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7

8 **03. AUTHORS:**

9 Jérémy Migliore^{1,2,3*}, Anne-Marie Lézine¹, Olivier J. Hardy²

10

11 **04. AUTHORS AFFILIATIONS:**

12 ¹Sorbonne Université, Laboratoire d'Océanographie et du Climat : Expérimentations et
13 Approches Numériques (LOCEAN/IPSL), CNRS UMR 7159, Paris, France.

14 ²Université Libre de Bruxelles, Faculté des Sciences, Service Evolution Biologique et
15 Ecologie, Bruxelles, Belgium.

16 ³Muséum départemental du Var, Toulon, France.

17

18 **05.* CORRESPONDING AUTHOR:**

19 Jérémy Migliore - Université Libre de Bruxelles, Faculté des Sciences, Service Evolution
20 Biologique et Ecologie, CP 160/12, 50 avenue F.D. Roosevelt, 1050 Bruxelles, Belgium.

21 Phone: +33 (0)681.112.891

22 Email addresses: jeremy.migliore@ulb.ac.be / jmigliore@var.fr

23

24 **06. RUNNING TITLE:**

25 Phylogenomics of Afromontane *Podocarpus*

1 **Abstract**

2 • **Background and Aims** Afromontane forests host a unique biodiversity distributed in
3 isolated high elevation habitats within a matrix of rain forests or savannahs. Yet, they share a
4 remarkable flora that raises questions about past connectivity between currently isolated
5 forests. Here, we focused on the *Podocarpus latifolius* - *P. milanjanus* complex
6 (Podocarpaceae), the most widely distributed conifers throughout sub-Saharan African
7 highlands, to infer its demographic history from genetic data.

8 • **Methods** We sequenced the whole plastid genome, mitochondrial DNA regions, and
9 nuclear ribosomal DNA of 88 samples from Cameroon to Angola in western Central Africa
10 and from Kenya to the Cape region in eastern and southern Africa to reconstruct time-
11 calibrated phylogenies and perform demographic inferences.

12 • **Key Results** We show that *P. latifolius* and *P. milanjanus* form a single species, whose
13 lineages diverged during the Pleistocene, mostly between *c.* 200 and 300 kyrs BP, after which
14 they underwent a wide range expansion leading to their current distributions. Confronting
15 phylogenomic and palaeoecological data, we argue that the species originated in East Africa
16 and reached the highlands of the Atlantic side of Africa through two probable latitudinal
17 migration corridors: a northern one towards the Cameroon volcanic line, and a southern one
18 towards Angola. Although the species is now rare in large parts of its range, no demographic
19 decline was detected, probably because it occurred too recently to have left a genetic signature
20 in our DNA sequences.

21 • **Conclusions** Despite the ancient and highly fluctuating history of podocarps in Africa
22 revealed by palaeobotanical records, the extended distribution of current *P.*
23 *latifolius/milanjanus* lineages is shown to result from a more recent history, mostly during

1 the mid-late Pleistocene, when Afromontane forests were once far more widespread and
2 continuous.

3 **Key words:** Afromontane forest, genome skimming, molecular dating, palaeoecology,
4 phylogenomics, phylogeography, plastome sequencing, Podocarpaceae, *Podocarpus*
5 *latifolius*, *Podocarpus milanjanus*

6

1 INTRODUCTION

2 Considered as hotspots of biodiversity (Gehrke and Linder 2014), African high mountains are
3 characterized by a complex dynamics of species diversity linked to their fragmented
4 distribution. They are described as islands, since low-elevation habitats (rain forests or
5 savannahs) act as dispersal barriers, facilitating the divergence of isolated populations
6 (McCormack *et al.* 2009). However, many species are shared between African mountains,
7 indicating long-distance dispersal between mountains and/or habitat connectivity in the past
8 (Hedberg 1969; Kebede *et al.* 2007; Gehrke and Linder 2009; Chala *et al.* 2017). To explain
9 such Afromontane diversity, one emerging hypothesis is the flickering connectivity system,
10 where Plio-Pleistocene climatic changes recurrently affected the distribution of montane
11 vegetation belts, generating new lineages (Flantua *et al.* 2019). An alternation of population
12 connectivity and fragmentation periods was thus suggested for *Prunus africana* (Kadu *et al.*
13 2011, 2013), *Hagenia abyssinica* (Ayele *et al.* 2009; Gichira *et al.* 2017), Rand Flora species
14 (Mairal *et al.* 2017), and Afroalpine species (Gizaw *et al.* 2013; Schwery *et al.* 2015; Chartier
15 *et al.* 2016). Unfortunately, genetic studies are still lacking for many Afromontane trees and it
16 remains difficult to know how and when populations/species have been able to disperse
17 among Afromontane 'islands'.

18 In Africa, Podocarpaceae, represented by the genera *Podocarpus* and *Afrocarpus*, are
19 typical montane forest trees. Among them, *Podocarpus latifolius* (Thunb.) R. Br. ex Mirb. is
20 often considered as a synonym of *Podocarpus milanjanus* Rendle (Barker *et al.* 2004) so that
21 we will refer to this taxon as *P. latifolius/milanjanus*. It is the most widespread species,
22 ranging from Cameroon to Angola in western Central Africa and from Kenya to the Cape
23 region in eastern and southern Africa (Fig. 1A). Despite being very extensive, its distribution
24 is extremely fragmented, and the species is nowadays rare and very localised in large parts of

1 its range, such as in western Central Africa. The origin of such an extensive but fragmented
2 distribution range remains unclear but could suggest past large-scale expansions of montane
3 forests through mid- to low elevation areas (White 1993). More specifically, western
4 populations have been postulated to originate from eastern populations following either a
5 northern dispersal corridor (Aubréville 1976) or a southern migratory track through the
6 Zambezi-Congo watershed (Maley *et al.* 1990; White 1993; Adie and Lawes 2011).

7 The most ancient fossil pollen data in Africa reported the sporadic occurrence of
8 podocarps during the (pre-) Cretaceous time (Goldblatt 1978; Salard-Chebouldaëff and Dejax
9 1991) and the late Oligocene (Coetzee 1980). Podocarps expanded during the Miocene in
10 eastern (Retallack 1992; Boaz *et al.* 1992) and southern Africa (Coetzee 1980; Dupont *et al.*
11 2011; Hoetzel *et al.* 2015), probably in response to the global climate change following the
12 setting of the high latitude ice sheets (Zachos *et al.* 2001) and the uplift of the East African
13 Plateau (Wichura *et al.* 2010). Podocarps were recorded in East Africa in most of the Plio-
14 Pleistocene hominid sites (Bonnefille 2010; Owen *et al.* 2018), and also in western Africa
15 (Morley 2000), where its presence coincides with the onset of northern hemisphere
16 glaciations at 2.7 Myrs (million years) (Knaap 1971; Adeonipekun *et al.* 2017). During the
17 last million years, the abundance of podocarp pollen underwent large amplitude variations
18 (Fig. 1B), generally increasing during the moist phases of forest development coinciding with
19 interglacial phases (Dupont *et al.* 2001). However, the chronology of palynological records is
20 less reliable for ancient periods, and as pollen grains of *P. latifolius/milanjanus* cannot be
21 distinguished from those of other *Podocarpus* or *Afrocarpus* species, it is difficult to assess
22 how individual podocarp species responded to glacial/interglacial cycles. In this context,
23 studying the genetic diversity of *P. latifolius/milanjanus* should provide key insights into the
24 demographic scenario associated with colonisation processes and routes, the role of refugia in

1 the preservation of genetic diversity, and the magnitude of gene flow, in such extremely
2 fragmented Afromontane forests (Sklenář *et al.* 2014; Wondimu *et al.* 2014; Gizaw,
3 Brochmann, *et al.* 2016).

4 Here, to gain insights into the past dynamics of Afromontane forests, we investigate
5 the phylogeography of *P. latifolius/milanjanus* using a genome skimming approach, by
6 sequencing plastid, mitochondrial and ribosomal DNA of a sampling representative of the
7 natural distribution of the species. Phylogenomic patterns from dated gene phylogenies will
8 be discussed against what we know from podocarp palaeorecords to examine the role of
9 Pleistocene climate oscillations on the demographic history of *P. latifolius/milanjanus*. We
10 will test the following hypotheses. (i) *P. latifolius* (southern Africa) and *P. milanjanus*
11 (western Central Africa, East Africa) are so closely related that they belong to the same
12 species. (ii) *P. latifolius/milanjanus* underwent a species range expansion, involving a
13 demographic expansion during the Pleistocene, followed by a demographic decline in parts of
14 its range where it is nowadays rare. (iii) *P. latifolius/milanjanus* populations from western
15 Central Africa (from Cameroon to Angola) originate from East Africa following a southern
16 and/or northern migration route.

17

18

19

20 **MATERIALS AND METHODS**

21 *Biological model*

22 The Podocarpaceae family is represented in Africa by six *Afrocarpus* species and seven
23 *Podocarpus* species (Barker *et al.* 2004; Adie and Lawes 2011). Species delimitation in
24 African *Podocarpus* is not yet completely resolved, since *P. latifolius* and *P. milanjanus* are

1 considered either as synonymous or as distinct species restricted to South Africa and
2 East/Central Africa respectively (Barker *et al.* 2004).

3 *Podocarpus latifolius/milanjanus* is a medium to large evergreen tree (20-30 m in
4 height) slowly growing in Afromontane forests, usually between 900 and 3,200 m asl,
5 although it sometimes occurs at lower elevation notably in coastal forests of South Africa
6 (Fig. 1A). Its ecology is not well documented, but it is a key component of montane forests,
7 where it dominates old-growth stages, being capable of establishing in relatively high light
8 conditions as well as in a fragmented landscape (Turner and Cernusak 2011). The leaves are
9 strap-shaped, with a bluntly pointed tip. The male cones of this dioecious tree are 10-50 mm
10 long and look like small pinkish catkins inflorescences adapted for wind pollination. The
11 female cones are fleshy, with a single (rarely two) 7-11 mm seed in apical position on an 8-14
12 mm pink, edible and sweet aril. Being animal dispersed (by birds, monkeys, bush pigs and
13 sometimes by humans) and capable of rapid germination after 4-6 weeks in a variety of
14 habitats, *P. latifolius/milanjanus* is an annual seeder fitting the model of a good disperser
15 (Geldenhuys 1993; Adie and Lawes 2011).

16

17 *Genomic libraries preparation, sequencing, and bioinformatic treatment*

18 A total of 88 field and herbarium specimens of *P. latifolius/milanjanus* and three outgroup
19 taxa (*Afrocarpus falcatus* from South Africa and Kenya, *A. usambarensis*, and *Podocarpus*
20 *elongatus*; see Supplementary Data Table S1) were selected for paired-end sequencing (2 ×
21 150 bp) of non-enriched genomic libraries. After CTAB extraction with QIAquick
22 purification, we performed genome skimming, following the NEBNext Ultra II DNA Library
23 Prep Kit for Illumina (details in Supplementary Data Appendix S1).

1 Three reference genomes were reconstructed for the whole plastome (cpDNA, using
2 MIRA 3.4.1.1 - MITOBIM 1.7; Chevreux *et al.* 1999; Hahn *et al.* 2013), the nuclear ribosomal
3 DNA (nrDNA, using NOVOplasty; Dierckxsens *et al.* 2017), and two mitochondrial DNA
4 regions (mtDNA NADH dehydrogenase *nad5* gene and the small subunit ribosomal RNA
5 gene SSU-RNA, using ARC 1.1.4; Hunter *et al.* 2015). Each genomic library was then
6 mapped on these reference genomes, before SNP calling, VCF filtering, and conversion to
7 fasta multi-alignment files after removing indels and heterozygous sites as detailed in
8 Appendix S2.

9

10 *Phylogenomic analyses*

11 To estimate the divergence time of current *P. latifolius/milanjanus* lineages, we generated
12 dated gene phylogenies in BEAST 1.8.2 (Drummond and Rambaut 2007) using the CIPRES
13 platform (Miller *et al.* 2011) for the cpDNA, nrDNA, and mtDNA datasets ($n = 71, 82, 60$
14 respectively, including outgroups taxa, as detailed in Tables 1 and S1), assuming the
15 following evolutionary sites models GTR, HKY+I, and GTR+G respectively, according to
16 jMODELTEST 2.1.7 (Darriba *et al.* 2012). A strict clock model and an “Extended Bayesian
17 Skyline Plot” coalescent tree model (‘mitochondrial data’ or ‘autosomal data’ with ‘linear
18 growth’ between population size change events) were applied. To time-calibrate the
19 phylogenies, the divergence between *Afrocarpus* and *Podocarpus* was set as a normally
20 distributed variable with a mean of 87.46 Myrs and a 95% HPD (Highest Posterior Density)
21 between 69.40 and 108.06 Myrs, following the divergence estimated by Quiroga *et al.* 2016
22 (nodes A, A’, and A’’ in Figs 2-3, S1-2, and S3-4). Five independent MCMC runs were
23 launched for 100 million generations each, sampling trees at 10,000 step intervals in order to
24 check the congruence of phylogenetic trees and divergence time estimations, before

1 combining them using LOGCOMBINER 1.8.2 (Drummond and Rambaut 2007). We used
2 TRACER 1.6 (Drummond and Rambaut 2007) to assess convergence, estimate Effective
3 Sample Sizes (ESS), and examine the posteriors of all the parameters. Mean heights were
4 taken in TREEANNOTATOR 1.4.8 (Drummond and Rambaut 2007), and trees were plotted in
5 FIGTREE 1.1.2 (<http://tree.bio.ed.ac.uk/software/figtree/>). Bayesian skyline plot were used to
6 graphically represent changes in coalescent rate through time (IICR, Inverse Instantaneous
7 Coalescence Rate; Chikhi *et al.* 2018), which is classically interpreted as underlying changes
8 of the effective size of a panmictic population (Ho and Shapiro 2011).

9 In addition, a network analysis of each reconstructed DNA region was undertaken
10 from a NeighborNet analysis using SPLITSTREE 4.14.2 (Huson and Bryant 2006), with
11 mapping of phylogroups using QGIS 2.18 (<http://qgis.osgeo.org>). Phylogroups were defined to
12 encompass closely related haplotypes (well supported clades in general) or isolated
13 haplotypes, combining phylogenetic and geographic coherence.

14 Three geographic groups of *P. latifolius/milanjanus* samples were also delineated
15 according to the main gene flow barriers detected: North-West (Cameroon), South-West
16 (Angola and Republic of the Congo), and East-South (remaining countries from Kenya to
17 South Africa). We computed Tajima's *D* statistics (Tajima 1989) and nucleotide diversity (π)
18 for each group, as well as mean genetic distances between groups using MEGA 10.0.5 (Kumar
19 *et al.* 2016).

20

21

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24

1 RESULTS

2 *Sequence data and reference genomes*

3 We obtained on average 3,089,119 R1-R2 reads per sample (SD = 1,465,677). Our
4 reconstructed references for *P. latifolius/milanjanus* reached 134,031 bp for the plastome,
5 which lacked one of the large inverted repeat regions as evidenced for the Neotropical *P.*
6 *lambertii* (Vieira *et al.* 2014), 7,890 bp for the nrDNA, 6,026 bp for the mtDNA *nad5* gene,
7 and 2,330 bp for the SSU-RNA region (Supplementary Data Tables S1-S2). After mapping
8 each sample on these four references, and filtering out samples with >10% missing data, the
9 average proportions of mapped reads reached, respectively, 1.96%, 0.19%, 0.08% and 0.11%,
10 and the depth reached, respectively, 52X ($n = 71$), 75X ($n = 82$), 47X ($n = 77$) and 33X ($n =$
11 61) (Table S2). The numbers of SNPs detected over all samples decreased a lot after
12 excluding outgroup taxa: from 4152 to 212 SNPs for cpDNA, from 206 to 18 SNPs for
13 nrDNA, from 583 to 35 SNPs for *nad5* and SSU-RNA mtDNA after concatenating data from
14 the two mtDNA references (Tables 1 and S3).

15

16 *Phylogeographic pattern and molecular dating*

17 The age of the crown node of the current lineages of *P. latifolius/milanjanus* ranged from
18 0.86 Myrs (95% HPD: 0.54-1.17 Myrs; Fig. 2) using plastomes, to 1.74 Myrs (95% HPD:
19 0.55-3.08 Myrs; Fig. S1) using nrDNA data, and to 1.17 Myrs (95% HPD: 0.52-1.87 Myrs;
20 Fig. S3) using mtDNA data. High support (PP>0.9) was obtained for 37% of the nodes of the
21 plastid phylogenetic tree (Fig. 2B) but for only 5% and 12% of the nodes of the nrDNA and
22 mtDNA trees (Figs S1B, S3B), reflecting their lower number of SNPs (Table 1). The
23 NeighborNet networks of the *P. latifolius/milanjanus* sequences presented star-like structures
24 for all the three genomes (Figs 3B, S2B, S4B).

1 For cpDNA, we identified six plastid phylogroups (CP) relatively well geographically
2 circumscribed (Fig. 3A), although two of them received low support (Fig. 2), plus five
3 isolated samples that were also numbered. The two oldest nodes (C dated to 0.86 Myrs and D
4 to 0.45 Myrs; Fig. 2B) isolated two samples coming from eastern D. R. Congo (CP 01) and
5 South Africa (CP 02) respectively, while the next oldest node (E is 0.31 Myrs, Fig 2B)
6 included all the phylogroups identified. Phylogroups CP 04, CP 05 and CP 09 were
7 distributed in eastern and south-eastern Africa with some degree of overlapping, whereas the
8 sister phylogroups CP 10 and CP 11 were exclusively found in the western part of Central
9 Africa, distributed in Cameroon (CP 10) and from Angola to the Republic of the Congo (CP
10 11). This main east-west genetic divergence was confirmed by the highest mean genetic
11 distances calculated between eastern and western populations of *P. latifolius/milanjianus*
12 (Table S4). The phylogroup CP 06 was more northerly distributed in Cameroon, Equatorial
13 Guinea, eastern D.R. Congo, and Tanzania. However, the low posterior probabilities for
14 phylogroups CP 06 (0.28) and CP 07-08 (0) prevented us from establishing their phylogenetic
15 relationship with other CP phylogroups. If we exclude the two most early-branching
16 haplotypes (CP 01 and CP 02), it is worth noting that all major phylogroups diverged from
17 each other between 0.2 Myrs (lower 95% HPD limit at 0.13 Myrs) and 0.31 Myrs (upper 95%
18 HPD limit at 0.43 Myrs). The most recent divergence time between west and east African
19 samples (between CP 03 in Cameroon and CP 04 or CP 05) was estimated at 0.24 Myrs (95%
20 HPD: 0.16- 0.33 Myrs).

21 Regarding nrDNA and mtDNA data, their low levels of polymorphism combined with
22 their star-like networks implied that posterior probabilities of nodes were extremely low and
23 generally null (due to a low number of informative SNPs). We could delineate the two
24 earliest-branching phylogroups of nrDNA: (i) a Central African phylogroup distributed until

1 the Albertine Rift (NR 01), and (ii) a northerly distributed phylogroup (NR 02) in Cameroon,
2 Uganda and Kenya (Fig. S2). According to mtDNA data, the delineation of some robust
3 phylogroups was highly congruent with those found from cpDNA data: MT 01-02 and CP 01-
4 02, MT03 and CP 09, MT 05b and CP 04, MT 06b and CP 10, MT 07b and CP 04 (Figs 2-3
5 and S3-4). This congruence could imply a same mode of uniparental transmission of the two
6 organelles, although paternal inheritance of chloroplasts (Vieira *et al.* 2014) and maternal
7 inheritance of mitochondria are often assumed in gymnosperms (Petit and Vendramin 2007).

8

9 *Demographic inferences and genetic diversity gradients*

10 Bayesian skyline plots were congruent between genomic datasets, showing a ten-fold increase
11 of coalescence rate between *c.* 200 kyrs ago compared to the present (i.e. inferred N_e ten
12 times lower 200 kyrs ago), which could result from a demographic and range expansion after
13 a bottleneck event (Figs 2C, S1C, S3C). There was no evidence of a recent increase in
14 coalescence rate (demographic decline) that we hypothesized given the low current population
15 sizes in several regions. Congruently with the apparent demographic expansion inferred by
16 the skyline plots, Tajima's D statistics were very negative across populations at the species
17 scale (most values < -2 ; Table 1) and also considering only East and South African samples
18 and, to a lower extent, western samples (Table S3). For all genomes, nucleotide diversity
19 decreased from South and East Africa to North-West (Cameroon) and to South-West (Angola
20 and Republic of the Congo) (Table S3), suggesting a more recent origin of the later
21 populations.

22

23

24 **DISCUSSION**

1 *Species delimitation*

2 Our phylogenomic data show that a large number of SNPs separates *Afrocarpus* from
3 *Podocarpus*, consistently with the ancient divergence dated between 82.6 and 87.5 Myrs
4 between *Podocarpus* and its sister group including *Afrocarpus*, *Nageia* and *Retrophyllum*
5 (Quiroga *et al.* 2016). By contrast, the number of SNPs per genomic region decreased 10 to
6 18 times when considering only *P. latifolius/milanjanus* samples (Table 1). As South-African
7 samples (*P. latifolius*) did not form a clade separated from the other ones (*P. milanjanus*), we
8 confirm that the two taxa can be considered as synonyms (Fig. 2B).

9

10 *Genetic diversification of P. latifolius/milanjanus during the Pleistocene*

11 The current lineages of *P. latifolius/milanjanus* started to diverge 0.86 Myrs ago (95% HPD:
12 0.54-1.17 Myrs) according to our cpDNA results (Fig. 2), coinciding with the mid-Pleistocene
13 period, when vegetation changes in Central Africa started to follow a clear glacial-interglacial
14 alternation (Dupont *et al.* 2001). However, most lineages diverged more recently, 0.31 Myrs
15 ago (95% HPD: 0.19-0.43 Myrs), and eventually reached locations distant by up to *c.* 4,500
16 km, implying wide dispersal. Recurrent widespread colonisation of Afromontane forests in
17 lowlands, followed by their fragmentation and decline driven by climate changes, could thus
18 have played a key role in connecting fragmented montane floras, during interglacial phases
19 (Ivory *et al.* 2018). However, this flickering connectivity pattern does not appear so simple
20 and synchronized throughout Africa. In East Africa, the expansion of mountain glaciers
21 during the last glacial period (Osmaston and Harrison 2005) led to the downward
22 displacement of the upper treeline and Afromontane forest, so that subalpine and alpine taxa
23 expanded into low- and midlands down to 470 m around Lake Malawi (Ivory *et al.* 2012) and
24 1,139 m around Lake Victoria (Pinklington Bay; Kendall 1969). Such a “glacial” expansion is

1 not observed in Cameroon where traces of Quaternary glaciers have never been observed and
2 podocarps populations seem to have remained at their current altitudinal range during the last
3 glacial period (Lézine *et al.* 2019). Southwards, however, pollen data report the presence of
4 *Podocarpus/Afrocarpus* in Congo and also probably in Gabon during the last glacial period
5 (Elenga and Vincens 1990; Dupont *et al.* 2000), suggesting that podocarps considerably
6 expanded in western Central Africa south of the Equator. As *P. latifolius/milanjanus* is today
7 the only podocarp growing in western Central Africa (except *Afrocarpus mannii*, endemic to
8 São Tomé island), it can be hypothesised that it was this species which is recorded in pollen
9 data in this area.

10 The divergence times between geographically isolated lineages and the Bayesian
11 skyline plots provide compelling clues that *P. latifolius/milanjanus* expanded substantially
12 from *c.* 200 kyrs BP to reach its current distribution range; the signal of demographic
13 expansion being congruent with geographic expansion of lineages (Figs 2, S1, S3). These
14 results fit into what we know from pollen data showing that *P. latifolius/milanjanus* widely
15 expanded in central Africa during the wet phases of the Quaternary (Elenga and Vincens
16 1990; Dupont *et al.* 2000; Lézine *et al.* 2019). Interestingly, the apparent demographic
17 bottleneck revealed by the skyline plot *c.* 200 kyrs BP (Fig. 2) closely coincides with a trend
18 of reduction of pollen abundance of podocarps in Atlantic marine cores between 400 and 250
19 kyrs BP, followed by an increase (with substantial variation) between 200 and 100 kyrs BP
20 (Fig. 1B). In *Prunus africana*, another Afromontane tree, coalescent simulations conducted on
21 chloroplast loci also suggested that this species expanded its range and reached its current
22 distribution during the late Pleistocene, within the last 100-180 kyrs (Kadu *et al.* 2011). The
23 relatively recent divergence time between disconnected populations of Afromontane trees
24 located thousands of kilometres apart sharply contrasts with the ancient divergence (several

1 million years) reported for parapatric populations of a central African mature rain forest tree
2 species (Migliore *et al.* 2019), probably highlighting the contrasted population dynamics of
3 lowland and montane tree species.

4

5 *Past migration routes between African mountains*

6 The centre of diversity and thus the putative centre of origin of *P. latifolius/milanjanus* was
7 likely centred in East Africa which hosts the highest number of plastid phylogroups (Fig. 3)
8 and the most divergent sample in the cpDNA and mtDNA phylogenies (phylogroups CP/MT
9 01 – Figs 2 and S3) as well as the highest genetic diversity (Table S3). Reconstructing
10 migration routes is difficult after a fast demographic expansion followed by a drastic
11 fragmentation because DNA sequences linking regions along migration paths may have
12 disappeared or may not have accumulated enough indicative mutations. Nevertheless, *P.*
13 *latifolius/milanjanus* plastome data reveal a clear east/west phylogeographic signal dated to
14 310 kyrs BP (node E; Fig. 2). Two phylogroups are restricted to western Central Africa, in
15 Cameroon (CP 10) and from Angola to the Republic of the Congo (CP 11). Three
16 phylogroups are exclusively distributed in East Africa, either in the Western branch of the
17 East African Rift (CP 04), or in the Eastern branch and the Lake Malawi branch (CP 05), or in
18 the Eastern branch and South Africa (CP 09). Central and East African phylogroups
19 diversified during the same temporal window between 200 and 210 kyrs BP (nodes J and I:
20 95% HPD of 130-280 kyrs; Fig. 2). Despite a lower phylogenetic resolution from nrDNA
21 sequences, the phylogroup NR01 is also restricted to Central Africa, from Cameroon to
22 Angola and Zambia (Figs S1-S2). This phylogeographic pattern suggests a barrier to gene
23 flow between Central and East/South Africa, as already detected for *Prunus africana*
24 (Dawson and Powell 1999; Kadu *et al.* 2011). Such east-west phylogeographic breaks were

1 also reported for non-montane species in *Adansonia digitata* (Pock Tsy *et al.* 2009), and in
2 several savannah ungulates and carnivores (Bertola *et al.* 2016). The convergence in the
3 timing of phylogeographic divergence might indicate that the same “recent” environmental
4 changes drove the distribution of these species.

5 Two migratory tracks between East/South and West Africa received support from our
6 data. Phylogroups CP 06 (Fig. 3) and NR 02 (Fig. S2) connect Cameroon to Tanzania and
7 Kenya, respectively, supporting the hypothesis of a “northern” migration corridor already
8 suggested by the plastid lineages shared between Cameroon and Uganda in *Prunus africana*
9 (Kadu *et al.* 2011). By contrast, phylogroups CP 05 and NR 01 support the hypothesis of a
10 southern corridor through the highlands and plateaus of Zambia, and an extension until
11 eastern D. R. Congo close to Malawi. Along the Congo-Zambezi watershed, patchily
12 distributed Afromontane forests in the southern D. R. Congo, Zambia, northern Angola and
13 Gabon could have acted as ‘stepping stones’ (White 1983, 1993). Although the exact
14 migration routes connecting Central and East/South Africa cannot be definitively determined,
15 our data are compatible with the occurrence of both northern and southern migration corridors
16 already hypothesised by White 1981, and also suggested for *Delphinium dasycaulon* (Chartier
17 *et al.* 2016).

18 Finally, plastome data suggest a barrier to gene flow between samples from Cameroon
19 (CP 10) and Angola/Congo (CP 11). This could account for the isolation of the Cameroon
20 highlands, as shown by pollen data from the last glacial period in western Central Africa. As
21 discussed by Dupont *et al.* (2000) and recently confirmed by Lézine *et al.* (2019) and
22 references therein, it is probable that the *Podocarpus* expansion in the lowlands during the
23 last glacial period never crossed the Equator to the North. Although several living trees of *P.*
24 *latifolius/milanjanus* currently occur at mid-altitude in Cameroon, as probable relicts of a

1 formerly wider distribution, there is no evidence that this expansion took place during the last
2 glacial period: *P. latifolius/milanjanus* was absent at that time from Lake Barombi Mbo (300
3 m asl; Maley and Brenac 1998) and Lake Monoun (1083 m asl; Lézine *et al.* submitted). Rare
4 pollen grains found at sites within the current elevation range of the species suggest that
5 *Podocarpus* populations survived only in the form of extremely restricted populations in the
6 Cameroon highlands. In this context, climate oscillations should not be considered as the only
7 driver of changes of range distribution; plant competition could also play a key role in the
8 succession of species during phases of forest expansion. It is likely that *P.*
9 *latifolius/milanjanus* does not occupy all its potential niche today.

10

11 *Past dynamics of Afroalpine forests*

12 Phylogeographic studies on Afroalpine flora showed that gene flow between mountains is low
13 but possible even across geographic barriers (Wondimu *et al.* 2014) with patterns of
14 differentiation coincident or predating the glacial cycles (Gizaw, Brochmann, *et al.* 2016;
15 Tusiime *et al.* 2017). Two Afroalpine species, *Erica arborea* and *Koeleria capensis*, that also
16 extend to lower vegetation belts in the mountains show no isolation-by-distance pattern
17 (Gizaw *et al.* 2013; Masao *et al.* 2013), possibly indicating that habitat connectivity through
18 lowland corridors has been more important than long-distance dispersal. By contrast to
19 Afroalpine vegetation, Afroalpine forests seem to have been better connected, as evidenced
20 by *P. latifolius/milanjanus* across South Africa (CP 09), across East Africa (CP 04-05),
21 across the Cameroon Volcanic Line (CP 10), between Congo and Angola (CP 11), and
22 between Central and East Africa (CP 06; Fig. 3).

23 In addition, the low levels of polymorphism detected, the star-like topology of
24 NeighborNet networks, and the wide distribution of each phylogroup are consistent with a

1 relatively recent and fast range expansion of *P. latifolius/milanjanus*. This hypothesis is
2 further supported by the apparent demographic expansion since *c.* 200 kyrs revealed by the
3 Bayesian skyline plots and Tajima's *D* statistics (Figs 2, S1, S3; Tables 1-S3). Extensive
4 lowland colonisation is consistent with palaeorecords which reveal high podocarp abundances
5 at low elevation (Ivory *et al.* 2018), while modern pollen samples of *Podocarpus* indicate that
6 high pollen grain concentration are only found close to the source trees (Maley *et al.* 1990;
7 Verlhac *et al.* 2018). Additional population genetics data would be necessary to better
8 estimate the intensity of gene flow and to assess if seed dispersal mechanisms play a crucial
9 role in shaping the distribution of genetic diversity and differentiation in Afromontane flora
10 (Gizaw, Brochmann, *et al.* 2016; Gizaw, Wondimu, *et al.* 2016; Minaya *et al.* 2017). We
11 should thus better evaluate the role of pollen dispersal by wind *versus* seed dispersal.
12 Potentially 36 frugivorous birds and mammals disperse seeds of *Prunus africana* over short-
13 and medium distances (Farwig *et al.* 2006), but whether they contributed to long-distance
14 dispersal at a subcontinental scale remains an open question.

15 Nowadays *P. latifolius/milanjanus* populations are generally very small, in particular
16 in western Central Africa, while the skyline plot does not display any recent increase in the
17 rate of coalescence as could be expected under strong demographic decline (Fig. 2). This
18 paradox is probably explained by two factors. First, if the demographic decline started
19 recently, during the major environmental crisis which occurred at the end of the Holocene
20 Humid Period (4.5-3.5 kyrs ago; Vincens *et al.* 1999; Lézine, Holl, *et al.* 2013) as suggested
21 by pollen records (Lézine, Assi-Kaudjhis, *et al.* 2013), it is maybe too early to detect a genetic
22 signature. Second, coalescence rate can increase between samples at a local scale due to
23 demographic decline but not between distant samples in the absence of gene flow, while our
24 samples are widely distributed. This is related to the problematic interpretation of skyline

1 plots in terms of demographic changes when there is a strong spatial genetic structure (Chikhi
2 *et al.* 2018). Hence, further studies must be conducted at a local scale to possibly detect recent
3 population declines.

4

5 **CONCLUSIONS**

6 A long-standing question in African biogeography is whether colonisation of the mountains
7 and subsequent intermountain gene flow mainly depend on long-distance dispersal across
8 unsuitable lowland habitat or on intermittent suitable habitat bridges (Kebede *et al.* 2007;
9 Mairal *et al.* 2017). *Podocarpus latifolius/milanjanus* seems to illustrate that migration has
10 been possible through habitat bridges under more suitable climates in the past, when the
11 montane forest habitat extended to lower elevation than today. Despite the ancient history of
12 podocarps in Africa revealed by palaeobotanical records, the extended distribution of current
13 *P. latifolius/milanjanus* lineages is shown to result from a more recent history, mostly during
14 the mid-late Pleistocene. Phylogenomic analyses support the hypothesis that the Afromontane
15 forests were once far more widespread and continuous, and that the current patches are a
16 result of recent fragmentation, probably too recent to be detected at the genomic scale.
17 Finally, this work highlights the resilience of Afromontane forests during previous drastic
18 climate changes but questions also the complex history of these hotspots of biodiversity such
19 as the Cameroon volcanic line where several lineages still persist and could represent
20 successive waves of migration more or less ancient.

21

22

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22

23

1 **DATA ACCESSIBILITY**

2 - Sampling locations are available as supporting information.

3 - Reference cpDNA, nrDNA, and mtDNA regions of *Podocarpus latifolius/milanjanus* are
4 available in GenBank and their accession numbers as supporting information.

5 - Fasta alignments are available on request.

6

7 **SUPPLEMENTARY DATA**

8 Supplementary data are available online at <https://academic.oup.com/aob> and consist of the
9 following.

10

11 **APPENDIX S1.** DNA extraction, genomic libraries preparation, and sequencing

12

13 **APPENDIX S2.** Bioinformatic treatment

14

15 **FIG. S1.** Phylogenetic relationships and tempo of diversification of *Podocarpus*
16 *latifolius/milanjanus*, using nuclear ribosomal DNA data

17

18 **FIG. S2.** Geographic distribution of nuclear ribosomal phylogroups (NR) of *Podocarpus*
19 *latifolius/milanjanus* with their NeighborNet representation

20

21 **FIG. S3.** Phylogenetic relationships and tempo of diversification of *Podocarpus*
22 *latifolius/milanjanus*, using mitochondrial DNA data

23

24 **FIG. S4.** Geographic distribution of mitochondrial phylogroups (MT) of *Podocarpus*
25 *latifolius/milanjanus* with their NeighborNet representation

26

27 **TABLE S1.** Characteristics of samples of *Podocarpus latifolius/milanjanus* used for genome
28 skimming

29

30 **TABLE S2.** Assembling and mapping statistics of plastid, ribosomal and mitochondrial DNA
31 regions reconstructed from genomic libraries of *Podocarpus latifolius/milanjanus*

32

33 **TABLE S3.** Diversity and Tajima statistics for plastid, ribosomal and mitochondrial DNA
34 regions reconstructed from genomic libraries of *Podocarpus latifolius/milanjanus*

35

36 **TABLE S4.** Mean distances between African geographic groups for plastid, ribosomal and
37 mitochondrial DNA regions of *Podocarpus latifolius/milanjanus*

38

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1 **FIGURES AND TABLE CAPTIONS**

2 **FIG. 1.** (A) Distribution map of African *Podocarpus* and *Afrocarpus* species. Data were
3 extracted from GBIF (GBIF.org - 26 January 2019 - GBIF Occurrence Download), field
4 missions, herbarium specimens (BRLU, BR, P), the Conifers of the world database
5 (<https://herbaria.plants.ox.ac.uk/bol/conifers>), and scientific literature. (B) Evolution of
6 Podocarp-type pollen abundance throughout the Quaternary in three Atlantic marine cores
7 located on the map: the gravity core GIK 16827-2 and the piston core GIK16827-3 from the
8 same location off Gabon (Dupont *et al.* 1998), and ODP 175-1075 from the Congo fan
9 (Dupont *et al.* 2001).

10

11 **FIG. 2.** Phylogenetic relationships and tempo of diversification of *Podocarpus*
12 *latifolius/milanjanus* lineages, using plastome data. (A) Dated phylogeny including four
13 outgroup taxa ($n = 71$). (B) Chronogram of *P. latifolius/milanjanus* plastomes, and molecular
14 dating of nodes A-M, including 95% highest posterior densities (HPD) and posterior
15 probabilities (PP). Nodes with a diamond symbol have a PP > 0.90. The main CP phylogroups
16 are represented by different coloured symbols, and five isolated samples are represented by
17 black stars and numbered as are phylogroups. (C) Inference of the inverse instantaneous
18 coalescence rate (IICR) through time using the Bayesian Skyline plot approach in BEAST,
19 expressed as the product of the effective population size N_e of panmictic population and the
20 generation time t (logarithmic scale).

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22 **FIG. 3.** Geographic distribution of plastome phylogroups (CP) of *Podocarpus*
23 *latifolius/milanjanus* (A) with their NeighborNet representation (B). Countries are
24 represented by their alpha-2-character alphabetic codes (ISO 3166).

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TABLE 1. Diversity and Tajima statistics for plastid, ribosomal and mitochondrial DNA regions of *Podocarpus latifolius/milanjanus*. The following genomic indices were calculated using MEGA 10.0.5 (Kumar *et al.* 2016) after pairwise deletion: total number of sites (n), number of sequences (m), number of segregating sites (S), proportion of variable sites ($p_s = S/n$), mean estimate of the expected number of single nucleotide polymorphisms between two DNA sequences under the neutral mutation model ($\Theta = p_s/a_1$), nucleotide diversity as the mean number of pairwise differences (π), and the Tajima statistic (D).

1 **TABLE 1**

2

Nb of sites <i>n</i>	Taxonomic groups	Sample size <i>m</i>	<i>S</i>	<i>p_s</i>	Θ	<i>Nucleotide diversity π</i>	<i>D Tajima</i>
Plastomes (cpDNA) 131770	<i>P. latifolius/milanjanus</i> including outgroups	71	4152	0.0315	0.0065	0.0019	-2.5166
	<i>P. latifolius/milanjanus</i>	67	212	0.0016	0.0003	0.0001	-2.5903
Ribosomal nuclear (nrDNA) 7810	<i>P. latifolius/milanjanus</i> including outgroups	82	206	0.0264	0.0053	0.0013	-2.5583
	<i>P. latifolius/milanjanus</i>	78	18	0.0023	0.0005	0.0002	-1.9823
Mitochondrial <i>nad5</i> and SSU-RNA (mtDNA) 8356	<i>P. latifolius/milanjanus</i> including outgroups	60	583	0.0413	0.0089	0.0026	-2.5084
	<i>P. latifolius/milanjanus</i>	56	35	0.0025	0.0005	0.0001	-2.5502

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