tree-bisection reconnection branch swapping with 10,000 random-addition replicates, with a limit of 1,000,000 swaps per replicate. A parsimony bootstrap search, with 1000 replicates, was also performed to obtain bootstrap support (bs) values.

Bayesian analysis was carried out using Mr Bayes version 3.2.1 (Ronquist & Huelsenbeck, 2003). Before running the Bayesian analysis, jModel Test version 2.1.4 (Darriba *et al.*, 2012) was used together with the Bayesian information criterion to select the optimum model of evolution for both the ITS and the plastid sequences. Once the models were chosen, the Markov chain Monte Carlo algorithm was run for 10,000,000 generations, with one cold and three heated chains starting from a random tree and sampling every 1000 generations. Tracer version 1.5 (Rambaut & Drummond, 2009) was used to check for convergence and to estimate burn-in. Trees falling within the burn-in were discarded and the remainder were used to construct either a Bayesian consensus or a maximum clade credibility (MCC) tree.

Before performing combined analyses, trees resulting from the individual region analyses were compared and visually inspected for strongly supported incongruence. Posterior probability (pp) values of ≥ 0.9 and bs values of $\geq 75\%$ were considered to indicate strong support. There were no examples of strongly supported incongruence between trees from either data set.

Morphological mapping

A Leica MZ75 standard binocular dissecting microscope (Leica, Wetzlar, Germany) was used to examine specimens from E, FHO and K, which were then scored for 34 morphological characters (Table 5). We scored a suite of characters used by both Aubréville and Pennington. Delimitation of states of quantitative characters were made in an arbitrary manner based on both examination of specimens and reference to the literature. Some online images of herbarium specimens (at BM, HBG, LISC and P) were also consulted to facilitate scoring of vegetative characters of species that were under-represented in the study material. Whenever floral material was available, this was boiled, dissected and mounted on card for analysis. In cases in which floral material was lacking, floral characters were scored from descriptions in the literature (Lecomte, 1928; Meeuse,

TABLE 5. Character states for all scored morphological characters

Character	State 1	State 2	State 3	State 4
1. Leaf length (cm)	0–15	> 15 to 30	> 30	NA
2. Leaf width (cm)	0–6	> 6 to 12	NA	NA
3. Leaf width:leaf length ratio	0.2–0.3	> 0.3 to 0.4	> 0.4 to 0.5	NA
4. Petiole length (cm)	0–1	> 1 to 2	> 2 to 3	NA
5. Petiole length:leaf length ratio	0–0.1	> 0.1	NA	NA
6. Distance between secondary veins (cm)	0–0.5	> 0.5 to 1	> 1 to 1.5	> 1.5
7. Distance between secondary veins:leaf length ratio	0–0.05	> 0.05	NA	NA
8. No. of secondary veins	0–27	≥ 28	NA	NA
9. No. of secondary veins per cm leaf length	0–1	> 1 to 2	> 2 to 3	> 3
10. Pattern of leaf venation	Brochidodromous	Eucamptodromous	NA	NA
11. Petiole shape	With closed groove	With open groove	Flat and thickened at base	NA
12. Intramarginal vein	Present	Absent	NA	NA
13. Midrib shape	Sunken above	Impressed above	Flat above	NA
14. Intersecondary veins	Present	Absent	NA	NA
15. Conspicuity of tertiary venation	Conspicuous	Inconspicuous	NA	NA
16. Pattern of tertiary venation	Parallel to secondary veins	Reticulate	Horizontal	Oblique
17. Stipules	Present	Absent	NA	NA
18. Trichomes on underside of midrib	Present	Glabrous or subglabrous	NA	NA
19. Trichomes on underside of lamina	Velvety	Sparse	Glabrous or subglabrous	NA
20. Pedicel	Pedicellate	Sessile or subsessile	NA	NA

TABLE 5. (Continued)

Character	State 1	State 2	State 3	State 4
21. Sepal length (cm)	0–2.5	> 2.5 to 5	NA	NA
22. Sepal fusion	Free	Fused	NA	NA
23. Petal tube length (mm)	0–2	> 2	NA	NA
24. Petal lobe length (mm)	0–2.5	> 2.5	NA	NA
25. Petal lobe length:petal tube length ratio	1–2	> 2	NA	NA
26. Filament fusion	Free	Partly fused	Completely fused	NA
27. Filament length (mm)	0–1.5	> 1.5	NA	NA
28. Anther dehiscence	Extrorse	Latrorse	NA	NA
29. Anther length (mm)	0–1	> 1 to 2	> 2	NA
30. Anther length:filament length ratio	0–0.4	> 0.4 to 0.8	> 0.8–1.2	> 1.2
31. Staminodes	Absent or vestigial	Medium (1–2.5 mm long)	Large (> 2.5 mm long)	NA
32. Style length (mm)	1–2	> 2 to 3	> 3	NA
33. Ovary length (mm)	1–2	> 2	NA	NA
34. Style length:ovary length ratio	0–1	> 1 to 2	> 2	NA

NA, not applicable.

1960; Aubréville, 1961a,b, 1964a,b; Baehni, 1965; Pennington, 1991; Swenson & Anderberg, 2005).

Once the character matrix was compiled, it was transferred into Mesquite version 2.75 (Maddison & Maddison, 2011). Morphological characters were mapped onto the MCC tree from the Bayesian analysis of the combined ITS and *trnH-psbA* data by using the Parsimony Ancestral States option in Mesquite. The resulting reconstructions were then analysed for morphological congruences and differences.

RESULTS

No hard incongruence was evident between the ITS and *trnH-psbA* data sets, so they were combined. Because the plastid region had fewer informative characters than the ITS region, it had relatively less impact on the final topology of the combined tree. The ITS alignment length was 952 bp, with 71% of the variable sites (259) being parsimony-informative. The plastid alignment length was 760 bp; 8.7% of the sites were variable, and only 53% of these were parsimony-informative. The consistency indices (CIs) and retention indices (RIs) showed that the plastid region (CI, 0.9; RI, 0.91) has less homoplasy than the ITS region (CI, 0.65; RI, 0.85). Representative most-parsimonious trees from the parsimony analysis are available from the corresponding author. The ITS analysis generated 16,496 equally most-parsimonious trees; the plastid analysis, 40,000; and the combined analysis, 29,319.

For the Bayesian analysis, the data were split into three partitions, representing the ITS region, the *trnH-psbA* region and binary gap data. The models chosen were SYM+G for ITS, F81+G for *trnH-psbA* and F81 for the binary gap data. In preliminary analyses the ITS region was partitioned into ITS1, 5.8S and ITS2. However, when the analysis was run using three ITS partitions, the topologies of the trees derived from the partitioned data analysis (not shown) and those with only one ITS partition were the same, with only very minor changes in support values. Therefore, the simpler model was chosen and the ITS data were not partitioned. The MCC tree from the combined ITS and *trnH-psbA* analysis is shown in Fig. 1. Trees from the separate Bayesian analyses are available from the corresponding author.

The monophyly of the ES clade was strongly supported in the analysis of the ITS and combined data sets (pp, 1; bs, 99% in all cases; see Fig. 1 and figures available from the corresponding author). However, the resolution in the plastid data set was too low to provide strong support (figures available from the corresponding author). In the plastid tree, the ES clade collapsed into a Chrysophylloideae polytomy in the strict consensus.

Six major lineages within the ES clade could be identified from the parsimony and Bayesian analysis of the ITS and combined data sets (see Fig. 1 and figures available from the corresponding author). These lineages are labelled clades A–F in the trees and are summarised in Table 6. Four of the lineages (clades A, B, D and F) consist exclusively of species currently accepted as belonging to the genus *Synsepalum* (although currently there is no combination for *Pseudopachystela lastoursvillensis* under *Synsepalum*; see discussion below), whereas the other two (clades C and E) consist of species belonging to

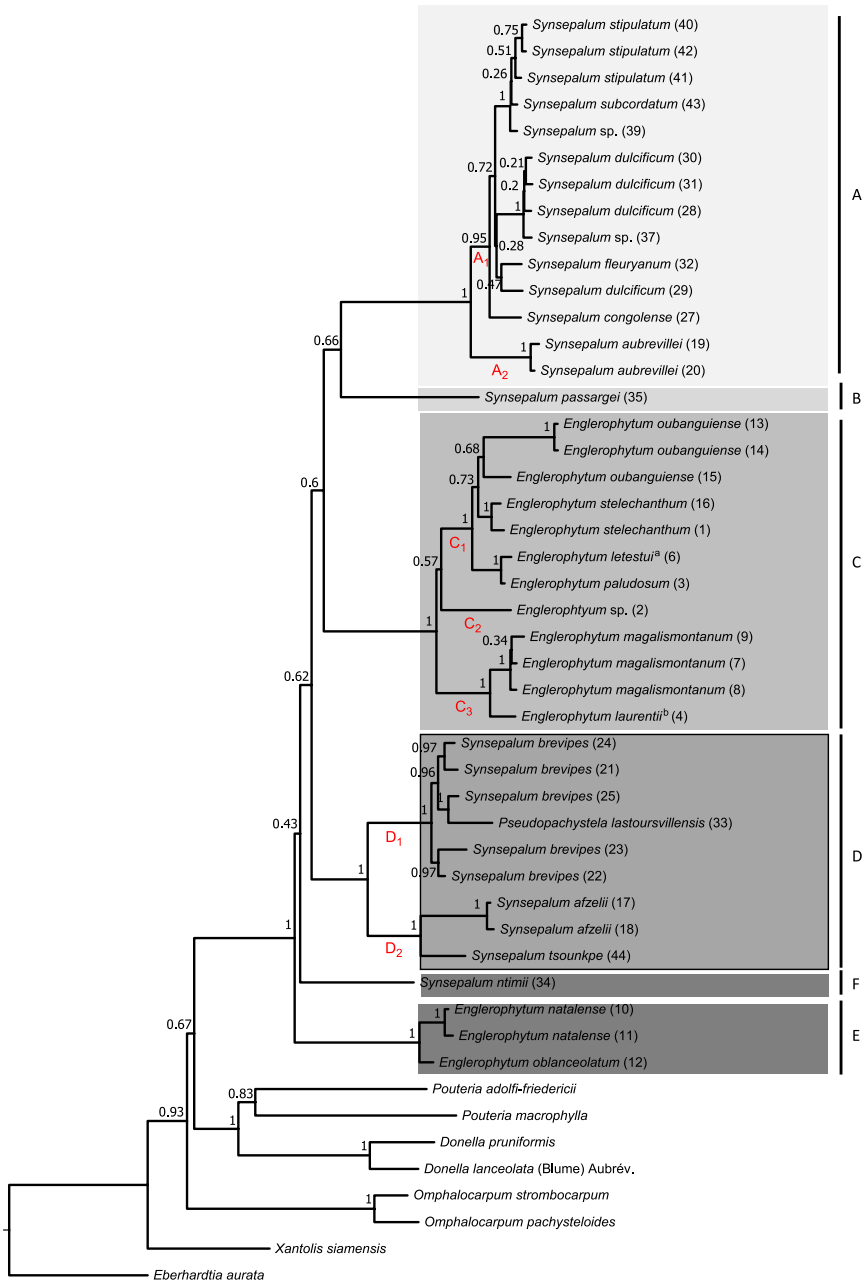


FIG. 1. The maximum clade credibility tree from the analysis of the ITS data set, showing the major subclades (clades A–E) within the *Englerophytum*–*Synsepalum* clade and their posterior probability values. Specimen numbers are shown in parentheses, thereby linking the specimens to information in Table 4. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

TABLE 6. The major lineages within the *Englerophytum*–*Synsepalum* clade, with their support values

Clade and constituent taxa	Posterior probability value, bootstrap support value (%)		
	Combined data set	Nuclear DNA data set	Chloroplast DNA data set
Clade A			
<i>S. aubrevillei</i> , <i>S. congolense</i> , <i>S. dulcificum</i> , <i>S. fleuryanum</i> , <i>S. stipulatum</i> , <i>S. subcordatum</i>	1.00, 1.00	1.00, 100	0.99, 74
Clade B			
<i>S. passargei</i>	NA, NA	NA, NA	NS, NS
Clade C			
<i>E. laurentii</i> ^a , <i>E. letestui</i> ^b , <i>E. magalismontanum</i> , <i>E. oubanguiense</i> , <i>E. paludosum</i> , <i>E. stelechanthum</i>	1.00, 100	1.00, 100	NS, NS
Clade D			
<i>Pseudopachystela lastoursvillensis</i> ^c , <i>S. afzelii</i> , <i>S. brevipes</i> , <i>S. tsoukpe</i>	1.00, 99	1.00, 98	NS, NS
Clade E			
<i>E. natalense</i> , <i>E. oblancoletum</i>	1.00, 100	1.00, 100	1.00, 94
Clade F			
<i>S. nimii</i>	NA, NA	NA, NA	NA, NA

NA, not applicable; NS, not supported.

^a New combination, made in this paper, for *Wildemaniodoxa laurentii*.

^b New combination, made in this paper, for *Zeyherella letestui*.

Englerophytum (including *Wildemaniodoxa laurentii* (De Wild.) Aubrév. & Pellegr. and *Zeyherella letestui* Aubrév. & Pellegr., newly combined below). None of the lineages (i.e. clades A–F) contain a mixture of species from both *Englerophytum* and *Synsepalum*. All resolve together in a six-way polytomy at the base of the strongly supported ES clade.

Some of the lineages were also present in the plastid trees. Clade A was particularly well resolved in the plastid data analysis, with a strong pp (0.99) in the Bayesian analysis and a bs of 74% in the parsimony tree. Clade E (pp, 1; bs, 94%) and clade F were also easily distinguishable from other clades in the plastid tree. In contrast, clades B–D were poorly resolved and appeared in a single clade with good support in the Bayesian analysis (pp, 0.97).

Three of the six major lineages (clades A, C and D) have well-supported subclades (summary provided in Table 7). These were clearly visible in the ITS and combined trees and are indicated by the red labels in Fig. 1. However, because the plastid tree was poorly resolved, the subclades were not mapped onto it. The evolution of several morphological characters that have utility in distinguishing clades A–F from each other are presented in Figs 2 and 3.

TABLE 7. Subclades of clades A, C and D within the *Englerophytum*–*Synsepalum* clade, with their support values^a

Clade	Subclade	Constituent taxa (collector and collector no.)	Posterior probability value	Bootstrap support value (%)
A	A ₁	<i>S. congolense</i> , <i>S. dulcificum</i> , <i>S. fleuryanum</i> , <i>S. stipulatum</i> , <i>S. subcordatum</i>	0.99	97
	A ₂	<i>S. aubrevillei</i>	1	100
C	C ₁	<i>E. letestui</i> ^a , <i>E. oubanguiense</i> , <i>E. paludosum</i> , <i>E. stelechanthum</i>	1	95
	C ₂	<i>Englerophytum</i> sp. (Jongkind 5084)	NA	NA
	C ₃	<i>E. magalismontanum</i> , <i>E. laurentii</i> ^b	1	100
D	D ₁	<i>Pseudopachystela lastoursvillensis</i> , <i>S. brevipes</i>	1	100
	D ₂	<i>S. afzelii</i> , <i>S. tsoukpe</i>	1	100

NA, not applicable.

^a Taken from the combined trees.

^a New combination, made in this paper, for *Zeyherella letestui*.

^b New combination, made in this paper, for *Wildemaniodoxa laurentii*.

DISCUSSION

The results of the analysis using our nuclear and combined data sets strongly support a monophyletic ES clade. This study does not support the hypothesis that *Englerophytum* and *Synsepalum*, as delimited by Pennington (1991), are monophyletic. Instead of two clades each representing these two genera, our trees showed six major lineages within the more inclusive ES clade (see Fig. 1 and figures available from the corresponding author); four of the lineages contain species exclusively from *Synsepalum* and two contain species exclusively from *Englerophytum*. This result can neither confirm nor reject the possibility that the current circumscription is based on monophyly, because there is no support for any of the relationships between the lineages at the base of the ES clade. However, despite not representing two clearly defined lineages, the six major lineages evident from the present analysis still provide useful information on the structure of the ES clade.

Clade A

Clade A consists of six species of *Synsepalum*: *S. aubrevillei* (Pellegr.) Aubrév. & Pellegr., *S. congolense* Lecomte, *S. dulcificum* (the type species of *Synsepalum*), *S. fleuryanum* A.Chev., *S. stipulatum* (Radlk.) Engl. and *S. subcordatum* De Wild. They range from small-leaved, short-petioled species such as *Synsepalum dulcificum* to large-leaved species such as *S. aubrevillei* and *S. subcordatum*. Members of clade A have several shared characters that, although variable, distinguish them from species within the other clades.

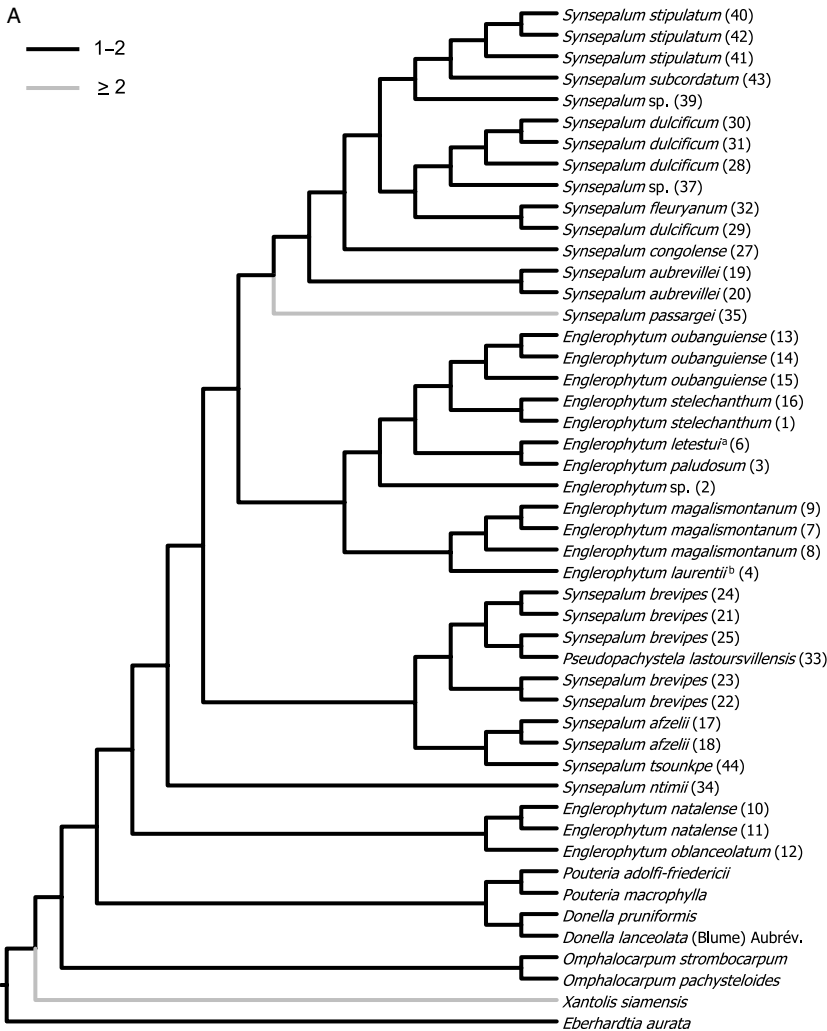


FIG. 2. A selection of morphological characters mapped onto the maximum clade credibility tree from the Bayesian analysis of the internal transcribed spacer data. A, Ratio of lobe length to tube length. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Species placed within clade A all have transverse-oblique tertiary venation (Fig. 3F); the tertiary veins form an oblique ladder-like pattern between two successive secondary veins. This character is not exclusive to clade A, because it is found in some other species (e.g. *Pseudopachystela lastoursvillensis* Aubrév. & Pellegr. and *Synsepalum tsoukpe* Aubrév. & Pellegr. in clade D). However, venation pattern, in combination with other characters, can be useful in providing a unique morphological definition for clade A.

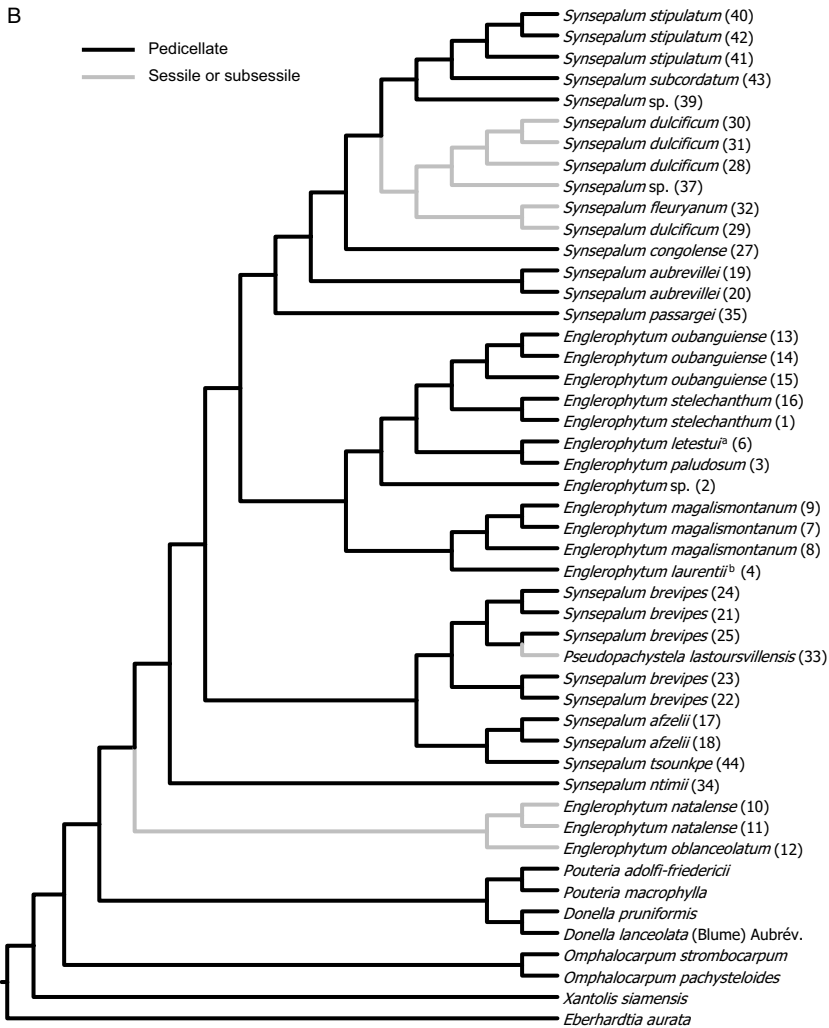


FIG. 2. (continued). B, Pedicel. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Another shared character of members of clade A is their relatively long style length (≥ 2.5 mm in most of these species). Some have exceptionally long styles (e.g. 7 mm in *Synsepalum aubrevillei* and *S. dulcificum*) not found elsewhere in the ES clade. Consequently, because ovary size (approximately 2 mm) shows little variation between species in the ES clade, the ratio of style length to ovary length exceeds 2 in all clade A species except that with the shortest style, *Synsepalum subcordatum* (1.8 mm).

All members of clade A have prominent (> 1 mm) antisepalous staminodes with denticulate margins. Staminodes in other clades are either small and vestigial (e.g. in

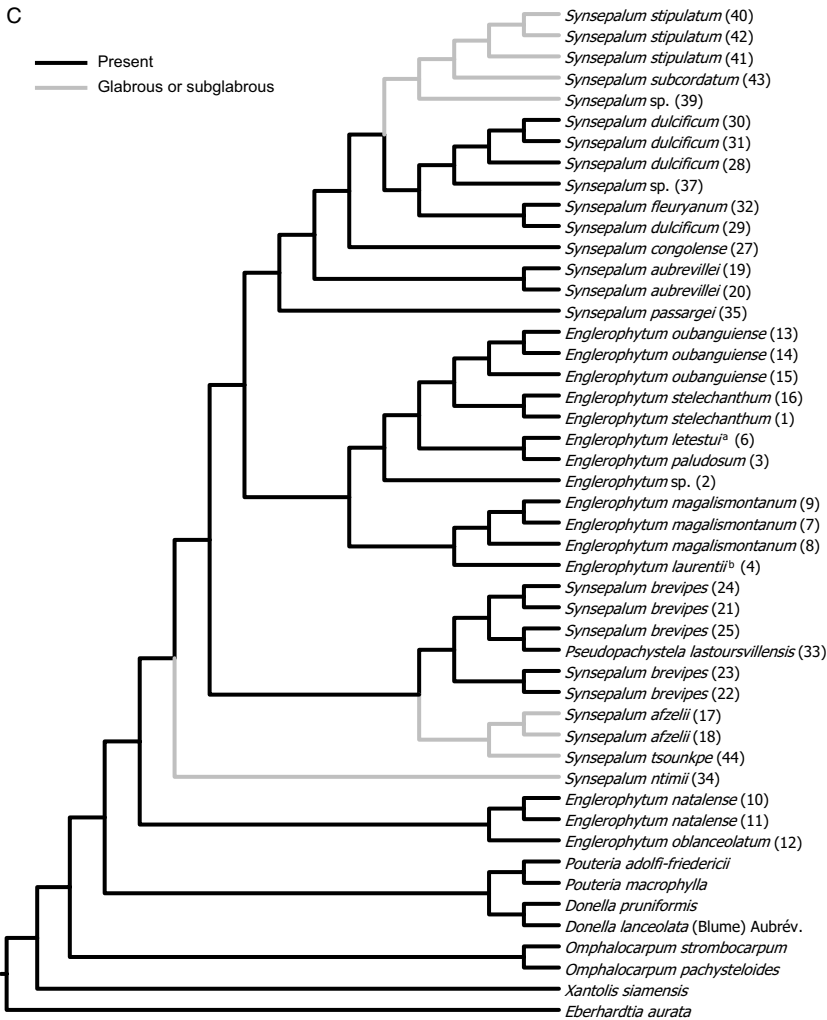


FIG. 2. (continued). C, Trichomes on the underside of the midrib. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Synsepalum afzelii and *S. brevipes* in clade D) or large but lacking denticulate margins (e.g. in *Englerophytum magalismontanum* in clade C and *Pseudopachystela lastoursvillensis* in clade D). Only *Pseudopachystela lastoursvillensis* and *Synsepalum tsounkpe* (both clade D) have staminodes that are similar to those of members of clade A.

The most significant shared character in clade A species is the presence of fused sepals, from which the name *Synsepalum* is derived. All members of this clade have sepals that are fused for at least a third of their length. The fused sepals usually form a tight cup around the petal tube and are very difficult to tease apart without making deep incisions. This character

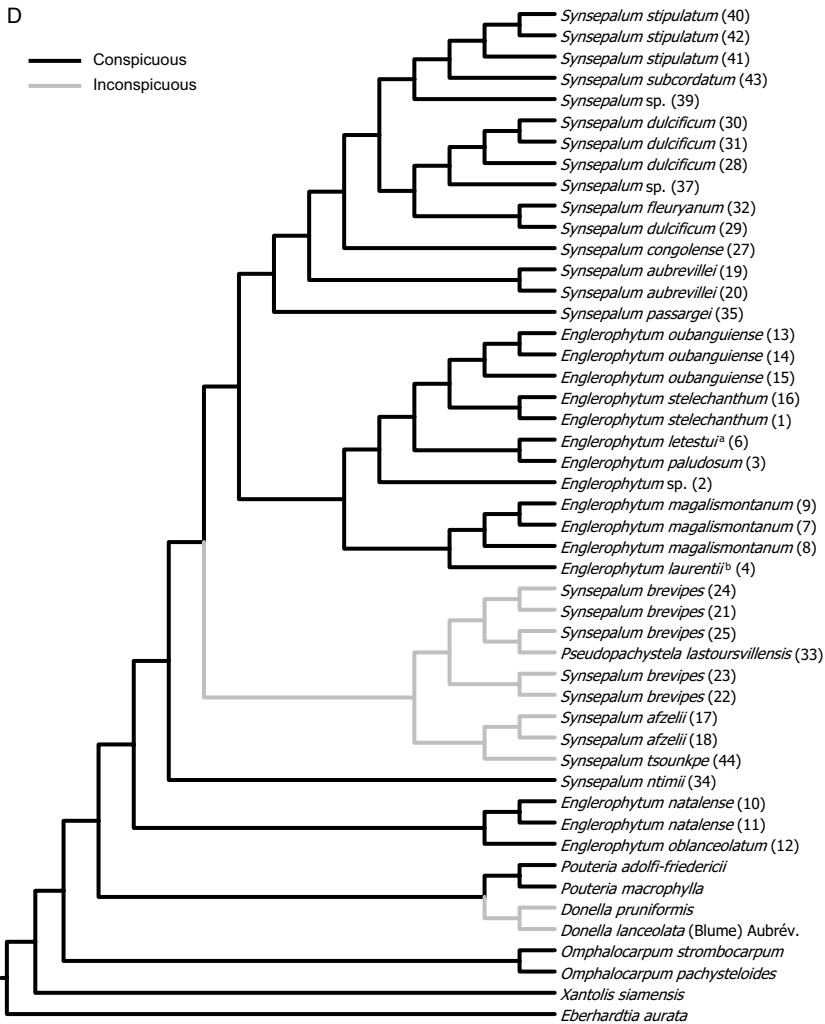


FIG. 2. (continued). D, Conspicuity of tertiary venation. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

is nearly exclusive to clade A (Fig. 2F). Other than in members of clade A, sepal fusion was present in only three other species in the analysis: *Englerophytum stelechanthum* Krause (clade C), *Synsepalum tsounkpe* (clade D) and *E. oblanceolatum* (S.Moore) T.D.Penn. (clade E). Unfortunately, none of the floral material of the above-mentioned species could be analysed at first hand, so the literature (Moore, 1907; Krause, 1914; Aubréville, 1959, 1961a; Liben, 1989) was consulted to obtain the information for sepal fusion.

The extent of sepal fusion in *Englerophytum oblanceolatum* and *E. stelechanthum* is difficult to determine from the literature because the relevant descriptions are unclear.

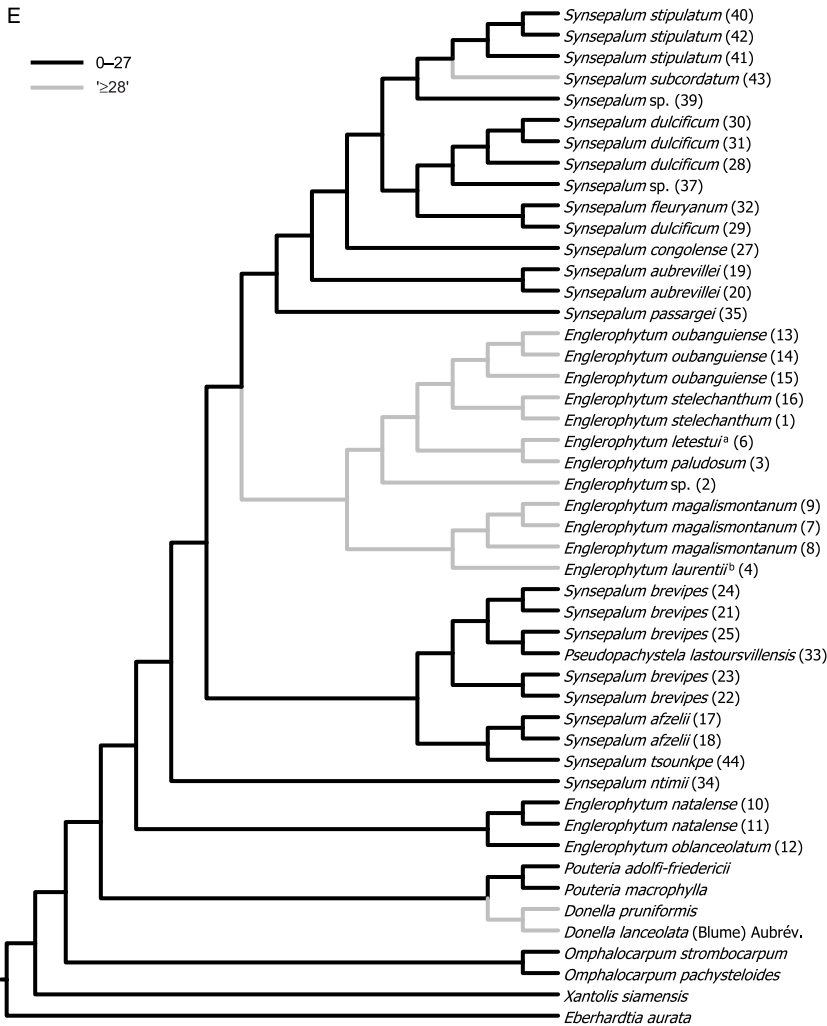


FIG. 2. (continued). E, Number of secondary veins. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

In the protologue of *Englerophytum oblanceolatum* (Moore, 1907), the calyx is described as “connate below” (inferne connatis), whereas in that of *E. stelechanthum* (Krause, 1914) the calyx is described as “slightly connate at base” (basi breviter connata). Therefore, it is difficult to say whether the extent of fusion is slight enough to be easily distinguishable from the type of sepal fusion in members of clade A. In contrast, the extent of sepal fusion in *Synsepalum tsounkpe* is clearly stated in Aubréville’s description of the species (1961a). *Synsepalum tsounkpe* has sepals fused for about half their length, a similar type of fusion to that of the flowers in clade A species. However, despite its morphological similarities,

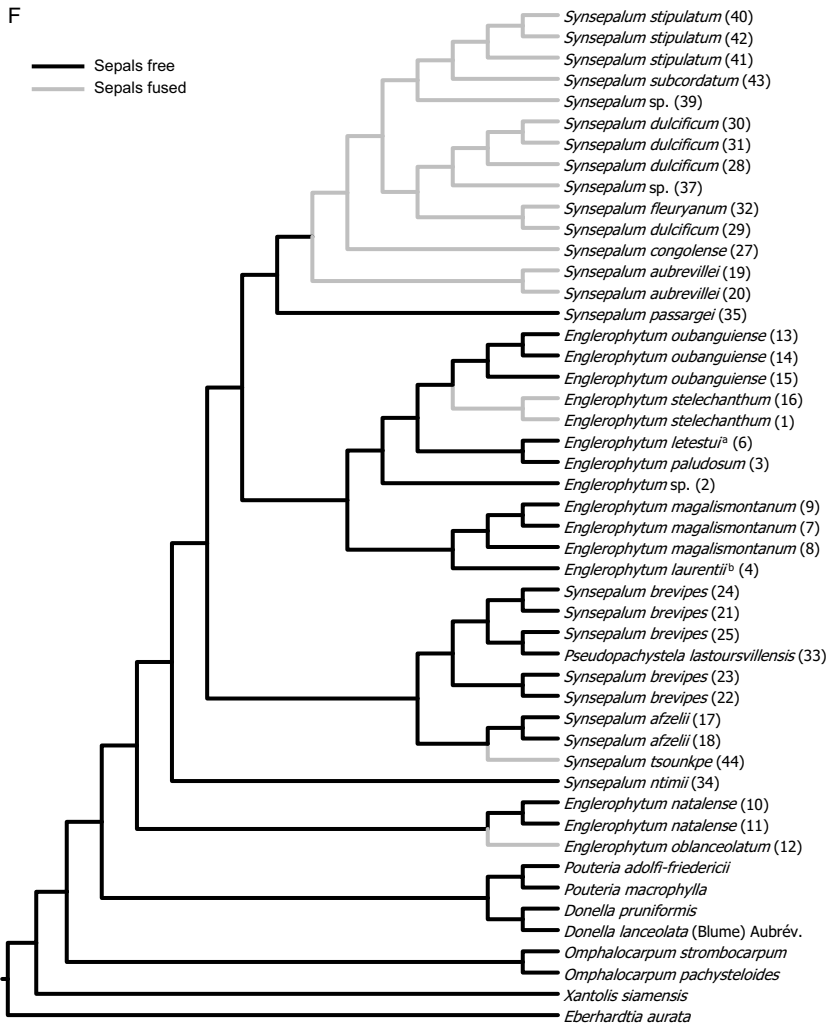


FIG. 2. (continued). F, Sepal fusion. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Synsepalum tsounkpe seems, based on molecular data, to be phylogenetically closer to members of clade D.

It is important to note that sepal fusion and some other synapomorphies mentioned here were the basis on which Aubréville (1961a) made his generic circumscription of *Synsepalum*. Consequently, clade A is nearly identical to Aubréville's circumscription of *Synsepalum*. In fact, it exclusively contains species from his concept of the genus, the only difference being in the placement of *Synsepalum tsounkpe*. Morphologically, this species fits into clade A (see Figs 2, 3), and it is also the only species in the phylogenetic

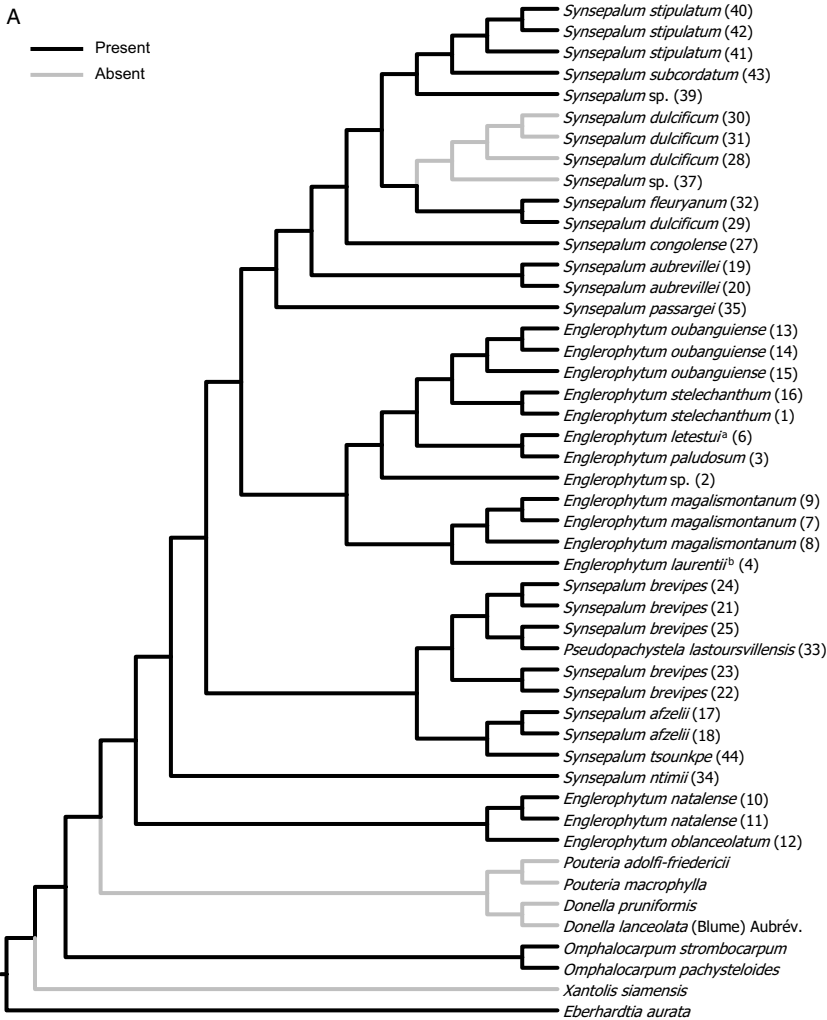


FIG. 3. A selection of morphological characters mapped onto the maximum clade credibility tree from the Bayesian analysis of the internal transcribed spacer data. A, Stipules. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

tree that has all the characters that define this clade, i.e. oblique tertiary venation pattern, long style, dentate staminodes and fused sepals. Therefore, it is not surprising that it was placed in *Synsepalum* by Aubréville. However, in this analysis and with strong support, this species appears in clade D as sister to *Synsepalum afzelii*. In the light of the morphological evidence, there seems to be a need to revisit the placement of *Synsepalum tsounkpe* in clade D. Unlike other species (e.g. *Synsepalum brevipes* and *S. dulcificum*), which were represented by more than one DNA sample in the analysis, *S. tsounkpe* was

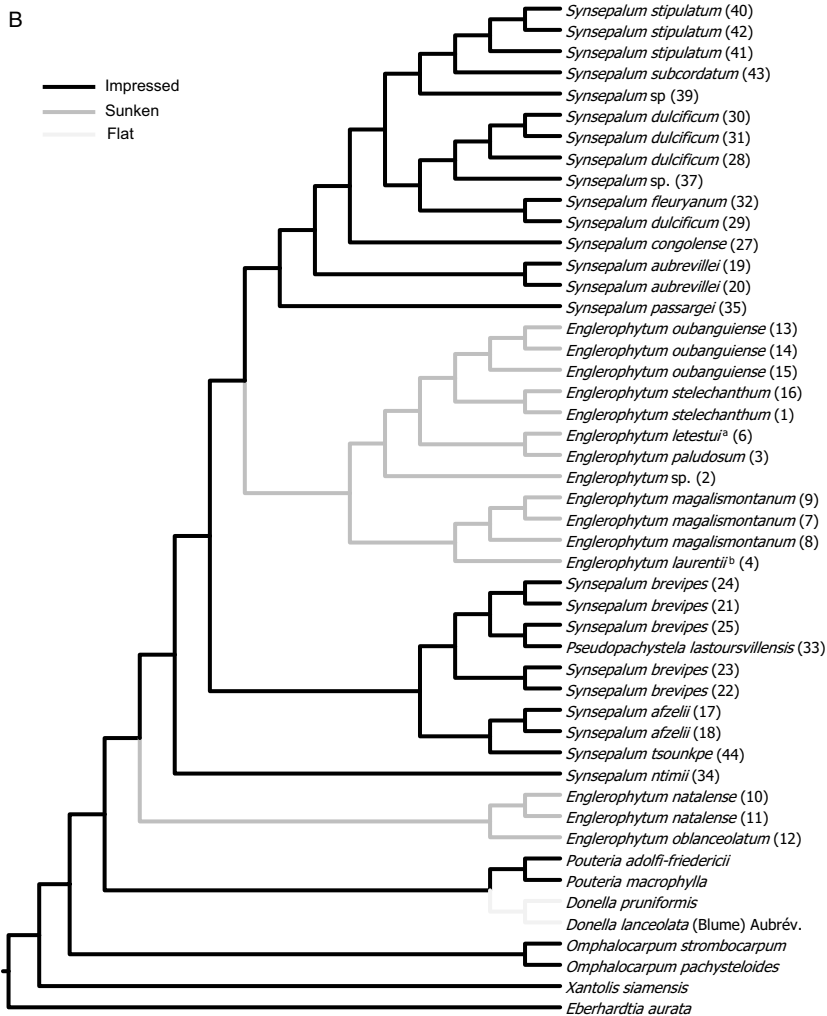


FIG. 3. (continued). B, Shape of midrib. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

represented by only a single exemplar. More samples of this species are needed to confirm its placement.

In summary, clade A in this analysis consists of six species (*Synsepalum aubrevillei*, *S. congolense*, *S. dulcificum*, *S. fleuryanum*, *S. stipulatum* and *S. subcordatum*) that share four consistent morphological characters, i.e. an oblique tertiary venation pattern, a long style, dentate staminodes and fused sepals. This clade, which includes the type species of the genus, shares strong similarities with Aubréville's delimitation of *Synsepalum*.

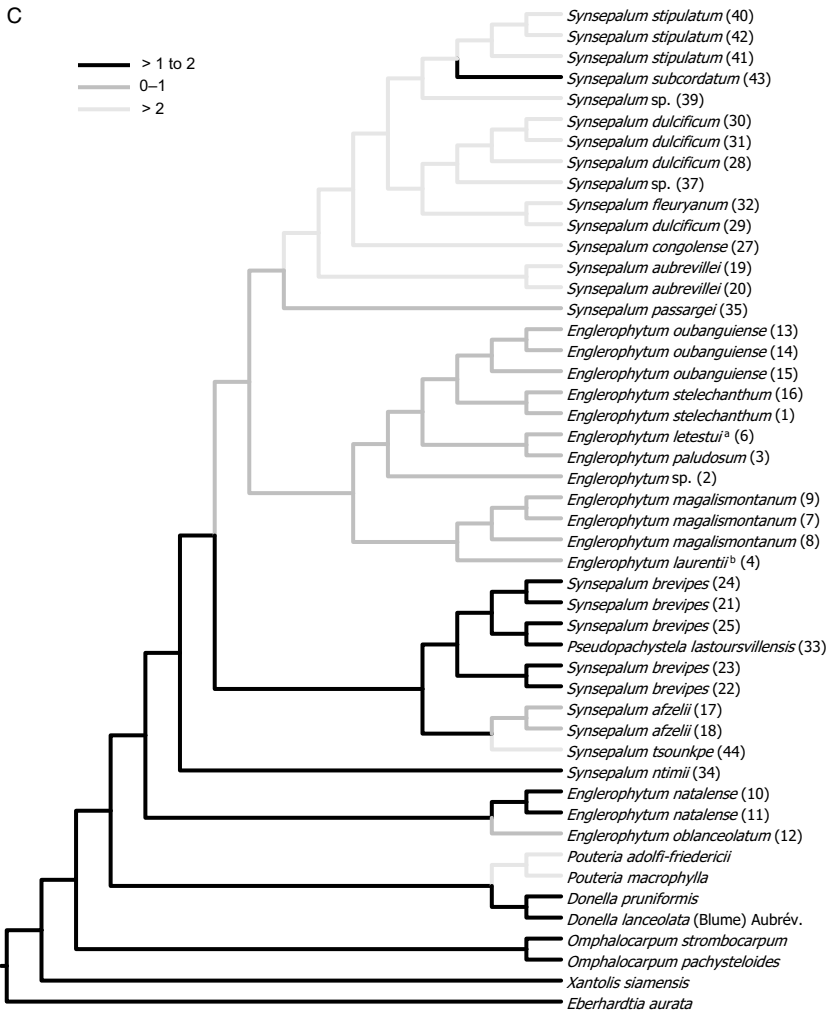


FIG. 3. (continued). C, Ratio of style length to ovary length. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Clade B

Clade B is represented by a single species, *Synsepalum passargei*. This species has unique floral characteristics not present in any of the other species analysed. It is characterised by an extremely small petal tube (< 1 mm); the tube is so small that in some literature, such as Aubréville (1961a), the tube is overlooked and the petals are termed “free”. Owing to the presence of a small tube, the ratio of lobe length to tube length for this species is large (lobes approximately 15 times longer than the tube, Fig. 2A). Additionally, the petals of

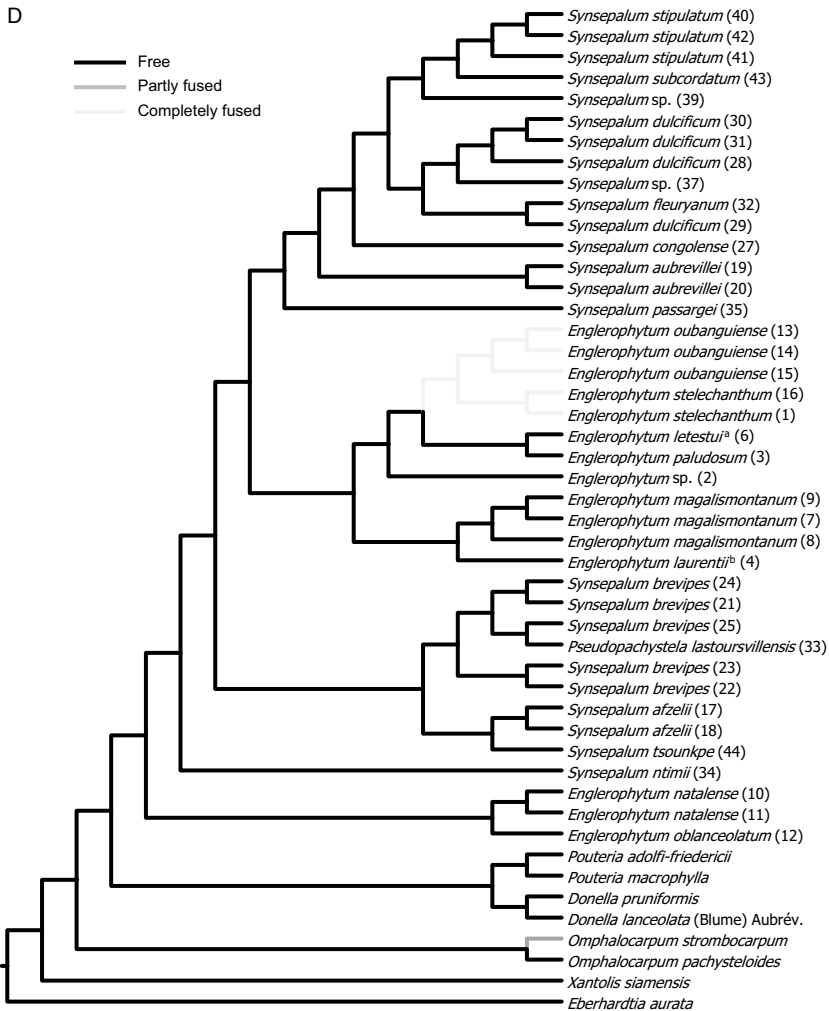


FIG. 3. (continued). D, Filament fusion. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Synsepalum passargei are unique in that they become strongly reflexed at maturity such that the apices of the corolla lobes nearly touch the pedicel when fully reflexed.

The second most prominent floral character of *Synsepalum passargei* is its large ovary relative to the size of the flower. The style is rather small, and therefore the ratio of style length to ovary length is less than 1 (Fig. 3C), which is rather unusual in the ES clade other than in clade C.

The androecium of *Synsepalum passargei* also has unique characters. Unlike the petals, which are reflexed, the alternipetalous staminodes and antepetalous stamens are erect and

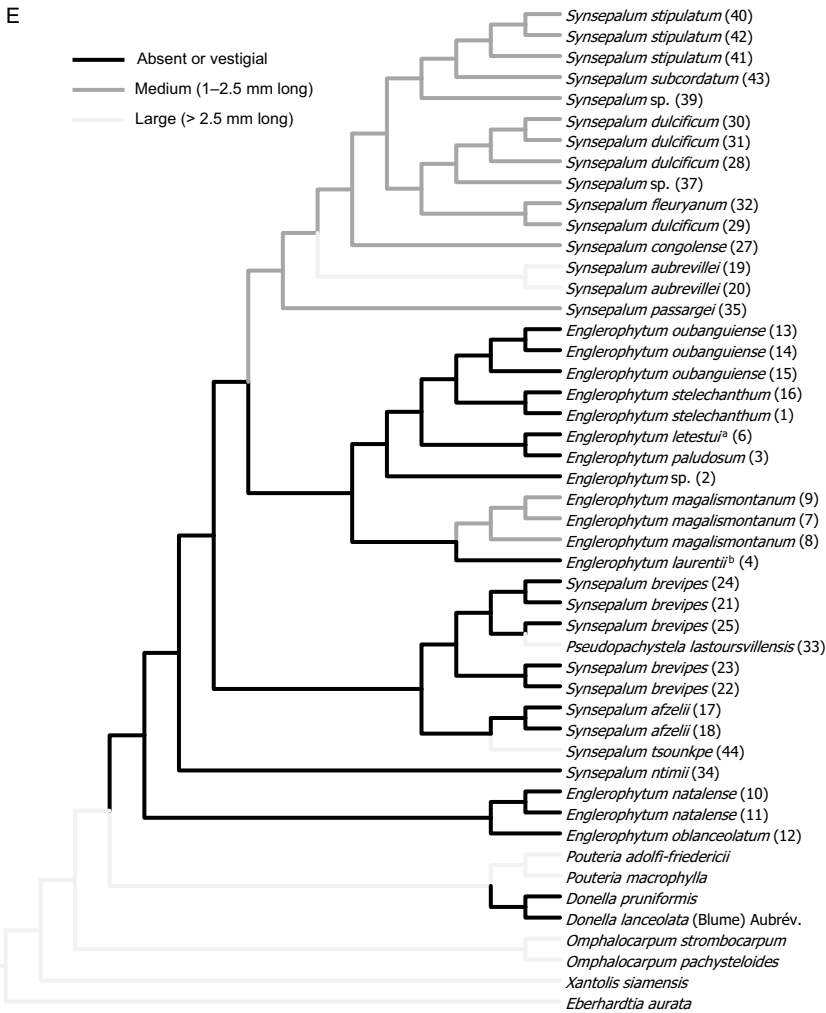


FIG. 3. (continued). E, Staminodes. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

immediately surround the massive ovary. The staminodes are linear, usually entire and approximately the same length as the petals. The presence of large, linear stamens was used by Aubréville to define *Vincentella*. The stamens of *Synsepalum passargei* have the smallest anthers of all the species included in the analysis, measuring less than 1 mm.

In this study, the anther dehiscence of *Synsepalum passargei* was noted to be latrorse. This observation agrees with depictions of *Synsepalum passargei* in the *Flora of East Tropical Africa* (Hemsley, 1968) but conflicts with other literature (Kupicha, 1978; Swenson & Anderberg, 2005), in which anther dehiscence is described as extrorse.

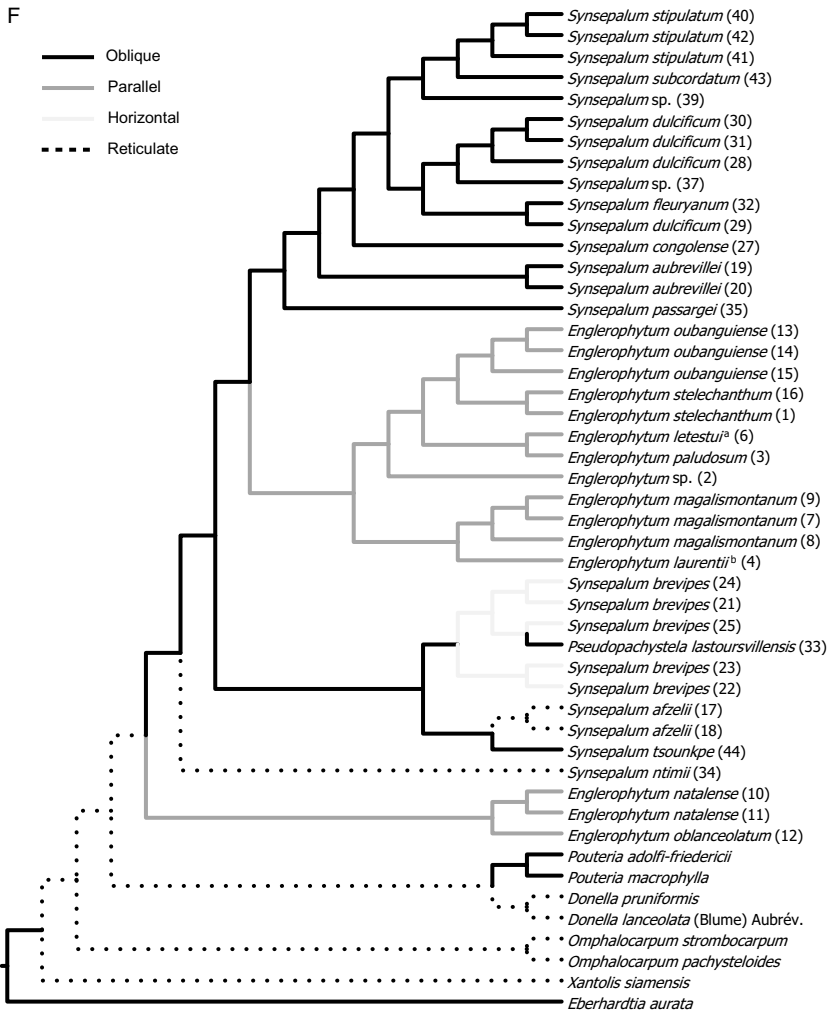


FIG. 3. (continued). F, Pattern of tertiary venation. Specimen numbers are shown in parentheses. ^aNew combination, made in this paper, for *Zeyherella letestui*. ^bNew combination, made in this paper, for *Wildemaniodoxa laurentii*.

Further material of the species, preferably fresh, should be analysed to resolve this discrepancy.

Although clade B was represented by only a single species in this analysis, several other species morphologically similar to *Synsepalum passargei* probably belong to this lineage, and further molecular work is required to verify this. They include *Synsepalum brenanii* (Heine) T.D.Penn., *S. muelleri* (Kupicha) T.D.Penn. and *S. revolutum*, morphologically similar species that have previously been grouped with *S. passargei* into a single genus, *Vincentella*, with *S. revolutum* as its type species (Aubréville & Pellegrin, 1934; Meeuse, 1960; Aubréville, 1964a; Kupicha, 1978). This genus was defined by slender

pedicels; sepals small, free nearly to the base, later patent or reflexed; corolla tube very short, the lobes many times longer; corolla lobes narrow and strongly reflexed; staminodes alternipetalous, narrowly linear, erect and as long as the corolla lobes; filaments erect, several times longer than the oblong-sagittate, minutely apiculate anthers; and ovary large, ovoid and villous (Meeuse, 1960). Most of the characteristics of *Vincentella* apply to *Synsepalum passargei* in clade B. Two other species, *Vincentella ogouensis* Aubrév. & Pellegr. and *V. ovatostipulata* (De Wild.) Aubrév. & Pellegr., which were also classified under *Vincentella* by Aubréville (1965) but which Pennington (1991) refrained from transferring to *Synsepalum*, also deserve further study to determine whether they belong to the clade B lineage.

In summary, clade B consists of the single species *Synsepalum passargei* and is characterised by (i) a reflexed corolla with a very short corolla tube, (ii) a large ovary, (iii) erect stamens with very small anthers (possibly latrorse), and (iv) erect, entire, linear alternipetalous staminodes.

Clades C and E

These two lineages consist solely of species currently belonging to the genus *Englerophytum* and are treated together because their members share several common morphological characters, especially in their vegetative parts. It is important to note that although clades C and E are collectively easily distinguishable from the other four major lineages, they are very difficult to distinguish from each other.

One of the reasons that Pennington (1991) grouped all members of clades C and E into *Englerophytum* was their characteristic leaf facies. Their leaves differ from those of *Synsepalum* (clades A, B, D and F) in two main aspects: (i) structure of the petiole and main vein, and (ii) pattern of venation. Leaves of *Englerophytum* have a sunken midrib that forms a channel along the lamina (Fig. 3B). This consequently affects the structure of the petiole as well as the apex of the leaf. The channelled petiole folds in on itself, forming a closed hollow groove, and at the apex the sunken main vein always extends slightly beyond the tip, forming a small mucro. This structure strongly contrasts with that of *Synsepalum*, in which the leaf has a petiole with an open groove and an impressed midrib, while lacking a mucronate tip. The pattern of venation in *Englerophytum* (clades C and E) is also very characteristic. The leaves have a brochidodromous (looping) pattern of venation with very closely parallel secondary veins. Owing to the close proximity of the looping veins to the leaf margin, the loops tend to form a submarginal vein. Additionally, all species in clades C and E have parallel intersecondary veins. These veins are situated between and initially of equal thickness to the secondaries but become thinner as they approach the leaf margin. The intersecondaries and secondaries are parallel to the tertiary veins, a pattern that is unique in the ES clade except for *Synsepalum nimii* W.D.Hawth. (clade F), which has reticulate tertiary veins.

Although vegetative characters clearly isolate clades C and E from the rest of the ES clade, floral characters are largely uniform within the ES clade. All members of subclades in the ES clade, except for *Wildemaniodoxa laurentii* and *Englerophytum paludosum* L.Gaut., Burgt & O.Lachenaud (the latter not represented in this study), have

5-merous flowers, and there is an irregular presence of staminodes and stamens attached at the top of the corolla throat. Therefore, it is more difficult to isolate clades C and E from the other clades by using floral characters.

A unique feature used by Pennington (1991) to separate *Englerophytum* from *Synsepalum* was the tendency towards fusion of the filaments into a cone-like structure enclosing the pistil. However, during the present study it became evident that filament fusion more appropriately defines a specific subgroup within clade C rather than both clades of *Englerophytum* together (Fig. 3D). This character, considered by Aubréville (1961a) as differentiating *Englerophytum* s. str. from the related *Wildemaniodoxa* Aubrév. & Pellegr. and *Zeyherella* (Pierre ex Baill.) Aubrév. & Pellegr., seems to have evolved within clade C. Of the species in our sampling, it is found in only *Englerophytum stelechanthum* (the type species of *Englerophytum*) and *E. oubanguiense* (Aubrév. & Pellegr.) Aubrév. & Pellegr. (see Fig. 3D). Nevertheless, filament fusion is still a very useful character for identification of some species, because it is rare in the ES clade. Other species from *Englerophytum* also showing filament fusion, and therefore included in *Englerophytum* s. str. by Aubréville (1961a), are *E. congolense* (De Wild.) Aubrév. & Pellegr. and *E. somiferanum* Aubrév. Furthermore, four of the five recently described species (Gautier *et al.*, 2016) also have fused filaments. All these should be included in a future study.

The remainder of clade C comprises species with free filaments and that were included by Aubréville (1961a, 1964a) in two genera: the monotypic *Wildemaniodoxa* (*W. laurentii*), separated on the basis of a 10-merous corolla, 10 stamens and a 10-celled ovary, and *Zeyherella* (comprising *Englerophytum magalismontanum*, *E. paludosum* and *Z. letestui*). In our study, *Wildemaniodoxa laurentii* is very close to *Englerophytum magalismontanum* and probably arose simply by doubling of the number of internal floral parts. Other species in *Zeyherella* not represented in this study include *Z. longepedicellata* (De Wild.) Aubrév. & Pellegr. and *Z. mayumbensis* (Greves) Aubrév. & Pellegr. Further work should focus on sampling these species to determine whether the doubling of number of floral parts evolved more than once in the ES clade. It is, however, important to note that although the two species with fused filaments included in this study group together in subclade C₁, this group also contains two species with free filaments, a character found in all members of clade C₃ (clade C₂ consists of a single unidentified sample for which no flowers were available for examination for filament fusion). Under such a topology, resurrecting *Zeyherella* based on free filaments or *Wildemaniodoxa* based on a 10-merous flower would make *Englerophytum* paraphyletic.

Although there are few differences in vegetative characters between clades C and E, there are two characters that, although not perfectly consistent throughout, might still prove helpful in some cases, namely number of secondary veins and leaf shape. Members of clade E usually have fewer than 28 secondary veins, whereas members of clade C usually have 28 or more (Fig. 2E). Leaf shape also shows some variation, being usually oblanceolate in clade C and obovate in clade E, although some overlap is present.

Distinguishing floral characters are also lacking between these clades. The most consistent differences include pedicel length and the ratio of style length to ovary length. Members of clade E usually have a very short pedicel, which appears sessile or subsessile

(Fig. 2B). In contrast, members of clade C have longer pedicels that raise the flower above the flowering branch or away from the trunk for cauliflorous species. The ratio of style length to ovary length varies because members of clade C usually have styles that are shorter than the ovary, whereas in clade E they are longer (see Fig. 3C). Aubréville & Pellegrin (1958) distinguished *Englerophytum natalense* (Sond.) T.D.Penn. and *E. oblanceolatum* (both clade E) as having petal lobes shorter than the petal tube. However, in the floral material analysed this character was not immediately evident, and more material is required to confirm it.

It is interesting to note that the two species in clade E have in the past been grouped together based on morphology. Aubréville & Pellegrin (1958) established the genus *Boivinella*, later changed to *Neoboivinella* Aubrév. & Pellegr. (Aubréville & Pellegrin, 1959), based on it being a later homonym. *Boivinella/Neoboivinella* contained *Englerophytum natalense* (the type species) and *E. oblanceolatum*, as well as *E. magalismontanum* (later removed from the genus, and clade C in our study). Members of the genus were distinguished by having (i) lobes smaller than the petal tube, (ii) shortly petiolate leaves, (iii) short filaments, (iv) absent or vestigial staminodia, and (v) a wide ventrifixed hilum. However, in a later publication Aubréville (1961a) changed the circumscription, leaving only *Englerophytum natalense* (then *Neoboivinella natalensis* (Sonder) Aubrév. & Pellegr.) in the genus. *Neoboivinella* was synonymised with *Englerophytum* by Pennington (1991). *Englerophytum natalense* and *E. oblanceolatum* (clade E) were also grouped together by Heine & Hemsley (1960) in *Bequaertiodendron* De Wild., a genus established by De Wildeman (1919) and including several other species now in *Englerophytum*, its type species being *B. congolense* De Wild. (= *E. congolense*). Heine & Hemsley distinguished these species on the basis of seed characters, namely the absence of endosperm and the presence of thick and fleshy planoconvex cotyledons. *Bequaertiodendron* is now also a synonym of *Englerophytum*. Should clade E be considered a separate genus, the correct name would be *Neoboivinella*.

In summary, clades C and E can collectively be distinguished from the rest of the ES clade by their distinctive leaf facies. The distinction between clades C and E is less obvious but the following characters may be useful: (i) leaf shape, (ii) number of secondary veins, (iii) pedicel length, and (iv) ratio of style length to ovary length.

Clade D

Clade D is composed here of four species: *Pseudopachystela lastoursvillensis*, *Synsepalum afzelii*, *S. brevipes* and *S. tsoukpe*. With the exception of *Synsepalum tsoukpe*, which has a number of morphological similarities with clade A species, members of this clade share a number of characters. They have inconspicuous tertiary venation (Fig. 2D), the veins being so fine that to determine their pattern, the leaves have to be viewed with transmitted light. This contrasts with leaves from clade A species, whose tertiaries are prominent and easily identifiable. Members of clade D, other than *Synsepalum tsoukpe*, have free sepals. Although this character is not exclusive to this clade, it is essential to distinguish its members from those of the morphologically similar clade A, in whose species the sepals

are fused. A third character shared by all members of clade D except *Synsepalum tsoumkpe* is the presence of staminodes with entire margins. Although *Synsepalum afzelii* and *S. brevipes* do not always have staminodes, when present, they are rudimentary with entire margins. *Pseudopachystela lastoursvillensis* also has staminodes with entire margins, but the staminodes of this species are larger than those of all other members of the clade. *Synsepalum tsoumkpe* differs from the other clade D species in having dentate staminodes.

In the available floral material of *Synsepalum afzelii* and *S. brevipes*, the anthers were found to be similarly narrowly sagittate. However, only line drawings of the other species in clade D were available, and it was unclear whether they shared the same anther structure. Therefore, further analysis is required to determine whether all members of this clade have narrowly sagittate anthers.

Clade D splits into two subclades. Subclade D₁ consists of *Synsepalum brevipes*, the type species of *Pachystela* Pierre ex Radlk., and *Pseudopachystela lastourvillensis*, the type species of *Pseudopachystela* Aubrév. & Pellegr. The latter was described by Aubréville (1961a) for two Gabonese species then sunk in synonymy with *Pachystela* three years later (Aubréville, 1964a) but without publication of the necessary combinations. Subclade D₂ consists of *Synsepalum afzelii* (the type species of *Afrosersalisia*) and *S. tsoumkpe* (included by Aubréville in his narrow circumscription of *Synsepalum* based on the morphological characters highlighted above). Further work on species included by Aubréville in *Pachystela* (*Pseudopachystela lastourvillensis*, *P. oyemensis* Aubrév. & Pellegr., *Synsepalum msolo*, *S. pobeguianum* (Dubard) Aké Assi & L.Gaut. and *S. seretii* (De Wild.) T.D.Penn.) and *Afrosersalisia* (*S. afzelii*) is needed before the taxonomy of this group can be finalised.

In summary, members of clade D have the following synapomorphies: (i) inconspicuous tertiary venation, (ii) free sepals, and possibly (iii) narrowly sagittate anthers.

Clade F

Clade F is composed of a single species, *Synsepalum ntimii*, that it has been suggested could represent an undescribed genus (W. D. Hawthorne, University of Oxford, personal communication, 2013). A short informal description of the species can be found in Hawthorne & Jongkind (2006), and it has since been formally described (Hawthorne, 2014).

The leaves of *Synsepalum ntimii* are clearly distinguishable from those of the other members of the ES clade. They differ from the leaves of other *Synsepalum* species (clades A, B and D) in their brochidodromous (rather than eucamptodromous) pattern of venation, which they share with the leaves of species in clades C and E. However, unlike the leaves of members of those clades, the leaves of *Synsepalum ntimii* have an impressed midrib and tertiary veins that are reticulate rather than parallel to the secondaries. Additionally, they have an average of 15 secondary veins, whereas those of members of clades C and E all have more than 15. Another distinguishing feature of *Synsepalum ntimii* is the glabrous and slightly shiny underside of the leaf, clearly distinguishing it from species of *Englerophytum* in clades C and E, the leaves of which generally have a velvety indumentum dorsally.

The lack of trichomes on the midrib is also very characteristic of the clade F lineage, because these are usually present in members of the ES clade (see Fig. 2C).

Hawthorne was unsure as to which genus would best accommodate this morphologically distinct species (personal communication, 2013), and its morphological uniqueness is indeed corroborated by its phylogenetic position. Of all the species examined, *Synsepalum ntimii* has the longest branch length in the ITS parsimony tree (figure available from the corresponding author). Hawthorne made the decision to place *Synsepalum ntimii* in *Synsepalum* only after Pennington advised him to recognise a very broad generic circumscription of this genus. Further study may reveal that the distinct lineages within the ES clade deserve generic status, in which case *Synsepalum ntimii* would represent an undescribed genus from tropical West Africa.

In summary, *Synsepalum ntimii* can be distinguished from the other clades by (i) brochidodromous venation with reticulate secondary venation, (ii) the average number of 15 secondary veins, and (iii) its glabrous, slightly shiny dorsal leaf surface.

CONCLUSIONS

A summary of all the major lineages within the ES clade and their diagnostic characters is shown in Table 8. Several lineages identified in this analysis have a number of shared features that enable them to be distinguished. However, more morphological and molecular support is required before it is possible to make final taxonomic decisions regarding the placement of species in this complex. Future studies should focus on resolving the lack of support at the base of the ES clade. This may provide further insights into whether the current circumscription of the genera is correct or if the taxonomy needs revision. Nevertheless, the six lineages we have described here within the ES clade are rather morphologically distinct entities, and if further study reveals additional synapomorphies, consideration should be given to the resurrection of some genera that in the past had very similar circumscriptions to some clades in our phylogenetic tree (e.g. *Afrosersalisia*, *Neoboivinella*, *Pachystela* and *Vincentella*). *Wildemaniodoxa* and *Zeyherella* are confirmed as synonyms of *Englerophytum*.

New combinations

As a result of this study, we propose five new combinations for the species of *Wildemaniodoxa* and *Zeyherella* that were not transferred to *Englerophytum* by Pennington (1991).

Englerophytum laurentii (De Wild.) L.Gaut., **comb. nov.**

Chrysophyllum laurentii De Wild., Miss. Em. Laur. 1: 429 (1907).

Wildemaniodoxa laurentii (De Wild.) Aubrév. & Pellegr., Notul. Syst. (Paris) 16: 251 (1961).

TABLE 8. Shared characters of the major lineages within the *Englerophytum*–*Synsepalum* clade

Clade	Constituent taxa	Characters shared within individual clade	Characters shared by across multiple clades
A	<i>S. aubrevillei</i> <i>S. congolense</i> <i>S. dulcificum</i> <i>S. fleuryanum</i> <i>S. stipulatum</i> <i>S. subcordatum</i>	(i) Oblique tertiary venation (ii) Fused calyx (iii) Long style (iv) Denticulate staminodes	NA
B	<i>S. passargei</i>	(i) Large ovary (ii) Strongly reflexed petal lobes (iii) Very short corolla tube (iv) Anthers < 1 mm (v) Erect linear staminodes	NA
C	<i>E. laurentii</i> ^a <i>E. letestui</i> ^b <i>E. magalismontanum</i> <i>E. oubanguiense</i> <i>E. paludosum</i> <i>E. stelechanthum</i>	(i) More than 28 secondary veins (ii) Style shorter than ovary (iii) Pedicellate flowers (iv) Usually oblanceolate leaves	Clades C and E (i) Brochidodromous venation with parallel tertiary veins (ii) Sunken midrib (iii) Petiole with closed groove (iv) Mucronate leaf tip (v) Submarginal vein (vi) Intersecondary veins
E	<i>E. natalense</i> <i>E. oblanceolatum</i>	(i) 17–27 secondary veins (ii) Style longer than ovary (iii) Sessile or subsessile flowers (iv) Usually obovate leaves	
D	<i>Pseudopachystela lastoursvillensis</i> <i>S. afzelii</i> <i>S. brevipes</i> <i>S. tsoukpe</i>	(i) Inconspicuous tertiary venation (ii) Calyx free (iii) Entire staminodes [(iv) Narrowly sagittate anthers]	NA
F	<i>S. ntimii</i>	(i) Brochidodromous venation with reticulate tertiary venation (ii) 15 or fewer secondary veins (iii) Glabrous leaf underside	NA

NA, not applicable.

^a New combination, made in this paper, for *Wildemaniodoxa laurentii*.

^b New combination, made in this paper, for *Zeyherella letestui*.

***Englerophytum laurentii* var. *lundense* (Cavaco) L.Gaut., comb. nov.**

Chrysophyllum laurentii var. *lundense* Cavaco, Comp. Diam. Angola, Public. Cultur. 42: 117 (1959).

Wildemaniodoxa laurentii var. *lundensis* (Cavaco) Liben, Bull. Jard. Bot. Natl. Belg. 58: 556 (1988).

Englerophytum letestui (Aubrév. & Pellegr.) L.Gaut., **comb. nov.**

Zeyherella letestui Aubrév. & Pellegr. Notul. Syst. (Paris) 16: 257 (1961), non *Englerophytum letestui* Aubrév. & Pellegr., Notul. Syst. (Paris) 16: 255 (1961), nom. inval., sine descr. lat.

Englerophytum longepedicellatum (De Wild.) L.Gaut., **comb. nov.**

Chrysophyllum longepedicellatum De Wild., Miss. Em. Laur. 1: 431 (1907).
Zeyherella longepedicellata (De Wild.) Aubrév. & Pellegr., Notul. Syst. (Paris) 16: 257 (1961).

Englerophytum mayumbense (Greves) L.Gaut., **comb. nov.**

Sideroxylon mayumbense Greves, J. Bot. 65 (Suppl. 2): 71 (1927).
Zeyherella mayumbensis (Greves) Aubrév. & Pellegr., Notul. Syst. (Paris) 16: 259 (1961).

The two species of *Pseudopachystela* (*P. lastoursvillensis*, the type of the genus and included in this study, and *P. oyemensis*) will have to be combined under *Afrosersalisia*, *Pachystela* or *Synsepalum* depending on the results of further phylogenetic studies and on the generic concepts that are chosen. For now, we have no objective reason to favour any of these solutions, and therefore refrain from making new combinations.

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