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REGULAR PAPER

Morphometrics and molecular phylogenetics of the continental African species of *Angraecum* section *Pectinaria* (Orchidaceae)

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Background and aims – Recent molecular studies suggest the polyphyly of *Angraecum* and the unnaturalness of some of its sections, as exemplified by sect. *Pectinaria*, which has species in two well-separated clades, one in Madagascar and the other in continental Africa. However, species delimitation among the five continental African members remained problematic due to morphological variability. In preparation for the taxonomic revision of this group, we used morphological and molecular data to re-assess the circumscription of each species, and to evaluate their monophyly and relationships to one another.
Methods – A total of 59 alcohol-preserved specimens were used to perform multivariate analyses on 37 morphological characters. DNA sequences from one nuclear (ITS-1) and five plastid regions (*matK*, *rps16*, *trnL–F*, *trnC–petN* and *ycf1*) were analyzed using Parsimony and Bayesian methods.
Key results – The morphometric study revealed five distinct morphospecies that correspond to the concepts of the currently recognized species. *Angraecum doratophyllum* and *A. subulatum* are the most distinct morphologically, whereas *A. atlanticum*, *A. gabonense* and *A. pungens* are most similar to one another. Phylogenetic analyses using a combined data set of the six markers yielded highly resolved, congruent trees with strong branch support. The polyphyly of *A.* sect. *Pectinaria* is confirmed, with continental African members appearing to be most closely related to sect. *Dolabrifolia*, found exclusively in Africa. The multiple accessions of *A. doratophyllum*, *A. gabonense*, *A. pungens* and *A. subulatum* each formed a well-supported clade. Parsimony and Bayesian analyses placed *A. atlanticum* and *A. pungens* in a subclade within which samples of *A. pungens* were nested but those of *A. atlanticum* formed a grade. These two species can be easily distinguished morphologically by leaf dimensions and flower length, but broader sampling in continental Africa is needed to test whether individuals recognized as *A. atlanticum* might represent two distinct taxa.

Key words – Angraecoid orchid, *Angraecum*, Bayesian analyses, continental Africa, molecular phylogeny, monophyly, morphometric analyses, parsimony, sect. *Pectinaria*.

INTRODUCTION

Orchidaceae are one of the largest and most diverse families of angiosperms, with more than 25,000 species (Govaerts et al. 2013). Describing this huge diversity represents a challenge for systematists, especially for tropical groups, and as a consequence, only a few genera have been treated in recent, authoritative revisions or monographs, such as *Stolzia* Schltr. (Droissart et al. 2009) and *Polystachya* Hook. (Russell et al. 2010). When conducting modern taxonomic revisions, a well resolved phylogenetic tree is useful because morphological characters traditionally used on their own for species delimitation have often proved to be of limited value because of an absence of variation and/or the presence of convergent evolution.

Angraecum Bory is a large and diverse orchid genus, with 229 recognized species (Govaerts et al. 2013) and several more that remain to be described. About 75% of *Angraecum* species are endemic to Madagascar and the Mascarene Islands (La Réunion, Mauritius and Rodrigues), and the remaining species occur in continental Africa and the Seychelles, with one species extending to Sri Lanka. Ten of the nineteen recognized sections have members both in continental Africa and Madagascar. Recent studies based on vegetative anatomy and morphology (Carlsward et al. 2006a) and DNA sequence data (Carlsward et al. 2006b, Micheneau et al. 2008) have suggested that the genus and several of its sections are polyphyletic. However, while the phylogenetics and biogeography of *Angraecum* in Madagascar and the Mascarenes have been investigated in some detail (Micheneau et al. 2008), members of the genus from Central Africa and adjacent islands in the Gulf of Guinea have received less attention to date.

One of the infrageneric groups within *Angraecum* that appears to be polyphyletic is *A*. sect. *Pectinaria*. Micheneau et al. (2008) showed that the Indian Ocean and continental African members of this group belong to two well-separated clades, although their taxon sampling included only three of the eleven currently recognized species: two from Africa and one from Madagascar.

Most African collections of *Angraecum* sect. *Pectinaria* can easily be assigned to one of the currently recognized species, but a modest number remain difficult or impossible to identify based on the currently available key (Stévart et al. 2010). As new material has become available over the last decade, the morphological distinctions between several species (e.g. between *A. atlanticum* Stévart & Droissart, *A. gabonense* Summerh. and *A. pungens* Schltr.) have become increasingly obscure, primarily reflecting significant morphological variability in floral characters within species. This lack of clarity with regard to species limits is in turn problematic for conducting molecular phylogenetic work aimed at clarifying relationships among the main groups of *Angraecum* and, perhaps more importantly, for testing the monophyly of species whose circumscription is based on morphological features.

As a precursor to conducting a taxonomic revision of the continental African species of *Angraecum* sect. *Pectinaria* and in order to provide an improved taxonomic framework for molecular phylogenetic investigations, we have attempted to clarify species delimitations within the group using a morphometric approach based on floral and vegetative characters. First, we defined coherent morphological groups (morphospecies), which we then correlated to currently recognized species based on their nomenclatural types, and then we identified which morphological features are informative for defining and differentiating these species. Using these morphometric results to provide an accurate identification of accessions, we finally carried out a molecular phylogenetic study using sequence data from six markers (one nuclear and five plastid regions) and several samples from each morphological group to clarify the relationships among the taxa currently assigned to *Angraecum* sect. *Pectinaria* and to test their monophyly.

MATERIAL AND METHODS

Morphometric analyses

Data matrix construction – Dried and spirit-preserved specimens of the five continental species of *Angraecum* sect. *Pectinaria* were examined from the following herbaria: BM, BR, BRLU, K, MA, MO, P, WAG and YA (herbarium abbreviations according to Thiers 2013). However, most of these collections are fragmentary or poorly preserved, with the flowers flattened and often deteriorated, making them nearly unusable for morphological studies. After excluding spirit-preserved specimens for which one or more key floral characters were missing or which were too brittle to dissect, we performed multivariate analyses based on morphological measurements of 59 high quality fertile specimens preserved in alcohol (table 1). Three type specimens (those of *A. atlanticum*, *A. gabonense* and *A. pungens*) were also included in the analyses, one conserved in spirit and the other two dried specimens, from which material was boiled to facilitate stereomicroscopic observation. Most of fertile specimens (36 of 59) were collected using a shadehouse cultivation system operated in Central Africa since 1997 (Stévart 2003, Droissart 2009), while 21 were collected in the field, including the type specimen of *A. atlanticum*.

A total of 37 characters were measured, of which 22 were quantitative and 15 qualitative (table 2). Quantitative characters were measured with graph paper and recorded as numeric (continuous or discrete) values (Cupido 2003, Poulsen & Nordal 2005), whereas qualitative characters were recorded as factors. Based on morphological features, all specimens were first grouped into morphospecies. After comparison with nomenclatural types, each morphospecies was then assigned to one of the currently accepted species. As some features comprised continuous characters while others were discrete, measurements were standardized to reduce the effects of different scales (Marcysiak et al. 2007). Qualitative characters were not standardized (Sneath & Sokal 1973).

Data analysis – To investigate independently whether groups of specimens could be assigned to species and to identify variables that discriminate among them, a Hill-Smith analysis was first performed (Hill & Smith 1976) using the function *dudi.hillsmith* of the library *ade4* in the R 3.0.1 (R Core Team 2013) software package (Chessel et al. 2004, Dray & Dufour 2007, Dray et al. 2007). This method,

Table 1 – **Material used for morphological study of continental African species of *Angraecum* sect. *Pectinaria*.** *A. = Angraecum*.

Taxon	Voucher	Location	Geographic origin
A. atlanticum 1	*Damen* 285	WAG	Gabon
A. atlanticum 2	*Jardin Botanique de Bambusa* 226	BRLU	Gabon
A. atlanticum 3	*Stévart* 1077 (isotype specimen)	BRLU	Equatorial Guinea
A. doratophyllum 1	*Stévart* 678	BRLU	São Tomé island
A. doratophyllum 2	*Stévart* 132	BRLU	Príncipe island
A. doratophyllum 3	*Primo & Stévart* 87	BRLU	São Tomé island
A. doratophyllum 4	*van der Laan* 307	WAG	São Tomé island
A. gabonense 1	*Simo M. et al. (Yaoundé shadehouse)* 2067	BRLU	Cameroon
A. gabonense 2	*Simo M. et al. (Yaoundé shadehouse)* 2878	BRLU	Cameroon
A. gabonense 3	*Simo M. et al. (Yaoundé shadehouse)* 2889	BRLU	Cameroon
A. gabonense 4	*Simo M. et al. (Yaoundé shadehouse)* 2852	BRLU	Cameroon
A. gabonense 5	*Droissart* 162	BRLU	Cameroon
A. gabonense 6	*Dessein et al.* 2641	BRLU	Cameroon
A. gabonense 7	*Stévart & Biteau* 47	BRLU	Gabon
A. gabonense 8	*Stévart & Biteau* 67	BRLU	Gabon
A. gabonense 9	*Stévart* 1631	BRLU	Gabon
A. gabonense 10	*Stévart* 1716	BRLU	Gabon
A. gabonense 11	*Stévart* 1764	BRLU	Gabon
A. gabonense 12	*Wieringa* 678	WAG	Gabon
A. gabonense 13	*Wieringa* 1073	WAG	Gabon
A. gabonense 14	*Damen* 215	WAG	Gabon
A. gabonense 15	*Damen* 216	WAG	Gabon
A. gabonense 16	*Missouri Botanical Garden* 524	BRLU	Gabon
A. gabonense 17	*Stévart et al.* 4015	MO	Gabon
A. gabonense 18	*Jardin Botanique de Bambusa* 110	BRLU	Gabon
A. gabonense 19	*De Wilde* 230	K	Gabon
A. gabonense 20	*Simo M. et al. (Yaoundé shadehouse)* 3162	BRLU	Cameroon
A. gabonense 21	*Arends* 906	WAG	Gabon
A. gabonense 22	*Arends* 957	WAG	Gabon
A. gabonense 23	*Arends* 953	WAG	Gabon
A. gabonense 24	*Arends* 884	WAG	Gabon
A. gabonense 25	*Arends* 912	WAG	Gabon
A. gabonense 26	*Troupin* 2453	K	Democratic Republic of the Congo
A. gabonense 27	*Jardin Botanique de Bambusa* 191	BRLU	Gabon
A. gabonense 28	*Jardin Botanique de Bambusa* 194	BRLU	Gabon
A. gabonense 29	*Jardin Botanique de Bambusa* 179	BRLU	Gabon
A. gabonense 30	*Droissart et al. (Yaoundé shadehouse)* 3601	BRLU	Cameroon
A. gabonense 31	*Cultivated at BR greenhouse*, Acc N° BR 20090378-29	BR	Gabon
A. gabonense 32	*LeTestu* 6384 (type specimen)	K	Gabon
A. pungens 1	*Simo M. et al. (Yaoundé shadehouse*) 2409	BRLU	Cameroon
A. pungens 2	*Simo M. et al. (Yaoundé shadehouse)* 2194	BRLU	Cameroon
A. pungens 3	*Jardin Botanique de Bambusa* 145	BRLU	Gabon
A. pungens 4	*Damen* 211	WAG	Gabon
A. pungens 5	*van der Laan* 440	WAG	Gabon
A. pungens 6	*Cable* 2434	K	Cameroon
A. pungens 7	*Cultivated at BR greenhouse*, Acc N° BR 20090383-34	BR	Gabon
A. pungens 8	*Westwood* 91	K	Equatorial Guinea
A. pungens 9	*Schlechter* 15774 (isotype specimen)	K	Cameroon

Table 1 (continued) – Material used for morphological study of continental African species of *Angraecum* sect. *Pectinaria*.

Taxon	Voucher	Location	Geographic origin
A. pungens 10	*Cultivated at BR greenhouse*, Acc N° BR 20090382-33	BR	Gabon
A. pungens 11	*Arends* 954	WAG	Gabon
A. pungens 12	*Damen* 192	WAG	Gabon
A. pungens 13	*van der Laan* 714	WAG	Gabon
A. subulatum 1	*Simo M. et al. (Yaoundé shadehouse)* 2503	BRLU	Cameroon
A. subulatum 2	*Simo M. et al. (Yaoundé shadehouse)* 2188	BRLU	Cameroon
A. subulatum 3	*Simo M. et al. (Yaoundé shadehouse)* 2350	BRLU	Cameroon
A. subulatum 4	*Simo M. et al. (Yaoundé shadehouse)* 2911	BRLU	Cameroon
A. subulatum 5	*Droissart & Simo M.* 996	BRLU	Cameroon
A. subulatum 6	*Jardin Botanique de Bambusa* 171	BRLU	Gabon
A. subulatum 7	*Simo M. et al. (Yaoundé shadehouse)* 2694	BRLU	Cameroon

Table 2 – List of variables assessed for the study of continental African species of *Angraecum* sect. *Pectinaria*. Continuous variables were measured in mm. For all the widths, the widest parts were measured.

N°	Variables	Codes	States
1	Internodes	INT	Continuous
2	Leaf orientation relative to the stem	LFO	LFO.parallel; LFO.skew
3	Leaf length	LFL	Continuous
4	Leaf width	LFW	Continuous
5	Leaf shape	LFS	LFS.ovate; LFS.subulate
6	Leaf apex	LFA	LFA.caudate; LFA.mucronate
7	Presence of mucronate apex	MUC	MUC.no; MUC.yes
8	Mucronate apex length	MUL	Continuous
9	Mucronate apex width	MUW	Continuous
10	Presence of black spots on the stem and the leaves	PBS	PBS.no; PBS.yes
11	Floral length	FLL	Continuous
12	Floral bract length	BRL	Continuous
13	Floral bract width	BRW	Continuous
14	Apex of the floral bract	BRA	BRA.acute; BRA.obtuse
15	Dorsal sepal length	DSL	Continuous
16	Dorsal sepal width	DSW	Continuous
17	Dorsal sepal shape	DSS	DSS.obovate; DSS.ovate
18	Dorsal sepal apex	DSA	DSA.acute; DSA.obtuse
19	Dorsal sepal veins number	DSN	Discrete
20	Lateral sepal length	LSL	Continuous
21	Lateral sepal width	LSW	Continuous
22	Lateral sepal shape	LSS	LSS.obovate; LSS.ovate
23	Lateral sepal apex	LSA	LSA.acute; LSA.obtuse
24	Lateral sepal veins number	LSN	Discrete
25	Lateral petal length	LPL	Continuous
26	Lateral petal width	LPW	Continuous
27	Lateral petal shape	LPS	LPS.obovate; LPS.ovate
28	Lateral petal apex	LPA	LPA.acute; LPA.obtuse
29	Lateral petal veins number	LPN	Discrete
30	Lip length	LIL	Continuous
31	Lip width	LIW	Continuous
32	Lip shape	LIS	LIS.obovate; LIS.ovate
33	Lip trilobated	LIT	LIT.1third; LIT.2third
34	Spur length	SPL	Continuous
35	Spur shape	SPS	SPS.curve; SPS.straight
36	Gynostemium length	GYL	Continuous
37	Pedicel and ovary length	POL	Continuous

which provides an estimation of the taxonomic distance between objects (samples), offers a useful compromise between principal component and multiple correspondence analyses. Data are ordinated on a biplot using a principal component analysis. It is possible to calculate the distance between the individual objects as measured in Euclidean space of the first few principal components (Hill & Smith, 1976) and then to illustrate these distances using a clustering dendrogram. It is also possible to classify the objects directly from the statistical triplet.

Prior to performing statistical tests among groups, the distribution of each quantitative variable was examined using the Shapiro-Wilk test of normality (Royston 1982) available in the package *stats* (R Core Team 2013). For six of the 22 quantitative characters, normality was confirmed and a one-way analysis of variance (ANOVA) (Chambers & Hastie 1992, Sokal & Rohlf 1995) was performed. For the 16 non normal variables, the non-parametric Kruskal-Wallis test was performed (Hollander & Wolfe 1973). For each test, the null hypothesis (H_0) was that there is no difference between means or medians for each group of morphospecies, while the alternative hypothesis (H_1) is that the mean or the median in one group differs from that of at least one other group.

If and when the null hypothesis of the ANOVA was rejected, then Tukey's Honestly Significant Difference test (Tukey's HSD or the Tukey-Kramer method, Tukey 1977) was used in conjunction with the ANOVA to find means that are significantly different from one another. If and when the null hypothesis of the Kruskal-Wallis test was rejected, then the *kruskalmc* test (Siegel & Castellan 1988), available in the package *pgirmess* (Giraudoux 2013), was used to perform multiple comparison tests between medians and to determine which groups were different, with pairwise comparisons adjusted appropriately. Those pairs of groups with observed differences higher than a critical value are considered statistically different at a given significance level. The function *multcompLetters* (Piepho 2004) of the package *multcompView* (Graves et al. 2012) was used to convert a logical vector or a vector of p-values into a character-based display in which common characters identify levels or groups that are not significantly different. This function is designed for use with the output of functions such as Tukey's HSD or *kruskalmc*. All analyses were performed using the R 3.0.1 software package.

Molecular analyses

Plant material and DNA purification – DNA was obtained from leaf samples taken from fertile specimens collected in the wild from Cameroon, Gabon, and São Tomé and Príncipe (electronic appendix 1). Plants that were not fertile at the time of collection were cultivated and monitored in the shadehouses in Cameroon and Gabon until they produce flowers, enabling accurate identification. Additional leaf and floral material was provided from the Gabonese orchid collection initially established at the Wageningen University Greenhouse (Netherlands) and now housed in the greenhouse of the National Botanical Garden of Belgium (Meise, Belgium). Specimens of Malagasy taxa (i.e. *A. pectinatum*, *A. panicifolium*, *A.* cf. *humblotianum* and *A. linearifolium*) were collected in the Andasibe region, Madagascar (electronic appendix 1). A total of thirty samples were used in the study: three per species for four of the five currently recognized members of *Angraecum* sect. *Pectinaria* in continental Africa (two samples from the recently described, range restricted *A. atlanticum*); one per species for three (out of six) species of *A.* sect. *Pectinaria* from Madagascar/Mascarenes; one from each of the four currently recognized species of *A.* sect. *Dolabrifolia*, along with two potential taxonomical novelties belonging to the group (all from continental Africa); one from the genus *Diaphananthe* (continental Africa); and two from the genus *Tridactyle* (continental Africa) (appendix). The vouchers for each sample are deposited at BRLU. Three taxa of *Polystacha* (*P. calluniflora*, *P. albescens* subsp. *imbricata* and *P. pyramidalis*) were selected as outgroups because subtribe Polystachyinae, to which they belong, has been identified as sister to the angraecoids (e.g. Chase et al. 2003, Freudenstein et al. 2004, Górniak et al. 2010).

Leaf or floral tissue was dried in silica gel for DNA extraction (Chase & Hills 1991). Total DNA was extracted from fresh (1 g) or silica-gel dried material (0.3 g) using one of two methods. The first method used 1 g of fresh leaves in a modified 2 × CTAB protocol (Doyle & Doyle 1987). Proteins were removed with SEVAG (chloroform/isoamyl alcohol 24:1) and DNA was precipitated with ethanol (-20°C). At the end of extraction, only turbid or colored DNA extracts were purified on Macherey-Nagel columns. The second extraction method used 0.3 g of dried material with the NucleoSpin® plant kit from Macherey–Nagel, following the manufacturer's protocol.

PCR amplification and DNA sequencing – The following primers were used for amplification and sequencing of each individual plastid region: Tab-C and Tab-D for the *trnL* intron and Tab-E and Tab-F for the *trnL–F* intergeneric spacer (Taberlet et al. 1991), *rps16-1F* and *rps16-2R* for the *rps16* intron (Oxelman et al. 1997), *19F* (Molvray et al. 2000), *1326R* (Cuénoud et al. 2002), *390F* (Cuénoud et al. 2002) and *trnK-2R* (Johnson & Soltis 1994) for *matK*, *trnC* and *petN-1R* for the *trnC–petN* intergenic spacer (Lee & Wen 2003), and *3720F*, *IntR*, *IntF* and *5500R* for *ycf1* (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 unknown 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 *trnL–F* region, *trnC–petN*, the *rps16* intron and ITS-1 consisted of an initial denaturation at 94°C for 3 min followed by 30 cycles of 30 s at 94°C, 30 s at 52°C, and 1 min at 72°C, with a final extension at 72°C for 10 min. Amplification of *matK* (*19F–1326R* and *390F–trnK2R*) and *ycf1* (*3720F–intR* and *intF–5500R*) involved an initial denaturation at 94°C for 3 min followed by 30 cycles of 30 s at 94°C, 30 s at 52°C and 1 min 30 s at 72°C, with a final extension at 72°C for 10 min. PCR products were 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 (unknown concentration) and 4.3–6.3 µL of H_2O for a total reaction volume of 10 µL. Cycle sequencing conditions were as follows: a premelt of 1 min (96°C), 25 cycles each with 10 s of denaturation (96°C), 5 s of annealing (52°C) and 4 min of elongation (60°C). For cleaning of cycle sequencing products, we used precipitation with ethanol. Sequences were generated on an ABI 3100 automated capillary DNA sequencer using BigDye terminator v3.1 chemistry following the manufacturer's protocols (ABI). Both strands were sequenced to assure accurate base calling. The two complementary and overlapping sequences were assembled using CodonCode Aligner (version 3.0.3, CodonCode Corporation) and Geneious (2013, version 6.1.6, Biomatters). Each individual base position was examined for agreement between the two strands. Consensus sequences were edited manually and aligned with the plugin MAFFT implemented within Geneious. For coding genes such as *matK* and *ycf1*, nucleotides were translated into amino acids to verify that the sequences corresponded to a protein using a reference sequence from NCBI. Gene datasets were concatenated using Geneious.

The number of taxa included in each of the six individual matrices was as follows: thirty for the *rps16* intron, thirty for the combined *matK* (*Angraecum aporoides*, *A. pectinatum* and all three samples of *A. gabonense* failed to amplify the region *390F–trnK-2R*), 29 for *ycf1* (*Tridactyle bicaudata* did not amplify and two samples, one of *A. doratophyllum* and the other of *A. subulatum* had incomplete sequences, represented by the fragments *3270F–IntR* and *IntF–5500R*, respectively), 29 for *trnL–F* (*A. panicifolium* failed to amplify), 29 for *trnC–petN* (*Polystacha calluniflora* failed to amplify), and 29 for ITS-1 (one sample of *A. subulatum* failed to amplify).

Parsimony analysis – Cladistic analyses using Fitch parsimony (Fitch 1971) were performed using PAUP* 4.0 beta 10Win (Swofford 2003). All characters were unordered with equal weight; gaps were coded as missing data. Heuristic searches were performed using tree bisection-reconnection (TBR) swapping with 1000 replicates of random-taxon addition, holding ten trees at each step, and saving twenty 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 (Efron 1979, Felsenstein 1985) with 1000 bootstrap replicates with simple-taxon addition and TBR branch swapping, holding ten trees at each step, and saving ten trees per replicate. Parsimony analyses were first run separately for each region (i.e. ITS-1, *matK*, *rps16*, *trnC–petN*, *trnL–F* and *ycf1*). 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 matrix (hereafter referred to as the plastid matrix) and then all plastid and ITS-1 regions into a second matrix (hereafter the combined matrix). Sequences that failed to amplify (5%) were coded as missing data in the plastid and combined matrices.

Bayesian analysis – Bayesian analyses were performed using MrBayes 3.2.1 (Ronquist & 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 2,000,000 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 (Rambaut & Drummond 2007) to assess stationarity, 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 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 1,000 (25%) were discarded as the burn-in phase. The majority-rule consensus tree was constructed using the function *sumt* in MrBayes.

RESULTS

Delimitation of continental African species of *Angraecum* sect. *Pectinaria*

The first three axes of the ordination using the Hill-Smith method explained 65% of the total variance among the specimens (electronic appendix 2, fig. 1). The variation along the first axis (32% of total variance) largely agrees with the highest positive loadings for the presence of an obovate lip and a caudate leaf apex, and the absence of black spots on the stem and the leaves; the highest negative loadings are for ovate lateral petals and a recurved spur. Except for the character states with the highest loadings in the first axis, the second axis (21% of total variance) has highest loadings for the absence of a mucronate leaf apex and an obtuse apex of the lateral sepals.

The projection using the two first axes shows five groups of specimens (fig. 1) that correspond respectively to the following species: *Angraecum atlanticum*, *A. doratophyllum*, *A. gabonense*, *A. pungens*, and *A. subulatum*. The most well defined characteristic groups are those formed by the material of *A. doratophyllum* and *A. subulatum*. The main discriminant variables for *A. doratophyllum* are the curved shape of the spur and the obovate shape of the lateral petals, whereas those for *A. subulatum* are the length, subulate shape and caudate apex of the leaf, as well as the obovate shape of the lip. Specimens of *A. atlanticum*, *A. gabonense* and *A. pungens* formed three groups that are, however, very close to one another. The clustering dendrogram obtained by constructing a taxonomic distance measure among the continental African specimens of *A.* sect. *Pectinaria* shows the same five groups (fig. 2) as the ordination diagram (fig. 1).

The pairwise comparisons of means using the Tukey-Kramer method revealed that, for each of the six variables

that followed a normal distribution, at least two groups were significantly different, with different letters displayed in superscript on the means (table 3). The multiple comparisons of medians obtained using the Kruskal-Wallis test also showed that all sixteen other variables except one (the number of veins in the lateral petals) were significantly different among the five groups (letters in superscript on the medians in table 4).

DNA sequences

The *trnL* intron was excluded from the analysis due to difficulties encountered while attempting to align the sequences of this highly variable region. The number of aligned characters contributed by each individual region retained are detailed in table 5. The plastid matrix contained 30 accessions and 5898 aligned characters of which 599 (10.15%) were parsimony-informative while the combined matrix contained thirty accessions and 6281 aligned characters of which 673 (10.71%) were parsimony-informative (table 5).

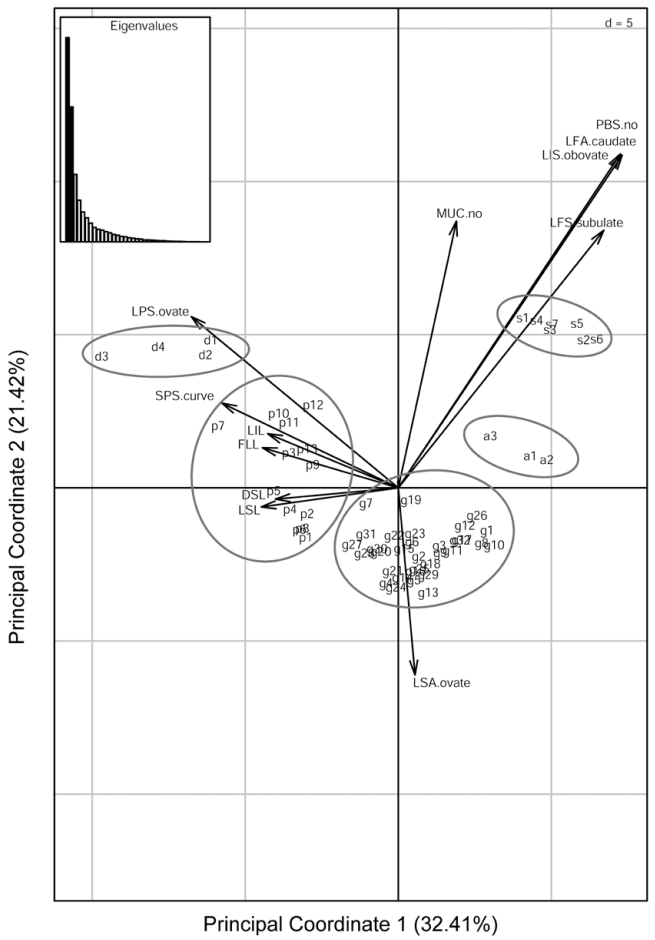

Figure 1 – Scatter plots of the two first axes of the principal coordinate analysis using the Hill-Smith method based on 37 characters scored from 59 specimens of continental African species of *Angraecum* sect. *Pectinaria*. The twelve variables showing the highest loadings are represented. Codes used for variables in the scatter plot are detailed in table 2. Circles summarize accessions from the same species. Codes used for multiple accessions per species in the scatter plot are detailed in table 1. a, *A. atlanticum*; d, *A. doratophyllum*; g, *A. gabonense*; p, *A. pungens*; s, *A. subulatum*.

Phylogenetic analyses

When individual markers were analyzed separately using parsimony, there was insufficient resolution to recover monophyletic species within *Angraecum* sect. *Pectinaria*

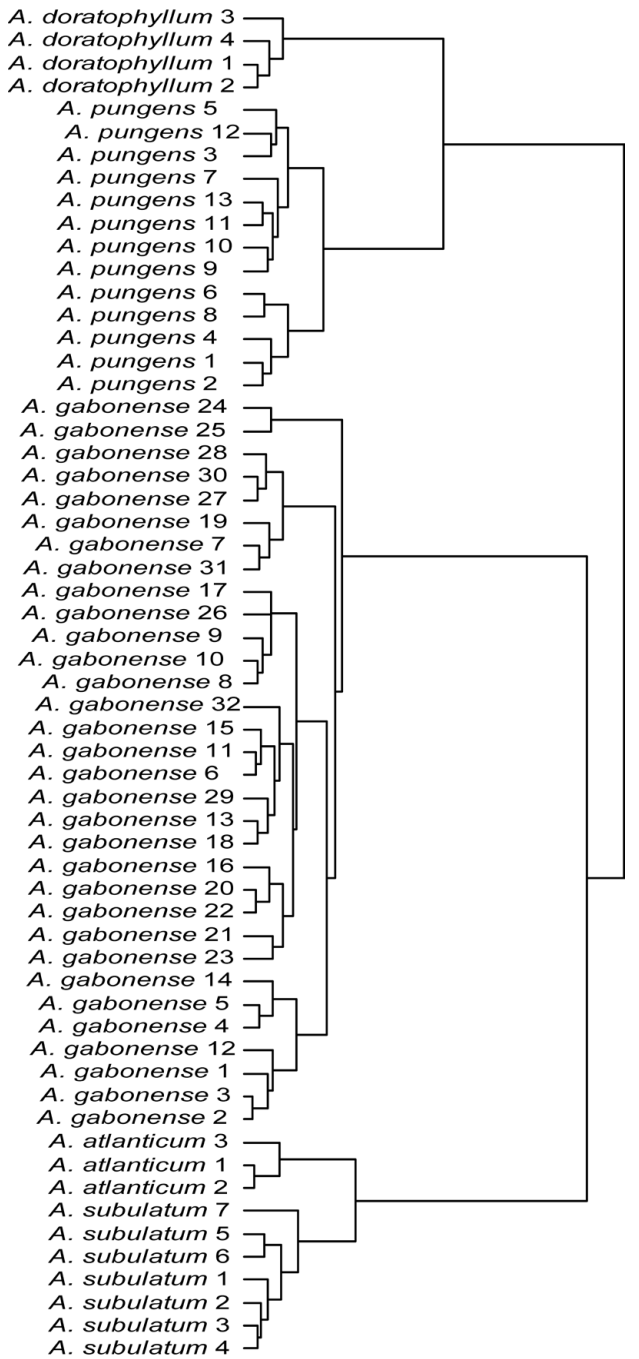

Figure 2 – Clustering dendrogram of 59 specimens of continental African species of *Angraecum* sect. *Pectinaria* obtained by calculating the taxonomic distance measured among 37 floral and vegetative characters using the Hill-Smith ordination test. The similarity matrix was calculated using the Gower index.

Table 3 – Significant differences resulting from multiple comparisons (using Tukey-Kramer HSD test) of means of the six numerical variables following a normal distribution, for the five described continental African species of *Angraecum* sect. *Pectinaria*.
The degrees of freedom are given as a subscript to Fs. The mean, the standard deviation, the range and the sample size are given for each group. For each variable, heterogeneous means among the five species are indicated by different letters. DSL, dorsal sepal length; DSW, dorsal sepal width; LSL, lateral sepal length; LSW, lateral sepal width; LPL, lateral petal length; LIW, lip width.

	$Fs_{4,54}$	P value	*A. atlanticum*	*A. doratophyllum*	*A. gabonense*	*A. pungens*	*A. subulatum*
DSL	41.808	< 0.0001	$4.6^{a} \pm 0.61$ [3.9–5] (3)	$10.1^{b} \pm 1.00$ [9.2–11.2] (4)	$6.51^{c} \pm 0.97$ [4.2–7.6] (32)	$8.33^{d} \pm 0.64$ [7.5–9.3] (13)	$4.33^{a} \pm 0.94$ [3–5.4] (7)
DSW	13.28	< 0.0001	$1.4^{a} \pm 0.26$ [1.2–1.7] (3)	$1.3^{a} \pm 0.22$ [1.1–1.6] (4)	$2.24^{b} \pm 0.35$ [1.5–3] (32)	$2.46^{b} \pm 0.50$ [1.4–3] (13)	$1.76^{a} \pm 0.13$ [1.6–2] (7)
LSL	47.88	< 0.0001	$4.68^{a} \pm 0.43$ [4.2–5] (3)	$9.25^{b} \pm 1.30$ [7.45–10.5] (4)	$6.62^{c} \pm 0.78$ [4.75–7.45] (32)	$8.47^{b} \pm 0.52$ [8–9.35] (13)	$4.63^{a} \pm 0.69$ [3.8–5.8] (7)
LSW	14.855	< 0.0001	$1.65^{ab} \pm 0.09$ [1.55–1.7] (3)	$1.3^{a} \pm 0.29$ [1–1.6] (4)	$2.04^{b} \pm 0.35$ [1.1–2.5] (32)	$2.57^{c} \pm 0.40$ [2–3.2] (13)	$1.88^{b} \pm 0.19$ [1.6–2.15] (7)
LPL	36.759	< 0.0001	$4.27^{a} \pm 0.29$ [4–4.5] (3)	$10.83^{b} \pm 0.64$ [10.3–11.75] (4)	$6.29^{c} \pm 0.99$ [4–8.2] (32)	$7.33^{d} \pm 1.34$ [5–9] (13)	$3.76^{a} \pm 0.67$ [3–5] (7)
LIW	18.981	< 0.0001	$3.53^{a} \pm 0.79$ [3.3–3.8] (3)	$7.68^{b} \pm 1.00$ [6.4–8.8] (4)	$5.23^{c} \pm 0.75$ [2.8–6.5] (32)	$5.43^{c} \pm 0.98$ [3.5–6.8] (13)	$3.87^{a} \pm 0.26$ [3.4–4.1] (7)

and the relationships among them (fig. 3A–F). However, in combination, these markers yielded well resolved trees with high values of branch support (fig. 3). The plastid matrix yielded two most-parsimonious trees of 993 steps each. The one illustrated here (fig. 3G), showing the same topology as the consensus tree, has a consistency index (CI) of 0.88 and a retention index (RI) of 0.94. Parsimony analysis of the combined matrix yielded a single most-parsimonious tree of 1173 steps (CI = 0.86; RI = 0.93) (table 5). Bayesian analyses reached convergence and a run length of 2,000,000 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 analysis provided a well resolved tree with high posterior probability (PP) values, and had the same topology as the tree obtained from the parsimony analysis (fig. 4).

The results based on parsimony and Bayesian analyses of the combined matrix confirmed that *Angraecum* sect. *Pectinaria* is polyphyletic, with species from Madagascar and continental Africa falling into two distinct and well-supported groups (compare fig. 4). The Malagasy taxa are sister to *A. linearifolium* (*A.* sect. *Arachnangraecum*), which also occurs in Madagascar, and this relationship is strongly supported with a bootstrap value (BS) of 100% and a PP of 1. In contrast, the species from continental Africa are placed in a clade that also includes other African species belonging to *A.* sect. *Dolabrifolia*, as well as the genera *Diaphananthe* and *Tridactyle* (BS = 100%; PP = 1; figs 3 & 4). Four of six analyses of single gene matrices placed the continental group of *A.* sect. *Pectinaria* as sister to sect. *Dolabrifolia*, but with weak BS support (i.e. 65–78%).

Within the continental African clade of *Angraecum* sect. *Pectinaria* (fig. 4), the monophyly of four of the five morphospecies recognized in the multivariate analyses is strongly supported, viz., *A. doratophyllum*, *A. gabonense*, *A. pungens* and *A. subulatum*, based both on parsimony (BS = 100%, 91%, 86% and 100%, respectively) and Bayesian analysis (PP = 1, 0.98, 1 and 1, respectively). *Angraecum atlanticum* (Gabon) and *A. pungens* (Gabon and Cameroon) form a well-supported group (BS = 100%; PP = 1) within which individuals of *A. pungens* form a subclade (BS= 86% and PP = 1) and those of *A. atlanticum* form a grade. While the placement of a single sample of *A. atlanticum* (2065) is not consistent with the monophyly of this species (fig. 4), support for this is weak (BS = 63%; PP = 0.94).

Concerning the relationships among the five continental African species (figs 3H & 4), *Angraecum subulatum* is sister to a clade comprising the other species sampled (BS = 100%; PP = 1), with *A. doratophyllum* in turn being sister to the three other species (BS = 100%; PP = 1), and then *A. gabonense* sister to *A. atlanticum* + *A. pungens* (BS = 100%; PP = 1). Concerning these latter species, samples of *A. atlanticum* and *A. pungens* are sisters in single marker analyses involving *trnC–petN* (fig. 3D) and *ycf1* (fig. 3F).

DISCUSSION

Polyphyly of *Angraecum* sect. *Pectinaria*

In the study of Micheneau et al. (2008), which used just plastid markers, *Angraecum* sect. *Pectinaria*, as defined by Garay (1973), was found to be polyphyletic, although sampling

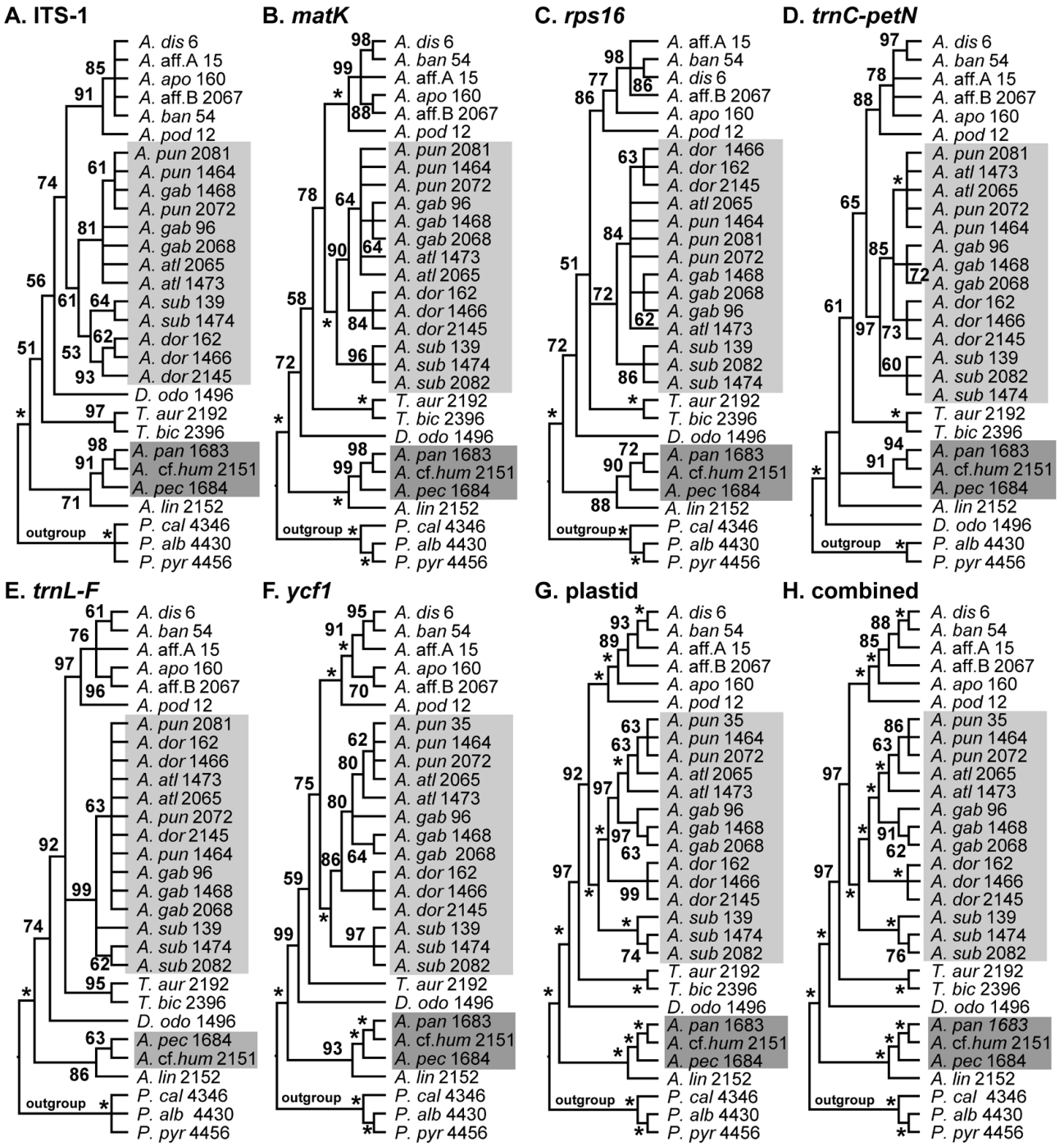

Figure 3 – Parsimony analysis (strict consensus tree with bootstrap percentages shown above or below branches) of ITS-1 (**A**), *matK* (**B**), *rps16* (**C**), *trnC–petN* (**D**), *trnL–F* (**E**), *ycf1* (**F**), plastid matrix (**G**) and combined matrix (**H**). Taxa: ***Angraecum* sect. *Dolabrifolia* (Africa)**: *A. apo* = *A. aporoides*; *A.* aff.A = *A.* aff. *aporoides*A; *A.* aff.B = *A.* aff. *aporoides*B; *A. ban* = *A. bancoense*; *A. dis* = *A. distichum*; *A. pod* = *A. podochiloides*; ***Angraecum* sect. *Pectinaria* (Africa)**: *A. atl* = *A. atlanticum*; *A. dor* = *A. doratophyllum*; *A. gab* = *A. gabonense*; *A. pun* = *A. pungens*; *A. sub* = *A. subulatum*; ***Angraecum* sect. *Pectinaria* (Madagascar)**: *A.* cf.*hum* = *A.* cf. *humblotianum*; *A. pan* = *A. panicifolium*; *A. pec* = *A. pectinatum*; ***Diaphananthe* (Africa)**: *D. odo* = *D. odoratissima*; ***Tridactyle* (Africa)**: *T. aur* = *T. aurantiopunctata*; *T. bic* = *T. bicaudata*; ***Angraecum* sect. *Arachnangraecum* (Madagascar)**: *A. lin* = *A. linearifolium*; **outgroups** (genus *Polystachya*): *P. cal* = *P. calluniflora*; *P. alb* = *P. albescens* subsp. *imbricata*; *P. pyr* = *P. pyramidalis*. Details of each analysis are given in table 5. Members of *Angraecum* sect. *Pectinaria* are represented by gray boxes: light gray for accessions from Africa and dark gray for material from Madagascar. * represent 100% bootstrap values.

Table 4 – Significant differences resulting from multiple comparisons (after Kruskal-Wallis test) of medians of the sixteen numerical variables not following a normal distribution, for the five described continental African species of *Angraecum* sect. *Pectinaria*.
The degree of freedom (df) for each variable is 4, except for MUL and MUW for which df = 3. The median, the standard deviation, the range and the sample size are given for each group. For each variable, heterogeneous medians among the five species are indicated by different letters. INT, Internodes; LFL, leaf length; LFW, leaf width; FLL, flower length; BRL, bract length; BRW, bract width; DSN, number of dorsal sepal veins; LSN, number of lateral sepal veins; LPW, lateral petal width; LPN, number of lateral petal veins; LIL, lip length; SPL, spur length; GYL, gynostemium length; POL, pedicel and ovary length.

	χ^2	P value	*A. atlanticum*	*A. doratophyllum*	*A. gabonense*	*A. pungens*	*A. subulatum*
INT	44.9278	< 0.0001	$6^{ab} \pm 1.04$ [4.5–6.5] (3)	$6^{ab} \pm 2.75$ [4–10.5] (4)	$3^{a} \pm 0.58$ [2–4] (32)	$6.5^{b} \pm 2.79$ [5–14] (13)	$9^{b} \pm 5.12$ [7–21.5] (7)
LFL	45.4683	< 0.0001	$18.5^{ab} \pm 0.75$ [17.75–19.25] (3)	$28.5^{ab} \pm 3.96$ [28–36.2] (4)	$16.18^{a} \pm 3.45$ [10.75–23] (32)	$32^{b} \pm 9.98$ [26.5–56] (13)	$71^{b} \pm 12.26$ [58–92.5] (7)
LFW	45.5398	< 0.0001	$1.5^{a} \pm 0.31$ [1.3–1.9] (3)	$4.25^{bc} \pm 0.90$ [4.1–6] (4)	$2.88^{ab} \pm 0.57$ [1.65–3.8] (32)	$5^{c} \pm 0.93$ [4.5–7.5] (13)	$2^{a} \pm 0.25$ [1.6–2.3] (7)
MUL	34.2484	< 0.0001	$1.75^{ab} \pm 0.53$ [1.1–2.15] (3)	$4.05^{a} \pm 0.15$ [3.85–4.2] (4)	$1.58^{b} \pm 0.34$ [1–2.1] (32)	$3.45^{a} \pm 0.86$ [2.5–5.65] (13)	/ / (/)
MUW	36.5904	< 0.0001	$0.4^{a} \pm 0.08$ [0.3–0.45] (3)	$1.83^{b} \pm 0.10$ [1.7–1.95] (4)	$0.85^{a} \pm 0.23$ [0.55–1.45] (32)	$1.75^{b} \pm 0.51$ [1.15–3.1] (13)	/ / (/)
FLL	44.4204	< 0.0001	$9.1^{a} \pm 1.52$ [8–11] (3)	$23^{b} \pm 3.37$ [19–27] (4)	$11.5^{a} \pm 1.40$ [10–14] (32)	$16^{b} \pm 1.06$ [15.5–19.5] (13)	$9^{a} \pm 1.14$ [8–11] (7)
BRL	38.7826	< 0.0001	$1.8^{a} \pm 0.57$ [1.5–2.6] (3)	$3.75^{b} \pm 0.43$ [3–4] (4)	$2.2^{a} \pm 0.24$ [1.7–2.5] (32)	$3.1^{b} \pm 0.33$ [2.6–3.7] (13)	$2.7^{ab} \pm 0.47$ [2–3.5] (7)
BRW	35.129	< 0.0001	$1.7^{ab} \pm 0.21$ [1.6–2] (3)	$2.6^{ac} \pm 0.65$ [2.2–3.7] (4)	$2^{b} \pm 0.26$ [1.3–2.3] (32)	$2.5^{c} \pm 0.18$ [2.5–3] (13)	$2^{abc} \pm 0.35$ [1.5–2.6] (7)
DSN	19.4849	0.0006	$5^{ab} \pm 0$ [5] (3)	$3^{a} \pm 0$ [3] (4)	$5^{ab} \pm 0.87$ [3–7] (32)	$5^{b} \pm 0.76$ [5–7] (13)	$5^{ab} \pm 1$ [3–6] (7)
LSN	23.7083	< 0.0001	$4^{ab} \pm 0.29$ [4–4.5] (3)	$3^{a} \pm 0.5$ [2–3] (4)	$5^{ab} \pm 0.88$ [3–6] (32)	$5^{b} \pm 0.87$ [5–7] (13)	$5^{b} \pm 0.69$ [4–6] (7)
LPW	25.3414	< 0.0001	$1.1^{ab} \pm 0.06$ [1.1–1.2] (3)	$1.13^{ab} \pm 0.39$ [0.8–1.7] (4)	$1.88^{a} \pm 0.37$ [1.1–2.4] (32)	$1.7^{a} \pm 0.45$ [0.7–2.3] (13)	$0.95^{b} \pm 0.09$ [0.8–1] (7)
LPN	10.5198	0.03	$3^{a} \pm 0$ [3] (3)	$3^{a} \pm 1$ [3–5] (4)	$3^{a} \pm 1.01$ [3–6] (32)	$3^{a} \pm 1.01$ [3–5] (13)	$3^{a} \pm 0.63$ [1.5–3] (7)
LIL	39.9963	< 0.0001	$2.8^{a} \pm 0.79$ [2.5–4] (3)	$7.6^{b} \pm 0.53$ [7.2–8.2] (4)	$4^{a} \pm 0.64$ [2.3–5] (32)	$5.5^{b} \pm 0.28$ [5.5–6.5] (13)	$3.2^{a} \pm 0.36$ [3–4] (7)
SPL	40.8179	< 0.0001	$3.2^{ab} \pm 0.67$ [2.8–4.1] (3)	$13^{a} \pm 1.32$ [12–15] (4)	$2.75^{b} \pm 0.75$ [1.6–4.5] (32)	$5^{a} \pm 1.18$ [4–7.2] (13)	$5^{a} \pm 0.76$ [3.6–5.5] (7)
GYL	22.3237	0.0002	$1.3^{ab} \pm 0.25$ [1–1.5] (3)	$2.3^{c} \pm 0.38$ [2–2.9] (4)	$1.6^{ab} \pm 0.31$ [1–2.2] (32)	$2^{ac} \pm 0.26$ [1.5–2.5] (13)	$1.5^{b} \pm 0.14$ [1.3–1.7] (7)
POL	37.3531	< 0.0001	$5.6^{ab} \pm 0.35$ [5–5.6] (3)	$9.75^{a} \pm 1.08$ [9–11.5] (4)	$5^{b} \pm 0.82$ [3.5–6.2] (32)	$7.2^{a} \pm 1.10$ [6.7–10] (13)	$5^{b} \pm 0.76$ [3.5–6] (7)

Table 5 – **Matrix values and statistics of parsimony analyses.**
CI, consistency index; RI, retention index; RC, rescaled consistency index.

Tree statistics	*ITS-1*	*matK*	*rps16*	*trnC-petN*	*trnL-F*	*ycf1*	plastid	combined
Length (aligned)	383	1792	987	878	480	1761	5898	6281
Parsimony- informative characters (%)	74 (19.32%)	142 (7.92%)	102 (10.33%)	119 (13.55%)	48 (10%)	193 (10.96%)	599 (10.15%)	673 (10.71%)
% of variability	27.93	10.16	15.09	15.72	12.70	15.62	13.65	14.52
Best trees found	2	6	6103	470	2	8	2	1
Tree length	177	226	175	163	68	353	993	1173
CI	0.77	0.88	0.90	0.94	0.96	0.85	0.88	0.86
RI	0.86	0.95	0.94	0.97	0.98	0.93	0.94	0.93
RC	0.66	0.83	0.84	0.91	0.94	0.79	0.83	0.80

Figure 4 – Phylogram obtained from Bayesian analysis of the combined molecular data set. Bootstrap values (BS) and posterior probabilities (PP) are given above or below the branches. Nodes with a combined support of 100% BS and 1.0 PP are indicated by a solid black circle. * BS/PP = 62/0.99; ** BS/PP = 91/0.98; *** PP: 0.98. *A.* = *Angraecum*; *T.* = *Tridactyle*; *D.* = *Diaphananthe*; *P.* = *Polystachya*.

of continental African *Angraecum* was limited to 12 of 138 species and only three taxa from *A*. sect. *Pectinaria* were included (one from Madagascar and two from the continent). Our study included all five described species from Africa, and in each analysis (i.e. those based on the six individual markers as well as the combined data set) they formed a well-supported clade whose position is not sister to the three Malagasy taxa included in our sampling that are historically assigned to the section. Our findings thus support the initial interpretation of Micheneau et al. (2008) that *A*. sect. *Pectinaria* does not comprise a natural group.

Species hypotheses in the continental group of *Angraecum* sect. *Pectinaria*

The combined morphometric approach used in our study, with the Hill-Smith ordination method and the clustering dendrogram of taxonomic distances, yielded clear delimitation of each of the five continental African species. In the molecular phylogenetic analyses, the multiple samples of both *Angraecum doratophyllum* and *A. subulatum* formed well supported clades that were distinct from the other continental species of *A*. sect. *Pectinaria*, namely *A. atlanticum*, *A. gabonense* and *A. pungens* (with the exception of the *trnL–F* analysis, which placed all five species in a polytomy; fig. 3E). Mean numerical variables measured among *A. atlanticum*, *A. gabonense* and *A. pungens*, while significantly different statistically, are still quite similar, probably because the flowers of these three species are similar morphologically. Of the 22 quantitative characters measured, only three (the lengths of the dorsal sepals, the lateral sepals and the lateral petals) showed significant differences for each species when compared to the other two (table 3). In the same manner, in the phylogenetic trees resulting from most of the single marker analyses, *A. atlanticum*, *A. gabonense* and *A. pungens* formed a clade but the positions of the accessions of *A. atlanticum* did not correspond to the species as currently circumscribed (figs 3 & 4).

The *Angraecum doratophyllum-A. subulatum* subgroup – *Angraecum doratophyllum*, endemic to the islands of São Tomé and Príncipe, possesses the longest petals of any African species of *A*. sect. *Pectinaria* and is also characterized by a deeply recurved spur with a wide mouth at the base of the lip. The multiple accessions sampled for this species formed a clade in all analyses except *trnL–F* (fig. 3E). *Angraecum subulatum*, which is abundant and widely distributed from Guinea Conakry to the Democratic Republic of the Congo, has the longest leaves of the group, along with a caudate leaf apex. The first species is easily distinguished by floral characters while the second is distinguished by foliar features. Results from molecular analyses indicate that *A. subulatum* is sister to all other African members of *A*. sect. *Pectinaria* (figs 3 & 4).

The *Angraecum pungens-A. gabonense-A. atlanticum* subgroup – *Angraecum pungens* is a poorly collected species that occurs in Nigeria, Cameroon, Equatorial Guinea, Gabon and the Democratic Republic of the Congo. It differs from *A. atlanticum* and *A. gabonense* by ten of the 22 morphological variables used in our study (tables 3 & 4). Within this subgroup, *A. pungens* has the longest and widest leaves, the longest flowers, the longest lateral sepals, lip, pedicel and ovary, and the widest floral bract. The combined matrix analysis placed *A. pungens* in a position nested within *A. atlanticum*, but *A. pungens* differs significantly from *A. atlanticum* on the basis of twelve morphological variables (see tables 3 & 4). Of these twelve variables, the most important providing a clear-cut separation of these two species are leaf width (LFW) and the lengths of the flower (FLL), the dorsal sepal (DSL), the lateral sepals (LSL), the lateral petal (LPL) and the lip (LIL). Moreover, several morphological features of *A. pungens*, including leaf (LFL) and pedicel and ovary (POL) lengths, show a rather clear separation, although the differences are not statistically significant.

Angraecum gabonense is distributed from Cameroon, Equatorial Guinea and Gabon to the Democratic Republic of the Congo, and is quite common in the field. Material of this species represents more than 50% (32 on 59) of accessions used in our morphological analyses. This large sample size reflects the fact that a preliminary examination of herbarium specimens showed that *A. gabonense* was highly variable with respect to the size and shape of its spur, prompting us initially to question whether more than one entity might be involved. Once all the alcohol preserved specimens were examined, it became clear, however, that significant variation also occurs in other characters measured, and that some specimens appear to resemble *A. pungens* while others look much like *A. atlanticum*. As a consequence, floral morphological differences between *A. gabonense* and its two closest relatives, *A. atlanticum* and *A. pungens*, are not obvious. Indeed, *A. gabonense* differs statistically from *A. atlanticum* by five of the six normal variables (table 3), i.e. the lengths of the dorsal sepal (DSL), the lateral sepals (LSL) and the lateral petals (LPL), and the widths of the dorsal sepal (DSW) and the lip (LIW). For the 17 remaining variables, the values seen in *A. gabonense* are similar to those in *A. atlanticum*. *Angraecum gabonense* differs statistically from *A. pungens* by fourteen of the 22 variables (tables 3 & 4), the dimensions of lateral sepals (LSL and LSW), the leaf (LFL and LFW), the mucronate apex of the leaf (MUL and MUW) and the floral bract (BRL and BRW), the lengths of flower (FLL), the dorsal sepal (DSL), the lateral petals (LPL), the lip (LIL), the spur (SPL) and the internodes (INT). For the eight remaining variables, *A. gabonense* resembles *A. pungens.*

Although expanded sampling of *A. gabonense* might reveal a phylogenetic pattern to the morphological variability detected here, its monophyly nevertheless was well supported in the phylogenetic analyses. *Angraecum gabonense* further differs from its close relatives in having its leaves held parallel to the stem so that the internodes appear to be very short. In the molecular analyses, the accessions of *A. gabonense* formed a clade when using three (*matK*, *rps16* and *trnC–petN*, figs 3B–D) of the six single markers, albeit weakly supported (62–72%), and when based on the plastid and combined data set.

Angraecum atlanticum, endemic to Gabon and Equatorial Guinea (Rio Muni), is currently known from only three populations. It differs from *A. gabonense* by five of the 22 morphological characters examined, although no quantitative feature easily distinguishes *A. atlanticum* from *A. gabonense*. However, the habitat requirements of these two spe-

cies are different: *A. atlanticum* is restricted to submontane forest whereas *A. gabonense* and *A. pungens* occur in dense lowland forest (Stévart et al. 2010). The phylogenetic tree resulting from analyses of the combined molecular data set placed the two accessions of *A. atlanticum* (one from each of the two Gabonese subpopulations) in a grade relative to *A. pungens*. Improved sampling, including from the Equatorial Guinean population of *A. atlanticum* where the type specimen was collected, might improve resolution and help clarify whether this species is monophyletic.

CONCLUSIONS

The comparison of results from our morphometric investigations with those from phylogenetic analyses using DNA sequence data allow us to clarify species circumscriptions, an indispensable prerequisite for the taxonomic revision of the continental group of *Angraecum* sect. *Pectinaria* (Simo-Droissart et al. in review). These results confirm that this group represents five species, notwithstanding the fact that the two accessions of *A. atlanticum* were not resolved as a clade. The three most closely related species, *A. atlanticum*, *A. gabonense* and *A. pungens*, resemble one another and were often confused, but they can be differentiated easily based on the criteria used in this study. Considering that *A. pectinatum*, the type species, belongs to the Malagasy clade, it will be necessary to remove the continental species from this section in order to maintain its monophyly. However, it would be premature at this point to attempt to recognize these species as a formal infrageneric group. A decision on how best to treat them within the infrageneric classification system of *Angraecum* must await results from a broader phylogenetic study of African angraecoids based on a more extensive sampling from throughout the genus, upon which improved sectional and generic limits can be established that meet the criterion of monophyly while also circumscribing morphologically coherent groups.

SUPPLEMENTARY MATERIAL

Supplementary data are available in pdf format at *Plant Ecology and Evolution*, Supplementary Data Site (http://www.ingentaconnect.com/content/botbel/plecevo/supp-data), and consist of (1) species names, distribution and voucher information for all taxa used in this study and (2) loadings of the three first axes of the principal component analysis with discrete characters on the 59 specimens of *Angraecum* sect. *Pectinaria*.

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Appendix – Voucher information and GenBank accession numbers for taxa used in the phylogenetic analysis of continental species of *Angraecum* sect. *Pectinaria* (including outgroups). For each taxon, voucher information (between brackets) is listed in the following order: voucher collector and collection number, institution where the voucher was deposited, country. Accession numbers are listed in the following order: ITS-1, *matK*, *rps16*, *trnC-petN*, *trnL-F*, *ycf1*. Hyphens indicate that no data are available. Further information on the locality of vouchers is available in electronic appendix 1 (see Supplementary Data).

Ingroup: *Angraecum aporoides* Summerh. (*Jardin Botanique de Bom Successo* 946, BRLU, São Tomé and Príncipe) KF672202, KF672276, KF672242, KF672292, KF662338, KF672340; *Angraecum* aff. *aporoides* A (*Droissart et al. (Yaoundé shadehouse)* 592, BRLU, Cameroon) KF672217, KF672266, KF672230, KF672308, KF662332, KF672336; *Angraecum* aff. *aporoides* B (*Jardin Botanique de Bambusa* 50, BRLU, Gabon) KF672200, KF672286, KF672232, KF672288, KF662340, KF672333; *Angraecum bancoense* Burg (*Droissart et al. (Yaoundé shadehouse)* 620, BRLU, Cameroon) KF672221, KF672280, KF672257, KF672311, KF662335, KF672320; *Angraecum distichum* Lindl. (*Droissart et al. (Yaoundé shadehouse)* 924, BRLU, Cameroon) KF672227, KF672265, KF672231, KF672289, KF662348, KF672337; *Angraecum podochiloides* Schltr. (*Droissart et al. (Yaoundé shadehouse)* 939, BRLU, Cameroon) KF672225, KF672281, KF672238, KF672293, KF662330, KF672339; *Angraecum atlanticum* Stévart & Droissart (*van der Laan* 1068, BRLU, Gabon) KF672213, KF672284, KF672243, KF672299, KF662343, KF672335; *Angraecum atlanticum* Stévart & Droissart (*Jardin Botanique de Bambusa* 130, BRLU, Gabon) KF672223, KF672274, KF672235, KF672312, KF662349, KF672326; *Angraecum doratophyllum* Summerh. (*Jardin Botanique de Bom Successo* 271, BRLU, São Tomé and Príncipe) KF672220, KF672275, KF672248, KF672291, KF662344, KF672334; *Angraecum doratophyllum* Summerh. (Accession N° of Cult. BR 20090375-26, BR, São Tomé and Príncipe) KF672224, KF672261, KF672247, KF672296, KF662351, KF672328; *Angraecum doratophyllum* Summerh. (Accession N° of Cult. *Jardin Botanique de Bom Successo* N° 1962.1, BRLU, São Tomé and Príncipe) KF672204, KF672258, KF672239, KF672297, KF662334, KF672318; *Angraecum gabonense* Summerh. (Accession N° of Cult. *Tchimbélé shadehouse, Gérald Brice* N° 189, BRLU, Gabon) KF672207, KF672264, KF672253, KF672316, KF662354, KF672325; *Angraecum gabonense* Summerh. (*Arends* 957, BRLU, Gabon) KF672209, KF672279, KF672237, KF672295, KF662333, KF672344; *Angraecum gabonense* Summerh. (Accession N° of Cult. *Tchimbélé shadehouse*, N° MBG 799, BRLU, Gabon) KF672222, KF672270, KF672250, KF672313, KF662342, KF672341; *Angraecum pungens* Schltr. (*Simo M. et al. (Yaoundé shadehouse)* 2817, BRLU, Cameroon) KF672216, KF672260, KF672249, KF672304, KF662328, KF672338; *Angraecum pungens* Schltr. (*Cultivated at BR greenhouse*, Accession N° BR 20090382-33, BRLU, Gabon) KF672203, KF672278, KF672254, KF672302, KF662329, KF672343; *Angraecum pungens* Schltr. (*Wilks* 3619, BRLU, Gabon) KF672226, KF672268, KF672240, KF672306, KF662353, KF672323; *Angraecum subulatum* Lindl. (*Jardin Botanique de Bambusa* 174, BRLU, Gabon) KF672218, KF672269, KF672233, KF672298, KF662337, KF672330; *Angraecum subulatum* Lindl., (*Cultivated at BR greenhouse*, Accession N° BR 20090388-39, BR, Ivory Coast) KF672206, KF672285, KF672251, KF672300, KF662355, KF672324; *Angraecum subulatum* Lindl. (*Simo M. et al. (Yaoundé shadehouse*) 2503, BRLU, Cameroon) -, KF672271, KF672241, KF672314, KF662347, KF672327; *Angraecum panicifolium* H.Perrier (*Simo M.* 215, BRLU, Madagascar) KF672205, KF672277, KF672244, KF672307, -, KF672322; *Angraecum pectinatum* Thouars (*Simo M.* 216, BRLU, Madagascar) KF672211, KF672267, KF672228, KF672309, KF662350, KF672342; *Angraecum* cf. *humblotianum* (*Simo M.* 217, BRLU, Madagascar) KF672199, KF672272, KF672255, KF672303, KF662345, KF672321; *Angraecum linearifolium* Garay (*Simo M.* 218, BRLU, Madagascar) KF672215, KF672273, KF672252, KF672301, KF662336, KF672331; *Diaphananthe odoratissima* (Rchb.f.) P.J.Cribb & Carlsward (*Cultivated at BR greenhouse*, Accession N° BR 19910192-69, BRLU, Rwanda) KF672208, KF672282, KF672256, KF672315, KF662341, KF672345; *Tridactyle aurantiopunctata* P.J.Cribb & Stévart (*Stévart* 656, BRLU, São Tomé and Príncipe) KF672201, KF672287, KF672236, KF672290, KF662356, KF672319; *Tridactyle bicaudata* (Lindl.) Schltr. (*Cultivated at Kisantu shadehouse*, BRLU, Democratic Republic of the Congo) KF672210, KF672263, KF672234, KF672305, KF662346, -; **Outgroups:** *Polystachya calluniflora* Kraenzl. (*Simo M. et al. (Yaoundé shadehouse)* 2527, BRLU, Cameroon) KF672214, KF672262, KF672229, -, KF662331, KF672329; *Polystachya albescens* Ridl. subsp. *imbricata* (Rolfe) Summerh. (*Simo M. et al. (Yaoundé shadehouse)* 2553, BRLU, Cameroon) KF672219, KF672259, KF672246, KF672310, KF662352, KF672317; *Polystachya pyramidalis* Lindl. (*Simo M. et al. (Yaoundé shadehouse)* 2497, BRLU, Cameroon) KF672212, KF672283, KF672245, KF672294, KF662339, KF672332.