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ORIGINAL ARTICLE

Malagasy *Dracaena* Vand. ex L. (Ruscaceae): an investigation of discrepancies between morphological features and spatial genetic structure at a small evolutionary scale

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Abstract Malagasy *Dracaena* (Ruscaceae) are divided into four species and 14 varieties, all of them showing a high level of morphological diversity and a putatively artefactual circumscription. In order to reveal relationships between those entangled entities, a span of Malagasy *Dracaena* were sampled and analyzed using cpDNA sequences and AFLP. The cpDNA analyses resolved three biogeographic clades that are mostly inconsistent with morphology, since similar phenotypes are found across the three clades. Bayesian inference clustering analyses based on the AFLP were not in accordance with the cpDNA analysis. This result might be explained by (1) a recent origin of the Malagasy species of *Dracaena* with an incomplete sorting of chloroplast lineages; (2) a high amount of hybridizations; (3) a complex migration pattern. Interestingly, when the AFLP are analyzed using the parsimony criterion, a trend towards a directional evolution of inflorescence types and ecological features was observed. This might be considered either as phenotypic plasticity and/or as the result of fast evolution in flower characters according to habitat preferences. Overall, our results point to the difficulty of defining evolutionarily significant units in Malagasy *Dracaena*, emphasizing the complex speciation processes taking place in tropical regions.

Keywords AFLP · Biogeography · cpDNA · Incomplete lineage sorting · Malagasy *Dracaena* · Phenotypic plasticity

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Introduction

Inconsistency between gross morphology and molecular phylogenies at the infrageneric level is uncommon in the literature. Nevertheless, incongruences have been published both in animals and plants. In animals, several cases have been described, e.g. in beetles (Normark and Lanteri 1998) and in *Daphnia* (Giessler 1997), in which no morphological characters showed variation corresponding to the phylogenetic patterns. In other cases, infrageneric lineages among Lepidoptera and Hymenoptera were highly divergent (>10 Mya) whereas no morphological differences could be identified (Molbo et al. 2003; Hebert et al. 2004).

In plants, Janssens et al. (2006) analyzed a survey of the highly diverse (>1,000 species) herbaceous genus *Impatiens* (Balsaminaceae), which was found to present complete incongruence between the molecular data set and the matrix of morphological characters, the latter being fully consistent with biogeography. Similarly, Wang et al. (2005) suggested that only geological and ecological factors could explain the phylogenetic clustering of taxa within Himalayan species of *Rheum* (Polygonaceae), in which no morphological characters were found to be informative. The authors invoked rapid speciation events in small and isolated demes, as well as independent parallel fixing of unique and/or rare morphological characters in

different populations. Whereas in these two studies, inconsistency between gross morphology and molecular phylogeny seemed to be directly related to rapid radiation (probably combined with introgression and reticulate evolution), biogeographic factors were relevant to explain the phylogenetic hypothesis. Cases of rapid speciation have also been addressed in tropical plants. For instance, Richardson et al. (2001) illustrated how quickly the mimosoid legume tree genus *Inga* radiated very early at the origin of this species-rich taxon (300 spp. of *Inga*), leading to a complex relationship between morphological and molecular characters. For decades, systematics in genera such as *Impatiens*, *Rheum* and *Inga*—in which species are vaguely segregated on the basis of continuous morphological characters—has been subject to numerous and contradictory taxonomic revisions.

Whereas the great majority of those studies highlighted complex evolutionary patterns at wide temporal and spatial scales, only few studies have addressed the question of incongruence between genetic structure and morphology at a smaller evolutionary level. In this study, we propose to investigate this problem, by examining the spatial genetic structure of Malagasy *Dracaena* Vand. ex L. (Ruscaceae)—a group of closely related taxa whose described entities show an extraordinary high level of morphological variation—and to correlate it with morphological features. Although the wide range of morphological diversity in Malagasy *Dracaena* has been described for seventy years (Perrier de la Bâthie 1937, 1938), only four species were circumscribed in the island. However, when Perrier de la Bâthie published the "Flore de Madagascar et des Comores" (1938), he eventually recognized 14 varieties within one single species (i.e. *Dracaena reflexa* Lam.). At that time, this author considered that those varieties were "linking" the three other species occurring in Madagascar (i.e. *D. angustifolia* Roxb., *D. elliptica* Thunb. and *D. xiphophylla* Baker) and proposed that all the taxa of Malagasy *Dracaena* were likely to compose a species complex. He was already aware of the poor morphological support for discriminating the Malagasy *Dracaena* and underlined that the delimitation of species and varieties was based on continuous morphological characters (mainly the shape and dimension of leaves) which were according to him "obviously arbitrary and contrary to the reality of facts" (Perrier de la Bâthie 1937).

At a wider spatial scale, the paleotropical genus *Dracaena* comprises approximately 60 species (Mabberley 2008) and ranges from Macaronesia to northern Australia (with the exception of one species recently introduced into the neotropics; see Marrero et al. 1998), showing a relatively high level of species diversity in tropical and subtropical Africa (23 species described; Bos 1984). This genus is able to colonize a large range of ecological habits from the thermophilous zone of Gran Canaria to the humid forests of tropical Africa and Madagascar. Surprisingly and despite the high diversity of African species, Bos (1984) did not encounter major problems to circumscribe species mainly on the base of leaf characters.

No molecular surveys have ever been carried out to unravel the complex relationships within the Malagasy *Dracaena*. In the present study, we consider data from chloroplast markers (cpDNA), amplified fragment length polymorphism (AFLP) and morphology to (1) test the consistency between gross morphology, biogeography and molecular phylogeny and (2) examine the implications of the molecular phylogeny for character evolution (especially the inflorescence types).

Material and methods

Taxon sampling

Representatives of Malagasy *D. angustifolia*, *D. elliptica*, *D. reflexa* and *D. xiphophylla* were included in our sampling (Table 1). Within the *D. reflexa* complex, nine of the 14 varieties were analyzed whereas the five remaining varieties—which are known from the type material only and are endemic to inaccessible forests—could not be collected. In total, 49 specimens (sampled in 18 sites) were included in the AFLP analysis. In addition, 24 specimens representing the morphological diversity of Malagasy *Dracaena* were selected to infer a plastid phylogeny. In addition to these samples, eight further [seven other Ruscaceae (AJ441158, AJ441175, AJ441177, AJ441178, AJ441179, AJ441181 and AJ441182) and one Asparagaceae (AJ441168)] *trnL-trnF* data sequences (Jang and Pfosser 2002) were included to the analysis.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from silica gel-dried tissue of single individuals (Chase and Hills 1991) using the QIAGEN DNeasy plant kit (Qiagen, Hilden, Germany) following the manufacturer's protocol, including a 1% RNase treatment during cell lysis. DNA extractions were purified using the Wizard® DNA clean-up (Promega, Madison, WI, USA). An herbarium voucher was prepared from each plant from which leaf material was sampled, except in cases where multiple individuals were collected from a single close-knit population.

Amplification and sequencing of the two following cpDNA regions was performed: *trn*L-*trn*F (Taberlet et al. 1991) and *rpl*12-*rps*20 (Hamilton 1999). The PCR reaction was performed in a 25 µl reaction mixture containing 5 µl 5× PCR buffer, 1.5 µl 25 mM $MgCl_2$, 0.5 µl 10 mM dNTPs, 0.5 µl 10 mM primers, 0.2 µl GoTaq polymerase

Table 1 Malagasy *Dracaena* taxa considered in this study, acronym (ID), population localities, voucher specimens and GenBank accessions

Taxon	ID	Location (province/locality)	Voucher	GenBank accessions	
				*rpl*12-*rps*20	*trn*L-*trn*F
D. angustifolia Roxb.	Da4	Antsiranana/Daraina, Antsahabe	Gautier 4713 (G, P, TAN)	–	–
D. elliptica Thunb.	Am1	Toamasina/Ambatovy	Ravokatra et al. 106 (NEU, TAN)	–	–
D. elliptica Thunb.	Am2	Toamasina/Ambatovy	Ravokatra et al. 105 (NEU, TAN)	EU032518	EU032494
D. reflexa Lam.	Am4	Toamasina/Ambatovy	Ravokatra et al. 102 (NEU, TAN)	–	–
D. reflexa Lam.	Am6	Toamasina/Ambatovy	Ravokatra et al. 109 (NEU, TAN)	–	–
D. reflexa Lam.	Rb1	Toliara/Ranobe Forest	Phillipson 5876 (G, K, MO, P, TAN)	–	EU032508
D. reflexa Lam. var. aff. *condensata*	Am3	Toamasina/Ambatovy	Ravokatra et al. 112 (NEU, TAN)	EU032526	EU032502
D. reflexa Lam. var. *angustifolia* Baker	An1	Antsiranana/Ankarana	Ravokatra et al. 4b (NEU, TAN)	EU032517	EU032493
D. reflexa Lam. var. *angustifolia* Baker	An2	Antsiranana/Ankarana	Ravokatra et al. 3e (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	An3	Antsiranana/Ankarana	Ravokatra et al. 3f (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	An4	Antsiranana/Ankarana	Ravokatra et al. 3a (NEU, TAN)	EU032521	EU032497
D. reflexa Lam. var. *angustifolia* Baker	An5	Antsiranana/Ankarana	Ravokatra et al. 3b (NEU, TAN)	EU032523	EU032499
D. reflexa Lam. var. *angustifolia* Baker	Da3	Antsiranana/Daraina, Ankaramy	Ranirison and Nusbaumer 466 (G, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	Mo1	Antsiranana/Montagne d'Ambre	Ravokatra et al. 22 (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	Mo2	Antsiranana/Montagne d'Ambre	Ravokatra et al. 15 (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	Mo3	Antsiranana/Montagne d'Ambre	Ravokatra et al. 8 (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	Mo4	Antsiranana/Montagne d'Ambre	Ravokatra et al. 29 (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	Mo5	Antsiranana/Montagne d'Ambre	Ravokatra et al. 28 (NEU, TAN)	–	–
D. reflexa Lam. var. *angustifolia* Baker	Mo6	Antsiranana/Montagne d'Ambre	Ravokatra et al. 27 (NEU, TAN)	EU032520	EU032496
D. reflexa Lam. var. *angustifolia* Baker	Mo7	Antsiranana/Montagne d'Ambre	Ravokatra et al. 9 (NEU, TAN)	EU032522	EU032498
D. reflexa Lam. var. *condensata* H. Perrier	Da14	Antsiranana/Daraina, Bekaraoka	Ranirison and Nusbaumer 964 (G, K, MO, P, TAN)	–	–
D. reflexa Lam. var. *condensata* H. Perrier	Da15	Antsiranana/Daraina, Solaniampilana-Maroadabo	Gautier et al. 4503 (G, MO, NEU, P, TAN)	–	–
D. reflexa Lam. var. *condensata* H. Perrier	Da7	Antsiranana/Daraina, Antsahabe	Nusbaumer and Ranirison 1325 (G, NEU, P, TAN)	–	EU032510
D. reflexa Lam. var. *lanceolata* H. Perrier	Da5	Antsiranana/Daraina, Antsahabe	Nusbaumer 948 (G, NEU, TAN)	EU032524	EU032500
D. reflexa Lam. var. *lanceolata* H. Perrier	Da6	Antsiranana/Daraina, Antsahabe	Nusbaumer and Ranirison 1287 (G)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Av1	Toliara/Ambovombe	Buerki and Phillipson 67 (TAN)	EU032529	EU032505
D. reflexa Lam. var. *linearifolia* Baker	Be1	Toamasina/Betampona	Buerki 147 (TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Da1	Antsiranana/Daraina, Ambohitsitondroina	Gautier et al. 4613 (G, MO, NEU, P, TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Da10	Antsiranana/Daraina, Antsahabe	Nusbaumer 990 (G, NEU, P, TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Da8	Antsiranana/Daraina, Antsahabe	Ranirison 746 (G, MO, NEU, P, TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Da9	Antsiranana/Daraina, Antsahabe	Nusbaumer and Ranirison 1324 (G, MO, NEU, P, TAN)	–	EU032507
D. reflexa Lam. var. *linearifolia* Baker	Ma1	Mahajanga/Mangindrano	Callmander et al. 405 (MO, TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Mo10	Antsiranana/Montagne d'Ambre	Ravokatra et al. 12 (NEU, TAN)	EU032513	EU032489
D. reflexa Lam. var. *linearifolia* Baker	Mo11	Antsiranana/Montagne d'Ambre	Ravokatra et al. 11 (NEU, TAN)	EU032514	EU032490
D. reflexa Lam. var. *linearifolia* Baker	Mo8	Antsiranana/Montagne d'Ambre	Ravokatra et al. 16 (NEU, TAN)	EU032511	EU032487
D. reflexa Lam. var. *linearifolia* Baker	Mo9	Antsiranana/Montagne d'Ambre	Ravokatra et al. 14 (NEU, TAN)	EU032512	EU032488

Table 1 continued

Taxon	ID	Location (province/locality)	Voucher	GenBank accessions	
				*rpl*12-*rps*20	*trn*L-*trn*F
D. reflexa Lam. var. *linearifolia* Baker	Mt1	Toamasina/Mantadia	Ravokatra et al. 113 (NEU, TAN)	EU032527	EU032503
D. reflexa Lam. var. *linearifolia* Baker	Ra1	Antsiranana/Ramena	Ravokatra et al. 2 (NEU, TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Ra2	Antsiranana/Ramena	Ravokatra et al. 1 (NEU, TAN)	–	–
D. reflexa Lam. var. *linearifolia* Baker	Rf1	Toamasina/Ranomafana	Ravokatra et al. 116 (NEU, TAN)	–	–
D. reflexa Lam. var. *nervosa* H. Perrier	Ai1	Toliara/Ambinanibe	Buerki and Phillipson 65 (MO, NEU, P, TAN)	EU032528	EU032504
D. reflexa Lam. var. *occidentalis* H. Perrier	An6	Antsiranana/Ankarana	Ravokatra et al. 5b (NEU, TAN)	EU032515	EU032491
D. reflexa Lam. var. *occidentalis* H. Perrier	An7	Antsiranana/Ankarana	Ravokatra et al. 5a (NEU, TAN)	EU032516	EU032492
D. reflexa Lam. var. *parvifolia* Thouars ex. H. Perrier	Da12	Antsiranana/Daraina, Bekaraoka	Nusbaumer and Ranirison 1195 (G, MO, NEU, P, TAN)	–	–
D. reflexa Lam. var. *parvifolia* Thouars ex. H. Perrier	Da13	Antsiranana/Daraina, Bekaraoka	Nusbaumer and Ranirison 1170 (G, MO, NEU, P, TAN)	EU032525	EU032501
D. reflexa Lam. var. *salicifolia* Baker	Am5	Toamasina/Ambatovy	Ravokatra et al. 101 (NEU, TAN)	EU032519	EU032495
D. reflexa Lam. var. *subelliptica* H. Perrier	Be2	Toamasina/Betampona	Buerki 148 (MO, TAN)	EU032530	EU032506
D. xiphophylla Baker	Da11	Antsiranana/Daraina, Antsahabe	Ranirison and Nusbaumer 862 (G, K, MO, P, TAN)	–	EU032509
D. xiphophylla Baker	Da2	Antsiranana/Daraina, Ampondrabe	Ranirison 580 (G, NEU, TAN)	–	–

G Geneva botanical garden, *K* Kew gardens herbarium, *MO* Missouri botanical garden, *NEU* Neuchâtel botanical garden, *P* Paris National Museum of Natural History, *TAN* Antananarivo herbarium

(5 U/µl) (Promega, Madison, WI, USA), and 14.5 µl ddH_2O. PCR was performed in a Biometra® T3 thermocycler. Initial template denaturation was programmed for 2 min at 95°C, followed by 35 cycles at 95°C for 45 s, 50°C for 45 s, 72°C for 1 min, plus a final extension of 10 min at 72°C. PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). Purified PCR products were automatically sequenced on an Applied Biosystems 310 DNA sequencer, using the dideoxy chain termination technique and a BigDye terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). A 5-µl sequencing labelling reaction was performed with 2 µl of premix provided by the manufacturer [containing the four labelled terminators, the deoxynucleotide triphosphates, the AmpliTaq DNA (Applied Biosystems, Foster City, CA, USA), $MgCl_2$ and Tris–HCI buffer], 0.2 µl of primer (10 mM) and 2.8 µl of amplified DNA. The cycle sequencing program was performed on a Biometra T3 thermocycler and consisted of 30 cycles of 10 s at 96°C and 4 min at 50°C.

Sequence alignment

Sequences were preliminary aligned with CLUSTAL X (Thompson et al. 1997) and subsequently manually adjusted using the similarity criterion (Morrison 2006). Gaps were coded as binary characters using GAP RECODER (Simmons and Ochoterena 2000; http://maen.huh.harvard.edu:8080/services/gap_recoder). A supermatrix combining the *trn*L-*trn*F and *rps*12-*rpl*20 matrices as well as the gap matrix was built using CONCATENATE (A. Criscuolo, http://www.lirmm.fr/~criscuol/).

Phylogenetic analyses based on sequence data

In order to preliminarily evaluate the genetic distance between the different taxa of Malagasy *Dracaena*, we calculated the pairwise Kimura-2P distance using MEGA version 3.1 (Kumar et al. 2004), both for *trn*L-*trn*F and *rps*12-*rpl*20 sequences. Afterwards, we considered a probabilistic approach by selecting the best-fit model for the concatenated cpDNA matrix using MRMODELTEST version.1.0 (Nylander 2002); analyses yielded to the selection of the general time reversible model with a proportion of invariable sites and a gamma distribution was selected (GTR + I + G). For the gap matrix the model was set as *restriction*. Considering the resulting supermatrix and the best-fit models as inputs, a partitioned Bayesian analysis was performed in MRBAYES version 3.1 (Huelsenbeck and Ronquist 2001). Four simultaneous Monte Carlo Markov Chains were run for 1,000,000 generations, saving a tree every 100 generations. Due to burn-in, 3,000 sample points were discarded until

stationarity in the likelihood value was established among the chains. The last 7,000 trees were used to calculate the Bayesian posterior probability (BPP) at each node. To corroborate patterns resulting from classical phylogenetic analyses, we computed a cpDNA network, using the *median network* algorithm (Bandelt et al. 1999), as implemented in SPLITSTREE version 4.6 (Huson and Bryant 2006).

AFLP analyses

After preliminary tests, two primer combinations which resulted in clear repeatable bands with sufficient variability were chosen: *Eco*RI-AGA/*Mse*I-CAC and *Eco*RI-ACT/*Mse*I-CAC. For the two AFLP datasets, the procedure followed Gaudeul et al. (2000). Selective PCR products (1 µl labelled products) were mixed and blended with 10 µl HiDi formamide and 0.1 µl ROX 500 size standard. Electrophoresis was carried out on an Applied Biosystems 310 capillary sequencer (Applied Biosystems, Foster City, CA, USA). PCR products from each primer combination were run separately.

Raw data were collected and sized using GENESCAN version 3.7 (Applied Biosystems, Foster City, CA, USA). The AFLP profiles were scored using the software GENOGRAPHER version 1.6 (available at http://hordeum.oscs.montana.edu/genographer/) and each band was coded as presence (1) or absence (0). Only variable markers in the size range 50–500 bp were scored. Eight replicates were included in all PCR plates. Based on these controls, reproducibility was estimated as the average proportion of correctly replicated bands. Markers with low reproducibility (<90%) were excluded; markers with a proportion of presences or absences lower than or similar to the error rate were checked carefully and only used if they were unambiguous.

Bayesian inference clustering analyses based on AFLP data

Population structure of the Malagasy *Dracaena* was analyzed through Bayesian inference clustering using STRUCTURE version 2.2 (Pritchard et al. 2000; Falush et al. 2007). Since no studies have yet addressed the occurrence of mixture events between different gene pools in the genus *Dracaena*, the two ancestral models ("no admixture" and "admixture") were analyzed, together with an assumption of independent allele frequencies. Five independent runs were carried out for each value of K (i.e. the number of clusters assumed) ranging between 1 and 10, with parameters and model likelihood estimated over 1,000,000 Monte Carlo Markov Chains generations following a burn-in period of 200,000 generations. For each value of K, only the run yielding the best likelihood was considered.

Maximum parsimony analyses based on AFLP data

Unrooted maximum parsimony (MP) analyses were conducted on the AFLP matrix using PAUP* version 4.0b10a (Swofford 2002). Heuristic searches were conducted with TBR branch swapping, MULPARS, ACCTRAN and 100 random addition-sequence replicates. Characters—which were of type "Dollo up" (in order to exclude convergences; see Le Quesne 1974)—were equally weighted. To assess the robustness of clades, bootstrap analyses (Felsenstein 1985) were performed in the MP analyses. Bootstrap values were calculated from 1,000 replicates using a heuristic search with simple addition, TBR branch swapping and MULTREE option.

Level of congruence between cpDNA and AFLP phylogenies

The topological incongruence between cpDNA and AFLP phylogenetic trees was assessed using the average normalised partition metric distance (hereafter NPM distance; also known as the Robinson–Foulds topological distance; Robinson and Foulds 1981) using PARTITIONMETRIC version 1.2.1 (O. R. P. Bininda-Emonds, http://www.unioldenburg.de/molekularesystematik/). The NPM distance is useful to calculate distances between phylogenetic trees that do not share the same number of taxa. The algorithm begins by pruning the phylogenetic tree to the same size and subsequently computes the sum of the components present in one but not both trees. A component refers to the relationships expressed by an internal branch, which separates the members of a clade from the non-members (including the root) (Wilkinson et al. 2005).

Inflorescence features

Since reproductive characters such as the type of inflorescence were poorly investigated by Perrier de la Bâthie (1937, 1938), we emphasized on those neglected characters in order to find morphological features compatible with the phylogenetic patterns. Three distinctive types of inflorescences were notably recognized in our specimens: panicles (Fig. 1a–c), racemes (Fig. 1d) and spikes (Fig. 1e). Moreover, three types of panicles are recognized according to the number of flower per glomerule (= cluster of flowers) and the development of the first axis A_0 of the inflorescence (Fig. 1a–c).

Character evolution

To detect synapomorphic features supporting the clades obtained with the AFLP based MP phylogeny, character

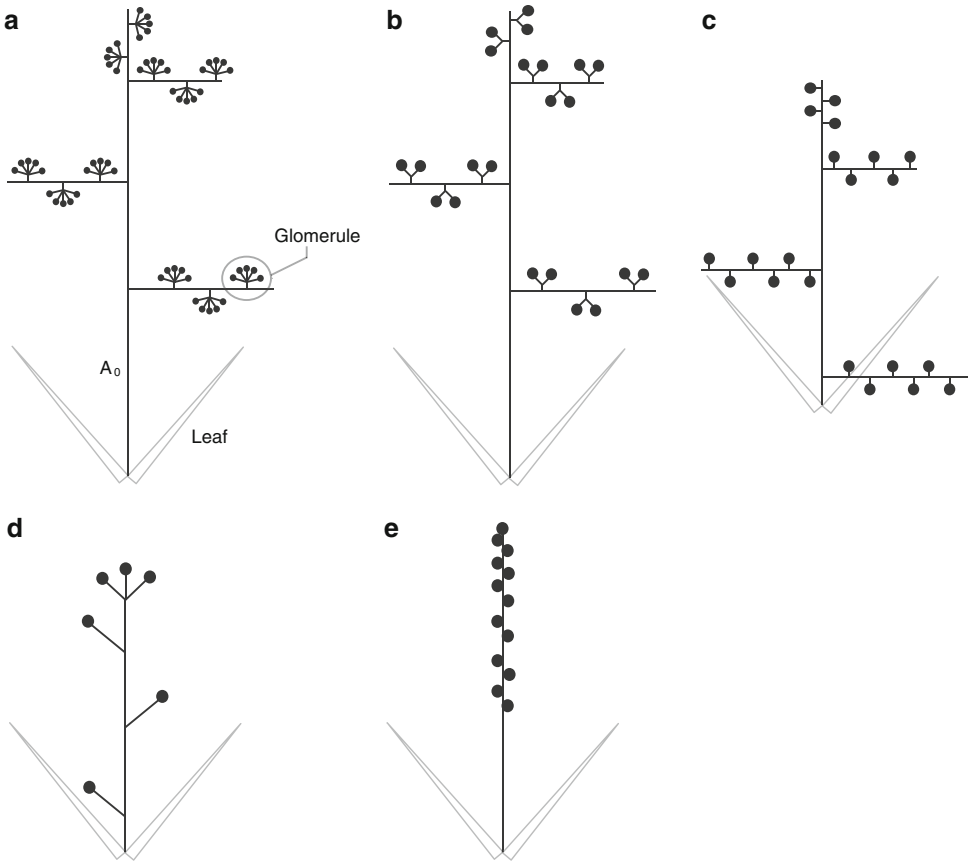


Fig. 1 Inflorescence types encountered in Malagasy *Dracaena*. Panicles (**a**–**c**), raceme (**d**) and spike (**e**). The panicles are subdivided into three types according to the number of flowers per glomerule (*grey circle*) as well as to the development of the principal axis of the inflorescence (axis A_0). **a** A-type panicle, **b** B-type panicle and **c** sessile panicle

reconstruction was realized according to a dozen of qualitative characters classically used to describe the taxa (e.g. habit, leaf shape, number of flowers per glomerules, inflorescence types and ecological features; data not shown). For instance, inflorescence types and ecological features were coded as multistate qualitative variables following the "A coding approach" proposed by Pleijel (1995). Coding was as follows: inflorescence types: 0 A-type panicle, 1 B-type panicle, 2 sessile panicle, 3 raceme and 4 spike, ? missing value; Ecological features: 0 Humid evergreen forest, 1 Ridges of humid evergreen forest, 2 Semi-deciduous forest and 3 Deciduous forest. In order to avoid homologous expressions among the different character states, all the characters were previously coded as absence/presence (following the "D coding approach" of Pleijel). Ancestral unordered parsimony reconstruction was performed for those two qualitative multistate characters on the midpoint-rooted most parsimonious AFLP tree, using the "trace character history" implemented in Mesquite (Maddison and Maddison 2004). Character coding for Malagasy *Dracaena* was largely based on personal observations of living and herbarium material.

Results

Phylogenetic hypothesis based on chloroplast sequences

Amplification of the two cpDNA regions (*trn*L-*trn*F and *rps*12-*rpl*20) resulted in alignments of 969 and 734 bp, respectively (see Table 1). When the outgroups were included, *trn*L-*trn*F had 132 variable characters and 41 potentially informative-characters, whereas *rps*12-*rpl*20 had only 4 variable characters and 3 of them were potentially parsimony-informative. Within *trn*L-*trn*F, 42 variable distinct indel positions were coded as gap characters. Although *rps*12-*rpl*20 sequences were missing in several specimens (for which amplification was not satisfactory), we analyzed the combined data set following Wiens (1998), who demonstrated, by performing multiple analyses of simulated data sets, that the addition of an incomplete data set to a data set complete for other markers is more likely to increase than decrease phylogenetic accuracy. The largest pairwise Kimura-2P distances were 0.019 and 0.004 for *trn*L-*trn*F and *rps*12-*rpl*20, respectively, whereas the average distances among all taxa were

0.009 and 0.001, respectively. The Bayesian inference majority-rule consensus tree (Fig. 2a) highlighted: (1) the para- or polyphyly of all taxa (with the minor exception of *D. reflexa* var. *occidentalis*) and (2) the presence of three major clades within Malagasy *Dracaena*, namely clades A, B and C. At a biogeographic scale, relationships between the three clades were weakly supported (BPP 0.36) and should be considered as a polytomy. Clades A (BPP 1.00) and C (BPP 0.99), which in contrast demonstrated a higher support, occurred in the North of Madagascar. In spite of their geographic proximity, these two clades colonized different vegetation types: clade A is principally distributed in humid evergreen forest (Montagne d'Ambre and humid zones of the northern part of Daraina) whereas clade C occurs in semi-deciduous to deciduous forest (Ankarana and dry zones of the southern part of Daraina). Clade B (BPP 1.00)—which is also strongly supported—covers a much larger area (in central and southern Madagascar) and is also ecologically distinct. This widespread clade is subdivided into two biogeographic groups. The first group (B1 which is monophyletic and nested into the second group; in bold black in Fig. 2a) colonizes the low (Betampona) to mid-elevation (Ambatovy and Mantadia) evergreen forest of the east coast of the island, whereas the second group (B2, which is paraphyletic; in bold grey in Fig. 2a) is distributed throughout the southernmost part of Madagascar from Fort-Dauphin (S-E) to Toliara (S-W). Individuals from this group grow in semi-deciduous (Ambovombe) to dry (Ranobe) forests. The median network analysis (Fig. 2b) was congruent with the Bayesian inference tree in supporting the three previously defined clades (Fig. 2a). However, the median network representation also indicated that specimens from clade C showed a transitional position between specimens of clades A and B. Within clade A, the network graph showed an intermediate position of Da7 and Mo7 in between the remaining specimens of clades A and C (Fig. 2b). These specimens were recognized as the most basal lineages in the Bayesian inference tree (Fig. 2a). Within clade B, Be2 is intermediate between specimens from clade B2 and the remaining specimens from clade B1 (Fig. 2b). This specimen was recovered as the most basal lineage in the clade B1 in the Bayesian inference tree (Fig. 2a).

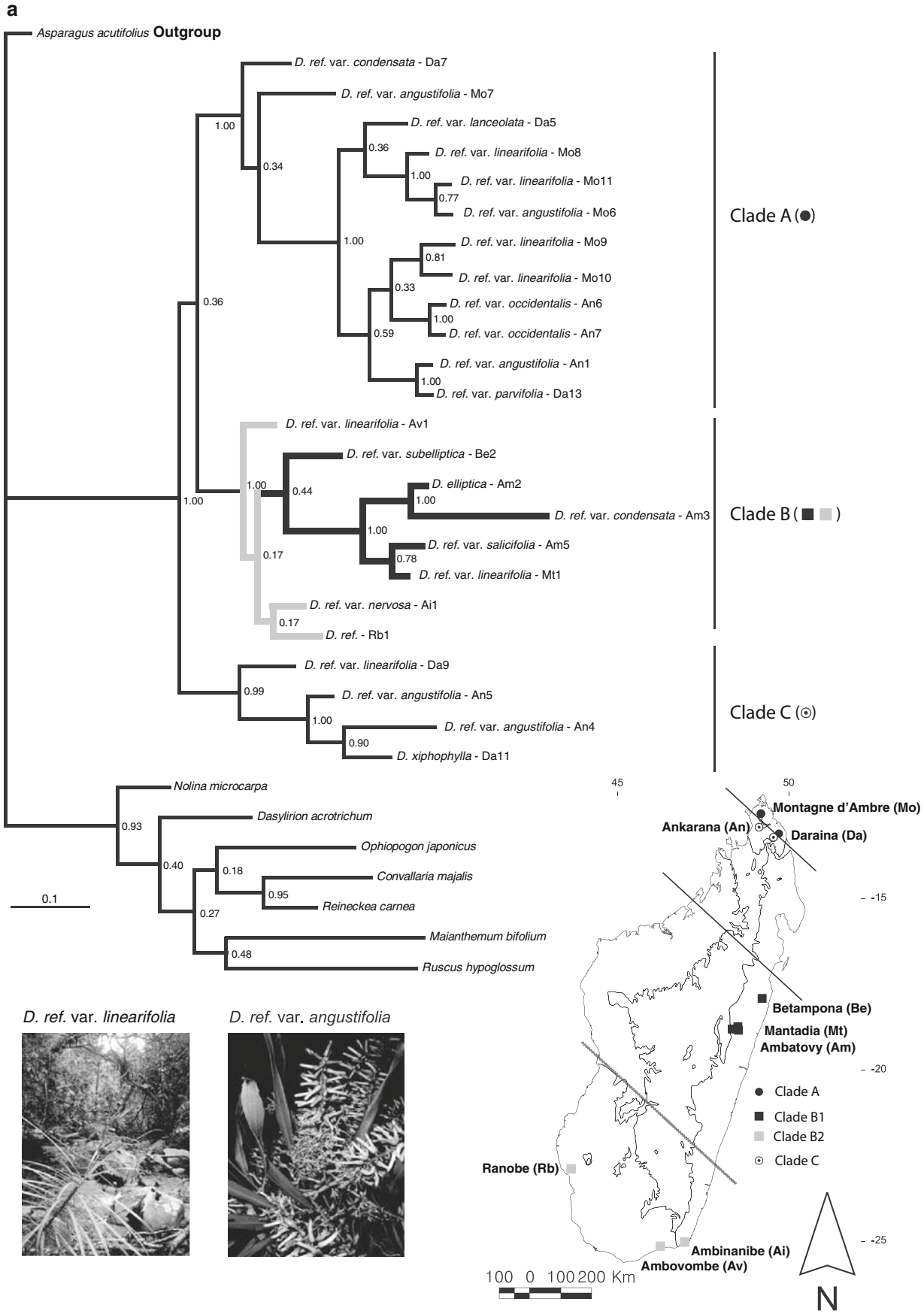
Genetic structure and phylogeny based on AFLP data

The two AFLP primer combinations yielded a total of 140 unambiguously scorable bands for the entire data set comprising 49 specimens. Among the 140 bands, 64 were generated by the primer combination *Eco*RI-AGA/*Mse*I-CAC, and 76 were generated by the primer combination *Eco*RI-ACT/*Mse*I-CAC. The matrix was analyzed both with Bayesian inference clustering and MP algorithms. Bayesian inference clustering analyses with STRUCTURE using the "no admixture" ancestry model maximized the likelihood when considering $K = 4$ [$-\ln(K = 3)$: $-2347.3 < -\ln(K = 4)$: $-2277.8 > \ln(K = 5)$: -2286.6], whereas considering the "admixture" ancestry model, $K = 6$ coincided with the highest likelihood [$-\ln(K = 5)$: $-2244.1 < -\ln(K = 6)$: $-2198.6 > \ln(K = 7)$: -2199.5]. In the "admixture" model, however, one group among six comprised only probabilities of assignment < 0.25 and we therefore considered that actually only five groups were assigned. Results obtained with the "no admixture" and "admixture" models were mostly akin, with probabilities of assignment being slightly higher in the "no admixture" model. The discrepancy in the optimal number of groups considered in the "no admixture" (i.e. four groups) and "admixture" (i.e. five groups) models is explained by the division, in the latter analysis, of one group into two, segregating Am1 and Am2 (both specimens belonging to *D. elliptica*) from the other individuals. As the two ancestry models gave similar results and because the "admixture" ancestral model provided a fifth group composed by the specimens of *D. elliptica*, only those results will be considered in the further discussion. The assignment probabilities to each group for each individual are represented as pie charts and plotted on the bioclimatic map of Madagascar (Fig. 3). At a first glance, no clear patterns of distribution are recognized among the five groups.

The AFLP based MP phylogeny resulted into two equally most parsimonious trees of 1,099 steps, in which only two samples exchanged positions (Mt and Ra2). Among 140 total characters, 115 characters were parsimony-informative. Bootstrap supports (= BS) were relatively low, since only the clade comprised by *D. elliptica* (Am1 and Am2) is strongly supported (BS: 91). When pruned to the same set of taxa, the NPM distance between the cpDNA and AFLP phylogenetic trees is 0.619 indicating a low level of congruence between the plastid and nuclear data sets.

Character evolution

Inflorescence features as well as ecological characteristics were optimized separately using ancestral parsimony on the AFLP based MP phylogeny and subsequently confronted using the mirror setting in MESQUITE (Fig. 4). Each single character allows the definition of circumscribed entities and a trend towards a correlation between them is observed: each particular inflorescence type is significantly associated with a specific forest type (Table 2; $\chi^2 = 25.744$, $P = 0.0001$). Types A and B panicles occur mainly in humid forests as well as semi-deciduous forests; sessile panicles are restricted to ridges; racemes are mainly


a
Asparagus acutifolius Outgroup
D. ref. var. condensata - Da7
D. ref. var. angustifolia - Mo7
D. ref. var. lanceolata - Da5
D. ref. var. linearifolia - Mo8
D. ref. var. linearifolia - Mo11
D. ref. var. angustifolia - Mo6
D. ref. var. linearifolia - Mo9
D. ref. var. linearifolia - Mo10
D. ref. var. occidentalis - An6
D. ref. var. occidentalis - An7
D. ref. var. angustifolia - An1
D. ref. var. parvifolia - Da13
Clade A (●)
D. ref. var. linearifolia - Av1
D. ref. var. subelliptica - Be2
D. elliptica - Am2
D. ref. var. condensata - Am3
D. ref. var. salicifolia - Am5
D. ref. var. linearifolia - Mt1
D. ref. var. nervosa - Ai1
D. ref. - Rb1
Clade B (■ ■)
D. ref. var. linearifolia - Da9
D. ref. var. angustifolia - An5
D. ref. var. angustifolia - An4
D. xiphophylla - Da11
Clade C (⊙)
Nolina microcarpa
Dasylirion acrotrichum
Ophiopogon japonicus
Convallaria majalis
Reineckea carnea
Maianthemum bifolium
Ruscus hypoglossum
0.1
D. ref. var. linearifolia
D. ref. var. angustifolia
45
50
-15
-20
-25
Montagne d'Ambre (Mo)
Ankarana (An)
Daraina (Da)
Betampona (Be)
Mantadia (Mt)
Ambatovy (Am)
Clade A
Clade B1
Clade B2
Clade C
Ranobe (Rb)
Ambinanibe (Ai)
Ambovombe (Av)
100 0 100 200 Km
N

◀ **Fig. 2** **a** Bayesian consensus tree based on *trn*L-*trn*F, *rps*12-*rpl*20 sequences data (including gaps). Three major biogeographical clades are well supported. *Clade B* is subdivided into two groups of taxa according to their ecology and biogeography: a first monophyletic group (in *black*) colonizes the low (Betampona) to mid-elevation (Ambatovy and Mantadia) evergreen forest of the eastern coast of the island; a second paraphyletic group (in *grey*) is distributed throughout the semi-deciduous forests of the southernmost part of Madagascar, from Fort-Dauphin (S-E) to Toliara (S-W). As an illustration of the high level of parallel evolution in the different clades, two frequent phenotypes are represented on the tree: *A* (*circles*): *Dracaena reflexa* var. *linearifolia*; *B* (*rectangle*): *Dracaena reflexa* var. *angustifolia*. **b** cpDNA-based median network. See above for the definition of the symbols

confined to deciduous forests and spikes are mostly found in humid forests. In general, the more arid is the environment, the greater is the tendency to reduce the size and development of the inflorescence from an A-type or B-type panicle (humid forest) to a sessile panicle (ridges) and to a spike (humid and deciduous forest) or a raceme (deciduous forest).

Discussion

Phylogenetic analysis based on chloroplast genes

Although the monophyly of the genus *Dracaena* (and consequently the monophyly of the Malagasy taxa) is not formally tested here, the small genetic distance values among the Malagasy *Dracaena*, together with the polyphyletic status of the varieties, constitute several lines of evidence supporting the close relationships between those terminal taxonomic units. Nonetheless, the monophyletic status of the genus, as well as the evolutionary relationships among taxa from different biogeographic regions are pending to be investigated by the Dractax project (see http://dractax.myspecies.info/?) involving authors from the present study. This project might also provide clues to confirm preliminary results by Rudall et al. (2000), who showed the close relationships of *Dracaena* with *Sansevieria* and *Pleomele* based on molecular markers and morphological evidence.

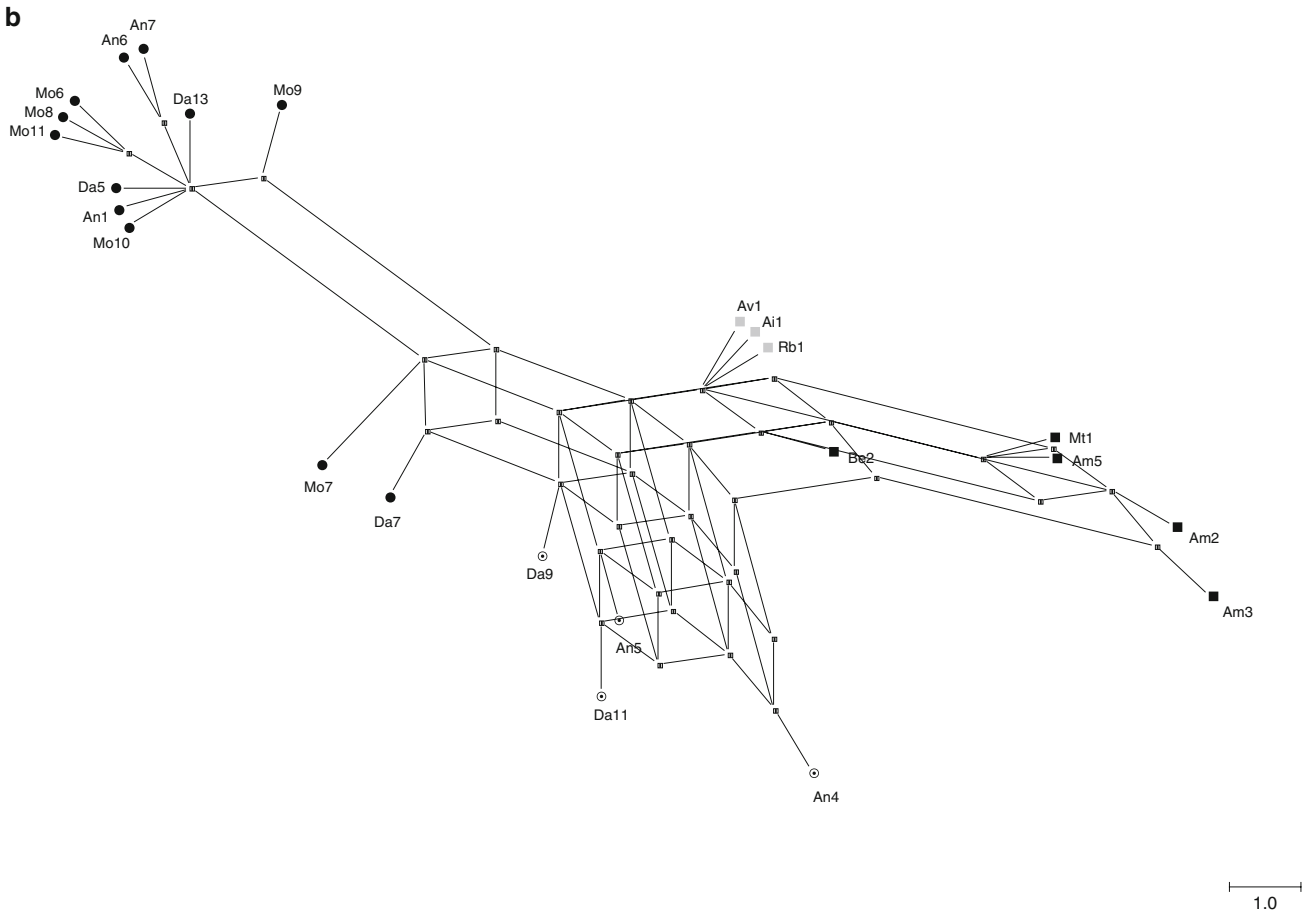


Fig. 2 continued

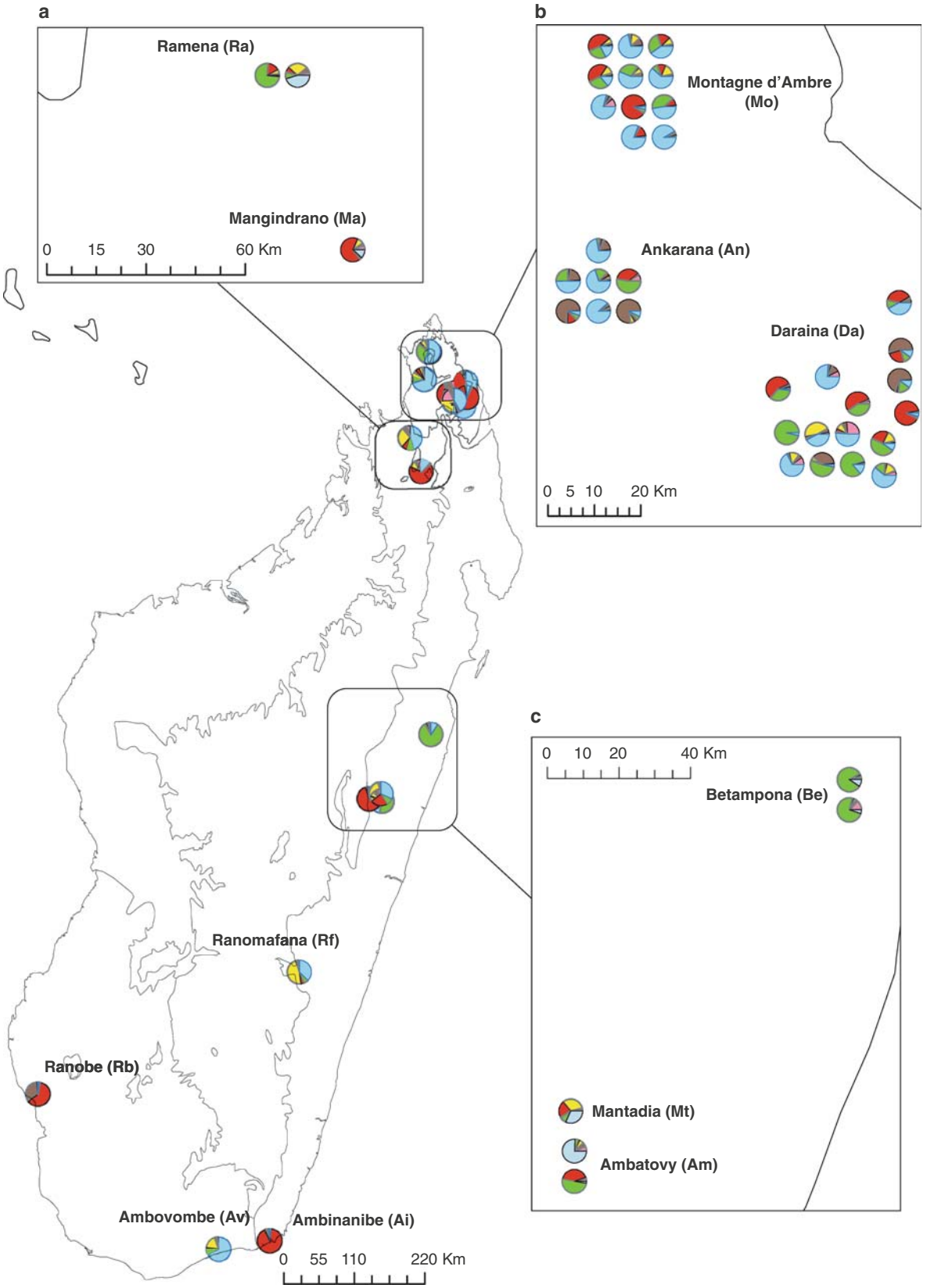


Fig. 3 Bayesian inference clustering results using an "admixture" ancestral model with five groups represented as *pie charts* on the bioclimatic map of Madagascar. To clearly visualize the pie charts of close populations, close-ups of three regions are represented (**a**–**c**)

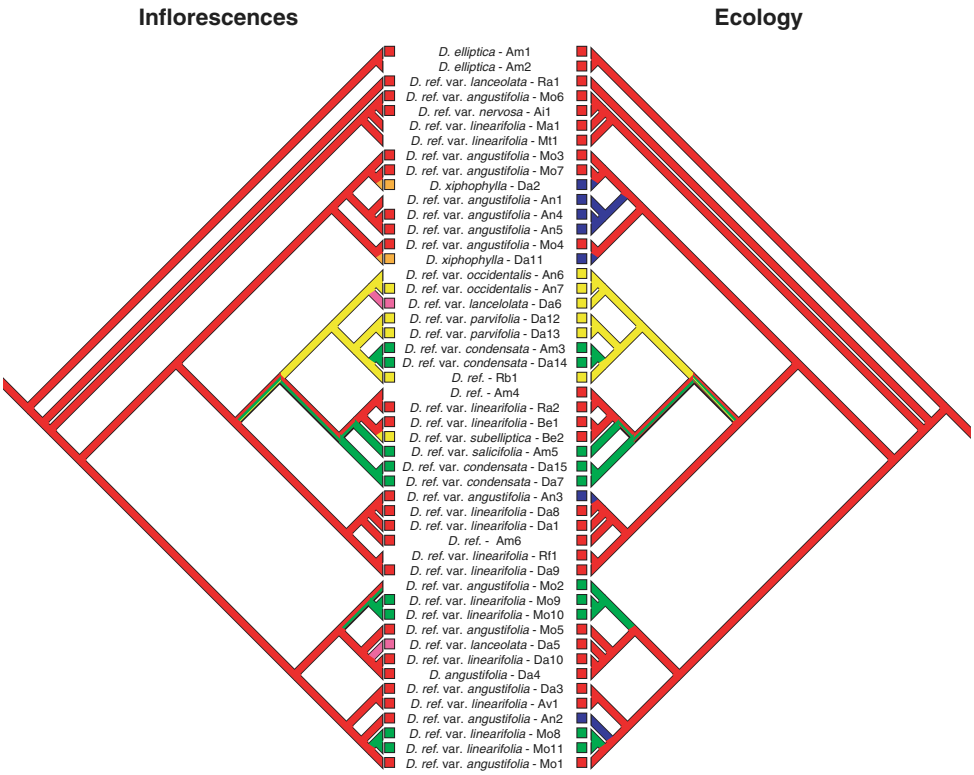


Fig. 4 Ancestral parsimony reconstruction of inflorescence types and ecological features on the MP tree (confronted in mirror). The tree was rooted using the midpoint rooting setting in PAUP. The different types of inflorescence are coded as follows (*right tree*): A-type panicle (in *orange*), B-type panicle (in *red*), sessile panicle (in *green*), raceme (in *yellow*), spike (in *pink*); missing data are indicated in *white*. The ecological features are coded as follows (*left tree*): Humid evergreen forests (in *red*), Ridges of humid evergreen forests (in *green*), Semi-deciduous forests (in *blue*) and Deciduous forest (in *yellow*)

Table 2 Relationships between inflorescence and ecology in 41 samples for which habitat has been described

Inflorescence\ ecology	Humid evergreen forests	Ridges of humid evergreen forests	Semi-deciduous forests	Deciduous forests
Panicle	19[a]	0	6[b]	0
Sessile panicle	0	9	0	0
Raceme	1	0	0	4
Spike	1	0	0	1

Values indicate the number of samples

[a] Divided into 17 type-A panicles and two type-B panicles

[b] All six are type-A panicles

Our results show that the topology of the chloroplast-based phylogeny of Malagasy *Dracaena* is better explained by biogeography than by any morphological characters, since identical phenotypes are distributed throughout the different clades of the tree [see the example of phenotypes such as *D. reflexa* var. *linearifolia* (A) and *D. reflexa* var. *angustifolia* (B) represented in Fig. 2]. Biogeographic distributions are relatively congruent with bioclimatic regions in Madagascar (Battistini 1996). For instance, clade A is distributed throughout mountainous, subhumid and dry regions, whereas clade C is distributed in subhumid and dry regions. The subdivision of clade B is also congruent with bioclimatic factors since group B1 is distributed throughout humid regions, whereas B2 is restricted to subarid regions. However, the relationships among the three clades are not well supported as shown by the Bayesian inference tree (Fig. 2a). In addition to identifying the three biogeographic clades, the chloroplast network recognizes clade C as transitional between clades A and B. This intermediate position is consistent with geographical features, since clade C is spatially distributed between clade A (which occurs in the far North of Madagascar) and clade B (which occurs from the middle-east to the southern part of Madagascar) (see Fig. 2). Although most of the specimens from clade A form a coherent group, the chloroplast network shows that some connexions still exist between this clade and specimens from clade C (i.e. Da7 and Mo7) (Fig. 2b). The same pattern is recovered with the Malagasy specimens from the subarid regions (clade B2), which are connected with those from the eastern part of the island (clade B1; especially regarding Be2; Fig. 2b). Interestingly, both chloroplast analyses recognized two clades in the far north of Madagascar. From an ecological perspective, this region was always considered as one of the most complex parts of the island (Battistini 1965) and is home to a complex mosaic of climates, orography and overlapping geology (Rossi 1976), due to important Pleistocene paleoclimatic shifts, which have certainly had an important role in influencing the distribution and speciation of organisms on continental landmasses today (Fjeldså and Lovett 1997; Hewitt 2000).

Although the phylogenetic information provided by the cpDNA data set allows the recognition of well-supported biogeographic clades, it also indicates that contemporary Malagasy *Dracaena* might result from complex evolutionary processes, as attested by the absence of consistent clustering of the different morphotypes on the phylogenetic hypothesis. Therefore, the recognition of evolutionary events potentially involved in the actual shape of distribution of these taxa must be considered as well in light of the AFLP analysis (which display mostly information from the nuclear genome).

Bayesian inference clustering analysis of the AFLP data

The distribution of the five AFLP-based clusters is strongly incongruent with the biogeographic clades highlighted by the cpDNA phylogeny (Figs. 2, 3): none of the three biogeographic clades highlighted by the cpDNA analyses are assessed by the AFLP markers. This incongruence is also characterized by a high NPM distance revealing that almost two-thirds (0.619) of the tree-components are not common to the two phylogenetic hypotheses. When studying myrmecophytic species of the tropical *Macaranga*, Bänfer et al. (2006) highlighted a similar pattern, in which the chloroplast genealogy reflected biogeography, whereas the information provided by the nuclear marker (in this case the *ITS* region) was congruent with the taxonomy. Interestingly, a few studies among temperate taxa also yielded the same pattern [e.g. oaks (e.g. Dumolin-Lapègue et al. 1997) and *Hordeum* (Jakob and Blattner 2006)]. According to some of these works (e.g. Bänfer et al. 2006; Jakob and Blattner 2006) such patterns might be explained by: (1) a recent origin of the species with an incomplete sorting of chloroplast lineages; (2) a high amount of hybridizations and (3) a complex migration pattern (involving vicariance and long-distance dispersals). Based on the results presented in this study, the pattern characterizing Malagasy *Dracaena* seems compatible with the hypothesis of a recently differentiated group, in combination with incomplete lineage sorting. Moreover, potential hybridizations events must not be ignored because our study indicates that several phenotypes (and even taxa) co-occur in the same areas. Finally, it must also be considered that the pattern observed here might be explained by a less parsimonious hypothesis involving a complex biogeographic history with long-distance dispersal events of pollen and local dispersion of the seeds. Accordingly, the long-distance dispersal of pollen might homogenize the genetic pools among populations, whereas the local dissemination of seeds might induce biogeographic barriers at the level of cpDNA. However, only very little information is available with respect to pollinators' and seed-dispersers' behavioural ecology and additional fieldwork missions are required to investigate this hypothesis.

Ecology and inflorescence type

Both the high level of phenotypic polymorphism and the distribution of intermediate specimens of *Dracaena* along a morphological continuum have impeded practical taxonomic revisions since early treatments of this genus in Madagascar (Perrier de la Bâthie 1937, 1938). Roughly 70 years after the last revision, our study underlines the close relationships among Malagasy *Dracaena*. Taxonomically, the problem posed by intermediate phenotypes was "resolved" by defining *D. reflexa*, a wide entity linking all the Malagasy species (*D. angustifolia*, *D. elliptica* and *D. xiphophylla*) throughout its 14 varieties (Lamarck 1786; Baker 1875, 1886; Perrier de la Bâthie 1937). Those artefactual varieties and species are puzzling for fieldworkers and their status is in great need of clarification. Our MP analysis of the AFLP matrix demonstrates that inflorescence types are likely to be good candidates for describing taxonomic entities (Fig. 4). Interestingly, this trend might be related to the kind of habitat, in terms of resources availability: the most extensively developed inflorescences (i.e. panicles; Fig. 4 and Fig. 1 for explanations) occur in humid environments—in which availability of resources is high—whereas the least developed ones (i.e. racemes) are found in dry environments—more likely to contain only scarce resources. Globally, the spatial distribution of inflorescence types could be related to (1) the phenotypic plasticity of *Dracaena* genomes, and/or (2) strong selective pressures acting on these traits. Regarding the first hypothesis (1), we might consider that the correlation between ecological factors and inflorescence types results from a substantial phenotypic plasticity: depending on the environmental conditions (defining resources availability), the same genotype could express different phenotypes. Phenotypic plasticity—which describes the capacity of a genotype to exhibit a range of phenotypes in response to environmental variation—has been widely documented in several plant families (e.g. Via 1993; Lortie and Aarssen 1996). In the case of *Dracaena*, Vladimirova et al. (1997) examined the response of a horticultural species, *D. sanderana* hort. Sander ex Mast. to different light intensities and demonstrated a wide range of developmental responses of several organs, such as leaves, internodes and root mass. Globally, the authors showed a strong negative correlation between light intensity and both leaf area and internode size. If those observations are extended to the inflorescence, one could also hypothesize that the striking morphological differences observed in Malagasy *Dracaena* may simply reflect the limits imposed by the environment. However, regarding the second hypothesis (2), we might consider that depending on the kind of environment, natural selection should favour different inflorescence types, as indicated by the presence of

identical inflorescence types in similar environments across the three biogeographic areas (Fig. 2). B-type panicles are widely distributed throughout humid evergreen forest and semi-deciduous forest, whereas sessile panicles and racemes are specifically found respectively in ridges of humid evergreen forest and deciduous forest. These two complementary hypotheses are fully compatible with the likely recent origin of the Malagasy *Dracaena*, in combination with incomplete lineage sorting and possible hybridizations.

Conclusion

Our study underlines the existence of complex genetic entities within Malagasy *Dracaena*—consistent with biogeography in the cpDNA phylogeny—that can sometimes be related to ecological and/or morphological variation. The future Dractax large scale phylogeny project, coupled with further fieldwork, a broader sampling (at the population level) and molecular analyses (e.g. using SSR) might be fundamental to confirm the evolutionary hypotheses presented here and to replace the Malagasy *Dracaena* in a broader concept. The level of plasticity of the different *Dracaena* genotypes should also be investigated in a long term experimental design. Such a study might however require several years before being able to describe the morphological variability in this long-lasting shrubby genus.

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