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https://doi.org/10.1016/j.ympev.2016.08.005

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Molecular Phylogenetics and Evolution 105 (2016) 126–138

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Phylogeography of the genus *Podococcus* (Palmae/Arecaceae) in Central African rain forests: Climate stability predicts unique genetic diversity

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ARTICLE INFO

Article history:
Received 24 October 2015
Revised 8 August 2016
Accepted 9 August 2016
Available online 10 August 2016

Keywords:
Phylogeography
Africa
Palms
Plastome
Refugia
Coastal forests

ABSTRACT

The tropical rain forests of Central Africa contain high levels of species diversity. Paleovegetation or biodiversity patterns suggested successive contraction/expansion phases on this rain forest cover during the last glacial maximum (LGM). Consequently, the hypothesis of the existence of refugia e.g. habitat stability that harbored populations during adverse climatic periods has been proposed. Understory species are tightly associated to forest cover and consequently are ideal markers of forest dynamics. Here, we used two central African rain forest understory species of the palm genus, *Podococcus*, to assess the role of past climate variation on their distribution and genetic diversity. Species distribution modeling in the present and at the LGM was used to estimate areas of climatic stability. Genetic diversity and phylogeography were estimated by sequencing near complete plastomes for over 120 individuals. Areas of climatic stability were mainly located in mountainous areas like the Monts de Cristal and Monts Doudou in Gabon, but also lowland coastal forests in southeast Cameroon and northeast Gabon. Genetic diversity analyses shows a clear North–South structure of genetic diversity within one species. This divide was estimated to have originated some 500,000 years ago. We show that, in Central Africa, high and unique genetic diversity is strongly correlated with inferred areas of climatic stability since the LGM. Our results further highlight the importance of coastal lowland rain forests in Central Africa as harboring not only high species diversity but also important high levels of unique genetic diversity. In the context of strong human pressure on coastal land use and destruction, such unique diversity hotspots need to be considered in future conservation planning.

1. Introduction

The tropical rain forests (TRF) of Central Africa represent the second largest continuous extent of this biome after the Amazon basin and contain high levels of species diversity and endemicity (Linder, 2001). Ancient climate change 33–3 million years ago, was shown to be an important driver of diversification in animals and plants (e.g. Couvreur et al., 2008; Couvreur, 2015; Faye et al., 2016; Menegon et al., 2014; Tolley et al., 2013; Voelker et al., 2010). The impact, however, of the last glacial maximum (LGM, 24 k – 12 k years ago) remains debated (Hardy et al., 2013). Our knowledge about the recent dynamics of Central African TRF has mainly been inferred from paleovegetation studies (Bonnefille, 2007; Maley and Brenac, 1998), biodiversity patterns (Diamond and Hamilton, 1980; Sosef, 1996) or past vegetation modeling (Cowling et al., 2008). Most of these studies suggested that African rain forests were affected by successive contraction/expansion events (Bonnefille, 2007) during the glacial cycles of the Pleistocene (in particular during the LGM). These repeated sequences of forest dynamics led to the hypothesis of forest refugia (Diamond and Hamilton, 1980; Maley, 1996; Sosef, 1994). Refugia are defined as geographical areas that operate on evolutionary time scales allowing viable populations of a species to persist throughout climatic oscillations. Because this definition is species-specific (Hewitt, 2000; Stewart et al., 2010), refugia refer to areas of species-specific habitat stability rather than to broadly defined climatically-stable regions (Ashcroft, 2010; Stewart et al., 2010). Maley (1996) identified several areas as potential refugia located along the West and Central African coasts and in the Congo Basin, mainly in montane regions (Fig. 1). These are recognized as the "traditional refugia" against which most data are compared to when discussing the existence of refugia in Africa (Anthony et al., 2007; Dauby et al., 2010; Droissart, 2009; Gomez et al., 2009).

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http://dx.doi.org/10.1016/j.ympev.2016.08.005
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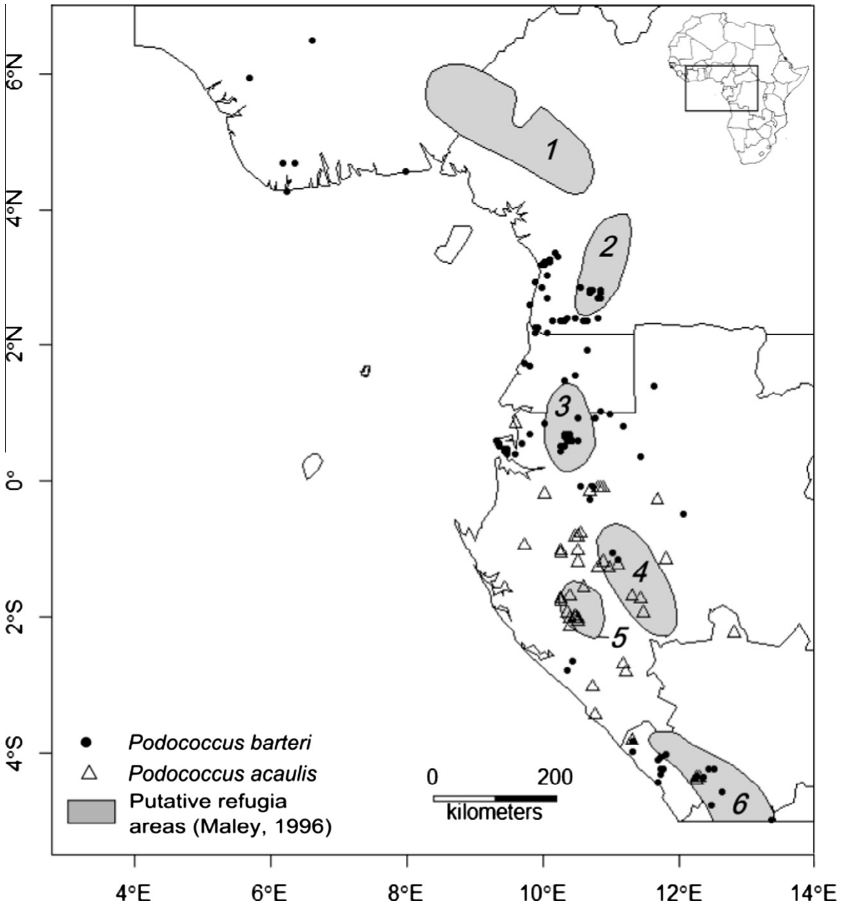

Fig. 1. Geographic distribution of the two species of *Podoccocus* used in this study based on field sampling and herbarium collection data. The widespread species *P. barteri* is distributed from the Niger Delta (south of Nigeria) to the Congo River up to 200 km inland. The sister species *P. acaulis* is mainly confined to the Gabonese-Congolese tierra firme rain forests from sea level to 800 m. Location of six putative refugia areas in Atlantic Central Africa as identified by Maley (1996) are indicated in grey as follows: (1) Mont Cameroon; (2) Campo Ma'an, (3) Monts de Cristal; (4) Chaillu mountains; (5) Monts Doudou; (6) Mayombe mountains.

Recent studies using genetic diversity analyses tested the hypothesis of forest refugia in Central African plant species (Hardy et al., 2013; Ley et al., 2014). Studies on several tree species reported a significant population structure within species population across the region (Hardy et al., 2013). Regions of high allelic diversity were partly concordant with some of the refugia defined by Maley (1996). A similar pattern was also found in Gabon for eight species of herbs and lianas of the Marantaceae family (Ley et al., 2014). Molecular dating studies have also provided evidence that tree species population structure could date back to the LGM or earlier glacial cycles (Debout et al., 2011; Duminil et al., 2015). In addition, a major probable contact zone, defined as a region where different populations meet during post glacial expansion (Taberlet et al., 1998), was suggested for several species in southern Cameroon separating northern populations from southern ones (Hardy et al., 2013; Heuertz et al., 2014). These results were taken as evidence that within-species diversity patterns are the result of historical population dynamics during the Pleistocene (Dauby et al., 2014a; Hardy et al., 2013; Gomez et al., 2009).

The hypothesis of forest refugia relates to areas of species specific habitat stability and, thus, inferring where such regions are located provides additional important data to test past rain forest dynamics (Carnaval et al., 2009; Svenning et al., 2011). Under the refuge theory, we should find a correlation between increasing area of habitat stability and genetic diversity as long term populations will tend to harbor more unique diversity than recently expanding ones (Svenning et al., 2011). Integrating phylogeography and paleoclimatic niche modeling (Richards et al., 2007) provides new insights in the history of rain forest dynamics. Such approaches provide a powerful way to locate and describe refugia across a region, as undertaken in similar studies in temperate biomes (Magri, 2008; Petit et al., 2002) and in other tropical blocks such as the Atlantic rain forests in Brazil (Carnaval et al., 2009) or the Wet Tropics in Australia (Moussalli et al., 2009).

To date, most studies looking at genetic structure and dynamics of African plant species have mainly focused on trees species (Hardy et al., 2013), but see Ley et al. (2014). Understory shade-tolerant species are dependent on rain forest cover and thus are also good markers to study past TRF dynamics. Moreover, understory trees or shrubs can allow for finer diversity assessments because of significant genetic differentiation even over short distances due to lower dispersal capacities when compared to trees (Lasso et al., 2011; Zeng et al., 2011). The understory stratum of TRFs contains an important diversity of shade tolerant plant species. For example, in a floristic study of one of the most diverse rain forests on earth, the Korup national park in Cameroon, just under half of all inventoried species were small trees (222 out of 493, smaller than 10 m) and the most abundant species were treelets (e.g. *Phyllobotryon spathulatum* and *Cola* spp.) (Kenfack et al., 2007). Thus, understanding how understory plant species have reacted to past climate change provides additional insights on how TRF diversity has reacted to past climate change.

Here, we infer the phylogeography of an understory Atlantic Central African palm genus: *Podococcus* G. Mann & H. Wendl. (Mann and Wendland, 1864). Palms are an interesting model to study rain forest evolution because they are one of the few tropical plant families for which refined taxonomical and phylogenetic data are available (Couvreur and Baker, 2013). The genus *Podococcus* includes two species, *P. barteri* G. Mann & H. Wendl. and *P. acaulis* Hua, endemic to the central Atlantic African rain forests distributed from the Niger Delta to the Congo River up to 200 km inland (Fig. 1). Until recently both these species were considered conspecific (Moore, 1973; Tuley, 1995). Both species present a mainly allopatric distribution with *P. barteri* centered in southern Cameroon, Equatorial Guinea and northern Gabon with a disjunct distribution in southern Nigeria and southern Republic of Congo (RC) and Cabinda (Angola). *Podococcus acaulis* occurs in central southern Gabon as well as the Mayombe forests in Republic of Congo. Only in RC are both species found to grow in sympatry (Faye et al., 2016).

We use near complete plastome sequence data for over 120 individuals and integrate this with past and present species distribution modeling (SDM) to gain insights into Central African rain forests dynamics. Specifically, we aim to answer the following questions: Has the potential distribution of *Podococcus* species been affected by past climate change and can we identify climatically stable areas? If yes, do these areas harbor more important unique genetic data than non stable areas, as hypothesized by the refuge model? Do our results on two understory species lead to similar conclusions about African rain forest dynamics as studies done on tree species?

2. Material and methods

2.1. Population sampling and DNA extraction

Sampling was carried throughout the area of distribution for both species. *P. barteri* is common to coastal forest swamps and mid elevation *tierra firme* rain forests (up to 800 m, Faye. pers. obs.) while *P. acaulis* is confined to Gabonese and Congolese *tierra firme* rain forests from sea level to 800 m (Valkenburg and Sunderland, 2008). *Podococcus barteri* is long lived with a slow growing stem and an estimated life span between 60 and 80 years often proliferating vegetatively via short stolons (Bullock, 1980). In contrast, *P. acaulis* does not have a stem (acaulescent) and no information is available on its life span.

We sampled 3–7 individuals per population across the distribution of both species. For *P. barteri*, 71 individuals representing eleven populations were sampled, whereas 36 individuals in five populations were sampled for *P. acaulis* (Supplementary Table S1). In addition, we sampled four individuals of the closely related species *Sclerosperma mannii* as an outgroup (Baker et al., 2009; Comer et al., 2015). Because *P. barteri* is known to clonally expand via the use of stolons (Bullock, 1980), sampled individuals were separated by at least 100 m. Population sites were distant by at least 10 km. Finally, because south Nigeria was not accessible at the time of this study we sampled four herbarium specimens (at the Royal Botanic Gardens, Kew) that were further treated as a single population. For each individual, GPS coordinates were recorded (Supplementary Table S1) and leaves were dried in the field using silicagel. The dried leaves were lyophilized in liquid nitrogen and were then grounded to a fine powder using Tissuelyser system. High quality DNA extractions were performed following the MATAB protocol (Risterucci et al., 2000).

2.2. De novo plastid genome reconstruction

In our study, DNA sequence data was preferred over alternative markers such as microsatellites or single nucleotide polymorphisms datasets (e.g. SNPs, restriction-site associated DNA (RAD-seq)) because such data provides a robust way of estimating spatial-temporal origins and demographics of populations (Drummond et al., 2006; Ho and Shapiro, 2011; McCormack et al., 2013). This data is also starting to be commonly used in phylogeography studies in plants in Central Africa (e.g. Dauby et al., 2010; Heuertz et al., 2014) especially because of the development of next generation sequencing and target enrichment approaches (e.g. McCormack et al., 2013; Garrick et al., 2015; Jones and Good, 2016).

At the time of our study, no chloroplast reference genome existed for the genus *Podococcus*. A reference genome was thus generated via two strategies. The first strategy was based on long range PCR (LR-PCR) amplification of the full chloroplast (Mariac et al., 2014). This approach was chosen as the resulting probes were also used for the next step of the study (see below). For that, we designed 12 pairs of primers based on the published plastome of *Phoenix dactylifera* (NC_013991_NCBI) (Yang et al., 2010). LR-PCRs were performed on a selected individual using the LongAmp Taq PCR kit (New England Biolabs) following the protocol of Mariac et al. (2014). The second approach used genome skimming on that same individual. In both cases libraries (see below) were sequenced on an Illumina MiSeq v3 platform. Resulting sequence data from both approaches were then used to reconstruct reference plastomes using the program Mitobim v1.7 (Hahn et al., 2013) using the published plastome of *Phoenix dactilifera* as initial reference. A consensus reference (see results) was finally built by aligning both resulting plastomes using Geneious Pro v.4.7.6. (Drummond et al., 2011).

2.3. Plastome sequencing

For the diversity study, we sequenced near full plastomes for all individuals using a hybridization capture enrichment protocol (Mariac et al., 2014). This cost-effective protocol allows the sequencing of plastomes for a hundred individuals in a single MiSeq run. Prior to sequencing, chloroplasts were enriched in-solution, and then hybridized to a species-specific biotinylated probe. We constructed a set of probes using the same LR-PCR primers as above. Genomic libraries were constructed for all sampled individuals following the protocol of Mariac et al. (2014). Approximately 10 µg of total genomic DNA and LR-PCR products are sheared into fragments with a mean of 400 bp using a Covaris S220. Fragments sized between 300 and 400 bp, equivalent to average read length of the Miseq sequencer (150 forward/reverse), were isolated based on a dual fragment size selection procedure (DFSS; Rohland and Reich, 2012) using Agencourt AMPure XP magnetic beads. Blunted and 5′ phosphorylated fragments are then used for adaptor ligation in probe construction followed by a PCR using biotinylated primers. Finally, each individual was barcoded using a 6 bp tag. Captured cpDNA are immobilized by streptavidin beads and enriched by real time PCR before sequencing in an Illumina MiSeq v3 platform.

2.4. Data mapping and Single Nucleotide Polymorphism (SNP) calling

Demultiplexing and reverse paired sequence matching were carried out using the freely available scripts “demultadapt.py” (without Levenshtein distance) and “compare_fastq_paired_v5.pl” (Mariac et al., 2014). Adapters were removed using Cutadapt (Martin, 2011) with the following options: - B (finding the whole adapter at the beginning of the read), minimum overlap of 7 bases between the reads and the adapter, minimum length of each read of 35 bases and base quality of 20. Mapping was performed on the reconstructed reference genome of *P. barteri* with the Burrows-Wheeler Aligner (BWA) mem (Li et al., 2009). Mismatch penalty of 4 and a “Mark shorter Split hits as secondary” were used to correctly map reads. Only paired mapped reads were kept using the option f = 0x0002 in Samtools view (Li et al., 2009). Even though the chloroplast is a haploid organelle, studies have identified a number of intra-individual polymorphisms from data generated via Next Generation Sequencing (NGS) (Sabir et al., 2014). A recent analysis of plastome NGS sequence data showed that these intra-individual polymorphisms are probably the result of plastid DNA transferred into the mitochondrial or the nuclear genome or both (Scarcelli et al., 2015). In order to avoid these erroneous calling of these intra-individual polymorphisms we followed the calling and mapping strategy of Scarcelli et al. (2015). In brief, SNP calling is carried out using VarScan (Koboldt et al., 2012) with a minimum base quality of 30, and a min coverage of 3. Calling for each individual was performed, and the output was recorded in Variant Call Format (VCF). We only considered polymorphic sites, SNPs and individuals with more than 20% of missing data were also removed, except for the herbarium specimens (see below).

2.5. Genetic structure, haplotype distribution and differentiation statistics

For each individual, haplotypes were inferred using DnaSp software v.5.10.01 (Librado and Rozas, 2009). Geographical visualization of haplotype diversity distribution was done via a haplotype network built with a median–joining network algorithm (Bandelt et al., 1999) using Pop-Art (Leigh and Bryant, 2015).

Genetic differentiation between populations was estimated based on haplotype identity using G_{st} and the proportion of polymorphic sites distinguishing each pair of haplotypes, N_{st} (Pons and Petit, 1996). These two statistics were calculated using SPAGeDi (Hardy and Vekemans, 2002). Nucleotidic distance matrices between haplotypes were generated using Arlequin v 3.5.1.3 (Excoffier, 2010). In order to test for isolation-by-distance, Gst and Nst were also estimated for each population pair. We computed averaged pairwise values for several intervals of distance. The existence of a phylogeographic signal was tested based on the difference between pairwise G_{st} and N_{st}. Phylogeographic signal occurs when $N_{st} > Gst$ indicating that distinct haplotypes within population are more related on average than haplotypes sampled between populations (Pons and Petit, 1996). To test whether N_{st} was correlated to geographical distance, we performed a Mantel test. Finally, to evaluate if N_{st} was significantly higher than G_{st} a randomization test permuting haplotype assignations in the genetic distance matrix was used. Distribution of N_{st} values derived from 20,000 randomizations were compared to observe N_{st} to obtain a unilateral test p-value.

2.6. Phylogenetic inference and molecular dating

Phylogenetic relationships between individuals were inferred using Bayesian phylogenetic inference as implemented in MrBayes v.3.2. Full plastome fasta sequences were derived from the VCF file (see above) for all individuals (see results, 71 *P. barteri*, 36 *P. acaulis* and four *Sclerosperma mannii* (outgroup taxon)). We then used a custom script (Supplementary file 1) that reads the VCF and compares each site with the *P. barteri* reference sequence to obtain an aligned plastome matrix. The substitution model for the single partition was estimated using MrModelTest 2.3 (Nylander, 2004) using the Akaike Information Criterion (AIC). Using the inferred best model, two independent runs were performed with four heated Metropolis-coupled Monte Carlo Markov chains. Chains were started from random trees and run for 7 million generations sampled every 1000 generations. Convergence between both runs and stationarity were checked using the program Tracer 1.4 (Rambaut and Drummond, 2013).

In order to estimate the divergence times between and within *Podococcus* species we used BEAST ver. 1.8.2 (Drummond and Rambaut, 2007). A two step “secondary calibration” approach was used. A subsample of the above matrix included two individuals of *P. barteri*, *P. acaulis* and *Sclerosperma mannii*. In addition, one individual of *Orania palindan* a sister genus of *Scleropserma*, which together with *Podoccocus* form the so called POS clade (Baker et al., 2009; Comer et al., 2015) GenBank accession number: KP221686 and one individual of *Phoenix dactilifera* (subfamily Coryphoideae, GenBank accession number GU811709) were added as outgroups. The sequence of *Orania palindan* and *Phoenix dactilifera* were manually added and aligned to the subdataset using Geneious Pro v.4.7.6. (Drummond et al., 2011).

The most recent common ancestor between *Phoenix dactilifera* and the POS clade is the stem node of subfamlillies Coryphoideae and Arecoideae (Baker et al., 2009). We used this node to calibrate the phylogeny using the fossil *Sabalites carolinensis* following Couvreur et al. (2011a). An exponential prior distribution was used with a hard lower bound set to 85.8 million years and a standard deviation of 1. Molecular dating was undertaken using an uncorrelated lognormal relaxed clock with tree prior “Yule model” as implemented in BEAST version 1.8.2 (Drummond and Rambaut, 2007). Two independent runs were undertaken of 20 and 40 million long each sampling trees and parameters every 2000 steps. Both the MrBayes and BEAST analyses were undertaken using the CIPRES Science Gateway V 3.3 portal (Miller et al., 2011).

The estimated age and its associated uncertainty for the crown node of *Podococcus* (split between *P. barteri* and *P. acaulis*) were then used for the second analysis as a second calibration point. Here, plastome data for all sequenced individuals of *P. barteri* and *P. acaulis* were included (107 total individuals) and no outgroup was used. Alignment of all sequences between the two species of *Podococcus* was done in Geneious Pro v.4.7.6. (Drummond et al., 2011). Three different combinations were tested reflecting different hypotheses about the evolution of the dataset: (1) clock prior: strict clock; tree prior: coalescent; (2) clock prior: uncorrelated relaxed clock (ucld); tree prior: Yule; (3) clock prior: ucld; tree prior: coalescent. A uniform prior was placed on the crown node of *Podococcus* covering the 95% Highest Posterior Distribution (HPD) inferred in the first step for that node. Each strategy was run for 40 million generations sampling trees and parameters every 5000 steps. In order to select the best fitting strategy the Bayes factor as implemented in Tracer 1.6 (Rambaut and Drummond, 2013) was estimated under the smoothed marginal likelihood and with 100 bootstrap replicates (Suchard et al., 2001). Finally, no prior constraint was applied about the monophyly of each species.

2.7. Current and paleoclimatic modeling

Regions that remained climatically suitable for each species were estimated by inferring current and past potential distributions using species distribution modeling (SDM) with the software MaxEnt v 3.3.3 (Phillips et al., 2006). Occurrence data was taken from our field population samplings and from herbarium specimens stored in the BRAHMS database of the Naturalis Biodiversity Center (Fig. 1) and identified within the framework of the taxonomic revision (Valkenburg and Sunderland, 2008). For training the models a set of 100 unique presence cells for *P. barteri* and 43 *P. acaulis* (Supplementary Table S2) covering the total known distribution were selected. However, in order to avoid overfitting linked to sampling bias (Boria et al., 2014) a spatial filtering approach was used with a custom R script authorizing only one occurrence per cell (Supplementary Figs. S3 A & D). Biologically meaningful climatic variables were acquired at 2.5 arc-minute resolutions (4.6 km) from the WorldClim (Hijmans et al., 2005) global dataset (www.worldclim.org). In order to avoid model over-fitting linked to correlated climatic parameters, non correlated variables according to Pearson's coefficient $R < 0.7$ were retained. The best predictors from each pair-wise correlation were selected using the R function rquery.cormat from the R package corrplot. MaxEnt models were run using default settings. Model performance was assessed using a cross-validation procedure (Elith et al., 2011) with a random set of 75% of occurrences data used to train the model which was subsequently assessed on the remaining 25%. Model evaluation was based on the area under the Receiver Operating Characteristic curve (AUC) statistics, a threshold-independent measure of performance (Elith et al., 2006). For each model binaries predictions of presence/absence were obtained using the maximum training sensitivity plus specificity threshold (Liu et al., 2005). Current evaluated species distributions models were then projected into the Last Glacial Maximum (LGM, 21,000 years ago). For that, climatic projections of MIROC global circulation model into the LGM were downscaled to the same resolution as that acquired from WorldClim (Hijmans et al., 2005).

Areas of climatic stability were inferred from current and LGM models as previously described (Graham et al., 2006; Hugall et al., 2002; VanDerWal et al., 2009). In brief, the mean predicted values of the two inferred distributions were calculated for individual cells. Area gain through time was estimated based on the presence/absence maps generated by applying a Maximum training sensitivity plus specificity threshold. The difference between pixels where the species occurred and those where it was absent in the two binary predictions allows us to estimate the loss/gain of area through time.

To test if areas of stability harbored significantly more unique haplotypes than unstable areas, a sampled-weighted linear regression analysis between extracted values of habitat stability and the unique haplotype frequencies per population was done. We first extracted per sample values of stability from the habitat stability map generated above, and then calculated the average value of stability across each population. Second, for each population we summed unique haplotype frequencies generated by Arlequin v 3.5.1.3 (Excoffier, 2010). Both indexes vary from 0 to 1.

3. Results

3.1. Reference genome construction

For the chloroplast genome reconstruction, we obtained two genomes from the two different strategies describe above. The first genome was reconstructed from the LR-PCR data using 1,034,492 reads of 100 bp. Four iterations of the MITOBIM protocol were sufficient to obtain the chloroplast genome. However, because only 11 out of 12 primers successfully amplified in *P. barteri* we only reconstructed a partial plastome (see below). The second genome was obtained using 15,822,176 reads of 100 bp from total genomic DNA data. A total of 208 iterations were needed to reconstruct the chloroplast genome in MITOBIM. This allowed us to cover the whole plastome. To generate the final reference genome *P. barteri* we used both generated genomes. This resulted in a plastome 157,688 bp long (Genebank Ref. NC_027276.1).

3.2. Enrichment and mapped reads

As indicated above, out of the 12 designed LR-PCR primers, 11 amplified successfully in *P. barteri*. The enriched probe was thus built on the 11 amplified LRPCR fragments. Consequently, we targeted roughly 144 kb of the chloroplast i.e. 92% of the whole plastome. An average of 394,197 reads were generated per individual for *P. barteri*, 385,924 reads for *P. acaulis* and 599,045 reads for *Sclerosperma* (Table 1, Supplementary Table S1). Around 40% of the sequence reads were mapped to the chloroplast for both *Podococcus* species, and 94% for *S. mannii*. Three out of four *P. barteri*'s herbaria samples were successfully sequenced with 32% of reads mapped to the plastome for these three samples.

We obtained on average 153,615 correctly mapped reads of 100 bp for *P. barteri* and 158,912 correctly mapped reads of 100 bp for *P. acaulis.* Between 77% and 80% of the targeted plastome had at least 5X coverage in all three species (Table 1). A total of 568,784 reads were correctly mapped for *Sclerosperma mannii* covering 80% of the targeted region with at least 5X coverage.

Our mapping and calling protocol identified a total of 286 SNPs for the 71 individuals of *P. barteri* and 153 SNPs for the 36 individuals of *P. acaulis*. Removal of individuals and SNPs with more than 20% of missing data resulted in 104 SNPs for *P. barteri* and 106 SNPs for *P. acaulis*.

3.3. Spatial distribution and haplotype endemism

A total of 28 haplotypes were inferred for *P. barteri* leading to a haplotype diversity (hd) of 0.93 and 19 haplotypes for *P. acaulis* with hd = 0.94.

The inferred haplotype network for *P. barteri* (Fig. 2B) separated two main haplotype groups (HG) by five mutational events. A haplogroup (here named HG1) regroups haplotypes found in the northern part of Central Africa from Nigeria to south Cameroon. HG1 includes populations of Fifinda, Campo, Ma'an and Akom referred to as northern populations (Fig. 2B). A second haplogroup (HG2) includes haplotypes found in the southern part of Central Africa from south Cameroun to the Republic of Congo, including the Gabonese populations Monts de Cristal, Mondah and Medouneu, and the Congolese populations Conkouati, Kola and Dimonika. In south Cameroun, several populations possess haplotypes shared between these two groups. Out of the 11 populations, ten contained unique haplotypes, e.g. a haplotype only found in that particular population. All haplotypes found in the Nigerian population were unique. Populations in Fifinda and Campo had three and two unique haplotypes, respectively, while the Ma'an population had just one unique haplotype. Akom was the only population not to have any unique haplotypes.

The HG2 group presents a more complex structure than HG1 in terms of haplotype occurrence across populations. First, four haplotypes (H2, H12, H18 and H19) are shared at least by three different populations. The common haplotype H2 is found in five populations: Akom, Campo, Fifinda, Mondah and Medouneu. The H12 haplotype is also found in Ma'an, Medouneu and Monts de

Table 1
Sampling size for each species and summary values of raw and mapped reads. Raw reads correspond to the number of reads obtained with the Miseq sequencing approach.

Species	Number of specimens	Enrichment percentage (%)	Mean raw number of reads	Mean number of mapped reads	Coverage of at least 5X (%)
Podococcus acaulis	36	40	385,924	158,912	80
Podococcus barteri	71	39	367,650	143,059	77
Sclerosperma mannii	4	94	599,045	568,784	80
Herbarium material	Tuley 600	32	182,202	57,491	63
	Onachie FHI32917	32	17,631	5712	17
	Okdodh	31	55,941	17,217	41

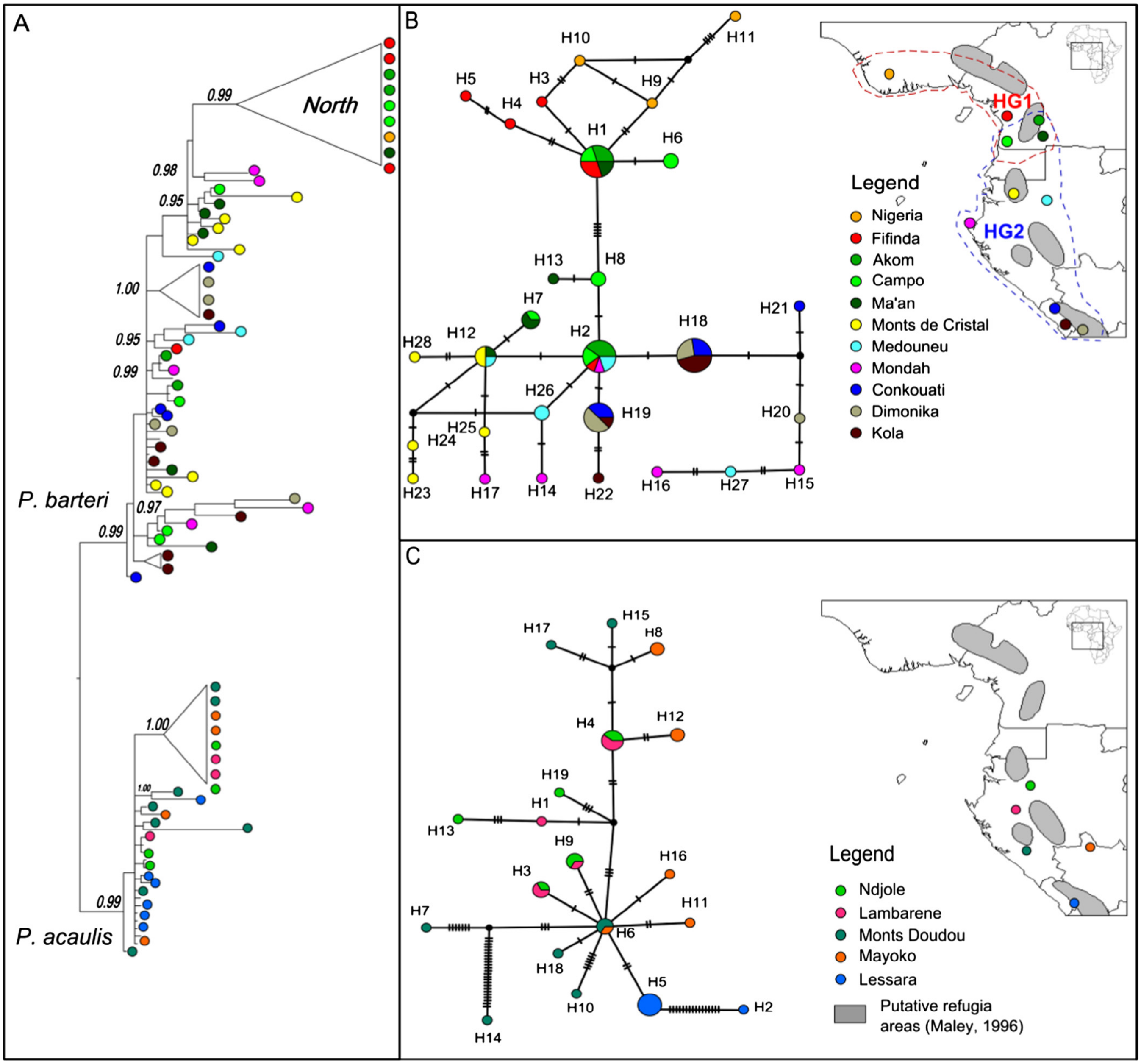

Fig. 2. Phylogenetic relationships and haplotype diversity in the palm genus *Podoccocus*. (A) Majority rule consensus tree of the last 30,000 trees from the Bayesian analysis showing the reciprocal monophyly of both species in *Podoccocus*. Posterior probabilities (PP) values above 0.95 are indicated above or next to nodes. The phylogeny strongly supports the monophyly of a northern group of individuals ("North" in figure) in *P. barteri*. Colored circles represent population origins (see B and C); B and C: Distribution of haplotypes in the two species of *Podoccocus*. Each chart presents a haplotype. Colors represent the location of that haplotype in the area map insert. Chart size is proportional to the number of individuals bearing that haplotype. The insert map shows the distribution of each population sampled in this study represented by its centroid position. Grey areas represent the putative location of six refugia as suggested by Maley (1996), see Fig. 1. (B) *P. barteri*: the two haplogroups HG1 and HG2 are separated by five mutational events (represented by the dashed line). Geographically, the two HG meet in south west Cameroon where the four populations Fifinda, Akom, Campo and Ma'an share haplotypes with the others populations in both HG. (C) *P. acaulis* shows a weak genetic structure with two main groups separated by three mutational events and a star-like structure centered on haplotype H6. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Cristal. The two haplotypes H18 and H19 occur mainly in Conkouati, Dimonika and Kola. Second, this common haplotype is also present in most northern populations (Campo, Ma'an and Akom). All populations in HG2 contain unique haplotypes: four in Mondah and Monts de Cristal (North West Gabon), three in Medouneu and one in Conkouati, Dimonika and Kola each. The Monts de Cristal population is the only one that does not share the majoritary haplotype H2 within the HG2.

In *P. acaulis* the network structure is more complex (Fig. 2C) and does not allow the identification of clear geographical genetic groups. We can, however, possibly distinguish two groups separated by three mutational events. One of the two groups reveals a star-like structure centered on haplotype H6. Two populations out of the five sampled (Monts Doudou in Gabon and Mayoko in RC) share this haplotype. These two latter populations have also 6 and 4 unique haplotypes, respectively. Populations Monts Doudou (Gabon) and Les sara (RC) also contain very divergent haplotypes with up to 24 mutational events for haplotype H14 (Monts Doudou) and 16 for haplotype H2 (Les sara). All haplotypes from Les sara are endemic to this southern part of RC. The lowest haplotype endemicity is found in Lambarene and Ndjole populations towards north Gabon.

3.4. Differentiation statistics

The G_{st} indicated a significant genetic differentiation within both species, (*P. barteri* $G_{st} = 0.15$, p-value = 0.0002; *P. acaulis* $G_{st} = 0.21$, p-value = 0.0001). A strong phylogenetic differentiation signal is found between populations of *P. barteri* with $N_{st} = 0.42$. In *P. acaulis* this value was much lower with $N_{st} = 0.15$. The randomization test indicated an average pairwise N_{st} significantly higher than the pairwise G_{st} (p-value = 0.0007) in *P. barteri* even for distances larger than 200 km, indicating a weak phylogeographical signal when considering distant populations (Supplementary Fig. S2). While in *P. acaulis* the test is not significant (p-value = 0.55).

The mean pairwise differentiation between populations (N_{st} and G_{st}) in *P. barteri* increases with geographic distance between populations from 0.10 for populations less than 200 km apart to 0.70 for populations more than 900 km apart. Between the north and south groups, corresponding to the two haplotype groups, a highly significant phylogeographic signal was found ($G_{st} = 0.19$, $N_{st} = 0.72$, p-value < 0.0001). The existence of this signal is driven by the differentiation of northern populations with 12 fixed SNPs. The Mantel test indicated a significant correlation between geographic distance and N_{st} for *P. barteri* (p-value = 0,0004), but was not significant for *P. acaulis* (p-value = 0.098).

3.5. Phylogenetic inference and molecular dating

Both independent runs for the Mr. Bayes analyses reached stationarity after just 200,000 runs and converged to similar values for all parameters. Both *Podococcus* species were resolved as monophyletic with maximum support (Posterior Probability, (PP) = 1.00). Overall, phylogenetic relationships within species were weakly supported with a few exceptions. Within *P. barteri* a large number of individuals sampled in northern populations (HG1) formed a well supported clade (Fig. 2A, Supplementary Fig. S1; PP = 0.99). Several, but not all, individuals from RC also formed a well supported clade, but with an unresolved position within the species (Fig. 2A). Within *P. acaulis* a single clade was strongly supported containing some individuals from all populations sampled, expect Les sara.

In the first level dating analysis (species level), both independent runs reached stationarity after 2 million generations and converged to similar values for all parameters as visualized in Tracer. In addition, all parameters had effective sampling sizes (ESS) above 100 and most above 200. The age for the crown group of *Podoccocus* (split between *P. barteri* and *P. acaulis*) was estimated to 2.4 million years ago (Mya) (95% HPD: 0.9–4.4 Mya). In the second analysis (population level) we applied a uniform prior distribution to the crown node of *Podococcus* ranging from 1.7 to 2.8 Mya. All three "tree/speciation prior" strategies reached stationary after 2 million runs. The Bayes factor strongly supported strategy 1 (tree prior: coalescent; clock prior: strict) over the two other strategies (Bayes Factor = 78 and 80 in favor of 1 over 2 and 3, respectively). Using this strategy, the maximum clade credibility tree was constructed using TreeAnnotator from the BEAST 1.8.2 package with a burn-in of 10% and node height set to the mean. The resulting tree (Fig. 2A) strongly supported both species as monophyletic (PP = 0.99) and was similar to the one obtained using MrBayes (Supplementary Fig. S1). Within *P. barteri*, the crown node of the northern individuals was well supported (PP = 0.99) and the age estimated to 0.57 Mya (95% HPD 0.75–0.33 Mya).

3.6. Predicted past and present distributions

The best predictors of the species distribution models selected were Annual Mean Temperature (Bio1), Annual Precipitation (Bio12), Precipitation Seasonality (Bio15), Precipitation of Warmest Quarter (Bio18), and Precipitation of Coldest Quarter (Bio19). Among the five predictors used Bio18, Bio12 and Bio19 mostly drove the species distribution (Supplementary Table S3). The predicted model for the current distribution shows an approximate representation of known geographic distribution for the two species (Supplementary Figs. S3A and D). Beyond the climatic range in the training data, model extrapolation was low within the areas of distribution according to the "multivariate environmental similarity surface" (MESS) (Supplementary Figs. S4 and S5). The mean values of the AUC for the training and test data in the modeled current distribution were 0.97 and 0.95 respectively for *P. barteri* and 0.98 and 0.97 for *P. acaulis*. Omission error rate corresponding to the proportion of presences incorrectly predicted and the commission error rate representing the proportion of absence incorrectly predicted were 0.042 and 0.106 for *P. barteri* and 0.000 and 0.055 for *P. acaulis*, respectively. Projected models showed a past distribution of *P. barteri* significantly reduced during the LGM leading to an area contraction of 23% compared to its present distribution. We identified one widespread climatic area of climatic stability in northwest Gabon and two smaller stable areas in southwest Cameroon (near Fifinda) and in the São Tomé Island. The inferred habitat of *P. acaulis* was affected by past climate change with a contraction of 34% of its inferred distribution during the LGM when compared to its current distribution. Two stable areas were inferred around Ndjole and massif du Chaillu montane range in Gabon and a less stable area in Monts Doudou in southwest Gabon.

In *P. barteri*, a significant correlation between the areas of climatic stability and unique haplotypes was inferred ($R^2 = 0.43$, p-value = 0.028). In *P. acaulis* this correlation was not significant ($R^2 = 0.37$, p-value = 0.28).

4. Discussion

4.1. Speciation in Podococcus

Podococcus was until recently considered as a monotypic genus (Tuley, 1995) but in the latest taxonomical revision two species were recognized, *P. barteri* and *P. acaulis* (Valkenburg and Sunderland, 2008). Our phylogenetic analyses clearly support this latter view (Fig. 2A). Even when individuals from both species were collected in sympatry (Mayombe region in the Republic of Congo) they clustered in separate clades, showing no signs of hybridization. We also did not detect cryptic species within *Podococcus* suggesting that species are well defined morphologically with a strong genetic barrier between them. The molecular dating results show that both species diverged at the start of or during the Pleistocene, ca. 2.4 Mya (highest posterior distribution (HPD) 4.4–0.9 Mya). This is a fairly recent speciation event, as several studies have shown pre Pleistocene divergences in plants of Central Africa (Auvrey et al., 2010; Couvreur et al., 2011b; Plana, 2004), although some studies suggest speciation events in African trees as recent as 600,000 years ago (Duminil et al., 2015). Both species occur in allopatry suggesting allopatric speciation confirming previous results for Central African trees (Couvreur et al., 2011b). However, the addition of nuclear sequence data and species tree analyses might provide more detailed and especially younger estimation of speciation processes (e.g. Duminil et al., 2015).

4.2. Refugia in Atlantic Central Africa

Our results show that both *Podococcus* species distributions were negatively impacted by past climate change during the LGM which is in line with the hypothesis of forest contraction in Central Africa during adverse climatic periods (Anhuf et al., 2006;

Maley, 1996). Our LGM simulations suggest that *P. barteri* retracted by 23% of its current distribution, while *P. acaulis* retracted by 34% of its current distribution (Supplementary Figs. S3C and S3F). In *P. barteri* we show a significant correlation between identified habitat stability and high and unique haplotype diversity (Fig. S6). The relationship between habitat stability and unique genetic diversity was not detected for the sampled populations of *P. acaulis* (Figs. 3B, D and S6). We only found one population (Monts Doudou) where habitat stability also harbored high levels of unique genetic diversity (Fig. 2B, Table 2). The star-like phylogeographic structure detected in *P. acaulis* (Fig. 2C) generally characterizes populations tightly connected by history with a shared ancestral haplotype (in this case H6). This pattern suggests that *P. acaulis* could be recently expanding (Avise, 2000; Rogers and

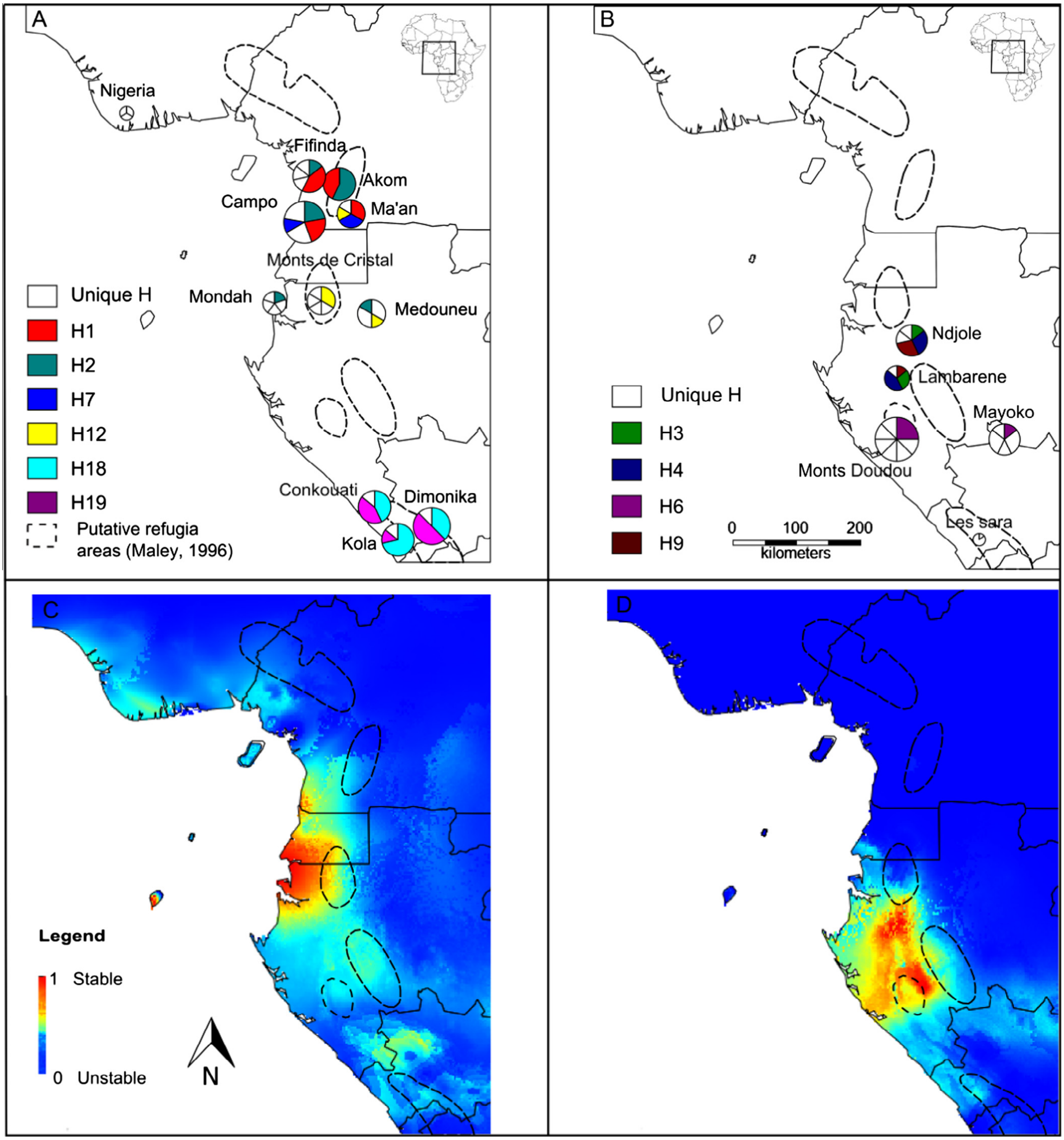

Fig. 3. Spatial distribution of haplotype diversity and modeled areas of climatic stability for both species of *Podococcus*. A–B: Pie charts represent populations sampled. Colored sectors on pie charts refer to shared haplotypes between different populations, while white sectors correspond to unique haplotypes (Unique H) in each population. A: haplotype distribution for *P. barteri*; B: haplotype distribution for *P. acaulis*. C–D: Modeled area of climatic stability since the LGM for both *P. barteri* (C) and *P. acaulis* (D) species. Red colors represent areas of modeled high climatic stability since the LGM, whereas blue represents areas of low or no climatic stability since the LGM. Areas indicated by hashed lines represent the putative location of six refugia as suggested Maley (1996). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Sample size, statistics of habitat stability and unique haplotype frequency per population within both *Podococcus* species.

Species	Population name	Country	Number of sampled individuals	Per sample values of habitat stability	Unique haplotype frequencies
P. barteri	Akom	Cameroon	7	0.35	0
	Campo	Cameroon	9	0.72	0.44
	Fifinda	Cameroon	7	0.35	0.43
	Ma'an	Cameroon	6	0.35	0.17
	Monts de Cristal	Gabon	6	0.74	0.67
	Medouneu	Gabon	6	0.22	0.5
	Mondah	Gabon	5	0.95	0.8
	Nigeria	Nigeria	3	0.40	1
	Conkouati	Republic of Congo	7	0.12	0.14
	Dimonika	Republic of Congo	8	0.17	0.12
	Kola	Republic of Congo	7	0.27	0.14
P. acaulis	Lambaréné	Gabon	7	0.54	0.14
	Ndjole	Gabon	7	0.33	0.29
	Doudou	Gabon	8	0.49	0.75
	Mayoko	Republic of Congo	7	0.17	0.86
	Les sara	Republic of Congo	7	0.25	1

Harpending, 1992; Takahata and Nei, 1990) which might explain why we did not detect a significant correlation between habitat stability and unique genetic diversity. In addition, our sampling of this species with only five populations was less extensive than for *P. barteri*. Our correlation test might be less powerfull under these conditons.

Stable species specific habitats harboring high genetic diversity in tropical rain forests has been shown in animals in the Coastal Atlantic Forests of Brazil (Carnaval et al., 2009) and the Wet Tropics in Australia (Moussalli et al., 2009). Similar results were found in other regions such as in the Iberian Peninsula (Abellán and Svenning, 2014) and the Himalayas (Qu et al., 2014). This is however, to our knowledge, the first time it has been shown in a plant genus in African rain forests. Stable species specific habitats since the LGM for *P. barteri* were restricted to three regions: a large area in north west Gabon and south west Equatorial Guinea, a smaller area in south west Cameroon and, interestingly São Tome, an island off the coast of Gabon (Fig. 3C). To date no species of *Podococcus* has been collected on São Tome (Valkenburg and Sunderland, 2008), thus this latter result probably reflects dispersal limitation. In northwest Gabon the inferred stable area extends from the coastal rain forests near Libreville to the mid altitude forests (400–800 m) mainly in Monts de Cristal area. Genetic diversity derived from near complete plastome sequences indicates a high level of unique haplotypes within each of these two regions. This genetic uniqueness is consistent with a long-term persistence of populations as expected under the forest refugia hypothesis (Bennett and Provan, 2008; Hewitt, 1996; Petit et al., 2003). Thus, our data support the idea of rain forest contraction during the LGM with species surviving in refugia in Central Africa. This result confirms previous studies on both plants (Dauby et al., 2014a; Duminil et al., 2015; Hardy et al., 2013) and animals (Anthony et al., 2007; Bohoussou et al., 2015; Nicolas et al., 2012; Quérouil et al., 2003). Because refugia are species specific (Ashcroft, 2010; Stewart et al., 2010), this study provides evidence of refugia for only two African palms. These results have to be replaced in the general context of African rain forests evolution (Duminil et al., 2015; Hardy et al., 2013; Heuertz et al., 2014). Our approach integrating SDM and phylogeography provide added evidence for the existence (or not) of certain regions as refugia for species within *Podococcus*. Maley (1996) outlined the location of several potential refugia in Atlantic Central Africa (Fig. 1), coinciding with mountainous areas. Our results support this hypothesis for the Monts de Cristal in *P. barteri* (Fig. 3A, Table 2) and the Monts Doudou area for *P. acaulis* (Fig. 3B, Table 2). Previous studies also highlighted high unique genetic diversity in Monts de Cristal such as in the tree species *Aucoumea klaineana* (Born et al., 2011), *Afrostyrax kamerunensis* (Dauby, 2012; Hardy et al., 2013) and in several liana and shrubs species within the Marantaceae family (Ley et al., 2014). To date, genetic data supporting Monts Doudou as a potential refugium for plants remains limited. Hardy et al. (2013) reported a possible refugial signal for that area in *Acoumea klaineana*. Born et al. (2011), however, suggested that the Monts Doudou refugium for *Aucoumea klaineana* must have been more severally affected than the Monts de Cristal, leading to an overall lower genetic diversity for Monts Doudou (see below).

The disjunct populations of *P. barteri* from the Republic of Congo (RC) show lower levels of haplotype diversity compared to Mondah or Monts de Cristal populations, but all the haplotypes are endemic to this region (Fig. 3A). This is incompatible with a hypothesis of recent dispersal into Congo after the LGM as we would expect some level of shared diversity. This idea is further supported by the fact that some individuals from RC clustered at the base of the Bayesian tree within a well supported clade also containing individuals from Mondah and Campo (Fig. 2A). This result suggests that these individuals might have once shared an ancestral haplotype forming a once widely distributed population, followed by climate induced contraction, isolation and differentiation. In addition, no area in RC is suggested as being stable (Fig. 3C and D) either for *P. barteri* or *P. acaulis*. Thus, although populations in south RC, which corresponds to the Mayombe refuge of Maley (1996), might have been able to persist throughout climatic oscillations, they must have been more severely affected by past climate change than in northwest Gabon. This severe impact might result in the lower observed genetic diversity within these southern populations. Few studies have sampled populations in the Mayombe, so it is unclear if this can be considered as a general pattern. A scenario in which northwest Gabon might have acted as a more efficient refugium compared to more southern ones (Monts Doudou, Massif du Chaillu, Mayombe) was also suggested in *Acoumea klaineana* (Born et al., 2011) and our data agree with this view. Hardy et al. (2013) reported unique genetic diversity within the tree species *Scorodophloeus zenkeri* in the Mayombe area, but sampling was actually restricted to south Gabon (Dauby, pers. comm.).

The coastal rain forests around the Libreville area contain high unique genetic diversity in *P. barteri* associated with an inferred high stable habitat (Fig. 3A and C). Interestingly, this region is just 100 km from the Monts de Cristal population, yet both regions are completely distinct genetically, with no shared haplotypes (Figs. 2B and 3A). This phylogeographical signal, even over a short distance between populations, was supported by the mean pairwise genetic differentiation between all populations

(Supplementary Fig. S2). A similar genetic discontinuity pattern between both areas has also been detected in several other studies for example in *Greenwayodendron suaveolens* (Dauby et al., 2010; Hardy et al., 2013), *Symphonia globulifera* (Dauby et al., 2014a) and *Erythrophelum ivorense* (Duminil et al., 2013), though individual and genetic sampling was in general low for that area. This genetic discontinuity was, however, not detected or highlighted in a comparative analysis of tree species across Central Africa (Hardy et al., 2013). In a recent study, both these regions (northern coastal forests and Monts de Cristal "hilly" forests) were also shown to be floristically distinct with high plant species turnover (Dauby et al., 2014b). These authors suggested that this pattern could be due to historical reasons, with both areas suggested as being distinct refugia (Dauby et al., 2014b). Our results add to this hypothesis by showing that there is also a strong infra specific genetic discontinuity, at least within the studied species *P. barteri*. Rapid genetic turnover between close regions is consistent with the hypothesis of two historically distinct and isolated areas (refugia) as shown in the Amazonian distributed *Inga* trees (Dexter et al., 2012). We suggest that both coastal and montane forests in northwest Gabon could have acted as refugia for *P. barteri*, and possibly many other species.

Individuals sampled from Nigeria revealed three endemic haplotypes suggesting a genetic discontinuity with the rest of the *P. barteri*'s population (Fig. 2B). This could be the result of the Cameroon Volcanic Line in northwest Cameroon, which has been shown to be a possible barrier to gene flow in animals between northwest Cameroon/Nigeria and Central Africa (Nicolas et al., 2012). Indeed, *Podococcus* is mainly adapted to lowland or pre montane habitats, and is not distributed around that area. Few studies have investigated genetic diversity of rain forest taxa in Nigeria and little is known about how rain forests reacted to past climate change in that region. It is also important to note that DNA post-mortem damage in herbaria samples can lead to erroneous sequence information and provide false SNPs (Staats et al., 2011). In this study we did not explore or correct for this artifact. Our results for Nigeria should thus be taken with caution. Finally, it is hard to advance any solid conclusions because we only sampled three herbaria individuals from different regions.

4.3. The north/south genetic discontinuity in Central Africa

A general pattern emerging from recent population genetic studies of tree species in Central Africa is a strong north/south genetic differentiation around southern Cameroon between 0° and 3° latitude north (Hardy et al., 2013). This region corresponds to a climatic hinge, with the inversion of the dry/rainy seasons between northern and southern regions. Our results show a similar pattern for understory plant species with a clear north and south divide detected within *P. barteri* (Fig. 2B). The origin of this genetic divide could have three possible explanations (Hardy et al., 2013): (1) disappearance of rain forest around the climatic hinge during adverse climatic periods, followed by recolonization from putative north and/or south refugia. (2) Pre zygotic isolation of north and south populations linked to an inversion of the rainy season at the climatic hinge leading to differences in flowering periods; (3) environmental constraints of population establishment across the climatic hinge. Hardy et al. (2013) suggested that all three hypotheses might not be mutually exclusive.

Most individuals sampled from northern populations of *P. barteri* form a strongly supported clade in our phylogenetic analysis (PP = 0.99; Fig. 2A). Based on our coalescent molecular dating approach using plastomes, this northern clade was inferred to be 517,000 years old (crown node; HPD: 759,000–337,000 years). This result suggests a significantly older origin of the north/south divide than the LGM (21,000 years ago). Populations sampled in the hypothesized refugium of the Ma'an area (Ma'an and Akom populations, in south west Cameroon, Fig. 3A) show average levels of genetic diversity with few or no unique haplotypes and with diversity mainly shared between northern and southern populations (Fig. 3A). This is reinforced by our distribution modeling results which show little habitat stability for *P. barteri* in this area (Fig. 3C). Taken together this would suggest that for *P. barteri*, southern Cameroon is not a refugium as suggested in general by Maley (1996) and other floristic studies in Campo Ma'an national park (Tchouto et al., 2006), but a zone of secondary contact between expanding northern and southern populations. This data would support hypothesis 1 of Hardy et al. (2013) whereby the genetic discontinuity between north and south Central Africa is related to historical events of isolation in refugia and recolonization. A possible northern refugium for *P. barteri* would be the coastal forests around Campo in south west Cameroon (Fig. 3A). Like in coastal Gabon, our simulations suggest that these forests provided past stable habitats and also contain several endemic haplotypes (Fig. 3A). The Fifinda population was also shown to have similar levels of genetic diversity than in Campo population with more unique haplotypes. This scenario would agree with the hypothesis of Anhuf et al. (2006) who suggested that coastal forests along the Atlantic coast served as refugia during the LGM, whereas forests more inland (e.g. towards Ma'an and Akom) were more severely affected by the LGM.

4.4. Conservation importance of Central African coastal forests

The coastal forests near the Gabonese capital Libreville contain high levels of plant species diversity including a large number of endemics (Lachenaud et al., 2013). In addition, this ecosystem is highly threatened because of human exploitation linked to the expansion of Libreville (Lachenaud et al., 2013). As indicated before, our results underline for the first time that these forests could also contain high and unique levels of genetic diversity. Surprisingly, few studies of Central African plants have sampled this region extensively (coastal forests around Libreville) in terms of number of individuals and/or genetic markers (Dauby et al., 2014a; Hardy et al., 2013; Heuertz et al., 2014; Ley et al., 2014). Dauby et al. (2014a) based on the genetic analyses of tree species from Central Africa found high distinctness for two out of three species for this region. For example, in the tree species *Greenwayodendron suaveolens*, out of three individuals sampled and for a short plastid fragment (Dauby et al., 2014a), one haplotype (H10) was unique for the region, and one (H16) shared with populations mainly from central Gabon. Based on nuclear microsatellites, Duminil et al. (2013) found for the rain forest tree species *Erythrophleum ivorense* a clearly delimited gene pool located along the Gabonese coast around Libreville. Taken together these results strongly suggest that the coastal forests around Libreville are not only an important region in terms of species diversity and endemism (Lachenaud et al., 2013), but also a hotspot for unique genetic diversity and a potential refugium for plant species in Central Africa. As indicated above, a similar situation might also be found in the coastal forests of Cameroon around Campo village and towards the north. However, genetic data on these regions remains very limited. Because population resilience is strongly linked to the level of genetic diversity that it contains (Pauls et al., 2013), such data should be carefully considered and taken into account in future conservation planning and management efforts in the region (Sgro et al., 2011).

4.5. Study limits

Our results are limited by the fact that we only included a single but very long non-recombinant marker in our analysis, the chloroplast. Ideally one should include numerous other independent (nuclear) marker sequences under a statistical phylogeography approach (Knowles, 2004, 2009). Nuclear markers can provide better phylogeographic resolution (Duminil et al., 2015; Zellmer et al., 2012), but they are generally harder to design and to work with, with numerous issues of paralogy, incongruence, and incomplete lineage sorting that have to be correctly addressed. Our study shows that, even within plant families known to have slower plastome substitution rates such as palms (Wilson et al., 1990), sequencing full plastomes using NGS approaches (Mariac et al., 2014) provides a relatively quick and cost effective way to achieve good levels of phylogeographic resolution. Full plastome sequences are, in general, more straight forward molecular markers to work with although some studies have shown that care has to be taken when calling SNPs (Scarcelli et al., 2015). In addition, our study, to a lesser extent, might also be limited by the number of sampled individuals per population (average of ~7 individuals per population, see Table 2). This value isn't too bad overall, but clearly for some regions, such as Nigeria, more individuals should be added. A larger scale study with more populations (especially in Nigeria) and more individuals sampled would no doubt bring further precisions in unravelling the phylogeographic history of this genus.

Species distribution modeling is another potential source of uncertainty in our study. This is especially true for the LGM projections which cannot be validated with independent occurrence data, such as fossil pollen (e.g. Gavin et al., 2014; Lima et al., 2014), as we did for the present. Because climatic data are predictions themselves, they bring their own uncertainty into the SDMs. This uncertainty comes from the interpolation of the scarce Central African weather station network, the LGM global circulation model, and the downscaling to 2.5 arc minute (Deblauwe et al., 2016). The consequence of this uncertainty will be to add noise in the inference of stable habitats. We were nevertheless able to find a correlation between predicted habitat stability and number of unique haplotypes (e.g. Waltari et al., 2007).

Acknowledgements

This study was funded by Agropolis Foundation – France through the «Investissements d'avenir» programme (ANR-10-LABX-0001-01) under the reference ID 1202-040 (CHRLORODIV) to the senior author, and the International Foundation for Science – Sweden (IFS) under grant number No. AI1D/30248 plus the International Palm Society (IPS) – U.S.A. to the first author. The study was additionally funded by a grant "Appuie aux Equipes des Recherches" of the Agence Universitaire de la Francophonie (AUF) – France 2013–2015 to Bonaventure Sonké. Finally, part of this project was funded by the IRD Regional Pluridisciplinary Programme – France "Global change, biodiversity and health in the central African rain forests" (PPR FTH-AC). We thank the GenSeq technical facilities of the labex Centre Méditerranéen de l'Environnement et de la Biodiversité for their help to carried out samples preparation for NGS library constructions. We thank the Centre National de la Recherche Scientifique et Technique (CENAREST) and the Agence National des Parcs Nationaux (ANPN) in Gabon (authorization AR0025/13) and the General Delegation for Scientific and Technical Research (DGRST) in the Republic of Congo for delivering the necessary research and collection permits in their respective countries. We are very grateful to Prof. Paul Henri Bourobou Bourobou, Prof. Jean-Marie Moutsamboté, Dr. Nestor Obiang and Dr. Mathieu Ndounga help with research permits. We thank Mr. Raoul Niangadouma, Mr. Narcisse Kandem, and the conservateurs Mr. Jean-Claude Ndingui (National Park Conkouati) and Mr. Elonsa Delphin (Dimonika Forest Reserve) for their assistance in the field. Bill Baker is thanked for sending us herbarium material from the Royal Botanic Gadens, Kew, UK. We thank Jason Comer and Jim Leebens-Mack for pre publication data sharing of a *Podococcus barteri* plastome. We are grateful to Olivier Hardy, Gilles Dauby, Ruksan Bose, Simon Loader and several anonymous reviewers for comments and improvements on earlier versions of this manuscript. Finally, we thank Jean Gregoire (a.k.a. Petit Jean) Kayoum and Valentin Ngoumi (IRD Yaoundé) for their driving skills and help in the field.

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.2016.08.005.

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