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Diversification and coexistence in the Madagascar olive (Noronhia, Oleaceae)

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DIVERSIFICATION AND COEXISTENCE IN THE MADAGASCAR OLIVE (*NORONHIA*, OLEACEAE)

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(*NORONHIA*, OLEACEAE)

TABLE OF CONTENTS

ABSTRACT

Evolution, speciation and distribution of “island” plants – whether oceanic or continental islands, or isolated mountains - can provide a rich source of information on the origin and maintenance of biological diversity. The long-isolated island of Madagascar provides a suitable setting for studying species diversification, with most groups of organisms there both radiating and showing a high level of endemism. *Noronhia* is one of these groups and represents the most successful radiation of the olive family (Oleaceae) in Madagascar, with ca. 80 species. Its phylogenetic position has, however, been largely unresolved and its evolutionary history has remained unexplored. In this study, using plastid and nuclear DNA sequences obtained from a comprehensive sampling both within *Noronhia* and the family, I show that *Noronhia*, together with Indian Ocean species of *Chionanthus*, form a monophyletic clade sister to African *Chionanthus*. Topological discordances between plastid and nuclear gene trees are likely accounted for by polyploidy and hybridization. Since *Noronhia* has long been established in Madagascar after a likely Cenozoic dispersal from Africa, any hybridization event between representatives of African and Malagasy taxa would predate those among the Malagasy ones.

Within the genus, relationships are mostly unresolved despite the species showing considerable ecological and phenotypic diversity. In most cases, analyses of bioclimatic, molecular and morphological data, interpreted in phylogenetic and geographic contexts, show support for the morphogroups, the initial species hypotheses defined based on qualitative morphological features, and offer new insights into species boundaries. Morphological data provide the strongest support while bioclimatic ones are the least

informative, suggesting that the broad-scale variation in bioclimatic data does not adequately capture the ecological processes driving the diversification of *Noronhia*. This is also supported by the poor fit between patterns of diversification within *Noronhia* and four models of species diversification of Madagascar's biota. It is very likely that several mechanisms, especially small-scale evolutionary processes, contributed to the radiation of this group, but current models and analyses carried out here are too simplistic to permit robust conclusions.

However, attempts to understand spatial patterns of richness and coexistence among species of *Noronhia* show that mountainous areas in the island harbor the highest concentrations of species and the highest endemism. Habitat heterogeneity likely explains how diversity is promoted and maintained in these topographically complex regions. Furthermore, analyses focused on a smaller spatial scale, the Montagne d'Ambre massif, again indicate that habitat heterogeneity plays an important role. Different groups of species grow in different habitats on the mountain, suggesting environmental filtering associated with rainfall and soil nutrient gradients. This environmental filtering leads to phylogenetic but not trait clustering, suggesting critical traits have been omitted from the analyses. Overall, the integrative approach applied in this study allows the identification of spatial, phylogenetic, ecological and morphological patterns of diversity and likely processes accounting for these patterns. It also highlights the importance of using different kinds of data analyzed at various scales to understand species diversification.

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CHAPTER 1

MOSAIC PATTERNS OF RELATIONSHIPS IN THE OLIVE FAMILY AS INFERRED FROM MULTI-LOCUS PLASTID AND NUCLEAR DNA SEQUENCE ANALYSES: A CLOSE-UP ON *CHIONANTHUS* AND *NORONHIA* (OLEACEAE)

1. Introduction

Noronhia, first described by Stadman in Du Petit-Thouars (1806) and the largest genus of the olive family (Oleaceae) in Madagascar, comprises 45 described species. Examination of herbarium material, however, suggests there may be as many as ca. 70 species (C. Hong-Wa, in prep.); many new specimens having been accumulated through intensive botanical exploration in Madagascar during the last two decades. The only taxonomic treatment of the genus is that of Perrier de la Bâthie (1949), revised by the same author in 1952 for the *Flore de Madagascar et des Comores* series. Forty-one species were recognized at that time and four others have been described since (Bosser, 1973; Callmander et al., 2009; Labat et al., 1999). As currently circumscribed, all species of *Noronhia* are endemic to Madagascar, except two that are found in the Comoro Islands.

Noronhia, together with ten other extant genera, belongs to tribe Oleeae and subtribe Oleinae where its position is uncertain (Wallander and Albert, 2000). Existing molecular phylogenies of Oleaceae have included only *Noronhia emarginata* (Lee et al., 2007; Wallander and Albert, 2000), a species that is commonly found in tropical botanical gardens. This species has also naturalized in different regions (e.g. Florida, French Polynesia, Hawaii, Reunion and Seychelles) and is even invasive in Hawaii (PIER, 2011). Recently, Besnard et al. (2009) included seven species of *Noronhia* and, for the first time, provided an idea of relationships within this genus. In their study, *Olea ambrensis* is nested within *Noronhia*, but the voucher specimen for *O. ambrensis* (Schatz 3605), wrongly identified by Green (2002), is in fact *N. linocerioides*. Given this, *Noronhia* is monophyletic in their study and is sister to *Chionanthus*.

A close relationship between *Noronhia* and the three Malagasy *Chionanthus* species has long been suspected. Indeed, Perrier de la Bâthie (1949, 1952) noted only subtle morphological differences between the two genera, mainly the presence of a corona in most species of *Noronhia*, and suggested that the *Noronhia* species lacking this feature would form a transition between the two genera. The most comprehensive phylogenetic study of *Noronhia* to date included only two *Chionanthus* species and neither is from Madagascar (Besnard et al., 2009). However, relationships of these *Chionanthus* species reflected geography more than phylogeny: *C. broomeana*, from La Réunion Island, was sister to *Noronhia*, and *C. retusus*, from China, was sister to an Asian clade of *Olea* (i.e. subgenus *Tetrapilus*). Therefore, inclusion of *Chionanthus* species from the Malagasy Floristic Region (MFR = Madagascar, Mascarene, Comoros, Seychelles, Aldabra, Amirantes and surrounding small islands; Takhtajan, 1986) and Africa is clearly of considerable interest in resolving the relationship between *Noronhia* and *Chionanthus*.

Phylogenetic inferences from molecular data within Oleaceae have used mainly plastid regions (Baldoni et al., 2002; Besnard et al., 2009; Guo et al., 2011; Lee et al., 2007; Wallander, 2008; Wallander and Albert, 2000; Yuan et al., 2010). The nuclear ribosomal internal transcribed spacer (ITS) has also been useful in resolving phylogenetic relationships within Oleaceae including *Fraxinus* (Jeandroz et al., 1997; Wallander, 2008), *Ligustrum* and *Syringa* (Li et al., 2002), *Olea* (Besnard et al., 2009) and *Osmanthus* (Yuan et al., 2010). Low-copy nuclear genes are increasingly being used to address phylogenetic questions, especially at lower taxonomic levels (Hughes et al., 2006; Small et al., 2004). Yet, such genes have been used only once within Oleaceae (Hamman-Khalifa et al., 2007). Also, a multi-locus approach to phylogenetic analysis

can increase the strength of the phylogenetic inference (Townsend, 2007; Aguileta et al., 2008), and the use of plastid and nuclear regions together allows the detection of evolutionary events such as hybridization or incomplete lineage sorting (Brysting et al., 2011; Lihová et al., 2006; Linder and Rieseberg, 2004).

In this study, we were interested in using genes that permit phylogenetic inferences within subtribe Oleinae, both at higher and lower taxonomic levels. In particular, we used plastid (*trnL-F*, *trnT-L*, *trnS-G* and *trnK-matK*) and nuclear (ITS, triose phosphate isomerase [TPI]) markers to: (1) examine the generic relationships within the subtribe with a particular focus on the placement of *Noronhia*, and (2) test the monophyly of this genus and infer its evolutionary history. In light of our phylogenetic results, we propose a revised generic circumscription for *Noronhia* based on both molecular and morphological evidence. We also evaluate possible explanations of incongruence between gene trees.

2. Materials and methods

2.1. Taxon and gene sampling

A total of 77 taxa were included in this study, of which 35 were named species and varieties of *Noronhia*, 38 represented 10 other genera within subtribe Oleinae, and four others were from tribe Oleeae (Supplementary Table S1). Whenever possible, multiple individuals per species were included, especially for *Noronhia*; species of the latter encompassed both the range of morphological variation within this group and its geographic distribution. Sampling for outgroups was mostly concentrated on the genus *Chionanthus*, and included 13 of the ca. 18 species occurring in Africa, Madagascar and the Comoro and Mascarene Islands; five representative species from the New World and

Asian-Pacific Old World were also included. The other genera within Oleinae were represented by a few species each except *Priogymnanthus* and *Hesperelaea*, for which adequate samples were unavailable. Total genomic DNA was extracted from silica gel-dried leaves collected in the field or from herbarium specimens using the DNeasy Plant Mini kit (Qiagen, Valencia, CA). Voucher specimens were deposited at the Missouri Botanical Garden-St. Louis (MO), Muséum National d'Histoire Naturelle-Paris (P) and Parc Botanique et Zoologique de Tsimbazaza-Antananarivo (TAN). DNA samples for some species were obtained from herbaria and botanical gardens in Geneva (G), Kew (K), Madrid (MA) and Paris (P).

Plastid regions previously used for phylogenetic inferences within Oleaceae with various degrees of resolution included *trnL-F*, *rps16*, *rbcL*, *ndhF*, *psbA-trnH*, *matK*, *trnT-L*, *trnS-G*, *rps16-trnQ*, *rpl32-trnL*, *psbJ-petA* (Baldoni et al., 2002; Besnard et al., 2009; Guo et al., 2011; Lee et al., 2007; Wallander, 2008; Wallander and Albert, 2000; Yuan et al., 2010). In general, plastid DNA sequence divergence was very low within *Noronhia* (0.03%), and so we used four of the most informative regions in this study (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*). Since ITS has already been successfully used in resolving phylogenetic relationships within Oleaceae and had higher informative variation, we also used it. In addition to ITS, we also surveyed low-copy nuclear genes. The nuclear nitrate reductase (NIA) gene has been successfully amplified in *Olea*, yielding two products of 900 bp and 250 bp (Hamman-Khalifa et al., 2007), but we inconsistently obtained only a small-sized product (< 300 bp) for *Noronhia* through standard polymerase chain reaction (PCR). We successfully amplified segments of genes encoding chalcone isomerase (CHI), chalcone synthase (CHS), glyceraldehyde 3-

phosphate dehydrogenase (G3PDH) and triose phosphate isomerase (TPI) (Strand et al., 1997). However, all low-copy nuclear genes, except TPI, were discarded for various reasons including doubtful sequence similarity, lack of informative variation and possible presence of multiple copies.

2.2. Laboratory protocols

Amplification of the four plastid DNA regions (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*) followed the protocol described in Besnard et al. (2009). PCR products were cleaned and directly sequenced using the same set of primers as for amplification.

Amplification of the internal transcribed spacer (ITS) region that encompasses ITS1, 5.8S and ITS2 was carried out using the primers ITSLeu1 (5'-GTC CAC TGA ACC TTA TCA TTT AG-3'; Baum et al., 1998) and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3'; White et al., 1990) in 25 μl reactions containing 2 μl of undiluted DNA template, 1.5 μl of each primer and 12.5 μl of GoTaq® Green Master Mix (Promega). Thermal cycling parameters consisted of an initial denaturation at 97˚C for 2 min, followed by 30 cycles of 1 min at 97˚C, 1 min at 50˚C, 1 min at 72˚C and a final extension of 7 min at 72˚C. PCR products were cleaned using the QIAquick PCR purification Kit (Qiagen, Valencia, CA) and cloned using pGEM-T® vector (Promega). Three to eight clones were sequenced in both directions using primers T7 and SP6.

The fourth intron of triose phosphate isomerase (TPI) was targeted for amplification. We used the primers TPIX4FN (5'- AAG GTC ATT GCA TGT GTT GG-3') and TPIX6RN (5'- CTT TAC CAG TTC CAA TAG CCC-3') developed by Strand et al. (1997). The PCR reaction was a 25 μl mixture of DNA template, primers and GoTaq®

Green Master Mix (Promega). Amplification was performed with an initial denaturation at 95˚C for 2 min, followed by 35 cycles of 1 min at 95˚C, 90 sec at 53˚C, 2 min at 72˚C and a final extension of 9 min at 72˚C, and yielded two distinct products of approximately 600 bp and 750 bp for most taxa. PCR products were gel-purified using Qiagen gel extraction system and were then ligated into the pGEM-T® vector (Promega). For each PCR band, four to eight clones were sequenced in both directions using primers T7 and SP6.

2.3. Data preparation

For each plastid DNA region, one sequence was generated for each accession. Since the plastid genome is inherited as a single linked unit, DNA sequences representing the four regions were combined into a single dataset of ca. 3800 bp for subsequent analyses. For nuclear genes, sequences with more than 80% overlap and base call accuracy ≥ 99% (Ewing et al., 1998) were assembled and edited using Seqman v4.00 (DNASTAR Inc., Madison, WI). BLAST searches were performed to confirm the authenticity of the amplified regions. Altered conserved motifs, lower GC content and higher minimum free energy (ΔG at 37˚C, estimated via the mFold website; Zuker, 2003) were used to assist in identification of putative pseudogenes in ITS. Multiple sequence alignments using MUSCLE (Edgar, 2004) implemented in MEGA v5 (Tamura et al., 2011), were followed by some manual adjustments where necessary. The final dataset contained only a single ITS sequence per accession, but up to four divergent TPI sequences per individual. Indeed, for each PCR band of TPI, two sequence types were identified and labeled TPI-L1 and TPI-L2 for the longer sequences (ca. 750 bp) and TPI-S3 and TPI-S4 for the shorter sequences (ca. 600 bp). Even longer sequences (ca. 900 bp) were obtained from

few outgroups (e.g. *Chionanthus virginicus*, *C. retusus*, *Phillyrea*, *Picconia* and *Osmanthus*) whereas only shorter sequences (ca. 600 bp) were found in *Olea*. Sequence data were deposited at GenBank (accession numbers in Supplementary Table S2 [to be completed later]).

2.4. Phylogenetic analyses

We used maximum likelihood (ML) and Bayesian inference (BI) in independent analyses of the combined plastid (CP) dataset and the ITS region. Model of nucleotide substitution for each dataset was assessed with jModeltest v0.1.1 (Posada, 2008), with the best-fit model selected from among 88 possible models based on the Akaike Information Criterion (AIC). The chosen models for the different datasets were: GTR + I + G for ITS and TVM + G for CP. Because the best model selected for the CP dataset is not implemented in the phylogenetic programs we used, the next best model was instead applied (GTR + G). This is acceptable since BI is known to be robust enough to over-parameterization (Huelsenbeck and Rannala, 2004).

Maximum likelihood analyses were performed with RAxML v7.2.6 (Stamatakis, 2006) using the rapid bootstrap algorithm for 1000 replicates combined with the search of the best-scoring ML tree under default parameters. Bayesian analyses were performed with MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) via the CIPRES portal (Miller et al., 2010). The analyses consisted of two parallel runs, each of four chains (one cold and three hot), initiated with random starting trees. Program parameters, mostly at default settings, were similar for the different datasets with the Markov chain Monte Carlo (MCMC) run for 15-20 million generations and trees sampled every 1000th generation. Preliminary analyses showed infrequent or no chain swapping under the

default temperature (T = 0.2), which was then adjusted along with the number of generations for optimal mixing (Table 1). Analyses of the two datasets applied the previously determined models of substitution with model parameters unlinked across different partitions. Stationarity and convergence between runs were assessed by plotting likelihood values against the number of generations, as well as with trace plots generated in Tracer v1.5 (Rambaut and Drummond, 2009) and with correlations of split frequencies between two runs using the online application AWTY (Nylander et al., 2008). The first 20% of trees before stationarity were discarded as burn-in and a 50% Majority-Rule Consensus Tree was generated with the remaining trees for each dataset. Trees were visualized using TreeGraph2 (Stöver and Müller, 2010).

For the TPI multigene family, we carried out a network analysis using the NeighborNet algorithm implemented in the SplitsTree program (Huson and Bryant, 2006) and tested for recombination among the different sequence types using the Phi test and five different algorithms (RDP, GENECONV, Chimaera, MaxChi and 3Seq) respectively implemented in SplitsTree and in the software RDP3 (Martin et al., 2010). Because sampling for any copy of the TPI genes was only comprehensive at the genus level, only generic relationships were assessed with this dataset.

Congruence among datasets was examined using the incongruence length difference test (ILD, Farris et al., 1995) as implemented by the partition homogeneity test in PAUP* 4.0b10 (Swofford, 2002). The test was applied to the CP and ITS datasets with 100 replicates. Topological congruence was also examined in a likelihood context. Site-wise log-likelihoods were obtained from RAxML for each dataset and run in CONSEL v0.1i (Shimodaira and Hasegawa, 2001) to evaluate the probability values of each

alternative topology using the approximately unbiased (AU) test (Shimodaira, 2002), the Kishino-Hasegawa (KH) test (Kishino and Hasegawa, 1989) and the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999).

2.5. Molecular dating

We obtained relative ages for each gene tree using a reduced dataset including only a single individual per species and accessions represented in both plastid and ITS datasets. We used BEAST v1.6.1 (Drummond and Rambaut, 2007) to estimate divergence times. The molecular clock hypothesis was tested using the likelihood ratio test (LRT) in MEGA v5 and was strongly rejected for each dataset. Data for BEAST analysis were first prepared with BEAUti v1.6.1 (Drummond and Rambaut, 2007) using a relaxed clock model (Drummond et al., 2006) and an uncorrelated lognormal model of rate variation. The same nucleotide substitution models applied for the Bayesian inference were used. The tree prior was set to a birth-death process and used a randomly generated starting tree. The other priors were left at default except for prior information on clade ages. Fossil evidence suggests a divergence time older than 37 Mya for *Fraxinus* and Oleinae (Call and Dilcher, 1992; Suzuki, 1982). Following Besnard et al. (2009), we implemented this age as a lower bound of a normal distribution with a mean of 40 Mya and a standard deviation of 3 Mya. Given our phylogenetic results, *Fraxinus* was constrained to be outside Oleinae in the ITS analysis as this position has been well supported in other studies (e.g. Besnard et al., 2009; Lee et al., 2007; Wallander and Albert, 2000). Divergence of *Olea sensu stricto* (excluding *Olea* subgenus *Tetrapilus*, represented here by *O. dioica*) occurred before 23 Mya (Muller, 1981; Palamerev, 1989; Terral et al., 2004) and was also applied as a calibration using a uniform distribution

constrained between 23 and 30 Mya. Results of two independent MCMC runs of 10 million generations with sampling frequency of 1000 generations were assessed with Tracer v1.5 (Rambaut and Drummond, 2009), combined with LogCombiner v1.6.1 (Drummond and Rambaut, 2007) and summarized in TreeAnnotator v1.6.1 (Drummond and Rambaut, 2007) into one Maximum Clade Credibility Tree using a burn-in of 2 million generations. The effective sample size (ESS), an indicator of effective independent draws from the posterior distribution (Drummond and Rambaut, 2007), was good (i.e. > 200) for all analyses. Node ages were estimated as mean node heights in million years. Trees were visualized using FigTree v1.3.1 (Rambaut, 2006).

3. Results

3.1. Data

This study analyzed a total of 136 individuals representing 77 taxa, of which 35 were named species and varieties of *Noronhia*, 38 members of subtribe Oleinae and four other members of tribe Oleeae. Statistics of the alignments and phylogenetic analyses of the three datasets (CP, ITS and TPI) are presented in Table 1. The entire molecular dataset included 5786 nucleotides. Although the CP dataset (3818 bp) had almost twice the number of nucleotides as the nuclear dataset (1968 bp), it had almost a similar number of variable sites, thus a much lower mean sequence divergence (Table 1). No evidence of recombination was detected by the RDP3 program for any of the nuclear genes (ITS, TPI) and the Phi test for recombination also yielded high p-values (> 0.05) for each. A pairwise ILD test comparing the different regions yielded p-values ≤ 0.01, indicating significant incongruence. Although the sensitivity of the ILD test is well-known and its use in testing data partition combinability has been discouraged (Barker and Lutzoni,

2002; Darlu and Lecointre, 2002; Yoder et al., 2001), these results and subsequent analyses nonetheless encouraged us to carry out only independent analyses of each dataset.

3.2. Phylogenetic analyses

For the CP or ITS dataset, the best-scoring ML tree and the BI consensus tree exhibited largely similar topologies and were combined in TreeGraph2 for better visualization. For the sake of clarity, clades composed of conspecific accessions in these two trees are presented with only a single taxon name, but are shown in full with all individuals in the Supplementary material (Fig. S1). Accessions from the same species usually clustered together (Fig. S1). Accessions of *Noronhia emarginata* introduced outside the MFR (i.e. Hawaii and Florida) were also included to verify their affinity and were found to form a strongly supported monophyletic group with accessions of that species from Madagascar and La Réunion (Fig. S1). We regard maximum likelihood bootstrap (MLBS) and Bayesian posterior probability (BPP) values of 100 – 85% and 1 – 0.95 respectively as strong, 84 – 75% and 0.94 – 0.85 moderate, and 74 – 50% and 0.84 – 0.70 low support.

3.2.1. Plastid regions (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*; Fig. 1A)

Both ML and BI analyses showed a strongly supported monophyletic Oleinae (MLBS = 100%, BPP = 1). Patterns of relationships within the subtribe were more structured than those shown in Wallander and Albert (2000), and several genera were found to be paraphyletic or polyphyletic, e.g. *Chionanthus*, *Olea*, *Osmanthus* and *Phillyrea*. The relationships among these genera corresponded more with geography than with taxonomy. For instance, *Osmanthus americanus* and *Chionanthus virginicus*, both

North American species, and *Chionanthus ramiflorus* and *Olea dioica*, both Asian, were sister to each other respectively.

Noronhia, together with *Chionanthus* species from Africa and Indian Ocean islands formed a strongly supported monophyletic clade, hereafter referred to as NCAIO (*Noronhia* and *Chionanthus* from Africa and Indian Ocean). *Chionanthus* species from the MFR were nested within *Noronhia* forming what we call the MFR clade. Relationships of the African *Chionanthus* with the MFR clade did not show a clear geographic patterning. Thus *C. battiscombei*, distributed in eastern and predominantly southern Africa, linked with the MFR clade together with the strictly southern African *C. foveolatus* and *C. peglerae* (Africa 2), whereas *C. richardsiae*, a strictly southern African species, clustered with the widely distributed central and eastern African species (*C. mannii*, *C. mildbraedii* and *C. niloticus*) in a separate clade (Africa 1) sister to the MFR and Africa 2 clades.

Various subclades were identified within the MFR clade, usually with moderate to strong BPP but moderate to low MLBS. Relationships within these subclades showed more geographic patterning than those between the MFR clade and the African *Chionanthus*. For instance, apart from *Noronhia comorensis*, species from the Comoro Islands fell within the same clade despite belonging to different genera (e.g. *N. cochleata* and *C. insularis*). Similarly, *N. buxifolia* and *N. myrtoides*, from southwestern Madagascar, were sister to each other, as were the northern species *N. linearifolia* and *N. longipedicellata*. These relationships were also strongly supported.

3.2.2. ITS (Fig. 1B)

In general, relationships obtained from the ITS dataset were not strongly supported. In contrast to previous studies (Besnard et al., 2009; Wallander and Albert, 2000) and our own CP tree, *Fraxinus* (subtribe Fraxininae) was nested within (instead of sister to) subtribe Oleinae but this topology received weak support (MLBS = 63%, BPP = 0.93). This shift in position has also been found elsewhere but with plastid markers, in which case *Fraxinus* was sister to the clade Schreberinae-Oleinae (Lee et al., 2007). The nested placement of *Fraxinus* in our ITS tree appeared to arise from the inclusion of *Comoranthus* and *Schrebera* (subtribe Schreberinae). Several genera (e.g. *Chionanthus*, *Olea* and *Osmanthus*) again appeared to be polyphyletic and showed the same geographic patterning in their relationships as found with the CP data.

The NCAIO clade was again recovered as strongly monophyletic with MLBS = 100% and BPP = 1, with the African *Chionanthus* species forming a monophyletic, albeit moderately supported, clade (MLBS = 77%, BPP = 0.9), sister to a monophyletic MFR clade (MLBS = 73%, BPP = 0.98). Within the NCAIO clade, subclades could be distinguished that also showed a geographic signature, but mostly they had support from BI only. In particular, the African and MFR clades were well separated, and within the African clade, southern mesic African species (*C. battiscombei*, *C. foveolatus*, *C. peglerae* and *C. richardsiae*) also formed a strongly supported clade sister to the central and eastern tropical African species (*C. mannii*, *C. mildbraedii* and *C. niloticus*).

Relationships within the MFR clade were largely unresolved. Any subclades that could be distinguished were weakly supported. Also, contrary to the CP topology, no strong geographic signal was observed. Indeed, species from the Comoro Islands or from

southwest Madagascar did not cluster. Instead, clustering of species showing morphological resemblance was recovered (see section 3.3).

3.2.3. TPI (Fig. 2)

The network analysis of the TPI genes suggested at least four sequence types within *Noronhia*. The presence of different sequence types, combined with a lack of monophyly of alleles and species (details not shown), suggests a complex pattern of evolutionary history for the TPI genes (e.g. gene duplication, possible reticulation events, incomplete lineage sorting). Moreover, it is certain that we failed to find all possible sequence variants within a species given our sample size of four to eight clones. Consequently, we decided not to conduct a thorough phylogenetic analysis on this dataset. Nonetheless, the network showed that the MFR clade was monophyletic within each sequence type. The only available African *Chionanthus* species (*C. foveolatus* and *C. peglerae*) were also sister to that clade in one of the copies (MFR clade – TPI-S4). Furthermore, the polyphyly of several genera was again recovered as in the case of *Chionanthus*, species of which clustered within *Noronhia*, within the African *Chionanthus* group and within the PPCO (*Phillyrea-Picconia-Chionanthus-Osmanthus*) and PPCON (*Phillyrea-Picconia-Chionanthus-Osmanthus-Nestegis*) groups.

3.3. Comparison of topologies

The ML and BI analyses of the CP and ITS datasets agreed in showing that *Noronhia* formed a strongly supported monophyletic clade with the African and Indian Ocean *Chionanthus* (Figs. 1A-B). They also found largely unresolved relationships in the backbone of subtribe Oleinae as well as within *Noronhia*, although, the CP region resolved more well-supported nodes (MLBS ≥ 85% and/or BPP ≥ 0.95) than did ITS

(Figs. 1A-B). However, inconsistencies were also apparent between the CP and ITS topologies. Incongruence patterns supported by MLBS ≥ 85% and/or BPP ≥ 0.95 (Figs. 1A-B) were considered hard incongruence. Conflicting relationships that were weakly supported in both CP and ITS trees or strongly supported in one tree (usually in the CP tree), but dissolved into polytomies or conflicted only weakly in the other tree (usually in the ITS tree) were considered soft incongruence.

Discordances within *Noronhia* were mostly soft incongruence. Noteworthy examples include *N. emarginata* and *N. crassiramosa*, both having very coriaceous leaf blades and relatively large fruit with a thick hard endocarp; they are sister to each other in the ITS but not in the CP phylogeny. Similarly, *N. humbertiana* and the morphologically similar *N. seyrigii* and *C. tropophyllus*, all (and the only) pubescent species formed a monophyletic clade in the ITS phylogeny but not in the CP tree. Likewise, *N. ovalifolia*, *N. densiflora* and *N. boivini* formed a strongly supported clade, characterized by reddish flowers, in the ITS tree, but were part of polytomies in the CP tree. Lastly, the clade formed by the species *N. decaryana* to *N. gracilipes* is characterized by the absence of the corona, although not all species without a corona clustered in that clade. Relationships in the ITS topology also correlated with ecogeographic features: *N. emarginata* and *N. crassiramosa*, *N. ovalifolia*, *N. densiflora* and *N. boivini*, and *N. decaryana* and the clade including it occur in humid areas of East or North Madagascar whereas the trio *N. humbertiana*, *N. seyrigii* and *C. tropophyllus* are species of dry areas of the West; these relationships are not present in the CP tree. In general there is greater correspondence between the ITS phylogeny and morphology or ecogeography than the CP phylogeny, however, it should be remembered that these incongruences are soft. The

only case of hard incongruence observed here concerns *N. myrtoides*, which is sister to *N. buxifolia* in the CP tree (MLBS = 96% and BPP = 1) but sister to *N. boinensis* in the ITS tree (MLBS = 99% and BPP = 1).

Major conflicts appeared in the placement of *Fraxinus* and some members of subtribe Oleinae (e.g. *Forestiera neomexicana, Olea paniculata,* African *Chionanthus*). For instance, *Olea paniculata* clustered with other *Olea sensu stricto* in the CP tree but not with ITS. The CP tree also showed two separate clades of African species that were not observed in the ITS topology (Figs. 1A-B). Even if relationships between African *Chionanthus* and MFR species were not significantly resolved in the ML topology of the ITS dataset, its BI counterpart showed that African *Chionanthus* species were clearly separated from the MFR taxa. Given the high support values (MLBS and/or BPP) for these inconsistent relationships, we considered these to be incongruence rather than a lack of phylogenetic signal. Likelihood-based tests comparing the best-scoring CP and ITS trees and using constraints in both directions mostly supported these conflicts as statistically significant (Table 2). Most constraints were rejected, although the SH test was more conservative by suggesting fewer conflicts (Table 2). In any case, the topology of the best-scoring CP and ITS trees were reciprocally rejected by each dataset with high confidence (Table 2), suggesting complex evolutionary histories.

3.4. Divergence times (Fig. 3)

For the CP data, both ML and BI suggested a migration from Africa to Madagascar. The reconstructed phylogeny in BEAST showed a topology similar to that obtained from ML and BI analyses except for a deeper placement of the second clade of African *Chionanthus*, which also suggested a possible migration from Madagascar back to Africa

although two independent colonization events of Madagascar cannot be excluded. Divergence time estimates indicated that the NCAIO clade split from the remaining Oleinae around 36.17 Mya with a 95% HPD (highest posterior density) ranging from 30.28 to 42.65 Mya, and the separation of the first clade of African *Chionanthus* species (Africa 1) and the remaining NCAIO occurred around 26.7 Mya (95% HPD = 21.06–32.47 Mya). Divergence between the second clade of African *Chionanthus* (Africa 2) and the MFR clade happened around 21.8 Mya (95% HPD = 18.61–29.08 Mya).

For ITS, the BEAST phylogeny was topologically identical to the ML and BI trees except where constraints were enforced for calibration. Indeed, *Fraxinus* was constrained to be outside Oleinae since this topology has been well supported in other studies (Besnard et al., 2009; Lee et al., 2007; Wallander and Albert, 2000). This constraint also resulted in the placement of *O. paniculata* together with African *Olea* (i.e. *Olea* subgenus *Olea*). The BEAST topology also suggested a migration from Africa to Madagascar although this pattern is not clear since the inverse could be true. Age estimations, with the position of *Fraxinus* constrained to be basal, indicated a separation of the NCAIO clade from the remaining Oleinae at about 33.74 Mya (this node is not supported and lacks 95% HPD) and a divergence time of 19.51 Mya (95% HPD = 14.06–25.27 Mya) between the African *Chionanthus* and the MFR clade.

4. Discussion

4.1. Phylogenetic utility of the plastid and nuclear DNA markers

In this study, we used plastid, ribosomal and low-copy nuclear genes to examine generic relationships within subtribe Oleinae, to infer the phylogenetic position of *Noronhia* and to test its monophyly and the hypothesis of its close relationship with

Malagasy *Chionanthus* suggested by Perrier de la Bâthie (1949, 1952). The ITS gene had the highest percentage of informative characters (Table 1), as in other studies in Oleaceae (Besnard et al., 2009; Wallander, 2008), but in the analyses here it performed comparably poorly as the CP region both within Oleinae and within *Noronhia*. This might be accounted for by its high level of homoplasy (Table 1), which may cause poor phylogenetic resolution (Levin et al., 2009). The low phylogenetic signal at the species level may not be surprising given the low CP mutation rate found within *Noronhia* and other members of Oleaceae (Besnard et al., 2009, 2011; Heuertz et al., 2004) and/or if rapid diversification or other evolutionary events are involved. The TPI region was the least useful, mainly because of the presence of at least four duplicated copies (Fig. 2), for which we were unable to obtain a comprehensive sample of sequences for each copy. Based on the network topology, which showed two independent clades of long and short TPI copies with members of both subtribes Oleinae and Fraxininae (Fig. 2), we can assume an ancient gene duplication event related to the allopolyploid origin of the tribe Oleeae (Taylor, 1945; Wallander and Albert, 2000). A detailed study of species-level phylogenetic relationships within *Noronhia* and its relatives using this gene family requires targeting individual copies using well designed primers.

4.2. Potential explanations for plastid and nuclear incongruence

Putting aside possible conflicts within Oleinae such as the placement of *Fraxinus*, *Forestiera* or *Olea paniculata* as well as those at shallower nodes within *Noronhia* that were mostly considered as soft incongruence, we focus on the relationships between the African *Chionanthus* and the MFR clade. Indeed, the ITS topology showed a clear separation between African and MFR species, even though the likelihood and Bayesian

procedures resolved this relationship slightly differently (Figs. 1B and 3B). In the TPI network, the placement of available species of African *Chionanthus* tends to agree with the ITS topology as the species pair *C. foveolatus* and *C. peglerae* were sister to the MFR species (MFR clade – TPI-S4 in Fig. 2) rather than ambiguously placed among them as in the CP tree (Figs. 1A and 3A). Statistically significant supports for this inconsistency suggest various potential causes.

4.2.1. Technical and statistical causes

Various technical causes, including taxon sampling, sample contamination or mix-up, PCR recombination, can affect phylogenetic inferences and result in conflicting topologies (Rautenberg et al., 2008; Wendel and Doyle, 1998). Thus, great care was taken (e.g. repeated PCRs, inclusion of multiple accessions, recombination test, etc) to ensure good quality data. In addition, our datasets included all but two African *Chionanthus* species; the existence of more *Chionanthus* from this region remains to be documented. And even though the present analyses included only the currently described species of *Noronhia*, inclusion of ca. 20 as yet undescribed species gave a topology similar to that presented here (data not shown), but with the denser sampling, there were more infrageneric polytomies. Therefore, focusing exclusively on the described species of *Noronhia* does not affect our interpretation. Moreover, the topologies were largely robust to different analytical procedures (e.g. likelihood, Bayesian and distance methods). Finally, the incongruence tests and the high support values also rejected the possibility that statistical uncertainties caused these inconsistencies (Figs. 1A-B; Table 2).

4.2.2. Biological explanations

Several evolutionary processes can account for discordances between gene trees and include - but are not limited to - rapid diversification, gene duplication/loss, hybridization, polyploidization and lineage sorting (Degnan and Rosenberg, 2009; Wendel and Doyle, 1998). Rapid diversification, characterized by the phenomenon of "short interior branches" can lead to phylogenetic incongruence (Seelanan et al., 1997; Wendel and Doyle, 1998). Within the MFR clade, rapid diversification likely accounts for most inconsistencies given the occurrence of both short interior branches and lack of statistical support, although hybridization cannot be entirely excluded (e.g. the case of *N. myrtoides*). However, alternative resolutions of relationships between African *Chionanthus* and the MFR clade displayed short internodes that were strongly supported (Supplementary Fig. S2) and were consequently inconsistent with the soft incongruence expected under the rapid diversification scenario. Instead, this incongruence is probably best explained by other evolutionary processes, three of which are discussed below.

First, in the absence of any other evolutionary process and with maternal inheritance, hybridization and introgression would result in the placement of taxa unrelated in their nuclear genomes close to each other in plastid-based phylogenies (Wendel and Doyle, 1998). In fact, other studies have found that relationships suggested by plastid genes tend to be more consistent with geography while nuclear genes provided better reflection of species relationships (McKinnon et al., 1999; Rautenberg et al., 2010; Zhang et al., 2010). Our CP topology, however, failed to show a clear geographic patterning consistent with these findings, although the three African *Chionanthus* species that fell with the MFR clade came from the southern part of the continent, thus closer to Madagascar (Fig. 1A; Supplementary Table S1). Second, gene duplication/loss and

lineage sorting, both producing the same pattern despite being completely different processes, may also explain this incongruence (Wendel and Doyle, 1998). In these cases, stochastic survivorship and/or differential sampling of duplicated copies would result in different placements of individual taxa in nuclear-based phylogenies. The separation of African and MFR taxa into two distinct clades in the ITS topology, contrary to the CP tree, showed better correspondence with taxonomy and geography (Fig. 1B). It is possible that different ITS copies were maintained in and/or sampled for these two groups, although homoplasy and concerted evolution could also obscure their true history (Álvarez and Wendel, 2003). Lastly, polyploidization can also account for incongruence between gene trees (Blöch et al., 2009; Lihová et al., 2006; Weiss-Schneeweiss et al., 2012). The occurrence of polyploids has been documented within tribe Oleeae, particularly in *Fraxinus*, *Olea* and *Osmanthus* (Besnard et al., 2008; Taylor, 1945; Wallander, 2008). Since *Olea* and *Osmanthus*, as well as *Chionanthus*, are widely scattered in our phylogenies (Figs. 1 and 2), it is therefore reasonable to assume polyploidy also occurs elsewhere within the tribe. However, our current data do not allow us to explore this possibility further.

The contribution of gene duplication/lineage sorting and hybridization as causes of incongruence can be assessed computationally through relative dating under the expectations of the coalescent theory (Frajman et al., 2009; Pelser et al., 2010; Rautenberg et al., 2008, 2010; Roos et al., 2011; Willyard et al., 2011). For 95% of nuclear loci, it takes 9-12Ng to reach reciprocal monophyly (Hudson and Coyne, 2002), N being effective population size and g generation time; for a haploid organelle gene, the effective population size is half that obtained from nuclear genes in hermaphroditic

organisms. Setting effective population size at 10 000 (although field observations suggest this could be high especially for tree species in fragmented habitats) and mean generation time of 10 years (reasonable, given that *Noronhia* species are shrubs or trees), the conservative coalescence time (CT) is estimated to be CT_N = 900 000–1 200 000 years for nuclear loci and CT_{CP} = 450 000–600 000 years for plastid genes. Hybridization is the most likely explanation if difference in divergence times is larger than the estimated conservative coalescence time, whereas an explanation of gene duplication/lineage sorting is preferred if difference in divergence times is smaller than the estimated conservative coalescence time. The time difference between the divergence of the African *Chionanthus* and *Noronhia* and the divergence of *Noronhia* in the ITS tree is far greater than the estimated conservative coalescence time for the plastid gene (3.7 My > CT_{CP}). Likewise, the time difference between the separation of the first clade of African *Chionanthus* (Africa 1) and *Noronhia* in the CP tree (3.1 My > CT_N) as well as between the second clade (Africa 2) and the remaining *Noronhia* (1.6 My > CT_N) is also much greater than the estimated conservative coalescence time for the nuclear gene. Thus, gene duplication and lineage sorting are the least likely explanations for the incongruent placement of the African *Chionanthus* with regard to *Noronhia* in the CP and ITS topologies. Instead, hybridization appeared to have played a major role, even when coalescence times are estimated with different values of effective population sizes and generation times. However, given the geologic history of the Malagasy region and the biology of these genera (see below, section 4.3), any hybridization event between African *Chionanthus* and *Noronhia* (and not between *Noronhia* and Indian Ocean *Chionanthus*) must have been ancient.

4.3. Biogeographic implications

Our relative dating was based on a few calibration points, and may suffer from stochastic errors given the low statistical support in some nodes; however, it yielded age estimates fairly consistent with those of other studies (Besnard et al., 2009; Lee et al., 2007). It also allowed a preliminary look into the biogeographic history of the MFR clade (Figs. 1 and 3). In particular, different colonization scenarios of the MFR are suggested. While both the CP and ITS topologies implied the existence of a common ancestor from which the MFR clade and the African *Chionanthus* diverged, the CP topology indicated a colonization of Madagascar between 26-23 Mya (95% HPD = [32–21]-[29–18] Mya). This was followed by a radiation of the clade within Madagascar starting around 23 Mya, a dispersal event back to Africa around 21 Mya or eventually a second independent migration to Madagascar, and later to the Indian Ocean islands. However, the node suggesting a migration back to Africa is not statistically supported (Fig. 3A); in fact, this second clade of African species is basal to *Noronhia* in the standard phylogenetic analyses (Fig. 1A). A scenario of migration back to Africa, although possible, is more difficult to reconcile with geological accounts and past water and wind circulations (Ali and Huber, 2010; McCall, 1997), which would have prevented westward and southbound migration from Madagascar before mid-to-late Miocene. Furthermore, species of *Noronhia* are predominantly dispersed by various kinds of lemurs (e.g. *Eulemur*, *Lemur*, *Microcebus*, *Varecia*, etc; Andriamaharoa et al., 2010; Birkinshaw, 1999, 2001; Donati et al., 1999; Martinez, 2010; Radespiel, 2007; Simmen et al., 2006; Thorén, 2011), making a lemur-mediated dispersal back to Africa unlikely for this time frame. Finally, most extant Malagasy migratory birds are not

frugivorous (Langrand, 1990). So, a more plausible scenario would be a separation of African *Chionanthus* into two distinct, tropical and southern clades followed by a migration to Madagascar giving rise to the MFR clade.

The ITS topology suggested a clear separation between the African *Chionanthus* and the MFR clade and a dispersal to Madagascar between 19-15 Mya (95% HPD = [25–14]-[20–11] Mya). The direction of the dispersal is rather equivocal but again we favor a migration from Africa based on the geological characteristics and water circulations of this region at that time. Diversification of the MFR clade started at about 15 Mya, the clade subsequently expanding towards the Indian Ocean islands. The younger ages obtained from the ITS dataset compared to those derived from the CP data can be explained by concerted evolution, which tends to homogenize different sequences and leads to an underestimation of divergence times (Teshima and Innan, 2004), or by the constraint on the position of *Fraxinus*, the first calibration point. Nevertheless, the timing of dispersal to Madagascar suggested by both the CP and ITS topologies overlaps the lower margin of the time frame of the Mozambique Channel land bridge [45-26 Mya] (McCall, 1997) or the eastward Mozambique palaeocurrent [Palaeogene period] (Ali and Huber, 2010) during which there were a number of major colonization events of Madagascar from Africa (Kuntner and Agnarsson, 2011; Russell et al., 2008; Yoder and Nowak, 2006). Colonization of other smaller Indian Ocean islands from Madagascar is also consistent with previous findings involving, e.g. spiders, dombeyoid Malvaceae, angraecoid orchids and chameleons (Kuntner and Agnarsson, 2011; Le Péchon et al., 2010; Micheneau et al., 2008; Raxworthy et al., 2002).

4.4. Taxonomic recommendations and revised classification

Traditional generic circumscriptions within tribe Oleeae, in particular, are largely artificial. This is probably because of the lack of distinctive morphologies in the clade itself. Data other than from macromorphology are still largely lacking and may not be available soon, hampering efforts in elucidating the taxonomy of members of this group. Indeed, most species of *Chionanthus*, for instance, were initially described in one of at least six genera. In particular, there has been much contention over the placement of the African *Chionanthus* species, which used to be included in genera such as *Linociera, Mayepea* and *Olea* (Stearn, 1980). Their placement in *Chionanthus* by Stearn (1976, 1980) was based largely on external morphology. Such taxonomic instability reflects the difficulty in interpreting convergent or homoplastic morphologies. Moreover, recent phylogenetic studies highlight the complexity of morphology-based taxonomy within Oleaceae in general by showing extensive cases of polyphyly and paraphyly of conventional genera (Besnard et al., 2009; Guo et al., 2011; Lee et al., 2007; Li et al., 2002; Wallander and Albert, 2000; Yuan et al., 2010). However, wood anatomy suggests the existence of two geographically structured groups (temperate and tropical) within *Chionanthus*, with perhaps three additional subgroups in the tropics: Neotropics and Africa, Asia-Pacific and S.E. Asia-Malesia (Baas et al., 1988). Our data agree with this anatomical study in finding a comparable number of geographic clades, but with a slightly different geographic distribution: Africa-Indian Ocean, Central America, North America and Asia-Pacific. Despite the absence of distinctive morphological characters within *Chionanthus*, other lines of evidence may support these anatomical and molecular results; in any case, we recommend restrictions in the use of the generic epithet "*Chionanthus*".

None of the type specimens of the previously used names subsumed under *Chionanthus sensu lato* occur in the Old World: *Chionanthus* (type *Chionanthus virginicus* L. [1753], USA), *Linociera* (type *Linociera ligustrina* Sw. [1797], Jamaica), and *Mayepea* (type *Mayepea guianensis* Aubl. [1775], French Guiana). These names would thus be available for other species previously called *Chionanthus* that occur in other clades. Instead we opted to apply the generic name "*Noronhia sensu lato*" to all species distributed in Africa and the MFR currently recognized as *Chionanthus* as well as species of *Noronhia* itself, despite the possible lack of robust morphological synapomorphies. Indeed, the datasets used in this study all very strongly support the monophyly of the NCAIO clade (i.e. *Noronhia* and *Chionanthus* from Africa and Indian Ocean islands) and the placement of *Chionanthus* species from the MFR deep within *Noronhia sensu stricto*. Recognition of several genera within this clade would not solve the problem of the absence of synapomorphies. Nevertheless, the extended *Noronhia* is characterized, with some degree of variation, by coriaceous evergreen foliage, woody petioles, and small flowers with a partially fused and fleshy corolla. These features distinguish members of this group from temperate and New World *Chionanthus*, e.g. *C. virginicus* and *L. ligustrina*. However, they are found in some tropical Asian representatives, in particular those from West Malesia, which have not yet been sampled and which lack separate generic names. The revised nomenclature applied to the African and Indian Ocean species of *Chionanthus* is presented in Appendix A; further extension of *Noronhia* is a possibility.

5. Conclusion

In all, the plastid and nuclear DNA markers used in this study provided us with new insights into relationships at various taxonomic levels within Oleinae. The most important of these is an extensive generic polyphyly, and in particular the distinctive geographic patterning within the polyphyletic *Chionanthus*, in which species from different continents are phylogenetically close to other genera of Oleaceae on those continents. We also gained new insights into the evolutionary history of *Noronhia*, for which a close relationship with African and Indian Ocean *Chionanthus* and a late Cenozoic dispersal from Africa to Madagascar have been found. However, within *Noronhia* as well as within Oleinae, some uncertainties remained and new questions arose. Additional molecular data (e.g. more nuclear markers), different approaches (e.g. next-generation genome sequencing, population genetic studies), and other types of data (e.g. anatomy, morphology) need to be used to further address these uncertainties and questions. Chromosomal and genomic studies would also contribute greatly to the understanding of the evolution of these groups. More botanical explorations and taxonomic studies are also needed to document the potential existence of additional species of *Chionanthus* in Africa.

Appendix A

The following presents the nomenclatural changes needed for species of *Chionanthus* from Africa and the Malagasy Floristic Region based on the results we obtained.

Noronhia africana (Knoblauch) Hong-Wa & Besnard, comb. nov.

Mayepea africana Knoblauch, *Botanische Jahrbücher*, 17: 529 (1893); Hiern, *Catalogue of African Plants collected by Dr Friedrich Welwitsch in 1853-1861*, 1: 658 (1898). – *Chionanthus africanus* Welw. ex Knoblauch, *Botanische Jahrbücher*, 17: 529 *pro syn.* – *Linociera africana* (Knoblauch) Knoblauch, *Beihefte zum Botanischer Centralblatt*, 61: 129 (1895); Gilg & Schellenb., *Botanische Jahrbücher*, 51: 69 (1913); Green in Hutch. & Dalziel, *Flora of West Tropical Africa*, 2nd ed. 2: 48 (1963); Liben, *Flore d'Afrique Centrale*, Oleaceae: 29 (1973). – *Linociera angolensis* Baker, *Flora of Tropical Africa*, 4: 20 (1902). – *Chionanthus africanus* (Knoblauch) Stearn, *Botanical Journal of the Linnean Society*, 80: 197 (1980). – Type: Angola, Pungo Andongo, *Welwitsch 941*.

Linociera johnsonii Baker, *Flora of Tropical Africa*, 4: 20 (1902); Eggeling & Dale, *Indigenous Trees of Uganda*: 285 (1952); Turrill, *Flora of East Tropical Africa*, Oleaceae 12, fig. 13 (1952). – Type: Ghana, Aburi Hills, *Johnson 453*.

Linociera mildbraedii Gilg & Schellenb. in Mildbraed, *Wissenschaftliche Ergebnisse der Deutschen Zentral Afrika-Expedition, 1907-1908, 2 (Bot.)*: 527 (1913); Gilg & Schellenb., *Botanische Jahrbücher*, 51: 70 (1913). – Type: Congo, Beni, *Mildbraed 2734*.

Linociera fragrans Gilg & Schellenb., *Botanische Jahrbücher*, 51: 70 (1913). – Type: Ghana, Aburi Hills, *Johnson 234*.

Linociera dasyantha Gilg & Schellenb. in Mildbraed, *Wissenschaftliche Ergebnisse der Deutschen Zentral Afrika-Expedition, 1907-1908, 2 (Bot.)*: 527 (1913). – Type: Congo, Beni, *Mildbraed 2286*.

Linociera oreophila Gilg & Schellenb., *Botanische Jahrbücher*, 51: 70 (1913). – Type: Cameroon, *Deistel 89*.

Noronhia ayresii (A. J. Scott) Hong-Wa & Besnard, comb. nov.

Olea obovata Baker, *Flora of Mauritius and the Seychelles*: 219 (1877), non *C. obovata* Rafin. (1836). – *Chionanthus ayresii* A. J. Scott nom. nov., *Kew Bulletin*, 33: 570 (1979). – Type: Mauritius, *Ayres s.n*.

Noronhia battiscombei (Hutch.) Hong-Wa & Besnard, comb. nov.

Dekindtia africana Gilg, *Botanische Jahrbücher*, 32: 139 (1902); Turrill, *Flora of Tropical East Africa*, Oleaceae, 15, fig. 5 (1952); non *Mayepea africana* Knoblauch (1893). – Type: Malawi, Nyasaland, *Buchanan 283*.

Linociera battiscombei Hutch., *Bulletin of Miscellaneous Information, Royal Botanic Gardens, Kew*: 17 (1914); Verdoorn, *Bothalia*, 6: 600, t. 26 (1956), *Flora of Southern Africa*, 26: 124, fig. 13 n. 4 (1963); Dale & Greenway, *Kenya Trees & Shrubs:* 346 (1961). – *Chionanthus battiscombei* (Hutch.) Stearn, *Botanical Journal of the Linnean Society*, 80: 197 (1980). – Type: Kenya, K4, Nairobi Dist. Nairobi Forests, *Battiscombe 517*.

Noronhia boutonii (A. J. Scott) Hong-Wa & Besnard, comb. nov.

Olea macrophylla Baker, *Flora of Mauritius and the Seychelles*: 219 (1877), non *C. macrophylla* (Wall. ex G. Don) Blume (1876). – *Linociera macrophylla* (Baker) H. Perrier, *Flore de Madagascar* famille 166 (Oléacées): 9 (1952), in adnot., non *L. macrophylla* Wall. ex G. Don (1837). – *Chionanthus boutonii* A. J. Scott nom. nov., *Kew Bulletin*, 33: 570 (1979). – Type: Mauritius, *Bouton s.n*.

Noronhia broomeana Horne ex Oliver in *Hooker's Icones Plantarum*, 14, t. 1365 (1881). – *Chionanthus broomeana* (Horne ex Oliver) A. J. Scott, *Kew Bulletin*, 33: 570 (1979). – Type: Mauritius, *Horne s.n.*

Linociera verrucosa Solereder, *Botanisches Centralblatt*, 45: 399 (1891), 46: 17 (1891). – *Mayepea verrucosa* (Solereder) Knoblauch, *Naturlichen Pflanzenfamilien*, 4: 10 (1892), *Botanische Jahrbücher*, 17: 527 (1893). – *Linociera broomeana* (Horne ex Oliver) Knoblauch, *Notizblatt des Königlichen Botanischen Gartens und Museums zu Berlin-Dahlem*, 11: 1028 (1934). **–** Type: Mauritius, *Sieber 125*.

Linociera coriacea Cordem., *Flore de l'île de la Réunion*: 458 (1895), non *L. coriacea* Vidal (1886). – Type: Réunion, *Cordemoy s.n*.

Linociera obscura Cordem., *Flore de l'île de la Réunion*: 457 (1895). – Type: Réunion, *Cordemoy s.n*.

Linociera cordemoyana Knoblauch, *Notizblatt des Königlichen Botanischen Gartens und Museums zu Berlin-Dahlem*, 11: 1031 (1934). – *Chionanthus broomeana* var. *cordemoyana* (Knoblauch) A. J. Scott, *Kew Bulletin*, 33: 570 (1979). – Type: Réunion, *Cordemoy s.n.*

Linociera cyanocarpa Cordem., *Flore de l'île de la Réunion*: 456 (1895). – Type: Réunion, *Cordemoy s.n*. – *Chionanthus broomeana* var. *cyanocarpa* (Cordem.) A. J. Scott, *Kew Bulletin*, 33: 570 (1979).

Noronhia camptoneura (Gilg & Schellenb.) Hong-Wa & Besnard, comb. nov.

Linociera camptoneura Gilg & Schellenb., *Botanische Jahrbücher*, 51: 68 (1913). – *Chionanthus camptoneurus* (Gilg & Schellenb.) Stearn, *Botanical Journal of the Linnean Society*, 80: 198 (1980). – Type: Cameroon, Bipinde, Urwaldgebiet, *Zenker 3149*.

Noronhia cordifolia (Labat, M. Pignal & O. Pascal) Hong-Wa & Besnard, comb. nov.

Chionanthus cordifolius Labat, M. Pignal & O. Pascal, *Novon*, 9: 68 (1999). – Type: Mayotte, Mlima Choungi, *Pascal 288*.

Noronhia foveolata (E. Meyer) Hong-Wa & Besnard, comb. nov.

Olea foveolata E. Meyer, *Commentariorum de plantis Africae australioris*: 176 (1837); Wright, *Flora Capensis*, 4.i: 485 (1907). – *Linociera foveolata* (E. Meyer) Knoblauch, *Repertorium Specierum Novarum Regni Vegetabilis*, *41*: 151 (1936); Verdoorn, *Bothalia*, 6: 591, t. 21-24 (1956), *Flora of Southern Africa*, 26: 120 (1963); Palmer & Pitman, *Trees of Southern Africa*, 3: 1832 (1972). – *Chionanthus foveolatus* (Meyer) Stearn, *Botanical Journal of the Linnean Society*, 80: 198-199 (1980). – Type: South Africa, *Drège s.n*.

Linociera marlothii Knoblauch, *Repertorium Specierum Novarum Regni Vegetabilis*, 41: 151 (1936). – Type: South Africa, Kwazulu-Natal, *Rudatis 1416*.

Linociera foveolata subsp. *tomentella* Verdoorn, *Bothalia*, 6: 597, t. 23 (1956), *Flora of Southern Africa*, 26: 122, fig. 13 n. 1 (1963). – *Chionanthus foveolatus* subsp. *tomentellus* (Verdoorn) Stearn, *Botanical Journal of the Linnean Society*, 80: 199 (1980). – Type: South Africa, *Burchell 5539*.

Linociera foveolata subsp. *major* Verdoorn, *Bothalia*, 6: 598, t. 24 (1956), *Flora of Southern Africa*, 26: 122, fig. 13 n. 3 (1963). – *Chionanthus foveolatus* subsp. *major* (Verdoorn) Stearn, *Botanical Journal of the Linnean Society*, 80: 199 (1980). – Type: South Africa, Graskop, Marieskop Forest, *Urry 28568*.

Noronhia incurvifolia (H. Perrier) Hong-Wa & Besnard, comb. nov.

Linociera incurvifolia H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série. B*, 2: 280 (1949), *Flore de Madagascar* famille 166 (Oléacées): 12, fig. 3 n. 4 (1952). – *Chionanthus incurvifolius* (H. Perrier) Stearn, *Botanical Journal of the Linnean Society*, 80: 199 (1980). – Type: Madagascar, Ankarana, *Humbert 19011*.

Linociera incurvifolia var. *planifolia* H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série. B*, 2: 280 (1949), *Flore de Madagascar* famille 166 (Oléacées): 14 (1952). – Type: Madagascar, Ankarana, *Humbert 18966*.

Noronhia insularis (Labat, M. Pignal & O. Pascal) Hong-Wa & Besnard, comb. nov.

Chionanthus insularis Labat, M. Pignal & O. Pascal, *Novon*, 9: 69 (1999). – Type: Mayotte, Bénara, *Pascal 713*.

Noronhia mannii (Solereder) Hong-Wa & Besnard, comb. nov.

Linociera mannii Solereder, *Botanisches Centralblatt*, 46: 17 (1891); Baker, *Flora of Tropical Africa*, 4: 19 (1902); Gilg & Schellenb., *Botanische Jahrbücher*, 51: 70 (1913); Green in Hutchinson & Dalziel, *Flora of West Tropical Africa*, 2nd ed., 2: 48 (1963). – *Mayepea mannii* (Solereder) Knoblauch, *Botanische Jahrbücher*,

17: 529 (1893). – *Chionanthus mannii* (Solereder) Stearn, *Botanical Journal of the Linnean Society*, 80: 199 (1980). – Type: Gabon, Gaboon River, *Mann 949*.

Linociera lingelsheimiana Gilg & Schellenb., *Botanische Jahrbücher*, 51: 72 (1913). – Type: Sierra Leone, Scarcies River, *Scott-Elliott 4717*.

Linociera macroura Gilg & Schellenb., *Botanische Jahrbücher*, 51: 72 (1913). – Type: Cameroon, *Preuss 1282a*.

Linociera congesta Baker, *Flora of Tropical Africa*, 4: 20 (1902), *quoad Mann, 1747*; *excl*. *Mann, 2214*; Gilg & Schellenb., *Botanische Jahrbücher*, 51: 72 (1913); Green in Hutchinson & Daziel, *Flora of West Tropical Africa*, 2nd ed., 2: 48 (1963); Liben, *Bulletin du Jardin Botanique National de Belgique*, 43: 358 (1973), *Flore d'Afrique Centrale*, Oleaceae, 31, t. 8 (1973). – *Chionanthus mannii* subsp. *congestus* (Baker) Stearn, *Botanical Journal of the Linnean Society*, 80: 201 (1980). – Type: Gabon, Muni, *Mann 1747*.

Noronhia mayottensis (H. Perrier) Hong-Wa & Besnard, comb. nov.

Linociera? mayottensis H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 280 (1949), *Flore de Madagascar* famille 166 (Oléacées): 12, fig. 3 n. 4 (1952). – Type: Mayotte, *Boivin 3196*.

Noronhia mildbraedii (Gilg & Schellenb.) Hong-Wa & Besnard, comb. nov.

Campanolea mildbraedii Gilg & Schellenb., *Botanische Jahrbücher*, 51: 74 (1913); non *Olea mildbraedii* Gilg & Schellenb. (1913). – *Olea mildbraedii* (Gilg & Schellenb.) Knoblauch, *Notizblatt des Königlichen Botanischen Gartens und Museums zu Berlin-Dahlem*, 11: 673 (1932); Turrill, *Flora of Tropical East*

Africa, Oleaceae: 8 (1952). – *Chionanthus mildbraedii* (Gilg & Schellenb.) Stearn, *Botanical Journal of the Linnean Society*, 80: 202 (1980). – Type: Cameroon, *Mildbraed 4409*.

Linociera giordani Chiovenda, *Atti della Reale Accademia Italiana Memorie della Classe di Scienze*, 11.ii.50 (1940); Friis, *Kew Bulletin*, 30: 16 (1975) as *L. giordanoi*. – Type: Ethiopia, *Giordano 2396 bis*.

Linociera latipetala Taylor, *Bulletin of Miscellaneous Informations, Royal Botanical Gardens, Kew*, 54 (1940); Eggeling & Dale, *Indigenous Trees of Uganda*: 285 (1952); Liben, *Bulletin du Jardin Botanique National de Belgique*, 43: 357 (1973), *Flore d'Afrique Centrale*, Oleaceae, 28, t. 7 (1973). – Type: Uganda, Lake Lutoto West of Ankole, *Eggeling 3186*.

Noronhia nilotica (Oliver) Hong-Wa & Besnard, comb. nov.

Linociera nilotica Oliver, *Transactions of the Linnean Society of London*, 29: 106, t. 117 (1875); Baker, *Flora of Tropical Africa*, 4: 19 (1902); Gilg & Schellenb., *Botanische Jahrbücher*, 51: 12 (1913); Eggeling & Dale, *Indigenous Trees of Uganda*: 285 (1952); Turrill, *Flora of Tropical East Africa*: 12 (1952); Dale & Greenway, *Kenya Trees & Shrubs*: 346 (1961); Green in Hutchinson & Daziel, *Flora of West Tropical Africa*, 2nd ed., 2: 48 (1963); Liben, *Flore d'Afrique Centrale*, Oleaceae: 30 (1973). – *Mayepea nilotica* (Oliver) Knoblauch, *Botanische Jahrbücher*, 17: 528 (1893). – *Chionanthus niloticus* (Oliver) Stearn, *Botanical Journal of the Linnean Society*, 80: 202 (1980). – Type: Cameroon, Briar, *Mildbraed 9431*.

Noronhia obtusifolia (Lam.) Hong-Wa & Besnard, comb. nov.

Olea obtusifolia Lam., *Tableau encyclopédique et méthodique*, 1: 28 (1791), *Encyclopédie Méthodique Botanique*, 4: 543 (1798). – *Linociera obtusifolia* (Lam.) H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 279 (1949), *Flore de Madagascar* famille 166 (Oléacées): 9, fig. 3 n. 1-3 (1952). – *Chionanthus obtusifolius* (Lam.) Stearn, *Botanical Journal of the Linnean Society*, 80: 203 (1980). – Type: Madagascar, *Commerson s.n.*

Linociera obtusifolia var. *minoriflora* H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 279 (1949), *Flore de Madagascar* famille 166 (Oléacées): 10 (1952). – Type: Madagascar, Tampina, *Louvel 126.*

Linociera obtusifolia var. *thouarsii* H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 279 (1949), *Flore de Madagascar* famille 166 (Oléacées): 10 (1952). – Type: Madagascar, *Thouars s.n.*

Noronhia peglerae (C. H. Wright) Hong-Wa & Besnard, comb. nov.

Olea peglerae C. H. Wright, *Flora Capensis*, 4.i: 485 (1907), as *O. pegleri*. – *Linociera peglerae* (C. H. Wright) Gilg & Schellenb, *Botanische Jahrbücher*, 51: 71 (1913); Verdoorn, *Bothalia*, 6: 599, t. 25 (1956), *Flora of Southern Africa*, 26: 22 (1963); Palmer & Pitman, *Trees of Southern Africa*, 3: 1833 (1972). – *Chionanthus peglerae* (C. H. Wright) Stearn, *Botanical Journal of the Linnean Society*, 80: 203 (1980). – Type: South Africa, Kentani, *Pegler 819.*

Noronhia richardsiae (Stearn) Hong-Wa & Besnard, comb. nov.

Chionanthus richardsiae Stearn, *Botanical Journal of the Linnean Society*, 80: 204 (1980). – Type: Zambia, *Richards 4144*.

Noronhia tropophylla (H. Perrier) Hong-Wa & Besnard, comb. nov.

Linociera tropophylla H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 280 (1949), *Flore de Madagascar* famille 166 (Oléacées): 14, fig. 3 n. 4 (1952). – *Chionanthus tropophyllus* (H. Perrier) Stearn, *Botanical Journal of the Linnean Society*, 80: 205 (1980). – Type: Madagascar, Boina, *Perrier 12340* (here designated).

Linociera tropophylla var. *angustata* H. Perrier, *Mémoires de l'Institut Scientifique de Madagascar, Série B*, 2: 281 (1949), *Flore de Madagascar* famille 166 (Oléacées): 15 (1952). – Type: Madagascar, Ankarafantsika, *Service Forestier 49* (here designated).

Supplementary materials

Table S1 - List of species included in this study.

Table S2 – GenBank accession numbers.

Fig. S1. Bayesian 50% majority-rule consensus tree inferred from analysis using (A) combined plastid (CP) DNA regions (*trnL-F*, *trnT-L*, *trnS-G* and *trnK-matK*), (B) nuclear ribosomal DNA (ITS) with all accessions included.

Fig. S2. Bayesian phylogram inferred from analysis using (A) combined plastid (CP) DNA regions (*trnL-F*, *trnT-L*, *trnS-G* and *trnK-matK*), (B) nuclear ribosomal DNA (ITS) with all accessions included.

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Table 1

Statistics of alignments and phylogenetic analyses of the different regions analyzed: combined plastid DNA regions (CP = *trnL-F*, *trnT-L*, *trnS-G*, *tnrK-matK*), internal transcribed spacer (ITS) and triose phosphate isomerase (TPI). PIC = parsimony-informative character; K2p = Kimura two-parameter; HI = homoplasy index; ln *L* = log-likelihood; ASDSF = average standard deviation of split frequencies.

	CP	ITS	TPI (all)
Number of terminals (redundant)	111	126	138
Aligned length (bp)	3818	765	1203
Variable characters	429	385	573
PIC/Percent	212/5.55	297/38.82	326/27.09
Mean K2p sequence divergence (%)	0.8	7.2	11.2
Mean GC content (%)	31.23	63.25	34.52
HI	0.35	0.68	0.24
Phi test (p-value)	-	0.23	0.98
Substitution model	TVM + G	GTR + I + G	
Alternative model	[GTR + G]		
-ln *L*	10484.76	10197.17	
	MrBayes/BEAST		
Number of generations	15/10	20/10	
Heating parameter	0.1	0.1	
ASDSF	0.003	0.006	

Table 2

P-values obtained from the approximately unbiased (AU), Kishino-Hasegawa (KH) and Shimodaira-Hasegawa (SH) tests for alternative topologies based on combined plastid (CP) and nuclear (ITS) datasets. Δln *L* = difference in log-likelihood. Boldfaced values indicate rejection of the corresponding alternative topology.

Dataset and constraints	Δln *L*	AU	KH	SH
CP				
Best ML tree (unconstrained)	0	0.995	0.981	0.998
Fraxinus nested within Oleinae	30.83	**0.007**	**0.010**	0.472
Olea s. str. polyphyletic (excluding *O. paniculata*)	69.39	**0.000**	**0.001**	0.136
African *Chionanthus* monophyletic	97.54	**0.000**	**0.003**	**0.034**
Topology mirroring ITS tree	246.28	**0.000**	**0.000**	**0.000**
ITS				
Best ML tree (unconstrained)	0	0.987	0.963	0.997
Fraxinus sister to Oleinae	17.53	**0.028**	**0.037**	0.547
Olea s. str. monophyletic (including *O. paniculata*)	25.00	**0.025**	**0.025**	0.411
African *Chionanthus* polyphyletic	33.5	0.057	0.061	0.061
Topology mirroring CP tree but with monophyletic African *Chionanthus*	210.41	**0.000**	**0.000**	**0.000**
Topology mirroring CP tree	391.67	**0.000**	**0.000**	**0.000**

Figure captions

Fig. 1. Bayesian 50% majority-rule consensus tree inferred from analyses using (A) combined plastid (CP) DNA regions (*trnL-F*, *trnT-L*, *trnS-G* and *trnK-matK*) and (B) nuclear ribosomal DNA (ITS). Values above branches denote maximum likelihood bootstrap support (MLBS %) and those below branches are Bayesian posterior probabilities (BPP). Boldfaced values are MLBS ≥ 85% and BPP ≥ 0.95. Shaded areas indicate members of subtribe Oleinae. Numbers after taxon names refer to vouchers listed in Supplementary Table S1. Abbreviations are: *Ch. m*. = *Chionanthus mannii*; *Ch. o*. = *Chionanthus obtusifolius*; *N* = *Noronhia*; *N. e*. = *Noronhia emarginata*; *N. l*. = *Noronhia luteola*; *O. c*. = *Olea capensis*; *O. e*. = *Olea europaea*.

Fig. 2. NeighborNet network of the nuclear triose phosphate isomerase (TPI) gene. Dashed line separates long (> 750 bp; TPI-L1 and TPI-L2) and short (ca. 600 bp; TPI-S3 and TPI-S4) sequences. Shaded areas indicate *Noronhia* and *Chionanthus* from the Malagasy Floristic Region (MFR clade). Dark thick lines represent species of *Chionanthus*. Abbreviations: PPCON = *Phillyrea-Picconia-Chionanthus-Osmanthus-Nestegis* group; PPCO = *Phillyrea-Picconia-Chionanthus-Osmanthus* group.

Fig. 3. Schematic diagrams showing the estimated relative divergence times within Oleinae obtained from analyses of (A) CP and (B) ITS datasets. Boldfaced values below branches refer to mean ages in million years of the nodes after the numbers. Values above branches are posterior probabilities. Mid-branch dots with associated

ages and support values indicate the start of diversification within a clade. MFR = Malagasy Floristic Region (comprising Madagascar, Comoros and Mascarene).

Fig. 1

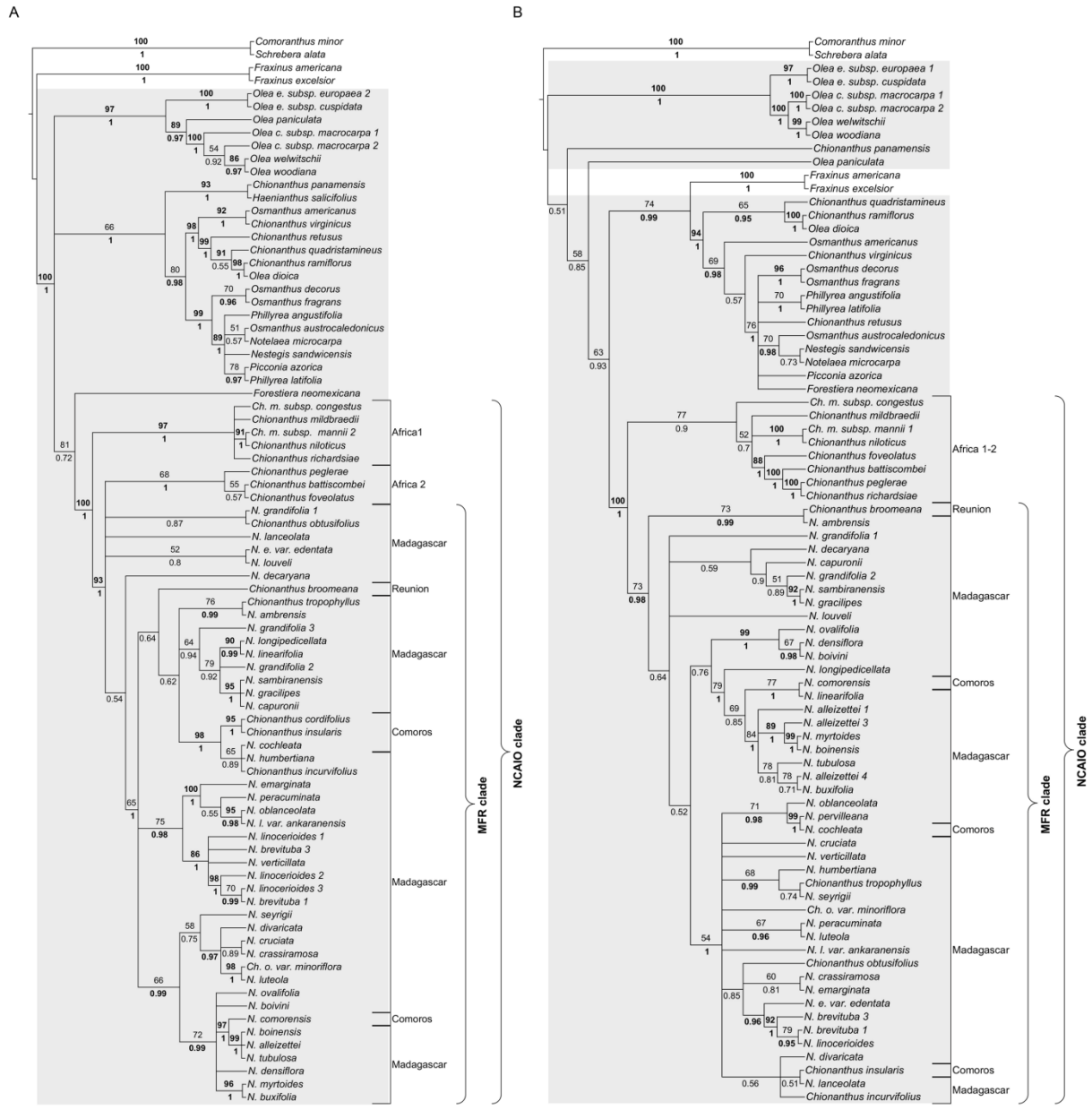

A
Comoranthus minor
Schrebera alata
Fraxinus americana
Fraxinus excelsior
Olea e. subsp. europaea 2
Olea e. subsp. cuspidata
Olea paniculata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus panamensis
Haenianthus salicifolius
Osmanthus americanus
Chionanthus virginicus
Chionanthus retusus
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Osmanthus decorus
Osmanthus fragrans
Phillyrea angustifolia
Osmanthus austrocaledonicus
Notelaea microcarpa
Nestegis sandwicensis
Picconia azorica
Phillyrea latifolia
Forestiera neomexicana
Ch. m. subsp. congestus
Chionanthus mildbraedii
Ch. m. subsp. mannii 2
Chionanthus niloticus
Chionanthus richardsiae
Chionanthus peglerae
Chionanthus battiscombei
Chionanthus foveolatus
N. grandifolia 1
Chionanthus obtusifolius
N. lanceolata
N. e. var. edentata
N. louveli
N. decaryana
Chionanthus broomeana
Chionanthus tropophyllus
N. ambrensis
N. grandifolia 3
N. longipedicellata
N. linearifolia
N. grandifolia 2
N. sambiranensis
N. gracilipes
N. capuronii
Chionanthus cordifolius
Chionanthus insularis
N. cochleata
N. humbertiana
Chionanthus incurvifolius
N. emarginata
N. peracuminata
N. oblanceolata
N. l. var. ankaranensis
N. linocerioides 1
N. brevituba 3
N. verticillata
N. linocerioides 2
N. linocerioides 3
N. brevituba 1
N. seyrigii
N. divaricata
N. cruciata
N. crassiramosa
Ch. o. var. minoriflora
N. luteola
N. ovalifolia
N. boivini
N. comorensis
N. boinensis
N. alleizettei
N. tubulosa
N. densiflora
N. myrtoides
N. buxifolia
Africa1
Africa 2
Madagascar
Reunion
Madagascar
Comoros
Madagascar
Madagascar
Comoros
Madagascar
MFR clade
NCAIO clade
B
Comoranthus minor
Schrebera alata
Olea e. subsp. europaea 1
Olea e. subsp. cuspidata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus panamensis
Olea paniculata
Fraxinus americana
Fraxinus excelsior
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Osmanthus americanus
Chionanthus virginicus
Osmanthus decorus
Osmanthus fragrans
Phillyrea angustifolia
Phillyrea latifolia
Chionanthus retusus
Osmanthus austrocaledonicus
Nestegis sandwicensis
Notelaea microcarpa
Picconia azorica
Forestiera neomexicana
Ch. m. subsp. congestus
Chionanthus mildbraedii
Ch. m. subsp. mannii 1
Chionanthus niloticus
Chionanthus foveolatus
Chionanthus battiscombei
Chionanthus peglerae
Chionanthus richardsiae
Chionanthus broomeana
N. ambrensis
N. grandifolia 1
N. decaryana
N. capuronii
N. grandifolia 2
N. sambiranensis
N. gracilipes
N. louveli
N. ovalifolia
N. densiflora
N. boivini
N. longipedicellata
N. comorensis
N. linearifolia
N. alleizettei 1
N. alleizettei 3
N. myrtoides
N. boinensis
N. tubulosa
N. alleizettei 4
N. buxifolia
N. oblanceolata
N. pervilleana
N. cochleata
N. cruciata
N. verticillata
N. humbertiana
Chionanthus tropophyllus
N. seyrigii
Ch. o. var. minoriflora
N. peracuminata
N. luteola
N. l. var. ankaranensis
Chionanthus obtusifolius
N. crassiramosa
N. emarginata
N. e. var. edentata
N. brevituba 3
N. brevituba 1
N. linocerioides
N. divaricata
Chionanthus insularis
N. lanceolata
Chionanthus incurvifolius
Africa 1-2
Reunion
Madagascar
Comoros
Madagascar
Comoros
Madagascar
Comoros
Madagascar
MFR clade
NCAIO clade

Fig. 2

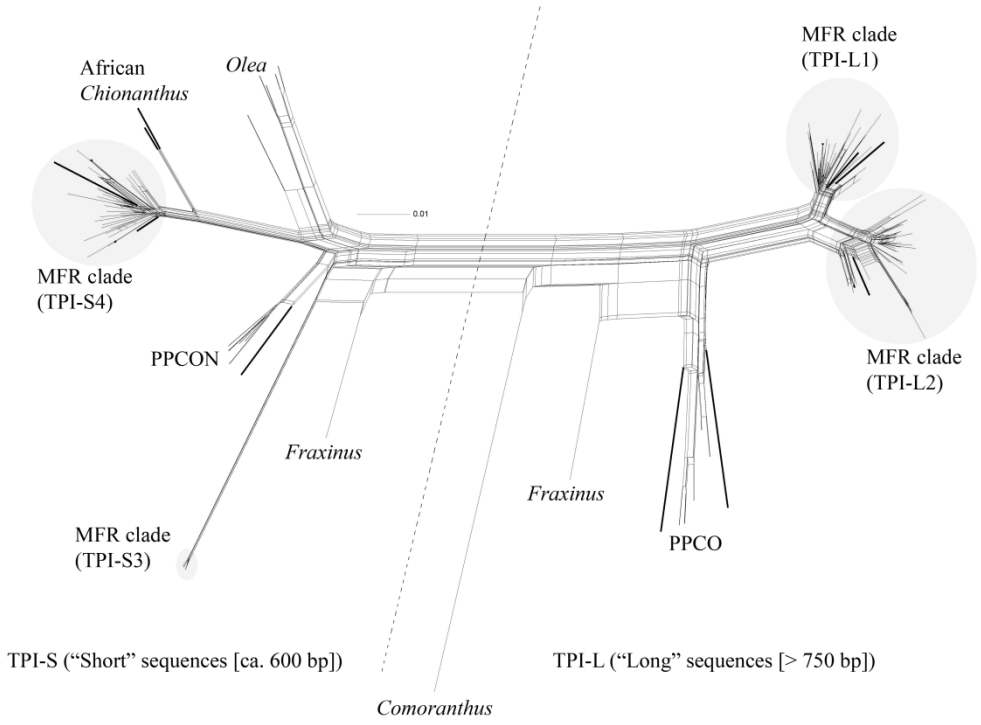
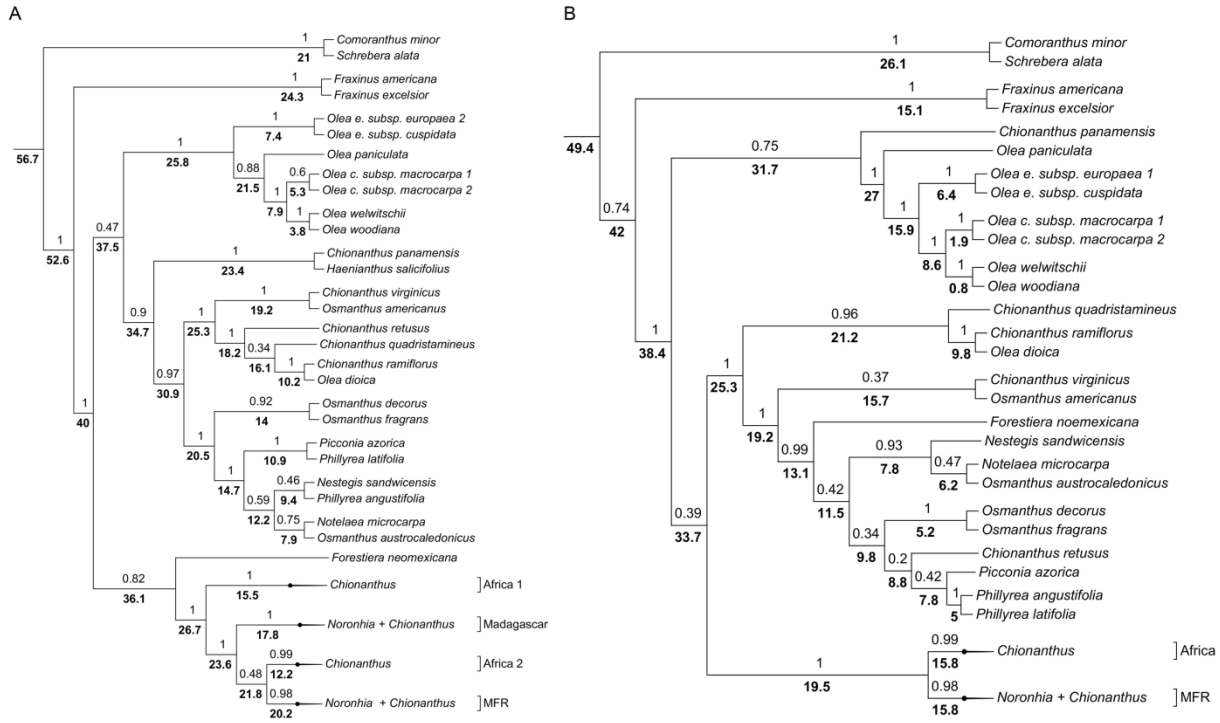

African
Chionanthus
Olea
MFR clade
(TPI-L1)
0.01
MFR clade
(TPI-S4)
PPCON
MFR clade
(TPI-L2)
Fraxinus
Fraxinus
MFR clade
(TPI-S3)
PPCO
TPI-S ("Short" sequences [ca. 600 bp])
TPI-L ("Long" sequences [> 750 bp])
Comoranthus

Fig. 3


A
Comoranthus minor
Schrebera alata
Fraxinus americana
Fraxinus excelsior
Olea e. subsp. europaea 2
Olea e. subsp. cuspidata
Olea paniculata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus panamensis
Haenianthus salicifolius
Chionanthus virginicus
Osmanthus americanus
Chionanthus retusus
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Osmanthus decorus
Osmanthus fragrans
Picconia azorica
Phillyrea latifolia
Nestegis sandwicensis
Phillyrea angustifolia
Notelaea microcarpa
Osmanthus austrocaledonicus
Forestiera neomexicana
Chionanthus
Noronhia + Chionanthus
Chionanthus
Noronhia + Chionanthus
Africa 1
Madagascar
Africa 2
MFR
B
Comoranthus minor
Schrebera alata
Fraxinus americana
Fraxinus excelsior
Chionanthus panamensis
Olea paniculata
Olea e. subsp. europaea 1
Olea e. subsp. cuspidata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Chionanthus virginicus
Osmanthus americanus
Forestiera noemexicana
Nestegis sandwicensis
Notelaea microcarpa
Osmanthus austrocaledonicus
Osmanthus decorus
Osmanthus fragrans
Chionanthus retusus
Picconia azorica
Phillyrea angustifolia
Phillyrea latifolia
Chionanthus
Noronhia + Chionanthus
Africa
MFR

Supplementary tables.

Table S1 - List of species included in this study. Abbreviations refer to herbaria and follow *Index Herbariorum* (Holmgren et al., 1990) and when in brackets indicate that the sample was taken from herbarium specimen, silica gel-dried leaf material or DNA extract deposited at those herbaria. BM: Natural History Museum; CP: combined plastid DNA regions (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*); ITS: internal transcribed spacer; K: Kew Garden; MA: Real Jardín Botánico Madrid; MO: Missouri Botanical Garden; MPU: Université Montpellier 2; P: Muséum National d'Histoire Naturelle Paris; TPI: triose phosphate isomerase.

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Chionanthus battiscombei (Hutch.) Stearn	Loveridge 1527	Zimbabwe [K]	x	x	
Chionanthus broomeana (Horne ex Oliver) A.J. Scott	Besnard	Reunion	x	x	x
Chionanthus cordifolius Labat, Pignal & Pascal	Pascal 288	Comoros [K]	x		
Chionanthus foveolatus (Meyer) Stearn	F557	South Africa [K]	x	x	x
Chionanthus incurvifolius 1 (H. Perrier) Stearn	Besnard 49-2006	Madagascar	x	x	x
Chionanthus incurvifolius 2	Ratovoson 1361	Madagascar	x	x	
Chionanthus incurvifolius 3	Andriamihajarivo 1401	Madagascar	x	x	x
Chionanthus insularis Labat, Pignal & Pascal	Barthelat 1069	Comoros [MO]	x	x	
Chionanthus mannii subsp. mannii 1 (Solereder) Stearn	White 886	Gabon [MO]		x	
Chionanthus mannii subsp. mannii 2	Leeuwenberg 2354	Ivory Cost [K]	x		
Chionanthus mannii subsp. congestus (Baker) Stearn	Schmidt 3487	Ghana [K]	x	x	
Chionanthus mildbraedii (Gilg & Schellenb.) Stearn	Friis 9842	Ethiopia [K]	x	x	

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Chionanthus niloticus (Oliver) Stearn	Fanshawe 4706	Rhodesia [K]	x	x	
Chionanthus obtusifolius (Lam.) Stearn	Hong-Wa 599	Madagascar	x	x	x
Ch. obtusifolius var. minoriflora (H. Perrier) Stearn	Hong-Wa 620	Madagascar	x	x	
Chionanthus panamensis 1 (Standl.) Stearn	Martinez 26308	Mexico [K]	x	x	
Chionanthus panamensis 2	Thomsen 1646	Costa Rica [MO]		x	
Chionanthus peglerae (C.H. Wright) Stearn	Maurin 1766	South Africa	x	x	x
Chionanthus quadristamineus F.Muell.	Papadopulos 366	Australia	x	x	
Chionanthus ramiflorus Roxb.	Flynn 6332	Hawaii, USA [MPU]	x	x	
Chionanthus retusus Lindl. & Paxton	Hong-Wa SN10	Cultivated-MO	x	x	x
Chionanthus richardsiae Stearn	Fanshawe 4052	Zambia [K]	x	x	
Chionanthus tropophyllus (H. Perrier) Stearn	Hong-Wa 630	Madagascar	x	x	x
Chionanthus virginicus 1 L.	Hong-Wa SN2	Cultivated-MO	x	x	x
Chionanthus virginicus 2	Miller 8217	Maryland, USA	x	x	
Comoranthus minor H. Perrier	Ratovoson 1457	Madagascar	x	x	x
Forestiera neomexicana A. Gray	Villemur 4	Cultivated-MA	x	x	
Fraxinus americana L.	Hong-Wa SN6	Cultivated-MO	x	x	x
Fraxinus excelsior 1 L.	Besnard 1-2007	Switzerland [G]	x		

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Fraxinus excelsior 2 L.	Wallander 353	Romania		x	
Haenianthus salicifolius Griseb.	Axelrod 9875	Puerto Rico [MO]	x		
Nestegis sandwicensis (A. Gray) O. Deg, I. Deg & L.A.S. Johnson	Flynn 6329	Hawaii, USA	x	x	x
Noronhia alleizettei 1 Dubard	Hong-Wa 632	Madagascar	x	x	x
Noronhia alleizettei 2	Hong-Wa 628	Madagascar	x	x	x
Noronhia alleizettei 3	Hong-Wa 622	Madagascar	x	x	x
Noronhia alleizettei 4	Hong-Wa 624	Madagascar	x	x	x
Noronhia ambrensis 1 H. Perrier	Hong-Wa 693	Madagascar	x	x	
Noronhia ambrensis 2	Hong-Wa 573	Madagascar	x	x	x
Noronhia boinensis H. Perrier	Phillipson 2277	Madagascar [MO]	x	x	
Noronhia boivini 1 Dubard	Hong-Wa 614	Madagascar		x	x
Noronhia boivini 2	Randriatafika 379	Madagascar [MO]	x	x	
Noronhia brevituba 1 H. Perrier	Hong-Wa 684	Madagascar	x	x	x
Noronhia brevituba 2	Hong-Wa 579	Madagascar	x	x	
Noronhia brevituba 3	Hong-Wa 638	Madagascar	x	x	x
Noronhia buxifolia 1	Andriamihajarivo 1488	Madagascar	x	x	x

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Noronhia buxifolia 2	Andriamihajarivo 1485	Madagascar	x	x	x
Noronhia capuronii 1 Bosser	Andriamihajarivo 1375	Madagascar	x	x	x
Noronhia capuronii 2	Hong-Wa 706	Madagascar	x	x	x
Noronhia capuronii 3	Trigui 536	Madagascar		x	
Noronhia cochleata 1 Labat, Pignal & Pascal	Labat 3258	Comoros [P]	x	x	
Noronhia cochleata 2	Labat 3308	Comoros [MO]			x
Noronhia cochleata 3	Pignal 1112	Comoros [P]	x	x	
Noronhia comorensis 1 S. Moore	Barthelat 537	Comoros [MO]			x
Noronhia comorensis 2	Labat 3257	Comoros [P]	x	x	
Noronhia crassiramosa 1 H. Perrier	Hong-Wa 669	Madagascar		x	x
Noronhia crassiramosa 2	Hong-Wa 658	Madagascar		x	x
Noronhia crassiramosa 3	Hong-Wa 640	Madagascar	x	x	x
Noronhia cruciata H. Perrier	Hong-Wa 654	Madagascar	x	x	x
Noronhia decaryana 1 H. Perrier	Hong-Wa 648	Madagascar	x	x	x
Noronhia decaryana 2	Hong-Wa 612	Madagascar		x	
Noronhia densiflora Bosser	Hong-Wa 611	Madagascar	x	x	
Noronhia divaricata 1 Scott-Elliott	Randrianaivo 1761	Madagascar	x	x	x

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Noronhia divaricata 2	Dumetz 1421	Madagascar [MO]		x	
Noronhia divaricata 3	Letsara 746	Madagascar	x	x	x
Noronhia divaricata 4	Rakotonasolo 2	Madagascar	x		
Noronhia emarginata 1 (Lam.) Thouars	Flynn 6331	Hawaii, USA	x	x	
Noronhia emarginata 2	Rakotonirina 464	Madagascar	x	x	x
Noronhia emarginata 3	Besnard	Reunion	x	x	x
Noronhia emarginata 4	Birkinshaw 506	Madagascar	x	x	
Noronhia emarginata 5	Miller 7216	Florida, USA	x		
N. emarginata var. edentata H. Perrier	Razanatsima 266	Madagascar	x	x	x
Noronhia gracilipes 1 H. Perrier	Besnard 46-2006	Madagascar	x	x	
Noronhia gracilipes 2 H. Perrier	Hong-Wa 686	Madagascar		x	x
Noronhia gracilipes 3	Hong-Wa 583	Madagascar	x	x	
Noronhia gracilipes 4	Hong-Wa 713	Madagascar		x	
Noronhia gracilipes 5	Hong-Wa 571	Madagascar	x	x	x
Noronhia grandifolia 1 H. Perrier	Hong-Wa 670	Madagascar	x	x	x
Noronhia grandifolia 2	Gautier 4803	Madagascar [MO]	x	x	
Noronhia grandifolia 3	Birkinshaw 468	Madagascar	x		

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Noronhia humbertiana H. Perrier	Hong-Wa 695	Madagascar	x	x	
Noronhia lanceolata 1 H. Perrier	Ratovoson 1475	Madagascar		x	x
Noronhia lanceolata 2	Lowry 6942	Madagascar	x	x	x
Noronhia lanceolata 3	Randrianaivo 1762	Madagascar	x	x	
Noronhia lanceolata 4	Hong-Wa 609	Madagascar	x	x	x
Noronhia lanceolata 5	Andriamihajarivo 1547	Madagascar		x	
Noronhia linearifolia 1 Boivin ex Dubard	Hong-Wa 526	Madagascar	x	x	x
Noronhia linearifolia 2	Claude 83	Madagascar		x	x
Noronhia linearifolia 3	Hong-Wa 546	Madagascar	x	x	x
Noronhia linocerioides 1	Schatz 3605	Madagascar [MO]	x	x	
Noronhia linocerioides 2	Birkinshaw 492	Madagascar	x	x	
Noronhia linocerioides 3	Birkinshaw 467	Madagascar	x		
Noronhia longipedicellata 1 H. Perrier	Besnard 53-2006	Madagascar	x	x	x
Noronhia longipedicellata 2	Hong-Wa 593	Madagascar	x	x	x
Noronhia longipedicellata 3	Hong-Wa 592	Madagascar	x	x	x
Noronhia longipedicellata 4	Hong-Wa 564	Madagascar	x	x	x
Noronhia louveli 1 H. Perrier	Ranaivojaona 1723	Madagascar	x	x	

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Noronhia louveli 2	Hong-Wa 647	Madagascar	x	x	x
Noronhia louveli 3	Hong-Wa 642	Madagascar	x	x	x
Noronhia luteola 1 H. Perrier	Hong-Wa 594	Madagascar	x	x	x
Noronhia luteola 2	Hong-Wa 596	Madagascar		x	
Noronhia luteola 3	Hong-Wa 598	Madagascar			x
N. luteola var. ankaranensis 1 H. Perrier	Besnard 51-2006	Madagascar	x	x	x
N. luteola var. ankaranensis 2	Hong-Wa 551	Madagascar		x	
N. luteola var. ankaranensis 3	Hong-Wa 545	Madagascar	x	x	x
Noronhia myrtoides H. Perrier	Sussman 153	Madagascar [MO]	x	x	
Noronhia oblanceolata 1 H. Perrier	Ranirison 1053	Madagascar [MO]		x	
Noronhia oblanceolata 2	Ranirison 756	Madagascar [MO]	x	x	
Noronhia ovalifolia 1 H. Perrier	Randrianaivo 1548	Madagascar	x	x	x
Noronhia ovalifolia 2	Randrianaivo 1760	Madagascar		x	x
Noronhia ovalifolia 3	Lowry 6955	Madagascar	x	x	
Noronhia peracuminata	Hong-Wa 720	Madagascar	x	x	x
Noronhia pervilleana 1	Hong-Wa 718	Madagascar		x	x
Noronhia pervilleana 2	Ranirison 867	Madagascar [MO]		x	

Taxa	Voucher	Geographic Origin	CP	ITS	TP-
Noronhia sambiranensis H. Perrier	Wohlhauser 60168	Madagascar [MO]	x	x	
Noronhia seyrigii 1 H. Perrier	Randrianasolo 1233	Madagascar		x	x
Noronhia seyrigii 2	Lowry 6940	Madagascar	x	x	x
Noronhia tubulosa H. Perrier	Hong-Wa 629	Madagascar	x	x	x
Noronhia verticillata H. Perrier	Hong-Wa 634	Madagascar	x	x	x
Notelaea microcarpa R. Br.	Streiman 731	Australia	x	x	
Olea dioica Roxb.	Munzinger 245	Laos [P]		x	x
Olea europaea subsp. e*uropaea* 1 L.	Hong-Wa SN1	Cultivated-MO			x
Olea europaea subsp. europaea 2	IRO-P	Sicily	x		
O. europaea subsp. cuspidata (Wall. ex G. Don) Cif.	INRA-M	Reunion	x	x	
Olea paniculata R. Br.	Lambrides 1	Australia	x	x	x
Olea capensis subsp. macrocarpa 1 (C. H. Wright) I. Verd.	Birkinshaw 1758	Madagascar	x	x	x
Olea capensis subsp. macrocarpa 2	Hong-Wa 557	Madagascar	x	x	x
Olea welwitschii (Knobl.) Gilg & Schellenb.	Besnard 1-2008	Kenya [G]	x	x	x
Olea woodiana Knobl.	Costa 2	South Africa	x	x	x
Osmanthus americanus (L.) Benth. & Hook. f. ex A. Gray		Cultivated-MO		x	x

Taxa	Voucher	Geographic Origin	CP	ITS	TPI
Osmanthus austrocaledonicus Knobl.	Munzinger 823	New Caledonia [MO]	x	x	x
Osmanthus decorus (Boiss. & Balansa) Kasipl.	Merello 2324	Georgia Rep. [MO]	x	x	x
Osmanthus fragrans (Thunb.) Lour.	Hong-Wa SN3	Cultivated-MO	x	x	x
Phillyrea angustifolia L.	Hong-Wa SN5	Cultivated-MO	x	x	x
Phillyrea latifolia L.	RJBM 27-95	Cultivated-MA	x	x	x
Picconia azorica (Tutin) Knobl.	Schaefer BM 2008-323	Azores [BM]	x	x	x
Schrebera alata (Hochst.) Welw.	Chase 3883	South Africa [K]		x	x

Table S2 – GenBank accession numbers. CP = combined plastid DNA regions (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*); ITS = internal transcribed spacer; TPI = triose phosphate isomerase; *Ch.* = *Chionanthus*; *N.* = *Noronhia*; *O.* = *Olea*.

Taxa	Voucher	CP	ITS	TPI
Ch. battiscombei	Loveridge 1527			
Ch. broomeana	Cultivated	AM931522, AM933079, AM933223, AM933426		
Ch. cordifolius	Pascal 288			
Ch. foveolatus	F557			
Ch. incurvifolius	Andriamihajarivo 1401			
Ch. incurvifolius	Besnard 49-2006	AM931529, AM933086, AM933230, AM933433		
Ch. incurvifolius	Ratovoson 1361			
Ch. insularis	Barthelat 1069			
Ch. mannii subsp. congestus	Schmidt 3487			
Ch. mannii subsp. mannii	Leewenberg 2354			
Ch. mannii subsp. mannii	White 886			
Ch. mildbraedii	Friis 9842			
Ch. niloticus	Fanshawe 4706			
Ch. obtusifolius	Hong-Wa 599			
Ch. obtusifolius var. minoriflora	Hong-Wa 620			
Ch. panamensis	Martinez 26308			
Ch. panamensis	Thomsen 1646			
Ch. peglerae	Maurin 1766			
Ch. quadristamineus	Papadopulos 366			
Ch. ramiflorus	Flynn 6332			
Ch. retusus	Hong-Wa SN10			
Ch. richardsiae	Fanshawe 4052			
Ch. tropophyllus	Hong-Wa 630			
Ch. virginicus	Hong-Wa SN2			
Ch. virginicus	Miller 8217			
Comoranthus minor	Ratovoson 1457			
Forestiera neomexicana	Villemur 4			
Fraxinus americana	Hong-Wa SN6			
Fraxinus excelsior	Besnard 1-2007	AM931523, AM933080, AM933224, AM933427		
Fraxinus excelsior	Wallander 353		EU314849	
Haenianthus salicifolius	Axelrod 9875			
Nestgeis sandwicensis	Flynn 6329	AM931525, AM933082, AM933226, AM933429		
N. alleizettei	Hong-Wa 622			

Taxa	Voucher	CP	ITS	TPI
N. alleizettei	Hong-Wa 624			
N. alleizettei	Hong-Wa 628			
N. alleizettei	Hong-Wa 632			
N. ambrensis	Hong-Wa 573			
N. ambrensis	Hong-Wa 693			
N. boinensis	Phillipson 2277			
N. boivini	Hong-Wa 614			
N. boivini	Randriatafika 379			
N. brevituba	Hong-Wa 579			
N. brevituba	Hong-Wa 638			
N. brevituba	Hong-Wa 684			
N. buxifolia	Andriamihajarivo 1485			
N. buxifolia	Andriamihajarivo 1488			
N. capuronii	Andriamihajarivo 1375			
N. capuronii	Hong-Wa 706			
N. capuronii	Trigui 536			
N. cochleata	Labat 3258			
N. cochleata	Labat 3308			
N. cochleata	Pignal 1112			
N. comorensis	Barthelat 537			
N. comorensis	Labat 3257			
N. crassiramosa	Hong-Wa 640			
N. crassiramosa	Hong-Wa 658			
N. crassiramosa	Hong-Wa 669			
N. cruciata	Hong-Wa 654			
N. decaryana	Hong-Wa 612			
N. decaryana	Hong-Wa 648			
N. densiflora	Hong-Wa 611			
N. divaricata	Dumetz 1421			
N. divaricata	Letsara 746			
N. divaricata	Rakotonasolo 2			
N. divaricata	Randrianaivo 1761			
N. emarginata	Cultivated	AM931526, AM933083, AM933227, AM933430		
N. emarginata	Birkinshaw 506			
N. emarginata	Flynn 6331			
N. emarginata	Miller 7216			
N. emarginata	Rakotonirina 464			
N. emarginata var. edentata	Razantsima 266			
N. gracilipes	Besnard 46-2006	AM931531, AM933088, AM933232, AM933435		

Taxa	Voucher	CP	ITS	TPI
N. gracilipes	Hong-Wa 571			
N. gracilipes	Hong-Wa 583			
N. gracilipes	Hong-Wa 686			
N. gracilipes	Hong-Wa 713			
N. grandifolia	Birkinshaw 468			
N. grandifolia	Gautier 4803			
N. grandifolia	Hong-Wa 670			
N. humbertiana	Hong-Wa 695			
N. lanceolata	Andriamihajarivo 1547			
N. lanceolata	Hong-Wa 609			
N. lanceolata	Lowry 6942			
N. lanceolata	Randrianaivo 1762			
N. lanceolata	Ratovoson 1475			
N. linearifolia	Claude 83			
N. linearifolia	Hong-Wa 526			
N. linearifolia	Hong-Wa 546			
N. linocerioides	Birkinshaw 467			
N. linocerioides	Birkinshaw 492			
N. linocerioides	Schatz 3605	AM931503, AM933059, AM933203, AM933406		
N. longipedicellata	Besnard 53-2006	AM931527, AM933084, AM933228, AM933431		
N. longipedicellata	Hong-Wa 564			
N. longipedicellata	Hong-Wa 592			
N. longipedicellata	Hong-Wa 593			
N. louveli	Hong-Wa 642			
N. louveli	Hong-Wa 647			
N. louveli	Ranaivojaona 1723			
N. luteola	Hong-Wa 594			
N. luteola	Hong-Wa 596			
N. luteola	Hong-Wa 598			
N. luteola var. ankaranrensis	Besnard 51-2006	AM931528, AM933085, AM933229, AM933432		
N. luteola var. ankaranrensis	Hong-Wa 545			
N. luteola var. ankaranrensis	Hong-Wa 551			
N. myrtoides	Sussman 153			
N. oblanceolata	Ranirison 1053			
N. oblanceolata	Ranirison 756			
N. ovalifolia	Lowry 6955			
N. ovalifolia	Randrianaivo 1548			
N. ovalifolia	Randrianaivo 1760			

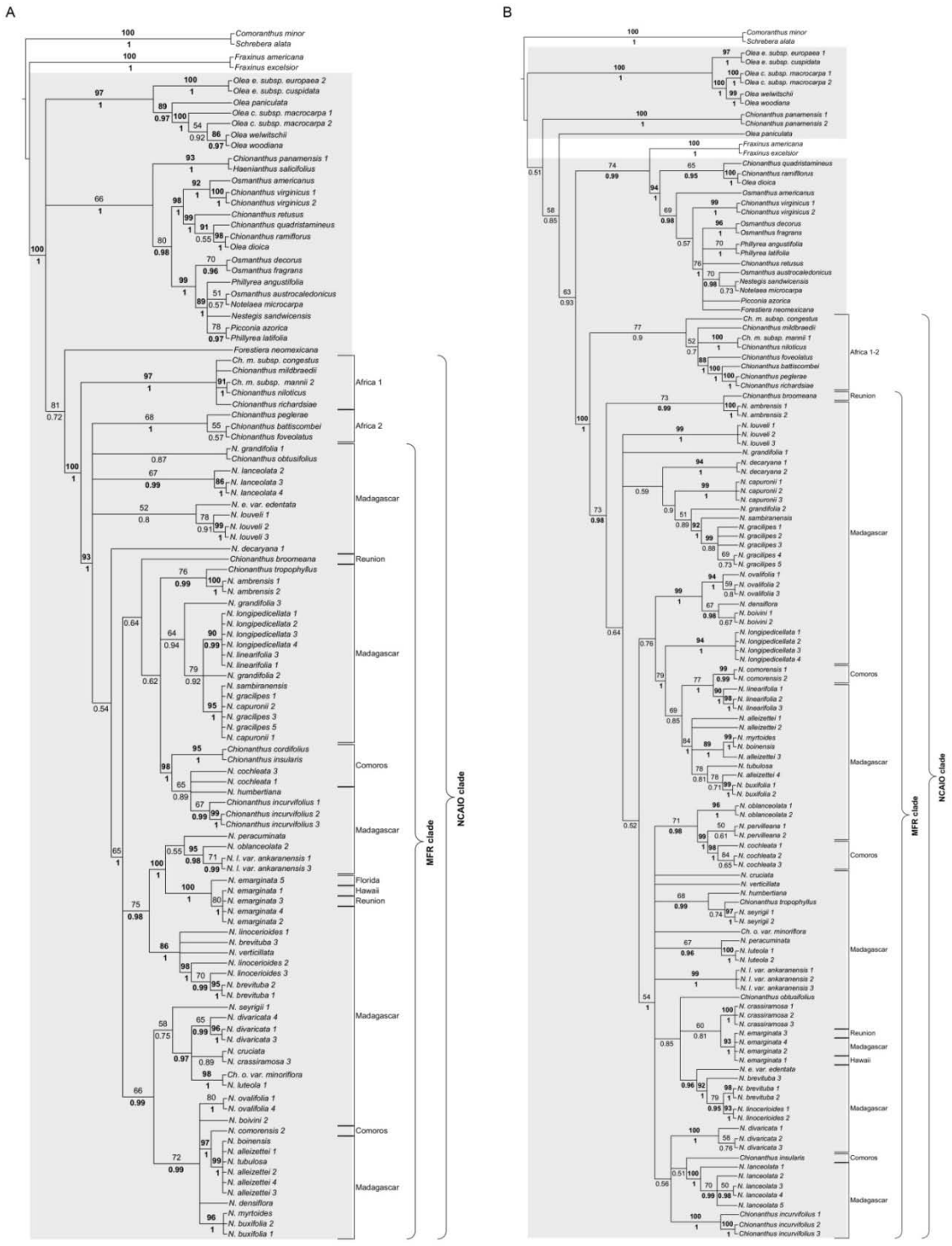
Taxa	Voucher	CP	ITS	TPI
N. peracuminata	Hong-Wa 720			
N. pervilleana	Hong-Wa 718			
N. pervilleana	Ranirison 867			
N. sambiranensis	Wohlhauser 60168			
N. seyrigii	Lowry 6940			
N. seyrigii	Randrianassolo 1233			
N. tubulosa	Hong-Wa 629			
N. verticillata	Hong-Wa 634			
Notelaea microcarpa	Streiman 731			
O. capensis subsp. macrocarpa	Birkinshaw 1758			
O. capensis subsp. macrocarpa	Hong-Wa 557			
O. dioica	Munzinger 245			
O. europaea subsp. cuspidata	Cultivated	AM931491, AM933048, AM933192, AM933395		
O. europaea subsp. europaea	Hong-Wa SN1			
O. europaea subsp. europaea	Cultivated	AM931476, AM933036, AM933180, AM933383		
O. paniculata	Lambrides 1	AM931519, AM933075, AM933219, AM933422		
O. welwitschii	Besnard 1-2008	AM931517, AM933073, AM933217, AM933420		
O. woodiana	Costa 2	AM931502, AM933058, AM933202, AM933405		
Osmanthus americanus	Cultivated			
Osmanthus austrocaledonicus	Munzinger 823			
Osmanthus decorus	Merello 2324			
Osmanthus fragrans	Hong-Wa SN3			
Phillyrea angustifolia	Hong-Wa SN5			
Phillyrea latifolia	Cultivated	FM208236, FM208226, FM208244, FM208252		
Picconia azorica	Schaefer BM 2008-323			
Schrebera alata	Chase 3883			

Supplementary figures

Fig. S1. Bayesian 50% majority-rule consensus tree inferred from analysis using (A) combined plastid (CP) DNA regions (*trnL-F*, *trnT-L*, *trnS-G* and *trnK-matK*), (B) nuclear ribosomal DNA (ITS) with all accessions included. Values above branches denote maximum likelihood bootstrap support (MLBS %). Boldfaced values below branches are Bayesian posterior probabilities (BPP). Numbers after taxon names refer to vouchers listed in Supplementary material Table S1. Abbreviations are: *Ch. m*. = *Chionanthus mannii*; *Ch. o*. = *Chionanthus obtusifolius*; *N* = *Noronhia*; *N. e*. = *Noronhia emarginata*; *N. l*. = *Noronhia luteola*; *O. c*. = *Olea capensis*; *O. e*. = *Olea europaea*.

Fig. S2. Bayesian phylogram inferred from analysis using (A) combined plastid (CP) DNA regions (*trnL-F*, *trnT-L*, *trnS-G* and *trnK-matK*), (B) nuclear ribosomal DNA (ITS) with all accessions included. Values below branches are Bayesian posterior probabilities (BPP). Only BPP ≥ 0.95 are shown. Numbers after taxon names refer to vouchers listed in Supplementary material Table S1. Abbreviations are: *Ch. m*. = *Chionanthus mannii*; *Ch. o*. = *Chionanthus obtusifolius*; *N* = *Noronhia*; *N. e*. = *Noronhia emarginata*; *N. l*. = *Noronhia luteola*; *O. c*. = *Olea capensis*; *Olea e*. = *Olea europaea*.

Fig. S1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Comoranthus minor
Schrebera alata
Fraxinus americana
Fraxinus excelsior
Olea e. subsp. europaea 2
Olea e. subsp. cuspidata
Olea paniculata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus panamensis 1
Haenianthus salicifolius
Osmanthus americanus
Chionanthus virginicus 1
Chionanthus virginicus 2
Chionanthus retusus
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Osmanthus decorus
Osmanthus fragrans
Phillyrea angustifolia
Osmanthus austrocaledonicus
Notelaea microcarpa
Nestegis sandwicensis
Picconia azorica
Phillyrea latifolia
Forestiera neomexicana
Ch. m. subsp. congestus
Chionanthus mildbraedii
Ch. m. subsp. mannii 2
Chionanthus niloticus
Chionanthus richardsiae
Chionanthus pegleraе
Chionanthus battiscombei
Chionanthus foveolatus
N. grandifolia 1
Chionanthus obtusifolius
N. lanceolata 2
N. lanceolata 3
N. lanceolata 4
N. e. var. edentata
N. louveli 1
N. louveli 2
N. louveli 3
N. decaryana 1
Chionanthus broomeana
Chionanthus tropophyllus
N. ambrensis 1
N. ambrensis 2
N. grandifolia 3
N. longipedicellata 1
N. longipedicellata 2
N. longipedicellata 3
N. longipedicellata 4
N. linearifolia 3
N. linearifolia 1
N. grandifolia 2
N. sambiranensis
N. gracilipes 1
N. capuronii 2
N. gracilipes 3
N. gracilipes 5
N. capuronii 1
Chionanthus cordifolius
Chionanthus insularis
N. cochleata 3
N. cochleata 1
N. humbertiana
Chionanthus incurvifolius 1
Chionanthus incurvifolius 2
Chionanthus incurvifolius 3
N. peracuminata
N. oblanceolata 2
N. l. var. ankaranensis 1
N. l. var. ankaranensis 3
N. emarginata 5
N. emarginata 1
N. emarginata 3
N. emarginata 4
N. emarginata 2
N. linocerioides 1
N. brevituba 3
N. verticillata
N. linocerioides 2
N. linocerioides 3
N. brevituba 2
N. brevituba 1
N. seyrigii 1
N. divaricata 4
N. divaricata 1
N. divaricata 3
N. cruciata
N. crassiramosa 3
Ch. o. var. minoriflora
N. luteola 1
N. ovalifolia 1
N. ovalifolia 4
N. bolvini 2
N. comorensis 2
N. boinensis
N. alleizettei 1
N. tubulosa
N. alleizettei 2
N. alleizettei 4
N. alleizettei 3
N. densiflora
N. myrtoides
N. buxifolia 2
N. buxifolia 1
Africa 1
Africa 2
Madagascar
Reunion
Madagascar
Comoros
Madagascar
Florida
Hawaii
Reunion
Madagascar
Comoros
Madagascar
MFR clade
NCAIO clade
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Olea e. subsp. cuspidata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus panamensis 1
Chionanthus panamensis 2
Olea paniculata
Fraxinus americana
Fraxinus excelsior
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Osmanthus americanus
Chionanthus virginicus 1
Chionanthus virginicus 2
Osmanthus decorus
Osmanthus fragrans
Phillyrea angustifolia
Phillyrea latifolia
Chionanthus retusus
Osmanthus austrocaledonicus
Nestegis sandwicensis
Notelaea microcarpa
Picconia azorica
Forestiera neomexicana
Ch. m. subsp. congestus
Chionanthus mildbraedii
Ch. m. subsp. mannii 1
Chionanthus niloticus
Chionanthus foveolatus
Chionanthus battiscombei
Chionanthus pegleraе
Chionanthus richardsiae
Chionanthus broomeana
N. ambrensis 1
N. ambrensis 2
N. louveli 1
N. louveli 2
N. louveli 3
N. grandifolia 1
N. decaryana 1
N. decaryana 2
N. capuronii 1
N. capuronii 2
N. capuronii 3
N. grandifolia 2
N. sambiranensis
N. gracilipes 1
N. gracilipes 2
N. gracilipes 3
N. gracilipes 4
N. gracilipes 5
N. ovalifolia 1
N. ovalifolia 2
N. ovalifolia 3
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N. bolvini 2
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N. linearifolia 2
N. linearifolia 3
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N. alleizettei 2
N. myrtoides
N. boinensis
N. alleizettei 3
N. tubulosa
N. alleizettei 4
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N. buxifolia 2
N. oblanceolata 1
N. oblanceolata 2
N. pervilleana 1
N. pervilleana 2
N. cochleata 1
N. cochleata 2
N. cochleata 3
N. cruciata
N. verticillata
N. humbertiana
Chionanthus tropophyllus
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N. seyrigii 2
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N. emarginata 3
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N. emarginata 2
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N. e. var. edentata
N. brevituba 3
N. brevituba 1
N. brevituba 2
N. linocerioides 1
N. linocerioides 2
N. divaricata 1
N. divaricata 2
N. divaricata 3
Chionanthus insularis
N. lanceolata 1
N. lanceolata 2
N. lanceolata 3
N. lanceolata 4
N. lanceolata 5
Chionanthus incurvifolius 1
Chionanthus incurvifolius 2
Chionanthus incurvifolius 3
Africa 1-2
Reunion
Madagascar
Comoros
Madagascar
Comoros
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Reunion
Madagascar
Hawaii
Madagascar
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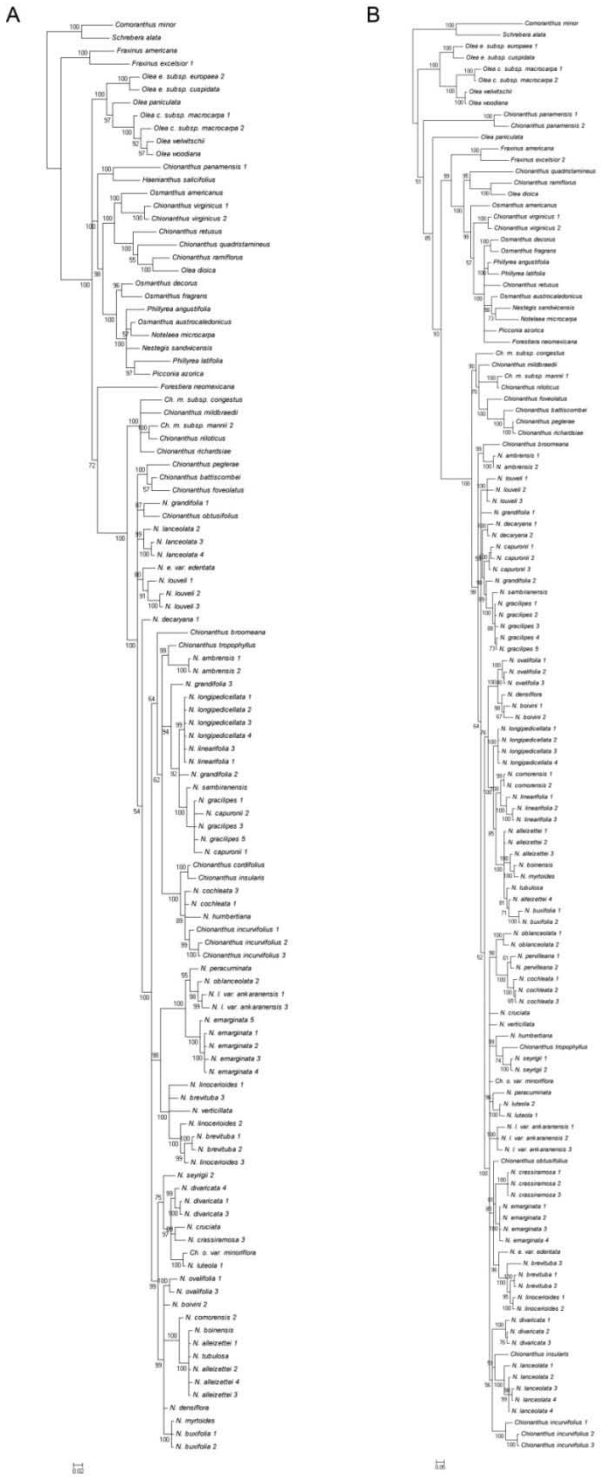
Madagascar
MFR clade
NCAIO clade

Fig. S2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Fraxinus americana
Fraxinus excelsior 1
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Olea paniculata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
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Olea woodiana
Chionanthus panamensis 1
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Osmanthus decorus
Osmanthus fragrans
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Osmanthus austrocaledonicus
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Nestegis sandwicensis
Phillyrea latifolia
Picconia azorica
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N. louveli 2
N. louveli 3
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Chionanthus tropophyllus
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N. ambrensis 2
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N. longipedicellata 2
N. longipedicellata 3
N. longipedicellata 4
N. linearifolia 3
N. linearifolia 1
N. grandifolia 2
N. sambiranensis
N. gracilipes 1
N. capuronii 2
N. gracilipes 3
N. gracilipes 5
N. capuronii 1
Chionanthus cordifolius
Chionanthus insularis
N. cochleata 3
N. cochleata 1
N. humbertiana
Chionanthus incurvifolius 1
Chionanthus incurvifolius 2
Chionanthus incurvifolius 3
N. peracuminata
N. oblanceolata 2
N. l. var. ank'aranensis 1
N. l. var. ank'aranensis 3
N. emarginata 5
N. emarginata 1
N. emarginata 2
N. emarginata 3
N. emarginata 4
N. linoceloides 1
N. brevituba 3
N. verticillata
N. linoceloides 2
N. brevituba 1
N. brevituba 2
N. linoceloides 3
N. seyrigii 2
N. divaricata 4
N. divaricata 1
N. divaricata 3
N. crociata
N. crassiramosa 3
Ch. o. var. minoriflora
N. luteola 1
N. ovalifolia 1
N. ovalifolia 3
N. boiviniana 2
N. comorensis 2
N. boiviniana
N. alleizettei 1
N. tubulosa
N. alleizettei 2
N. alleizettei 4
N. alleizettei 3
N. densiflora
N. myrtoides
N. buxifolia 1
N. buxifolia 2
B
Comoranthus minor
Schrebera alata
Olea e. subsp. europaea 1
Olea e. subsp. cuspidata
Olea c. subsp. macrocarpa 1
Olea c. subsp. macrocarpa 2
Olea welwitschii
Olea woodiana
Chionanthus panamensis 1
Chionanthus panamensis 2
Olea paniculata
Fraxinus americana
Fraxinus excelsior 2
Chionanthus quadristamineus
Chionanthus ramiflorus
Olea dioica
Osmanthus americanus
Chionanthus virginicus 1
Chionanthus virginicus 2
Osmanthus decorus
Osmanthus fragrans
Phillyrea angustifolia
Phillyrea latifolia
Chionanthus retusus
Osmanthus austrocaledonicus
Nestegis sandwicensis
Notelaea microcarpa
Picconia azorica
Forestiera neomexicana
Ch. m. subsp. congestus
Chionanthus mildbraedii
Ch. m. subsp. mannii 1
Chionanthus niloticus
Chionanthus foveolatus
Chionanthus battiscombei
Chionanthus peglerae
Chionanthus richardsiae
Chionanthus broomeana
N. ambrensis 1
N. ambrensis 2
N. louveli 1
N. louveli 2
N. louveli 3
N. grandifolia 1
N. decaryana 1
N. decaryana 2
N. capuronii 1
N. capuronii 2
N. capuronii 3
N. grandifolia 2
N. sambiranensis
N. gracilipes 1
N. gracilipes 2
N. gracilipes 3
N. gracilipes 4
N. gracilipes 5
N. ovalifolia 1
N. ovalifolia 2
N. ovalifolia 3
N. densiflora
N. boiviniana 1
N. boiviniana 2
N. longipedicellata 1
N. longipedicellata 2
N. longipedicellata 3
N. longipedicellata 4
N. comorensis 1
N. comorensis 2
N. linearifolia 1
N. linearifolia 2
N. linearifolia 3
N. alleizettei 1
N. alleizettei 2
N. alleizettei 3
N. boiviniana
N. myrtoides
N. tubulosa
N. alleizettei 4
N. buxifolia 1
N. buxifolia 2
N. oblanceolata 1
N. oblanceolata 2
N. perrieriana 1
N. perrieriana 2
N. cochleata 1
N. cochleata 2
N. cochleata 3
N. crociata
N. verticillata
N. humbertiana
Chionanthus tropophyllus
N. seyrigii 1
N. seyrigii 2
Ch. o. var. minoriflora
N. peracuminata
N. luteola 2
N. luteola 1
N. l. var. ank'aranensis 1
N. l. var. ank'aranensis 2
N. l. var. ank'aranensis 3
Chionanthus obtusifolius
N. crassiramosa 1
N. crassiramosa 2
N. crassiramosa 3
N. emarginata 1
N. emarginata 2
N. emarginata 3
N. emarginata 4
N. e. var. edentata
N. brevituba 3
N. brevituba 1
N. brevituba 2
N. linoceloides 1
N. linoceloides 2
N. divaricata 1
N. divaricata 2
N. divaricata 3
Chionanthus insularis
N. lanceolata 1
N. lanceolata 2
N. lanceolata 3
N. lanceolata 4
N. lanceolata 4
Chionanthus incurvifolius 1
Chionanthus incurvifolius 2
Chionanthus incurvifolius 3

CHAPTER 2

SPECIES LIMITS AND DIVERSIFICATION IN THE MADAGASCAR OLIVE (*NORONHIA*, OLEACEAE)

Introduction

Madagascar's unique and diverse biota testify to adaptive radiations of a variety of groups of animals and plants. The island has been isolated from major landmasses since at least 90 Ma (de Wit, 2003) with most colonization events hypothesized to have occurred in the Cenozoic (Yoder and Nowak, 2006; Russel et al., 2008; Kuntner and Agnarsson, 2011). While some of the island's biota are assumed to be Gondwanan relicts, most are thought to be derived from Tertiary African and Asian colonizers (Yoder and Nowak, 2006; Warren et al., 2010; Reddy et al., 2012). Levels of taxonomic endemism and species diversity are high (Goodman and Benstead, 2003), endemism being estimated to be above 90% for non-volant and non-marine vertebrates and above 80% for vascular plants (Goodman and Benstead, 2003; Callmander et al., 2011). The spatial pattern of endemism is even more impressive, with many species having narrow ranges and being known from only one or a few localities (Goodman and Benstead, 2003; Vences et al. 2009).

Patterns of diversification and endemism - mainly faunal - within Madagascar have been explained by various hypotheses. In particular, Yoder and Heckman (2006) proposed the ecogeographic constraint hypothesis to explain the east-west vicariance that follows the sharp bioclimatic division of Madagascar into humid east and dry west. Raxworthy and Nussbaum (1995) found mountain massifs of northern Madagascar to be centers of endemism and claimed they have a large role in the generation and maintenance of diversity in this region. Wilmé et al. (2006) suggested that watershed contractions during past climatic oscillations led to zones of isolation, thus promoting microendemism. Angel (1942) and Martin (1972) proposed a zoogeographical zonation

of Madagascar based on the distributions of its reptiles and lemurs respectively and the role of rivers as barriers to dispersal. Recent population genetic analyses confirm that large rivers and geographic distance are primary factors structuring some rodent and lemur populations, although they may not act as strict barriers to dispersal (e.g. Quéméré et al., 2010; Rakotoarisoa et al. 2010). All of these hypotheses emphasize the importance of physical barriers to gene flow in species divergence. By contrast, the current climate hypothesis (Pearson and Raxworthy, 2009) invokes a strong influence of environmental gradients in driving species divergence and in generating local endemism. Overall, these hypotheses suggest a major role of ecological diversification in lineage separation, and propose that adaptive speciation dominates. Moreover, the environment of Madagascar is particularly heterogeneous (Dewar and Richards, 2007), past changes may have led to successive population fragmentations and reconnections for many taxa (allopatry-sympatry oscillations). Such habitat dynamic may promote speciation events by reinforcement during secondary contacts (e.g. Aguilée et al., 2011).

The Malagasy plant genus *Noronhia* (Oleaceae), recently extended to include African relatives to accommodate phylogenetic relationships (Hong-Wa and Besnard accepted, see chapter 1 of this dissertation), may contain ca. 80 species although only 54 have names (taxonomic revision in progess, C. Hong-Wa, in prep.). Species of *Noronhia* distributed in the Malagasy Floristic Region (MFR, including Madagascar, Comoros and Mascarenes; Takhtajan, 1986) form a monophyletic radiation derived from an African ancestor (Hong-Wa and Besnard accepted, see chapter 1 of this dissertation). Colonization of Madagascar may have occurred in the late Cenozoic (ca. 23 Ma) followed by a burst of diversification. Relationships within this group are currently

largely unresolved, and basal divergences seem to have been rapid, leaving little signal of lineage separation. In contrast, the extent of morphological variation within this group (e.g. variation in leaf shape, size, texture and venation pattern, flower size, color and arrangement, fruit shape, size and ornamentation) and ecological diversity are extremely high. Indeed, *Noronhia* grows in arid to humid habitats from sea level to above 2000 m, and specializes in both karst and quartzite areas. The mechanisms by which diversity within *Noronhia*, and in fact most groups of organisms in Madagascar, arose are largely unknown. However, the various hypotheses that explain species diversity, endemism and diversification in Madagascar can shed light on the diversification within *Noronhia*. In particular, the ecogeographic constraint (ECH), the riverine barrier (RBH), the watershed contraction (WCH) and the current climate (CCH) hypotheses, proposed to explain the observed patterns of faunal diversification and endemism on the entire island, may also be broadly applicable to its flora. The radiation of *Noronhia*, therefore, provides an opportunity to examine these four hypotheses from a plant perspective.

The signature of low genetic differentiation contrasting with high morphological and ecological diversity within *Noronhia* raises questions about the boundaries of the species it contains. Morphology was initially used to recognize species within this group, which resulted in a fairly good, but now outdated and unsatisfactory, taxonomy (Perrier de la Bâthie, 1952). Most morphological plant species may correspond to reproductively independent lineages and represent biologically real entities (Rieseberg et al., 2006), but species boundaries may be obscure, for instance, in the case of cryptic, plastic or polymorphic phenotypes (Duminil and Di Michele, 2009). As separately evolving lineages with contingent properties such as morphological distinctiveness, reciprocal

monophyly, reproductive isolation and ecological divergence, species can be recognized using these properties, independently or together, as evidences of their boundaries (de Queiroz, 2007). As such, an integrative approach using multiple criteria (e.g. morphology, ecology and genetics) has been increasingly applied to the species delineation problem (Dayrat, 2005; Yoder et al., 2005; Raxworthy et al., 2007; Rissler and Apodaca, 2007; Bond and Stockman, 2008; Leaché et al., 2009; Rivera et al., 2011). The "integration by cumulation" approach (Padial et al., 2010) acknowledges that lines of evidence are contingent inr their existence, their order of appearance and their magnitude, thus there may not be concordance between them, and any single or combined line of evidence can provide evidence of species. This approach can also be useful when dealing with recently evolved lineages or recent and/or rapid radiations (Sites and Marshall, 2003; Padial et al., 2010). It is opposed to "integration by congruence" in which agreement between at least two lines of evidence is necessary to recognize species (Padial et al., 2010).

The Malagasy *Noronhia*, being a monophyletic radiation and being morphologically, genetically and ecologically diverse, represents an ideal setting to study species diversification. Our approach to understanding diversification in *Noronhia* focused on phylogeny, taxonomy and biogeography. In particular, we used a multifaceted, integrated approach to (1) reassess phylogenetic relationships among species of *Noronhia* distributed in the MFR with a denser taxon sampling, (2) examine patterns of morphological variation and species limits in a phylogenetic context and across geographical scales, and (3) evaluate the congruence between the predictions of the four

hypotheses of diversification mentioned above with phylogenetic patterns within *Noronhia*.

Methods

Phylogenetic analyses

Chloroplast (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*) and nuclear (ITS) DNA sequences were obtained from 39 of the 54 currently described species of *Noronhia* distributed in the MFR (Hong-Wa and Besnard accepted, see chapter 1 of this dissertation). Three described subspecies and varieties, whose rank may warrant elevation to that of species, were also included. Sequences from African relatives as well as sequences from other members of Oleaceae were also included as outgroups. This dataset was complemented with sequences from three other described species, four taxa of uncertain identity and 19 of the 32 as yet undescribed species of *Noronhia* (C. Hong-Wa, in prep.). Thus this dataset of 157 accessions included a total of 68 species of *Noronhia* from the MFR, of which 40 were represented by multiple individuals. Voucher specimens are listed in Supplementary Table S1 and sampling localities are shown in Fig. 1. Described species of *Noronhia* known to occur in the MFR and absent from this dataset are: *N. ayresii* and *N. boutonii* from la Réunion; *N. mayottensis* from the Comoros (this taxon is also of dubious status); and *N. ecoronulata*, *N. crassinodis*, *N. jeremii*, *N. leandriana*, *N. populifolia*, *N. urceolata*, *N. verrucosa* and *N. verticilliflora* from Madagascar. The Malagasy species *N. ecoronulata*, *N. populifolia* and *N. verticilliflora* are each known only from a single collection 100 to 110 years old and from a single locality. However, the status of *N. ecoronulata* and *N. verticilliflora* as full species is uncertain as they

cannot obviously be distinguished from the sampled species *N. alleizettei* and *N. verticillata* respectively. Thirteen undescribed species remain to be sampled for their DNA.

Laboratory protocols, primers used and data preparation are described in Hong-Wa and Besnard (accepted, see chapter 1 of this dissertation). All sequences were submitted to GenBank (accession numbers are given in Supplementary Table S1). Despite inconsistencies between the chloroplast and ITS datasets (p-value < 0.05 from the Partition Homogeneity test), topological discordances among MFR species were considered to be soft incongruences. Therefore we chose to follow a total evidence approach and combined the two datasets into a single matrix for subsequent analyses. The combined dataset had 4500 bp, of which 691 bp were variable and 438 bp were parsimony-informative; the overall mean sequence divergence was 5.3%. Phylogenetic analyses of the combined dataset used maximum likelihood (ML) conducted on RAxML v7.2.6 (Stamatakis, 2006) and Bayesian inference (BI) carried out on MrBayes v.3.1.2 (Ronquist and Huelsenbeck, 2003) on the CIPRES portal (Miller et al., 2010). Phylogenetic analyses and program settings were replicated from Hong-Wa and Besnard (accepted, see chapter 1 of this dissertation) except for the Markov chain Monte Carlo (MCMC) of the BI, which used an exponential prior of 1 for the shape parameter and was run for 50 million generations with a sampling frequency of 5000 generations. Temperature was also reduced to 0.02 to allow optimal chain swapping. Convergence of runs was checked with the online application AWTY (Nylander et al., 2008).

Patterns of morphological variation and niche differentiation

For this study, we measured 973 herbarium specimens deposited at G, MO, P, TAN and TEF (abbreviated according to the Index Herbariorum, Holmgren *et al*., 1990) to evaluate patterns of morphological variation within the MFR *Noronhia* and assess the species boundaries. We first sorted specimens into narrowly defined groups based on the presence of diagnostic, mainly qualitative, vegetative and reproductive features (e.g. plant habit, presence of indumentum, color of stem, leaf, flower and fruit, texture of leaf and fruit, venation pattern, location of flowers, inflorescence type). Initial discriminant analysis suggested that seven of these groups were not statistically different from others and were lumped together. In total 87 groups ("hypotheses of species", abbreviated from here onwards as species) were distinguished and named when they corresponded to the 54 currently described species. Twelve groups included only one or two specimens; 33 specimens lacking distinctive characters or exhibiting intermediacy could not be assigned to any named or unnamed groups. For each specimen, we measured 14 leaf, 11 flower and 11 fruit variables (Table S2). Three measurements per variable were taken from each specimen and averaged. In general, there were very few specimens of each group with flowers and fruits, so analyses of flower and fruit variables were carried out separately. Measurements were taken from organs from similar developmental stages to reduce size bias, which led to additional instances of missing values. Overall, we chose to maximize the number of groups and individuals in each analysis when missing data were an issue, thus occasionally excluding some characters. In addition, 19 bioclimatic variables and elevation were obtained from the WorldClim database (Hijmans et al., 2005) and extracted for each herbarium specimen to identify the species' climate space and assess patterns of ecological variation. Both morphological and bioclimatic variables

were used in independent Principal Component Analysis (PCA) in R 2.10.1 (R Development Core Team, 2009). The PCA of bioclimatic variables provided a quick assessment of the relative positions of each species in climate space (Zhu et al., 2012).

Patterns of morphological variation and niche differentiation were assessed in phylogenetic and geographic contexts. The phylogenetic context was established by focusing on clades with bootstrap (BP) ≥ 70% and/or posterior probability (PP) ≥ 0.90. In total, 16 such clades were identified (Fig. 2), and PCAs of morphological and bioclimatic variables were carried out independently on these clades to estimate patterns of variation among closely related species. For species that were part of polytomies or that were not available for molecular study, analyses were conducted in a biogeographical context. In particular, we used the ecoregions proposed by Wilmé et al. (2006) as biogeographic units. Independent PCAs of morphological characters and bioclimatic variables were performed within each of the 12 biogeographic ecoregions to assess patterns of variation among co-occurring species.

Patterns of diversification

We used different biogeographic zonations of Madagascar to represent the four hypotheses of diversification in the island: the bioclimatic zones of Schatz (2000) for ECH, the zoogeographical zonations of Martin (1972) for RBH, the centers of endemism of Wilmé et al. (2006) for WCH and the climate clusters of Pearson and Raxworthy (2009) for CCH (Fig. 1). Phylogenetic predictions could be derived for the four hypotheses of diversification (Vences et al., 2009). In particular, the ECH predicts an east-west partition between clades or sister species given the sharp bioclimatic

distinction between these two regions, or by extension a genetic break between clades or species from different bioclimatic zones. The RBH predicts genetic differentiation between clades or sister species occurring on either side of major rivers, or by extension a genetic break between clades or species from areas separated by any biogeographic barrier such as mountain ranges. The WCH predicts a genetic differentiation among watersheds that served as zones of isolation. It also predicts that sister species would occupy contiguous watersheds. Likewise, the CCH predicts a genetic break between clades or species from different climate clusters. In the humid eastern escarpment and the central highlands, sister species are expected to occupy adjacent climate clusters along elevational gradients whereas in the dry western lowlands, sister species are expected to be separated along the north-south gradient.

Overall, genetic differentiation is expected to increase with geographic distance or the presence of physical barriers to gene flow. We thus tested for correlation between pairwise genetic and pairwise geographic distances using a Mantel test for each clade. In light of each species' range (Fig. S1), we then assessed whether the observed phylogenetic patterns supported the phylogenetic and spatial predictions of each of the four hypotheses. Since these hypotheses were formulated only for mainland Madagascar (excluding smaller islands such as Nosy Be or Ste Marie), species and individuals occurring in these smaller islands were removed from this analysis of diversification.

Results

Phylogenetic analyses

The analyses of the combined plastid (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*) and nuclear (ITS) dataset resulted in a highly concordant Bayesian and likelihood phylogenetic hypothesis within *Noronhia* (Fig. 2) even if the Bayesian analyses suffered from a lack of convergence between independent runs. The monophyly of *Noronhia* was strongly supported. The MFR taxa clearly separated from African species, supporting the single radiation of the genus within Madagascar, with subsequent colonization of surrounding islands. This radiation was highly supported in ML (BS = 96%) and BI (PP = 1). Relationships within this MFR clade were characterized by short internodes and resolved into only 16 clades with moderate to high support values (BS ≥ 70% and/or PP ≥ 0.85) and large polytomies. These clades are identified here with capital letters (A-P) and will be referred to accordingly.

Within each clade, species, usually represented by multiple individuals, separated clearly from each other (Fig. 2). For instance, the three species (*N. capuronii*, *N. gracilipes* and *N. sambiranensis*) forming the clade B were all reciprocally monophyletic. This was also the case for the clades C, E, F, I, J, K, L, N and P. However, ambiguous relationships were observed within the clades A, D, G, H, M and O. Thus within clade D, the three individuals representing *N. linearifolia* did not cluster together. Similarly, the two individuals of *N. boivini* in clade H as well as the individuals of *N. pervilleana* and *N. sp32* in clade O failed to form monophyletic groups but instead were either paraphyletic or formed parts of polytomies. Finally, individuals of *N. brevituba* and *N. linocerioides* within clade M were not reciprocally monophyletic but instead formed a strongly supported mixed clade. There were also instances where different individuals of one species occurred in more than one clade or were parts of

polytomies (e.g. *N. grandifolia*). However, in general, individuals of the same species still clustered together (e.g. *N. crassiramosa*, *N. comorensis*, *N. decaryana* and *N. louveli*) with high support values within the large polytomy (Fig. 2).

The 19 undescribed species of *Noronhia* included here showed overall good genetic differentiation from each other and from the described species (Fig. 2). For instance, clade C was only composed of two undescribed species (*N. sp11* and *N. sp13*) that were well separated. The clades L and P also included undescribed species (*N. sp38* and, *N. sp2* and *N. sp22* respectively) that were reciprocally monophyletic and distinct from the described species. However, such clear patterns were not always recovered. In some cases, individuals of these undescribed species did not cluster (*N. sp28* and *N. sp30*), or if they did, they were part of the large polytomy (*N. sp5*, *N. sp15*, *N. sp27*).

Patterns of morphological variation and niche differentiation

Results of the PCAs of morphological and environmental variables on the 16 phylogenetic clades and 12 biogeographic zones are summarized in Tables 1 and 2 and Supplementary Figs. S3-S32. Overall, species of *Noronhia* showed distinct patterns of variation. Individuals of most species formed a cluster distinct from such other clusters in multivariate analyses of vegetative characters alone or a combination of vegetative and flower or fruit characters. In some cases, distinction among species was observed only in separate analyses of flower or fruit characters (Tables 1 and 2, Figs. S3, S11 and S20). In other cases, patterns of variation were obscured by the presence of a widely distinct species, the exclusion of which clarified the patterns of the remaining species (e.g. Figs. S10, S15 and S17). Species that included one or two specimens only were also

mostly distinguishable. Analyses of bioclimatic and elevation variables showed a high degree of overlap between the climate space of different species. This is not surprising since species of *Noronhia* often co-occur in several forested areas. However, species occupying the same or similar climatic spaces could usually be differentiated in morphospace (e.g. Figs. S4, S5 and S14).

Of the 50 species belonging to the various clades and thus analyzed in a phylogenetic context, five (*N. ambrensis*, *N. sp9*, *N. sp22*, *N. sp25* and *N. sp38*) and three (*N. sp20*, *N. sp22* and *N. sp38*) lacked information on flowers and fruits respectively (Table 1). Species distinguishable by vegetative characters alone included *N. densiflora*, *N. sp22* and *N. sp38*. All but six species (*N. boivini*, *N. brevituba*, *N. linocerioides*, *N. luteola* var. *ankaranensis* to be considered as a species, *N. mangorensis* and *N. sp9*) formed discrete morphological clusters in at least one analysis based on vegetative, flower or fruit features only or some combination of these three datasets.

Of the 37 species falling into large polytomies or lacking molecular data, and thus analyzed in a geographic context, 13 (*N.* aff. *candicans*, *N. crassiramosa*, *N. verrucosa*, *N. sp4*, *N. sp6*, *N. sp8*, *N. sp17*, *N. sp18*, *N. sp19*, *N. sp28*, *N. sp30*, *N. sp39* and *N. sp40*) and three (*N.* aff. *candicans*, *N. sp14* and *N. sp40*) were missing information on flowers and fruits respectively (Table 2). Four species (*N. verrucosa*, *N. sp5*, *N. sp15* and *N. sp40*) could not be clearly distinguished. Species that were morphologically similar were analyzed separately to see if they could really be differentiated. For instance, *N.* aff. *candicans* and *N. candicans* were analyzed together and appeared distinct although only vegetative characters were available (Fig. S31A). These two species were

phylogenetically unrelated but geographically sympatric. *Noronhia* aff. *candicans* was also distinct from other species occurring in the same area (Fig. S31B).

Patterns of diversification

There was a lack of correlation between genetic and geographic distances among species within each clade (Table 3) suggesting that geographic distance or presence of physical barriers to gene flow alone was not a sufficient predictor of genetic distance. Indeed, nucleotide diversity was fairly high within clades J, M, N and P (4.5%, 5%, 4.9% and 4.8% respectively) regardless of geographic distance between members of the clades (16 km, 32 km, 217 km and 314 km respectively). Similarly, nucleotide diversity was relatively low within clades D and O (1.8% and 1.2% respectively), but their members were 43 km and 383 km apart respectively.

There was also an overall lack of evidence in support of the ECH, as there was no evidence of strong phylogenetic and spatial fit with the predictions of the RBH, WCH and CCH (Fig. S2). Indeed, the ECH predicted four bioclimatic subdivisions or at least an east-west partition, none of which was apparent in the phylogeny either among or within clades. Instead, species from the same region or occupying the same bioclimatic zone tended to cluster together within clades, but the overall pattern of the phylogeny was a mosaic. Likewise, the correspondence between the phylogeny and major genetic breaks predicted by the RBH was rather weak. In many instances, species occupying different sides of a river were more closely related than species occurring on the same side. For example, *N. capuronii* and *N. ambrensis*, both occurring north of Mahavavy

river, belonged to different clades within which they were respectively related to *N. sambiranensis* and *N. sp20*, both found south of Mahavavy river (Fig. S2).

Similar patterns were also observed for the WCH. However, even if species from the same watershed were not always closely related, and species from different watersheds were not always particularly distinct (Fig. S2), geographic proximity seems to be of some importance. There was no clade with species from northern and southern Madagascar for instance. Instead, related species were found within clusters of geographically close watersheds, e.g. clade B (CE1, CE10 and CE12) or clade H (CE2, CE4 and CE5) (Fig. S2), suggesting a broad biogeographic differentiation. The CCH also did not obtain strong support from the data; sister species did not always occur in adjacent clusters but more often they would occupy the same cluster, e.g. clade C, or one species would straddle two or more zones, e.g. clade J (Fig. S2).

Discussion

Evidence for multiple distinct species

Debates over the nature and definition of species have dominated the systematic world for decades. Species have been defined according to different, but not mutually exclusive, concepts such as biological, morphological, phylogenetic and evolutionary. However, sound delineation of species boundaries, regardless of the conceptual framework, is critical if species are to be used as a unit of evolution (Mayr, 1969), a measure of biodiversity (Gaston, 2000; Tilman and Lehman, 2001), or as currency for conservation (Myers et al., 2000). Meaningful recognition of species, considered as separately evolving lineages, can be based on multiple lines of evidence, but a single

form of evidence can be sufficient in any one case (de Queiroz, 2007). Each line of evidence, resulting from an evolutionary process affecting lineage splitting and divergence, may or may not appear with the same order and magnitude or at the same level (de Queiroz, 2007; Padial and De la Riva, 2010). Therefore, congruence between lines of evidence, although desirable, is not necessary to recognize species and incongruence is even expected since the changes during speciation are contingent and vary in their order of appearance and their magnitude (de Queiroz, 2007; Padial and De la Riva, 2010). Recognition of species that follows an integrative framework without the necessity of congruence has been referred to as "integration by cumulation" by Padial et al. (2010), and depends on the assumption that differences in any taxonomic character suggests the existence of distinctions between groups of specimens, and thus of a species. This approach is also most appropriate in cases of recent adaptive radiations (Padial et al., 2010).

In this study, we considered three lines of evidence as operational criteria to recognize species: bioclimatic/ecological, molecular and morphological distinctions. Twenty-two species lacked molecular data, and so congruence with morphology could not be assessed. Moreover, the combined dataset of chloroplast (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*) and nuclear (ITS) DNA regions resulted in large polytomies, which meant that congruence between molecular and morphological characters could not be easily assessed. Consequently, we incorporated various lines of evidence for recognizing species within *Noronhia* using the integration-by-cumulation approach. The strength of the various operational criteria to evaluate the limits among the 87 species varied, the bioclimatic data being the least informative. Indeed, phylogenetically related species

usually occupied the same climatic niche (Table 1, Figs. S3-S18); analyses carried out in the geographic context obviously focused on species from the same region, and so could hardly show bioclimatic differentiation (Table 2, Figs. S19-S30). By contrast, morphological data provided the strongest support for species limits, with phylogenetically or geographically related species usually showing clear morphological differentiation. Molecular data also provided good support of species limits (Tables 1 and 2) despite unresolved relationships at deeper levels (Fig. 2) since species sharing similar morphology or environment were usually not immediately related. Given the contingency of each line of evidence within separately evolving lineages, concurrent support for the same species is not expected nor does the lack of support from a particular criterion justify the rejection of that species as long as it is likely to represent a distinct evolutionary trajectory (de Queiroz, 2007; Padial and De la Riva, 2010).

Overall, 84 of 87 species could be recognized using the integration-by-cumulation approach (Table 4). Species that failed to differentiate ecologically, genetically or morphologically include *N. boivini* and *N. mangorensis*, *N. luteola* var. *ankaranensis* and *N. sp9*, *N. brevituba* and *N. linocerioides* (Table 1, Figs. S10, S14 and S15). *Noronhia mangorensis* and *N. brevituba* appear to be inland forms of the more littoral species *N. boivini* and *N. linocerioides* respectively (Fig. S1), from which they differ mostly by the presence of smaller leaf blades with longer acumen, and furthermore by the length of the sepals and corona for *N. brevituba* and *N. linocerioides*. Conversely, *N. sp9* did not differ morphologically from *N. luteola* var. *ankaranensis* but showed some genetic distinction (Table 1, Figs. S14). These two species have overlapping ranges (Fig. S1), so geographic distance is not an explanation for the genetic distinction. However, only a single

accession of *N. sp9* (versus three for *N. luteola* var. *ankaranensis*) was available for molecular study. Further molecular sampling will provide better understanding of the relationship between these two species. Similarly, *N. sp5* and *N. sp15* were not clearly distinct in both climatic and morphological spaces (Table 2, Fig. S20) but were genetically different (Fig. 2) and geographically distant. *Noronhia sp15* is littoral and *N. sp5* is inland, occurring above 1000 m elevation; both grow in the eastern part of Madagascar. *Noronhia sp40* also did not clearly differ from *N. sp15* (Table 2, Fig. S20) and could be a variant of this species, but this is based only on vegetative characters. Finally, *N. verrucosa* could not be distinguished from *N. sp5* (Table 2, Fig. S20) despite distinctive diagnostic features such as obtrullate leaf blades and verrucose fruit versus obovate leaf blades and smooth fruit. However, only a single specimen lacking molecular data was available for analysis. Furthermore, its flower is unknown and qualitative characters like those just mentioned were not used in the multivariate analyses. For described species for which the status has been compromised by insufficient data, notably small sample size, recognition is not rejected until further samples are available. This is the case for *N. verrucosa*. However, other described species that have been initially combined with other species as a result of a discriminant analysis and a lack of distinctive features, and which did not further distinguish from those in the PCAs will be subjected to taxonomic reevaluation (C. Hong-Wa, in prep.). The new species will also be described.

Mechanisms of species diversification

The results presented here provided evidence for extensive morphological variation within *Noronhia* despite low genetic differentiation among species. The disconnect

between morphological and molecular changes is not surprising considering that the genetic markers used here are *a priori* neutral and not involved in the evolution of morphological differences (Campagna et al., 2012). This contrasting pattern suggests a rapid, recent and/or incomplete radiation (Parchman et al., 2006; Campagna et al., 2012). Whether this rapid diversification is ecologically-mediated remains to be determined, but the lack of differentiation in climatic space indicates that factors other than climate have played a major role in driving this pattern.

The four models of diversification considered here proposed explicit mechanisms by which species divergence could arise (Martin, 1972; Wilmé et al., 2006; Yoder and Heckman, 2006; Pearson and Raxworthy, 2009; Vences et al., 2009). Our analytical assessment of support for these models is rather simplistic and does not warrant robust conclusions. Nonetheless, the overall fit between the phylogeny and the physical and ecological barriers to gene flow is poor (Table 3, Fig. S2). The Mantel test showed that geographic distance was not a good predictor of genetic differentiation (Table 3).

The overall poor correspondence between the predictions of various diversification models and the observed patterns suggests that a combination of several factors promotes diversification. In particular, geographic isolation alone is not sufficient to explain patterns of diversification within *Noronhia*. Models invoking parapatric divergence along environmental gradients such as the CCH (Pearson and Raxworthy, 2009) also account for some of the observed patterns. Indeed, some closely related species of *Noronhia* exhibit some differentiation along elevational bands (e.g. *N. boivini* vs. *N. mangorensis*, *N. brevituba* vs. *N. linocerioides*, and *N. sp5* vs. *N. sp15*). However, this is not always the case. Overall, the apparent lack of differentiation between different

biogeographic zones or the pronounced differentiation within the same zone may reflect the signature of past climatic fluctuations and forest dynamics (e.g. Aguilée et al. 2011). Moreover, the four models considered here have been formulated for terrestrial faunal diversification in Madagascar and highly vagile species did not support these models (Weyeneth et al., 2011). Thus they may not be suitable for plants dispersed by these animals. However, the CCH closely resembles the phytogeographic subdivisions of Humbert (1955) and would presumably be more appropriate than the other three models. In any case, it is likely that fine-scale ecological processes such as habitat specialization or biotic interactions also contributed to the diversification of *Noronhia* in addition to the coarse-scale processes invoked by the four models.

Implications for diversity estimates

This study indicates an almost two-fold increase in species richness within this group since the last taxonomic treatment 60 years ago (Perrier de la Bâthie, 1952). This increase results mainly from many new collections accumulated since then but involves also some generic rearrangements (Hong-Wa and Besnard accepted, see chapter 1 of this dissertation) and rank changes (C. Hong-Wa, in prep.). Although taxon sampling is still an issue (e.g. sampling for molecular and morphological analyses differed; small sample size), the integrated analyses of bioclimatic, molecular and morphological data, interpreted in phylogenetic and geographic contexts, provide useful insights into the complex nature of divergence within this group and allow better assessment of species boundaries. Anatomical and chromosomal data would provide additional information for better separating the species of *Noronhia*.

This study also shows that in *Noronhia*, most – if not all - species delimited on morphological grounds likely correspond to independent lineages and thus emphasizes the importance of morphology for proposing initial hypotheses of species. These hypotheses can then be tested with further data from ecology, geography or molecular sequences (Meudt et al., 2009; Valcárcel and Vargas, 2010; Zapata, 2010; Barrett and Freudenstein, 2011). As new data and approaches become available, more robust and more stable hypotheses of species will be generated. In the meantime, it seems crucial to document these species hypotheses as we endeavor to inventory, understand and protect the biodiversity on Earth, especially in poorly known areas.

Supplementary materials

Table S1. List of species included in this study with corresponding GenBank accession numbers for the different loci.

Table S2. List of characters measured for morphometric analyses.

Fig. S1. Maps showing the distribution of species analyzed in a phylogenetic context (Figs. S3-S18).

Fig. S2. Biogeographic zones recognized under the ecogeographic constraint (ECH), riverine barrier (RBH), watershed contraction (WCH) and current climate (CCH) hypotheses mapped onto the phylogenetic tree.

Figs. S3-S32. Results of principal component analyses of bioclimatic and morphological data carried out in phylogenetic (Figs. S3-S18) and geographic (Figs. S19-S32) contexts. Abbreviations: BIO, FL, FR, VEG, VEG + FL and VEG + FR are respectively

bioclimatic, flower, fruit, vegetative, vegetative and flower and vegetative and fruit variables. Number (2) after an abbreviation refers to a second analysis with the same set of variables but excluding the species indicated in brackets.

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Table 1

Summary of the results of PCA on quantitative environmental and morphological data of species belonging to clades A-P (Figs. S3-18). Signs indicate presence (+) or absence (-) of distinction. Number in brackets [1] means that only a single specimen was available in the analysis. Abbreviations are: BIO = bioclimate; MOLEC = molecules; MORPHO = morphology; FL = flower; FR = fruit; VEG = vegetative; mi = material insufficient; nk = not known. Some distinctive floral features such as inflorescence type, flower color and presence of corona are shown. Flower color refers to the dominant hue of the outer side of the corolla; outer and inner sides often have different colorations.

CLADE	SPECIES	BIO	MOLEC	MORPHO			FLOWER		
				VEG	FL	FR	TYPE	COLOR	CORONA
A	grandifolia	-	-	-	+	-	cyme	orange	no
	introversa	-	+	-	+	-	cyme	pink	yes
B	capuronii	-	+	+	+	+	cyme	reddish	no
	gracilipes	-	+	+	+	+	cyme	reddish	no
	sambiranensis	-	+	+	+	+	cyme	red	no
C	sp11	-	+	+	+	+	fascicle	cream	yes
	sp13	-	+	+	+	+	cyme	white	yes
D	candicans	-	-	+	+	+	fascicle	purplish	yes
	linearifolia	-	+-	+	+	+	solitary	red	yes
E	ambrensis	+	+	+	nk	+	cyme	nk	nk
	broomeana	+	+	+	+ [1]	+ [1]	cyme	white	no
	sp20	+	+	+	+	nk	cyme	nk	no
F	buxifolia	-	+	+	+	+	fascicle	red	yes
	myrtoides	-	+	+	+	+	fascicle	ivory	yes
G	alleizettei	-	+-	-	+	-	solitary	white	no
	boinensis	-	-	+	+ [1]	+	fascicle	yellow	yes
	tubulosa	-	-	-	+	-	solitary	orange	yes
	sp21	-	-	+	+ [1]	- [1]	cyme	pink	yes
H	boivini	-	-	-	-	-	fascicle	purplish	yes
	densiflora	-	+	+	mi	mi	cyme	red	no
	mangorensis	-	+	-	-	-	fascicle	purplish	yes
	ovalifolia	-	+	+	+	+	fascicle	pinkish	yes
I	tropophylla	-	+	-	+	-	cyme	white	no
	seyrigii	-	+	-	+	-	cyme	red	yes

J	divaricata	-	+	+	+	+	cyme	yellow	yes
	sp34	-	+	+ [1]	+ [1]	+ [1]	cyme	purple	yes
K	minoriflora	+	+	+	+	+	cyme	white	no
	luteola	+	+	+	+	+	cyme	ivory	yes
L	ankaranensis	-	+	-	+	-	cyme	whitish	yes
	emarginata	+	+	+	+	+	cyme	yellow	yes
	oblanceolata	-	+	+	mi	+	solitary	white	yes
	peracuminata	-	-	+	+	+	cyme	nk	yes
	sp9	-	-	-	nk	-	cyme	nk	nk
	sp38	-	+	+	nk	nk	nk	nk	nk
M	brevituba	-	-	-	+-	-	cyme	yellow	yes
	linocerioides	-	-	-	+-	-	cyme	yellowish	yes
	verticillata	-	+	+	+	+	cyme	yellow	yes
	sp25	-	+	-	nk	+	cyme	nk	nk
N	obtusifolia	-	-	+	+	mi	cyme	white	no
	edentata	-	-	+	+	+	cyme	cream	yes
	lanceolata	+	+	+	+	+	cyme	white	no
O	cochleata	-	+	-	+	-	cyme	yellow	yes
	humbertiana	-	+	-	+ [1]	+	cyme	orange	yes
	pervilleana	-	-	-	+	-	cyme	cream	yes
	sp32	-	-	+	+	+	cyme	pinkish	yes
P	incurvifolia	-	+	-	+	-	cyme	white	no
	insularis	+	+	+	+ [1]	+ [1]	cyme	yellow	no
	sp2	-	+	-	+	-	cyme	cream	yes
	cordifolia	-	+	-	mi	+ [1]	cyme	yellowish	no
	sp22	-	+	+	nk	nk	nk	nk	nk

Table 2

Summary of the results of PCA on quantitative environmental and morphological data of species found within the 12 centers of endemism (CE) and northern (RDN) and southern (RDS) retreat-dispersion watersheds of Wilmé et al. (2006), and the Comoros (COM) (Figs. S19-30). Signs indicate presence (+) or absence (-) of distinction. Number in brackets [1] means only a single specimen was available in the analysis. Abbreviations are: BIO = bioclimate; MOLEC = molecules; MORPHO = morphology; FL = flower; FR = fruit; VEG = vegetative; mi = material insufficient; na = not available; nk = not known. Some distinctive floral features such as inflorescence type, flower color and presence of corona are shown although they were not used in the PCA. Flower color refers to the dominant hue of the outer side of the corolla; outer and inner sides often have different colorations. Species occurring in more than one watershed are shown in bold.

ZONES	SPECIES	BIO	MOLEC	MORPHO			FLOWER		
				VEG	FL	FR	TYPE	COLOR	CORONA
CE1	aff. candicans	-	-	+	nk	nk	nk	nk	nk
	crassinodis	-	na	+	+	-	fascicle	orangish	yes
	aff. crassinodis	-	-	+	+	- [1]	fascicle	pinkish	yes
	longipedicellata	+	-	+	mi	- [1]	cyme	purplish	yes
	louveli	-	+	+ [1]	+ [1]	mi	cyme	red	yes
	sp17	-	+	+	nk	+	cyme	nk	nk
	sp28	-	-	+ [1]	nk	+ [1]	solitary	nk	nk
	sp30	-	-	+	nk	+ [1]	fascicle	nk	nk
CE2	**thouarsii**	-	na	-	mi	+ [1]	cyme	nk	no
	crassiramosa	-	+	+	nk	+ [1]	cyme	nk	nk
	decaryana	-	+	-	+	-	cyme	orangish	no
	jeremii	-	na	-	mi	+ [1]	cyme	yellow	no
	louveli	-	+	-	+	-	cyme	red	yes
	verrucosa	-	na	- [1]	nk	- [1]	cyme	nk	nk
	sp4	-	na	+	nk	+ [1]	cyme	nk	nk
	sp5	-	+	+-	+-	-	cyme	orangish	yes
	sp6	-	na	+	nk	+	cyme	nk	nk
	sp8	-	na	-	nk	-	cyme	nk	nk
	sp15	-	+	+-	+-	- [1]	cyme	cream	yes

	sp26	-	na	-	+	-	cyme	pinkish	yes
	sp36	-	na	-	+ [1]	mi	cyme	nk	yes
	sp40	-	na	-	nk	nk	nk	nk	nk
CE3	**crassiramosa**	-	+	+	nk	mi	cyme	nk	nk
	decaryana	-	+	+ [1]	mi	mi	cyme	orangish	no
	louveli	+	+	+ [1]	mi	mi	cyme	red	yes
	sp31	-	na	+	+	mi	cyme	pinkish	yes
	sp37	-	na	+	+	mi	cyme	reddish	yes
CE4	**decaryana**	+	+	+	mi	mi	cyme	orangish	no
	sp15	+	+	+	mi	mi	cyme	cream	yes
	sp26	+	na	+	mi	mi	cyme	pinkish	yes
CE5	sp14	-	na	+	+	nk	cyme	red	no
	sp15	-	+	+	+	mi	cyme	cream	yes
	sp16	-	na	+	+	+ [1]	cyme	white	yes
	sp18	-	+	+ [1]	nk	+ [1]	cyme	nk	nk
	sp26	-	na	+ [1]	+ [1]	+ [1]	cyme	pinkish	yes
CE8	**leandriana**	-	na	+	+	mi	cyme	nk	yes
	louveli	+	+	+ [1]	+ [1]	mi	cyme	red	yes
	urceolata	-	na	+	+	mi	cyme	nk	yes
CE9+COM	comorensis	+	+	+	+	mi	fascicle	yellow	yes
	leandriana	+ [1]	na	+ [1]	+ [1]	mi	cyme	nk	yes
CE10	**louveli**	- [1]	+	+ [1]	mi	+ [1]	cyme	red	yes
	populifolia	+ [1]	na	+ [1]	mi	+ [1]	cyme	pink	yes
	sp19	-	na	+	nk	+	cyme	nk	nk
CE11	**humblotiana**	+ [1]	na	+ [1]	mi	+ [1]	fascicle	red	yes
	jeremii	-	na	+	mi	+ [1]	cyme	yellow	no
	sp19	-	na	+	nk	-	cyme	nk	nk
	sp36	+ [1]	na	+ [1]	mi	- [1]	cyme	nk	yes
	sp39	-	na	+	nk	+	cyme	nk	nk
CE12	**thouarsii**	-	na	+	mi	+	cyme	nk	no
	crassinodis	-	na	+	+	mi	fascicle	orangish	yes
	aff. crassinodis	-	-	-	mi	+	fascicle	pinkish	yes
	humblotiana	-	-	- [1]	+ [1]	mi	fascicle	red	yes
	longipedicellata	+	-	-	+	- [1]	cyme	purplish	yes
	sp27	-	+	+	+	+ [1]	cyme	cream	yes
	sp30	- [1]	-	- [1]	nk	+ [1]	fascicle	nk	nk
RDN	**decaryana**	-	+	+	+ [1]	+ [1]	cyme	orangish	no
	humblotiana	-	na	+	+	mi	fascicle	red	yes
	longipedicellata	-	-	+	mi	mi	cyme	purplish	yes
	louveli	-	+	+ [1]	mi	+ [1]	cyme	red	yes
	planifolia	-	na	+	mi	+	cyme	nk	nk
	urceolata	-	na	-	+ [1]	mi	cyme	nk	yes
	sp4	-	na	+ [1]	nk	+ [1]	cyme	nk	nk

	sp7	-	na	- [1]	+ [1]	+ [1]	cyme	yellow	yes
	sp8	-	na	+ [1]	nk	mi	cyme	nk	nk
	sp19	-	na	+	nk	mi	cyme	nk	nk
	sp36	-	na	-	mi	+	cyme	nk	yes
RDS	**decaryana**	+	+	-	mi	-	cyme	orangish	no
	leandriana	+	na	-	+	-	cyme	nk	yes
	sp1	+	-	+	+	+	cyme	white	no

Table 3

Results of Mantel tests between geographic and genetic distances among species within each phylogenetic clade. Signs represent support for (+), contradictions with (-), or uncertainty over (+-) the predictions of the four hypotheses. CCH = current climate hypothesis; ECH = ecogeographic constraint hypothesis; RBH = riverine barrier hypothesis; WCH = watershed contraction hypothesis; r = Pearson's correlation coefficient.

Clade	Mean geographic distance (km)	Genetic distance (%)		r	p-value	ECH	RBH	WCH	CCH
		Mean	Maximum						
B	55	2	3.3	0.06	0.29	+	+	+	+
C	62	1.5	3	0.75	0.15	-	-	-	-
D	43	1.2	1.8	0.06	0.30	-	-	-	-
E	110	1.9	2.8	0.99	0.17	-	+	+	-
F	80	0.3	0.5	0.98	0.35	-	-	-	-
G	169	1.5	3	0.58	0.05	-	-	-	-
H	307	1.7	3	0.34	0.03	+-	+-	+-	+-
I	554	1.7	3.1	0.91	0.13	-	-	-	-
J	16	3.1	4.5	-0.70	0.85	-	-	-	-
K	139	1.8	2.5	0.93	0.32	+	+	+	+
L	53	2.1	4.1	-0.33	0.80	+-	+-	+-	+-
M	32	3	5	0.15	0.21	-	-	-	-
N	217	2.5	4.9	0.61	0.06	+	-	-	+
O	383	0.8	1.2	0.12	0.17	-	-	-	-
P	314	2.6	4.8	-0.23	0.75	-	-	-	-

Table 4

Number of species recognized based on different data types and their combinations.

Lines of evidence	Number of species recovered out of 87 initial morphogroups	Number of species recovered with additional data
Bioclimatic only	19	
+ Molecular	50	31
+ Morphological	79	60
+ Molecular + Morphological	82	63
Molecular only	43 out of 68 with molecular data	
+ Morphological	82	39
Morphological only	79	
Insufficient data/specimens	2	

Figure captions

Fig 1. (A) Geographic locations of samples used for molecular analysis superimposed on different biogeographic zonations of Madagascar (B to E). (B) Bioclimatic zones of Schatz (2000); letters refer to different zones: D = dry, H = humid, SA = subarid, SH = subhumid. (C) Zoogeographical zonations/riverine barriers of Martin (1972); abbreviations are: CP = central plateau, E1 and E2 = east 1 and east 2, N = north, NW = north-west, W1 and W2 = west 1 and west 2, Sb = Sambirano. (D) Centers of endemism of Wilmé et al. (2006); numbers and letters respectively indicate centers of endemism and zones of retreat-dispersion (also hatched). (E) Climate clusters of Pearson and Raxworthy (2009), the different clusters are shown with different symbols in the legend.

Fig. 2. Maximum-likelihood tree for *Noronhia* based on the combined plastid and ITS dataset. Numbers above and below branches are PP and BS respectively. Bold letters refer to supported clades. Dark gray represents African species and light gray indicates species occurring in Madagascar and the surrounding islands (Comoros and Mascarenes).

Fig. 1

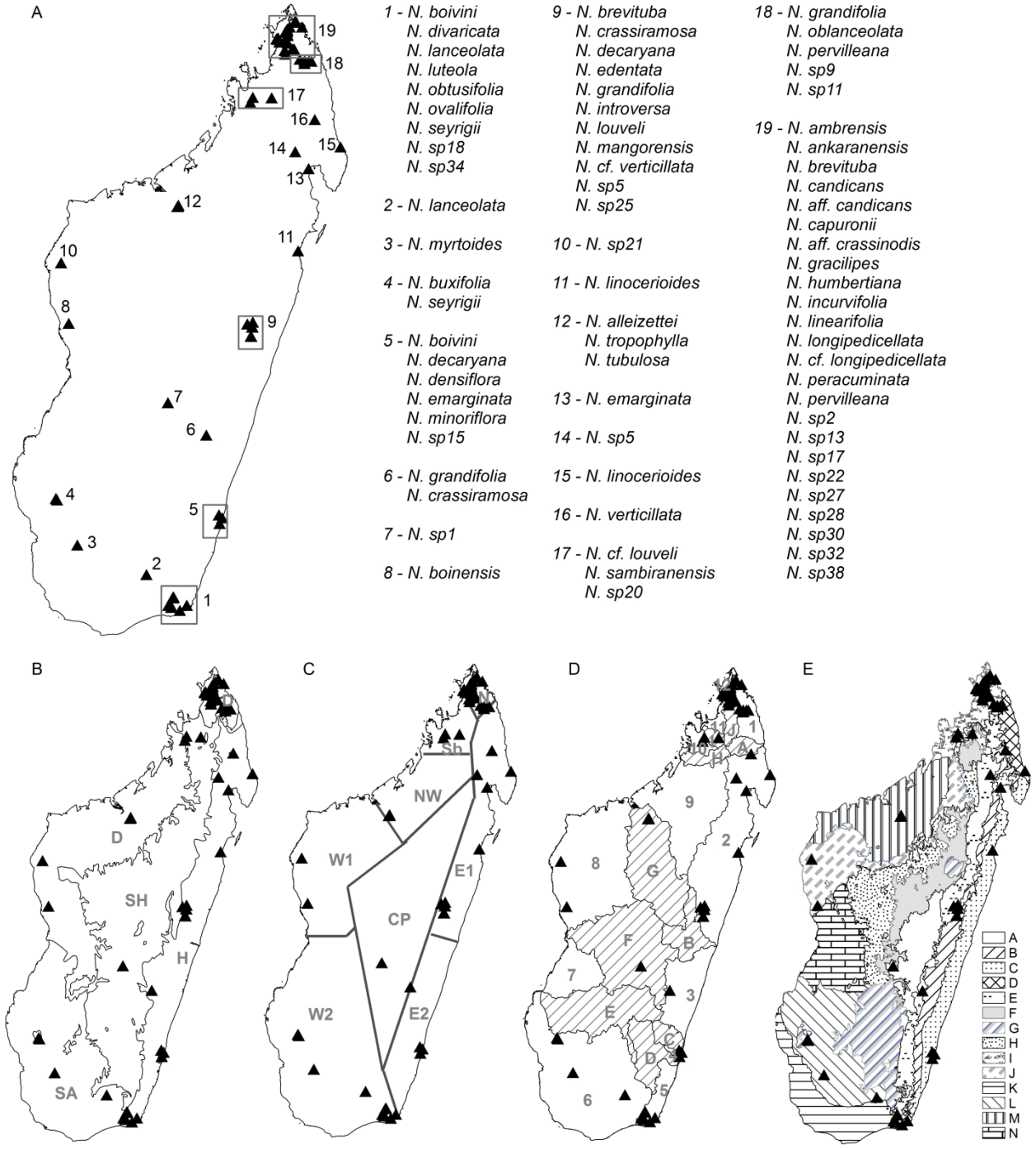

A
B
C
D
E
1 - N. boivini
N. divaricata
N. lanceolata
N. luteola
N. obtusifolia
N. ovalifolia
N. seyrigii
N. sp18
N. sp34
2 - N. lanceolata
3 - N. myrtoides
4 - N. buxifolia
N. seyrigii
5 - N. boivini
N. decaryana
N. densiflora
N. emarginata
N. minoriflora
N. sp15
6 - N. grandifolia
N. crassiramosa
7 - N. sp1
8 - N. boinensis
9 - N. brevituba
N. crassiramosa
N. decaryana
N. edentata
N. grandifolia
N. introversa
N. louveli
N. mangorensis
N. cf. verticillata
N. sp5
N. sp25
10 - N. sp21
11 - N. linocerioides
12 - N. alleizettei
N. tropophylla
N. tubulosa
13 - N. emarginata
14 - N. sp5
15 - N. linocerioides
16 - N. verticillata
17 - N. cf. louveli
N. sambiranensis
N. sp20
18 - N. grandifolia
N. oblanceolata
N. pervilleana
N. sp9
N. sp11
19 - N. ambrensis
N. ankaranensis
N. brevituba
N. candicans
N. aff. candicans
N. capuronii
N. aff. crassinodis
N. gracilipes
N. humbertiana
N. incurvifolia
N. linearifolia
N. longipedicellata
N. cf. longipedicellata
N. peracuminata
N. pervilleana
N. sp2
N. sp13
N. sp17
N. sp22
N. sp27
N. sp28
N. sp30
N. sp32
N. sp38
D
SH
H
SA
Sh
NW
W1
E1
CP
W2
E2
A
B
C
D
E
F
G
H
I
J
K
L
M
N

Fig. 2

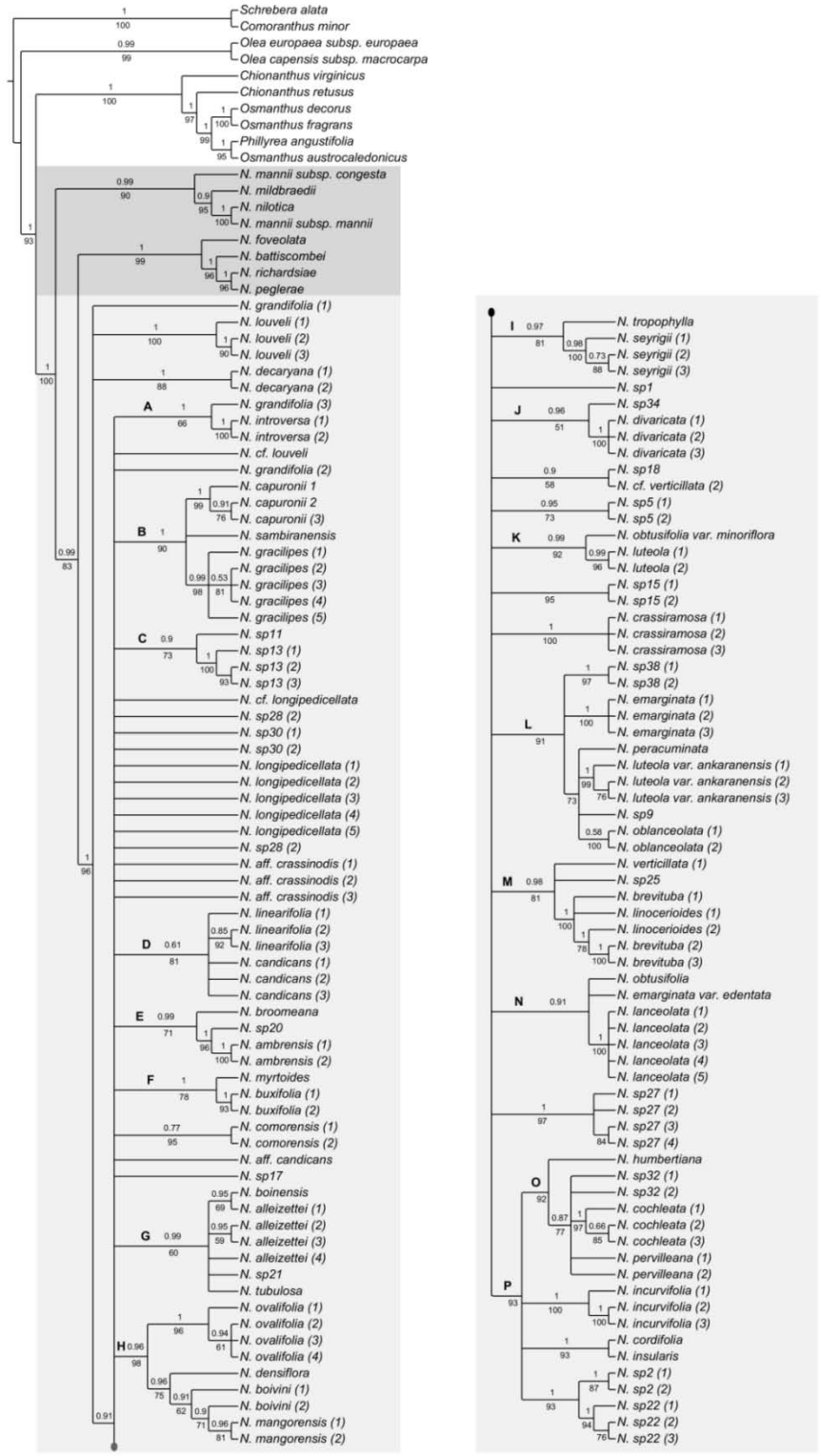

Schrebera alata
Comoranthus minor
Olea europaea subsp. europaea
Olea capensis subsp. macrocarpa
Chionanthus virginicus
Chionanthus retusus
Osmanthus decorus
Osmanthus fragrans
Phillyrea angustifolia
Osmanthus austrocaledonicus
N. mannii subsp. congesta
N. mildbraedii
N. nilotica
N. mannii subsp. mannii
N. foveolata
N. battiscombei
N. richardsiae
N. peglerae
N. grandifolia (1)
N. louveli (1)
N. louveli (2)
N. louveli (3)
N. decaryana (1)
N. decaryana (2)
A
N. grandifolia (3)
N. introversa (1)
N. introversa (2)
N. cf. louveli
N. grandifolia (2)
N. capuronii 1
N. capuronii 2
N. capuronii (3)
B
N. sambiranensis
N. gracilipes (1)
N. gracilipes (2)
N. gracilipes (3)
N. gracilipes (4)
N. gracilipes (5)
C
N. sp11
N. sp13 (1)
N. sp13 (2)
N. sp13 (3)
N. cf. longipedicellata
N. sp28 (2)
N. sp30 (1)
N. sp30 (2)
N. longipedicellata (1)
N. longipedicellata (2)
N. longipedicellata (3)
N. longipedicellata (4)
N. longipedicellata (5)
N. sp28 (2)
N. aff. crassinodis (1)
N. aff. crassinodis (2)
N. aff. crassinodis (3)
N. linearifolia (1)
N. linearifolia (2)
D
N. linearifolia (3)
N. candicans (1)
N. candicans (2)
N. candicans (3)
N. broomeana
E
N. sp20
N. ambrensis (1)
N. ambrensis (2)
F
N. myrtoides
N. buxifolia (1)
N. buxifolia (2)
N. comorensis (1)
N. comorensis (2)
N. aff. candicans
N. sp17
N. boinensis
N. alleizettei (1)
N. alleizettei (2)
G
N. alleizettei (3)
N. alleizettei (4)
N. sp21
N. tubulosa
N. ovalifolia (1)
N. ovalifolia (2)
N. ovalifolia (3)
H
N. ovalifolia (4)
N. densiflora
N. boivini (1)
N. boivini (2)
N. mangorensis (1)
N. mangorensis (2)
I
N. tropophylla
N. seyrigii (1)
N. seyrigii (2)
N. seyrigii (3)
N. sp1
J
N. sp34
N. divaricata (1)
N. divaricata (2)
N. divaricata (3)
N. sp18
N. cf. verticillata (2)
N. sp5 (1)
N. sp5 (2)
K
N. obtusifolia var. minoriflora
N. luteola (1)
N. luteola (2)
N. sp15 (1)
N. sp15 (2)
N. crassiramosa (1)
N. crassiramosa (2)
N. crassiramosa (3)
N. sp38 (1)
N. sp38 (2)
N. emarginata (1)
L
N. emarginata (2)
N. emarginata (3)
N. peracuminata
N. luteola var. ankaranensis (1)
N. luteola var. ankaranensis (2)
N. luteola var. ankaranensis (3)
N. sp9
N. oblanceolata (1)
N. oblanceolata (2)
N. verticillata (1)
M
N. sp25
N. brevituba (1)
N. linocerioides (1)
N. linocerioides (2)
N. brevituba (2)
N. brevituba (3)
N. obtusifolia
N
N. emarginata var. edentata
N. lanceolata (1)
N. lanceolata (2)
N. lanceolata (3)
N. lanceolata (4)
N. lanceolata (5)
N. sp27 (1)
N. sp27 (2)
N. sp27 (3)
N. sp27 (4)
N. humbertiana
O
N. sp32 (1)
N. sp32 (2)
N. cochleata (1)
N. cochleata (2)
N. cochleata (3)
N. pervilleana (1)
N. pervilleana (2)
P
N. incurvifolia (1)
N. incurvifolia (2)
N. incurvifolia (3)
N. cordifolia
N. insularis
N. sp2 (1)
N. sp2 (2)
N. sp22 (1)
N. sp22 (2)
N. sp22 (3)

Table S1 – GenBank accession numbers. CP = combined plastid DNA regions (*trnL-F*, *trnT-L*, *trnS-G*, *trnK-matK*); ITS = internal transcribed spacer; *Ch.* = *Chionanthus*; *N.* = *Noronhia*; *O.* = *Olea*.

Taxa	Voucher	CP	ITS
Ch. retusus	Hong-Wa SN10		
Ch. virginicus	Hong-Wa SN2		
Comoranthus minor	Ratovoson 1457		
N. alleizettei	Hong-Wa 622		
N. alleizettei	Hong-Wa 624		
N. alleizettei	Hong-Wa 628		
N. alleizettei	Hong-Wa 632		
N. ambrensis	Hong-Wa 573		
N. ambrensis	Hong-Wa 693		
N. battiscombei	Loveridge 1527		
N. boinensis	Phillipson 2277		
N. boivini	Hong-Wa 614		
N. boivini	Randriatafika 379		
N. brevituba	Hong-Wa 579		
N. brevituba	Hong-Wa 638		
N. brevituba	Hong-Wa 684		
N. broomeana	Cultivated	AM931522, AM933079, AM933223, AM933426	
N. buxifolia	Andriamihajarivo 1485		
N. buxifolia	Andriamihajarivo 1488		
N. aff. *candicans*	Hong-Wa P10_1		
N. capuronii	Andriamihajarivo 1375		
N. capuronii	Hong-Wa 706		
N. capuronii	Trigui 536		
N. cochleata	Labat 3258		
N. cochleata	Labat 3308		
N. cochleata	Pignal 1112		
N. comorensis	Barthelat 537		
N. comorensis	Labat 3257		
N. cordifolia	Pascal 288		
N. aff. *crassinodis*	Hong-Wa 694		
N. aff. *crassinodis*	Hong-Wa 696		
N. aff. *crassinodis*	Hong-Wa 708		
N. crassiramosa	Hong-Wa 640		
N. crassiramosa	Hong-Wa 658		
N. crassiramosa	Hong-Wa 669		
N. decaryana	Hong-Wa 612		
N. decaryana	Hong-Wa 648		

Taxa	Voucher	CP	ITS
N. densiflora	Hong-Wa 611		
N. divaricata	Dumetz 1421		
N. divaricata	Letsara 746		
N. divaricata	Rakotonasolo 2		
N. divaricata	Randrianaivo 1761		
N. emarginata	Birkinshaw 506		
N. emarginata	Cultivated	AM931526, AM933083, AM933227, AM933430	
N. emarginata	Flynn 6331		
N. emarginata	Miller 7216		
N. emarginata	Rakotonirina 464		
N. emarginata var. *edentata*	Razantsima 266		
N. foveolata	F557		
N. gracilipes	Besnard 46-2006	AM931531, AM933088, AM933232, AM933435	
N. gracilipes	Hong-Wa 571		
N. gracilipes	Hong-Wa 583		
N. gracilipes	Hong-Wa 686		
N. gracilipes	Hong-Wa 713		
N. grandifolia	Birkinshaw 468		
N. grandifolia	Gautier 4803		
N. grandifolia	Hong-Wa 662		
N. grandifolia	Hong-Wa 670		
N. humbertiana	Hong-Wa 695		
N. incurvifolia	Andriamihajarivo 1401		
N. incurvifolia	Besnard 49-2006	AM931529, AM933086, AM933230, AM933433	
N. incurvifolia	Ratovoson 1361		
N. insularis	Barthelat 1069		
N. introversa	Hong-Wa 656		
N. introversa	Hong-Wa 659		
N. lanceolata	Andriamihajarivo 1547		
N. lanceolata	Hong-Wa 609		
N. lanceolata	Lowry 6942		
N. lanceolata	Randrianaivo 1762		
N. lanceolata	Ratovoson 1475		
N. linearifolia	Claude 83		
N. linearifolia	Hong-Wa 526		
N. linearifolia	Hong-Wa 546		
N. linocerioides	Birkinshaw 467		
N. linocerioides	Birkinshaw 492		

Taxa	Voucher	CP	ITS
N. linocerioides	Schatz 3605	AM931503, AM933059, AM933203, AM933406	
N. longipedicellata	Besnard 53-2006	AM931527, AM933084, AM933228, AM933431	
N. longipedicellata	Hong-Wa 564		
N. longipedicellata	Hong-Wa 592		
N. longipedicellata	Hong-Wa 593		
N. cf. *longipedicellata*	Razafitsalama 1231		
N. louveli	Hong-Wa 642		
N. louveli	Hong-Wa 647		
N. louveli	Ranaivojaona 1723		
N. cf. *louveli*	Wohlhauser 408		
N. luteola	Hong-Wa 594		
N. luteola	Hong-Wa 596		
N. luteola	Hong-Wa 598		
N. luteola var. *ankaranrensis*	Besnard 51-2006	AM931528, AM933085, AM933229, AM933432	
N. luteola var. *ankaranrensis*	Hong-Wa 545		
N. luteola var. *ankaranrensis*	Hong-Wa 551		
N. mangorensis	Antilahimena 6043		
N. mangorensis	Antilahimena 6044		
N. mannii subsp. *congesta*	Schmidt 3487		
N. mannii subsp. *mannii*	Leewenberg 2354		
N. mannii subsp. *mannii*	White 886		
N. mildbraedii	Friis 9842		
N. myrtoides	Sussman 153		
N. nilotica	Fanshawe 4706		
N. oblanceolata	Ranirison 1053		
N. oblanceolata	Ranirison 756		
N. obtusifolia	Hong-Wa 599		
N. obtusifolia var. *minoriflora*	Hong-Wa 620		
N. ovalifolia	Lowry 6955		
N. ovalifolia	Randrianaivo 1548		
N. ovalifolia	Randrianaivo 1760		
N. peglerae	Maurin 1766		
N. peracuminata	Hong-Wa 720		
N. pervilleana	Hong-Wa 718		
N. pervilleana	Ranirison 867		
N. richardsiae	Fanshawe 4052		
N. sambiranensis	Wohlhauser 60168		

Taxa	Voucher	CP	ITS
N. seyrigii	Lowry 6940		
N. seyrigii	Randrianassolo 1233		
N. tropophylla	Hong-Wa 630		
N. tubulosa	Hong-Wa 629		
N. verticillata	Hong-Wa 634		
N. cf. *verticillata*	Hong-Wa 654		
N. sp1	Lowry 5906		
N. sp2	Hong-Wa 554		
N. sp2	Hong-Wa 702		
N. sp5	Hong-Wa 663		
N. sp5	Ravelonarivo 2865		
N. sp9	Ranirison 762		
N. sp11	Razafimandimbison 979		
N. sp13	Hong-Wa 517		
N. sp13	Hong-Wa 539		
N. sp13	Ratovoson 1331		
N. sp15	Hong-Wa 616		
N. sp15	Rakotonirina 452		
N. sp17	Hong-Wa 549		
N. sp18	Hong-Wa 600		
N. sp20	SF 24980		
N. sp21	Rakotonasolo 1433		
N. sp22	Hong-Wa 547		
N. sp22	Hong-Wa 556		
N.sp22	Hong-Wa 697		
N. sp25	Hong-Wa 643		
N. sp27	Hong-Wa 578		
N. sp27	Hong-Wa 680		
N. sp27	Hong-Wa 711		
N. sp27	Trigui 383		
N.sp28	Hong-Wa 524		
N. sp28	Hong-Wa 544		
N. sp30	Hong-Wa 525		
N. sp30	Hong-Wa 548		
N. sp34	Randrianaivo 1564		
N. sp32	Hong-Wa 514		
N. sp32	Hong-Wa 698		
N. sp38	Hong-Wa 542		
N. sp38	Hong-Wa 543		
O. capensis subsp. *macrocarpa*	Hong-Wa 557		
O. europaea subsp. *europaea*	Hong-Wa SN1		

Taxa	Voucher	CP	ITS
Osmanthus austrocaledonicus	Munzinger 823		
Osmanthus decorus	Merello 2324		
Osmanthus fragrans	Hong-Wa SN3		
Phillyrea angustifolia	Hong-Wa SN5		
Schrebera alata	Chase 3883		

Table S2 – Characters measured for morphometric analyses

Characters	Abbreviations
Vegetative	
Twig diameter	TWIGD
Petiole length	PETL
Petiole diameter	PETD
Petiole cork length	CORKL
Leaf total length	LEAFTL
Leaf total width	LEAFTW
Leaf total length/Leaf total width	LEAFTLTW
Leaf length at widest part	LEAFTLATW
Leaf total length/Leaf length at widest part	LEAFTLLATW
Vein number	VEINNUMB
Distance between secondary veins	VEINDIST
Vein density	VEINDENS
Loop distance from margin	LOOPD
Acumen length	ACUML
Flower	
Pedicel length	PEDIL
Corolla length	COROL
Tube length	TUBEL
Lobe length	LOBEL
Sepal length	SEPL
Sepal width	SEPW
Corona length	CORONL
Stamen length	STAML
Anther length	ANTHL
Pistil length	PISTL
Stigma length	STIGML
Fruit	
Fruit pedicel length	FRPEDIL
Fruit pedicel diameter	FRPEDID
Fruit length	FRL
Fruit diameter	FRD
Fruit length/diameter	FRLD
Pericarp thickness	PERIT
Seed length	SEEDL
Seed diameter	SEEDD
Fruit sepal length	SEPFRL
Fruit sepal width	SEPFRW

Figure captions

Fig. S1. Maps showing the distribution of species falling into clades, so subsequently analyzed in a phylogenetic context (Figs. S3-S18).

Fig. S2. Biogeographic zones recognized under the ecogeographic constraint (ECH), riverine barrier (RBH), watershed contraction (WCH) and current climate (CCH) hypotheses mapped onto the phylogenetic tree.

Figs. S3-S32. Results of principal component analyses of bioclimatic and morphological data carried out in phylogenetic (Figs. S3-S18) and geographic (Figs. S19-S32) contexts. Abbreviations: BIO, FL, FR, VEG, VEG + FL and VEG + FR are respectively bioclimatic, flower, fruit, vegetative, vegetative and flower and vegetative and fruit variables. Number (2) after an abbreviation refers to a second analysis with the same set of variables but excluding morphospecies indicated in brackets.

Fig. S1

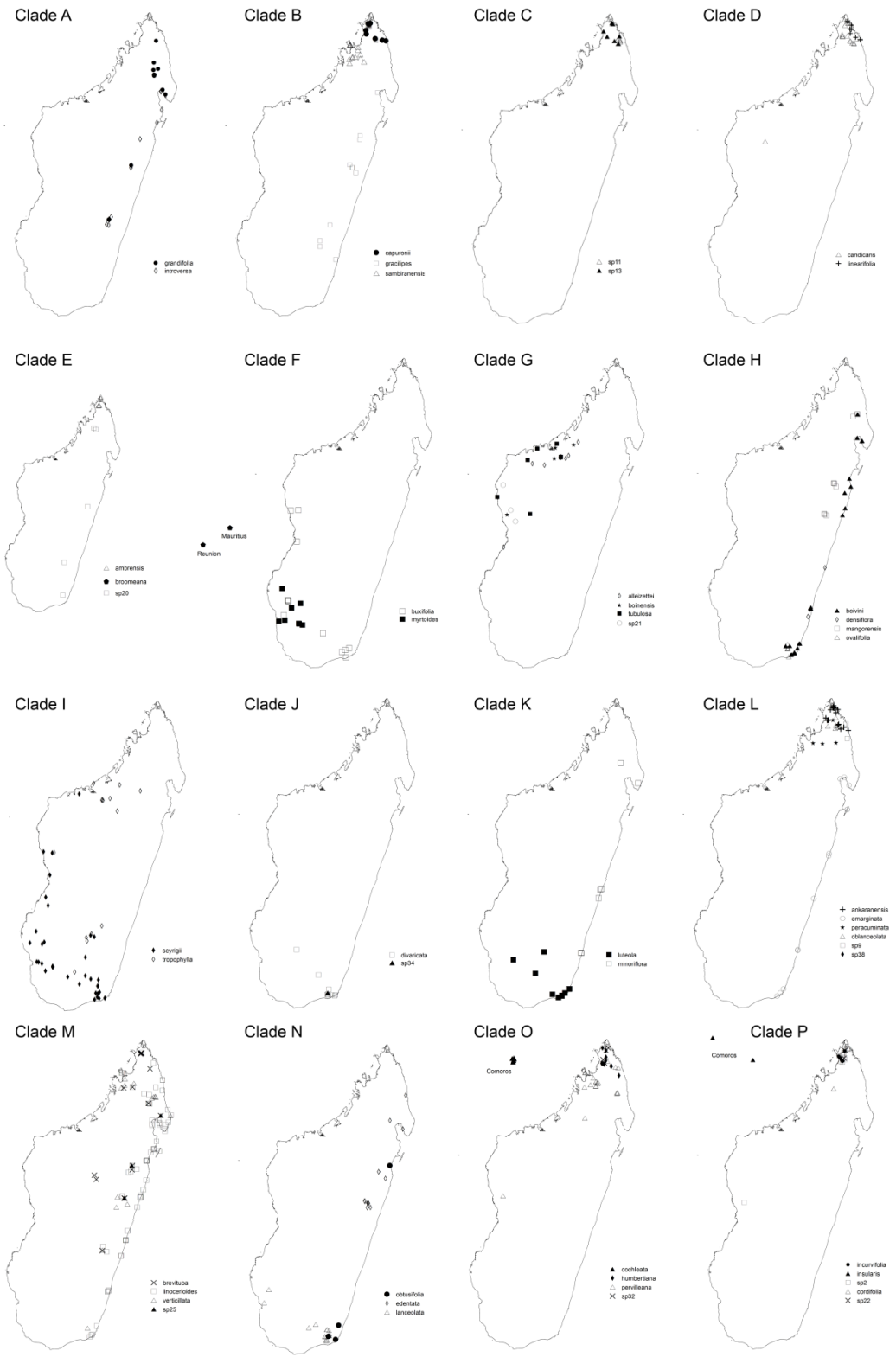

Clade A
grandifolia
introversa
Clade B
capuronii
gracilipes
sambiranensis
Clade C
sp11
sp13
Clade D
candicans
linearifolia
Clade E
ambrensis
broomeana
sp20
Clade F
Mauritius
Reunion
buxifolia
myrtoides
Clade G
alleizettei
boinensis
tubulosa
sp21
Clade H
boivini
densiflora
mangorensis
ovatifolia
Clade I
seyrigii
tropophylla
Clade J
divaricata
sp34
Clade K
luteola
minoriflora
Clade L
ankaranensis
emarginata
peracuminata
oblanceolata
sp9
sp38
Clade M
brevituba
linocerioides
verticillata
sp25
Clade N
obtusifolia
edentata
lanceolata
Clade O
Comoros
cochleata
humbertiana
pervilleana
sp32
Clade P
Comoros
incurvifolia
insularis
sp2
cordifolia
sp22

Fig. S2

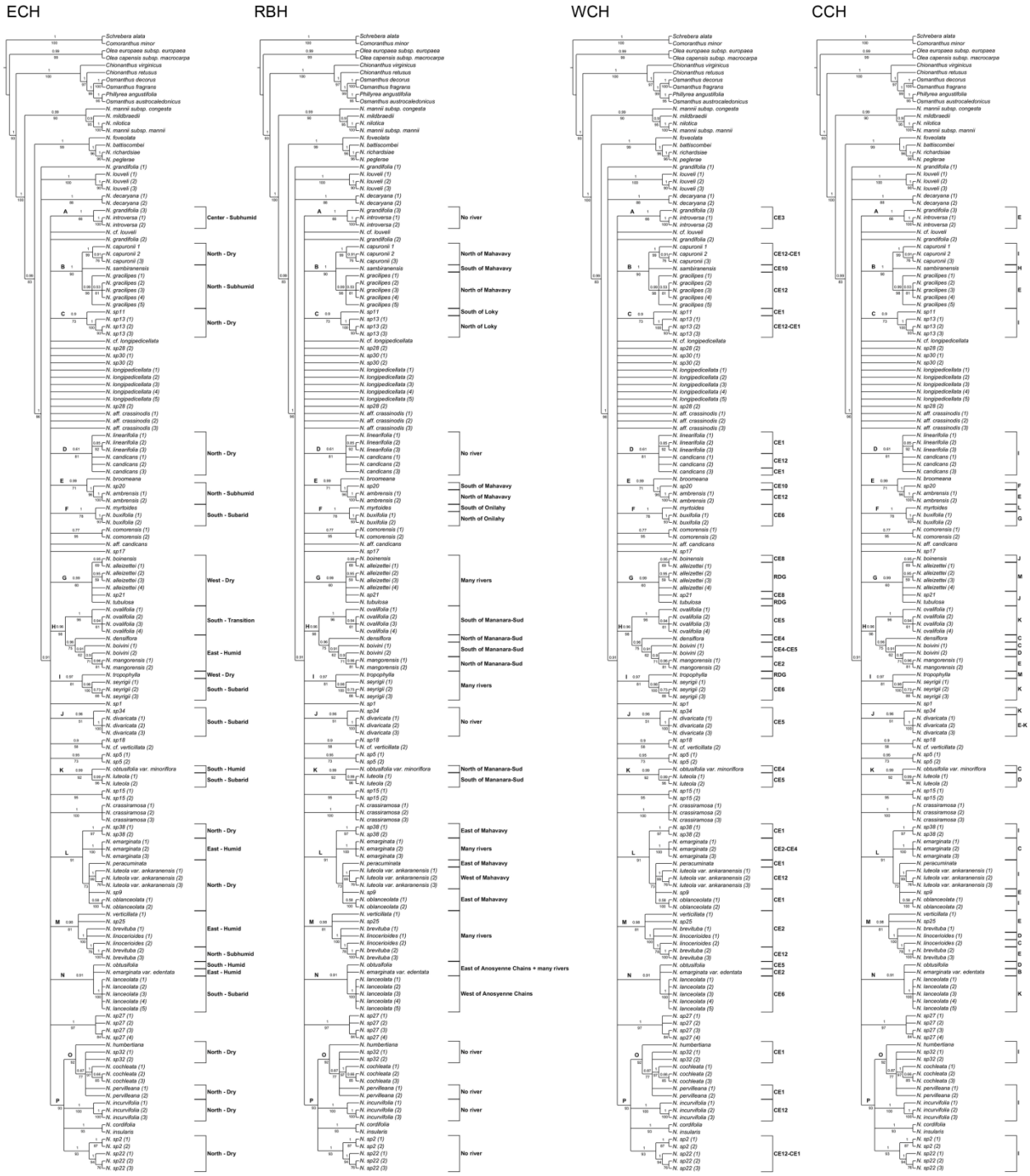
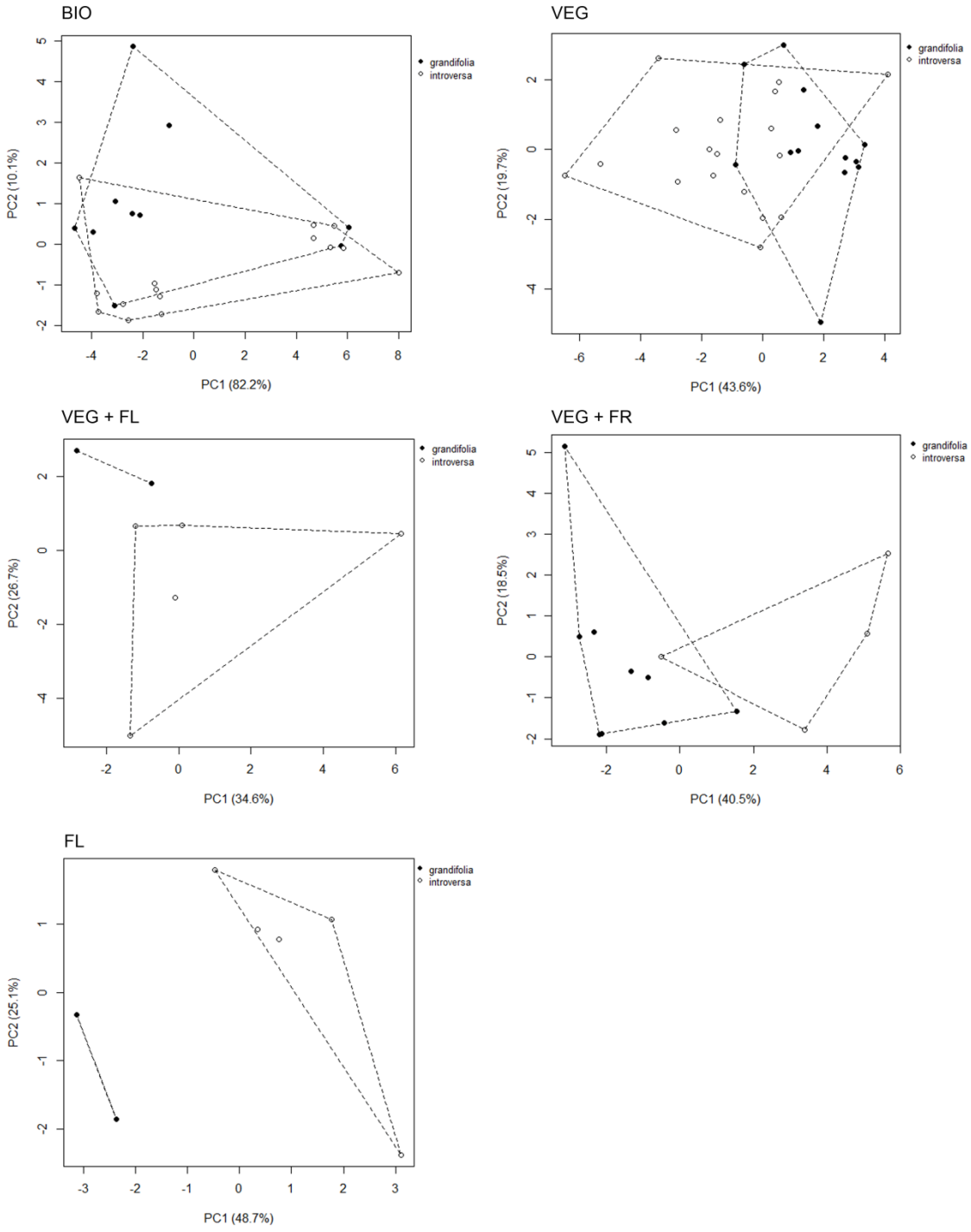


Fig. S3: CLADE A



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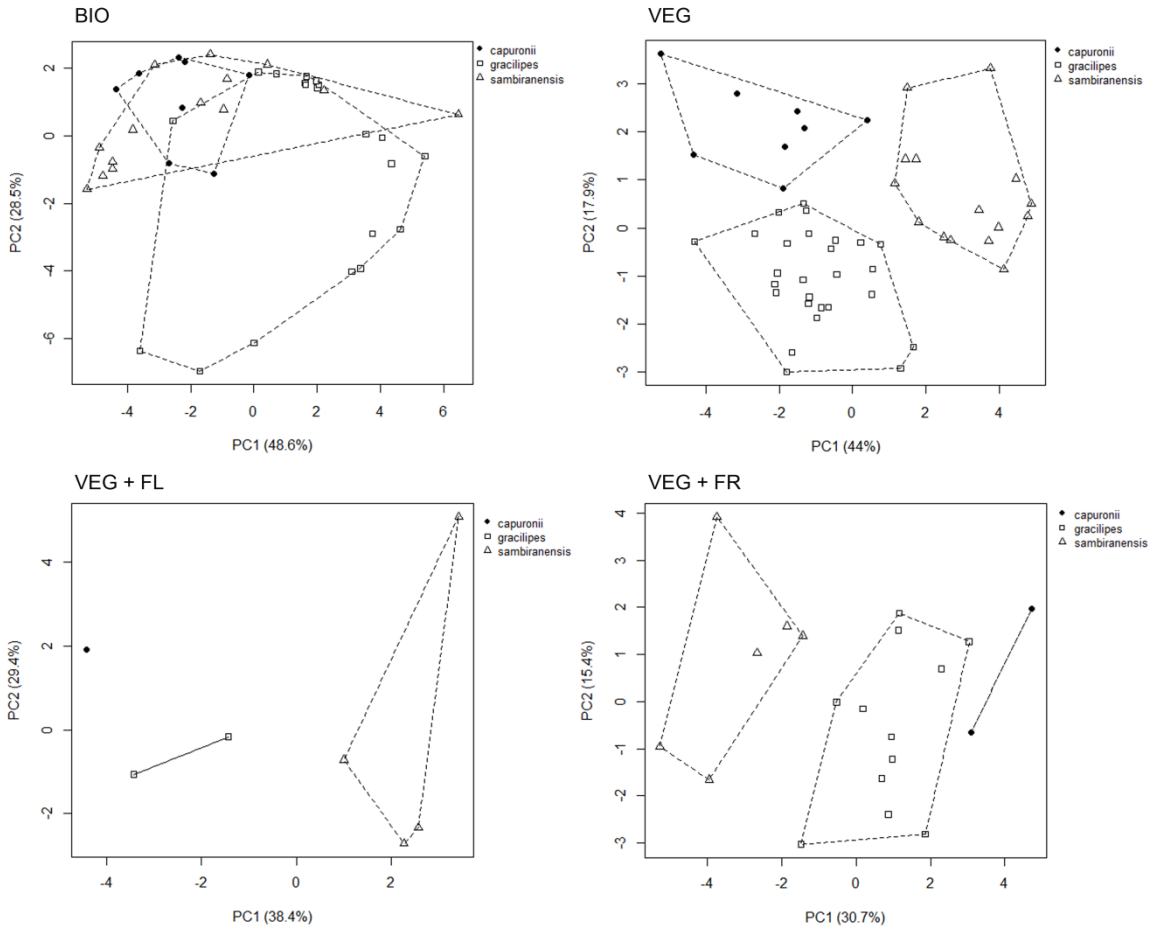


Fig. S5: CLADE C

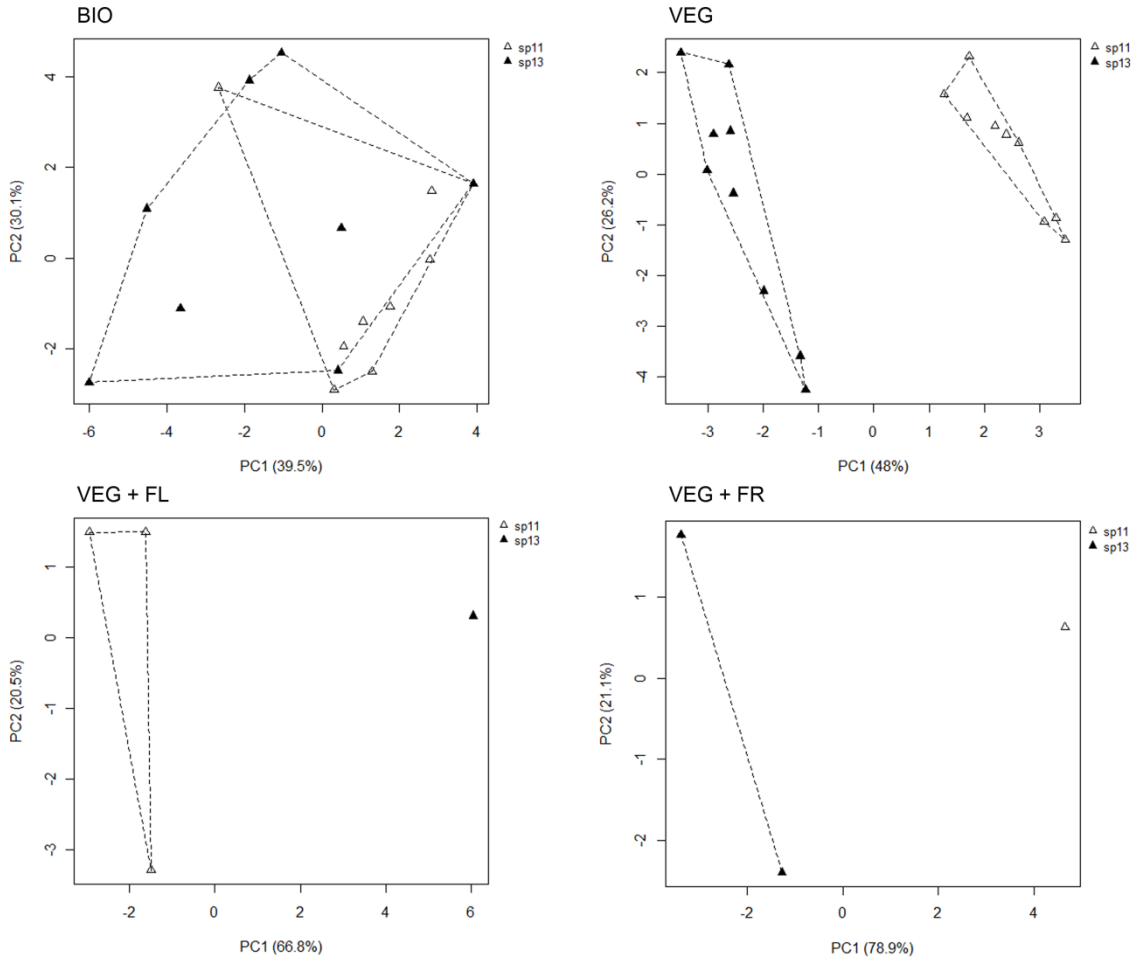
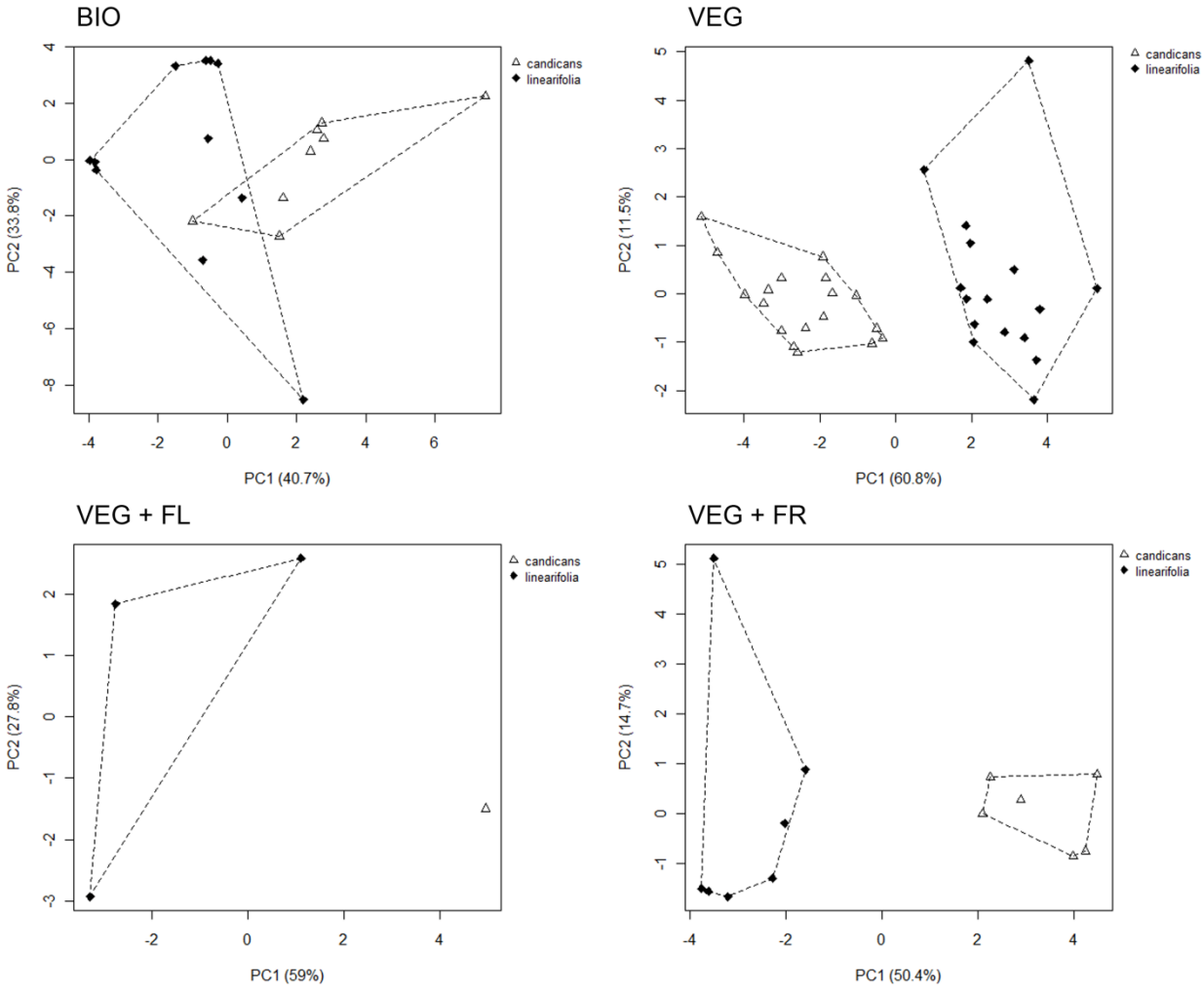


Fig. S6: CLADE D



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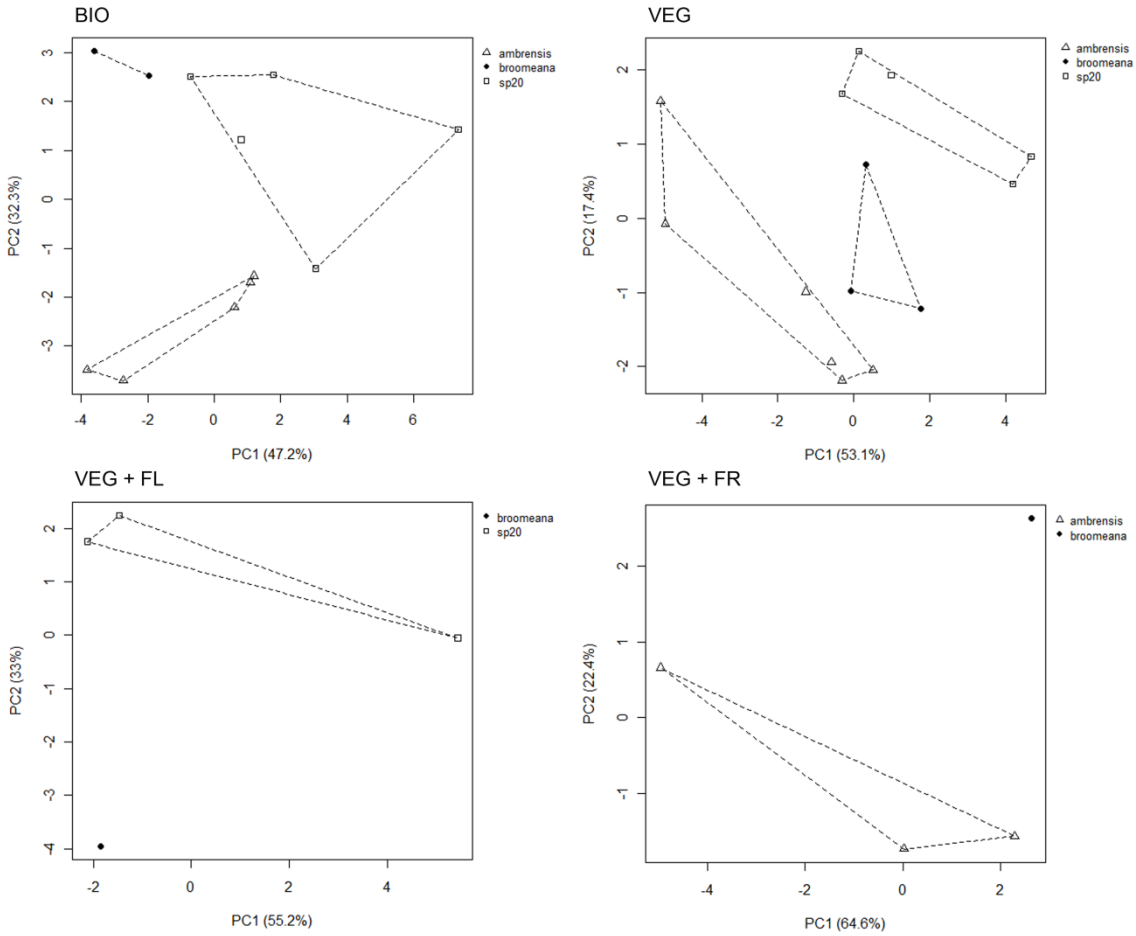
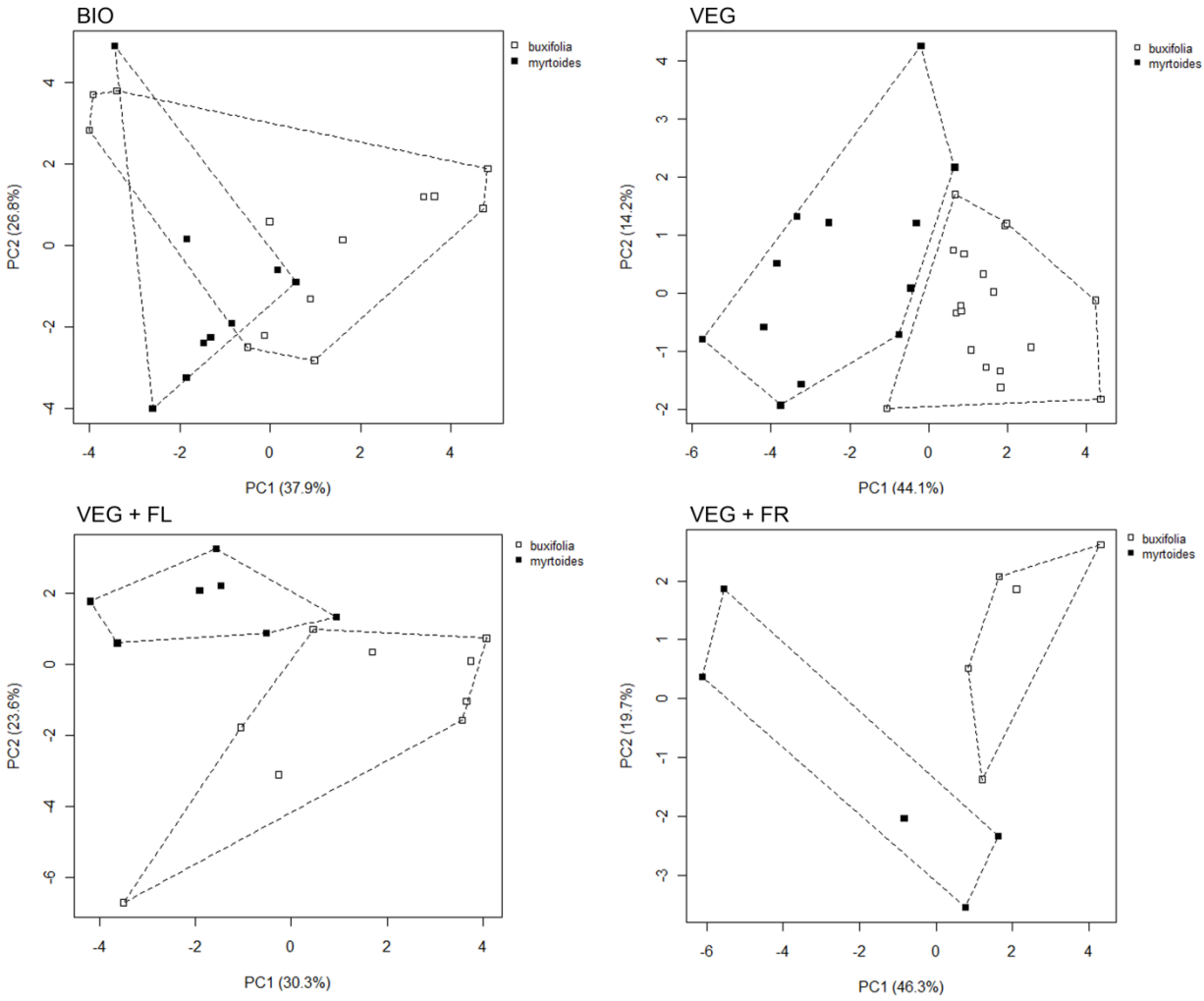


Fig. S8: CLADE F



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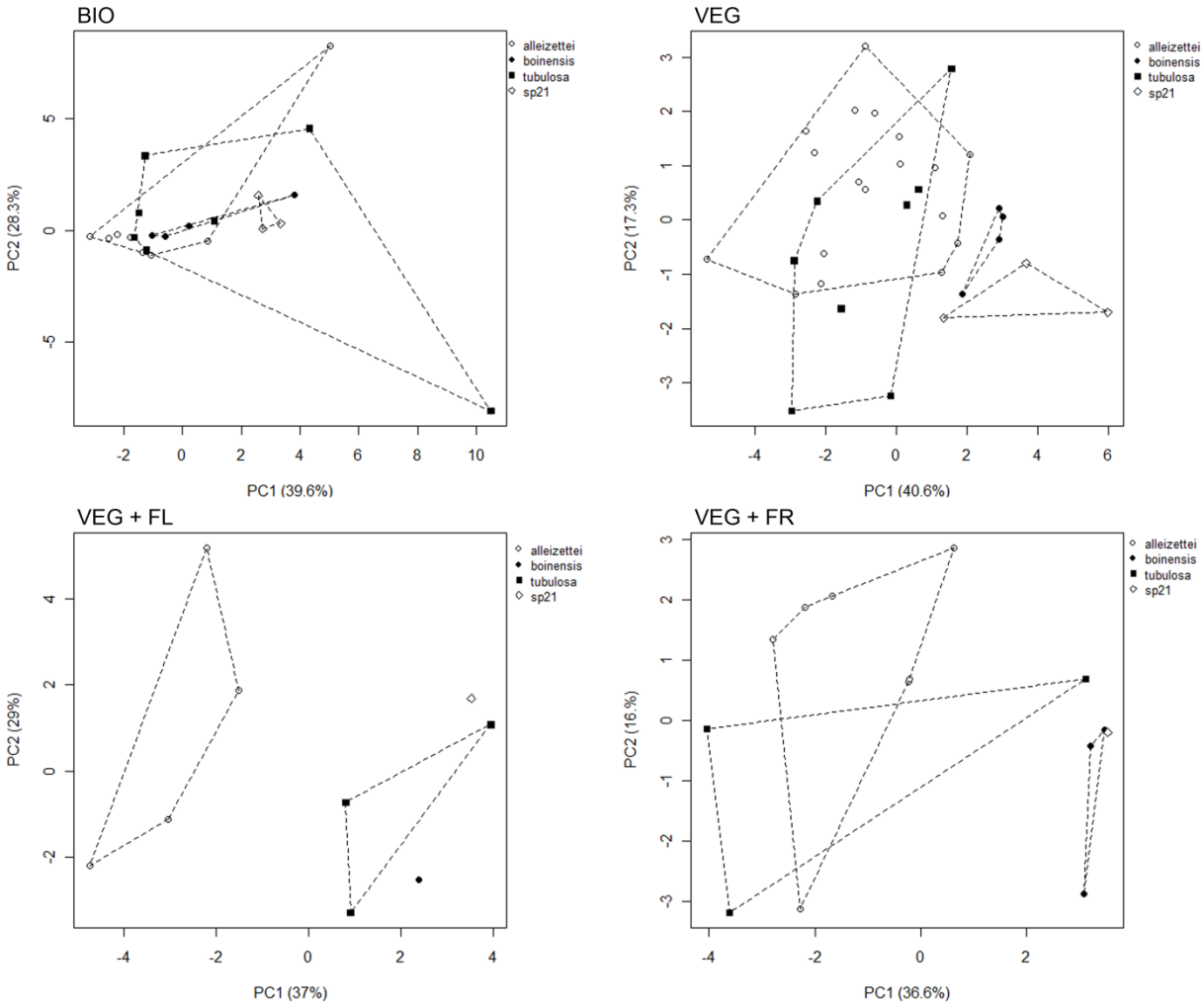

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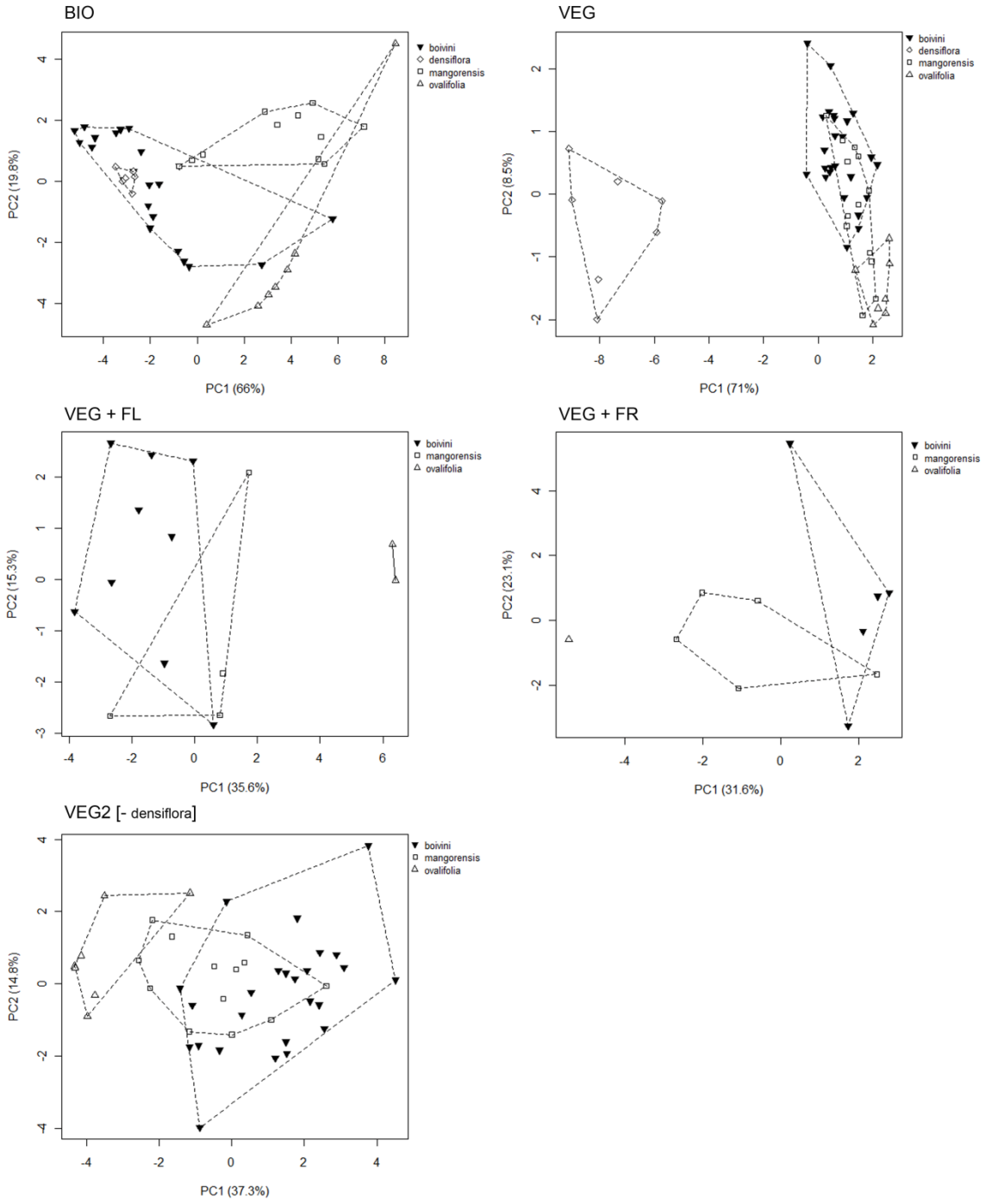
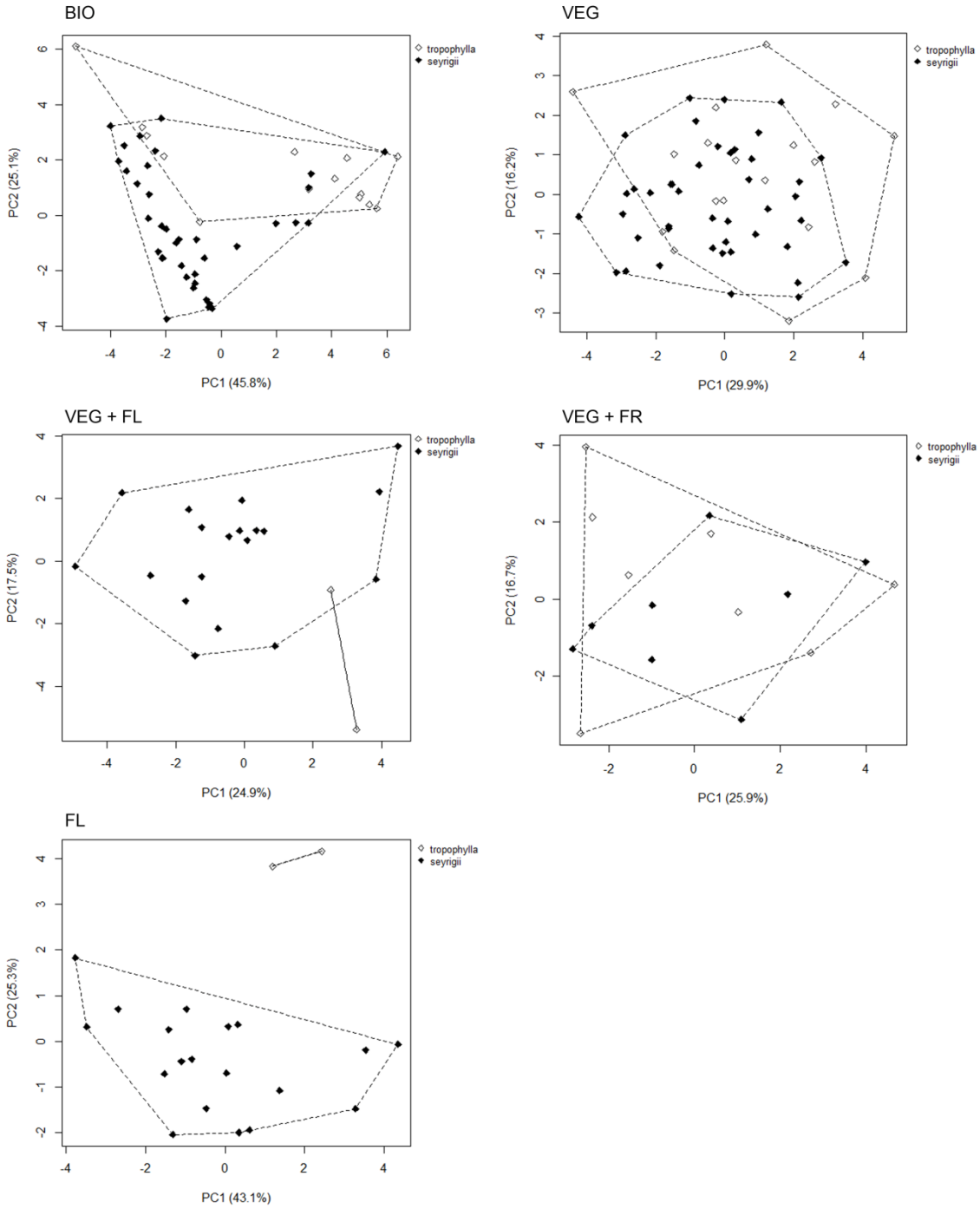


Fig. S11: CLADE I



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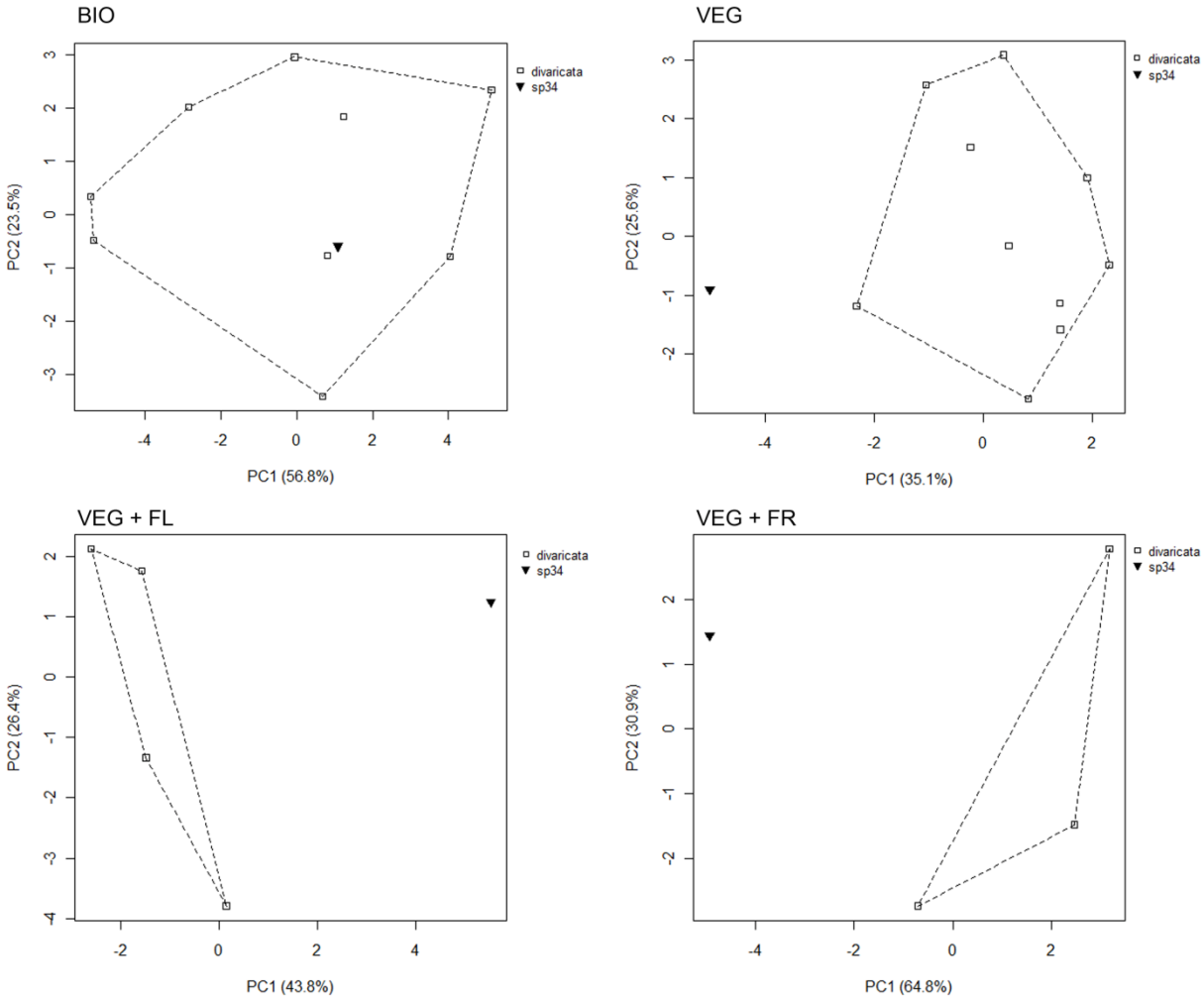

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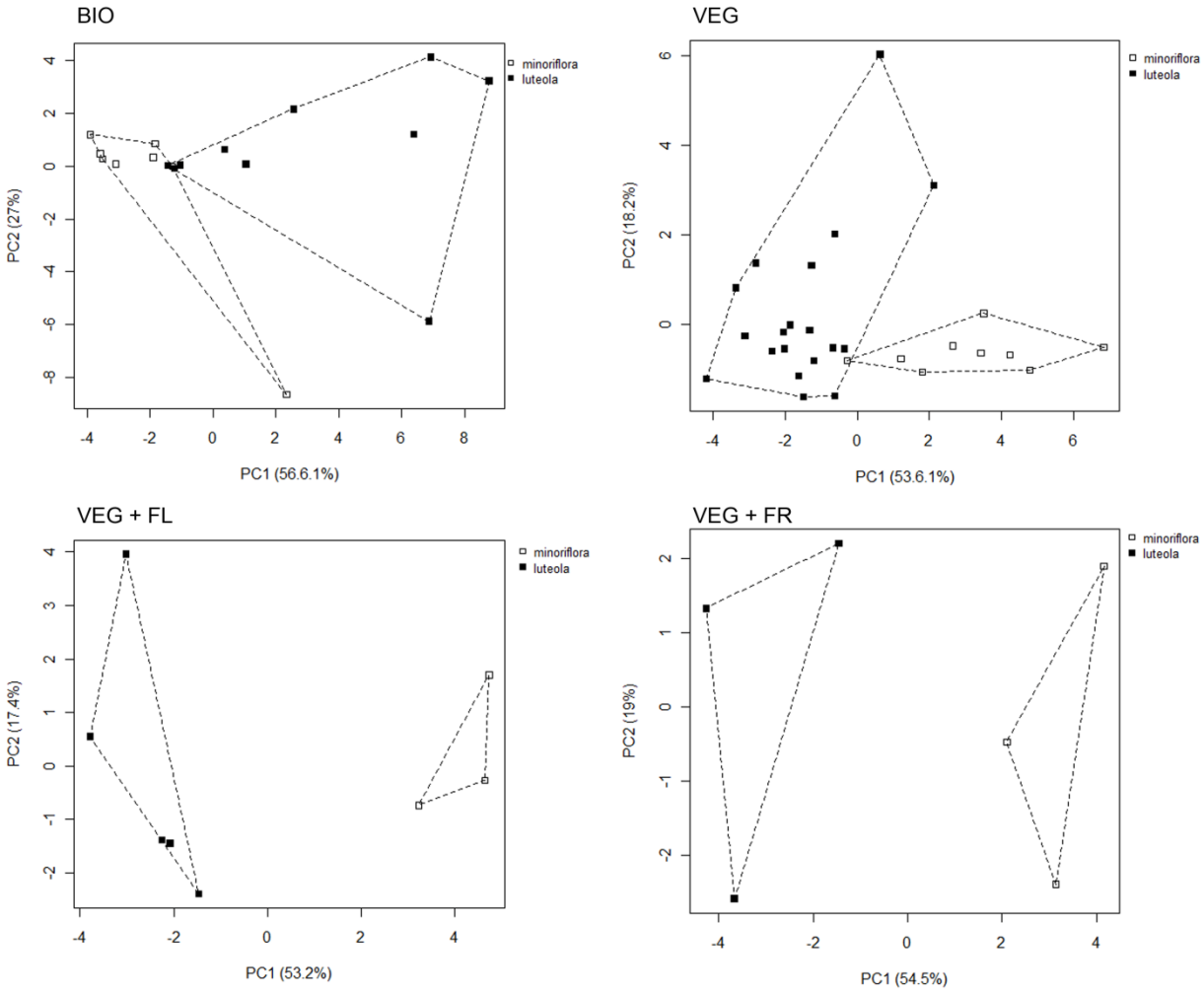

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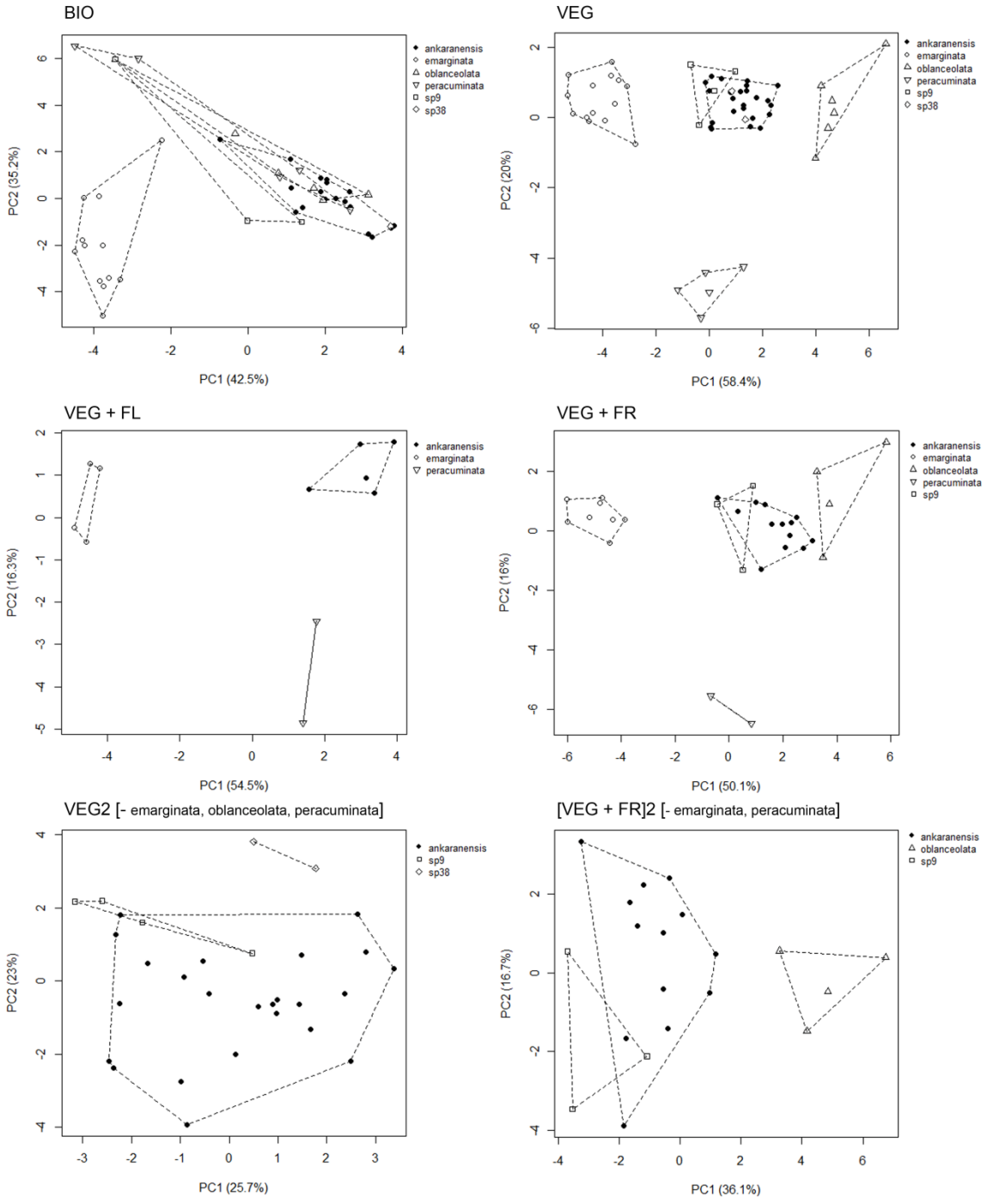
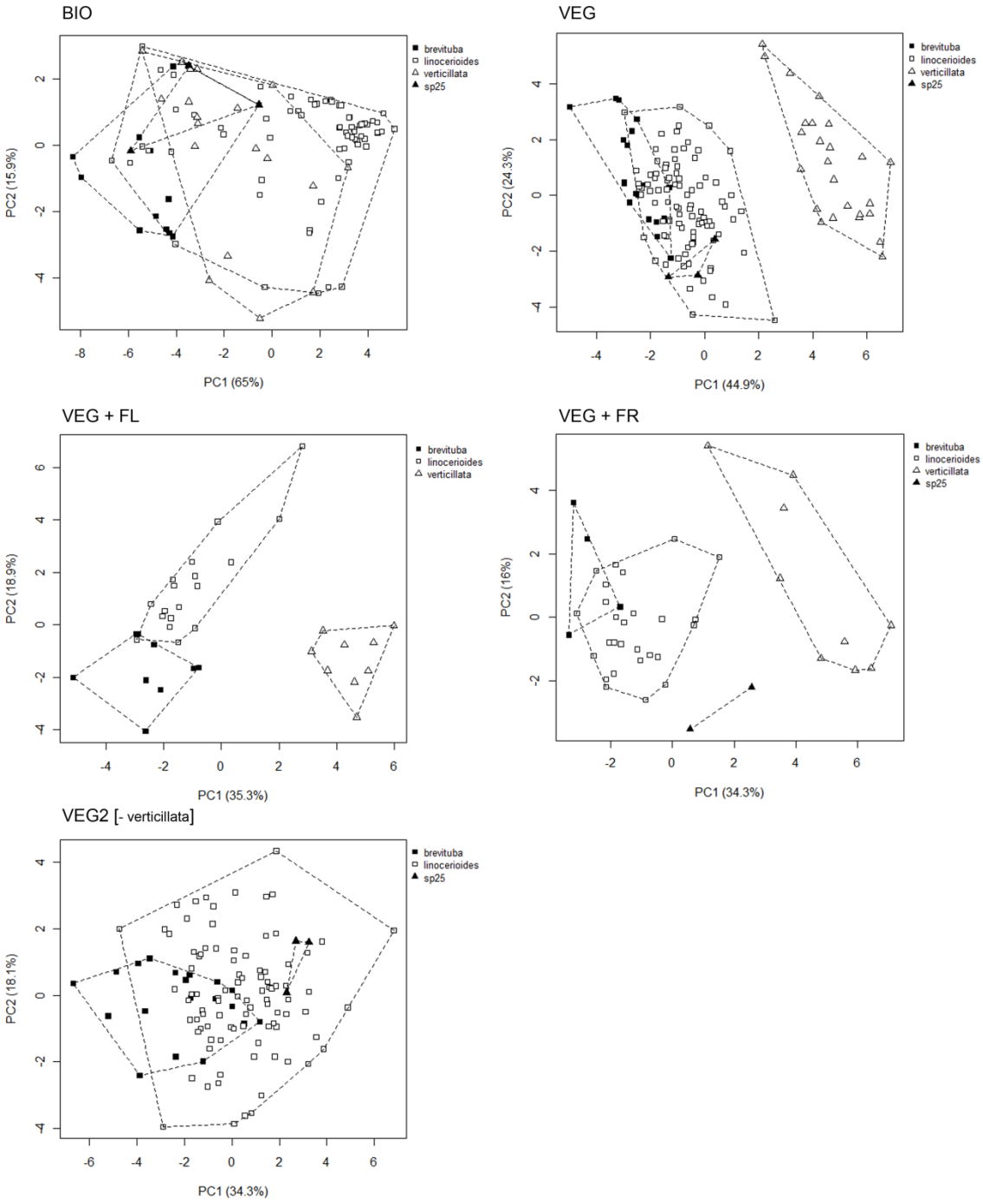


Fig. S15: CLADE M



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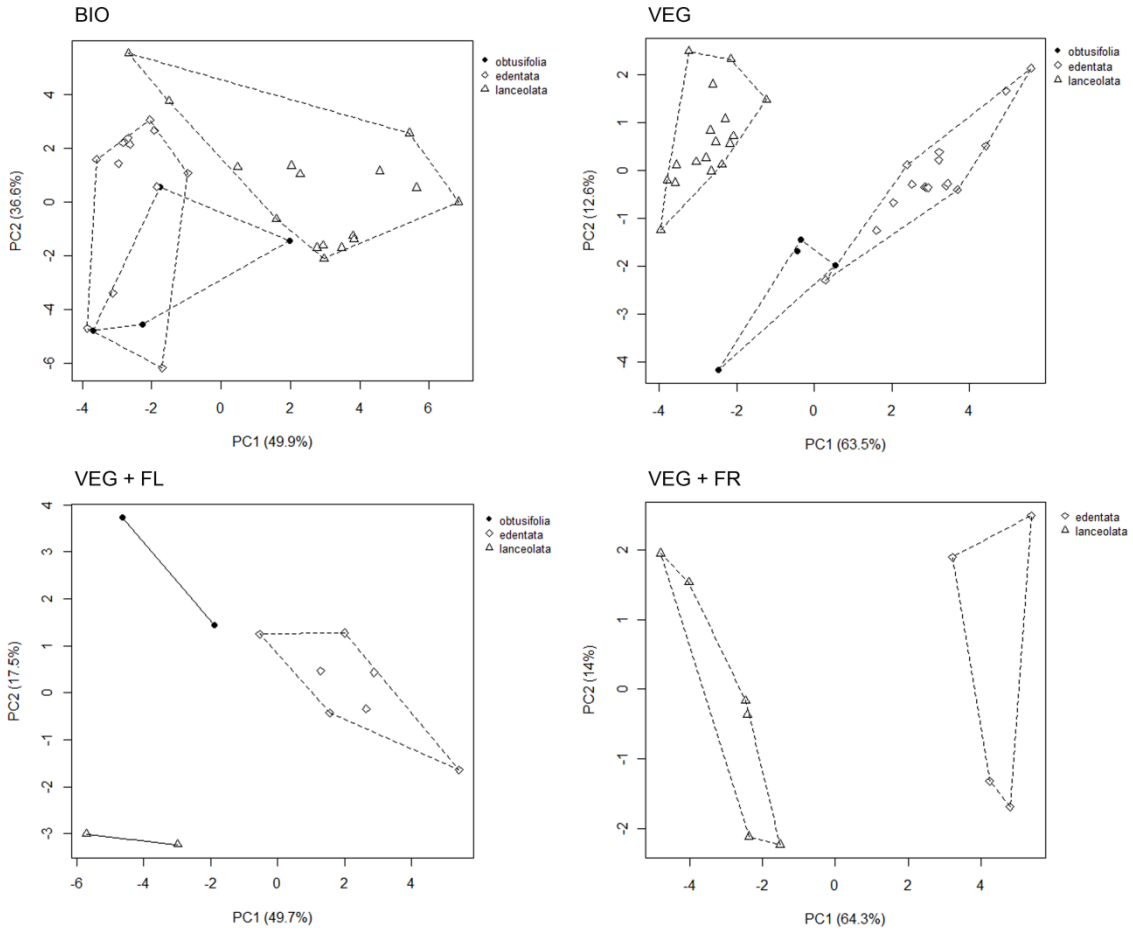


Fig. S17: CLADE O

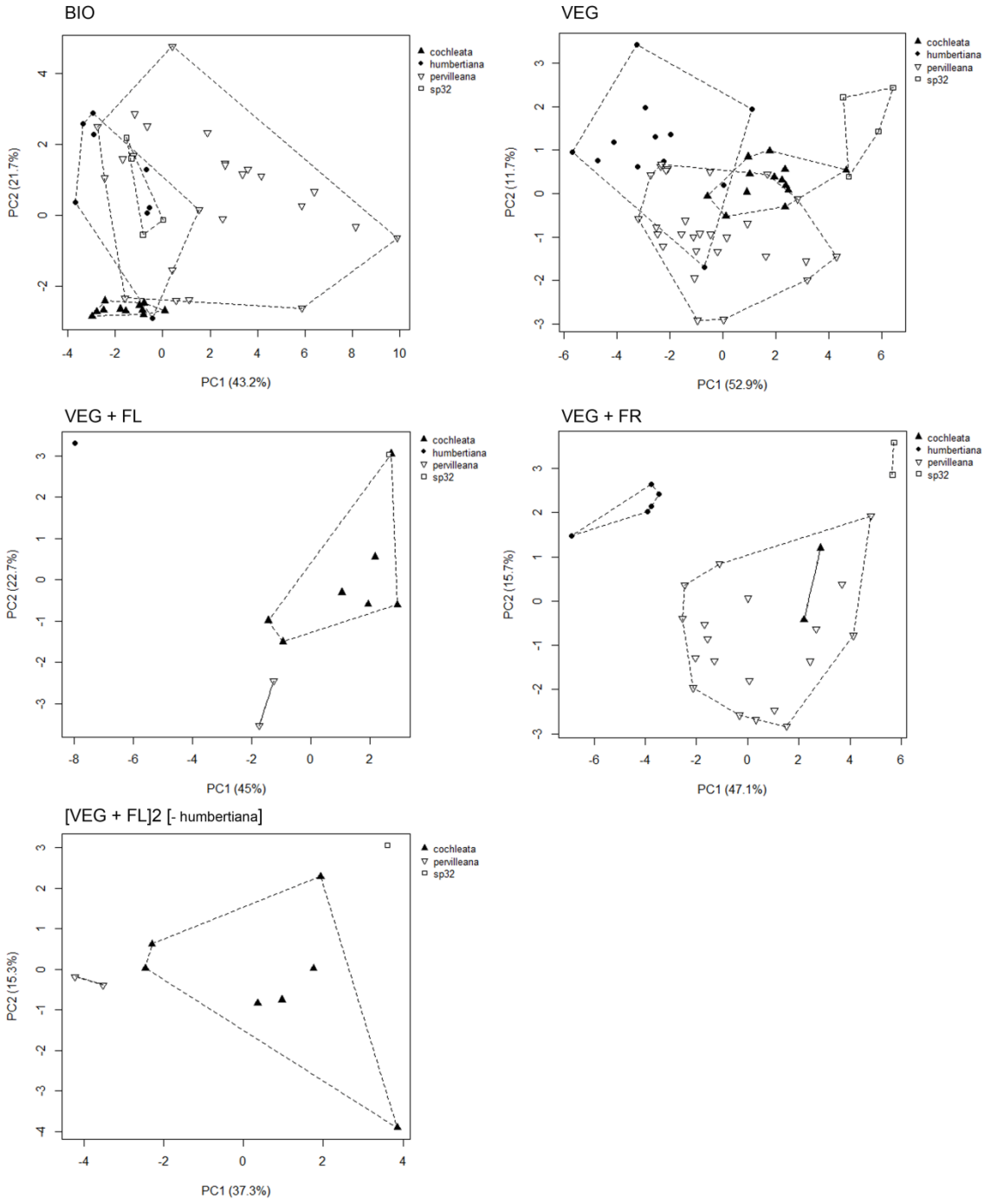


Fig. S18: CLADE P

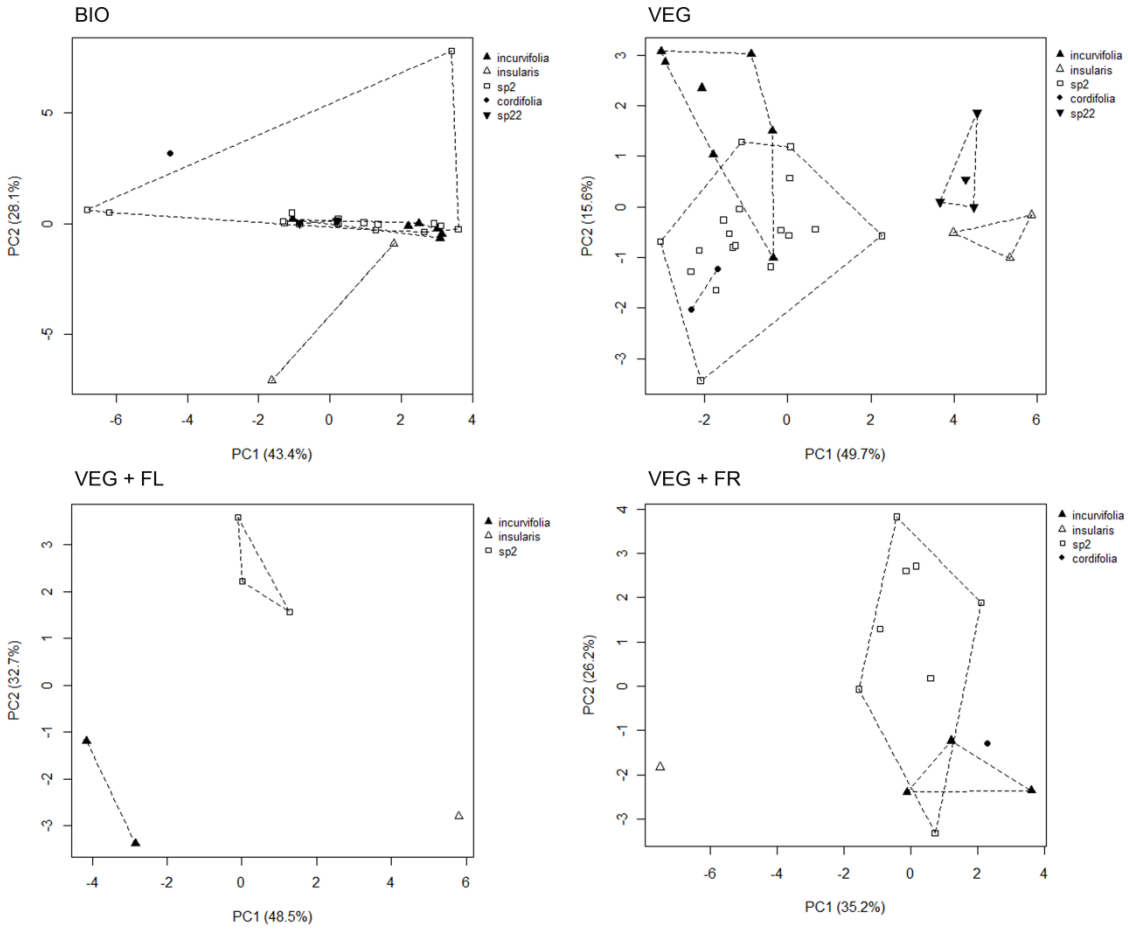


Fig. S19: ZONE CE1

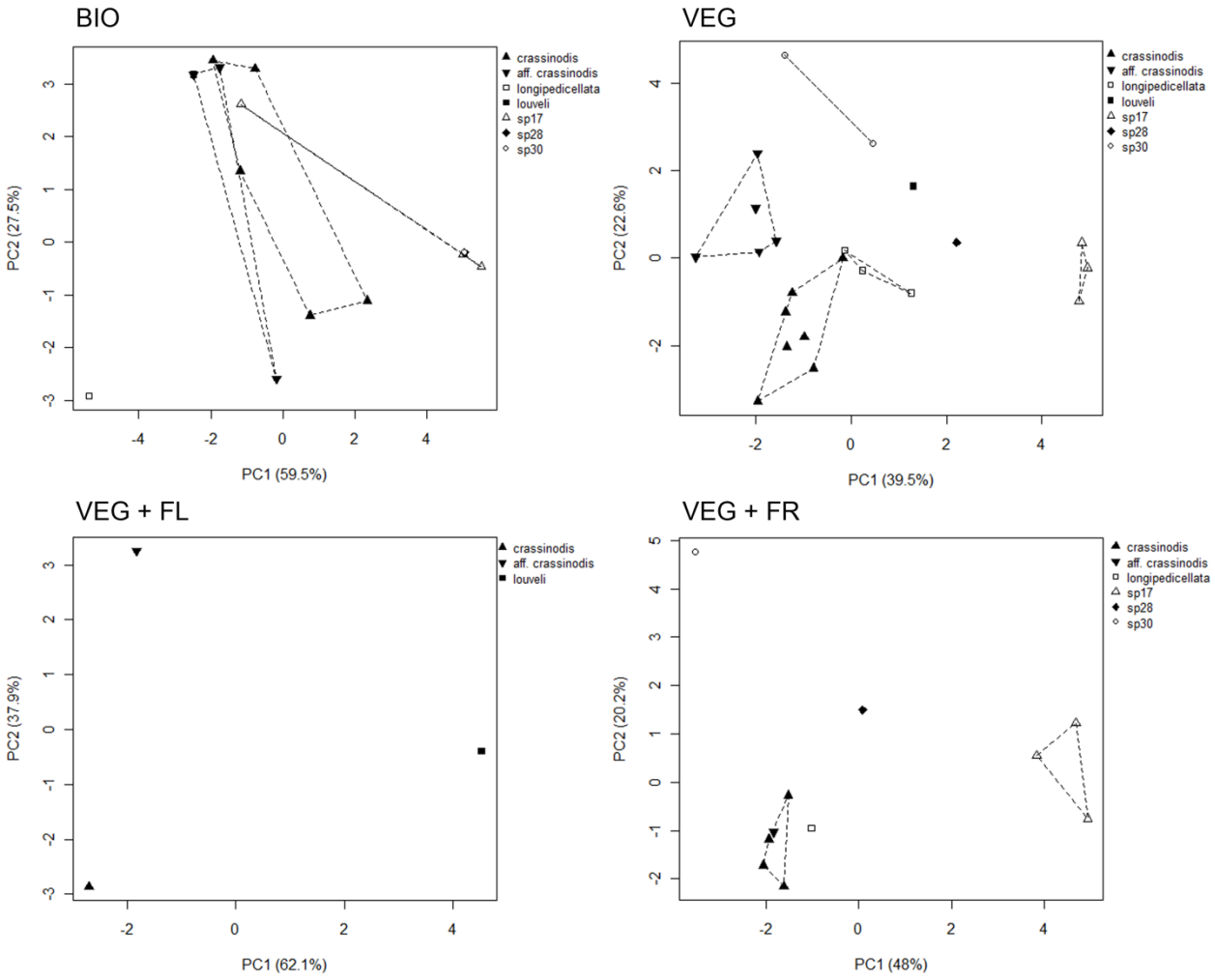


Fig. S20: ZONE CE2

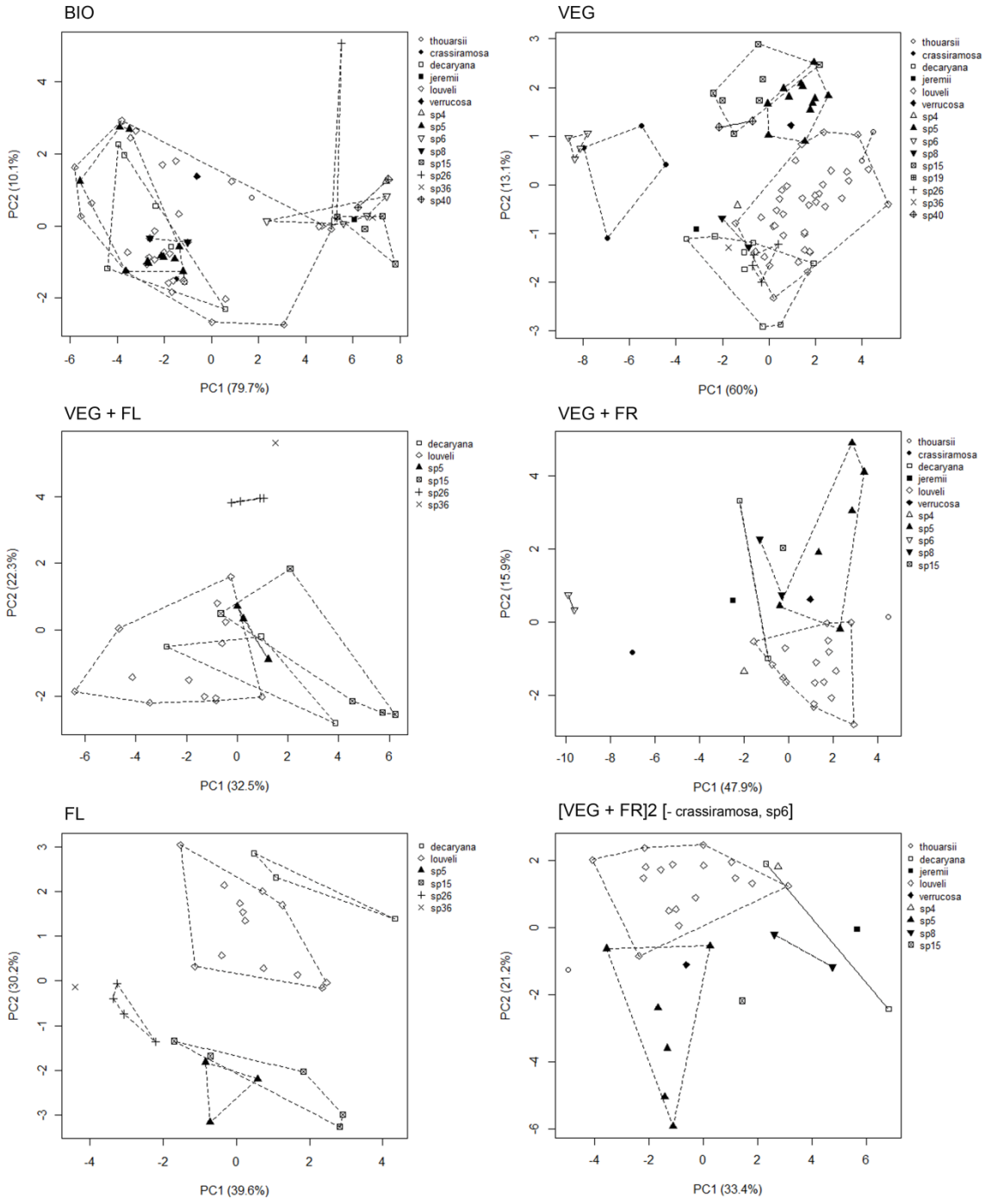


Fig. S21: ZONE CE3

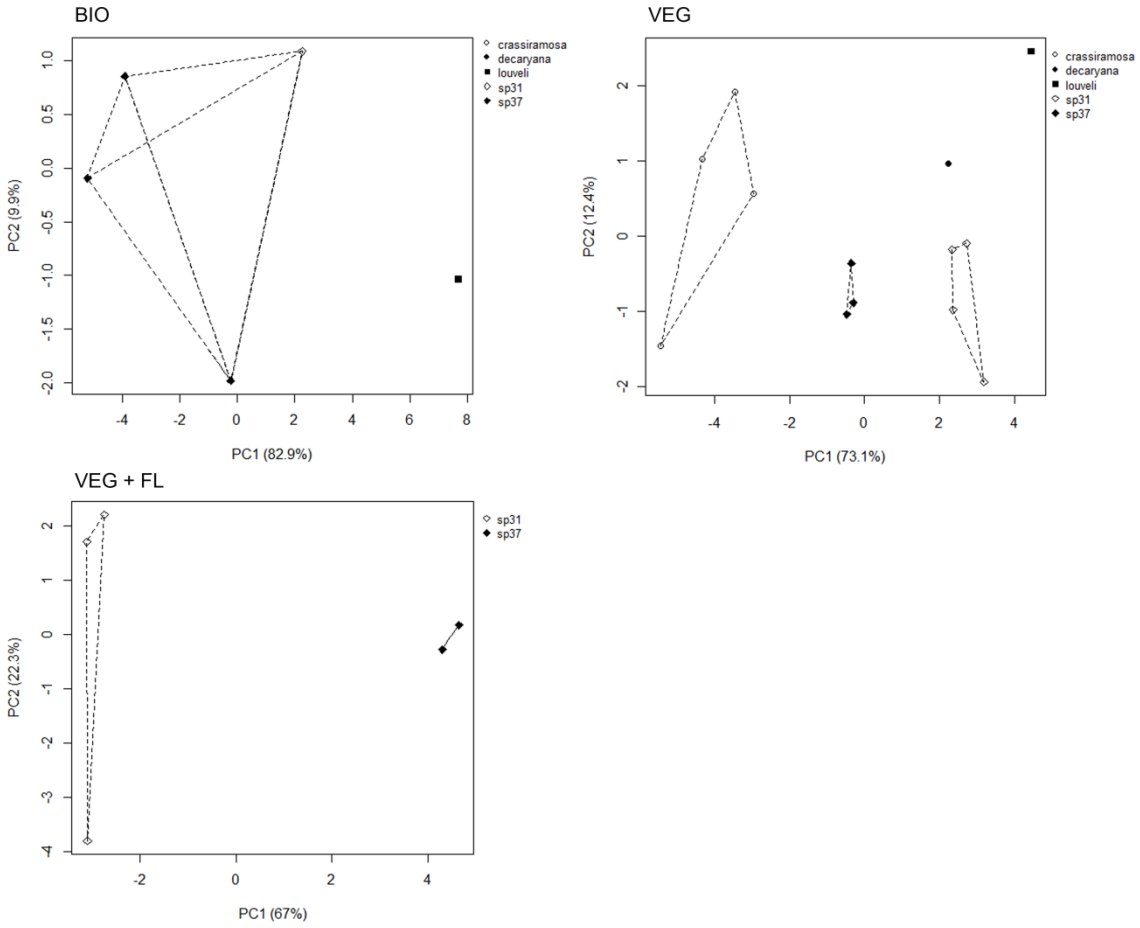


Fig. S22: ZONE CE4

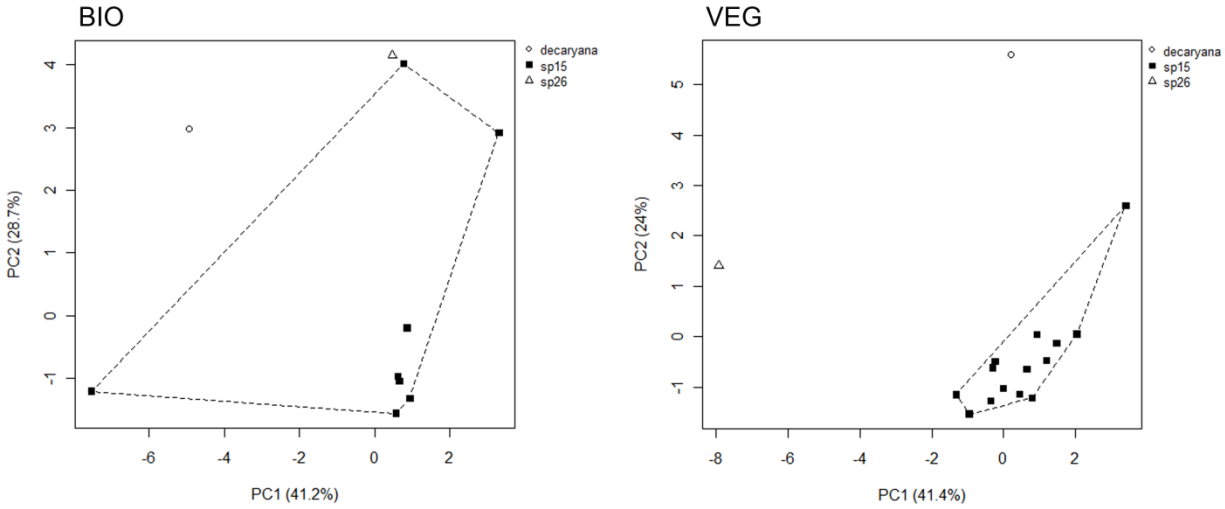


Fig. S23: ZONE CE5

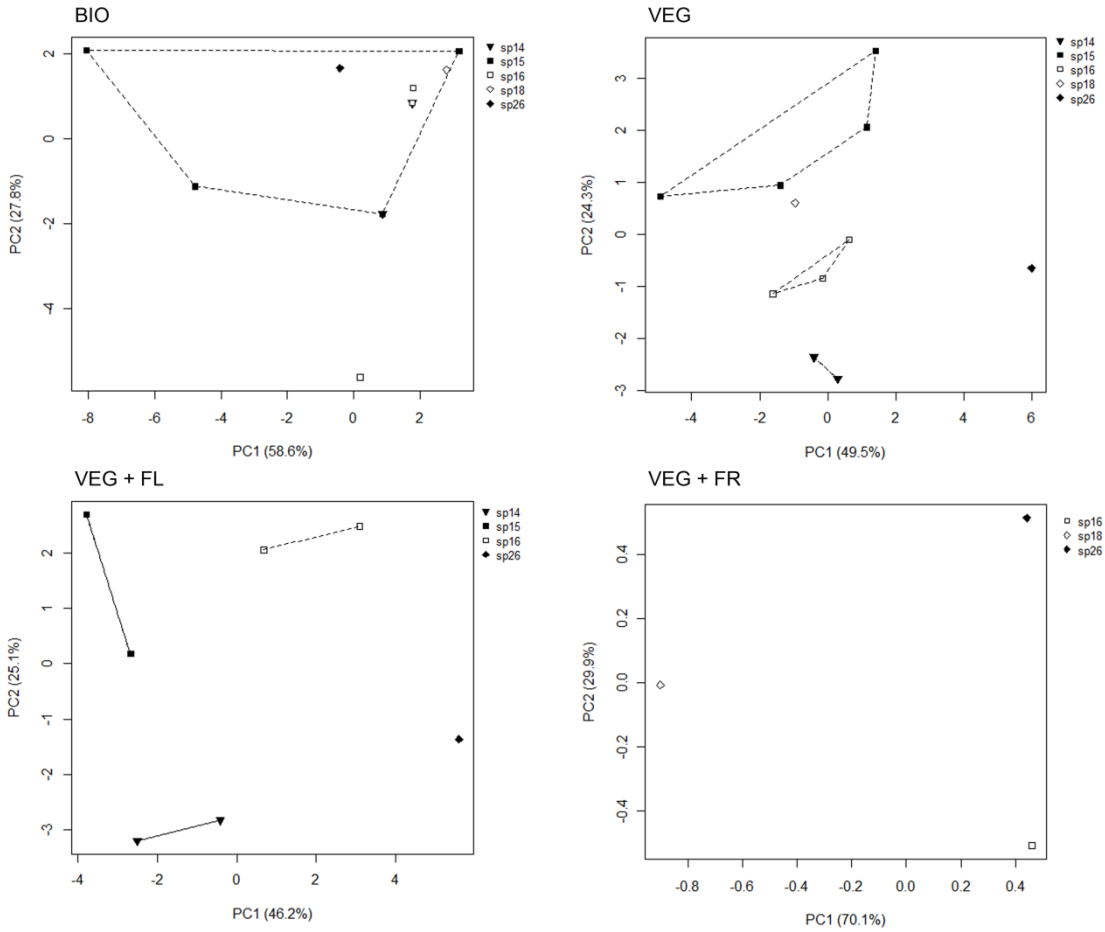


Fig. S24: ZONE CE8

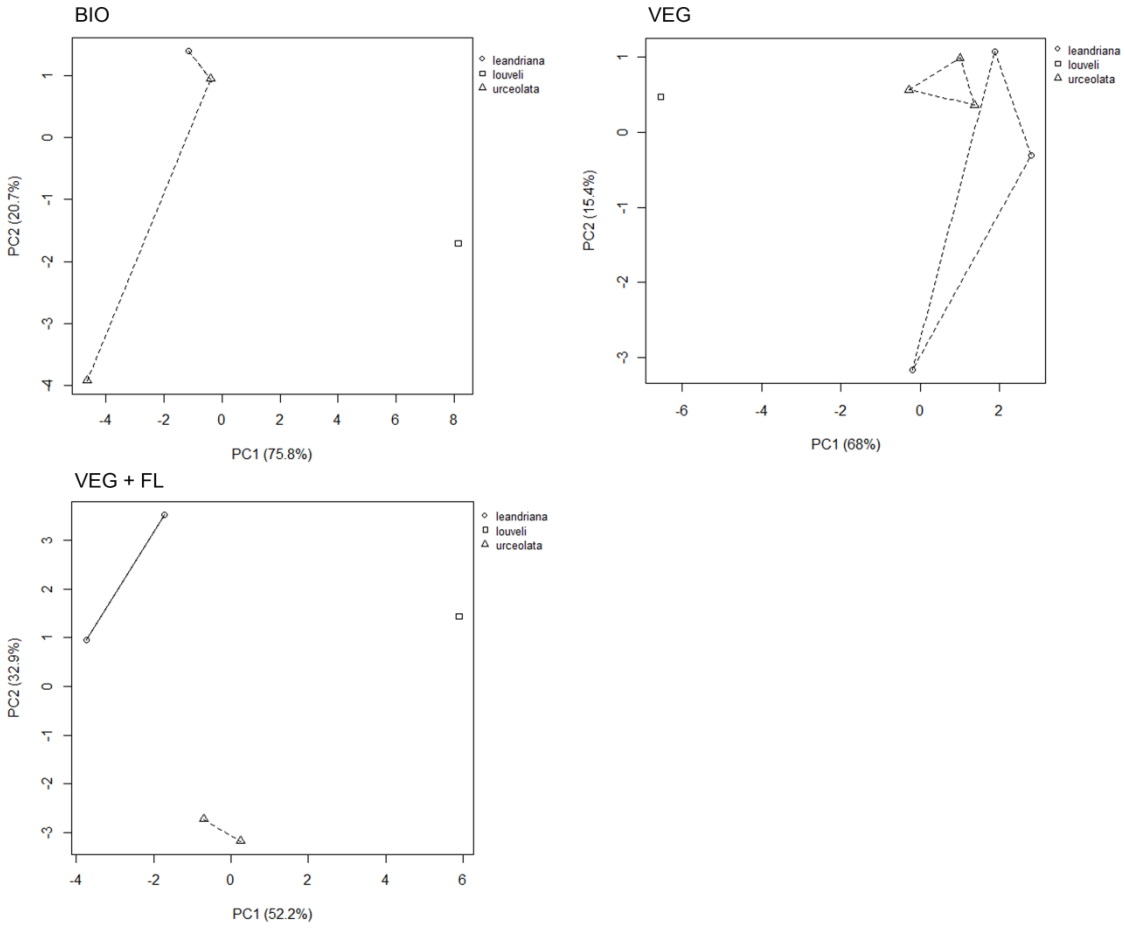


Fig. S25: ZONE CE9+COMOROS

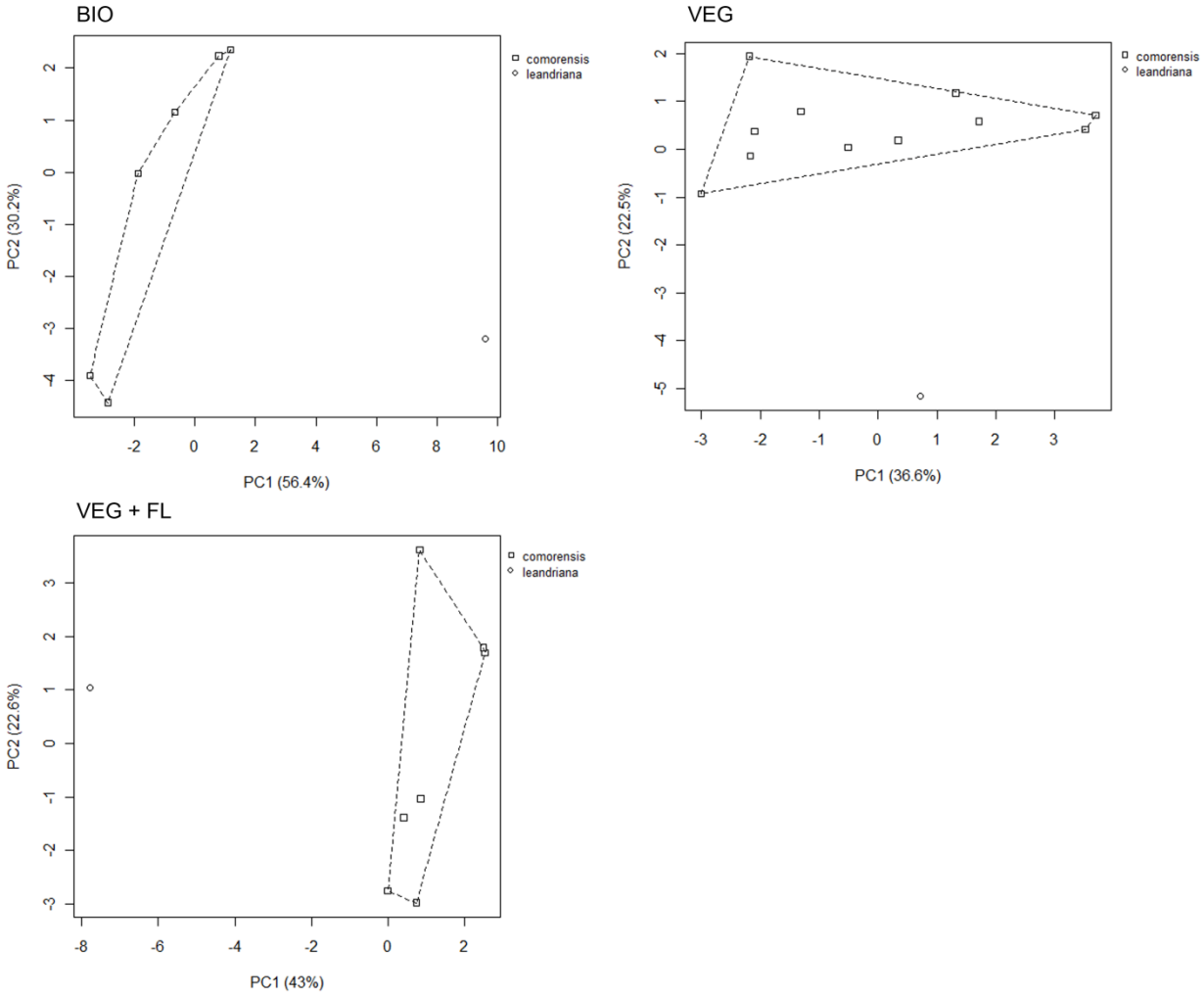


Fig. S26: ZONE CE10

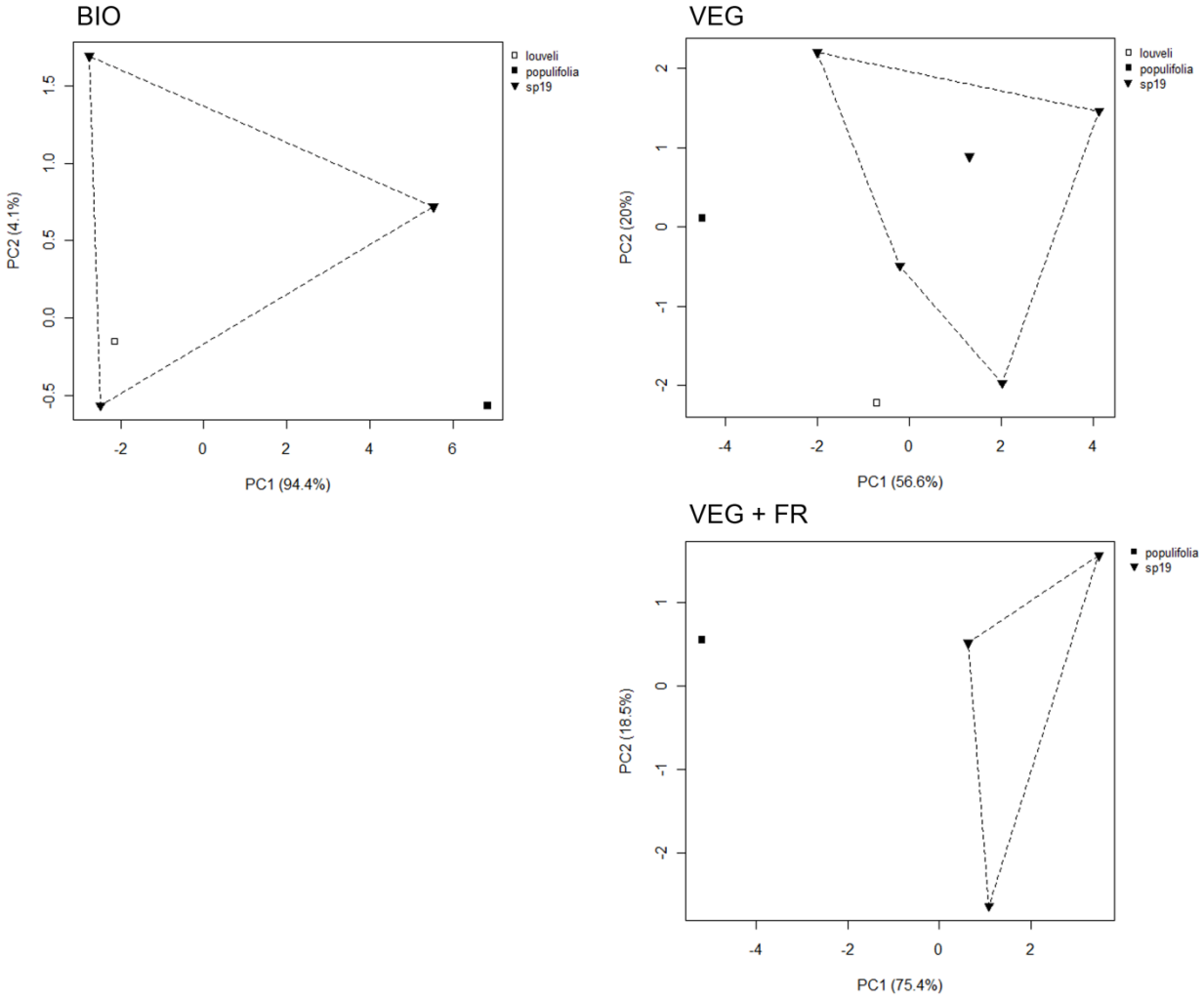


Fig. S27: ZONE CE11

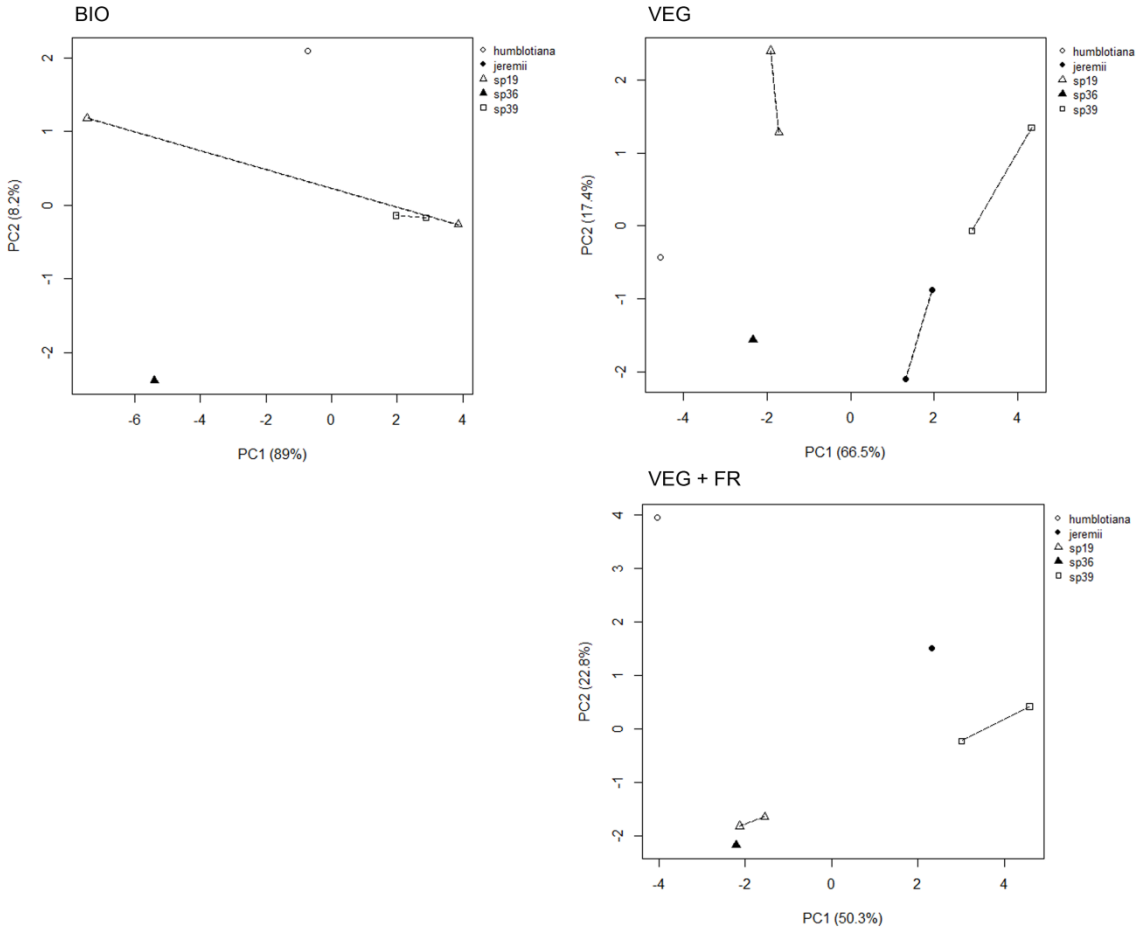


Fig. S28: ZONE CE12

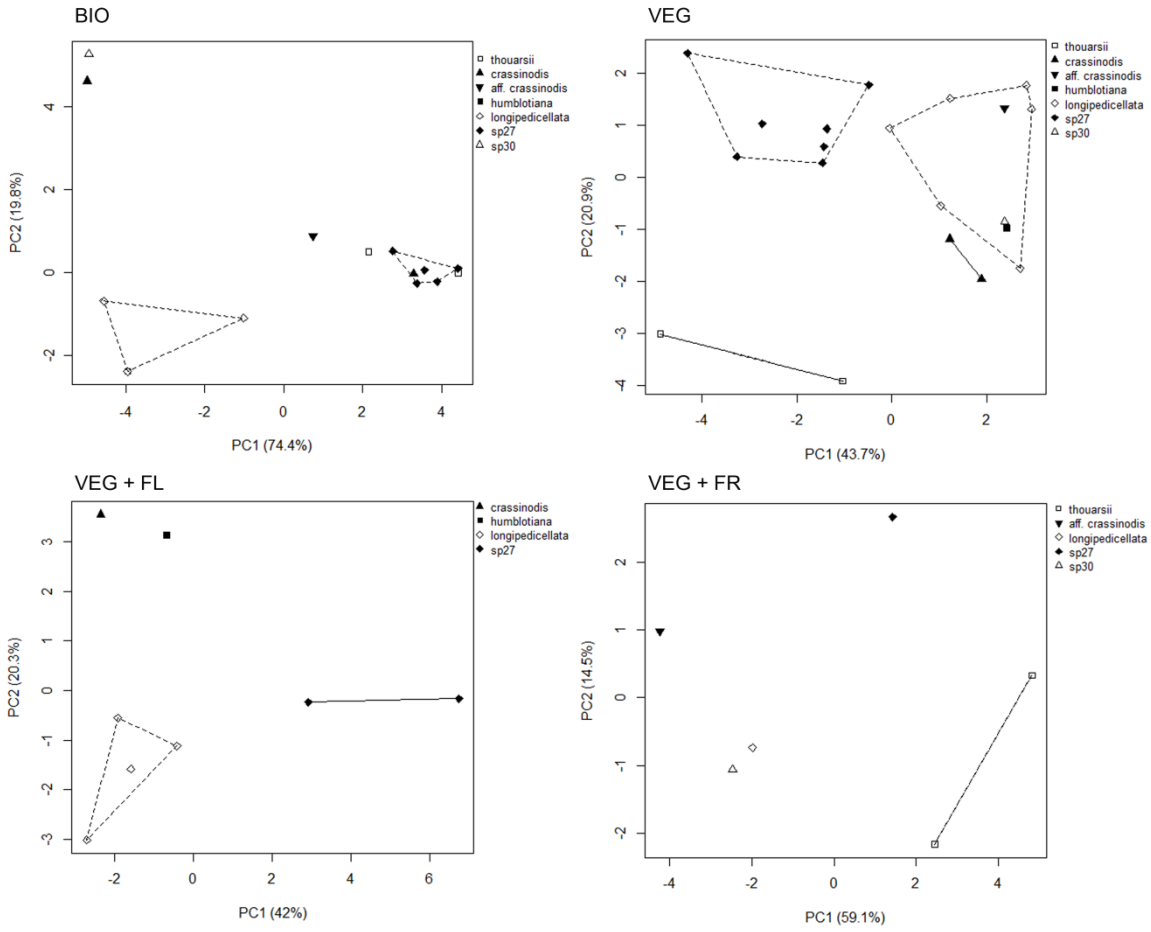


Fig. S29: ZONE RDN

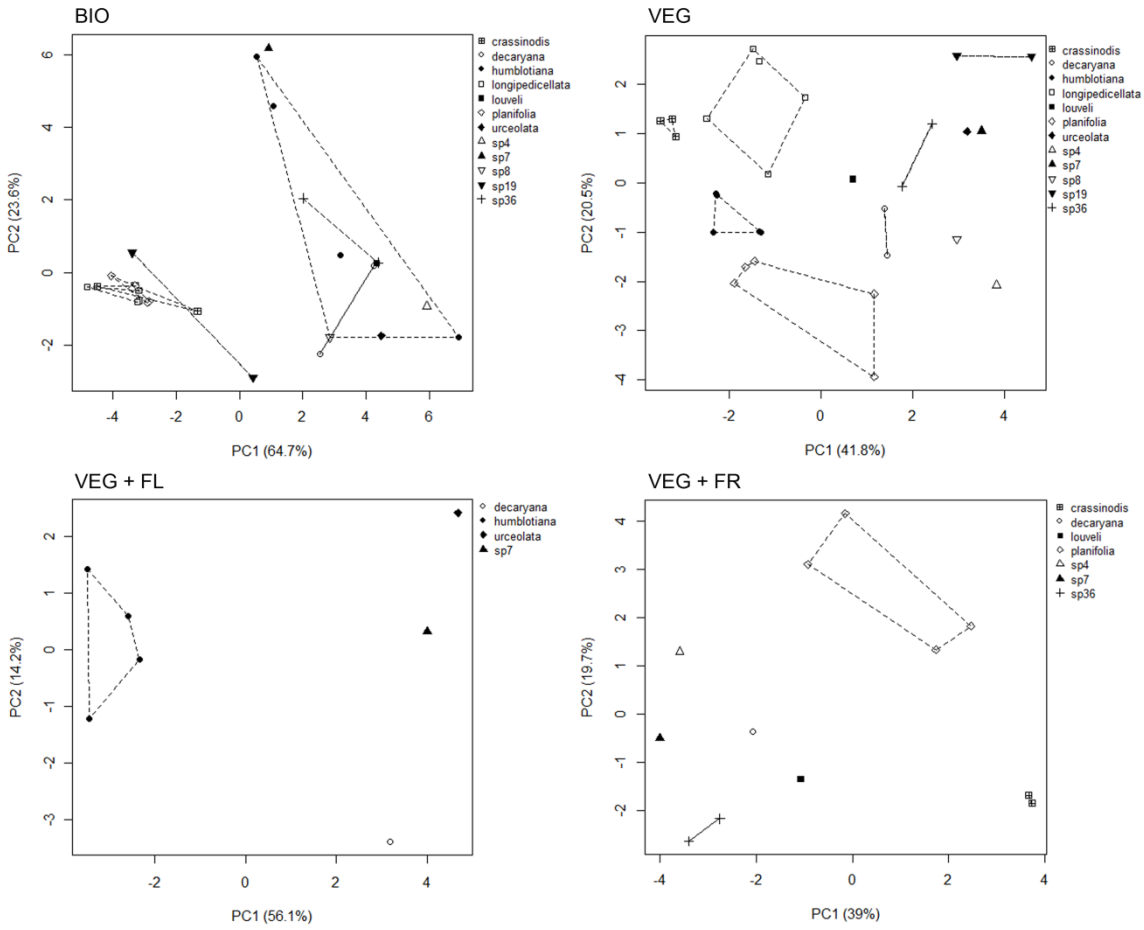


Fig. S30: RDS

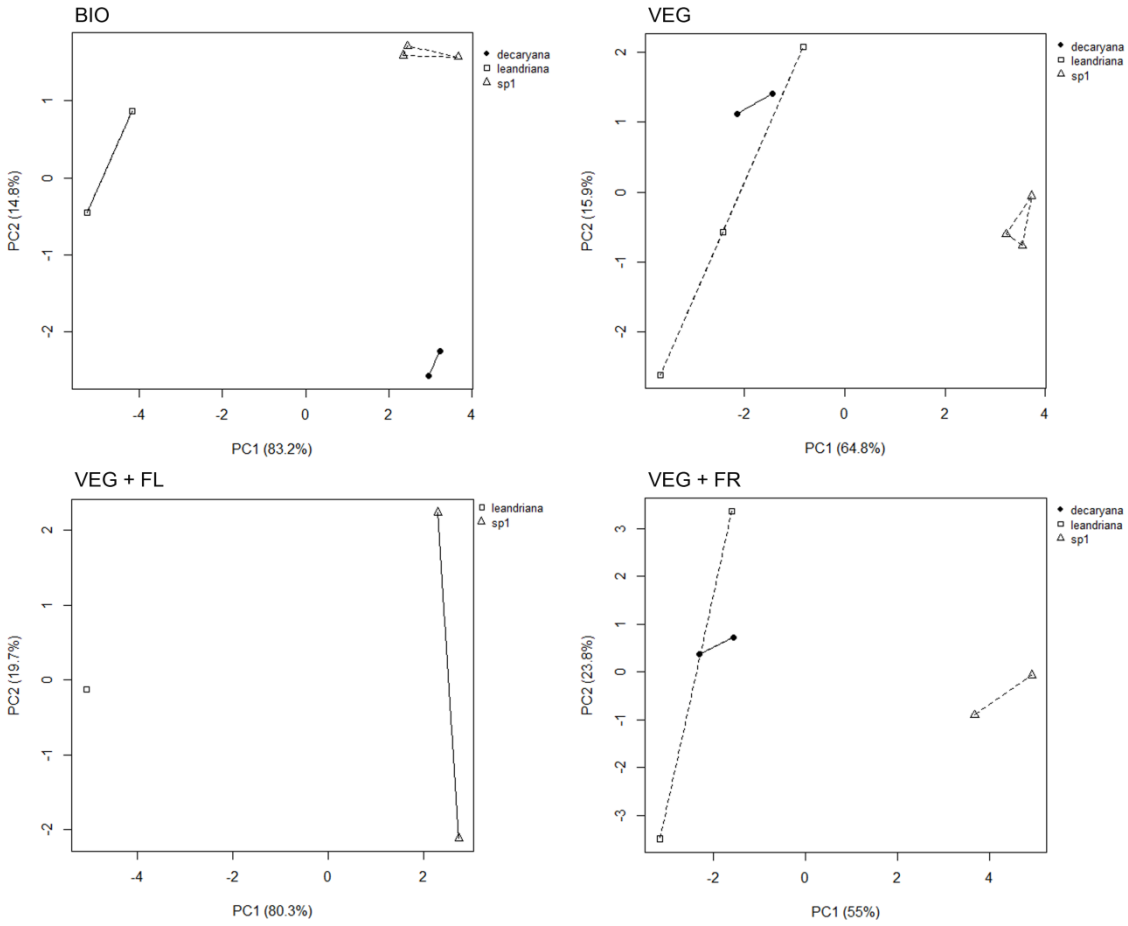

BIO
PC2 (14.8%)
PC1 (83.2%)
decaryana
leandriana
sp1
VEG
PC2 (15.9%)
PC1 (64.8%)
decaryana
leandriana
sp1
VEG + FL
PC2 (19.7%)
PC1 (80.3%)
leandriana
sp1
VEG + FR
PC2 (23.8%)
PC1 (55%)
decaryana
leandriana
sp1

Fig. S31: A - *N*. aff. *candicans* vs. *N*. *candicans*; B – *N*. aff. *candicans* vs. CE1 species

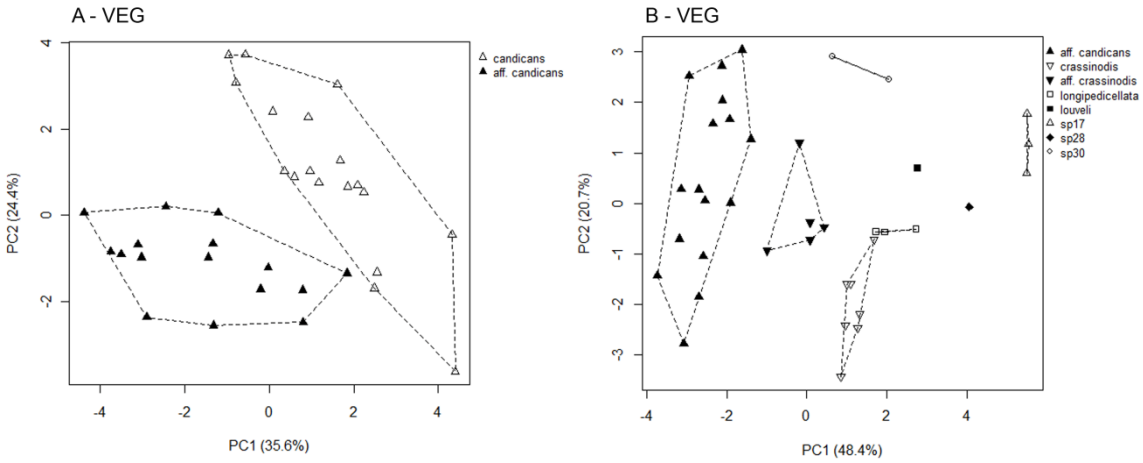
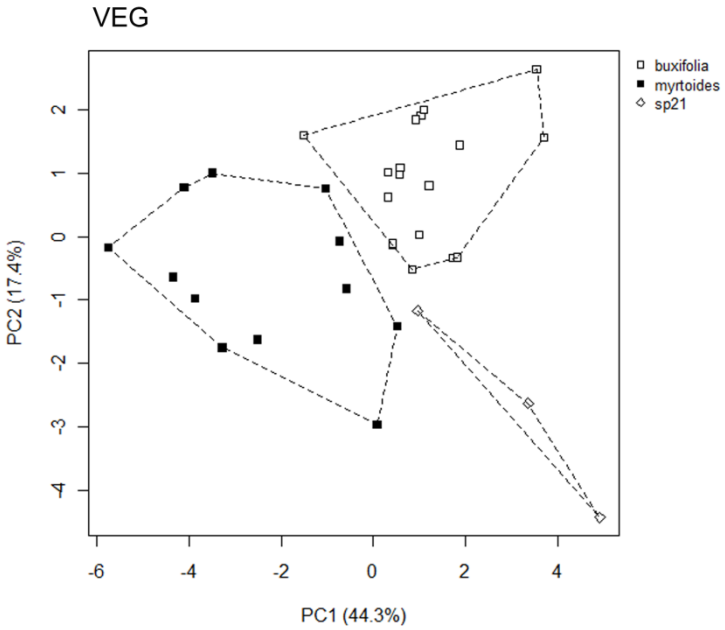


Fig. S32: *N*. sp21 vs. *N*. *buxifolia* and *N*. *myrtoides*



CHAPTER 3

RICHNESS, ENDEMISM AND COEXISTENCE IN THE MADAGASCAR OLIVE (*NORONHIA*, OLEACEAE)

Introduction

Species coexistence concerns the richness of species that occur together in space and time (Tokeshi 1999) and results from the interaction between ecological and evolutionary processes (Ricklefs 1987). The mechanisms by which coexistence is maintained remain the source of ongoing debate (Chesson 2000), but emphasis has been given to niche-based assembly rules (Diamond 1975, Webb et al. 2002, Cavender-Bares et al. 2004) and more recently to neutral assembly (Hubbell 2001). On one hand, niche-based models usually explain co-occurrence of species by the processes of limiting similarity and environmental filtering, which generate contrasting patterns of community assembly. Limiting similarity promotes niche differentiation through phylogenetic or trait diversity in the community. Alternatively, environmental filtering creates a local assemblage of species with similar tolerance to abiotic or biotic factors, leading to less phylogenetic or trait diversity in the community. On the other hand, community assembly can also be random, in which case species co-occur by chance alone and phylogenetic and trait patterns are neither clustered nor overdispersed.

If explaining species coexistence in general has been a challenge in community ecology, understanding the coexistence of closely related species becomes an even more daunting task (Mooney et al. 2008, Zhang et al. 2010). Existing methodological frameworks have mostly focused on the coexistence of a broad range of taxonomic groups (Webb et al. 2002) and may not sufficiently address the special case of coexistence patterns of closely related taxa, e.g. within-genus patterns. Indeed, closely related species are likely to share many phenotypic and ecological attributes owing to their recent common ancestry and they may also use a similar set of resources (Mooney

et al. 2008). A high level of phenotypic and ecological similiraty among closely related species can imply similar environmental tolerance suggesting that community assembly may involve environmental filtering. It can also imply exploitation of similar resources suggesting that community assembly may be governed by competition for limiting resources. However, studies of phylogenetic community structures at fine spatial and taxonomic scales found evidence for phylogenetic overdispersion (Cavender-Bares et al. 2004, Slingsby and Verboom 2006) indicating that interspecific competition, and not habitat preferences, shaped the structure of the community.

In this study, I focused on the genus *Noronhia* (Oleaceae), whose species often co-occur both at small spatial scales, growing literally side by side, and at broad spatial scales within Madagascar (C. Hong-Wa pers. obs.), which makes them a good model system to assess processes that maintain coexistence and promote species richness. There are about 80 species of *Noronhia* in Madagascar, of which ca. 30 are new and are referred to hereafter as *Noronhia sp* followed by a number (see chapter 2 of this dissertation). *Noronhia* species vary greatly in their morphology and ecology; however some are cryptic (indicated here with the qualifier "aff.") and were only detected after detailed analyses of morphological and molecular data (see chapter 2 of this dissertation). *Noronhia* is the largest radiation of the olive family in Madagascar and represents an important component of the Malagasy flora. It is also ecologically important showing adaptations to different environmental conditions, e.g. sclerophylly or indumentum to resist drought, or drip-tips to tolerate high humidity. Physiological and anatomical adaptations to particular edaphic conditions are also likely. Such adaptive traits may have facilitated the colonization of different habitats in Madagascar and could

explain the overall success of this genus relative to the other Malagasy members of the family.

To understand the spatial patterns of richness, endemism and coexistence within *Noronhia*, I used a combination of herbarium, field and laboratory data. Specifically, a study of species coexistence that integrates environmental, spatial, trait and phylogenetic information presents a unique opportunity to examine the influences of ecological and evolutionary factors involved in the spatial organization and assembly of local communities. Species are spatially distributed with respect to environmental factors that act upon the environmentally sensitive traits they possess. A spatial signal in the distribution of traits and species can thus be detected when environmental factors that filter some traits are spatially autocorrelated (Fortin and Dale 2005, Pavoine et al. 2011).

Overall, my specific objectives for this study were to (1) describe the patterns of *Noronhia* species richness and endemism within the island, (2) determine the pattern of phylogenetic structure in a local community, (3) examine the association between species and their habitat, (4) identify the combinations of environmental variables contributing to the assembly of the local community, and (5) detect the lineages on which, and the geographic locations where, these environmental variables act.

Methods

Spatial patterns of species richness and endemism

Occurrence data for all species of *Noronhia* in Madagascar were compiled based on field surveys (global positioning system - GPS - records), herbarium specimens and the TROPICOS database of the Missouri Botanical Garden (http://www.tropicos.org).

Georeferencing used the Gazetteer to Malagasy Botanical Collecting Localities (http://www.mobot.org/MOBOT/Research/madagascar/gazetteer/) and maps. I divided Madagascar into grid cells of 82 x 63 km = 5166 km^2, the scale on which ecological parameters are suggested to vary on the island (Wollenberg et al. 2008). Indeed, the size of grid cells is an important variable when identifying centers of species richness and endemism as it can either inflate the number of these centers when too small or confound explanatory ecological factors that vary on a smaller scale when too large (Crisp et al. 2001). For each grid cell, I calculated values of species richness and endemism of *Noronhia* in Madagascar (Figs. 1a and b). Species richness is just the number of species per grid cell whereas endemism is scored according to the species range, i.e. the smaller the range, the higher the endemism score. For example, a score of 1 if the species occurs in a single grid cell, a score of 0.5 if it occurs in two grid cells and so on. I then calculated the weighted endemism for each grid cell as the sum of the endemism score for that grid cell. I used the corrected weighted endemism (Crisp et al. 2001), which is obtained by dividing the weighted endemism score of a cell by the total number of species in the cell and is thus somewhat insensitive to the effect of species richness. All spatial analyses were done using ArcGIS (ESRI Inc., Redlands, CA) and the ArcView extension "Endemicity Tools".

Patterns of species coexistence

Study site and sampling

At least 10 species of *Noronhia* co-occur in Montagne d'Ambre (12°32'S, 49°10'E) in the northern tip of Madagascar, on which forests range from 200 m to 1475 m elevation (Nicoll and Langrand 1989). The mountain, approximately 30 x 10 km, is volcanic and is

some 14 million years old (IUCN/UNEP/WWF 1987). It has a distinctive humid microclimate, with mean annual temperature ranging from 17°C to 25°C, and precipitation averaging 3500 mm/year. Montagne d'Ambre encompasses mostly primary rainforests above 800 m, surrounded by a belt of lowland transitional rainforest (Raxworthy and Nussbaum 1994) and lies within a matrix of dry forests and savannas (0 to 300 m). It is now completely isolated from other rainforests in Madagascar (Fig. 1c), and the isolation may date to millions of years given the age of the mountain.

Twenty-four 50 x 20 m plots were set up in Montagne d'Ambre (Figs. 1c and d). I established these plots randomly in three different sites of increasing humidity and elevation gradients, from north to south: five around Lac Mahery, eight around Station des Roussettes and 11 around Lac Texier (Figs. 1c and d). In particular, annual precipitation is 1000-1500 mm around Lac Mahery where elevation is 300-500 m, 1500-2000 mm around Station des Roussettes between 800-1200 m elevation, and above 3000 mm around Lac Texier between 1000-1300 m elevation (Barat 1958). Species were separated in geographic space between dry areas at lower elevations and humid areas at mid to higher elevations. Within each plot, I recorded the presence and abundance of each species of *Noronhia*. Four leaf traits, presumably related to environmental filters such as water limitation and soil nutrient stress, were also noted as categorical variables: sclerophylly, indumentum, drip-tips (measured as the length of the laminar acumen) and domatia presence. Sclerophylly and indumentum are known to play a role in drought resistance whereas domatia presence has been found to correlate positively with foliar carbon concentration (O'Connell et al. 2010), which facilitates plant growth despite soil nutrient stress (Oren et al. 2001, Ma et al. 2007). Drip-tips are adaptations to extreme

humidity to increase water shedding and reduce fungal growth (Ivey and DeSilva 2001 and references therein).

Each plot was divided into 10 quadrats of 10 x 10 m. Within each plot, I recorded variables characterizing the forest structure, soil properties and topographic features. In particular, I quantified the forest structure as: abundance of all trees with diameter at breast height (DBH) equal or greater than 10 cm, percentage of forest canopy cover measured at the four corners and the middle of the plot with a spherical densiometer, forest canopy height estimated with a graduated pole and measured along the central line of the plot, and litter depth measured with a ruler at the four corners and the middle of the plot. These characteristics distinguish the dry forests of low elevations from the rainforests at high elevations, where values for each variable are usually higher suggesting that the rainforest is taller, denser and richer in organic matters. Variables characterized by five measurements were averaged before analyses. Soil samples were taken at the four corners and the middle of the plot at 20 cm depth and were homogenized before being sent to the Laboratoire de Pédologie at the Centre National de la Recherche Appliquée au Développement Rural (Antananarivo, Madagascar) for analysis of the following variables, which vary between the dry and humid areas and across topography (Barat 1958): pH, electrical conductivity (EC), which measures the ability of the soil to conduct electrical current by measuring the dissolved material (i.e. quantity of available nutrients) in the soil solution, Kjeldahl nitrogen (N), organic carbon (C), C/N ratio, organic matter (OM), available phosphorus (P), exchangeable calcium (Ca), exchangeable potassium (K) and exchangeable magnesium (Mg). I removed the Kjeldahl nitrogen (N) and organic carbon (C) from subsequent analyses because they

were highly correlated with each other and with organic matter (Pearson's $R > 0.95$). Topography was represented by elevation, which I recorded on site with a GPS.

Phylogenetic community structure

To examine the phylogenetic structure of the community of *Noronhia* in Montagne d'Ambre, I used the data from the 24 plots. Since the number of species sampled per plot was commonly low, I carried out the analyses only at the level of the sites (Lac Mahery, Station des Roussettes, Lac Texier). I also ran an analysis in which I considered Montagne d'Ambre as a single community, thus pooling all the data. I used the software Phylocom 4.2 (Webb et al. 2008) to determine the net relatedness index (NRI) and nearest taxon index (NTI) with the null hypothesis that the phylogenetic pattern is random. These indices respectively quantify the relatedness of taxa over the phylogeny of the whole pool of species and the relatedness of taxa within particular terminal clades (Webb 2000). I generated null communities using the null model 2 (Webb et al. 2008), in which species richness in the sample is maintained and species are random draws from the whole phylogeny pool (i.e. species in Madagascar) in a total of 9999 randomizations. Each species is assumed to have equal probability of presence in the study area. Phylogenetic clustering is indicated by high positive NRI and NTI, whereas negative values reflect evenness (Webb et al. 2002). Significance was assessed at a p-value of 0.05. Given the environmental differences between sites at low and high elevations, I expect significant positive NRI and NTI indicative of phylogenetic clustering at the level of the mountain, but negative or non-significant NRI and NTI indicative of phylogenetic dispersion at the level of the sites where competition for limiting resources would be

stronger. The phylogenetic tree used in this study came from the second chapter of this dissertation).

Drivers of community assembly

To estimate the degree of environmental filtering in the community of *Noronhia* in Montagne d'Ambre, I followed the procedure described in Pavoine et al. (2011). This is an ordination technique that connects five matrices representing the spatial positions (matrix S), environmental variables (matrix E), biological traits (matrix T), phylogenetic positions (matrix P) and presence or abundance of species within a site (matrix L), and is an extension of the RLQ ordination, in which the matrix R (sites) is linked with the matrix Q (traits) through the matrix L (species composition in sampling units). This approach explores and identifies environmental filters that organize communities (Pavoine et al. 2011) by assessing the combinations of traits and environmental characteristics that covary the most (Dolédec et al. 1996, Batalha et al. 2011).

The five matrices included different kinds of data and were prepared separately. First, the spatial matrix S, defined as the eigenvectors of a neighbor matrix (Thioulouse et al. 1995) that includes a value of one for neighboring sites and zero for all others (Fig. 1d), used the spatial coordinates of each plot (Figs. 1c and d). Second, the environmental matrix E used the plots in rows and the environmental variables in columns; quantitative variables were log-transformed and the entire matrix was standardized by the range. I also tested for spatial autocorrelation in the environmental variables using a Moran's test (Thioulouse et al. 1995). Third, the trait matrix T had species in rows and traits in columns. Traits variables were measured in an ordinal scale and were used to generate a distance matrix. Fourth, the phylogenetic matrix P was based on the pairwise distances

among species in the phylogenetic tree (Fig. 3), which was extracted from the master tree used in the phylogenetic community structure (see chapter 2 of this dissertation). I checked for phylogenetic signal both in the composite trait (i.e. all traits together) and in each trait separately using the root-skewness test (Pavoine et al. 2010). I also assessed the trait clustering using the TQE test developed by Pavoine et al. (2010), which measures trait diversity in an assemblage using Rao's quadratic entropy on distance matrices. Finally, the last matrix (matrix L) used the plots as rows, the species as columns and their abundance as entries.

Before linking the five matrices, I first analyzed each one using a factorial method; in particular, I used principal component analyses for the matrices S and E, principal coordinate analyses for the distance matrices T and P, and correspondence analyses for matrix L. The new matrices with reduced dimensionality - X_E, X_S, X_T and X_P - were subsequently standardized using the square root of the first eigenvalue of each analysis to ensure their comparability at the same scale. I combined the standardized matrices X_E^*, X_S^*, X_T^* and X_P^* by juxtaposition to form the matrices R as $[X_E^*|X_S^*]$ and Q as $[X_T^*|X_P^*]$ linked by matrix L, which were then analyzed in the RLQ framework with centered principal component analysis (Dolédec et al. 1996, Pavoine et al. 2011). I carried out all analyses in R 2.15.0 (R Development Core Team 2012) using the packages ade4 (Dray and Dufour 2007), ape (Paradis et al. 2004), spdep (Bivand 2012) and the different functions provided in Pavoine et al. (2011).

Results

Species richness and endemism

Noronhia species grow over almost the entire island of Madagascar, the large gaps in the center and south (Fig. 1a), are in areas dominated mainly by grasslands and spiny forests. Centers of species richness occur mainly in the north and in the east, the former harboring the highest species diversity (Fig. 1a). These centers tended to be confined to mountainous areas, e.g. in the north, Montagne d'Ambre, Montagne des Français, Manongarivo and Marojejy, and in the south, Andohahela and Anosy-Vohimena. By contrast, centers of endemism were more widespread and were found mainly in the north, west and south (Fig. 1b) at both high and low elevations. There was also a center of endemism in the Central Highlands, which had the highest value of corrected weighted endemism. Only in the north and south did the centers of species richness and centers of endemism correspond. The west had low species richness but showed higher scores for endemism whereas the central east (the region around Andasibe) had higher species richness but low endemism (Fig. 1a and b). While centers of species richness coincided mostly with mountainous regions, centers of endemism did not show any topographic pattern (Fig. 1a and b).

Community structure

Twelve species were recorded within the plots established in Montagne d'Ambre (Fig. 2). Species richness was highest in the dry habitats with a total of eight species (*N*. aff. *candicans*, *N*. aff. *crassinodis*, *N*. *candicans*, *N*. *capuronii*, *N*. *humbertiana*, *N*. *sp2*, *N*. *sp22* and *N*. *sp32*), seven of which could be found in just a single plot of 0.1 ha. The other four species (*N*. *ambrensis*, *N*. *brevituba*, *N*. *gracilipes* and *N*. *sp27*) were found at mid to higher elevations in humid habitats; *N*. *ambrensis* was recorded only around Station des Roussettes. The average number of species per plot ranged from two in the

humid habitats to five in the dry habitats. Species of the dry habitats were shrubs to small trees ≤ 5 m high whereas those of the humid areas were medium to large trees > 5m high.

The phylogenetic structure of *Noronhia* communities did not differ among the three sites in Montagne d'Ambre. Both NRI and NTI had positive values that were generally not significantly different from the expectation of the null hypothesis (Table 1) suggesting that co-occurring species did not belong to the same clades and were not closely related within particular clades. The NRI of the Lac Mahery community, however, was significantly higher than expected by chance alone indicating that species within this community were clustered within the same or few clades without being immediately related. Indeed, the species in the whole study area belong to only six of the 16 major clades recovered for *Noronhia* (see Fig. 2 in chapter 2 of this dissertation). Clustering of closely related species may also be suggested for this community given the marginal significance of the NTI (= 1.435, p = 0.058). This significant positive NRI of the Lac Mahery community suggests that species membership to this community was filtered by the environmental variables examined here and to which they are adapted. The lower significance of NTI relative to NRI may result from the lack of phylogenetic resolution near the tips, thus reducing the ability to detect any patterns, whether clustering or overdispersion, among sister taxa (Vamosi and Vamosi 2007). By contrast, analyses that considered Montagne d'Ambre as a single community showed a significant pattern of phylogenetic clustering both at deeper nodes (NRI = 1.852, p = 0.017) and near the tips (NTI = 1.771, p = 0.021).

Community assembly

Independent tests on the different matrices showed significant spatial autocorrelation in the environmental variables, in particular, pH ($p = 0.008$), electrical conductivity (EC, $p = 0.023$), organic matter (OM, $p = 0.001$), exchangeable magnesium (Mg, $p = 0.002$), elevation ($p = 0.001$), litter depth ($p = 0.003$) and tree abundance ($p = 0.001$). I did not detect a phylogenetic signal in the composite trait ($p = 0.148$) and only one biological trait (sclerophylly) had a significant phylogenetic signal ($p = 0.015$) when analyzed separately, although domatia presence also showed marginally significant signal ($p = 0.055$). Likewise no overall trait clustering or dispersion was observed (TQE, $p = 0.109$).

The integrated analysis of the five matrices (ESLTP) according to the RLQ framework allows detection of associations between the species attributes (trait and phylogeny) and their habitats (space and environment). In the RLQ analysis, 83% of the total variation was picked up by the first axis alone. Positive correlations were with areas with higher pH and higher concentration of exchangeable calcium, available phosphorus and exchangeable potassium (Fig. 4b). These areas, located in the drier areas at lower elevations around Lac Mahery (Fig. 5a), were rich in soil base cations but poor in organic matter, and harbored plants with traits such as sclerophylly, indumentum and domatia presence (Fig. 4a). All but three species of *Noronhia* in Montagne d'Ambre had at least one of these traits (Fig. 5b). The first axis was also negatively correlated with elevation, organic matter, canopy cover, canopy height, litter depth, electrical conductivity, carbon-to-nitrogen ratio and concentration of exchangeable magnesium (Fig. 4b). These environmental variables characterized wetter areas in central and southern Montagne d'Ambre (Fig. 5a), which were rich in organic matter. The only plant trait that was negatively correlated with the first axis was laminar acumen length (Fig.

4a), which was found to be long (i.e. presence of drip-tips) in the three species (*N. brevituba*, *N. gracilipes* and *N. sp27*) growing in moist habitats (Fig. 5b).

Discussion

Species richness and endemism

The geographic analyses indicated that northern Madagascar was the center of species richness for *Noronhia* (Fig. 1a). Although this pattern may reflect a collection bias toward well-sampled localities in the northern biogeographic region (e.g. Montagne d'Ambre, Montagne des Français, Ankarana), other well-sampled areas (e.g. Ankarafantsika, Ranomafana, Zahamena) harbored fewer species. Species richness mostly coincided with topographically complex areas throughout the island, suggesting a substantial role of mountainous areas in the diversification of *Noronhia*. Similar patterns have been found in other groups of organisms in Madagascar such as cophyline frogs (Wollenberg et al. 2008), leaf chameleons (Townsend et al. 2009) and vascular plants (Hong-Wa et al. 2008). In particular, the northern massifs of Madagascar, including Tsaratanana, Manongarivo, Marojejy and Montagne d'Ambre, as well as the southeastern massifs of Andohahela and Anosy-Vohimena have been suggested to act as species pumps, promoting adaptive and vicariant speciation (Raxworthy and Nussbaum 1995, Wollenberg et al. 2008, Vences et al. 2009).

Centers of endemism coincided with only a few centers of species richness. These are the grid cells containing Montagne d'Ambre in the north, Manongarivo in the northwest, and Andohahela and Anosy-Vohimena in the south (Fig. 1a and b). Overlapping centers of endemism and species richness may represent historical centers

of cladogenesis (Ricklefs and Schluter 1993, Jetz et al. 2004). Although it is unclear whether current distributions of species reflect the original geography of speciation or postspeciation range shifts (Losos and Glor 2003), the coincidence of endemism and richness in Montagne d'Ambre, Manongarivo, Andohahela and Anosy-Vohimena supports the idea that these massifs are centers of diversification for *Noronhia*. Centers of endemism were also found in less known areas in the west and northeast that are mostly characterized by historical collections. Many species have indeed been collected in only a few localities, so their actual range may be underestimated. Nevertheless, the pattern of microendemism suggests an evolution by specialization to particular environments or fine-scale environmental variables (Vences et al. 2009), as with species growing on karst mountains in the north (Ankarana) and the west (Bemaraha). Indeed, a variety of speciation mechanisms may have contributed to the generation of this pattern of local endemism (Pearson and Raxworthy 2009, see chapter 2 of this dissertation).

Community structure

Species of *Noronhia* often co-occur. Indeed, figure 1 shows high concentrations of species in small areas. For instance, at least 10 species coexist in areas such as Montagne d'Ambre, Andasibe and Andohahela. If Montagne d'Ambre were a center of diversification, one would expect close relationships among the species it harbors. Despite low phylogenetic resolution at deeper levels, several small clades could be recovered within *Noronhia* (see chapter 2 of this dissertation) and the phylogeny as a whole showed that the 12 species occurring in Montagne d'Ambre belong to six clades only. The analyses of the phylogenetic structure of *Noronhia* communities in Montagne d'Ambre showed a pattern no different than random in two of the three sites, although a

signal of clustering emerged when Montagne d'Ambre was considered as a single community (Table 1).

Montagne d'Ambre is divided into dry and humid habitats. Although species occurring in each habitat are not immediately related, in the big picture they belong to a few clades only, thus the positively significant NRI (Table 1). The signal of phylogenetic clustering suggests that communities are structured along the moisture gradient from low to high elevations. Therefore, water limits are an environmental filter at the scale of the mountain. Within the drier habitat (i.e. Lac Mahery), however, other factors may influence species assembly.

The interpretation of the phylogenetic structure of a community can be influenced by the taxonomic and geographic scales at which the analysis was carried out (Cavender-Bares et al. 2006, Swenson et al. 2006). Specifically, phylogenetic overdispersion characterizes finer taxonomic (e.g. a single phylogenetic lineage) and spatial (e.g. one plot or one forest type) scales whereas phylogenetic clustering appears at broader scales. Although most species on Montagne d'Ambre belonged to different but few clades, the phylogenetic clustering there seemed to result from the comparison of the local assemblage to a species pool that encompassed a broader spatial scale (the entire island). However, using a null model that drew species from the local sample only (model 1, Webb et al. 2008), there was the same pattern of phylogenetic underdispersion, although it was only marginally significant (NRI = 1.35, $p = 0.094$; NTI = 1.385, $p = 0.085$). The focus on a single phylogenetic lineage also resulted in a pattern of underdispersion that is inconsistent with previous findings (Cavender-Bares et al. 2006, Swenson et al. 2006). There might be some power issues to detect patterns of clustering or dispersion with a

sample size of 12 species although this sampling is hardly different from other studies finding phylogenetic dispersion, e.g. 17 species of Floridian oaks (Cavender-Bares et al. 2004), 15 species of South African reticulate-sheathed schoenoid sedges (Slingsby and Verboon 2006) and 11 species of Caribbean anoline lizards (Losos et al. 2003). However, the polytomies at basal and terminal nodes in the master tree (see chapter 2 of this dissertation) may have influenced the outcome of the analyses since NRI and NTI are known to be sensitive to phylogenetic resolution (Swenson 2009).

Community assembly

The RLQ analysis that integrated space, environment, phylogeny and traits highlighted the role of soil nutrients in addition to humidity gradients as dominant environmental filters organizing the distribution of *Noronhia* species on Montagne d'Ambre (Fig. 4b). The high number of spatially autocorrelated environmental variables in Montagne d'Ambre suggests a spatial structure in which particular combinations of variables reflect habitat heterogeneity (Batalha et al. 2011); species in this community are thus spatially clumped. Indeed, there was a geographic separation between species of dry areas at lower elevations and humid areas at mid to higher elevations (Fig. 5a). Finer segregation may be determined by soil nutrients, forest structure and topography since most variables representing these characteristics were spatially autocorrelated.

In addition, there was also a spatial signal in the distribution of the species traits such as sclerophylly, indumentum and domatia presence (Fig. 4a). These traits characterized species growing in the dry, nutrient-poor habitats, which were *N*. aff. *candicans*, *N*. aff. *crassinodis*, *N. candicans*, *N. capuronii*, *N. humbertiana*, *N. sp2*, *N. sp22* and *N. sp32* (Fig. 5b). Another species (*N. crassinodis*) was also observed in these habitats but lacked

molecular data and was therefore excluded from this study. It has the same traits as the other species of the dry habitats and I suspect it would be part of the polytomy as well.

The overall phylogenetic clustering indicated that environmental factors such as water and soil nutrient availability filtered closely related species. This result substantiates the idea that environmental filtering was the dominant process allowing species coexistence in Montagne d'Ambre. Among the traits included here, only sclerophylly exhibited a strong phylogenetic signal, indicating that it was conserved in some lineages (the clade containing *N. humbertiana* to *N. sp22*). However, it was also found in other species (e.g. *N.* aff. *candicans*, *N.* aff. *crassinodis*, *N. candicans*). Thus it appeared that both trait conservatism and trait convergence have influenced the coexistence of species in Montagne d'Ambre.

However, this pattern of phylogenetic clustering coupled with randomness in traits suggested that critical conserved traits were omitted from the analysis (Pavoine et al. 2010, 2011). Indeed, physiological traits (e.g. specific leaf area, leaf mineral content, wood density), pollination syndromes (e.g. flower shape, size, color) or fruit dispersal syndromes (e.g. fruit color, mass, persistence) can potentially influence community assembly (Kraft et al. 2008, Sargent and Ackerly 2008, McEwen and Vamosi 2010). The power to detect patterns in traits also depends on scale, and is largely reduced when scale is large (Kraft and Ackerly 2010). The scale used in this study is much larger than the commonly 20 x 20 m used for tropical forests (Webb 2000, Kembel et al. 2006, Kraft et al. 2008), especially because the data were pooled at the scale of the mountain. Therefore, the random pattern obtained in the trait-based analysis in this study could

indeed have resulted from a lack of power in the statistical analyses rather than showing a true pattern of randomness.

Conclusion

Habitat heterogeneity largely explains the patterns of species richness within the Madagascar olive (*Noronhia*) at different spatial scales. At large scales, the highest concentrations of species were located on topographically complex areas across the island. In addition, the pattern of microendemism may also indicate a spatially structured diversification, probably correlated with habitat heterogeneity. At small scale, variation in environmental characteristics acting as filters permitted species coexistence across habitats in local communities. However, it is not clear how the coexistence of seven congeneric species could be maintained in an area of just 0.1 ha. It is possible that biotic factors prevail in the species assembly when scale is small (Mooney et al. 2008, Sargent and Ackerly 2008). Indeed, these species vary mostly in habit and the shape of their leaves; but also in the shape, size and color of their flowers, thus potentially attracting different pollinators, and in the shape, texture and color of their fruits, thus attracting different dispersers (Fig. 2).

An issue of conservation concern emerging from this study relates to species inclusion within protected areas (Fig. 1a and b). Species-rich areas mostly include protected areas, which may reflect a bias in sampling effort towards protected areas. It may also suggest that given the current habitat degradation in Madagascar, species will in the future be found only in areas benefiting from some kind of protection. Unfortunately, most range-restricted species of *Noronhia* are not represented within protected areas (Fig. 1b). Thus they face a substantial risk of extinction, which will also

lead to a considerable loss of phylogenetic diversity. *Noronhia* is an ecologically important genus, being adapted to different environmental conditions and being part of the diet of several lemur species (Donati et al. 1999, Birkinshaw 2001, Simmen et al. 2006, Radespiel 2007). It is also an evolutionary important genus, being the largest genus of the olive family in Madagascar (the other three genera include only ca. 20 species altogether). It is an important component of the Malagasy flora, having colonized various habitats and thriving in most of them. In addition, its pattern of diversification, likely driven by several mechanisms (see chapter 2 of this dissertation), offers useful insights into the diversification of the Malagasy biota. *Noronhia* is therefore a taxon of high conservation value. Based on this study, I recommend both richness and rarity as critical criteria guiding conservation strategies.

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Table 1. Phylogenetic structure of *Noronhia* communities in Montagne d'Ambre

Community	NRI	NTI
Montagne d'Ambre	1.852 (p = 0.017)	1.771 (p = 0.021)
Lac Mahery	1.498 (p = 0.045)	1.435 (p = 0.058)
Station des Roussettes	1.028 (p = 0.144)	1.053 (p = 0.122)
Lac Texier	0.902 (p = 0.171)	0.960 (p = 0.115)

Figure captions

Figure 1. (a) Spatial patterns of species richness in *Noronhia*, quantified as the number of species present per grid cell. (b) Patterns of endemism across Madagascar measured as the corrected weighted endemism, for which values close to zero and one means low and high endemism respectively. Numbers refer to areas discussed in the text: 1 = Montagne des Français, 2 = Montagne d'Ambre, 3 = Ankarana, 4 = Manongarivo, 5 = Tsaratanana, 6 = Marojejy, 7 = Ankarafantsika, 8 = Zahamena, 9 = Bemaraha, 10 = Andasibe, 11 = Ranomafana, 12 = Andohahela, 13 = Anosy-Vohimena. (c) Bioclimatic map of the northern tip of Madagascar showing the location of Montagne d'Ambre and the study plots. (d) Neighborhood graph of the 24 study plots labeled with numbers. PA = protected areas, PN = parc national, RS = réserve spéciale.

Figure 2. Species of *Noronhia* observed on Montagne d'Ambre. (a) *N*. aff. c*rassinodis*. (b) *N*. *ambrensis*. (c) *N*. *capuronii*. (d) *N*. *gracilipes*. (e) *N*. *sp2*. (f) *N*. *sp27*. Photo credit: C. Hong-Wa.

Figure 3. Phylogenetic tree of *Noronhia* species occurring in Montagne d'Ambre extracted from a phylogeny of the whole pool of species. The elevation at which each species occurs is given.

Figure 4. (a) Spearman correlations between the ordinal species traits and the coordinates of species on the first axis of the RLQ analysis. (b) Pearson correlations between the environmental variables and the coordinates of the sites on the first axis of the RLQ analysis. Ca = exchangeable calcium, C.N = carbon to nitrogen ratio, cover = percentage of canopy cover, EC = electrical conductivity, height = canopy height, litter = litter

depth, K = exchangeable potassium, Mg = exchangeable magnesium, OM = organic matter, P = available phosphorus, tree = abundance of trees with dbh ≥ 10 cm.

Figure 5. Geographic and phylogenetic representation of the results of the RLQ analysis based on the coordinates of sites and species on the first axis. (a) Global coordinates of sites defined as the sum of a combination of both environmental and spatial variables. White and black squares indicate negative and positive coordinates respectively; their size is proportional to the absolute values of the site coordinates. (b) Global coordinates of species defined as the sum of a combination of both trait and phylogenetic variables. Species names are given in Figure 3.

Figure 1

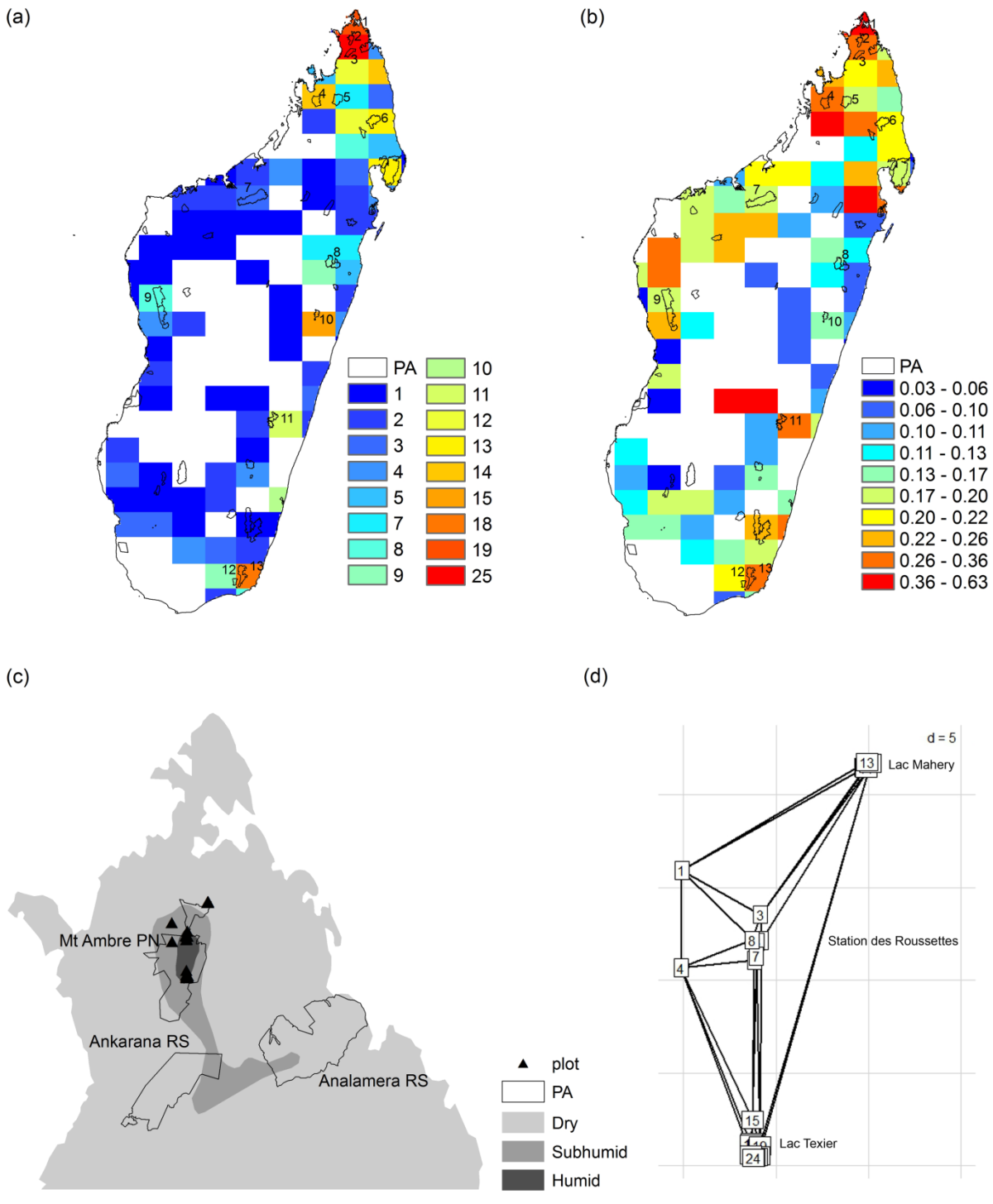

(a)
PA
1
2
3
4
5
7
8
9
10
11
12
13
14
15
18
19
25
(b)
PA
0.03 - 0.06
0.06 - 0.10
0.10 - 0.11
0.11 - 0.13
0.13 - 0.17
0.17 - 0.20
0.20 - 0.22
0.22 - 0.26
0.26 - 0.36
0.36 - 0.63
(c)
Mt Ambre PN
Ankarana RS
Analamera RS
plot
PA
Dry
Subhumid
Humid
(d)
d = 5
Lac Mahery
Station des Roussettes
Lac Texier

Figure 2

Figure 3

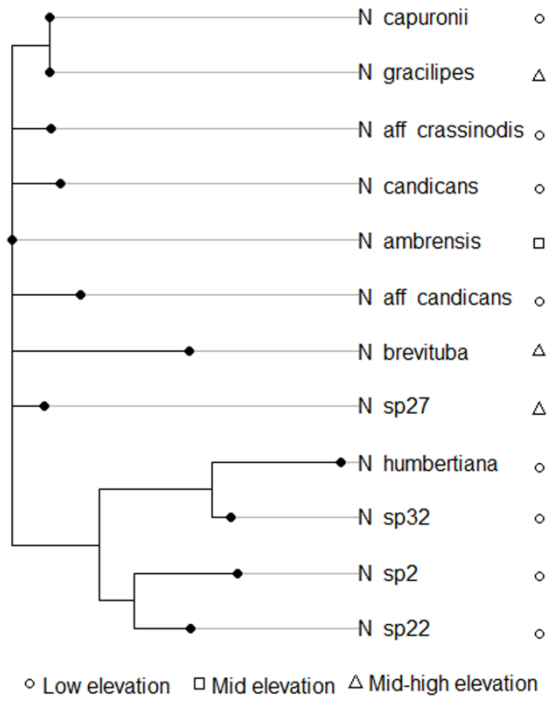

N capuronii
N gracilipes
N aff crassinodis
N candicans
N ambrensis
N aff candicans
N brevituba
N sp27
N humbertiana
N sp32
N sp2
N sp22
Low elevation
Mid elevation
Mid-high elevation

Figure 4

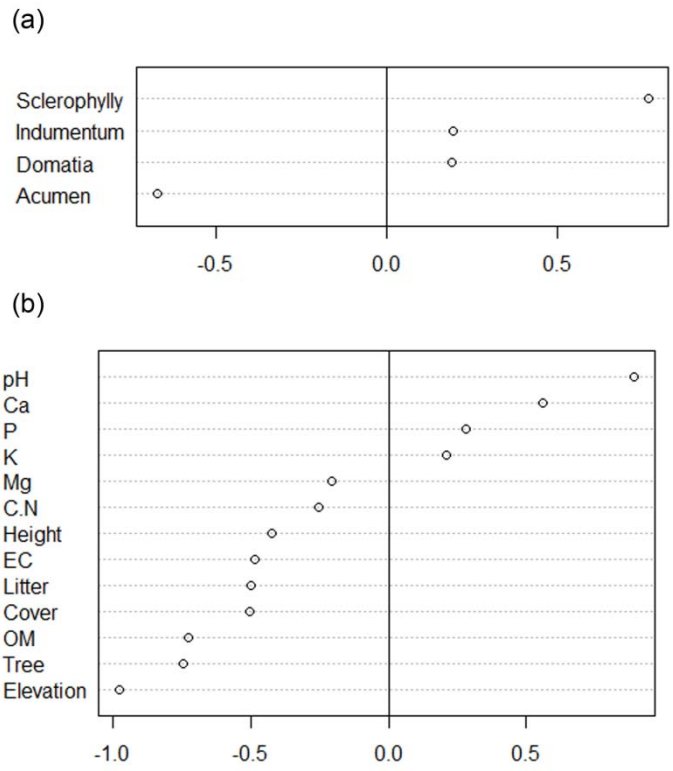
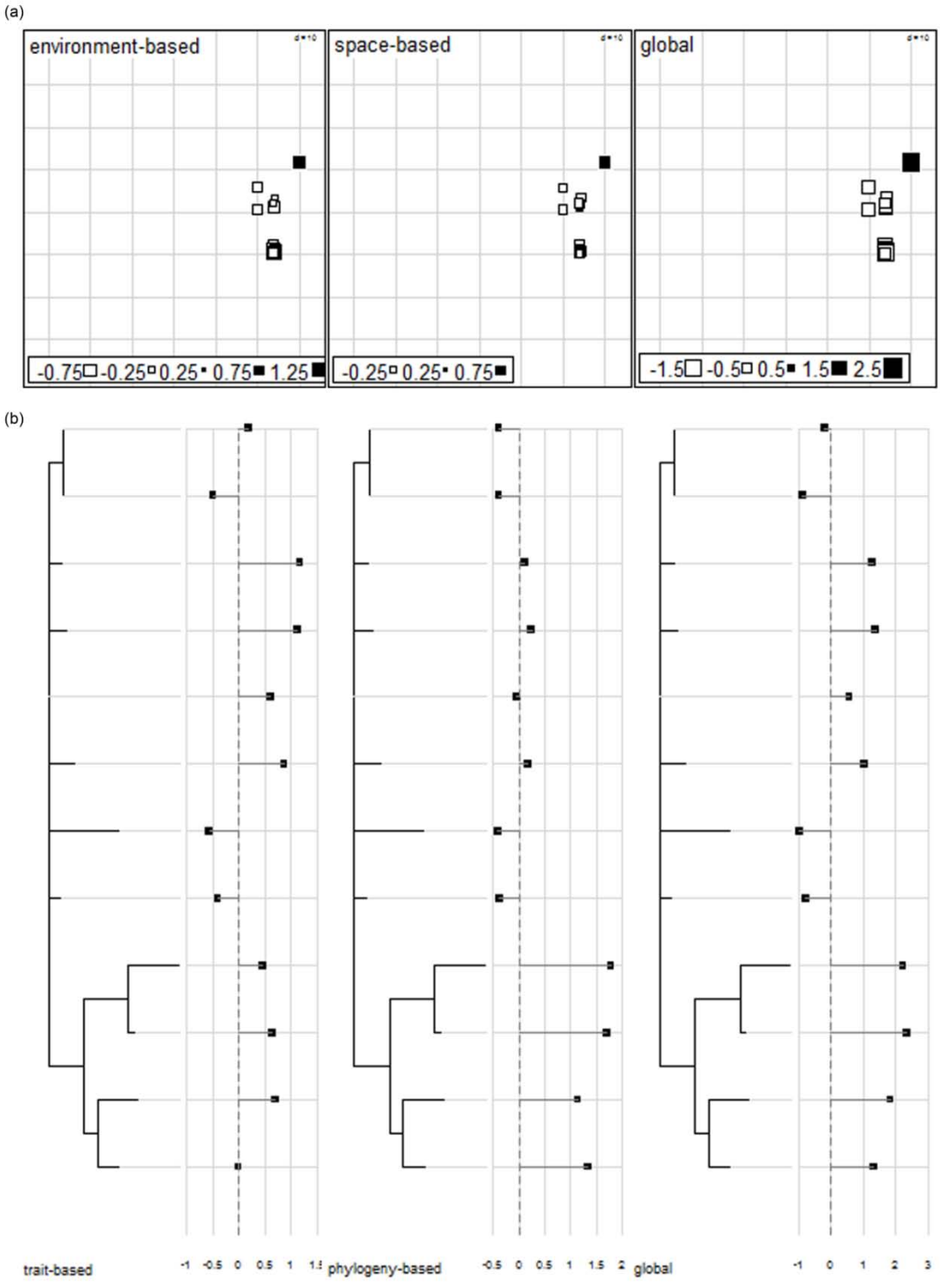

(a)
Sclerophylly
Indumentum
Domatia
Acumen
-0.5
0.0
0.5
(b)
pH
Ca
P
K
Mg
C.N
Height
EC
Litter
Cover
OM
Tree
Elevation
-1.0
-0.5
0.0
0.5

Figure 5


(a)
environment-based
space-based
global
-0.75 -0.25 0.25 0.75 1.25
-0.25 0.25 0.75
-1.5 -0.5 0.5 1.5 2.5
(b)
trait-based
phylogeny-based
global