

# Molecular systematics and evolutionary trends and relationships in the genus *Jumellea* (Orchidaceae): Implications for its species limits

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**Abstract** *Jumellea* is an orchid genus centered on Madagascar but also occurs on some of the neighboring oceanic islands (the Mascarenes and Comoros) and in southern and eastern Africa. Prior to our study the genus contained ca. 55 morphologically distinct species, of which six are found in the Comoros (three endemic, three shared with Madagascar), nine in the Mascarenes (four endemic, four shared with Madagascar), two in southern and eastern Africa (both endemic), and 41 species endemic to Madagascar. We perform Bayesian and parsimony phylogenetic analyses of *Jumellea* based on combined chloroplast (*matK*, *trnL-F*, *rps16*, *ycf1*) and nuclear (nrITS) data from 60 specimens representing 47 species, four subspecies, and two varieties of *Jumellea*: (1) to assess the phylogenetic value of growth form, leaf, bract and lip shape, and spur length, presently used for recognizing informal groups within the genus; and (2) to test the monophyly of some variable species (e.g., *J. gracilipes*, *J. lignosa*). We find no support for the informal groups of *Jumellea*, as all the characters tested are evolutionarily labile. *Jumellea lignosa* (comprising *J. lignosa* subsp. *lignosa*, subsp. *tenuibracteata*, subsp. *acutissima*, and subsp. *latilabia*) is not monophyletic unless subsp. *tenuibracteata* is excluded. *Jumellea gracilipes* s.l. (including *J. ambongensis*, *J. imerinensis*, and *J. unguicularis*) is polyphyletic. As a result, we resurrect these three latter species, and recognize *J. lignosa* subsp. *tenuibracteata* at species level. Furthermore, we propose new circumscriptions for the following species: the Comorian *J. arachnantha* (including the Malagasy *J. sagittata*); the Reunionese *J. exilis* (including the Malagasy *J. flavescens*); the Reunionese *J. recta* (including the Malagasy *Jumellea* sp. 1); the Reunionese *J. recurva* (including the Malagasy *J. pandurata*); and the Reunionese *J. stenophylla* (including the Malagasy *J. gracilipes* 2 and 3). Finally, *Jumellea arborescens* and *J. maxillarioides* are recorded from Madagascar and the Comoros. Finally, the number of species of *Jumellea* has now increased from 55 to 57: seven species in the Comoros (four shared with Madagascar), nine species in the Mascarenes (four shared with Madagascar), two species in Africa, and 39 species, three subspecies, and two varieties restricted to Madagascar.

**Keywords** chloroplast data; *Jumellea*; molecular phylogeny; new species circumscriptions; nuclear data; Orchidaceae; western Indian Ocean

**Supplementary Material** Figure S1 (in the Electronic Supplement) and the alignment files are available in the Supplementary Data section of the online version of this article (<http://www.ingentaconnect.com/content/iapt/tax>).

## ■ INTRODUCTION

*Jumellea* Schltr. (Orchidaceae) is a predominantly Malagasy genus that has diversified on some island groups of the western Indian Ocean and in southern and eastern Africa. Its position in subtribe Angraecinae (tribe Vandae, subfamily Epidendroideae) has been confirmed by molecular data (e.g., Chase & al., 2003; Hidayat & al., 2005; Carlsward & al., 2006; Micheneau & al., 2008). Since its establishment (Schlechter, 1914), prior to our study, the number of species of *Jumellea* had increased to ca. 55, of which 41 species are found in Madagascar (Cribb & Hermans, 2009), nine in the Mascarenes (four shared with Madagascar, Rakotoarivelo & al., unpub.), six in the Comoros (three shared with Madagascar, Rakotoarivelo & al., unpub.), and two in southern and eastern Africa (Wood,

1982; La Croix, 2000). Micheneau & al. (2008), who used sequence data from chloroplast markers (*matK*, *rps16*, *trnL-F*) and investigated 20 species of *Jumellea*, demonstrated the monophyly of the genus and revealed its close relationships to *Angraecum* Bory s.l. and *Aeranthus* Lindl. The phylogenetic relationships within *Jumellea* were not addressed in this study due to the lack of resolution and limited sampling.

So far, no formal infrageneric classification of *Jumellea* has been proposed. Perrier de La Bâthie (1941) established the first identification key for the Malagasy and Comorian species of *Jumellea*, which were grouped into many small informal groups, using a number of characters, namely growth habit, leaf, bract and lip shapes, and spur length. He utilized spur length (species with spurs less than 2 cm long [Group 1] versus those with spurs more than 10 cm long) as the primary character and leaf

shape (species with cylindrical leaves [Group 2] versus those with non-cylindrical leaves) to further divide the species with spurs longer than 10 cm into two groups. The presence/absence of stems (acaulescent species [Group 3] versus caulescent species) was used to further divide the species with non-cylindrical leaves into two groups. The caulescent species were divided into two groups based on thickness of peduncle sheaths and bracts (species with thick peduncle sheaths and bracts [Group 4] versus those with thin ones); this latter group was sorted into two groups based on lip shape (species with lip with two enlargements [Group 5] versus species with lip without two enlargements). Finally, the species without lip with two enlargements were classified into two groups based on leaf length: those with large leaves (16–35 mm long; Group 6) and those with small leaves (5–15 mm long; Group 7). It is worth noting that dividing the Malagasy species of *Jumellea* into two groups (Group 1 and Group 2) on the basis of spur lengths as done by Perrier de la Bâthie is problematic, because this grouping excludes the species with spur lengths between 2 and 10 cm. Stewart & al. (2006) proposed another identification key to Malagasy, Comorian, and Mascarene species of *Jumellea* and divided the genus into six groups, using leaf and stem length as primary characters and spur length as secondary character. They divided the species of *Jumellea* with short stems into two groups: those with very large stems and inflorescences arising from the fan of leaves (Group 1) and those with rather short stems bearing small leaves very close together (Group 2). Plants with elongated stems were divided into four groups based on spur length. Ten species having spurs shorter than 1.5 cm were put in Group 3; three species having spur lengths between 2.5 and 4.5 cm were put in Group 4; one species having spur lengths between 6.5 and 8.0 cm were placed in Group 5; and lastly 18 species having spurs longer than 10 cm were grouped in Group 6. Like Perrier de la Bâthie's grouping, Stewart & al.'s groupings based on spur lengths are also problematic, as there are few species with elongated stems that cannot be placed in their Groups 3–6. It is important to note that neither Perrier de la Bâthie (1941) nor Stewart & al. (2006) claimed that their informal groups of *Jumellea* were natural. The phylogenetic value of the characters used for recognizing the informal groups in *Jumellea* is here tested with molecular data.

Species limits in *Jumellea* have been controversial for some Malagasy species, namely *Jumellea gracilipes* Schltr. and *J. lignosa* (Schltr.) Schltr. as defined by Perrier de la Bâthie (1938). The Malagasy *Jumellea ambongensis* Schltr., *J. exilipes* Schltr., *J. imerinensis* Schltr., *J. unguicularis* Schltr., and *J. gracilipes* Schltr. were all described by Schlechter (1922, 1925) as different species, but Perrier de la Bâthie (1941) merged all of them in his broadly delimited *J. gracilipes*, based on similarities in spur length and growth habit. This has rendered the latter species morphologically highly heterogeneous. The Malagasy *Jumellea lignosa* as delimited by Perrier de la Bâthie (1938) contains four subspecies: subsp. *acutissima*, subsp. *latilabia*, subsp. *tenuibracteata*, and subsp. *lignosa*. These subspecies differ in leaf shape, spur length, and geographic distribution. Therefore, the monophyly of *J. gracilipes* and *J. lignosa* is here tested with molecular data.

The objective of the present study is to reconstruct a robust molecular phylogeny of *Jumellea* based on sequence data from four chloroplast regions (*matK*, *trnL-F*, *rps16*, *ycf1*) and the nuclear ribosomal internal transcribed spacer (nrITS). The resulting phylogenetic tree is used: (1) to assess the phylogenetic value of the characters used by Perrier de la Bâthie (1941) and Stewart & al. (2006) for delimiting their informal groups (i.e., to test if these characters define monophyletic groups); and (2) to test the monophyly of some variable species (e.g., *J. gracilipes* and *J. lignosa*).

## ■ MATERIALS AND METHODS

**Taxon sampling.** — We sampled a total of 62 specimens, representing 47 species, four subspecies, and two varieties of *Jumellea*, and two outgroups. Each *Jumellea* taxon was represented by one specimen, with the exception of the species shared by either Madagascar and the Comoros archipelago or Madagascar and the Mascarene archipelago. The species with problematic delimitations, *J. lignosa* and *J. gracilipes*, respectively, were represented by four and five specimens. All the voucher information about the investigated taxa is summarized in the Appendix.

**DNA extraction and amplification.** — The collected samples of leaves were preserved in silica gel prior to DNA extraction. Total DNA of each specimen was extracted from 20 mg of the dried leaf sample using the DNeasy Plant Tissue Kit (Qiagen, Valencia, California, U.S.A.). DNA amplification and sequencing were carried out using primers listed in Table 1. We chose the chloroplast markers *matK*, *rps16* and *trnL-F*, as they revealed variation among the Angraecoids species (Carlswald & al., 2006; Micheneau & al., 2008); this allowed us to include the published sequences of *Jumellea*. We added *ycf1* and nrITS to increase the phylogenetic resolution within *Jumellea*. PCR reactions were carried out in a reaction volume of 25  $\mu$ L, containing 10 ng of DNA, 2.5  $\mu$ L PCR buffer  $\times$ 10 (ABgene, Epsom, Surrey, U.K.), 1.5 mM MgCl<sub>2</sub> (ABgene), 0.2 mM dNTP, 2 U *Taq* DNA Polymerase (Red Hot DNA polymerase, ABgene), and 0.2  $\mu$ M of each primer. PCR amplifications were performed with the GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, California, U.S.A.), and the details of the protocols are summarized in Table 2. The PCR products were run electrophoretically in a 2% agarose TBE gel stained with GelRed Nucleic Acid Gel (FluoProbes, Montluçon, France).

**Sequence editing and alignment.** — Purified PCR products were subsequently sequenced in both directions using the same primers (Table 1). The resulting chromatograms were assembled, edited, and aligned using the 65% similarity cost matrix and the global alignment with free end gaps in Geneious v.5.1 (Drummond & al., 2010). Indels in the plastid sequences were coded with the “simple gap coding” method described by Simmons & Ochoterena (2000) implemented in SeqState v.1.4.1. (Müller, 2005). GenBank accession numbers are provided in the Appendix.

**Phylogenetic analysis.** — We performed separate and combined analyses of the sequence data from the four chloroplast genes (*matK*, *trnL-F*, *rps16*, *ycf1*) and the nrITS region,

using both maximum parsimony (MP) and Bayesian methods. MP analyses were performed with PAUP\* v.4.0b10 (Swofford, 2002) using the following settings: heuristic searches, tree bisection-reconnection (TBR), 1000 replicates, and random sequence addition. Best trees per replicate were saved to reduce time spent in swapping on large islands of trees. All characters were equally weighted and unordered (Fitch, 1971). A total of 1000 parsimony bootstrap replicates were performed to evaluate the internal support of clades (Felsenstein, 1985), using TBR branch swapping and saving all trees from replicates.

Bayesian phylogenetic inferences of the separate and combined data were performed using MrBayes v.3.1.2 (Ronquist & Huelsenbeck, 2003) implemented in the AIR package (Kumar & al., 2009). For the combined analyses the data were partitioned by gene, for a total of five data partitions (*matK*, *trnL-F*, *rps16*, *ycf1*, nrITS). The best-fitting model for each partition was selected using jModeltest v.0.1 (Posada, 2008) under the corrected Akaike information criterion (AIC; Akaike, 1973). The nucleotide substitution models attributed to each gene partition are summarized in Table 3. Two independent

**Table 1.** Primers used for amplification and sequencing of new sequences in this study.

Gene region	Primer	Primer sequence	Reference
nrITS	17SE (forward)	5' ACG AAT TCA TGG TCC GGT GAA GTG TTC 3'	Sun & al., 1994
	26SE (reverse)	5' TAG AAT TCC CCG GTT CGC TCG CCG TTA C 3'	Sun & al., 1994
<i>matK</i>	matK-19F	5' CGT TCT GAC CAT ATT GCA CTA TG 3'	Molvray & al., 2000
	matK-1326R	5' TCT AGC ACA CGA AAG TCG AAG T 3'	Cuénoud & al., 2002
	matK-390F	5' CGA TCT ATT CAT TCA ATA TTT C 3'	Cuénoud & al., 2002
	trnK-2R	5' AAC TAG TCG GAT GGA GTA G 3'	Johnson & Soltis, 1994
<i>trnL-F</i>	trnL-c (forward)	5' CGA AAT CGG TAG ACG CTA CG 3'	Taberlet & al., 1991
	trnL-d (reverse)	5' GGG GAT AGA GGG ACT TGA AC 3'	Taberlet & al., 1991
	trnL-e (forward)	5' GGT TCA AGT CCC TCT ATC CC 3'	Taberlet & al., 1991
	trnL-f (reverse)	5' ATT TGA ACT GGT GAC ACG AG 3'	Taberlet & al., 1991
<i>rps16</i>	rps16-1F	5' GTGGTAGAAAGCAACGTGCGACTT 3'	Oxelman & al., 1997
	rps16-2R	5' TCG GGA TCG AAC ATC AAT TGC AAC 3'	Oxelman & al., 1997
<i>ycf1</i>	ycf1-3720F	5' TAC GTA TGT AAT GAA CGA ATG G 3'	Neubig & al., 2009
	ycf1-5500R	5' GCT GTT ATT GGC ATC AAA CCA ATA GCG 3'	Neubig & al., 2009

**Table 2.** PCR reaction programs and evolution models of the amplified gene regions.

	nrITS	<i>matK</i>	<i>rps16</i>	<i>trnL-F</i>	<i>ycf1</i> (touchdown PCR)	
Initialization	94°C – 2 min 30 s	94°C – 2 min 30 s	94°C – 2 min 30 s	94°C – 2 min 30 s	94°C – 3 min	
Denaturation	94°C – 1 min	94°C – 1 min	94°C – 1 min	94°C – 30 s	94°C – 30 s	94°C – 30 s
Annealing	55°C – 30 s	52°C – 45 s	58°C – 30 s	56°C – 30 s	60 à 51°C – 1 min	50°C – 1 min
Extension	72°C – 1 min	72°C – 30 s	72°C – 1 min	72°C – 1 min	72°C – 3 min	72°C – 3 min
Final extension	72°C – 7 min	72°C – 7 min	72°C – 7 min	72°C – 7 min		72°C – 3 min
Number of cycles	26	29	28	29	8+18	
jModelTest	HKY+G	GTR+G	GTR+G	GTR+G	GTR+G	

**Table 3.** Matrix values, parsimony statistics, and Model of evolution used in Bayesian analysis of the nrITS, *trnL-F*, *matK*, *rps16*, and *ycf1* gene regions, and the combined dataset.

	nrITS	<i>matK</i>	<i>rps16</i>	<i>trnL-F</i>	<i>ycf1</i>	Combined
Number of taxa (number of <i>Jumellea</i> )	61 (59)	62 (60)	62 (60)	61 (59)	54 (52)	62 (60)
Aligned lengths	564	1372	780	1275	1578	5569
Parsimony non-informative characters	66	82	41	103	144	398
Parsimony-informative characters	67	58	27	81	87	312
% informative characters	11.88	4.22	3.46	6.35	5.51	5.60
Consistency index (CI)	0.62	0.85	0.84	0.76	0.81	0.68
jModelTest	HKY+G	GTR+G	GTR+G	GTR+G	GTR+G	GTR+G

Markov chain Monte Carlo (MCMC) runs were conducted for 10 million generations, sampled every 1000 generations. Each run was distributed across four chains with heating parameter of 0.2. Convergence of the individual chains was confirmed by inspecting the average standard deviation of split frequencies, and by plotting likelihood values across generations using Tracer v.1.5.0 (Rambaut & Drummond, 2009). The two runs were combined after the removal of the first 5000 trees as burn-in. Majority-rule consensus trees were constructed from the remaining trees to estimate Bayesian clade credibility and visualized with FigTree v.1.3.1 (Rambaut, 2010).

**Ancestral state reconstruction.** — To assess the phylogenetic value of spur length, growth habit, and leaf, bract and lip shapes as used by Perrier de La Bâthie (1941) and Stewart & al. (2006) for keying out the Malagasy and Comorian species of *Jumellea*, ancestral state reconstructions of these characters were performed by tracing their respective states over the 50% Bayesian majority-rule consensus tree from the combined analyses. We used the parsimony reconstruction method as implemented in Mesquite v.2.01 (Maddison & Maddison, 2010), as our main goal was to test if the characters used by Perrier de La Bâthie (1941) and Stewart & al. (2006) define monophyletic groups. Character states used in this study were taken from herbarium specimens and/or from the literature (Perrier de La Bâthie, 1941; Stewart & al., 2006). We coded spur length as follows:  $\leq 8$  cm (short = 0) and  $> 8$  cm (long = 1). In addition, *Jumellea* contains three types of growth habit: species with short, erect stems up to 5 cm in length (sometimes described as acaulescent) (= 0); long, creeping (scrambling) stems (= 1); and species with long, erect stems between 6 and 100 cm (= 2). The leaves of the species of *Jumellea* can be either oblong (= 0) or elliptic (= 1). Floral bracts were coded as tubular (= 0) or sigmoid, curving in the upper part and acuminate at the apex (= 1). The sigmoid bracts are mostly persistent and intact in dried plants. Finally, we coded the lip shape as ovate (= 0), lanceolate (= 1), or sagittate (= 2).

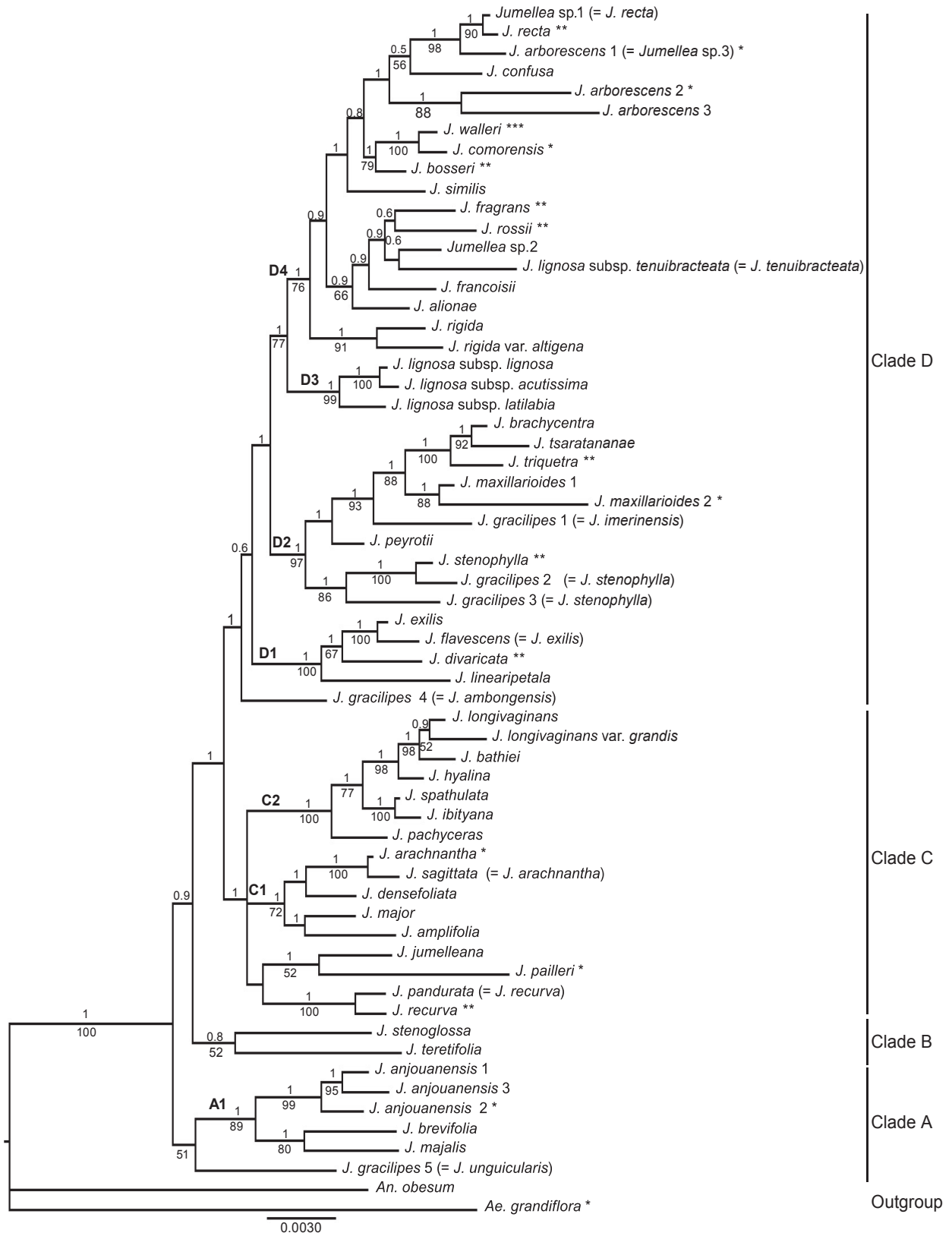
**Species delimitation.** — Our main criterion for evaluating the status of species of *Jumellea* is based on the principle of monophyly. Specimens that are similar morphologically and form a clade in our phylogenetic analysis (de Queiroz & Donoghue, 1988; Cracraft, 2000) are recognized as one species. If these specimens, however, do not form a monophyletic group despite their morphological similarities, they are recognized as different species. This is also coupled with relative sequence divergence.

## ■ RESULTS

**Sequence characteristics.** — The sequences from nrITS are the shortest (564 bp) and those from *ycf1* the longest (1578 bp); nrITS is the most variable region with 11.88% informative sites (Table 3); and *rps16* is the least variable (3.46% informative sites). The alignment is straightforward with the exception of the *trnL-F* matrix, which contains many gaps and indels, consistent with other orchids groups (e.g., Bellstedt & al., 2001; Bytebier & al., 2007; Micheneau & al., 2008; Waterman & al., 2009).

Given that the repeated sequences vary in length and contain very little phylogenetic information, they were not coded as indels. As a result, only the informative indels, varying among sequences, were coded at the end of the aligned matrices. The shortest sequence for all combined datasets is about 3210 bases long (*J. walleri* (Rolfe) la Croix—due to missing *ycf1* and nrITS sequences) and the longest is about 5329 bases long (*J. longivaginata* H. Perrier). Overall, the percentage of informative characters is relatively low compared to the total length of sequence (5.6% informative sites). We were not able to obtain sequences of *trnL-F* for *J. stenoglossa* H. Perrier, nrITS for *J. walleri*, and *ycf1* for *J. arborescens* H. Perrier 1, 2 and 3, *J. bosseri* Paillet, *J. lignosa* subsp. *lignosa*, *J. lignosa* subsp. *tenuibracteata*, *Jumellea* sp. 1, *J. recta* (Thouars) Schltr., and *J. walleri*.

**Phylogenetic analyses.** — The tree data and statistics from the separate and combined MP analyses are summarized in Table 3. The separate MP analyses for each dataset (*matK*, *trnL-F*, *rps16*, *ycf1*, nrITS) show that the trees have similar overall topologies (results not presented). Hence, we combined all datasets in one large matrix. The combined Bayesian analysis shows that the average standard deviation of split frequencies reaches 0.005 after 10 million generations. Credible sets of trees contain 4865 samples, which are used for calculating tree probabilities. The two runs converged with a potential scale reduction factor (PSRF) tending to 1.001 (estimated marginal likelihood =  $-14,837.66$ ). The 50% majority-rule consensus tree from the Bayesian analysis of the combined data is shown in Fig. 1 (strict consensus MP is depicted in Fig. S1, Electronic Supplement). The genus *Jumellea* forms a strongly supported clade (bootstrap support, BS = 100%; posterior probability, PP = 1). Out of 59 nodes 32 are supported with BS  $\geq 75\%$ . Out of 59 nodes 50 are supported by PP  $\geq 0.9$ . *Jumellea* consists of four major lineages, clades A–D (Fig. 1). Clade A (*J. gracilipes* 5 to *J. anjouanensis* 1 (Finet) H. Perrier) forms a poorly supported monophyletic group which is sister to the remainder of *Jumellea* (BS = 51). Clade B (*J. stenoglossa*, *J. teretifolia* Schltr.; PP = 0.8; BS = 52) is sister group of the large clades C and D. Clade C (*J. recurva* (Thouars) Schltr. to *J. longivaginata*; PP = 1) and clade D (*J. gracilipes* 4 to *Jumellea* sp. 1, PP = 1) form sister groups. In addition, none of the informal groups used in the keys to species of *Jumellea* (Perrier de La Bâthie, 1941; Stewart & al., 2006) are supported as monophyletic. However, a number of small lineages are well supported: subclades A1, C1–2, and D1–4. Furthermore, *J. lignosa* subsp. *tenuibracteata* does not form a clade with the other three subspecies (*J. lignosa* subsp. *acutissima*, *J. lignosa* subsp. *latilabia*, *J. lignosa* subsp. *lignosa*), which form a highly supported group (subclade D3); *J. lignosa* subsp. *tenuibracteata* is nested in subclade D4 and forms a clade with *Jumellea* sp. 2, *J. fragrans* (Thouars) Schltr., *J. rossii* Senghas, and *J. francoisii* Schltr. (BS < 50%; PP = 0.9). The sampled *J. gracilipes* (1–5), as circumscribed by Perrier de la Bâthie (1941), are not resolved as a clade. *Jumellea gracilipes* 4 is resolved as sister to the remainder of clade D (BS < 50%; PP = 1). *Jumellea gracilipes* 1, 2 and 3 are nested within subclade D2, while *J. gracilipes* 5 is poorly supported as sister to subclade A1. Several species from the oceanic islands that exhibit striking morphological affinities with some Malagasy species are shown to be closely



**Fig. 1.** Fifty percent majority-rule consensus tree resulting from Bayesian analysis of the combined dataset of *trnL-F*, *matK*, *rps16*, *ycf1* and nrITS sequences. Bootstrap and posterior probability values are shown below and above branches, respectively. Clades discussed in the text are indicated to the right (clades A, B, C, and D). Subclades discussed in the text are indicated to the left (A1, C1, C2, D1, D2, D3, D4). Names of newly accepted species in this study are in parentheses. \*: specimens collected from the Comoros; \*\*: specimens collected from the Mascarenes; \*\*\*: specimen collected from Africa.

related. The following pairs of species are resolved as sisters in our analyses (Fig. 1); the Comorian *J. arachnantha* (Rchb. f.) Schltr. and the Malagasy *J. sagittata* H. Perrier form a clade (BS = 100%; PP = 1); the Comorian *J. arborescens* 2 and the Malagasy *J. arborescens* 3 (BS = 88%; PP = 1); the Reunionese *J. exilis* Schltr. and the Malagasy *J. flavescens* H. Perrier (BS = 100%; PP = 1); the Malagasy *J. maxillarioides* (Ridl.) Schltr. 1 and the Comorian *J. maxillarioides* 2 (BS = 88%; PP = 1); the Malagasy *Jumellea* sp. 1 and the Reunionese *J. recta* (BS = 90%; PP = 1); the Reunionese *J. recurva* and *J. pandurata* Schltr. (BS = 100%; PP = 1); and the Reunionese *J. stenophylla* (Frapp. ex Cordem.) Schltr. and the Malagasy *J. gracilipes* 2 and 3 (BS = 100%; PP = 1). Finally, *J. arborescens* 1 from the Comoros (Mayotte) does not group with the Malagasy *J. arborescens* 3 and the Comorian *J. arborescens* 2.

**Ancestral state reconstruction.** — The results from the ancestral character state optimizations are presented in Fig. 2A–E. The putative ancestor of *Jumellea* is inferred to have had long spurs, very short stems, oblong leaves, tubular bracts, and lanceolate lips. Each of the selected characters (spur length, growth habit, and leaf, bract and lip shape) evolved independently numerous times in *Jumellea*. Short spurs appear to have evolved at least seven times from long spurs (Fig. 2A), long stems at least three times, and creeping stems at least two times both from very short stems (Fig. 2B). Elliptic leaves evolved at least six times from oblong leaves (Fig. 2C), while the sigmoid bracts arose independently three times from tubular bracts (Fig. 2D). Finally, ovate and sagittate lips, respectively, evolved at least five and three times, respectively, from lanceolate lips (Fig. 2E).

## DISCUSSION

**Phylogenetic value of the characters used for recognizing informal groups on *Jumellea*.** — This is the first phylogenetic study of *Jumellea* using a large sample. We found no support for any of the informal groups established by Perrier de La Bâthie (1941) and Stewart & al. (2006). For example, Group 1 as defined by Perrier de La Bâthie (1941), represented by 14 species (*J. amplifolia* Schltr., *J. anjouanensis*, *J. arachnantha*, *J. bathiei* Schltr., *J. brachycentra* Schltr., *J. fragrans*, *J. francoisii*, *J. hyalina* H. Perrier, *J. ibityana* Schltr., *J. major* Schltr., *J. maxillarioides*, *J. pachyceras* Schltr., *J. sagittata* and *J. spathulata*

(Ridl.) Schltr.) in our analysis (Fig. 2A), is polyphyletic, as its sequenced representatives are nested in five separate subclades (A1, C1, C2, D2, D4). Similarly, Group 6 as delimited by Stewart & al. (2006), represented by 13 species (*J. arborescens*, *J. brevifolia* H. Perrier, *J. comorensis* (Rchb. f.) Schltr., *J. confusa*, *J. flavescens*, *J. jumelleana* (Schltr.) Summerh., *J. lignosa*, *J. longivaginans*, *J. majalis* (Schltr.) Schltr., *J. pandurata*, *J. rigida* Schltr., *J. similis* Schltr. and *J. stenoglossa*), is resolved as polyphyletic, because these species are nested in five distinct subclades (A1, C2, D1, D3, D4). None of the five characters (spur length, habit, and leaf, bract, and lip shape) can be used for recognizing the major lineages of *Jumellea* (clades A, B, C, and D, Figs. 1–2). In other words, these characters are all evolutionarily labile and should not be used for recognizing any groups in *Jumellea*. On the other hand, many subclades (e.g., A1, C1, C2, D1, D2, D3, D4, Figs. 2A–E) can be characterized by a combination of some of these characters (Table 4). Subclades A1 and C2 each consist of species of *Jumellea* with erect stems and small and oblong leaves (2–8 × 0.2–1.0 cm). However, the former has sigmoid and the latter tubular bracts.

Stewart & al. (2006) divided the short-stemmed (or stemless) species into two groups based on their leaf size (very large and thin leaves). The species from these two groups are scattered across the phylogenetic tree of *Jumellea* (Figs. 1, 2C), but the majority is found within subclades C1 and D2. Subclade C1 is composed of species with a very short stem, very large leaves (width > 20 mm) and sagittate lips. Subclade D2 contains species with very short or without stems and long and narrow (width ≤ 20 mm) leaves.

Subclades D3 and D4 each contain species with long stems and large and oblong leaves (6–12 × 1.5–2.5 cm). Subclade D3 has species with thicker stems and larger flowers with ovate lips, while subclade D4 is characterized by thinner stems and smaller flowers with ovate, lanceolate, or sagittate lips. Despite the fact that subclades A1, C1, C2, D1, D2, D3 and D4 can be recognized morphologically, some *Jumellea* species with the same character combinations do not belong into these groups. This makes it impossible for now to establish a formal infrageneric classification for *Jumellea*. Moreover, the support values for clades A and B are low.

**Comments on species limits in *Jumellea* and taxonomic implications.** — The results of our analyses indicate that the species limits of some species of *Jumellea* as recognized by Perrier de la Bâthie (1941) are untenable. The Malagasy

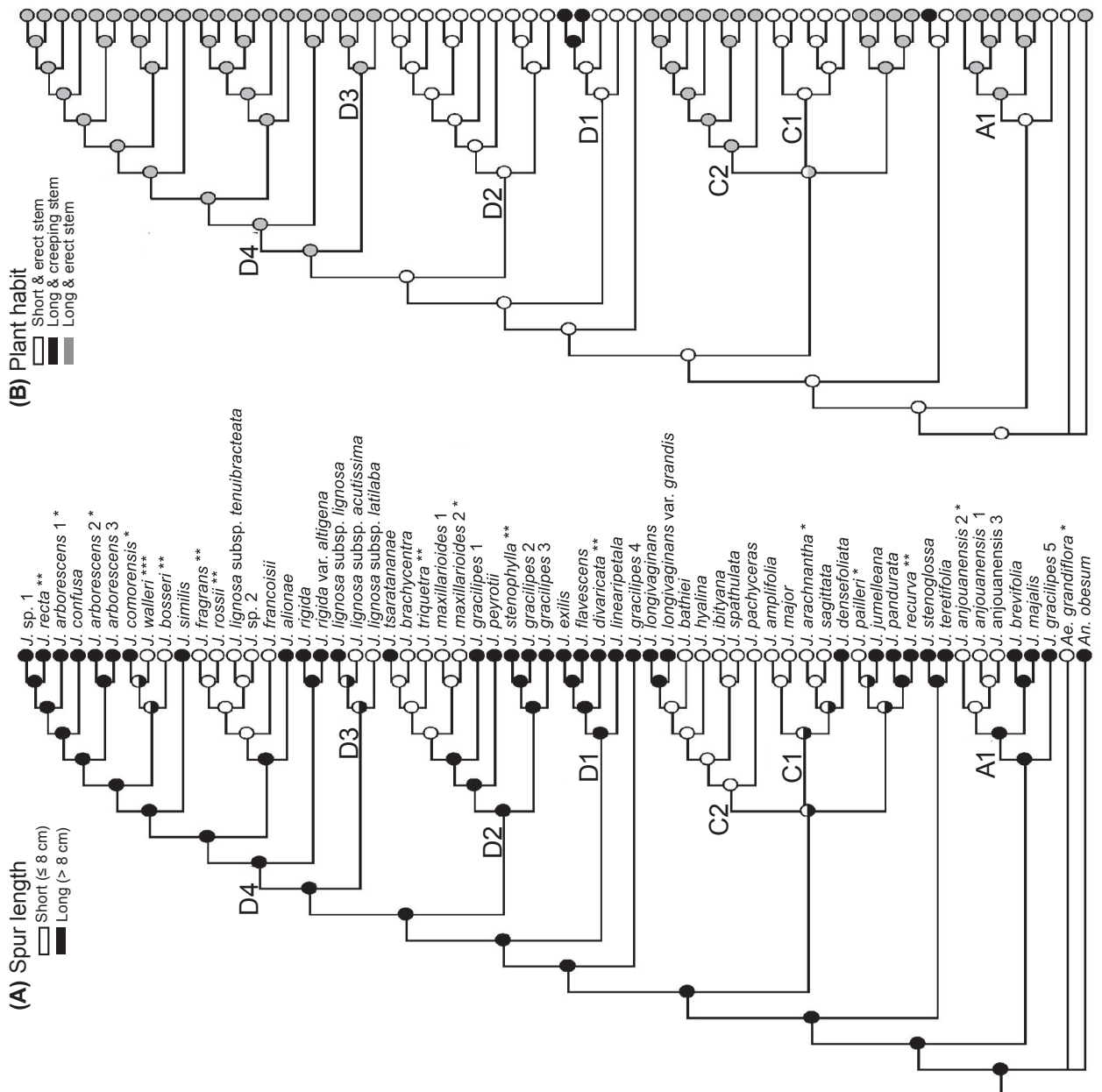
**Table 4.** Morphological characteristics of the subclades.

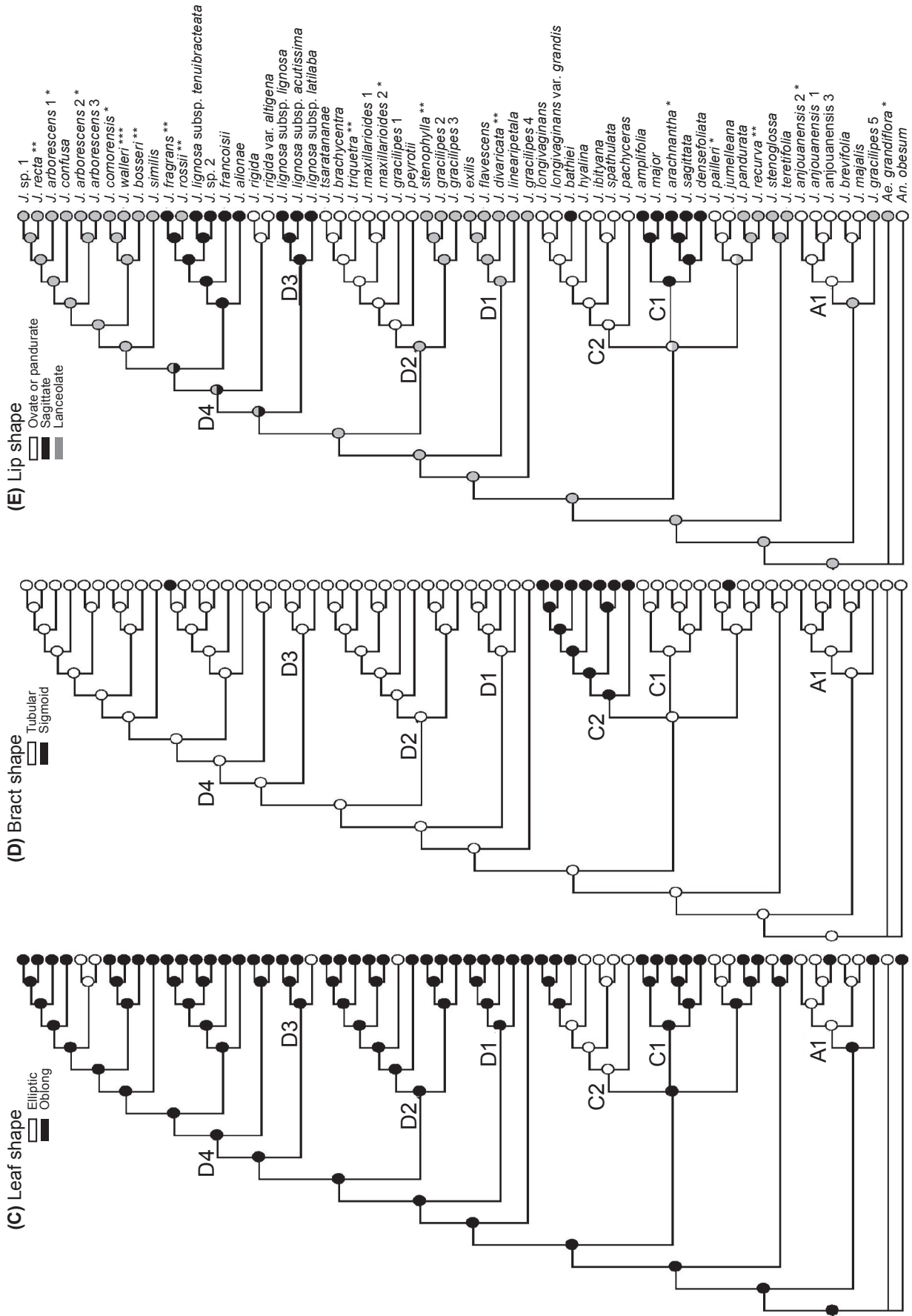
Subclade	Characteristics
Subclade A1	Erect and long stems, small and elliptic leaves, tubular bracts, ovate or pandurate lips
Subclade C1	Erect and short stems, oblong leaves, tubular bracts, sagittate lips
Subclade C2	Erect and long stems, small and elliptic leaves, sigmoid bracts, ovate or pandurate lips
Subclade D1	Long spurs, oblong leaves, tubular bracts, lanceolate lips
Subclade D2	Erect and short stems, oblong leaves, tubular bracts, lanceolate lips
Subclade D3	Erect and long stems, tubular bracts, sagittate lips
Subclade D4	Erect and long stems

*Jumellea lignosa* appears to be non-monophyletic, as subsp. *tenuibracteata* is nested in subclade D4, whereas subsp. *acutissima*, subsp. *latilabia*, and subsp. *lignosa* all fall in subclade D3. Therefore, subsp. *tenuibracteata* should be recognized at species level (see Taxonomic synopsis). Furthermore, the five specimens of *J. gracilipes* s.l. are scattered across the phylogenetic tree (Fig. 1). *Jumellea gracilipes* 1, 2 and 3 do not form a monophyletic group, but they are all nested in subclade D2; *Jumellea gracilipes* 4 and 5 are not placed in any of the main subclades. Considering their morphological differentiation together with their phylogenetic positions, we suggest that *J. gracilipes* be divided into four different species as originally proposed by Schlechter (1925). *Jumellea gracilipes* 1 corresponds to *J. imerinensis*, *J. gracilipes* 2 and 3 to *J. stenophylla*, *J. gracilipes* 4 to *J. ambongensis*, and *J. gracilipes* 5

to *J. unguicularis*. These four species vary greatly in leaf size and shape. *Jumellea imerinensis* has short and elliptic leaves (0.6 × 8–11 cm); *J. gracilipes* has very thin and oblong leaves (0.3–0.6 × 25–30 cm); *J. ambongensis* has large, thick, and oblong leaves (0.8–1.3 × 18.5–26.0 cm) and finally *J. unguicularis* has large and basally conduplicate leaves (1.5–2.5 × 19–30 cm).

The Comorian and Malagasy *Jumellea arborescens* (1–3) do not form a clade; *Jumellea arborescens* 1 from Mayotte (the Comoros) is more closely related to the Reunionese *J. recta* and the Malagasy *J. confusa* (Schltr.) Schltr. and *Jumellea* sp. 1 than to *J. arborescens* 2 and 3 from Grande Comore and Madagascar, respectively. Consequently, *J. arborescens* 1 should be resurrected at species level (*Jumellea* sp. 3). Moreover, our analyses confirm the occurrence of *J. maxillarioides* (*J. maxillarioides* 1) and *J. arborescens* (*J. arborescens* 2) in





**Fig. 2.** Ancestral state reconstructions of **(A)** spur length, **(B)** plant habit, **(C)** leaf shape, **(D)** bract shape, and **(E)** lip shape traced over the 50% majority-rule consensus tree using parsimony reconstruction methods in the Trace Character option implemented in Mesquite. Short spurs are inferior to 8 cm and long spurs are superior or equal to 8 cm. \*: specimens collected from the Comoros; \*\*: specimens collected from the Mascarenes; \*\*\*: specimen collected from Africa.



the Comoros (Pailler & al., 2009; Rakotoarivelo & al., unpub.). Some *Jumellea* species from the Comoros and Réunion islands form monophyletic groups with some Malagasy *Jumellea* species. These species are morphologically similar, and therefore we consider them to be conspecific. The Malagasy *J. sagittata* is synonymised with in the Comorian *J. arachnantha*, while *J. flavescens*, *J. gracilipes*, *Jumellea* sp. 1 and *J. pandurata* from Madagascar are proposed as synonyms of *J. exilis*, *J. stenophylla*, *J. recta*, and *J. recurva*, respectively, all from Réunion.

In sum, the number of species of *Jumellea* has now increased from 55 to 57: seven species in the Comoros (four shared with Madagascar), nine species in the Mascarenes (four shared with Madagascar), two species in Africa, and 39 species, three subspecies, and two varieties restricted to Madagascar.

## ■ TAXONOMIC SYNOPSIS

The present study demonstrates that the circumscription of *J. gracilipes* and *J. lignosa* as defined by Perrier de la Bâthie (1938) is untenable. As a result, we formally merge *J. gracilipes* sensu Schlechter in *J. stenophylla* and resurrect *Jumellea ambongensis*, *J. imerinensis*, and *J. unguicularis*, as originally proposed (Schlechter, 1922, 1925), at species level. In addition, *J. lignosa* subsp. *tenuibracteata* is here recognized at species level, as it does not group with the other subspecies of *J. lignosa* in our analyses. Finally, *J. sagittata*, *J. flavescens*, and *J. pandurata* are, respectively, placed in synonymy of *J. arachnantha*, *J. exilis*, and *J. recurva*.

***Jumellea arachnantha*** (Rchb. f.) Schltr. in Beih. Bot. Centralbl. 33: 428. 1915 ≡ *Aeranthus arachnanthus* Rchb. f. in Flora 68: 539. 1885 – Type: Comoros, Grande Comore, Combani, *Humblot 423* (holotype: W; isotypes: P00090526, P00090527, P00090528, images!).

= *Jumellea sagittata* H. Perrier in Notul. Syst. (Paris) 7: 52. 1938, **syn. nov.** – Type: Madagascar, Antananarivo, Mandraka, *Perrier de la Bâthie 18423* (holotype: P00094238, image!; isotype: P00094239, image!).

***Jumellea exilis*** (Cordem.) Schltr. in Beih. Bot. Centralbl. 33: 429. 1915 ≡ *Angraecum exile* Cordem. in Rev. Gen. Bot. 11: 429. 1899 – Type: Réunion, Cilaos, Tapcal, *Cordemoy s.n.* (holotype: P00750181, image!).

= *Jumellea flavescens* H. Perrier in Notul. Syst. (Paris) 7: 63. 1938, **syn. nov.** – Type: Madagascar, Ankeramadinika. *Perrier de la Bâthie 18470* (holotype: P00090549, image!; isotype: P00090550, image!).

***Jumellea recurva*** (Thouars) Schltr. in Beih. Bot. Centralbl. 33: 430. 1915 ≡ *Angraecum recurvum* Thouars, Hist. Orchid.: pl. 56. 1822 – Type: Réunion, s.loc., *Thouars s.n.* (holotype: P00112436, image!).

= *Jumellea pandurata* Schltr. in Beih. Bot. Centralbl. 34: 334. 1916, **syn. nov.** – Type: Madagascar, Antananarivo, Betafo, *Perrier de la Bâthie 8105* (holotype: P00094216, image!; isotype: P00094217, image!).

***Jumellea stenophylla*** (Frapp. ex Cordem.) Schltr. in Beih. Bot. Centralbl. 33: 430. 1915 ≡ *Angraecum stenophyllum* Frapp. ex Cordem., Fl. Réunion: 200. 1895 – Type: Réunion, Plaine des Palmistes, *J. Cordemoy s.n.* (holotype: P00750184, image!).

= *Jumellea gracilipes* Schltr. in Repert. Spec. Nov. Regni Veg. 18: 324. 1922, **syn. nov.** – Type: Madagascar, Antananarivo, Manjakandriana, *R. Viguier & H. Humbert 1216* (holotype: P00090562, image!; isotype: P00090563, image!).

***Jumellea tenuibracteata*** (H. Perrier ex Hermans) F. Rakotoar. & Pailler, **comb. et stat. nov.** ≡ *Jumellea lignosa* subsp. *tenuibracteata* H. Perrier ex Hermans in Orchids Madagascar ed.2: 289. 2007 – Type: Madagascar, Antsiranana, Tsaratanana Massif, *Perrier de la Bâthie 15331* (holotype: P00090581, image!).

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**Appendix.** Species sampled with voucher information and GenBank accession numbers of the new sequences from *matK*, *rps16*, *trnL-F*, *ycf1*, and nrITS used in this study.

*Aeranthus grandiflora* Lindl., *Rakotoarivelo & al.* 045 (TAN), Madagascar, JQ905330, JQ905452, JQ905513, JQ905277, JQ905391; *Angraecum obesum* H. Perrier, *Rakotoarivelo & al.* 008 (TAN), Madagascar, JQ905332, JQ905454, JQ905515, JQ905279, JQ905393; *Jumellea alionae* Schltr., *Rakotoarivelo & al.* 200 (TAN), Madagascar, JQ905383, JQ905505, JQ905566, JQ905322, JQ905444; *Jumellea amplifolia* Schltr., *Rakotoarivelo & al.* 312 (TAN), Madagascar, JQ905331, JQ905453, JQ905514, JQ905278, JQ905392; *Jumellea anjouanensis* (Finet) H. Perrier 1, *Rakotoarivelo & al.* 306 (TAN), Madagascar, JQ905334, JQ905456, JQ905517, JQ905281, JQ905395; *Jumellea anjouanensis* (Finet) H. Perrier 2, *Rakotoarivelo & al.* 072 (REU), Grande Comore, JQ905333, JQ905455, JQ905516, JQ905280, JQ905394; *Jumellea anjouanensis* (Finet) H. Perrier 3, *Rakotoarivelo & al.* 203 (TAN), Madagascar, JQ905370, JQ905492, JQ905553, JQ905311, JQ905431; *Jumellea arachnantha* (Rchb. f.) Schltr., *Rakotoarivelo & al.* 042 (REU), Grande Comore, JQ905335, JQ905457, JQ905518, JQ905282, JQ905396; *Jumellea arborescens* H. Perrier 1 (= *Jumellea* sp. 3), *Jubault 001* (REU), Mayotte, JQ905338, JQ905460, JQ905521, –, JQ905399; *Jumellea arborescens* H. Perrier 2, *Rakotoarivelo & al.* 058 (REU), Grande Comore, JQ905336, JQ905458, JQ905519, –, JQ905397; *Jumellea arborescens* H. Perrier 3, *Rakotoarivelo & al.* 202 (TAN), Madagascar, JQ905337, JQ905459, JQ905520, –, JQ905398; *Jumellea bathiei* Schltr., *Rakotoarivelo & al.* 035 (TAN), Madagascar, JQ905339, JQ905461, JQ905522, JQ905283, JQ905400; *Jumellea bosseri* Pailler, *Pailler 270* (REU), Réunion, JQ905340, JQ905462, JQ905523, –, JQ905401; *Jumellea brachycentra* Schltr., *Rakotoarivelo & al.* 241 (TAN), Madagascar, JQ905341, JQ905463, JQ905524, JQ905284, JQ905402; *Jumellea brevifolia* H. Perrier, *Rakotoarivelo & al.* 300 (TAN), Madagascar, JQ905342, JQ905464, JQ905525, JQ905285, JQ905403; *Jumellea comorensis* (Rchb. f.) Schltr., *Rakotoarivelo & al.* 040 (REU), Grande Comore, JQ905343, JQ905465, JQ905526, JQ905286, JQ905404; *Jumellea confusa* (Schltr.) Schltr., *Rakotoarivelo & al.* 088 (TAN), Madagascar, JQ905379, JQ905501, JQ905562, JQ905318, JQ905440; *Jumellea densefoliata* Senghas, *Rakotoarivelo & al.* 109 (TAN), Madagascar, JQ905344, JQ905466, JQ905527, JQ905287, JQ905405; *Jumellea divaricata* (Frapp. ex Cordem.) Schltr., *Pailler 267* (REU), Réunion, JQ905345, JQ905467, JQ905528, JQ905288, JQ905406; *Jumellea exilis* (Cordem.) Schltr., *Pailler 216* (REU), Réunion, JQ905346, JQ905468, JQ905529, JQ905289, JQ905407; *Jumellea flavescens* H. Perrier, *Rakotoarivelo & al.* 027 (TAN), Madagascar, JQ905348, JQ905470, JQ905531, JQ905291, JQ905409; *Jumellea*

## Appendix. Continued.

*fragrans* (Thouars) Schltr., *Paillet 203* (REU), Réunion, JQ905349, JQ905471, JQ905532, JQ905292, JQ905410; *Jumellea francoisii* Schltr., *Rakotoarivelo & al. 230* (TAN), Madagascar, JQ905350, JQ905472, JQ905533, JQ905293, JQ905411; *Jumellea gracilipes* (Schltr.) H. Perrier 1 (= *J. imerinensis* Schltr.), *Rakotoarivelo & al. 317* (TAN), Madagascar, JQ905347, JQ905469, JQ905530, JQ905290, JQ905408; *Jumellea gracilipes* (Schltr.) H. Perrier 2 (= *J. stenophylla* (Frapp. ex Cordem.) Schltr.), *Rakotoarivelo & al. 204* (TAN), Madagascar, JQ905351, JQ905473, JQ905534, JQ905294, JQ905412; *Jumellea gracilipes* (Schltr.) H. Perrier 3 (= *J. stenophylla* (Frapp. ex Cordem.) Schltr.), *Rakotoarivelo & al. 90* (TAN), Madagascar, JQ905352, JQ905474, JQ905535, JQ905295, JQ905413; *Jumellea gracilipes* (Schltr.) H. Perrier 4 (= *J. ambongensis* Schltr.), *Rakotoarivelo & al. 131* (TAN), Madagascar, JQ905329, JQ905451, JQ905512, JQ905276, JQ905390; *Jumellea gracilipes* (Schltr.) H. Perrier 5 (= *J. unguicularis* Schltr.), *Rakotoarivelo & al. 216* (TAN), Madagascar, JQ905389, JQ905511, JQ905571, JQ905328, JQ905450; *Jumellea hyalina* H. Perrier, *Rakotoarivelo & al. 006* (TAN), Madagascar, JQ905354, JQ905476, JQ905537, JQ905297, JQ905415; *Jumellea ibityana* Schltr., *Rakotoarivelo & al. 031* (TAN), Madagascar, JQ905355, JQ905477, JQ905538, JQ905298, JQ905416; *Jumellea jumelleana* (Schltr.) Summerh., *Rakotoarivelo & al. 099* (TAN), Madagascar, JQ905356, JQ905478, JQ905539, JQ905299, JQ905417; *Jumellea lignosa* subsp. *acutissima* H. Perrier ex Hermans, *Rakotoarivelo & al. 036* (TAN), Madagascar, JQ905357, JQ905479, JQ905540, JQ905300, JQ905418; *Jumellea lignosa* subsp. *latilabia* H. Perrier ex Hermans, *Rakotoarivelo & al. 228* (TAN), Madagascar, JQ905359, JQ905481, JQ905542, JQ905301, JQ905420; *Jumellea lignosa* subsp. *tenuibracteata* H. Perrier ex Hermans (= *J. tenuibracteata* (H. Perrier ex Hermans) F. Rakotoar. & Paillet), *Rakotoarivelo & al. 321* (TAN), Madagascar, JQ905360, JQ905482, JQ905543, –, JQ905421; *Jumellea lignosa* subsp. *lignosa* H. Perrier, *Rakotoarivelo & al. 148* (TAN), Madagascar, JQ905358, JQ905480, JQ905541, –, JQ905419; *Jumellea linearipetala* H. Perrier, *Rakotoarivelo & al. 108* (TAN), Madagascar, JQ905361, JQ905483, JQ905544, JQ905302, JQ905422; *Jumellea longivaginanans* H. Perrier, *Rakotoarivelo & al. 328* (TAN), Madagascar, JQ905362, JQ905484, JQ905545, JQ905303, JQ905423; *Jumellea longivaginanans* var. *grandis* H. Perrier, *Rakotoarivelo & al. 150* (TAN), Madagascar, JQ905353, JQ905475, JQ905536, JQ905296, JQ905414; *Jumellea majalis* (Schltr.) Schltr., *Rakotoarivelo & al. 307* (TAN), Madagascar, JQ905366, JQ905488, JQ905549, JQ905307, JQ905427; *Jumellea major* Schltr., *Rakotoarivelo & al. 322* (TAN), Madagascar, JQ905363, JQ905485, JQ905546, JQ905304, JQ905424; *Jumellea maxillarioides* (Ridl.) Schltr. 1, *Rakotoarivelo & al. 095* (TAN), Madagascar, JQ905364, JQ905486, JQ905547, JQ905305, JQ905425; *Jumellea maxillarioides* (Ridl.) Schltr. 2, *Rakotoarivelo & al. 061* (REU), Grande Comore, JQ905365, JQ905487, JQ905548, JQ905306, JQ905426; *Jumellea pachyceras* Schltr., *Rakotoarivelo & al. 311* (TAN), Madagascar, JQ905367, JQ905489, JQ905550, JQ905308, JQ905428; *Jumellea pailleri* F. Rakotoar., *Rakotoarivelo & al. 060* (REU), Grande Comore, JQ905368, JQ905490, JQ905551, JQ905309, JQ905429; *Jumellea pandurata* Schltr. (= *J. recurva* (Thouars) Schltr.), *Rakotoarivelo & al. 009* (TAN), Madagascar, JQ905371, JQ905493, JQ905554, JQ905312, JQ905432; *Jumellea peyrotii* Bosser, *Rakotoarivelo & al. 144* (TAN), Madagascar, JQ905369, JQ905491, JQ905552, JQ905310, JQ905430; *Jumellea recta* (Thouars) Schltr., *Paillet 205* (REU), Réunion, JQ905373, JQ905495, JQ905556, –, JQ905434; *Jumellea recurva* (Thouars) Schltr., *Paillet 204* (REU), Réunion, JQ905372, JQ905494, JQ905555, JQ905313, JQ905433; *Jumellea rigida* Schltr., *Rakotoarivelo & al. 220* (TAN), Madagascar, JQ905376, JQ905498, JQ905559, JQ905315, JQ905437; *Jumellea rigida* var. *altigena* Schltr., *Rakotoarivelo & al. 032* (TAN), Madagascar, JQ905375, JQ905497, JQ905558, JQ905314, JQ905436; *Jumellea rossii* Senghas, *Paillet 293* (REU), Réunion, JQ905377, JQ905499, JQ905560, JQ905316, JQ905438; *Jumellea sagittata* H. Perrier (= *J. arachnantha* (Rechb. f.) Schltr.), *Rakotoarivelo & al. 136* (TAN), Madagascar, JQ905378, JQ905500, JQ905561, JQ905317, JQ905439; *Jumellea similis* Schltr., *Rakotoarivelo & al. 100* (TAN), Madagascar, JQ905381, JQ905503, JQ905564, JQ905320, JQ905442; *Jumellea sp. 1* (= *J. recta* (Thouars) Schltr.), *Paillet 220* (TAN), Madagascar, JQ905374, JQ905496, JQ905557, –, JQ905435; *Jumellea sp. 2*, *Rakotoarivelo & al. 149* (TAN), Madagascar, JQ905382, JQ905504, JQ905565, JQ905321, JQ905443; *Jumellea spathulata* (Ridl.) Schltr., *Rakotoarivelo & al. 209* (TAN), Madagascar, JQ905380, JQ905502, JQ905563, JQ905319, JQ905441; *Jumellea stenoglossa* H. Perrier, *Paillet 239* (TAN), Madagascar, JQ905384, JQ905506, –, JQ905323, JQ905445; *Jumellea stenophylla* (Frapp. ex Cordem.) Schltr., *Paillet s.n.* (REU), Réunion, JQ905385, JQ905507, JQ905567, JQ905324, JQ905446; *Jumellea teretifolia* Schltr., *Rakotoarivelo & al. 160* (TAN), Madagascar, JQ905386, JQ905508, JQ905568, JQ905325, JQ905447; *Jumellea triquetra* (Thouars) Schltr., *Paillet 292* (REU), Réunion, JQ905387, JQ905509, JQ905569, JQ905325, JQ905448; *Jumellea tsaratananae* Schltr., *Rakotoarivelo & al. 326* (TAN), Madagascar, JQ905388, JQ905510, JQ905570, JQ905327, JQ905449; *Jumellea walleri* la Croix, unvouchered (Micheneau & al., 2008a), Africa.