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ORIGINAL ARTICLE

Morphometrics and molecular phylogenetics of *Angraecum* section *Dolabrifolia* (Orchidaceae, Angraecinae)

Murielle Simo-Droissart[1] · Bonaventure Sonké[1,2,3] · Vincent Droissart[1,2,4,5] · Claire Micheneau[6] · Porter P. Lowry II.[2,7] · Olivier J. Hardy[3] · Gregory M. Plunkett[8] · Tariq Stévart[2,5,9]

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Abstract Species delimitation within *Angraecum* section *Dolabrifolia* is problematic due to morphological variability coupled with overlap in many of the characters traditionally used to distinguish species. Recent molecular phylogenetic studies of the genus included three of the five currently described species of the *Dolabrifolia* group, placing them as sister to continental African species of *Angraecum* sect. *Pectinaria*. In preparation for a taxonomic revision of section *Dolabrifolia*, we analyzed morphological and molecular data to re-assess the circumscription of each of the five currently described species, examined the relationships among members of the section, and assessed their position within the genus. We used 172 alcohol-preserved specimens to perform multivariate analyses on 15 morphological characters. We also collected molecular sequence data from 16 taxa including all members of the section using six DNA regions, and analyzed these data with parsimony and Bayesian methods. The morphometric study revealed five distinct groups, of which four correspond to currently recognized species, while the fifth represents a taxonomic novelty. *Angraecum podochiloides* is the most distinctive morphologically, recognizable by its narrow leaves bearing white-yellowish flowers. The often confused species *Angraecum distichum* and *Angraecum bancoense* are clearly distinguishable by flower size. Molecular phylogenetic analyses indicated that section *Dolabrifolia* forms a well-supported clade related to the continental African members of section *Pectinaria*. Four species are well delimited, while the accessions of

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✉ Murielle Simo-Droissart
murielle_simo@yahoo.fr

[1] Plant Systematics and Ecology Laboratory, Higher Teachers' Training College, University of Yaoundé I, P. O. Box 047, Yaoundé, Cameroon

[2] Missouri Botanical Garden, Africa and Madagascar Department, P. O. Box 299, St. Louis, MO 63166-0299, USA

[3] Evolutionary Biology and Ecology, Faculty of Sciences, Université Libre de Bruxelles, 50 Avenue F. Roosevelt, CP 160/12, 1050 Brussels, Belgium

[4] Institut de Recherche pour le Développement (IRD), Unité Mixte de Recherche AMAP (Botanique et Bioinformatique de l'Architecture des Plantes), Boulevard de la Lironde, TA A-51/PS2, 34398 Montpellier CEDEX 5, France

[5] Herbarium et Bibliothèque de Botanique africaine, Université Libre de Bruxelles, Boulevard du Triomphe, CP 265, 1050 Brussels, Belgium

[6] Université de La Réunion, Peuplements Végétaux & Bioagresseurs Milieu Tropical, Unité Mixte de Recherche C53 Centre International de Recherche Agronomique pour le Développement, Université Saint Denis 9, La Réunion, France

[7] Institut de Systématique, Évolution, Biodiversité (ISYEB), Unité Mixte de Recherche 7205, Centre National de la Recherche Scientifique/Muséum National d'Histoire Naturelle/École Pratique des Hautes Études, Université Pierre et Marie Curie, Sorbonne Universités, 57 rue Cuvier, C.P. 39, 75231 Paris CEDEX 5, France

[8] Cullman Program for Molecular Systematics, The New York Botanical Garden, Bronx, NY 10458-5126, USA

[9] Agentschap Plantentuin Meise, Domein van Bouchout, Nieuwelaan 38, 1860 Meise, Belgium

Angraecum aporoides formed two well-supported clades corresponding to two subclusters revealed by the morphometric study. The recently published *Angraecum poppendickianum* is shown to be a synonym of *A. distichum*.

Keywords Angraecoid orchids · Bayesian analyses · Continental Africa · Monophyly · Morphometrics · Parsimony

Introduction

With 28,349 recognized taxa (The Plant List 2013), Orchidaceae are one of the largest and most diverse angiosperm families. Historically a great deal of controversy has surrounded the phylogenetic relationships in the family and its higher-level classification (Fay and Chase 2009). Starting with Chase et al. (1994), numerous molecular studies have provided an increasingly detailed phylogenetic framework for the orchids (e.g., Cameron and Chase 1999; Cameron et al. 1999; Cameron 2004; Carlsward et al. 2003, 2006b; Goldman et al. 2001; Górniak et al. 2010; Martos et al. 2014; Micheneau et al. 2008; Rakotoarivelo et al. 2012; Russell et al. 2010; van den Berg et al. 2000), and some authors have also incorporated morphological data to aid in the circumscription of subordinate taxa (see Mytnik-Ejsmont et al. 2013; Simo-Droissart et al. 2013).

With the publication of the most recent classification of Orchidaceae (see Chase et al. 2015), a great deal of progress has been made in understanding the phylogenetics of Epidendroideae, its largest subfamily, which currently comprises 16 tribes, of which Vandeae is the third most speciose. Within Vandeae, the angraecoid orchids are of particular interest because of their spectacular white, nectariferous, long-spurred flowers, which make them especially prized by hobbyists. However, they remain one of the most taxonomically problematic groups (Pridgeon et al. 2014). Historically, angraecoid species were divided into two subtribes: Aerangidinae (ca. 350 spp.) and Angraecinae (ca. 410 spp.) (see Summerhayes 1966). These two subtribes were distinguished from other groups based on rostellum shape and chromosome number. While both subtribes, as traditionally circumscribed, have since proven to be polyphyletic based on molecular analyses, together they form a well-supported clade now recognized as a more broadly defined subtribe Angraecinae (Carlsward et al. 2006b; Chase et al. 2015). The generic name *Angraecum* Bory (1804), on which the terms "Angraecinae" and by extension "angraecoid" are based, is the oldest within this group. *Angraecum* is both species-rich and morphologically diverse, with 221 currently recognized species (The Plant List 2013), about 75 % of which are endemic to Madagascar and the Mascarene Islands of La Réunion, Mauritius, and Rodrigues, while most of the remaining taxa occur in continental Africa. Recent studies have shown that *Angraecum* is polyphyletic (Carlsward et al. 2006a, b; Micheneau et al. 2008), with several species scattered across other African angraecoid genera or clades. The phylogenetic relationships of species from the Western Indian Ocean Islands have been examined in detail (see Micheneau et al. 2008), but this is not the case for members of the genus from continental Africa and the adjacent islands in the Gulf of Guinea.

Among the 19 sections recognized within *Angraecum* by Garay (1973), the last author who treated the group as a whole, section *Dolabrifolia* occurs exclusively in continental Africa and in the Gulf of Guinea Islands. Its members are easily identifiable by their laterally compressed and densely imbricate leaves with a groove on the upper surface, the lateral compression being a unique feature within the genus. This prompted Szlachetko and Romowicz (2007) to recognize *Dolabrifolia* as a distinct genus, an interpretation followed by Szlachetko et al. (2013) based on molecular studies (which included three of the five currently described species in the group) and morphological data. However, the relationships between the *Dolabrifolia* group and other groups generally regarded as belonging to *Angraecum* remain unclear, and a broader study is needed before any firm taxonomic decisions can be made.

In the study of Micheneau et al. (2008), the *Dolabrifolia* group appeared to be monophyletic, although only two of the five currently described species were included. They were placed as sister to the continental African group *Angraecum* sect. *Pectinaria*, a result supported by the phylogenetic study of Simo-Droissart et al. (2013). Despite sampling limitations, these recent studies lend support to the recognition of the *Dolabrifolia* group at the sectional level within *Angraecum*. Among its five published species, only four are currently recognized; the status of the recently described *A. poppendickianum* Szlachetko and Olszewski (2001) has been controversial because of its morphological similarity to *A. distichum* Lindl., under which it was regarded as a synonym by Govaerts in 2003 according to Govaerts et al. (2016) and by Droissart et al. (2006).

During the last two decades, many additional specimens of plants belonging to *Angraecum* sect. *Dolabrifolia* have been collected, and as new material has accumulated the morphological distinction between most species (in particular *A. aporoides* Summerh., *A. distichum*, and *A. bancoense* Burg) has become increasingly blurred, primarily reflecting significant overlap in the morphological variability of leaf and floral characters within and among species. Previously, Arends et al. (1980) had pointed out that *A. bancoense* and *A. distichum* share the same

characteristic foliage and could not be distinguished based on vegetative traits. In turn, the lack of clarity in species delimitation presents problems for molecular phylogenetic studies that aim to clarify relationships among members of the section and to test the delimitation of morphologically defined species. In order to rectify this situation, we have taken a three-step approach in this study. First, we investigated species delimitations in *A.* sect. *Dolabrifolia* by visual inspection to define coherent morphological groups. For that, we used the available collections and included nomenclatural types, applying the appropriate species name to each distinct group (when available) or otherwise treating it as a potentially new entity. Second, we used a morphometric approach to identify the most informative quantitative and qualitative traits for defining and differentiating these species, especially *A. distichum* and *A. bancoense*. Finally, we investigated the delimitation of each species using DNA sequences from six markers (obtained from one nuclear and five plastid regions).

Materials and methods

Data matrix construction for morphometric analyses

Dried and spirit-preserved specimens representing all described species of *Angraecum* sect. *Dolabrifolia* were examined from the following herbaria: BM, BR, BRLU, K, MA, MO, NY, P, WAG, and YA (acronyms according to Thiers (continuously updated)). Specimens were first grouped based on overall morphology. The groups obtained were assigned when possible to one of the four currently accepted species after comparison with the diagnoses provided in the protologue and with nomenclatural types. Herbarium specimens were used only for the initial recognition of species. Following this step, data for the morphometric analyses were collected only from alcohol-preserved material (from BR, BRLU, K, MO, P, and WAG; see Online Resource 1) because flowers on herbarium specimens are typically flattened and/or deteriorated, which obscures key features and precludes accurate measurements for morphometric analyses.

After grouping based on morphology, it was found that four of the five distinct groups corresponded to the following four accepted species: *A. aporoides*, *A. bancoense*, *A. distichum*, and *A. podochiloides* Schltr. In addition, we identified a fifth group that was vegetatively similar to *A. aporoides* (these two entities have the longest leaves in the section) but differs in by flower length and the shape of the leaf apex. This fifth group is hereafter referred to as *A.* aff. *aporoides*. While the type specimens of all four accepted species were used to help circumscribe the initial species, three of them could not be used in the morphometric analysis. The type of *A. distichum* is represented only by a drawing, while those of *A. bancoense* and *A. podochiloides* are preserved only on herbarium sheets.

After excluding spirit-preserved specimens for which one or more key vegetative or floral characters were missing as well as those that were too brittle to dissect, morphological measurements were taken from 172 high-quality fertile specimens preserved in alcohol (including the type material of *A. aporoides*) to perform multivariate analyses (Online Resource 1). Most of the fertile specimens (148 out of 172) were recently collected by our team either in the field or in a shadehouse cultivation system operated in Central Africa since 1997 (see Droissart 2009; Simo 2014; Stévart 2003). Thirteen quantitative characters (Table 1) were measured with graph paper (1 mm^2 grid) and standardized by subtracting the character mean and dividing by the standard deviation to eliminate the distorting effects of different scales of measurement on the output results (Cupido 2003; Marcysiak et al. 2007; Poulsen and Nordal 2005). Two qualitative characters (Table 1) were also recorded as factors (i.e., with each discrete character state).

Data analysis

All morphometric analyses were performed using the R 3.0.1 software package (R Core Team 2013). To investigate the morphological variation within and among species, an extended principal component analysis (PCA) (Hill and Smith 1976) that included multistate discrete characters (i.e., a multivariate analysis allowing mixed quantitative variables and factors) was performed using the function *dudi.hillsmith* of the library *ade4* (Chessel et al. 2004; Dray and Dufour 2007; Dray et al. 2007). The principal components of this analysis are centered and normalized vectors maximizing the sum of squared correlation coefficients with quantitative variables and correlation ratios with factors. Using the function *dist* of the package *stats*, with the "Euclidean" method, we obtained a distance matrix to compute the distances between individual objects (samples) of our data matrix (Borg and Groenen 1997). We used the function *hclust* of the package *stats* to perform a hierarchical cluster analysis using a set of dissimilarities for the 172 individuals being analyzed. Initially, each individual was assigned to its own cluster and then the algorithm proceeded iteratively, at each stage joining the two most similar clusters, continuing until there was just a single cluster (Sneath and Sokal 1973). The clustering method used was the *Ward's* minimum variance, where the criterion for choosing the pair of clusters to merge at each step is based on the optimal value of an objective function, such as the error sum of squares. The Ward's minimum variance method is designed to find compact, spherical clusters.

Table 1 List of variables assessed for the study of *Angraecum* sect. *Dolabrifolia*

No	Variables	Codes	States
1	Leaf length	LFL	Continuous
2	Leaf width	LFW	Continuous
3	Leaf apex	LFA	LFA.acute; LFA.obtuse
4	Flower color	FLC	FLC.white; FLC.whiteyellow
5	Flower length	FLL	Continuous
6	Dorsal sepal length	DSL	Continuous
7	Dorsal sepal width	DSW	Continuous
8	Lateral sepals length	LSL	Continuous
9	Lateral sepals width	LSW	Continuous
10	Lateral petals length	LPL	Continuous
11	Lateral petals width	LPW	Continuous
12	Lip length	LIL	Continuous
13	Lip width	LIW	Continuous
14	Spur length	SPL	Continuous
15	Pedicel and ovary length	POL	Continuous

Continuous variables were measured in mm. For all widths, the widest parts were measured

Prior to performing statistical tests among groups obtained after the extended PCA, the distribution of each quantitative variable was examined using the Shapiro–Wilk test of normality (Royston 1982) through the function *shapiro.test* available in the package *stats* (R Core Team 2013). None of the 13 quantitative variables followed a normal distribution. We therefore performed nonparametric Kruskal–Wallis tests (Hollander and Wolfe 1973) to assess significant differences between the five groups obtained after the extended PCA for each variable, using the *kruskal.test* function available in the package *stats*. When a character differed significantly among groups, we performed multiple comparison tests between groups using the function *kruskalmc* (Siegel and Castellan 1988) available in the package *pgirmess* (Giraudoux 2013), with pairwise comparisons adjusted appropriately. The function *multcompLetters* (Piepho 2004) of the package *multcompView* (Graves et al. 2012) was then used to convert a logical vector into a character-based display in which common characters identify levels or groups that are not significantly different.

Plant material and DNA purification

DNA was obtained from leaf tissues taken from fertile specimens collected in Guinea-Bissau, Ivory Coast, São Tomé and Príncipe, Cameroon, Gabon, Democratic Republic of the Congo and Rwanda (Online Resource 2). Plants that were not fertile at the time of collection in Cameroon and Gabon were cultivated and monitored in shadehouses until they produce flowers, enabling accurate identification. Additional leaf and flower material was provided from the Gabonese orchid collection initially established at the Wageningen University Greenhouse (Netherlands) and now housed in the greenhouse of the Botanic Garden Meise (Belgium). A total of 37 accessions were used in the study: ten for *Angraecum aporoides*, two for the potential novelty *A.* aff. *aporoides*, four for *A. bancoense*, and five for each of the two remaining species in section *Dolabrifolia*, viz., *A. distichum* and *A. podochiloides*; one from each of the five currently recognized species of *A.* sect. *Pectinaria* from continental Africa; one from the genus *Diaphananthe* (continental Africa); and two from the genus *Tridactyle* (continental Africa). Unlike for the morphometric study, we did not attempt to include the type collection of *A. aporoides* (*Cooper 82/3*) in the phylogenetic analysis because it comprises only a liquid-preserved specimen, and previous attempts to obtain DNA extracts from such material proved unsuccessful, presumably due to poor DNA quality. Three taxa of *Polystacha* (*P. albescens* subsp. *imbricata*, *P. calluniflora*, and *P. pyramidalis*) were also included as out-groups because subtribe Polystachyinae, to which they belong, has been identified as the sister clade to the angraecoids (Chase et al. 2015; Górniak et al. 2010). Vouchers for each accession are deposited either at BR or BRLU (Online Resource 2).

Leaf and flower tissue were dried in silica gel for DNA extraction (Chase and Hills 1991). Total DNA was extracted from fresh (1 g) or silica-gel-dried material (0.3 g) using one of the two methods detailed in Simo-Droissart et al. (2013).

PCR amplification and DNA sequencing

The following primers were used for amplification and sequencing of each individual plastid region: (1) Tab-E and

Tab-F for the *trn*L-F intergenic spacer (Taberlet et al. 1991); (2) *rps*16-1F and *rps*16-2R for the *rps*16 intron (Oxelman et al. 1997); (3) 19F (Molvray et al. 2000), 1326R (Cuenoud et al. 2002), 390F (Cuenoud et al. 2002), and *trn*K-2R (Johnson and Soltis 1994) for *mat*K; (4) *trn*C and *pet*N-1R for the *trn*C-*pet*N intergenic spacer (Lee and Wen 2003); and (5) 3720F, *Int*R, *Int*F, and 5500R for *ycf*1 (Neubig et al. 2009). The nuclear marker ITS-1 was amplified using ITS-A and ITS-C designed for angiosperms (Blattner 1999).

PCR amplifications were carried out in one of three thermocyclers (Biometra TProfessional thermocycler, PTC-100 or PTC-200, Bio-Rad Laboratories, Inc.) in a total volume of 25 μL, with 1–2 μL of template DNA extract (of unquantified concentration), 0.125 μL (5 U/μL) of Taq polymerase (Qiagen), 2.5 μL PCR buffer, 1 μL $MgCl_2$ (25 mM), 0.5 μL dNTPs (10 μM), 0.25 μL of each primer (10 μM), and 18.375–19.375 μL of H_2O. The PCR amplification profiles used for the six DNA regions are detailed in Simo-Droissart et al. (2013). PCR products were then purified by enzymatic digestion using Exosap (Qiagen).

Cycle sequencing was carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Inc., ABI, Lennik, Netherlands) with the same primers used for PCR amplification: 1.5 μL of sequencing buffer, 1 μL of BigDye terminator with 0.2 μL of 10 μM primer, 1–3 μL of amplified product (unquantified concentration), and 4.3–6.3 μL of H_2O for a total reaction volume of 10 μL. Cycle sequencing conditions used are detailed in Simo-Droissart et al. (2013). Sequencing products were cleaned by ethanol precipitation and then separated on an ABI 3100 automated capillary DNA sequencer following the manufacturer's protocols (ABI). Both strands were sequenced to ensure accurate base calling. Sequence chromatograms were imported into Geneious Pro v.6 (Drummond et al. 2005). They were automatically trimmed at both ends using 5 % chance of error per base, after which the sense and antisense chromatograms were assembled to generate a consensus sequence. All incongruities and ambiguities were manually checked and edited. Consensus sequences were then aligned with the plugin MAFFT (Katoh et al. 2002) implemented within Geneious. Alignments were visually checked and edited wherever necessary. For coding regions, such as *mat*K and *ycf*1, nucleotides were translated into amino acids to verify that the sequences corresponded to a protein, using a reference sequence from NCBI.

The number of accessions included in each of the six individual matrices was as follows: 37 accessions for ITS-1 (Online Resource 3), the *rps*16 intron (Online Resource 4), and *trn*L-F (Online Resource 5); 37 accessions for the combined *mat*K regions (Online Resource 6), i.e., 19F–1326R and 390F–*trn*K-2R (although amplification of the region 390F–*trn*K-2R failed in *Angraecum gabonense,* two accessions of *A. aporoides* and two accessions of *A. distichum*); 36 accessions for the combined *ycf*1 regions (Online Resource 7), i.e., 3270F–*Int*R and *Int*F–5500R (*Tridactyle bicaudata* did not amplify while *A. gabonense* and one accession of *A. aporoides* had incomplete sequences, represented by the fragments *Int*F–5500R and 3270F–*Int*R, respectively); and 34 accessions for *trn*C-*pet*N (Online Resource 8) (*Polystachya calluniflora*, one accession of *A. aporoides* and one accession of *A. distichum* failed to amplify).

Parsimony and Bayesian analyses

Cladistic analyses using Fitch parsimony (Fitch 1971) were performed using PAUP* 4.0 beta 10 (Swofford 2003). All characters were unordered with equal weight; gaps were coded as missing data. Heuristic searches were performed using tree bisection–reconnection (TBR) branch swapping with 1000 replicates and random taxon addition, holding ten trees at each step, and saving 20 trees per replicate to reduce time spent in swapping on large islands of trees. In a second round of analysis, we used all trees found in the tree-limited analysis as starting trees, with a limit of 10,000 trees, which were then swapped to completion. Levels of internal support were estimated using the bootstrap method (Efron 1979; Felsenstein 1985) with 1,000 bootstrap replicates with random taxon addition and TBR branch swapping, retaining ten trees at each step, and saving ten trees per replicate. Parsimony analyses were first run separately for each region (i.e., ITS-1, *mat*K, *rps*16, *trn*C-*pet*N, *trn*L-F, and *ycf*1). Consensus trees and bootstrap values generated from each region were then compared visually for congruence. As there were no conflicts involving any of the well-supported clades, we combined data for all plastid regions into an initial alignment matrix (hereafter referred to as the plastid matrix, Online Resource 9) and then all plastid and ITS-1 regions into a second alignment matrix (hereafter the combined matrix, Online Resource 10). For taxa that had missing sequence regions, those partitions were coded as missing data (4 %) in the plastid and combined matrices.

Bayesian analyses were performed using MrBayes 3.2.1 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012) on the combined matrix, with one partition per gene (six partitions in total). Two independent analyses were run for 2 million generations with four chains (default temperatures) using a model-jumping approach that allows sampling across the entire general time reversible (GTR) model space (i.e., no best-fitting models were defined a priori, Huelsenbeck et al. 2004) and with model parameters unlinked between partitions. The separate runs were

analyzed and compared using TRACER v1.5 (Drummond and Rambaut 2007) to assess stationarity and convergence, and to verify that the effective sample size for all parameters was sufficiently high (ESS > 200). Convergence of runs was also assessed by a graphical exploration of the posterior split probabilities (hereafter PP) using the online version of AWTY (Nylander et al. 2008). Trees were sampled every 500 generations, resulting in a total of 4,001 trees per run from which the first 1000 (25 %) were discarded as the burn-in phase. The majority-rule consensus tree was constructed using the function *sumt* in MrBayes.

The species were defined using a combination of the morphological (Mayr 1969) and phylogenetic (Purvis et al. 2005) species concepts. In this study, we thus circumscribed species to comprise morphologically coherent entities that included, whenever possible, accessions belonging to a single clade.

Results

Preliminary species delimitation within *Angraecum* sect. *Dolabrifolia* based on morphometric analyses

The first three axes of the extended PCA (the Hill-Smith ordination) explained 93 % of the total variance among the 172 specimens included in the multivariate analysis (Online Resource 11, Fig. 1a, b). The variation explained by the first axis (68 % of the total) largely correlates with the highest negative loadings for lateral sepal length (LSL), dorsal sepal length (DSL), lateral petal length (LPL), and flower length (FLL). The second axis (16 % of total variance) has the highest negative loadings for the white-yellowish color of the flower (FLC.whiteyellow) and the acute apex of the leaves (LFA.acute). The variation along the third axis (9 % of total variance) correlates with the highest negative loading for the white-yellowish color of the flower (FLC.whiteyellow) and the highest positive loadings for the width and the length of the leaves (LFW and LFL, respectively).

The projection using the first two axes reveals four groups of specimens (Fig. 1a). The first group corresponds to a mix of all specimens assigned to *Angraecum aporoides* and *A. distichum*, whereas the three other groups correspond, respectively, to *A.* aff. *aporoides*, *A. bancoense,* and *A. podochiloides*. The projection using the first and the third axes reveals four groups of specimens, with *A. aporoides* and *A. distichum* forming separate albeit neighboring groups (Fig. 1b). Again, three of these four groups correspond to species, albeit not the same set as above (*A. aporoides*, *A.* aff. *aporoides* and *A. bancoense*), and the fourth group comprises specimens assigned to *A. distichum* and *A. podochiloides*. The ordination on the three PC axes is thus consistent with the delimitation of five species. The main discriminant variables for *A.* aff. *aporoides* are the dimensions of the leaf along with its acute apex, whereas those for *A. bancoense* are the dimensions of the flower (i.e., small lengths of the sepals and lateral petals, see also Table 2).

The clustering dendrogram based on distance measures between specimens likewise reveals five groups that correspond to the five species (Fig. 2). These are cluster 1: *Angraecum* aff. *aporoides*, with eight specimens; cluster 2: *A. podochiloides*, with ten specimens; cluster 3: *A. aporoides*, with 25 specimens; cluster 4: *A. distichum*, with 63 specimens; and cluster 5: *A. bancoense*, comprising 66 specimens. Boxplots (Fig. 3) for each of the six numerical variables showing the highest loadings in the Hill-Smith ordination (based on the first three axes, see Online Resource 11) provide a graphical depiction of variation among the five species.

The multiple comparisons of medians obtained using the Kruskal–Wallis test showed that all 13 quantitative variables studied differed significantly among the five groups (Table 2). Of these 13 variables, leaf width (LFW) and flower length (FLL) provide clear-cut separation among them. Indeed, *Angraecum aporoides* and *A.* aff. *aporoides* have the widest leaves, while *A. bancoense* has the smallest flowers. Considering those species possessing the longest leaves, specimens assigned to *A. aporoides* differ from those of *A.* aff. *aporoides* by eight of the 13 numeric variables used in our study (Table 2). Of these eight variables, the most informative, providing a clear-cut separation between these two taxa, are the lengths of the lateral petals (LPL), the lip (LIL) and the spur (SPL). Among the species possessing the smallest leaves, *A. podochiloides* is clearly distinct from the two other species (i.e., *A. bancoense* and *A. distichum*) by having the narrowest leaves, which are lanceolate and have an acute or subacute apex. *Angraecum podochiloides* is also the only species of section *Dolabrifolia* to possess yellow or orange tips on its perianth parts.

The detailed examination of herbarium material showed that around 70 % of specimens of *Angraecum distichum*—described by Lindley (1836)—have been confused with the recently described *A. bancoense*—described by Burg (Arends et al. 1980), although it was first collected in 1860. *Angraecum distichum* differs from *A. bancoense* by all 13 of the numeric variables used in our study ($p < 0.0001$; Table 2). The seven most important variables that provide a clear-cut separation of these two species are flower, dorsal sepal, lateral sepal, lip and spur lengths (FLL, DSL, LSL, LIL and SPL, respectively), and lateral sepal and petal widths (LSW and LPW, respectively). The most distinctive feature differentiating the two species is FLL. Indeed, *A. distichum* has longer flowers than *A. bancoense* (11–17 vs. 6–9 mm) and is also easily distinguishable by its curved lip and the slender apex of its spur.

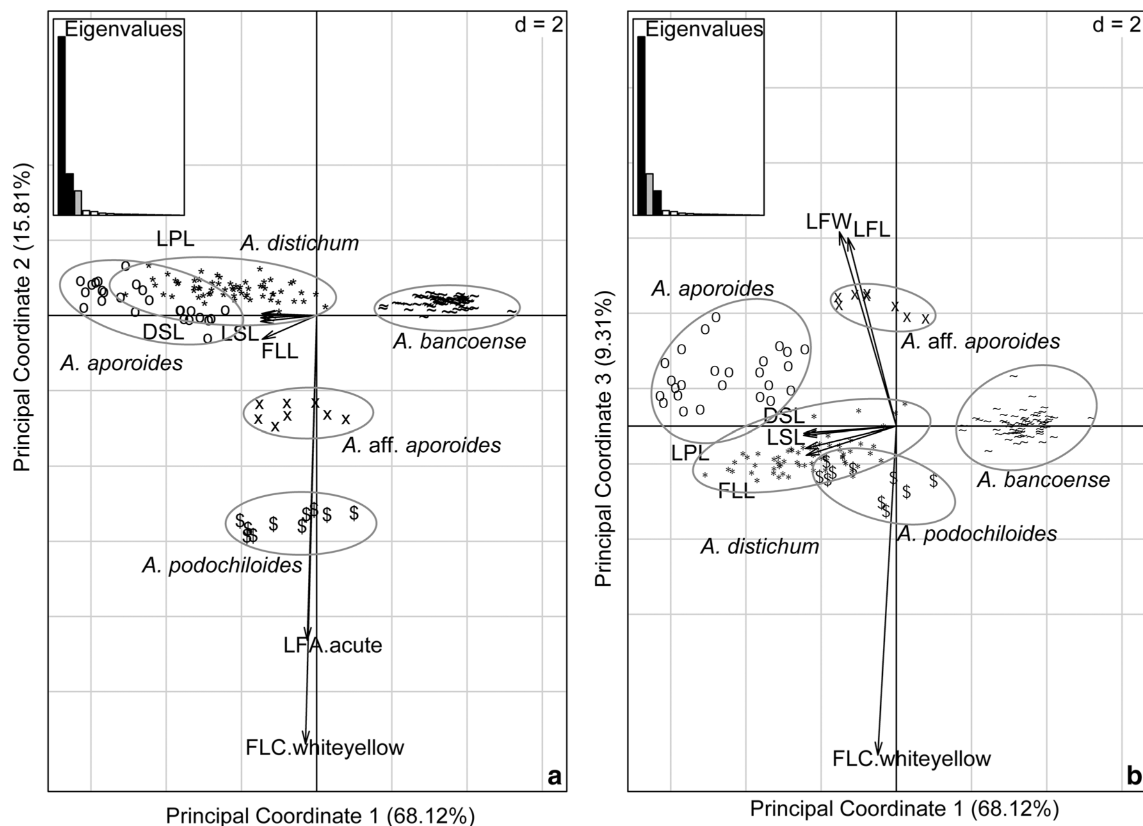

Fig. 1 *Scatter plots* of the first three axes of the extended principal component analysis based on 15 characters scored from 172 specimens of *Angraecum* sect. *Dolabrifolia*. **a** Axes 1 and 2; **b** axes 1 and 3. *Variables* showing the highest loadings are represented (see also Online Resource 11). Codes used for variables are detailed in Table 1. *Circles* summarize specimens from the same species

Phylogenetic analyses based on DNA sequences

The number of aligned characters in the matrices of each of the six markers examined (i.e., ITS-1, *mat*K, *rps*16, *trn*C-*pet*N, *trn*L-F, and *ycf*1) is detailed in Table 3. The plastid matrix contained 37 accessions, and 6224 aligned characters of which 931 (14.9 %) were potentially parsimony-informative. The combined matrix also contained 37 accessions, with an aligned length of 6591 characters, of which 998 (15.1 %) were potentially parsimony-informative (Table 3).

When analyzed separately, each marker produced a consensus tree (Fig. 4a–f) with insufficient resolution to evaluate the circumscription of the individual species recognized in *Angraecum* sect. *Dolabrifolia*, although the results did provide strong support for the monophyly of the section, with moderate to high bootstrap values (hereafter BS) of 78–100 %. Indeed, the five accessions of *A. podochiloides* formed a clade with weak support (BS = 64 %) in the analysis with the *trn*L-F matrix and with strong support using the ITS-1, *mat*K, *trn*C-*pet*N, and *ycf*1 matrices (BS = 85–100 %). The four accessions of *A. bancoense* formed a clade with weak support (BS = 61–62 %) in the analyses using the *ycf*1 and *trn*L-F matrices, respectively, and with strong support using ITS-1 (BS = 95 %). The five accessions of *A. distichum* formed a weakly supported clade (BS = 62 %) only in the analyses using the *ycf*1 matrix. The two accessions of *A.* aff. *aporoides* formed a clade with weak support (BS = 62 %) in the analysis using the *trn*C-*pet*N matrix and with strong support using *ycf*1 and *rps*16 (BS = 75 % and 86 %, respectively). The ten accessions of *A. aporoides* did not form a clade in any of the six single-marker analyses.

When analyzed together, the markers yielded more fully resolved trees that generally had higher support values (Figs. 4g–h, 5). Parsimony analysis of the combined matrix yielded 36 most parsimonious trees of 1424 steps each, with a consistency index (CI) of 0.91 and a retention index (RI) of 0.96 (Fig. 4h; Table 3). In the Bayesian analyses, a run length of 2 million generations appeared to be sufficient to obtain a satisfactory sampling of the posterior distribution (average standard deviation of split frequencies <0.001; ESS > 200 for all parameters). AWTY plots of the posterior split probabilities showed that the two independent runs were close in parameter (tree) space and confirmed the convergence diagnostic. The Bayesian

Table 2 Median, standard deviation, and range of the 13 numeric characters measured in this study

	A. aporoides ($N = 25$)	*A.* aff. *aporoides* ($N = 8$)	*A. bancoense* ($N = 66$)	*A. distichum* ($N = 63$)	*A. podochiloides* ($N = 10$)
LFL****	$17.50^{a} \pm 2.83$	$24.13^{a} \pm 2.08$	$6.50^{b} \pm 0.91$	$7.95^{c} \pm 1.13$	$14.35^{ac} \pm 4.07$
	[13.00–23.75]	[23.00–28.50]	[5.50–10.90]	[6.05–12.50]	[6.85–19.25]
LFW****	$7.35^{a} \pm 0.69$	$6.43^{a} \pm 0.61$	$3.40^{b} \pm 0.42$	$4.00^{c} \pm 0.51$	$2.75^{d} \pm 0.24$
	[6.00–8.75]	[5.75–7.50]	[2.75–5.00]	[2.90–5.00]	[2.30–3.00]
FLL****	$14.00^{a} \pm 1.31$	$12.00^{a} \pm 1.10$	$7.50^{b} \pm 0.71$	$13.15^{a} \pm 1.27$	$14.25^{a} \pm 1.61$
	[12.00–17.00]	[11.00–14.00]	[6.00–9.00]	[10.80–17.00]	[11.80–16.50]
DSL****	$5.00^{a} \pm 0.56$	$3.30^{bc} \pm 0.34$	$2.00^{b} \pm 0.26$	$4.00^{c} \pm 0.50$	$3.50^{c} \pm 0.58$
	[3.70–6.20]	[2.80–3.70]	[1.60–2.80]	[3.00–5.20]	[2.60–4.50]
DSW****	$2.20^{a} \pm 0.38$	$1.30^{bc} \pm 0.19$	$1.00^{b} \pm 0.12$	$1.90^{ac} \pm 0.25$	$0.95^{b} \pm 0.08$
	[1.50–2.90]	[1.00–1.60]	[0.50–1.30]	[1.30–2.50]	[0.90–1.10]
LSL****	$5.50^{a} \pm 0.57$	$3.93^{bc} \pm 0.36$	$2.35^{b} \pm 0.25$	$4.60^{c} \pm 0.55$	$4.10^{c} \pm 0.57$
	[4.50–7.00]	[3.40–4.50]	[1.90–2.90]	[3.50–6.00]	[3.50–5.20]
LSW****	$2.70^{a} \pm 0.50$	$1.50^{bc} \pm 0.24$	$1.10^{b} \pm 0.14$	$2.20^{ac} \pm 0.31$	$0.90^{b} \pm 0.10$
	[1.50–3.30]	[1.10–1.90]	[0.80–1.35]	[1.50–2.90]	[0.80–1.10]
LPL****	$4.30^{a} \pm 0.55$	$2.90^{bc} \pm 0.23$	$2.00^{b} \pm 0.21$	$3.80^{ac} \pm 0.57$	$3.15^{ac} \pm 0.51$
	[3.40–5.30]	[2.60–3.20]	[1.20–2.50]	[2.25–5.00]	[2.50–4.00]
LPW****	$1.50^{a} \pm 0.31$	$0.95^{bc} \pm 0.11$	$0.65^{b} \pm 0.11$	$1.20^{ac} \pm 0.19$	$0.70^{b} \pm 0.20$
	[0.90–2.10]	[0.70–1.00]	[0.30–0.85]	[0.90–1.75]	[0.40–1.00]
LIL****	$4.10^{a} \pm 0.44$	$2.70^{bc} \pm 0.36$	$1.70^{b} \pm 0.30$	$3.55^{cd} \pm 0.43$	$4.35^{ad} \pm 0.45$
	[3.10–5.00]	[2.10–3.00]	[1.00–2.60]	[2.70–4.50]	[3.50–5.00]
LIW****	$4.50^{a} \pm 0.76$	$3.15^{a} \pm 0.42$	$2.30^{b} \pm 0.24$	$4.00^{a} \pm 0.50$	$3.50^{a} \pm 0.35$
	[2.70–5.50]	[2.80–3.90]	[1.50–2.80]	[2.70–5.00]	[3.00–4.00]
SPL****	$7.00^{a} \pm 0.64$	$3.50^{bc} \pm 0.42$	$2.45^{b} \pm 0.47$	$6.60^{a} \pm 0.70$	$5.75^{ac} \pm 0.56$
	[6.00–8.50]	[3.00–4.20]	[1.80–3.70]	[4.80–8.00]	[5.30–7.00]
POL****	$10.00^{a} \pm 0.90$	$8.00^{a} \pm 0.82$	$5.40^{b} \pm 0.75$	$9.00^{a} \pm 1.04$	$11.00^{a} \pm 1.02$
	[7.50–11.00]	[8.00–10.00]	[4.00–8.00]	[6.75–11.50]	[8.50–11.50]

Significant differences and heterogeneous groups from multiple comparisons (Kruskal–Wallis test) are indicated by **** (p value <0.001) and letters ($^{a, b, c, d}$), respectively. See Table 1 for variable codes

analysis provided a well-resolved tree (Fig. 5), which showed the same topology as the consensus tree obtained from the parsimony analysis.

Phylogenetic analyses conducted on the combined matrix (Figs. 4h, 5) confirmed that *Angraecum* sect. *Dolabrifolia* is monophyletic (BS = 100 %; PP = 1). Similarly, strong support was provided for the delimitation of four of the five entities recognized, viz., *A.* aff. *aporoides* (BS = 94 %; PP = 1; clade C, Fig. 5), *A. bancoense* (BS = 99 %; PP = 1; clade D, Fig. 5), *A. distichum* (BS = 74 %; PP = 0.99; clade E, Fig. 5), and *A. podochiloides* (BS = 100 %; PP = 1; clade A, Fig. 5), but the accessions of *A. aporoides* were placed into two distinct and well-supported clades (each with BS = 100 % and PP = 1; clades B and F, respectively, Fig. 5). The first clade of *A. aporoides* (clade B), comprising three accessions from Príncipe Island and Rabi (Gabon), is sister (BS = 97 %; PP = 1) to the clade of *A.* aff. *aporoides* (clade C), known only from Gabon. The second clade of *A. aporoides* (clade F), comprising seven accessions from Bifa and Bipindi (southern Cameroon) and Gabon, is sister (BS = 97 %; PP = 1) to the subclade uniting *A. bancoense* and *A. distichum*. All three accessions of clade B were represented in the morphometric analyses (see cluster 3A in Fig. 2) while only four of the seven accessions of clade F were represented (see cluster 3B in Fig. 2).

Within *Angraecum* sect. *Dolabrifolia* (Figs. 4h, 5), *A. podochiloides* (clade A, Fig. 5) is sister (BS = 100 %; PP = 1) to a clade comprising the four other members of the group, namely *A. aporoides*, *A.* aff. *aporoides*, *A. bancoense*, and *A. distichum* (clades B–F, Fig. 5). Clade B (Fig. 5) of *A. aporoides* is sister to *A.* aff. *aporoides* (clade C, Fig. 5), and the subclade formed by clades B and C is in turn sister (BS = 100 %; PP = 1) to a subclade uniting the three other clades (clades D–F, Fig. 5). Finally, the subclade comprising *A. bancoense* and *A. distichum* (clades D and E, respectively, Fig. 5) is sister (BS = 97 %; PP = 1) to the clade F of *A. aporoides*.

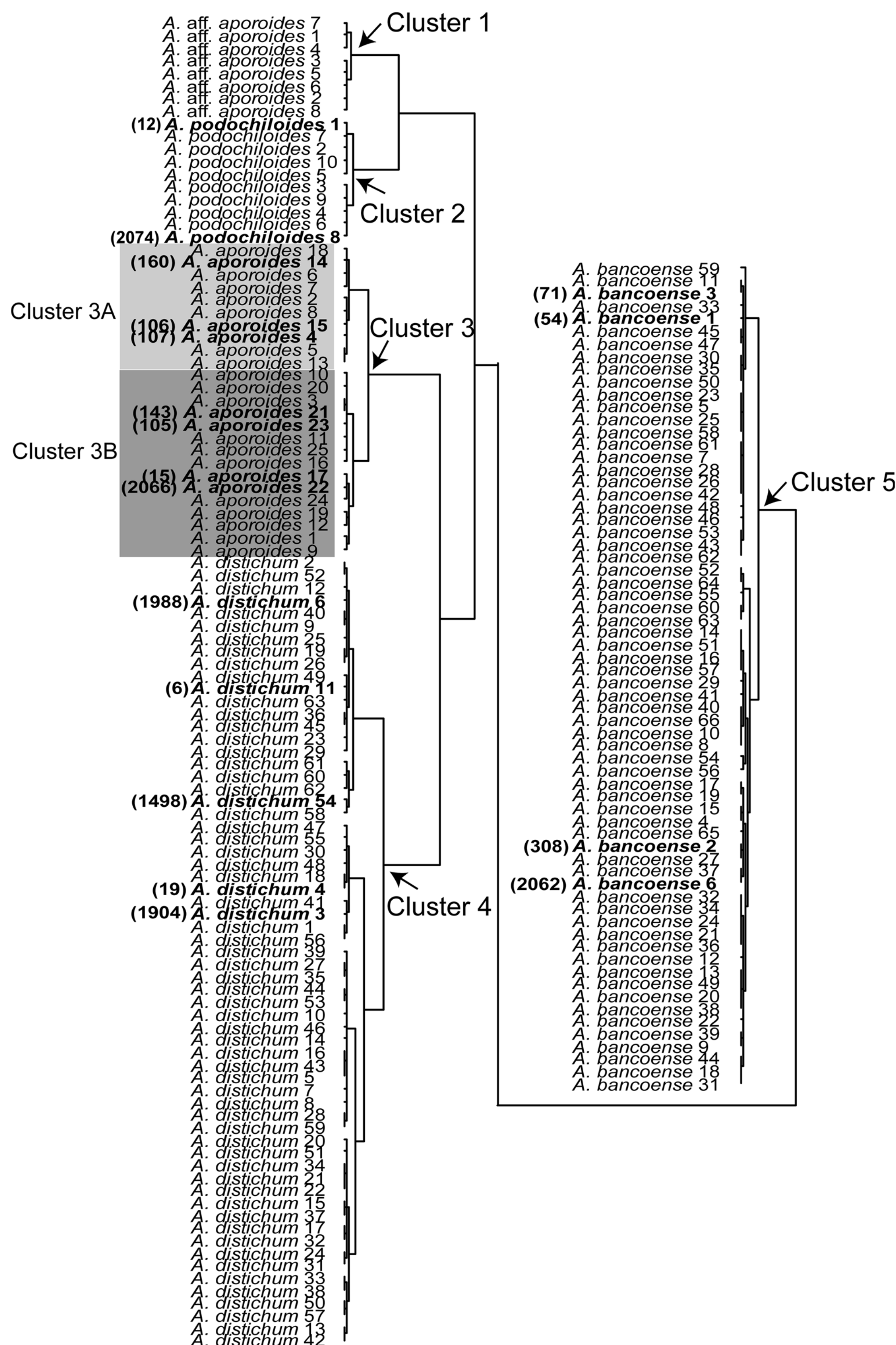

Fig. 2 Clustering dendrogram obtained by computing the distance matrix measured between the 172 specimens of *Angraecum* section *Dolabrifolia*. In *bold*, specimens with representatives in molecular analyses. Members of the two subclusters of *A. aporoides* are represented by *light gray* and *dark gray boxes*, respectively

Comparison between morphometric and phylogenetic analyses of *Angraecum aporoides*

Accessions of *Angraecum aporoides* do not appear to form a monophyletic group in our phylogenetic analyses. The type of this species could not be included in the molecular study, but in the morphometric analyses, it was placed in cluster 3B of the dendrogram (Fig. 2), which comprises 15 specimens, four of which are represented in the phylogenetic analyses. In an attempt to elucidate why the ten accessions identified as *A. aporoides* form two clades that are not sister to one another, multiple comparisons of medians using the Kruskal–Wallis test were performed on the two subclusters of *A. aporoides* observed in the clustering dendrogram (see light and gray

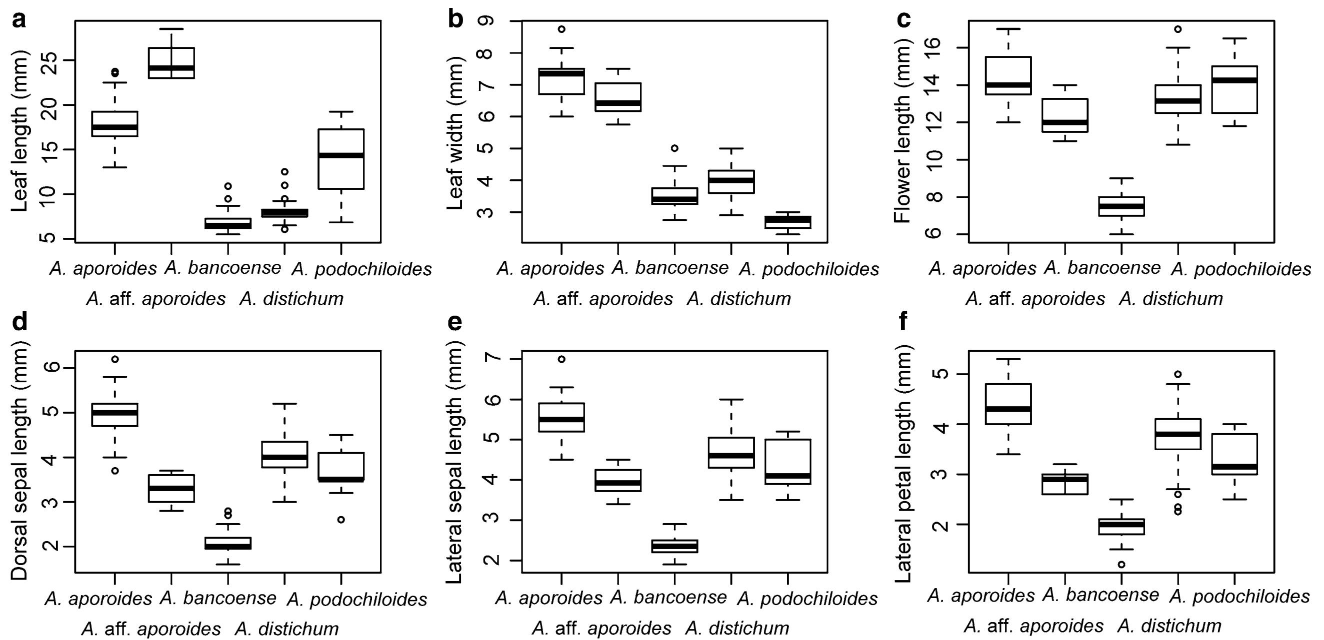

Fig. 3 *Box* and *whisker plots* depicting the character variation ranges among the five species in section *Dolabrifolia*. **a** Leaf length, **b** leaf width, **c** flower length, **d** dorsal sepal length, **e** lateral sepal length, **f** lateral petal length

Table 3 Matrix values and statistics of parsimony analyses

Tree statistics	ITS-1	*mat*K	*rps*16	*trn*C-*pet*N	*trn*L-F	*ycf*1	Plastid	Combined
Length (aligned)	367	1855	1124	949	505	1791	6224	6591
Parsimony-informative characters (%)	67 (18.26 %)	131 (7.06 %)	459 (40.84 %)	114 (12.01 %)	45 (8.91 %)	182 (10.16 %)	931 (14.96 %)	998 (15.14 %)
% of variability	24. 52	8.95	44.4	13.91	11.88	14.24	17.87	18.24
Best trees found	1	2,012	19,940	8417	2	5	9	36
Tree length	127	200	546	150	65	306	1289	1424
Consistency index	0.85	0.91	0.97	0.96	0.98	0.90	0.91	0.91
Retention index	0.91	0.96	0.98	0.98	0.99	0.96	0.96	0.96
Rescaled consistency index	0.77	0.87	0.95	0.94	0.98	0.86	0.89	0.87

boxes in Fig. 2). These comparisons indicated that nine of the 13 numeric variables are significantly different between the two subgroups, namely the dimensions of dorsal (DSL, DSW) and lateral (LSL, LSW) sepals, lateral petal (LPL, LPW), lip (LIL, LIW) and the spur length (SPL) (Table 4). However, none of these nine variables showed a clear-cut separation between the two subclusters of *A. aporoides*. Using box and whisker plots (Fig. 6), these nine numeric variables were used to graphically depict the variation among the two subclusters (cluster 3A with 10 specimens and cluster 3B with 15 specimens) of *A. aporoides*. Since none of the nine variables with significant differences was informative in distinguishing the two subclusters, we thus performed multiple comparisons of medians using the Kruskal–Wallis test, but restricted to the seven specimens represented in the molecular analyses, i.e., the three accessions of clade B and four (out of seven) of clade F (Fig. 5). The new multiple comparisons based on these collections showed that only three variables were significantly different between the two clades, lateral sepal width (LSW, 1.8–2 mm $\neq$ 2.2–3.2 mm), lip width (LIW, 3.3–3.5 mm $\neq$ 4.5–5.3 mm), and spur length (SPL, 6–6.5 mm $\neq$ 7.3–7.6 mm) for clades B and F, respectively.

Discussion

Monophyly of *Angraecum* sect. *Dolabrifolia*

Based on a phylogenetic analysis using four plastid DNA regions, Micheneau et al. (2008) found that *Angraecum* sect. *Dolabrifolia*, as defined by Garay (1973), was monophyletic, although their sampling included only a single accession from each of just two taxa belonging to the

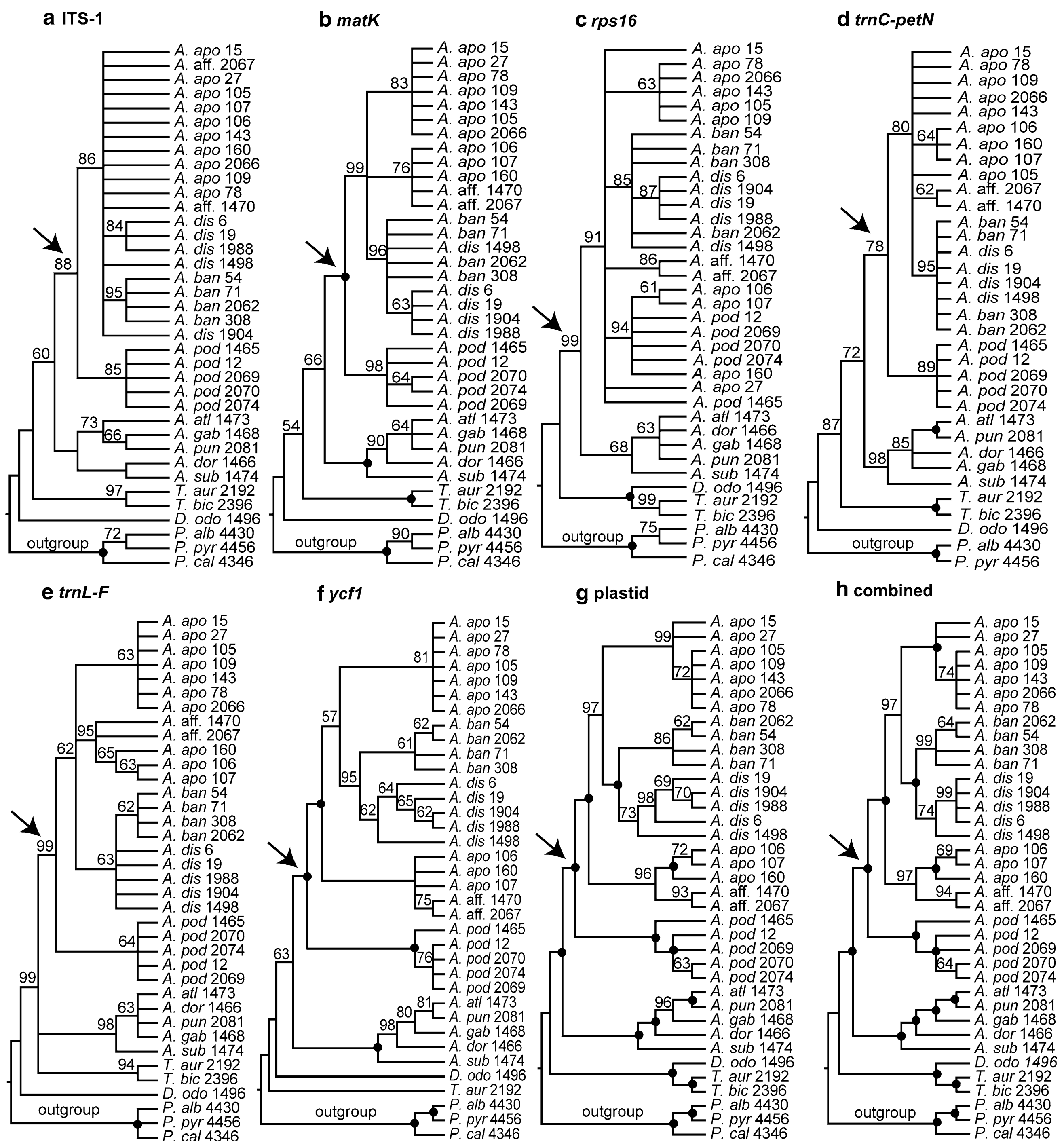

Fig. 4 Parsimony analysis (strict consensus tree with bootstrap percentages shown above or below branches) of ITS-1 (**a**), *mat*K (**b**), *rps*16 (**c**), *trn*C-*pet*N (**d**), *trn*L-F (**e**), *ycf*1 (**f**), plastid matrix (**g**), and combined matrix (**h**). Taxa: *Angraecum* sect. *Dolabrifolia*: *A. apo* = *A. aporoides*; *A.* aff. = *A.* aff. *aporoides*; *A. ban* = *A. bancoense*; *A. dis* = *A. distichum*; *A. pod* = *A. podochiloides*; *Angraecum* sect. *Pectinaria*: *A. atl* = *A. atlanticum*; *A. dor* = *A. doratophyllum*; *A. gab* = *A. gabonense*; *A. pun* = *A. pungens*; *A. sub* = *A. subulatum*; *Diaphananthe*: *D. odo* = *D. odoratissima*; *Tridactyle*: *T. aur* = *T. aurantiopunctata*; *T. bic* = *T. bicaudata*; out-groups (genus *Polystachya*): *P. alb* = *P. albescens* subsp. *imbricata*; *P. cal* = *P. calluniflora*; *P. pyr* = *P. pyramidalis*. Details of each analysis are given in Table 3. The *arrow* indicates the section *Dolabrifolia*. Nodes with 100 % bootstrap values are indicated by a *solid black circle*

group. Our study confirms their finding, but with much more comprehensive sampling from sect. *Dolabrifolia*. Each of our analyses (i.e., those based on the six individual markers as well as the combined data set) placed all members of the five recognized species of section *Dolabrifolia* in a well-supported clade that is sister to the

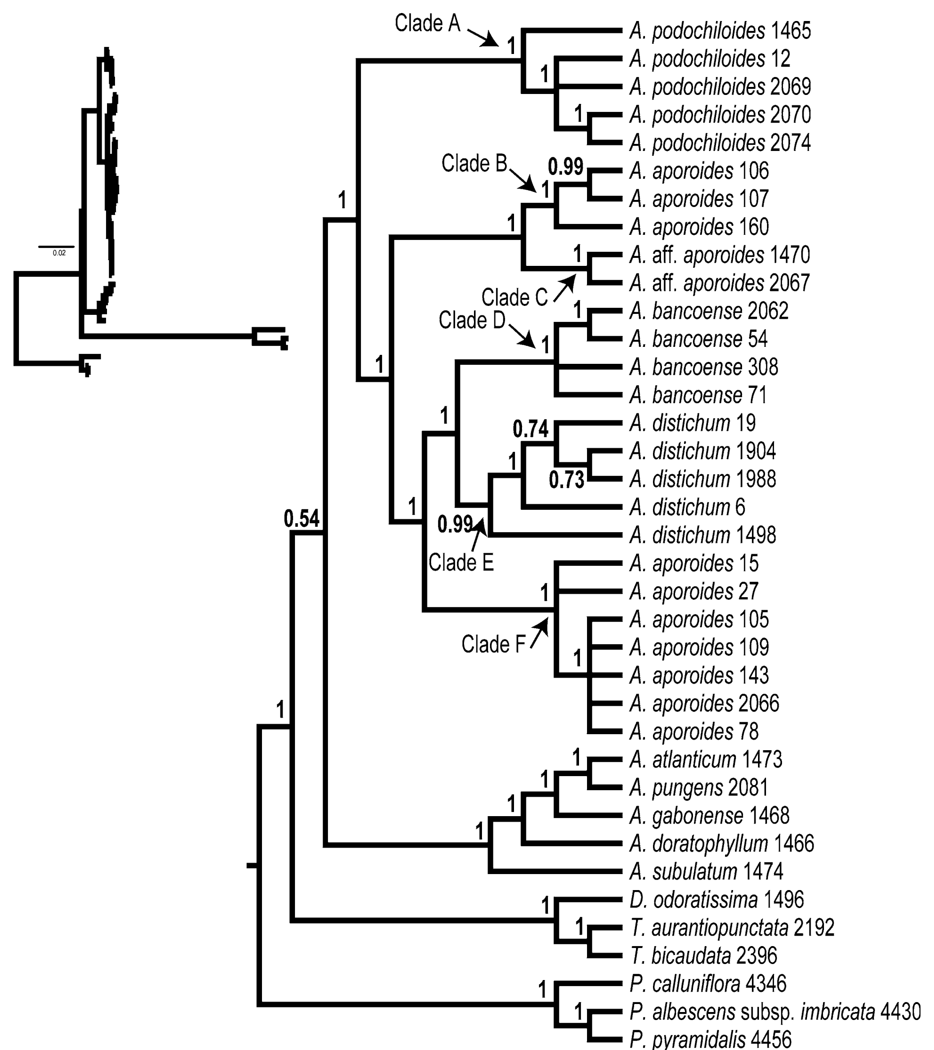

Fig. 5 Consensus tree obtained from Bayesian analysis of the combined molecular data set. Posterior probabilities (PP) are given above or below the branches. Scaled phylogram obtained from Bayesian analysis is shown in the *upper left corner*, demonstrating the relative branch lengths. *A.* = *Angraecum*; *D.* = *Diaphananthe*; *T.* = *Tridactyle*; *P.* = *Polystachya*

continental African group comprising *A.* sect. *Pectinaria*. The results of the morphometric and phylogenetic approaches used in this study to circumscribe taxa and test their delimitation provide a robust foundation for conducting a taxonomic revision of section *Dolabrifolia* (M. Simo-Droissart et al. submitted), as was recently done for *A.* sect. *Pectinaria* (Simo-Droissart et al. 2014).

Szlachetko and Romowicz (2007) raised *Angraecum* sect. *Dolabrifolia* to the rank of genus based on the unique foliar characters of the group (viz., the laterally compressed and densely imbricate leaves), and this treatment was followed by Szlachetko et al. (2013) based on molecular and morphological data. The results presented in our study do not support this interpretation, but a final decision on how best to treat this group must await the resolution of phylogenetic relationships within the entire genus *Angraecum* and allies, in the context of our efforts to develop a robust generic/infrageneric classification system that takes into account all members of the broader angraecoid orchid clade.

Species hypotheses in *Angraecum* sect. *Dolabrifolia*

When considered together with the clustering dendrogram constructed using the distance matrix of the 172 specimens of *Angraecum* sect. *Dolabrifolia*, the combination of the first three axes of the extended PCA yielded clear delimitations of each of the five species-level entities identified prior to conducting the morphometric analyses. The results of the molecular phylogenetic analyses support the delimitation of four of these species, while accessions of the fifth, *A. aporoides*, were placed in two separate, well-supported clades. Below, we discuss the circumscription of each of these taxa.

Angraecum podochiloides

This species, native to the Guineo-Congolian Region (see White 1979), possesses the narrowest leaves in *Angraecum* sect. *Dolabrifolia*, which are lanceolate and have an acute or subacute apex. It also has the narrowest lateral sepals of any

Table 4 Median, standard deviation, and range of the 13 numeric characters measured in this study for the two clusters of *A. aporoides*

	A. aporoides cluster 3A ($N = 10$)	*A. aporoides* cluster 3B ($N = 15$)
LFL	17.75[a] ± 2.85	17.50[a] ± 2.91
	[13.60–22.50]	[13.00–23.75]
LFW	7.08[a] ± 0.53	7.40[a] ± 0.76
	[6.00–7.50]	[6.00–8.75]
FLL	13.88[a] ± 1.08	15.00[a] ± 1.35
	[12.00–16.00]	[12.00–17.00]
DSL****	4.70[a] ± 0.50	5.20[b] ± 0.48
	[3.70–5.20]	[4.20–6.20]
DSW****	1.80[a] ± 0.12	2.40[b] ± 0.28
	[1.50–1.90]	[1.80–2.90]
LSL****	5.25[a] ± 0.40	5.80[b] ± 0.60
	[4.50–5.90]	[4.50–7.00]
LSW****	2.00[a] ± 0.20	2.90[b] ± 0.30
	[1.50–2.20]	[2.20–3.30]
LPL****	4.00[a] ± 0.33	4.80[b] ± 0.53
	[3.60–4.75]	[3.40–5.30]
LPW****	1.20[a] ± 0.12	1.70[b] ± 0.24
	[0.90–1.30]	[1.20–2.10]
LIL****	4.00[a] ± 0.34	4.50[b] ± 0.37
	[3.10–4.20]	[4.00–5.00]
LIW****	3.50[a] ± 0.59	4.80[b] ± 0.48
	[2.70–5.00]	[4.00–5.50]
SPL****	6.50[a] ± 0.41	7.30[b] ± 0.62
	[6.00–7.00]	[6.00–8.50]
POL	9.25[a] ± 0.94	10.00[a] ± 0.85
	[7.50–11.00]	[8.00–11.00]

Significant differences and heterogeneous groups from multiple comparisons (Kruskal–Wallis test) are indicated by **** (p value <0.001) and letters ([a, b]), respectively. See Table 1 for variable codes

species in the group. While the other members of the section have entirely white flowers, *A. podochiloides* is easily recognized by the yellow or orange tips of its perianth parts (see Simo et al. 2010). In the morphometric study, the projections based on the extended PCA placed *A. podochiloides* and *A.* aff. *aporoides* close to one another, primarily because the leaves of both species have an acute apex (Figs. 1a, 2). The trees resulting from the phylogenetic analyses based on molecular sequence data placed the five accessions of *A. podochiloides* in a well-supported clade sister to the remaining clades of section *Dolabrifolia*, with the exception of the *rps*16 tree, which lacked resolution (placing all *Dolabrifolia* species in a polytomy; Fig. 4c).

The *Angraecum aporoides* complex

The ten accessions of *Angraecum aporoides* included in our molecular phylogenetic analyses form two clades that are not sisters (clades B and F, Fig. 5). These two clades correspond precisely to the two subclusters of *A. aporoides* in the dendrogram resulting from the extended PCA performed on morphological data (clusters 3A and 3B in Fig. 2), cluster 3A comprising 10 specimens and cluster 3B comprising 15 specimens. Although nine of the 13 numeric variables are significantly different between the two subclusters, none of them shows any clear-cut separation. Moreover, there is no apparent difference in geographic distribution among the members of clades B and F. Indeed, accessions of clade B (and their representative specimens) were collected on Príncipe Island and in Gabon (with other specimens of cluster 3A coming from Cameroon and São Tomé Island) while those belonging to clade F came from Cameroon and Gabon (with other specimens of cluster 3B also coming from Cameroon and Gabon, as well as Nigeria, where the type specimen was collected). Multiple comparisons of morphometric characters from the seven specimens of *A. aporoides* represented in the molecular analyses (clades B and F) revealed that three of the 13 variables (LSW, LIW, and SPL) are significantly different, with a clear-cut separation between the two clades.

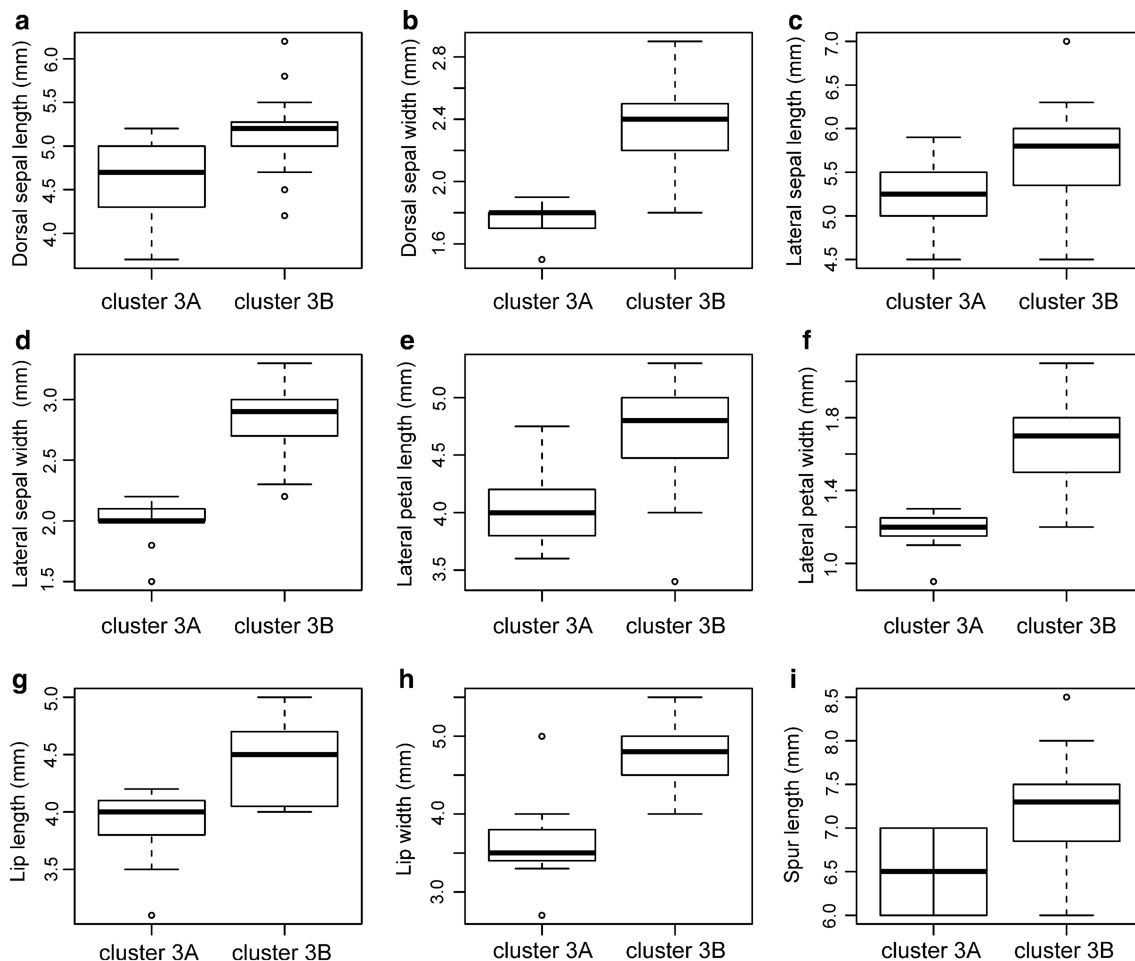

Fig. 6 *Box* and *whisker plots* depicting the character variation ranges among the two subclusters of *Angraecum aporoides*. **a** Dorsal sepal length, **b** dorsal sepal width, **c** lateral sepal length, **d** lateral sepal width, **e** lateral petal length, **f** lateral petal width, **g** lip length, **h** lip width, **i** spur length

However, these morphological differences are observed only when considering the reduced sample size of *A. aporoides* (seven out of 25), and not with the broader sampling of 25 specimens used in the dendrogram. As such, these features alone are thus not sufficient to distinguish between the two subclusters of this species. The type specimen of *A. aporoides* (specimen *A. aporoides* 9) is placed in cluster 3B in the clustering dendrogram. The distinction between clades B and F appears based solely on plastid data, since this portion of the ITS tree is not resolved. Indeed, all five plastid sequences are derived from a single, non-recombining genome, and therefore represent a single marker. Another possible explanation of the non-sister placement of clades B and F (Fig. 5) could be that hybridization has taken place and that the tree obtained from the combined data set reflects lateral transfer of the plastid genome. Further studies including additional specimens for both morphometric and DNA-based phylogenetic analyses (especially using nuclear markers) will be needed to clarify whether the two clades of *A. aporoides* recovered in our molecular phylogenetic analyses warrant taxonomic recognition.

Angraecum aporoides versus *A.* aff. *aporoides*

As indicated above, while examining material of *Angraecum* sect. *Dolabrifolia* in order to define species, some specimens were found to be similar to *A. aporoides* in general appearance but to have leaves that clearly differed in shape and by the presence of an acute apex (a unique feature among the members of the section with entirely white flowers). All of these atypical specimens were collected in Gabon, while material of typical *A. aporoides* was obtained from São Tomé and Príncipe, Gabon, and Cameroon. The material of *A.* aff. *aporoides* thus appears to represent a new entity best recognized at the species level, which is being described in a separate paper (M. Simo-Droissart et al. submitted).

The specimens assigned to *Angraecum* aff. *aporoides* clearly differ from those of *A. aporoides* by eight of the 13

numeric variables, of which the length of the lateral petals (LPL), the lip (LIL), and the spur (SPL) are the most informative, providing a clear-cut separation between these two entities. With their relatively small flowers, plants of *A.* aff. *aporoides* resemble *A. bancoense*, which is widely distributed in the Guineo-Congolian Region, but they differ clearly by leaf length (LFL) and width (LFW), and the length of the flower (FLL), the lateral sepals (LSL), and the lateral petals (LPL) (see Table 2). None of the trees resulting from the phylogenetic analyses placed the accessions of *A.* aff. *aporoides* (clade C) and those of the *A. aporoides* complex (clades B and F) as sisters.

Angraecum bancoense versus *A. distichum*

These two species are clearly distinct from one another on the basis of flower size, but are difficult to distinguish vegetatively. In their original description of *Angraecum bancoense*, Arends et al. (1980) pointed out this difficulty, noting the similarities in leaf shape and concluding that the two species were vegetatively indistinguishable. For this reason, we used the largest possible sample size to observe and quantify variation in leaf shape. Taken together, the specimens of *A. bancoense* (66 specimens) and *A. distichum* (63 specimens) represent 75 % (129 out of 172) of the specimens used in our morphometric analyses. This is particularly important because variation in leaf shape between specimens of these two species often leads to misidentifications in the absence of floral parts, as Arends et al. (1980) had suggested. In fact, most fruiting collections that retained remnant floral parts, notably the spur (whose length is informative in distinguishing the two species), were erroneously identified as *A. distichum*, long regarded as the most widespread and abundant species in section *Dolabrifolia*. These numerous misidentifications may also have resulted from the fact that these two species are sometimes found growing together, which has confused collectors and orchid taxonomists alike.

The numerous misidentifications involving material of *Angraecum distichum* and *A. bancoense* prompted us to question the taxonomic status of these two species and to consider whether other morphological features, together with geographic distribution, could be used to differentiate them. According to Arends et al. (1980), *A. distichum* differs from *A. bancoense* by its larger flowers, which are longer than the leaves. However, we found several specimens clearly referable to *A. bancoense* that also possess flowers longer than the leaves. All of the accessions used in the molecular analyses were unambiguously assigned to either *A. bancoense* or *A. distichum* and were placed in the corresponding clades, which were well supported as sister groups (Figs. 4, 5), confirming that they represent divergent lineages, but also share a most recent common ancestor.

As currently circumscribed, *Angraecum bancoense* and *A. distichum* are widely distributed in West and Central Africa. Preliminary tests of autonomous self-pollination in these two species have shown that they both require a pollinator to set fruits, and self-pollination carried out by hand showed that they are self-compatible. While *A. bancoense* and *A. distichum* occur in sympatry and flower throughout the year, no intermediate forms have been observed, suggesting the presence of one or more reproductive barriers between them. This interpretation is further supported by the fact that preliminary cross-pollination tests between the two species failed to yield any fruit.

Angraecum poppendickianum

The status of the recently described *Angraecum poppendickianum* Szlachetko and Olszewski (2001) has been controversial since its publication in the Flore du Cameroun, particularly because it was not accepted in the world checklist (Govaerts et al. 2016). The description suggests that *A. poppendickianum* may be an intermediate form between *A. distichum* (which it resembles vegetatively) and *A. aporoides* (which has similar flowers), and Szlachetko and Olszewski (2001) commented that the material on which they based their novelty might indeed be of hybrid origin. However, examination of these specimens (*N. Hallé 872*, P 00259997!, the holotype, and *Merle 32*, P 00259998!, a paratype) clearly shows that they belong to *A. distichum* because the sizes of their leaves, flowers, and floral parts fall within the range of material belonging to that species. In light of this information, we therefore regard *A. poppendickianum* as a synonym of *A. distichum*.

Conclusions

Comparison of the results from the morphometric investigations of the members of *Angraecum* section *Dolabrifolia* with those from phylogenetic analyses based on DNA sequence data has helped to clarify species circumscriptions within this group, an indispensable prerequisite for undertaking a taxonomic revision. Our results confirm the monophyly of the section and indicate that it comprises at least five species, namely *A. aporoides*, *A. bancoense*, *A. distichum, A. podochiloides*, and a new taxon, provisionally referred to as *A.* aff. *aporoides*. The delimitation of four of these five species is well supported, but the multiple accessions of the fifth, *A. aporoides*, appear to form a paraphyletic group, comprising two well-supported clades. The members of these two clades differ morphologically by the size of the flowers (lateral sepal width, lip width, and spur length), but these differences do not show a clear-cut separation between the two subclusters obtained in the

morphometric analyses. Moreover, no geographic pattern was detected in either the two clades or the two subclusters. In an attempt to assess whether the two clades of *A. aporoides* can be differentiated morphologically, a broader morphometric analysis may be needed that would include expanded sampling from throughout the geographic range of this species. Also, molecular phylogenetic analyses including more nuclear markers should be performed.

Results from the present study have also helped to differentiate two frequently confused species, *Angraecum bancoense* and *A. distichum*, which can be easily distinguished based on the length of their flowers and sepals, as well as the length of their lip and spur, along with the width of their lateral sepals and petals. The new species, currently referred to as *A.* aff. *aporoides*, is closely related to *A. aporoides* but differs based on both morphometric and molecular data. This new taxon is only known from Gabon and is being described as part of a broader taxonomic revision of the entire section (M. Simo-Droissart et al. submitted).

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Appendix

Voucher information and GenBank accession numbers for taxa used in the phylogenetic analysis of *Angraecum* sect. *Dolabrifolia* (including out-groups). For each taxon, voucher information (between brackets) is listed in the following order: locality, date, collector name and collection number, herbarium acronym. Accession numbers are listed in the following order: ITS-1, *mat*K, *rps*16, *trn*C-*pet*N, *trn*L-F, *ycf*1. Hyphens indicate that no data are available.

In-group: *Angraecum aporoides* Summerh. (South Cameroon, Bifa, 12 Jul 2007, *Droissart* et al. (*Yaoundé shadehouse*) *592*, BRLU) KF672217, KF672266, KF672230, KF672308, KF662332, KF672336; *A. aporoides* Summerh. (South Cameroon, Bipindi, 1 Oct 2009, *Simo* et al. (*Yaoundé shadehouse*) *1839*, BRLU) KX060061, KX060081, KX060101, –, KX060139, KX060159; *A. aporoides* Summerh. (Gabon, Andok Foula, 2 Feb 2008, *Jardin Botanique de Bambusa 144*, BRLU) KX060058, KX060078, KX060098, KX060117, KX060136, KX060156; *A. aporoides* Summerh. (Gabon, Rabi, 8 Jul 2011, *Jardin Botanique de Bambusa 193*, BRLU) KX060057, KX060077, KX060097, KX060116, KX060135, KX060155; *A. aporoides* Summerh. (Gabon, *s.loc.*, 25 May 2007, *Jardin Botanique de Bambusa 83*, BRLU) KX060055, KX060075, KX060095, KX060114, KX060133, KX060153; *A. aporoides* Summerh. (Gabon, *s.loc.*, 6 Feb 2012, *Jardin Botanique de Bambusa 259*, BRLU) KX060054, KX060074, KX060093, KX060113, KX060132, KX060152; *A. aporoide*s Summerh. (Gabon, *s.loc.*, 2 Mar 2010, *Jardin Botanique de Bambusa 131*, BRLU) KX060046, KX060066, KX060086, KX060106, KX060124, KX060144; *A. aporoides* Summerh. (Gabon, Mont Songo, 3 Mar 2008, *Accession N° of Cult. Tchimbélé shadehouse MBG 161*, BRLU) KX060059, KX060079, KX060099, KX060118, KX060137, KX060157; *A. aporoides* Summerh. (São Tomé and Príncipe, Praia da Lapa, 1 Feb 2008, *Accession N° of Cult. Bom Successo* shadehouse *946*, BRLU) KF672202, KF672276, KF672242, KF672292, KF662338, KF672340; *A. aporoides* Summerh. (Gabon, *s.loc.*, 25 May 2007, Cultivated at *JardiGab shadehouse N° BTO 30*, BRLU) KX060056, KX060076, KX060096, KX060115, KX060134, KX060154; *Angraecum* aff. *aporoides* (Gabon, Rabi, 3 Jun 2010, *Accession N° of Cult.* BR 20090387-38, BR) KX060051, KX060071, KX060091, KX060110, KX060129, KX060149; *A.* aff. *aporoides* (Gabon, *s.loc., s.d., Jardin Botanique de Bambusa 50*, BRLU) KF672200, KF672286, KF672232, KF672288, KF662340, KF672333; *Angraecum atlanticum* Stévart and Droissart (Gabon, Doudou Mountains, 12 Sep 1986, *van der Laan 1068*, BRLU) KF672213, KF672284, KF672243, KF672299, KF662343, KF672335; *Angraecum bancoense* Burg (South Cameroon, Nkolembonda, 14 Oct 2010, *Simo* et al. (*Yaoundé shadehouse*) *2462*, BRLU) KX060047, KX060067, KX060087, KX060107, KX060125, KX060145; *A. bancoense* Burg (Littoral Cameroon, Douala-Edéa Reserve, 6 Jun 2009, *Simo* et al. (*Yaoundé shadehouse*) *1581*, BRLU) KX060053, KX060073, KX060093, KX060112, KX060131, KX060151; *A. bancoense* Burg (South Cameroon, Monts des Eléphants, 26 Jul 2007, *Droissart* et al. (*Yaoundé shadehouse*) *620*, BRLU), KF672221, KF672280, KF67225, KF672311, KF662335, KF672320; *A. bancoense* Burg (South Cameroon, Nkoltsia, 28 Jun 2010, *Simo* et al. (*Yaoundé shadehouse*) *2207*, BRLU) KX060060, KX060080, KX060100, KX060119, KX060138, KX060158; *Angraecum distichum* Lindl.

(Centre Cameroon, Mbam Minkom, 2 Aug 2004, *Droissart* et al. (*Yaoundé shadehouse*) *62*, BRLU) KX060062, KX060082, KX060102, KX060120, KX060140, KX060160; *A. distichum* Lindl. (South Cameroon, Yokadouma-Moloundou road, 23 Aug 2010, *Simo* et al. (*Yaoundé shadehouse*) *2309*, BRLU) KX060049, KX060069, KX060089, KX060108, KX060127, KX060147; *A. distichum* Lindl. (South Cameroon, Ngoyla, 17 Sep 2010, *Simo* et al. (*Yaoundé shadehouse*) *2394*, BRLU) KX060048, KX060068, KX060088, −, KX060126, KX060146; *A. distichum* Lindl. (East Cameroon, Dja Forest Reserve, 24 Jan 2008, *Droissart* et al. (*Yaoundé shadehouse*) *924*, BRLU) KF672227, KF672265, KF672231, KF672289, KF662348, KF672337; *A. distichum* Lindl. (Guinea-Bissau, Mounts Kalikouma, 3 Jun 2010, *Accession N° of Cult. BR 19540481,* BR) KX060050, KX060070, KX060090, KX060109, KX060128, KX060148; *Angraecum doratophyllum* Summerh. (São Tomé and Príncipe, *s.loc.*, 3 Jun 2010, *Accession N° of Cult. BR 20090375-26*, BR) KF672224, KF672261, KF672247, KF672296, KF662351, KF672328; *Angraecum gabonense* Summerh. (Gabon, between Rabi 49 and 50, 22 Jun 1992, *Arends 957*, BRLU) KF672209, KF672279, KF672237, KF672295, KF662333, KF672344; *Angraecum podochiloides* Schltr. (*s.loc.*, 3 Jun 2010, *Accession N° of Cult. BR 2009380-31*, BR) KX060052, KX060072, KX060092, KX060111, KX060130, KX060150; *A. podochiloides* Schltr. (Southwest Cameroon, Banyang Mbo wildlife sanctuary, 8 Feb 2008, *Droissart* et al. (*Yaoundé shadehouse*) *939*, BRLU) KF672225, KF672281, KF672238, KF672293, KF662330, KF672339; *A. podochiloides* Schltr. (Gabon, *s.loc., s.d.*, *Accession N° of Cult. Tchimbélé shadehouse MBG* 656, BRLU) KX060045, KX060065, KX060085, KX060105, KX060123, KX060143; *A. podochiloides* Schltr. (Gabon, *s.loc.*, 15 Feb 2012, *Accession N° of Cult. Tchimbélé shadehouse*, *MBG* 703, BRLU) KX060044, KX060064, KX060084, KX060104, KX060122, KX060142; *A. podochiloides* Schltr. (Gabon, *s.loc.*, 11 Jan 2011, *Jardin Botanique de Bambusa 159*, BRLU) KX060043, KX060063, KX060083, KX060103, KX060121, KX060141; *Angraecum pungens* Schltr. (Southwest Cameroon, Banyang Mbo wildlife sanctuary, 31 May 2011, *Simo* et al. (*Yaoundé shadehouse*) *2817*, BRLU) KF672216, KF672260, KF672249, KF672304, KF662328, KF672338; *Angraecum subulatum* Lindl. (Ivory Coast, *s.loc.*, 3 Jun 2010, *Cultivated at BR Greenhouse Accession N° BR 20090388-39*, BR) KF672206, KF672285, KF672251, KF672300, KF662355, KF672324; *Diaphananthe odoratissima* (Rchb.f.) P.J.Cribb and Carlsward (Rwanda, Bugesera, 3 Jun 2010, *Cultivated at BR Greenhouse*, *Accession N° BR 19910192-69*, BR) KF672208, KF672282, KF672256, KF672315, KF662341, KF672345; *Tridactyleaurantiopunctata* P.J.Cribb and Stévart (São Tomé and Príncipe, Pico Papagaio, 1 Sep 1999, *Stévart 656*, BRLU) KF672201, KF672287, KF672236, KF672290, KF662356, KF672319; *Tridactyle bicaudata* (Lindl.) Schltr. (Democratic Republic of the Congo, Kisantu, *s.d.*, *Cultivated at Kisantu shadehouse Accession N° KIS 135*, BRLU) KF672210, KF672263, KF672234, KF672305, KF662346, −; **Out-groups**: *Polystachya albescens* Ridl. subsp. *imbricata* (Rolfe) Summerh. (South Cameroon, Akom II, 8 Nov 2010, *Simo* et al. (*Yaoundé shadehouse*) *2553*, BRLU) KF672219, KF672259, KF672246, KF672310, KF662352, KF672317; *Polystachya calluniflora* Kraenzl. (Southwest Cameroon, Rumpi Hills, 2 Nov 2010, *Simo* et al. (*Yaoundé shadehouse*) *2527*, BRLU) KF672214, KF672262, KF672229, −, KF662331, KF672329; *Polystachya pyramidalis* Lindl. (South Cameroon, Eboundja, 25 Oct 2010, *Simo* et al. (*Yaoundé shadehouse*) *2497*, BRLU) KF672212, KF672283, KF672245, KF672294, KF662339, KF672332.

Information on Electronic Supplementary Material

Online Resource 1. Material used for morphological study of species of *Angraecum* section *Dolabrifolia*
Online Resource 2. Species names with ID DNA, distribution and voucher information for all taxa used in this study
Online Resource 3. Alignment used to produce the tree with the ITS-1 marker
Online Resource 4. Alignment used to produce the tree with the *rps16* marker
Online Resource 5. Alignment used to produce the tree with the *trnL-F* marker
Online Resource 6. Alignment used to produce the tree with the *matK* marker
Online Resource 7. Alignment used to produce the tree with the *ycf1* marker
Online Resource 8. Alignment used to produce the tree with the *trnC-petN* marker
Online Resource 9. Alignment used to produce the tree with the five plastid markers
Online Resource 10. Alignment used to produce the tree with the six markers
Online Resource 11. Loadings of the first three axes of the extended principal component analysis on the 172 specimens of section *Dolabrifolia*, eigenvalues, percentage of variance, and cumulative percentage of variance explained by these first three axes. In bold, the highest loadings of each principal component. See Table 1 for variable codes

References

Arends JC, van der Burg WJ, van der Laan FM (1980) Notes on African orchids. In: Arends JC, Boelema G, de Groot CT,

Leewenberg AJM (eds) Miscellaneous papers Landbouwhogeschool 19. Wageningen, Veenman H & Zonen BV, pp 23–36
Blattner FR (1999) Direct amplification of the entire ITS region from poorly preserved plant material using recombinant PCR. Biotechniques 27:1180–1186
Borg I, Groenen P (1997) Modern multidimensional scaling: theory and applications. Springer series in statistics. Springer, New York
Bory de Saint Vincent JBGM (1804) Voyage dans les quatre principales îles des mers d'Afrique. F. Buisson, Paris
Cameron KM (2004) Utility of plastid psaB gene sequences for investigating intrafamilial relationships within Orchidaceae. Molec Phylogen Evol 31:1157–1180. doi:10.1016/j.ympev.2003.10.010
Cameron KM, Chase MW (1999) Phylogenetic relationships of Pogoniinae (Vanilloideae, Orchidaceae): an herbaceous example of the eastern North America–eastern Asia phytogeographic disjunction. J Pl Res 112:317–329
Cameron KM, Chase MW, Whitten WM, Kores PJ, Jarrell DC, Albert VA, Yukawa T, Hills HG, Goldman DH (1999) A phylogenetic analysis of the Orchidaceae: evidence from rbcL nucleotide sequences. Amer J Bot 86:208–224
Carlsward BS, Whitten WM, Williams NH (2003) Molecular phylogenetics of Neotropical leafless Angraecinae (Orchidaceae): reevaluation of generic concepts. Int J Pl Sci 164:43–51. doi:10.1086/344757
Carlsward BS, Stern WL, Bytebier B (2006a) Comparative vegetative anatomy and systematics of the angraecoids (Vandeae, Orchidaceae) with an emphasis on the leafless habit. Bot J Linn Soc 151:165–218. doi:10.1111/j.1095-8339.2006.00502.x
Carlsward BS, Whitten WM, Williams NH, Bytebier B (2006b) Molecular phylogenetics of Vandeae (Orchidaceae) and the evolution of leaflessness. Amer J Bot 93:770–786. doi:10.3732/ajb.93.5.770
Chase MW, Hills HG (1991) Silica gel: an ideal desiccant for preserving field-collected leaves for use in molecular studies. Taxon 40:215–220. doi:10.2307/1222975
Chase MW, Cameron KM, Hills HG, Jarrell D (1994) Molecular systematics of the Orchidaceae and other lilioid monocots. In: Pridgeon AM (ed) Proceedings of the 14th world orchid conference. Her Majesty's Stationary Office, London, pp 61–73
Chase MW, Cameron KM, Freudenstein JV, Pridgeon AM, Salazar G, Berg C, Schuiteman A (2015) An updated classification of Orchidaceae. Bot J Linn Soc 177:151–174
Chessel D, Dufour A-B, Thioulouse J (2004) The ade4 package. I. One-table methods. R News 4:5–10
Cuenoud P, Savolainen V, Chatrou LW, Powell M, Grayer RJ, Chase MW (2002) Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid rbcL, atpB, and matK DNA sequences. Amer J Bot 89:132–144
Cupido CN (2003) Systematic studies in the genus Merciera (Campanulaceae): a re-assessment of species boundaries. Adansonia 25:33–44
Dray S, Dufour A-B (2007) The ade4 package: implementing the duality diagram for ecologists. J Stat Soft 22:1–20
Dray S, Dufour A-B, Chessel D (2007) The ade4 package II: two-table and K-table methods. R news 7:47–52
Droissart V (2009) Étude taxonomique et biogéographique des plantes endémiques d'Afrique centrale atlantique: le cas des Orchidaceae. PhD thesis, Université Libre de Bruxelles
Droissart V, Sonké B, Stévart T (2006) Les Orchidaceae endémiques d'Afrique centrale atlantique présentes au Cameroun. Syst & Geogr Pl 76:3–84
Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214
Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Duran C, Field M, Heled J, Kearse M, Markowitz S, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A (2005) Geneious Pro, version 6. Program distributed by Biomatters. Available at: http://www.geneious.com. Accessed 25 Oct 2015
Efron B (1979) Bootstrap methods: another look at the jackknife. Ann Stats 7:1–26
Fay MF, Chase MW (2009) Orchid biology: from Linnaeus via Darwin to the 21st century. Ann Bot 104:359–364. doi:10.1093/aob/mcp190
Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791
Fitch WM (1971) Toward defining the course of evolution: minimum change for a specific tree topology. Syst Zool 20:406–416
Garay LA (1973) Systematics of the genus *Angraecum* (Orchidaceae). Kew Bull 28:495–516
Giraudoux P (2013) Pgirmess: data analysis in ecology. R package version 1.5.7. Available at: http://CRAN.R-project.org/package=pgirmess. Accessed 10 Mar 2015
Goldman DH, Freudenstein JV, Kores PJ, Molvray M, Jarrell DC, Whitten WM, Cameron KM, Jansen RK, Chase MW (2001) Phylogenetics of Arethuseae (Orchidaceae) based on plastid matK and rbcL sequences. Syst Bot 26:670–695
Górniak M, Paun O, Chase MW (2010) Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, Xdh: congruence with organellar and nuclear ribosomal DNA results. Molec Phylogen Evol 56:784–795. doi:10.1016/j.ympev.2010.03.003
Govaerts R, Bernet P, Kratochvil K, Gerlach G, Carr G, Alrich P, Pridgeon AM, Pfahl J, Campacci MA, Holland Baptista D, Tigges H, Shaw J, Cribb PJ, George A, Kreuz K, Wood J (2016) World Checklist of Orchidaceae The Board of Trustees of the Royal Botanic Gardens, Kew. Available at: http://www.kew.org/wcsp/. Accessed 20 Feb 2016
Graves S, Piepho H-P, Selzer L, with help from Dorai-Raj S (2012) multcompView: visualizations of paired comparisons. Available at: http://CRAN.R-project.org/package=multcompView. Accessed 10 Oct 2015
Hill MO, Smith AJE (1976) Principal component analysis of taxonomic data with multi-state discrete characters. Taxon 25:249–255
Hollander M, Wolfe DA (1973) Nonparametric statistical methods. Wiley, New York
Huelsenbeck JP, Larget B, Alfaro ME (2004) Bayesian phylogenetic model selection using reversible jump Markov chain Monte Carlo. Molec Biol Evol 21:1123–1133. doi:10.1093/molbev/msh123
Johnson LA, Soltis DE (1994) Matk DNA-sequences and phylogenetic reconstruction in Saxifragaceae S-Str. Syst Bot 19:143–156
Katoh K, Misawa K, Kuma K, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucl Acids Res 30:3059–3066
Lee C, Wen J (2003) Phylogeny of Panax using chloroplast trnC-trnD intergenic region and the utility of trnC–trnD in interspecific studies of plants. Molec Phylogen Evol 31:894–903. doi:10.1016/j.ympev.2003.10.009
Lindley J (1836) Edwards's botanical register, vol 21. James Ridgway and sons, London
Marcysiak K, Mazur M, Romo A, Montserrat JM, Didukh Y, Boratyńska K, Jasińska A, Kosiński P, Boratyński A (2007) Numerical taxonomy of *Juniperus thurifera*, *J. excelsa* and *J. foetidissima* (Cupressaceae) based on morphological characters. Bot J Linn Soc 155:483–495. doi:10.1111/j.1095-8339.2007.00730.x
Martos F, Johnson SD, Peter CI, Bytebier B (2014) A molecular phylogeny reveals paraphyly of the large genus Eulophia

(Orchidaceae): a case for the reinstatement of Orthochilus. Taxon 63:9–23. doi:10.12705/631.6
Mayr E (1969) The biological meaning of species. Biol J Linn Soc 1:311–320
Micheneau C, Carlsward BS, Fay MF, Bytebier B, Pailler T, Chase MW (2008) Phylogenetics and biogeography of Mascarene angraecoid orchids (Vandeae, Orchidaceae). Molec Phylogen Evol 46:908–922. doi:10.1016/j.ympev.2007.12.001
Molvray MP, Kores J, Chase MW (2000) Polyphyly of mycoheterotrophic orchids and functional influences on floral and molecular characters. In: Wilson KL, Morrison DA (eds) Monocots: systematics and evolution. CSIRO Publishing, Collingwood, pp 441–448
Mytnik-Ejsmont J, Szlachetko DL, Baranow P, Górniak M (2013) A phylogenetic and morphological study of Polystachya sect. Superpositae (Orchidaceae) with description of a new species from Cameroon. Pl Syst Evol. doi:10.1007/s00606-013-0856-0
Neubig KM, Whitten W, Carlsward BS, Blanco M, Endara L, Williams NH, Moore M (2009) Phylogenetic utility of ycf 1 in orchids: a plastid gene more variable than mat K. Pl Syst Evol 277:75–84. doi:10.1007/s00606-008-0105-0
Nylander JAA, Wilgenbusch JC, Warren DL, Swofford DL (2008) AWTY (are we there yet?): a system for graphical exploration of MCMC convergence in Bayesian phylogenetics. Bioinformatics 24:581–583. doi:10.1093/bioinformatics/btm388
Oxelman B, Liden M, Berglund D (1997) Chloroplast rps16 intron phylogeny of the tribe Sileneae (Caryophyllaceae). Pl Syst Evol 206:393–410
Piepho H-P (2004) An algorithm for a letter-based representation of all-pairwise comparisons. J Comput Graph Stat 13:456–466. doi:10.1198/1061860043515
Poulsen AD, Nordal I (2005) A phenetic analysis and revision of Guineo-Congolean rain forest taxa of Chlorophytum (Anthericaceae). Bot J Linn Soc 148:1–20. doi:10.1111/j.1095-8339.2005.00386.x
Pridgeon AM, Cribb PJ, Chase MW, Rasmussen FN (2014) Genera Orchidacearum, volume 6: Epidendroideae (Part 3). Oxford University Press, New York
Purvis A, Gittleman JL, Brooks T (2005) Phylogeny and conservation. Cambridge University Press, New York
R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing. Available at: http://www.R-project.org/. Accessed 10 Oct 2015
Rakotoarivelo FP, Razafimandimbison SG, Mallet B, Faliniaina L, Pailler T (2012) Molecular systematics and evolutionary trends and relationships in the genus Jumellea (Orchidaceae): implications for its species limits. Taxon 61:534–544
Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574. doi:10.1093/bioinformatics/btg180
Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. doi:10.1093/sysbio/sys029
Royston JP (1982) An extension of Shapiro and Wilk's W test for normality to large samples. Appl Stat 31:115–124
Russell A, Samuel R, Rupp B, Barfuss MHJ, Safran M, Besendorfer V, Chase MW (2010) Phylogenetics and cytology of a pantropical orchid genus Polystachya (Polystachyinae, Vandeae, Orchidaceae): evidence from plastid DNA sequence data. Taxon 59:389–404
Siegel S, Castellan NJ (1988) Non parametric statistics for the behavioral sciences, 2nd edn. McGraw-Hill Humanities/Social Sciences/Languages, New York
Simo TMM (2014) Étude taxonomique et phylogénétique de deux sections du genre Angraecum (Orchidaceae) en Afrique continentale et dans les îles du Golfe de Guinée. PhD thesis, University of Yaoundé I, Cameroun
Simo M, Sonké B, Droissart V, Stévart T (2010) Little-known Angraecums. The genus in Central Africa with a focus on two sections. Orchids 79:690–695
Simo-Droissart M, Micheneau C, Sonké B, Droissart V, Lowry PP II, Plunkett GM, Hardy OJ, Stévart T (2013) Morphometrics and molecular phylogenetics of the continental African species of Angraecum section Pectinaria (Orchidaceae). Pl Ecol Evol 146:295–309. doi:10.5091/plecevo.2013.900
Simo-Droissart M, Sonké B, Droissart V, Geerinck D, Micheneau C, Lowry PP II, Plunkett GM, Hardy OJ, Stévart T (2014) Taxonomic revision of the continental African species of Angraecum section Pectinaria (Orchidaceae). Syst Bot 39:725–739. doi:10.1600/036364414X682184
Sneath PHA, Sokal RR (1973) Numerical taxonomy. The principles and practice of numerical classification. A series of books in biology. Freeman WH & Co, San Francisco
Stévart T (2003) Étude taxonomique, écologique et phytogéographique des Orchidaceae en Afrique centrale atlantique. PhD thesis, Université Libre de Bruxelles
Summerhayes VS (1966) African orchids: XXX. Kew Bull 20:165–199
Swofford DL (2003) PAUP* phylogenetic analysis using parsimony (and other methods) version 4.0 b10. Sinauer Sunderland, Massachussets
Szlachetko DL, Olszewski TS (2001) Orchidacées 3. In: Achoundong G, Morat P (eds) Flore du Cameroun 36. MNHN, Paris, pp 666–948
Szlachetko DL, Romowicz A (2007) *Dolabrifolia*, un nouveau genre d'orchidées de l'alliance *Angraecum*. Richardiana 7:53–54
Szlachetko DL, Tukałło P, Mytnik-Ejsmont J, Grochocka E (2013) Reclassification of the Angraecum-alliance (Orchidaceae, Vandoideae) based on molecular and morphological data. Biodivers Res Conservation 29:1–23. doi:10.2478/biorc-2013-0004
Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of 3 noncoding regions of chloroplast DNA. Pl Molec Biol 17:1105–1109
The Plant List (2013) Version 1.1. Available at: http://www.theplantlist.org. Accessed 3 Mar 2016
Thiers B (continuously updated) Index Herbariorum: a global directory of public herbaria and associated staff New York Botanical Garden. Available at: http://sciweb.nybg.org/science2/IndexHerbariorum.asp. Accessed 25 Oct 2015
van den Berg C, Higgins WE, Dressler RL, Whitten WM, Arenas S, Culham A, Chase MW (2000) A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA. Lindleyana 15:96–114
White F (1979) The Guineo-Congolian Region and its relationships to other phytochoria. Bull Jard Bot Natl Belg 49:11–55