Multi-locus phylogenies of the genus Barteria (Passifloraceae) portray complex patterns in the evolution of myrmecophytism

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A B S T R A C T

The four species of the central African genus Barteria show variation in habitat and in degree of association with ants. Whereas B. solida, restricted to submontane forests, attracts opportunistic ants to extrafloral nectar, the three other species, found in lowland rainforests (B. fistulosa, B. dewevrei) and in littoral scrub (B. nigritana), possess stem domatia of varying shapes and degrees of specialisation, hosting either non-specific arboreal ants (B. nigritana, some B. dewevrei) or two large species of ants of the genus Tetraponerina Smith, 1852 that are specific to some species of Barteria (B. fistulosa, some B. dewevrei). We aimed to investigate whether this variation represents an evolutionary trend toward increasing specialisation of mutualism or the reduction or loss of myrmecophytic traits. For this, we determined phylogenetic relationships within the genus using DNA sequences (primarily nuclear ITS) and microsatellite genotypes (11 loci) on a large sample of individuals, mostly from Cameroon and Gabon. The two types of markers support an initial dichotomy that groups B. dewevrei with B. nigritana and B. fistulosa with B. solida respectively. Within these pairs, species do not appear reciprocally monophyletic. At microsatellite loci, B. nigritana forms a clade embedded within B. dewevrei; and within both B. solida and B. fistulosa, geographical populations show levels of differentiation similar to that observed between populations of B. solida and B. fistulosa. Geographic distance alone does not account for genetic differentiation between species, which indicates reproductive isolation. Divergence in each of the two pairs implies evolutionary transitions in habitat and in myrmecophytism. Specialised mutualism with specific ant species of the genus Tetraponera has been lost in species found in more marginal habitats.

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1. Introduction

The tree genus Barteria Hook. f. (Passifloraceae) is endemic to the forests of Lower Guinea and the Congo basin. It comprises four recognised species of small trees that show variable degrees of symbiotic association with ants. Three species are myrmecophytic: they have swollen, hollow cavities in lateral branches, called domatia, that are used by ants as nesting sites. The four species were recognised as such only recently (Breteler, 1999). Photographs in Breteler (1999) illustrate the considerable variation in stem morphology related to myrmecophytism among these taxa. Barteria solida Breteler, the most recently described species, is essentially restricted to submontane forest in a few isolated sites in the Lower Guinea forest block (from extreme south-eastern Nigeria through Cameroon and Equatorial Guinea to Gabon). This species produces extrafloral nectar on its leaf margins and on the deciduous leaf extensions along the branches, which attracts opportunistic ants, but does not display specialised structures for hosting ants. Barteria nigritana Hook. f. is mostly restricted to a very narrow band of land along the Atlantic coast, where it grows on sandy beaches and other Holocene deposits. In southern Gabon, however, it also occurs in the interior, reaching 650 m altitude (So-
ferences in myrmecophytic traits between species. Tetraponera, T. aethiops F. Smith, 1877 or T. latifrons (Emery, 1912). These species are the largest (~1 cm long) of their genus (Wheeler, 1922) and are only found in association with individuals of Barteria presenting wide (broad) domatia. Barteria fistulosa also depends strongly on these ants, which protect their host tree against herbivores and prune plants adjacent to their host tree, and vines growing on it (Dejean et al., 2008; Janzen, 1972; McKey, 1974). Only rarely are trees of this species found without a colony of Tetraponera ants. The distribution of the fourth species, B. dewevrei De Wild. & T. Durand broadly overlaps with that of B. fistulosa, although the former species was previously thought to be absent from Cameroon and Gabon (Breteler, 1999). Lateral branches of B. dewevrei are hollow over their entire length, but the diameter of the cavity is more variable than in B. fistulosa. In the eastern part of its range it seems that most individuals are occupied by small, non-specialist ants of the genus Crematogaster (~4 mm long), whereas in the western part the large ants of the genus Tetraponera (T. aethiops and T. latifrons) are by far the most frequent inhabitants. In the course of this study, we discovered the presence of B. dewevrei much further west (Cameroon and north-western Gabon) than its previously known range. In these western populations, its domatia are very similar to those of B. fistulosa. Whether the morphotype with wide domatia occupied by Tetraponera occurs outside Cameroon, Gabon and Central African Republic still remains to be investigated. Fig. 1 illustrates the differences in myrmecophytic traits between species.

Obligate symbiosis with ants has appeared many times in the evolution of angiosperms, but myrmecophytes do not constitute large clades (Davidson and McKey, 1993). This suggests frequent acquisitions of myrmecophytic traits. It also suggests their frequent disappearance, which could result from two quite different processes. The first is the “evolutionary dead end scenario”, whereby ecological specialisations, such as species-specific obligate mutualisms, arise and evolve but eventually end in extinction (for discussion see Althoff et al., 2012; Tripp and Manos, 2008). If this is so, myrmecophytic specialisation may be irreversible, and specialised myrmecophytes may be prone to extinction when conditions no longer favour them. Alternatively, specialisation may be reversible; in this case the lineages may survive but lose their myrmecophytic traits (Davidson and McKey, 1993; Janzen, 1974). Phylogenetic analyses suggest instances of reversal from obligate myrmecophytism to nonmyrmecophytism, and loss of variant specificity in myrmecophytes, in two of the largest myrmecophyte radiations, Macaranga (Euphorbiaceae) (Blattner et al., 2001; Davies et al., 2001) and Neonauclea (Rubiaceae) (Razafimandimbison et al., 2005), but not in a third, neotropical Acacia (Fabaceae: Mimosoideae) (Gomez-Acevedo et al., 2010). The evolutionary scenarios and environmental conditions leading to gains and losses of myrmecophytic traits are largely unknown and must be investigated by reconstructing phylogenies of closely related species. The contrasting degree of association with ants in Barteria makes this genus a good model system for investigating such questions.

Currently, there is no hypothesis regarding the phylogeny of the four currently recognised taxa of Barteria, whose status as biological species (in terms of reproductive isolation) is not strictly established. We thus determined phylogenetic relationships within the genus using both DNA sequences and microsatellite data on a large sample of individuals, mostly from Cameroon and Gabon. The phylogenies obtained allowed us to highlight an unexpectedly complex pattern of evolution of myrmecophytic traits.

2. Materials and methods

2.1. Sample collection

Large sample sizes are required to clarify the species status and relatedness of taxa for which reproductive isolation has not been tested specifically. For this study, we obtained genotypes for 696 specimens of the four recognised species of Barteria. Out of the 53 specimens provided by herbaria, 40 were used in this study (we could not obtain genotypes for the others). Samples of leaves for 656 individual trees were collected in the field by the authors or their colleagues specifically for genetical analyses. These samples consisted in a piece of leaf 10 × 5 cm that was dried in silica gel immediately after collection. A very small fraction of each sample was used for DNA extraction. The remaining material is stored in the Centre d’Ecologie Fonctionnelle et Evolutive (Montpellier, France). Apart from the four species names currently recognised in the genus Barteria (Breteler, 1999), four others have been published. Barteria acuminata Baker f. and B. stuhlmannii Engl. & Gilg., both junior synonyms of B. dewevrei, were described respectively.
from Uganda and Tanzania. We did not include the type specimen of B. acuminata in our analysis, but we included a specimen collected in the same area of Tanzania as the neotype of B. stuhlmannii. Barteria braunii Engl., a junior synonym of B. nigritana, was described from the village of Batanga, Cameroon. For this study we collected 30 specimens in Batanga, the type locality of B. braunii.

Barteria urophylla Mildbr. is a nomen nudum. Fig. 2 shows the geographical origin of each Barteria used. The genus Barteria belongs to the tribe Paropsieae, which is composed of six genera. We obtained specimens of two other genera in the Paropsieae: Paropsia edulis Thou. and Androsiphonia adenostegia Stapf. We also obtained specimens of three other genera in the Passifloraceae to be used as outgroups: Adenia cynanchifolia Harms, Eulensia clematoides C.H. Wright and Passiflora viridescens L.K. Escobar. Table 1 summarises the samples used. A Supplementary Table (Table S1) provides detailed information on individual samples.

2.2. Molecular methods

For each specimen, DNA was isolated from 0.2 g of dry leaves. Extractions were completed using the DNeasy Plant Mini Kit (Qia-gen, Venlo, Netherlands) or the Extract-N-Amp PCR ReadyMix (Sigma–Aldrich, St. Louis, USA) following the manufacturer’s instructions.

DNA sequence variation was investigated at nuclear (the Internal Transcribed Spacer locus comprising part of ITS1, 5.8S and ITS2) and plastid markers (matK and spacer 5'trnK-matk, trnH-psbA, rbcL, trnL), using standard Polymerase Chain Reaction (PCR) and Sanger sequencing. Table 1 indicates only those markers whose variation is detailed in Section 3. Table 2 presents the set of all markers investigated (including those in which variation was too low for a meaningful analysis), together with the primers used to amplify them.

Amplification of the ITS marker with primers “itsRYf” and “its-RYr” used 50 μl of solution containing 1X buffer (Q-Biogene, Montreal, Canada), 0.2 U of Taq polymerase (Q-Biogene), 2.5 mM of MgCl2, 0.25 mM of dNTPs (Promega Corp, Fitchburg, USA.), 0.2 μM of each primer and 2 μl of DNA template. Amplifications took place in a thermal cycler programmed for an initial denaturation step of 5 min at 94 °C, followed by 35 cycles of 60 s at 94 °C, 45 s at 50–55 °C and an elongation step of 60 s at 72 °C.

Sequences obtained with this procedure allowed designing more specific internal primers for increased PCR yields (ITS-Bar2F and ITS-Bar2R, see Table 2). Amplifications were performed in a 30 μl solution containing 1X PCR mix (Qiagen multiplex kit), 0.2 μM of each primer and 2 μl of DNA template. They took place in a thermal cycler programmed for an initial denaturation step of 15 min at 94 °C, followed by 35 cycles of 60 s at 95 °C, 90 s at 58 °C and 60 s at 72 °C. The last elongation step lasted 11 min.

Products obtained with the “itsRYf” and “itsRYr” primers were purified by the PEG precipitation protocol (Rosenthal et al., 1993) and both strands were sequenced using the same primer combination as for PCR amplifications. Cycle sequencing products were run on ABI capillary sequencers (Applied Biosystems, Foster City, USA). Products obtained with the internal primers were purified and sequenced by Genoscreen (Lille, France).

Eleven microsatellite markers were genotyped for all individuals of Barteria according to a protocol described previously (Molecular Ecology Resources Primer Development Consortium et al., 2012). These markers, initially developed for B. fistulosa, showed reliable amplification in the other species (Molecular Ecology Resources Primer Development Consortium et al., 2012) and are: Bar6, Bar12, Bar16, Bar27, Bar50, Bar51, Bar53, Bar61, Bar62, Bar64 and Bar69. However, these markers failed to amplify in individuals belonging to the other genera.

2.3. Analysis of genetic data

Plastid markers did not show sufficient variation in Barteria for an informative phylogeny. Thus, we used only the ITS locus for phylogenetic reconstructions.
Sequence data for the ITS marker were obtained for 145 individuals of *Barteria*. Sequence chromatograms were trimmed, assembled (for each individual), aligned using the Muscle algorithm and visually checked under Geneious Pro 5 (Drummond et al., 2011). Many individuals showed ambiguities in base calling at one or several polymorphic sites. Ambiguities might have resulted from heterozygosity, but also from variation among the numerous repeats of ribosomal DNA gene clusters (Alvarez and Wendel, 2003). This uncertainty prevented us from inferring the gametic phase of the genotypes, and from recovering sequences of every gene copy via cloning of PCR products. We instead scored any ambiguous nucleotide in a given individual as such.

Maximum likelihood phylogenies were constructed with the PhyML (Guindon and Gascuel, 2003) Geneious plug-in and assumed the Hasegawa–Kishino–Yano (Hasegawa et al., 1985)+Gamma model of nucleotide evolution favoured by the Bayesian Information Criterion calculated with jModelTest (Posada, 2008). The PhyML algorithm was set to estimate transition/transversion ratios and the gamma parameter and to optimise the tree topology, branch lengths and the substitution rate. One thousand trees were generated by bootstrapping to test node support.

Bayesian inference phylogenies were constructed with the MrBayes (Ronquist and Huelsenbeck, 2003) Geneious plug-in and assumed the same model of evolution. The same sequence data were used as for reconstruction of maximum likelihood phylogeny. Four Markov chains were run simultaneously and sampled every 1000 iterations for a total of five million iterations. Stationarity was reached around 10⁶ generations; the first 20% of trees generated were thus discarded.
membership and geographical origin. We then built a Neighbour-Joining phylogeny of these genetic groups using the same distance measure. We also used the Average Square Distance (Goldstein et al., 1995; Slatkin, 1995), which assumes closer relatedness between microsatellite alleles of similar sizes (the stepwise mutation model of microsatellite evolution, Ohta and Kimura, 1973). For each analysis, 1000 trees were generated by bootstrapping over loci.

Because some taxa of Barteria were not sampled from the same site (they grow in different habitats), genetic differentiation might merely reflect distance between sampled individuals and not necessarily reproductive isolation. To investigate barriers to gene flow beyond spatial distance, we examined whether genetic differentiation between any two individuals, as measured by Rousset’s \( a_r \) (Rousset, 2000), was correlated with the natural logarithm of distance separating them. We then examined whether taxon membership could explain differentiation better than distance alone. We achieved this by correlating the \( a_r \) matrix to the distance matrix and then by correlating the matrix of residuals to a binary matrix with “0” for pairs of individuals of the same taxon and “1” for heterospecific pairs. Significance of correlations was tested by permuting data in matrices 5000 times (partial Mantel test) in FSTAT (Goudet, 1995). We restricted this test to the two pairs of taxa for which it was necessary (see Section 3).

3. Results

Fig. 3 shows the Maximum Likelihood (ML) tree of ITS sequences obtained from 145 individuals (Genbank accession numbers KC207253 to KC207402). We note that the limited level of sequence variation in this phylogeny limits the impact of the chosen model of nucleotide evolution (HKY with gamma) on the generated topology and contributes to the low bootstrap support of most clades. The Bayesian consensus tree is shown as a Supplementary figure (Fig. S1). Maximum Likelihood and Bayesian inference produced compatible phylogenies with respect to the well-supported clades. An initial dichotomy groups Barteria dewevrei with B. nigritana and B. fistulosa with B. solida respectively, with relatively good support. Within these two clades, individuals of each species do not form monophyletic groups. Barteria nigritana and B. dewevrei are admixed throughout most of the upper clade in Fig. 3 and share many ITS sequences. Individuals of Barteria fistulosa and B. solida are found in distinct, albeit poorly supported, groups in several parts of the lower clade. In this clade, some geographical grouping can be noted, as individuals from Gabon are paraphyletic with respect to samples from Cameroon. Variation at chloroplast sequences (four markers, see Table 2, Genbank accession numbers KC207127 to KC207252) obtained on a subsample of the four taxa was extremely low, with at most two polymorphic positions separating the same taxa pairs at the ITS marker.

Fig. 4 shows the microsatellite phylogeny grouping the 11-locus genotypic of all individuals compared with Nei’s minimum distance (Saitou and Nei, 1987). The microsatellite phylogeny could not be rooted because our microsatellites failed to amplify in the other genera of Passifloraceae we tested, including two genera of the tribe Paropsisae. Notably, all individuals from different taxa of Barteria constitute different clusters outlined on the figure, with the sole exception of a few possible hybrids of intermediate genotype. The grouping of the two pairs of taxa by ITS sequences (Fig. 3) is also found at microsatellite loci. These highly variable markers further differentiate genotypes of B. nigritana and B. dewevrei. Individuals from different geographic regions within taxa form very distinct clusters, such that, except for B. nigritana, the recognised species of Barteria do not appear monophyletic. Among individuals of B. nigritana and B. dewevrei, the logarithm of spatial distance explained 35.4% of the variance in genetic differentiation (Rousset’s \( a_r \)) while membership in different taxa explained a further 39%.
For *B. fistulosa* and *B. solida*, the percentages of variance explained were respectively 11.4% and 27.9%. All correlations were highly significant (none of the 5000 permutations produced stronger correlation), showing that membership in different taxa explained genetic differentiation better than mere distance between samples.

We used the seven genotypic clusters delineated in Fig. 4 to build population-based trees using two measures of genetic distance (Fig. 5), in order to decipher and statistically gauge their evolutionary relationships. Topologies grouping *B. nigritana* and *B. dewevrei* are consistent across trees (Figs. 4 and 5) and present relatively good statistical support. Populations of *B. solida* and *B. fistulosa* are also grouped in each tree with varying, poorly supported topologies.

4. Discussion

4.1. Generic and specific status in Barteria

The phylogeny based on ITS sequence data is consistent with monophyly of *Barteria*, an hypothesis that requires further testing by including the genus *Smeathmannia*, which appears to be the closest relative to *Barteria* (Tokuoka, 2012). Our broad sampling across the genus *Barteria* supported taxonomic decisions made in the most recent taxonomic revision (Breteler, 1999). For example, the phylogeny based on microsatellite loci showed that samples of *Barteria* from the type locality of one of the synonyms of *B. nigritana*, *B. braunii* (Batanga, Cameroon), fitted within the clade composed of individuals of *B. nigritana*. Similarly, the sample collected in the same district as the neotype specimen of *B. stuhlmannii* was included among *B. dewevrei*. Sleumer (1974) considered *fistulosa* as a sub-species of *B. nigritana* but Breteler (1999) treated them as two species. The genetic distance between the two taxa clearly establishes that *B. fistulosa* and *B. nigritana* are two separate species.

The microsatellite phylogeny of individuals (Fig. 4) separates genotypes belonging to different taxa into distinct genetic clusters, suggesting that gene flow between these groups is restricted or absent. Gene flow between *B. fistulosa* and *B. dewevrei* must be limited by strong or complete reproductive isolation, since these two taxa form clearly distinct genetic clusters at both ITS sequences and microsatellite markers, although they frequently grow at the same sites in Cameroon and in Gabon. The other two taxa of *Barteria*, i.e. *B. nigritana* and *B. solida*, have colonised distinct habitats and each only rarely grows in sympathy with any other *Barteria*. They may be isolated from related taxa by eco-geographic barriers (geographic isolation resulting from distance between habitats). Because genetic differentiation between individuals of different taxa is not explained by sampling distance alone, additional barriers to gene flow must exist. These barriers may be only historical, representing traces of some past range fragmentation that may in the future vanish through hybridisation. However, for a given pair of taxa of *Barteria*, populations collected in the same region (countries noted in Fig. 4) are not genetically closer than geographically distant populations. For instance, Gabonese *B. solida* are not genetically closer to Gabonese *B. fistulosa* than they are to Cameroonian *B. fistulosa* (Fig. 4). Observations thus indicate the absence of effective gene flow and high levels of reproductive isolation between all taxa of *Barteria*. In three of them, *B. dewevrei*, *B. fistulosa* and *B. solida*, microsatellite data also show deep divergences between geographically separate populations within each of the two countries, Cameroon and Gabon. In the last two species, the geographical differentiation is even visible at ITS sequences (Fig. 3). It is unclear at this stage whether such genetic differentiation only results from gaps in sampling, or whether barriers to gene flow are/were involved. For convenience, we shall refer to each of the four described taxa of *Barteria* as a “species”, even though each may not have a unique origin and may actually encompass cryptic geographical species.
Barteria species do not appear monophyletic based on ITS sequence data (Fig. 3). In terms of number of generations, species of Barteria thus appear to have diverged recently from their respective sister groups in comparison to their effective population sizes at the analysed loci (Avise, 2001), a result that is consistent with their overall morphological similarity. Despite their presumed recent divergence, species of Barteria show marked differences in respect to symbiotic traits and provide insight into the evolution of myrmecophytic traits. The small number of taxa of Barteria prevents statistical inference on ancestral states and on the probability of homoplaspy of myrmecophytic traits. However, based on the limited genetic divergence among taxa, this probability seems low, assuming these traits have high heritability (limited plasticity). In the great majority of myrmecophytes, production of domatia has been shown to be inherited, and these structures are produced in the absence of ants (Beattie, 1985; Beccari, 1884–86; Jolivet, 1996). In fact, induction of domatia-like structures in the presence of ants is a highly exceptional phenomenon (Blüthgen and Wesenberg, 2001; Edwards et al., 2009). In Barteria, several observations suggest that some crucial myrmecophytic traits, in particular the size and shape of domatia, are inheritable. Domatia are produced even in the absence of ants and can, in rare cases, harbour ant species usually associated with other types of domatia (Breteler, 1999; D. Mckeay, R. Blatrix, C. Djiéto-Lordon, personal observations). In a few instances (e.g., near Mamfé [South-West Region] and at Nkolo [South Region] in Cameroon), individuals of B. nigritana were found among trees of B. fistulosa and still presented their typical domatia and ants. As domatia shape is likely to have high heritability and taxa of Barteria appear to have diverged only recently, the evolution of myrmecophytism in the genus Barteria (developed in the following sections) can be discussed with an a priori limited risk of homoplaspy in domatia shape.

4.2. Initial divergence between species pairs

The rooted ITS phylogeny shows an initial divergence of two pairs of species: B. dewevrei and B. nigritana versus B. fistulosa and B. solida. The genetic distance at highly variable microsatellite loci also rapidly reaches saturation and may not reflect increasing divergence between species pairs. However, we note that 6 of the 17 microsatellites initially developed for B. fistulosa failed to amplify in both B. dewevrei and B. nigritana (Molecular Ecology Resources Primer Development Consortium et al., 2012), while all markers could be genotyped in B. solida. Amplification failures likely result from mutations at microsatellite flanking regions, which are much less frequent than mutations within the microsatellite themselves (Weber and Wong, 1993). This observation supports the hypothesis of significantly greater genetic divergence between the two pairs of species than between species within each pair. We therefore consider the ancestral divergence indicated by the ITS phylogeny as real.

Interestingly, this initial divergence led to two species of similar phenotype. Barteria fistulosa and western B. dewevrei both present long, wide domatia that can harbour the large Barteria-specific ant species of the genus Tetraponera. This phenotype would thus constitute the ancestral state of all sampled Barteria populations, should the monophyly of Barteria be confirmed after considering relationships with its close relative Smeathmannia (Tokuoka, 2012).

4.3. Relationships between B. fistulosa and B. solida

Phylogenies group B. fistulosa and B. solida, but show different, poorly supported topologies that fail to clarify the relationships between the two taxa. The paraphyly of Gabonese B. fistulosa in respect to B. solida and B. fistulosa from other countries at the ITS gene (Fig. 3) does not appear in the microsatellite tree (Fig. 4), which groups conspecific individuals according to their source regions. Possibly, patterns of historical species paraphyly are being erased by local gene flow, to which multilocus microsatellite genotypes are highly sensitive.

These two morphological taxa present extreme phenotypes: B. solida completely lacks domatia and is essentially restricted to widely scattered patches of submontane forest, whereas B. fistulosa has long, wide domatia, lives in symbiosis with Tetraponera, and is common in lowland forest throughout the region. Our results imply profound evolutionary transitions in habitat and in myrmeco-
4.5. Phylogenetic value of morphological characters

Our aforementioned scenario of ancestral symbiosis with Tetraponera would imply a loss (or several losses) of myrmecophytism in B. solida. Such loss would be consistent with the adaptation of B. solida to higher-elevation habitats that are unfavourable to ants, and with the fact that ITS lineages of B. solida are embedded within the diversity of B. fistulosa.

4.4. Relationships between B. dewevrei and B. nigritana

ITS-based phylogeny (Fig. 3) showed a large polytomy in the clade composed of B. dewevrei and B. nigritana, with specimens of both species interwoven. In contrast, microsatellite markers are much more variable, and accordingly, separate the two species in the phylogeny. Microsatellite-based phylogenies consistently show the paraphyly of B. dewevrei with respect to B. nigritana, with well-supported topologies (Figs. 4 and 5). Genotypes of Barteria nigritana constitute a sister group of Cameroonian B. dewevrei. This topology may reflect recent ancestry or genetic convergence due to local interspecific gene flow. Genetic convergence is not supported by the few Gabonese B. nigritana samples, which appear genetically closer to Cameroonian B. dewevrei than to Gabonese B. dewevrei sampled at much closer distances. Derivation of B. nigritana from a population of B. dewevrei is also consistent with its lower diversity at microsatellite markers (Table S1), and constitutes a more likely scenario.

Under this scenario, parsimony posits the phenotype of B. nigritana, with horizontal branches swollen only at their basal part, as a derived state. The candidate ancestral state found in B. dewevrei corresponds to domatia that extend over the whole length of the branch and vary from narrow to wide. Within B. dewevrei, the width of domatia does not seem to be correlated with genetic variation at microsatellite loci, providing no additional information to infer the ancestral phenotype of this species. In the western part of the distribution of B. dewevrei, only the phenotype with the widest domatia harbouring Tetraponera is present. Because B. nigritana is related to Cameroonian B. dewevrei and is restricted to the western, coastal part of the region, this species is most likely derived from a population of B. dewevrei presenting wide and long domatia. Such a scenario implies a shift from obligate symbiosis with Tetraponera to mutualism with less specific “opportunist” ants of various genera (Djéto-Lordon et al., 2004). This transition in myrmecophytism would have co-occurred with an ecological shift adapting B. nigritana to more open coastal habitats.

4.5. Phylogenetic value of morphological characters

The most relevant morphological characters used to differentiate recognised taxa of Barteria are: (i) the shape of the apex of floral bracts, (ii) the number of flowers per inflorescence, (iii) the shape of the fruits, and (iv) the shape of domatia. This last character appears to be of less value than proposed by Breteler (1999) in differentiating the species, as discussed above. For instance, although B. fistulosa and B. dewevrei belong to clades that are clearly differentiated, they can have similar domatia and host the same specialised ants. In contrast, domatia restricted to the basal part of the lateral branches is an autapomorphy of B. nigritana. Both fruit shape and number of flowers per inflorescence follow the same pattern of variation between species: B. fistulosa has ellipsoidal fruits and numerous flowers per inflorescence, whereas the three other taxa have subglobose fruits and fewer flowers per inflorescence. The distribution of these two characters is thus in contradiction with the main separation between the two species pairs identified by molecular data in our study. Interestingly, shape of floral bracts is rounded in both B. fistulosa and B. solida, and acute in both B. dewevrei and B. nigritana. This character follows the initial divergence of the species pairs, and thus reflects the molecular data better than the other characters discussed here.

4.6. Implications for the evolution of myrmecophytism

Myrmecophytes have appeared many times in the course of evolution (Davidson and McKey, 1993) but only very few constitute evolutionary radiations of any size. This pattern may reflect frequent extinction of specialised myrmecophytes (the “specialisation as evolutionary dead end” hypothesis, Althoff et al., 2012; Tripp and Manos, 2008) and/or reduction or loss of mutualistic traits in the course of evolution (Janzen, 1974). As in Macaranga (Blattner et al., 2001; Davies et al., 2001), Neonauclea (Razafiman-dimboison et al., 2005) and Tococa (Michelangi, 2005) our results corroborate the latter hypothesis, suggesting dramatic reductions and loss of a myrmecophytic trait in the course of species evolution. In particular, non-myrmecophytism (as seen in B. solida) does not appear a likely ancestral state for the sampled populations of Barteria, even though it must be, at a deeper evolutionary scale, because Barteria is the only genus of the tribe Paropsieae to bear domatia (Tokuoka, 2012).

Barteria encompasses few species but is successful in an evolutionary sense, since its two myrmecophytic species are abundant throughout Lower Guinea and the Congo basin. Possibly, mutualism between Barteria and Tetraponera enabled B. fistulosa and B. dewevrei to colonise a large core area, from which some descendants dispersed to habitats that favoured reduced investment in myrmecophytic traits (B. nigritana, B. dewevrei with the narrowest domatia) or even their loss in derived taxa (B. solida), resulting in the pattern we see today. This proposed evolutionary scenario is still tentative, but provides working hypotheses for future studies. The discrepancies between myrmecophytic traits and phylogenetic grouping should help to identify the loci controlling these traits, via genetic association studies and population genome scans (Storz, 2005). Detailed study of these loci should provide more definitive insights into the evolution of myrmecophytism.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2012.11.006.