The Madeiran laurel forest endemic *Goodyera macrophylla* (Orchidaceae) is related to American orchids

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Abstract. Macaronesian laurel forests harbour many herbs and lauro-phyllous trees with Mediterranean/European or Macaronesian affinities. Traditionally, the origin of these taxa has been explained by the relict hypothesis interpreting these taxa as relics of formerly wide-spread laurel forests in the European continent and the Mediterranean. We analysed the phylogenetic relationships of the Madeira laurel forest endemic *Goodyera macrophylla* (Orchidaceae) using sequences from the nuclear ribosomal DNA Internal Transcribed Spacers (ITS) and plastid DNA regions. The results were incongruent, either the two Central American *G. brachyceras* and *G. striata* (ITS) or the North American *G. oblongifolia* (plastid DNA) were sister group to *G. macrophylla*. Nonetheless, biogeographic analyses indicated an American origin of this nemoral laurel forest plant in the two data sets. Molecular clock analyses suggest a colonisation of Madeira in the span of the upper Miocene/lower Pliocene to the Pleistocene. Although the relict hypothesis cannot be ruled out by our data when assuming extinction events on the European and northern African mainland, dispersal from Central or North America to the archipelago of Madeira is a much more likely explanation of the data.

Keywords. Biogeography, laurisilva, Macaronesia, orchids, phylogeny.

Resumen. La laurisilva de la Macaronesia alberga muchos árboles lauró-filos y hierbas con afinidades mediterráneo/europeas o macaronésicas. El origen de estos taxones se ha explicado por la hipótesis del bosque relicto que interpretaba estos taxones como relictos de bosques de laurel previa-mente extendidos en Europa y el Mediterráneo. Analizamos las relaciones filogenéticas de *Goodyera macrophylla* (Orchidaceae), especie endémica de la laurisilva de Madeira, utilizando secuencias del ADN ribosómico (ITS) y de regiones plastidiales. Los resultados fueron incongruentes: las dos especies de Centroamérica, *G. brachyceras* y *G. striata*, emergieron como grupo hermano de *G. macrophylla* en el análisis de ITS, mientras que *G. oblongifolia*, de Norteamérica, resultó hermana de *G. macrophylla* en el análisis plastidial. No obstante, los análisis biogeográficos indicaron un origen en norte america para *G. macrophylla* en los dos conjuntos de datos. Los análisis del reloj molecular sugieren una colonización de Madeira en el lapso del Mioceno superior/Plioceno inferior al Pleistoceno. Aunque nuestros datos no pueden descartar la hipótesis del bosque relic-to, cuando se asumen eventos de extinción en Europa y norte de África, la dispersión desde Centro o Norteamérica al archipiélago de Madeira es una explicación mucho más probable de los datos.

Palabras clave. Biogeografía, filogenia, laurisilva, Macaronesia, orquídeas.

INTRODUCTION

Macaronesian laurel forests (MLF) consist of trees with a laurophyll habit and occur in different species compositions on the Canary Islands, Madeira and the Azores (Kondraskov & al. 2015; Fernández-Palacios & al. 2018). On the Canary Islands and Madeira, laurel forests retain most of their water supply by NE trade winds (Prada & al. 2012; Figueira & al. 2013) and are characterised by evergreen leaves often with thick cuticles. Because of the resemblance of their laurophyllous leaves to Paleogene and Neogene fossils, this vegetation type has traditionally been regarded as an old biome and as a relict remnant of Tertiary (65–2.6 Ma) laurel forests (Hooker 1867; Fernández-Palacios & al. 2011). Using molecular divergence times, Kondraskov & al. (2015) found that many key taxa of MLF originated in the Plio-Pleistocene with only a few taxa dating back to the Miocene. This was especially surprising for MLF Laura-ceae, namely *Laurus novocanariensis* Rivas Mart., Lousã, Fern.Prieto, E.Días, J.C.Costa & C.Aguiar, *Ocotea foetens*

(Aiton) Baill., *Persea indica* (L.) Spreng. and *Apollonias barbujana* (Cav.) A.Braun, with Pleistocene and Pliocene stem node ages. Close taxonomic links to lauraceous Palaeogene and Neogene fossils attributed to morpho-taxa like *Laurus abchasica* (Kolakovsky & Shakryl) Ferguson or *Laurophyllum* Goeppert (Ferguson 1974; Kvaček & Teodoridis 2007; Worobiec 2007) were therefore not supported. Based on this evidence Kondraskov & al. (2015) interpreted MLF to have underwent a high species turn over during time and/or to be relatively newly formed. Other studies analysing *Hedera canariensis* Willd. (Valcárcel & al. 2017), *Ranunculus cortusifolius* Willd. (Williams & al. 2015), *Solanum vespertilio* Aiton, *S. trisectum* Dunal (Echeverría-Londoño & al. 2020), *Gesnouinia arborea* (L.f.) Gaudich. (Schüßler & al. 2019) corroborated evolutionary divergence times for MLF taxa from the Miocene to the Pleistocene. Recently, the finding of fossil fruits of *Melanoselinum decipiens* (Schrad. & J.C.Wendl.) Hoffm. from Madeira dating 1.3 Ma (Góis-Marques & al. 2019) is in accordance with a possible Pleistocene/Pliocene origin of this taxon (Spalik & al. 2010). As to the spatial patterns of MLF, several geographic regions have been identified as sources (Kondraskov & al. 2015). Europe served as a major source area, including the Mediterranean. MLF elements also often originated as parts of Macaronesian radiations. Minor biogeographic links with tropical Asia and the Americas were revealed.

An element of MLF with a very restricted area of distribution is the Madeira island endemic *Goodyera macrophylla* Lowe (Orchidaceae). Described by R. T. Lowe (1831), it is very rare, although locally abundant, growing in forest clearings or ravines in the stink-laurel forest (Clethro arboreae-Ocoteetum foetentis; Costa & al. 2004) between 300 and 1000 m a.s.l (Press & Short 1994; Gouveia & al. in prep.). Its preferences for habitats associated with *Ocotea foetens* dominated forests was already stated by Lowe (1831): "Hab. gregaria in declivibus sylvarum Maderae humidis. umbrosis. Rariss.". *Goodyera macrophylla* is a herb with creeping branching rhizomes which forms more or less dense clonal aggregates, with ovate to lanceolate or narrowly elliptic leaves up to 20 cm long and spikes with 25 to 80 flowers as illustrated in the original water-colour drawings by Lowe kept at Kew (Mesquita & al. 2020). Rankou (2011) evaluated *G. macrophylla* as "Critically Endangered" although with a stable population trend and called for more research concerning population size, distribution and trends.

Of the more than 200 described taxa in the genus *Goodyera* R.Br., about 100 are currently accepted species (Chen & al. 2009; POWO 2019). Schlechter (1914) originally described two sections of *Goodyera*, sect. *Otosepalum* Schltr. with reflexed outer lateral tepals and sect. *Eu-Goodyera* Schltr. with parallel outer tepals. According to the criteria

of this classification, *G. macrophylla* should belong to sect. *Eu-Goodyera*. The shape of the outer tepals, however, was found to vary and not to match clades of molecular trees (Hu & al. 2016). According to Hu & al. (2016), *Goodyera* s.l. is polyphyletic. They recognised four sections of *Goodyera*: *Otosepalum* and *Goodyera* in a different morphological circumscription compared to Schlechter (1914), *Reticulum* S.W.Chung & C.H.Ou and a still undescribed section with *G. procera* Hook. Within the sections *Otosepalum*, *Goodyera* and *Reticulum* several subsections were created based on the topology of the molecular trees. In another study by Chen & al. (2019), the *Goodyera* clade consisted of two major groups, the subclade including *Goodyera* and the subclade including *Microchilus* C.Presl. Several of these *Goodyera* s.l. taxa included in the *Microchilus* subclade have subsequently been excluded from *Goodyera* and transferred to other genera by Pace (2020). Despite these previous studies, the current understanding of both the taxonomy and of the phylogenetic relationships within the genus is far from being complete. In special, the neotropical taxa are largely underrepresented in molecular genetic analyses and the delimitation of species and infrageneric groups is hampered by convergence of morphological characters (Pace 2020). *Goodyera macrophylla* has, so far, not been analysed genetically yet and no explicit hypothesis on its systematic affinities has been published.

Goodyera in its wide definition, including the *Microchilus* subclade, is widely distributed, especially in Asia, but also in northern and Central America, Europe, and it extends to northeast Australia, South Africa, Madagascar and the southwestern Pacific islands (Chen & al. 2009; Hu & al. 2016; POWO 2019). Therefore, it seems plausible to assume that *G. macrophylla* could have originated from one of the subareas of the entire distribution of the genus. *Goodyera macrophylla* is the only member of Goodyerinae in Macaronesia. Frey & Pickering (1975) regarded it as a relict species of a former 'Atlantic [island] vegetation' without giving evidence for this statement and no other hypotheses on the biogeographic affinities of *G. macrophylla* are known. Considering the general patterns observed in the Macaronesian flora, biogeographic relationships to European, American or Asian *Goodyera* species could occur. The aim of this paper is to provide hypotheses on the phylogenetic relationships and the biogeography of *G. macrophylla* based on molecular data.

MATERIAL AND METHODS

Taxon sampling

DNA sequence data of *Goodyera macrophylla* was analysed in a phylogenetic framework including *Goodyera* and other genera of subtribe Goodyerinae (Appendix 1). To test if *G. macrophylla* is part of other groups be-

sides *Goodyera* in its wide circumscription, other genera of Goodyerinae; namely *Erythrodes* Blume, *Kreodanthus* Garay and *Aspidogyne* Garay were also included. *Pterostylis* R.Br. (Pterostylidinae; sistergroup of Goodyerinae; Givnish & al. 2015) was used as outgroup. Seventeen DNA sequences of *G. macrophylla* (Sequeira 9073, 9074, 9114, 10600), *G. repens* (L.) R.Br. (Thiv 6213) and *G. striata* Rchb.f. [García 127 (P) MNHN-P-P01019179] were newly generated in the context of this study.

Laboratory protocols and data matrices

DNA extraction, PCR and sequencing protocols followed Schüßler & al. (2019). Genetic markers were chosen following Hu & al. (2016), Chen & al. (2019) and Shin & al. (2002). Accordingly, the nuclear ITS region and the plastid *trnL* intron, *trnL-F* spacer and *mat*K coding region were used for phylogenetic reconstruction (Appendix 1). Primers were obtained from Hu & al. (2016) except for *G. striata*, for which we designed new *mat*K primers: matK 225R ACCAAAAATTTCCACAGGTT-CGT, matK 225F ACGAACCTGTGGAAATTTTTG-GT, matK 578R TCCAGATGGATGGGATGGGG, *mat*K 592F TGCTGGATCAAAGATGTTCCT, matK 1031F GGTCTCAACCTTATAGGATCCATAT, matK 1053R TGGATCCTATAAGGTTGAGACCA, matK 1356R TGAGGATCCGCTGTGATAACG, matK 1345F CGT-TATCACAGCGGATCCTCA, matK 1850R ACCGTGC-TTGCAGTTTTCAT, matK 1831F ATGAAAACTGCAA-GCACGGT. These primers yielded fragments of lengths between ca. 200 and 450 bp using 55°C as annealing temperature and 1 min. as elongation time.

For ITS and the plastid DNA markers available sequences of *Goodyera* species and related taxa (see taxon sampling) were downloaded from GenBank of the National Center for Biotechnology Information (NCBI). Identical and very similar sequences of the same taxon, often grouping together in a clade in a preliminary analysis, and doubtful, possibly incorrect determined sequences were not included. All newly generated sequences were deposited at GenBank, the corresponding accession numbers as well as a detailed list of the analysed samples are given in Appendix 1. DNA sequences were aligned using MAFFT v7.388 (Katoh & al. 2002) in Geneious 11.1.5.

Data analyses and divergence time estimation

The ITS data set was 751 bp in length and included 45 species of *Goodyera* and 13 of Goodyerinae. In the cpDNA matrix 37 species of *Goodyera* and 8 other representatives of Goodyerinae were included. For non-coding parts of the cpDNA dataset several poly-A/T, very variable, repetitive regions or unique insertions were excluded from the analysis. This concerns the positions 72–96, 245–344, 412–512,

535–544, 576–599, 623–650, 721–730, 753–759, 782–790, 819–824, 1003–1016, 1069–1077, 1124–1149, 1206–1224, 1300–1305, 1341–1350, 1374–1388, 1421–1430, 3157–3180, 3301–3310, 3314–3318 of the original dataset (3400 bp) yielding a new matrix with 2929 bp.

Several methods were applied to reconstruct the phylogenetic relationships. Maximum likelihood (ML) trees were calculated using RaxML v8 (Stamatakis 2014). Bayesian inference (BI) trees were generated using MrBayes 3.2.6 (Huelsenbeck & Ronquist 2001). We used the GTR substitution model with four Gamma categories and the shape being estimated. Four runs of 20,000,000 generations with samples taken every 2,000 generations provided Effective Sample Size values > 200 in TRACER (Rambaut & Drummond 2007).

To evaluate divergence times for *G. macrophylla* we used BEAST v2.5 (Drummond & al. 2012) on the ITS and cpDNA data set. Because these data sets consisted mostly of different species of *Goodyera,* we used the Yule model and lognormal relaxed clock. The data sets were analysed separately because the position of *G. macrophylla* differed in the two data sets (see results). Assuming equal probabilities in the 95% HPD, we applied in our analyses two uniform priors following Givnish & al. (2015) as secondary calibration points with 15.13–27.13 my to the most recent common ancestor (mrca) of *Pristiglottis* (plus other Goodyerinae genera) and all *Goodyera* species and 12.00–23.07 my to the mrca of *Goodyera, Erythrodes* and *Kreodanthus* (Figs. 1, 2). Several analyses have used fossils to date the origin of the orchid family. As example, Ramírez & al. (2007) dated the origin of the orchid family to the Cretaceous. Using a broad taxon sample, Givnish & al. (2015) also dated the orchid family to the Cretaceous. They used a total of 17 calibration points for their family dating, among them several monocot fossils which were revised by Iles & al. (2015). Moreover, they included some calibration points inferred from angiosperm phylogenies and the three fossil orchids from the Miocene belonging to *Dendrobium* Sw., *Earina* Lindl. (Conran et al. 2009) and *Meliorchis* S.R.Ramirez, Gravend., R.B.Singer, C.R.Marshall, N.E.Pierce (Ramírez & al. 2007). According to Givnish's & al. (2015) results, within Goodyerinae, the group of *Pristiglottis* Cretz. & J.J.Sm and *Goodyera* is 20.68 my (95% HPD ca. 15.13–27.13) old and the split between *Goodyera, Erythrodes* and *Kreodanthus* is dated to 17.34 my (95% HPD ca. 12–23.07).

Biogeographic analyses

For biogeographic analyses, the following areas were coded: A: Eastern and South-Eastern Asia, B: temperate-boreal Asia, C: temperate North America, D: tropical Central America, E: Pacific region, F: Madeira (Macarone-

sia) and G: Europe. In a second alternative approach, temperate North America (C) and tropical Central America (D) were combined to America.

As input, the dated maximum clade credibility (MCC) consensus trees from the BEAST analyses were used after being reduced to taxa of the *Goodyera* subclade using Mesquite 3.70 (Maddison & Maddison 2021). Different biogeographical models, i.e. dispersal-extinction-cladogenesis (DEC), Dispersal-Vicariance Analysis (DIVALIKE) and BayArea (BAYAREALIKE) were tested using the BioGeoBEARS R package (Matzke 2013a; Matzke 2013b). The inclusion of founder-event speciation (+ J) was also tested for each of these models.

Additionally, Bayesian Binary MCMC (BBM) analyses were conducted using RASP 3.2 (Ronquist & Huelsenbeck 2003; Yu & al. 2015). The maximum number of ranges was set to two and nodes supported with posterior probabilities (PP) < 0.90 were excluded from analyses. The default setting of fixed state frequencies (JC) with equal among-site variation was chosen. For the MCMC settings, the default was used.

RESULTS

Phylogenetic relationships

Identical topologies and very similar support values were obtained using MrBayes (not shown), RAxML (not shown) and BEAST (Figs. 1, 2, 3). Accordingly, the overall patterns of phylogenetic reconstructions based on nuclear and plastid DNA largely correspond to the results of Hu & al. (2016) and Chen & al. (2019). Within subtribe Goodyerinae, the *Goodyera* clade with the *Goodyera* and *Microchilus* subclades, and the *Cheirostylis* Blume clade with the *Cheirostylis* and *Ludisia* A.Rich. subclades were recovered. In all analyses, *G. macrophylla* was part of the *Goodyera* subclade, supporting its attribution to the genus *Goodyera*. It did not group with Asian or Eurasian taxa, but with two different American taxa.

For the ITS region, the BEAST analyses yielded trees with a mean log-likelihood of -5879.59 with a standard deviation of 8.95, and lower 95% HPD of -5897.17, upper 95% was HPD -5862.56. In this analysis, *Goodyera macrophylla* was sister to the Central American *G. striata*/*G. brachyceras* (A.Rich. & Galeotti) Garay & G.A.Romero with a PP of 1.00 (Fig. 1). These three taxa formed a clade together with the North American *G. oblongifolia* Raf. (PP 1.00). The ITS sequences of all four accessions of *G. macrophylla* were identical.

The plastid DNA analyses resulted in trees with a mean log-likelihood of -12766.71 with a standard deviation of 7.82, and lower 95% HPD of -12781.53, upper 95% was

HPD -12750.91. Here, *G. macrophylla* grouped together with *G. oblongifolia* (PP 1.00; Fig. 2). Uncorrected pairwise distances among accessions of *G. macrophylla* varied between 0.0002 and 0.0020.

The position of the conflicting lineage including *Goodyera striata* and *G. brachyceras* in different clades was highly supported by PP in the ITS and plastid trees. We still combined the data to follow a total evidence approach. This analysis of combined data using BEAST with the same settings as for the single data sets resulted in mean log-likelihood of -18918.65 with a standard deviation of 10.56, lower 95% HPD of -18938.95 an upper 95% was HPD -18887.11. Here, *G. macrophylla* was also sister to *G. oblongifolia*, largely corroborating the results of the plastid DNA analyses (Fig. 3).

Divergence time estimation

In the ITS data set the mrca of *Goodyera-Erythrodes-Kreodanthus* was dated to 16.75 my (mean), 16.46 my (median), 12.04–21.84 my (95% HPD), the split between *Pristiglottis* and *Goodyera* to 19.25 my (mean), 18.71 my (median), 15.13–25.02 my (95% HPD), the mrca of *G. macrophylla* and *G. oblongifolia* to 6.09 my (mean), 5.88 my (median), 2.93–9.89 my (95% HPD) and the split between *G. macrophylla* and *G. striata*/*G. brachyceras* to 4.33 my (mean), 4.13 my (median), 1.61–7.33 my (95% HPD). The crown node of *G. macrophylla* is 0.7 my (mean), 0.58 my (median), 0.04–1.7 my (95% HPD) (Fig. 1).

The divergence time calculations yielded the following ages for the plastid DNA: the mrca of *Goodyera-Erythrodes-Kreodanthus* 16.54 my (mean), 16.05 my (median), 12.17–21.79 my (95% HPD), the mrca of *Pristiglottis-Goodyera* 19.24 my (mean), 18.55 my (median), 15.13–25.04 my (95% HPD), the split between *G. macrophylla* and *G. oblongifolia* 6.65 my (mean), 6.44 my (median), 2.80-10.73 my (95% HPD), the crown node of *G. macrophylla* 0.86 my (mean), 0.79 my (median), 0.18-1.85 my (95% HPD) (Fig. 2).

The results of the combined data set yielded the following ages: for *Goodyera macrophylla* and *G. oblongifolia* 7.46 my (mean), 7.22 my (median), 3.26–12.36 my (95% HPD), the crown node of *G. macrophylla* 0.56 my (mean), 0.79 my (median), 0.11–1.16 my (95% HPD) (Fig. 3).

Biogeographic analyses with ITS data

According to the AICc criterion, DEC + J was the most likely model for all tested data sets. To infer the origin of *G. macrophylla*, the ancestral areas for the node of *G. macrophylla* and *G. striata*/*G. brachyceras* were reconstructed. DEC + J analyses yielded Madeira with a probability of 0.5 as ancestral area and Central America with 0.5 when coding
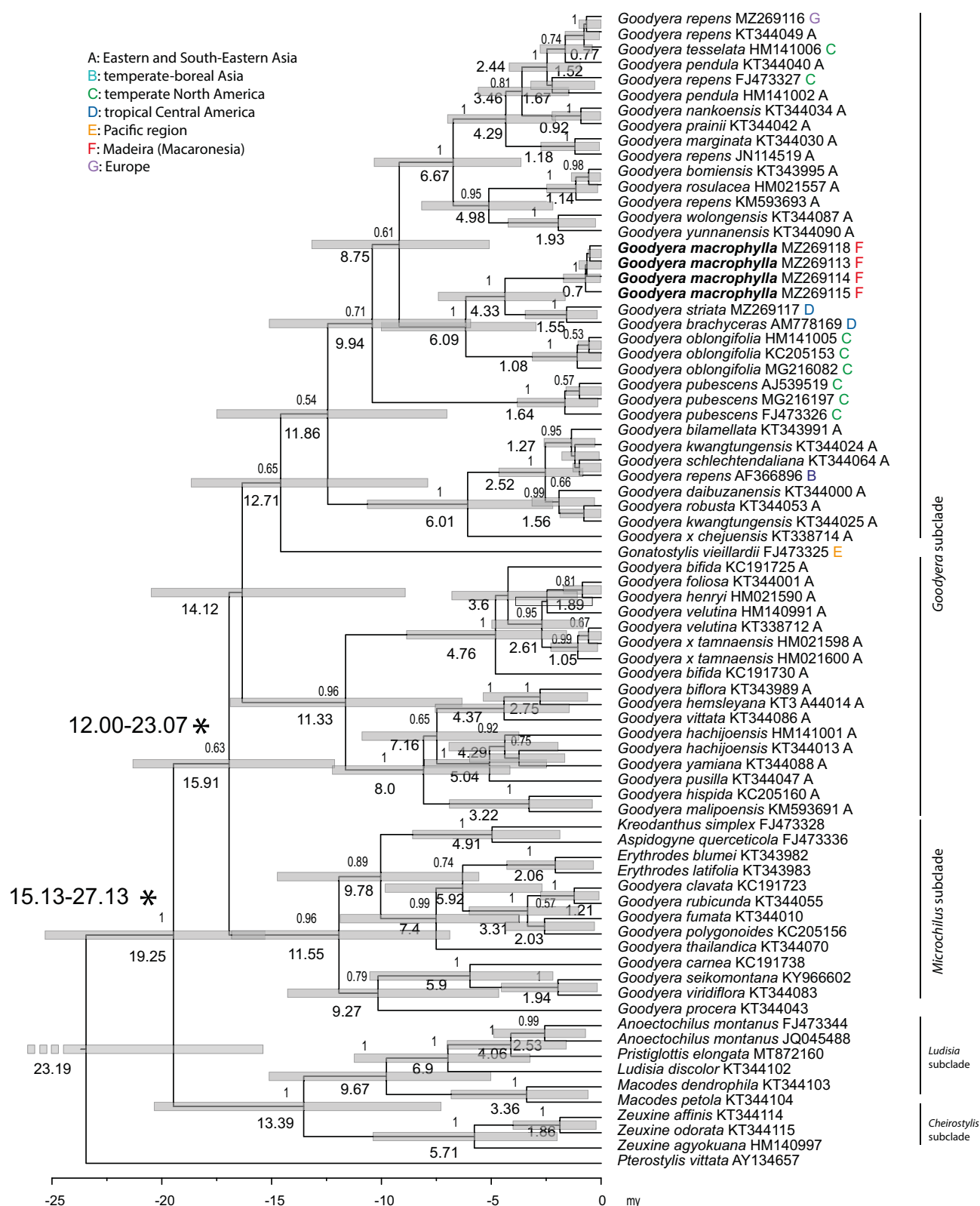
Fig. 1. Phylogenetic Maximum Clade Credibility (MCC) tree of the BEAST analyses of *Goodyera* and related taxa based on nuclear ribosomal ITS. Numbers at the branches are posterior probabilities. Genbank accession numbers are given behind the taxon names. Capital letters behind Genbank accession numbers of the *Goodyera* subclade indicate coded areas of distribution. Bars indicate 95% HPD of age estimations. Mean ages are shown below branches for most of the clades. Calibration points with used dates are given before asterisks (see text).
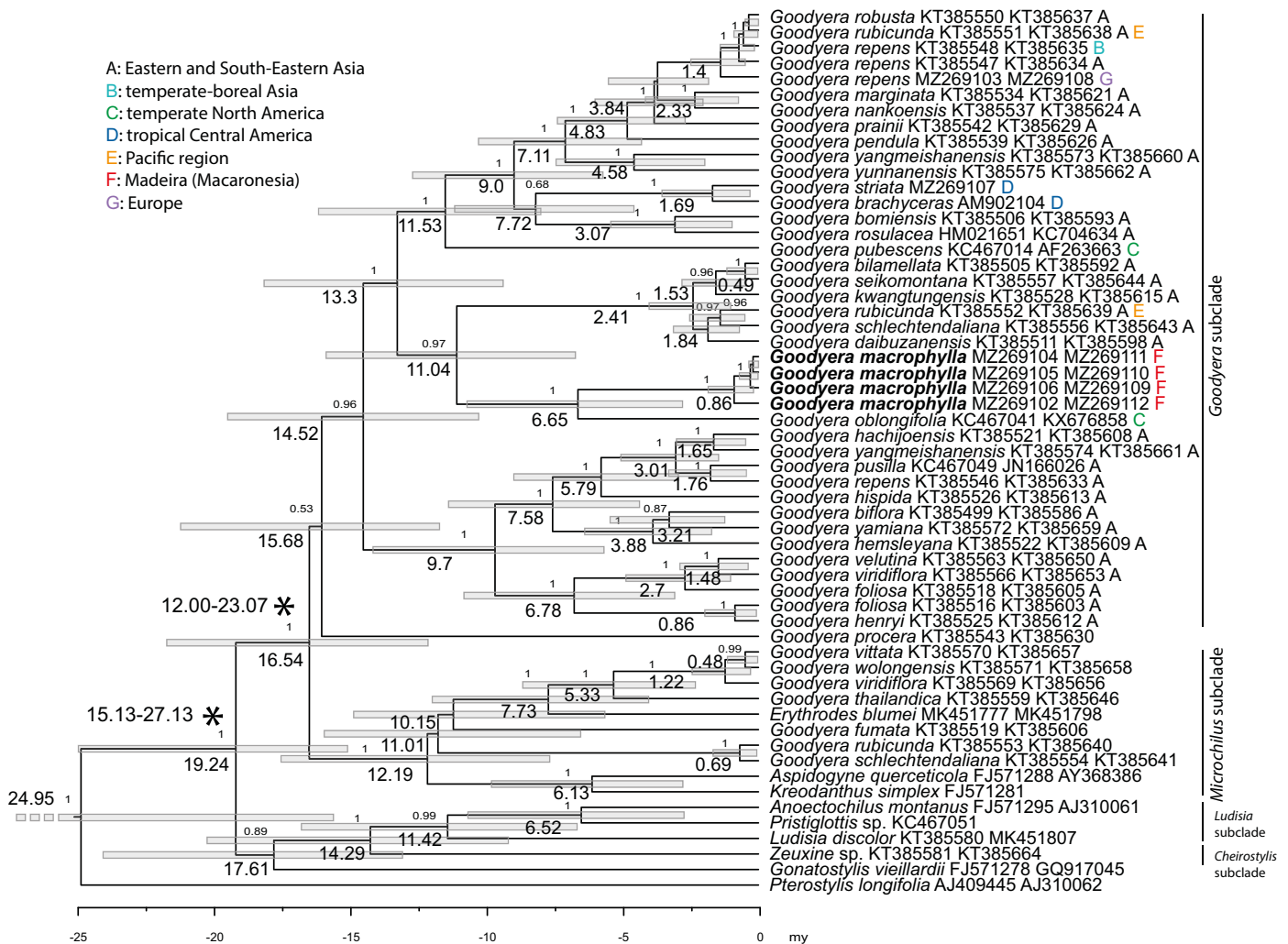
Fig. 2. Phylogenetic MCC tree of the BEAST analyses of *Goodyera* and related taxa based on plastid DNA (*trnL* intron, the *trnL-F* spacer and *matK*). Numbers at the branches are posterior probabilities. Genbank accession numbers are given behind the taxon names. Capital letters behind Genbank accession numbers of the *Goodyera* subclade indicate coded areas of distribution. Bars indicate 95% HPD of age estimations. Mean ages are shown below branches for most of the clades. Calibration points with used dates are given before asterisks (see text).

distinguished between Central and North America (Fig. 4). When only coding America, this region received a probability of 0.98 for this node. BBM analyses resulted in Central America (0.44), Madeira (0.35), North America (0.11) as ancestral areas. Alternatively, when coding only America, this continent (America, 0.93) and Madeira-America (0.07) were revealed as ancestral areas (Fig. 5).

Biogeographic analyses with the plastid data

For the node of *Goodyera macrophylla* and *G. oblongifolia*, North America had a probability of 0.54 and Madeira of 0.35 as ancestral area using DEC + J analyses. When coding America, America and Madeira had slightly higher values of 0.64 and 0.36, respectively (Fig. 6). BBM analyses yielded east/south East Asia (0.40), North America (0.29) and Madeira (0.21) for this node (Fig. 7).

DISCUSSION

Phylogenetic relationships

The position of *Goodyera macrophylla* varied in phylogenetic analyses based on ITS and cpDNA data. While the Madeiran endemic is sister to Central American *G. brachyceras* and *G. striata* with North American *G. oblongifolia* being sister to this clade in the ITS analysis, it appears as sister group to *G. oblongifolia* in the cpDNA trees. These phylogenetic relationships are supported by high PP values. Incongruence between the plastid and nuclear datasets was already observed by Hu & al. (2016). Possible explanations for this include (ancient) events of hybridisation, chloroplast capture or concerted evolution. One of these mechanisms may also account for the case of *G. macrophylla*, but further evidence like chromosome numbers or ploidy
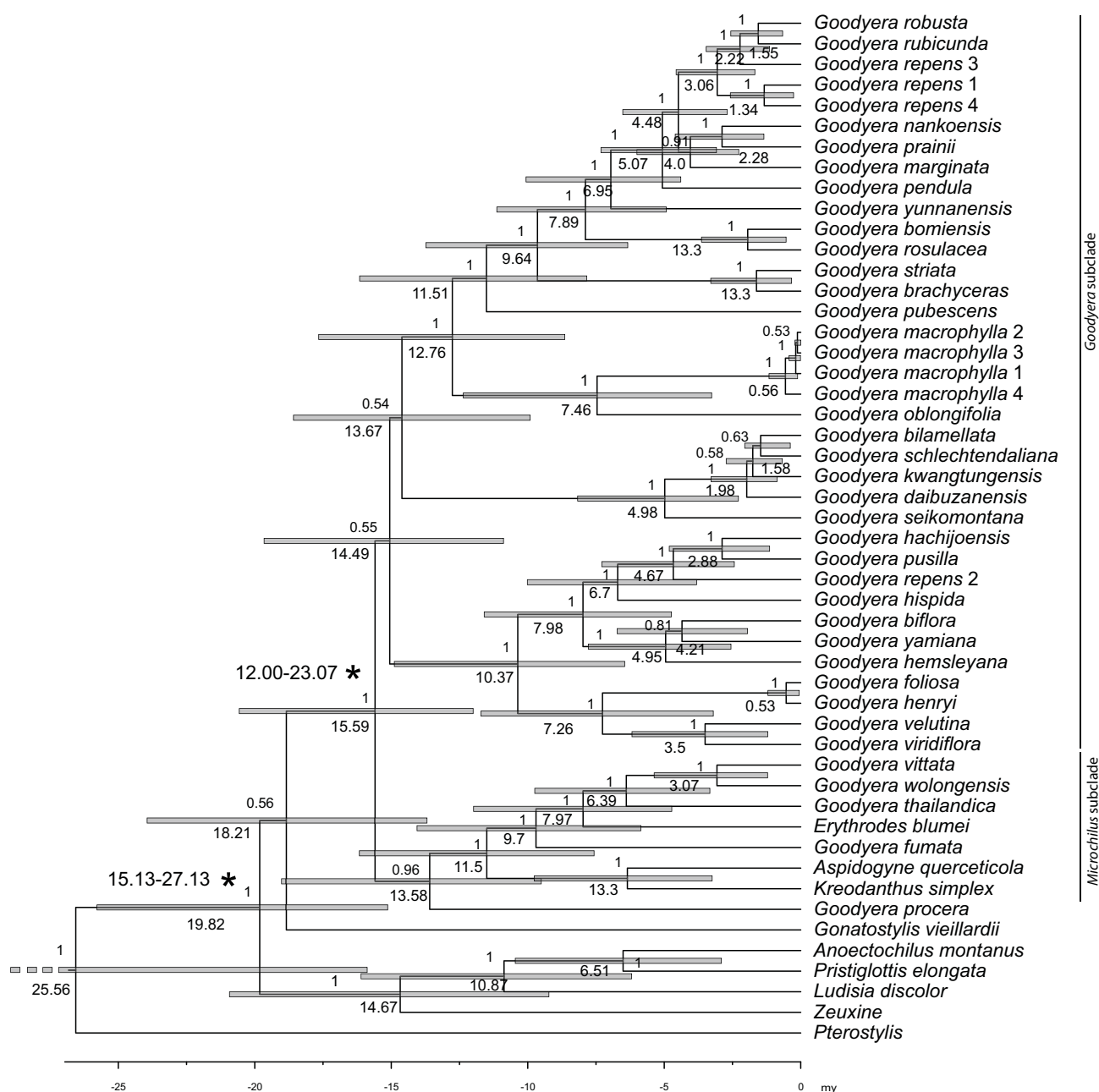
Fig. 3. Phylogenetic consensus tree of the BEAST analyses of *Goodyera* and related taxa based on combined ITS and plastid DNA (*trnL* intron, the *trnL-F* spacer and *mat*K). Numbers at the branches are posterior probabilities. Bars indicate 95% HPD of age estimations. Mean ages are shown below branches. Calibration points with used dates are given before asterisks (see text). Numbers behind taxon names refer to superindices in Appendix 1.

levels is not available. A discussion about the phylogenetic pattern of *Goodyera* is outside of the scope of this study which focuses on *G. macrophylla*. Despite the incongruence, *G. macrophylla* always appears as sister to an American group. *Goodyera macrophylla* shares rather oblong leaves with *G. striata* and *G. brachyceras* which are sometimes regarded as synonyms (Garay & Romero-González 1998; POWO 2019), and *G. oblongifolia*. Based on the present evidence, we exclude a close relationship of the Madeiran

species to Asian or central European taxa, in special to *G. repens* and hypothesise that it is part of a Central or North American clade.

The species-rich genus *Goodyera* is far from being sufficiently represented in phylogenetic analyses (Pace 2020). Although, all North American species are included in this study, several of the Central American species have not been sequenced for phylogenetic analysis yet and it
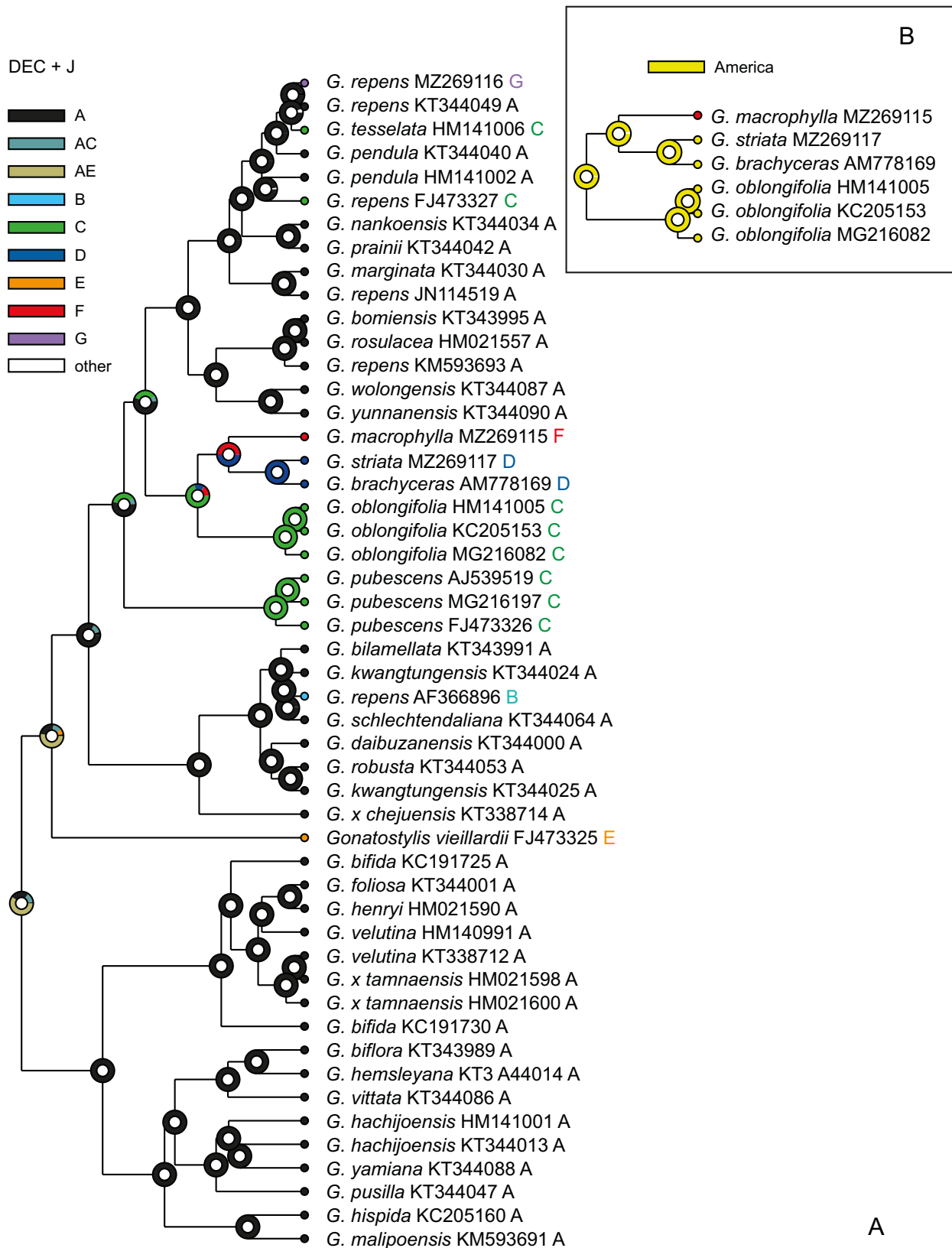
Fig. 4. Ancestral range reconstruction of *Goodyera macrophylla* and the *Goodyera* subclade based on the ITS MCC tree and the best fit DEC+J model using BioGeoBears. Single and compound areas are indicated in the chart: **a**, coded areas refer to Figure 1; **b**, reconstruction for *G. macrophylla* and closest relatives when America was coded as a single area.
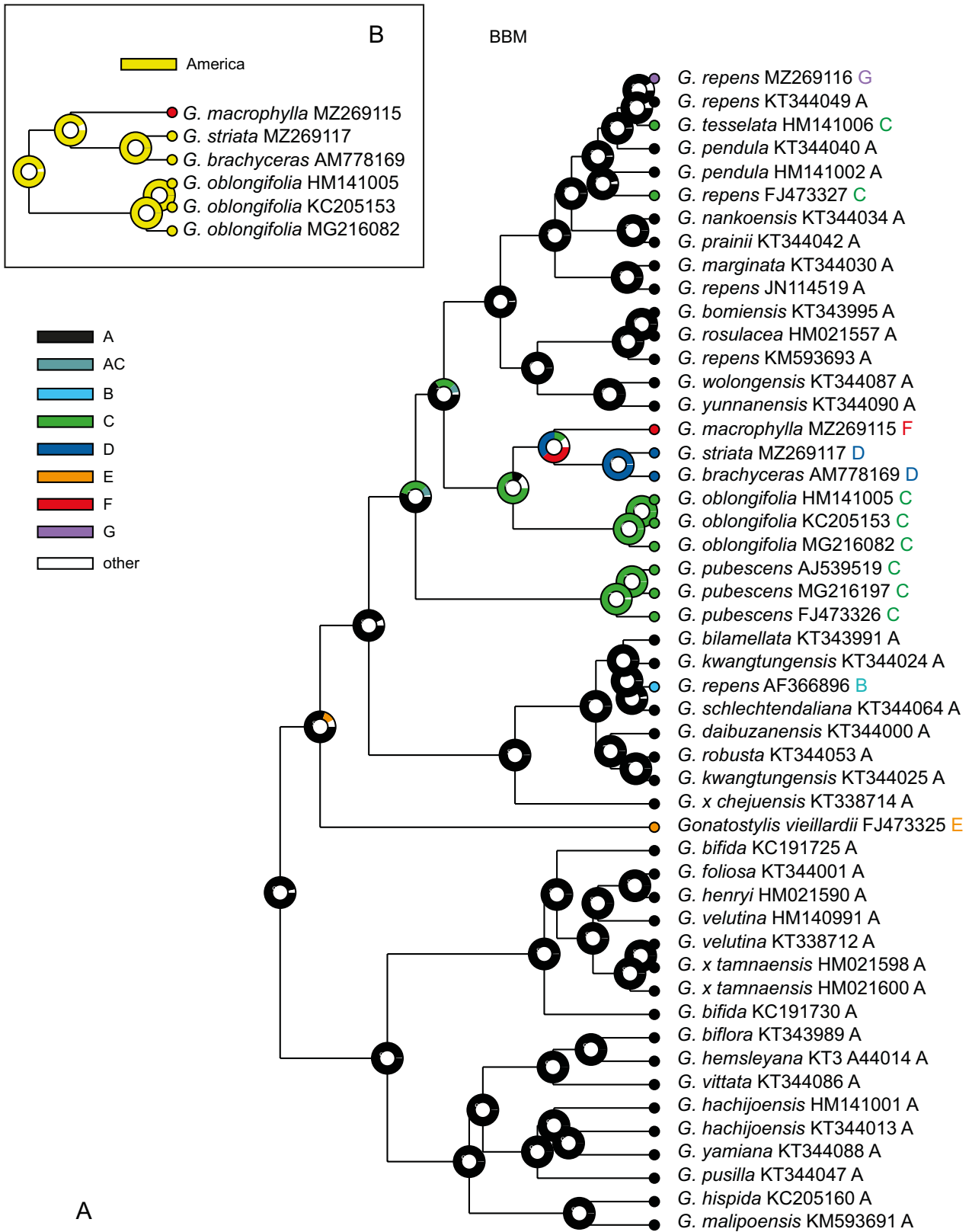
Fig. 5. Ancestral range reconstruction of *Goodyera macrophylla* and other *Goodyera* species based on Bayesian Binary MCMC (BBM) analyses of the ITS data conducted with RASP 3.2. Single and compound areas are indicated in the chart: **a**, coded areas refer to Figure 1; **b**, reconstruction for *G. macrophylla* and closest relatives when America was coded as a single area (colours refer to A, except for America).
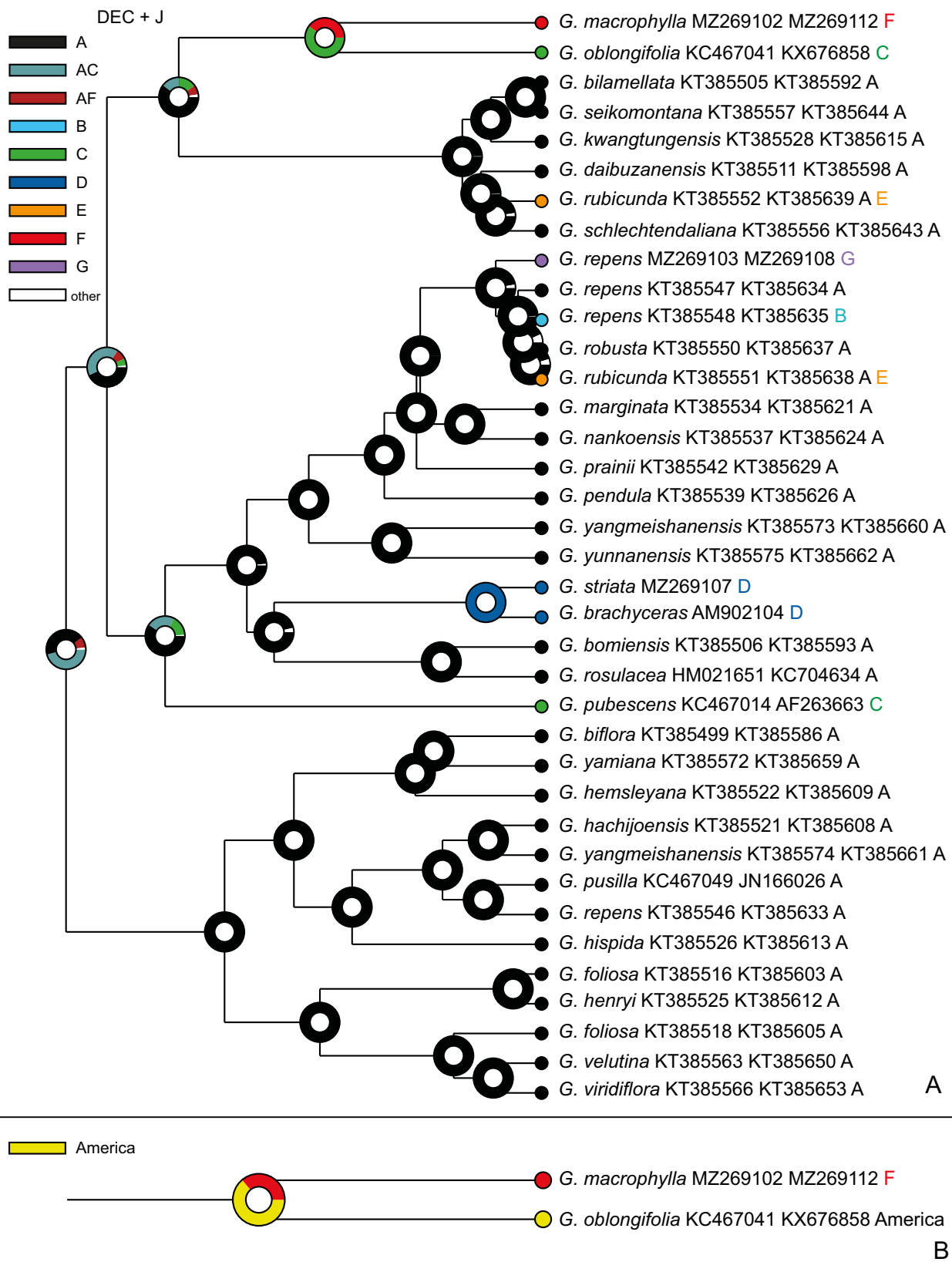
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Fig. 6. Ancestral range reconstruction of *Goodyera macrophylla* and the *Goodyera* subclade based on the plastid DNA MCC tree and the best fit DEC+J model using BioGeoBears. Single and compound areas are indicated in the chart: **a**, coded areas refer to Figure 2; **b**, reconstruction for *G. macrophylla* and its sister group when America was coded as a single area.

Fig. 7. Ancestral range reconstruction of *Goodyera macrophylla* and other *Goodyera* species based on Bayesian Binary MCMC (BBM) analyses of the plastid DNA data conducted with RASP 3.2. Single and compound areas are indicated in the chart: **a**, coded areas refer to Figure 2; **b**, reconstruction for *G. macrophylla* and its sister group when America was coded as a single area (colours refer to A, except for America).
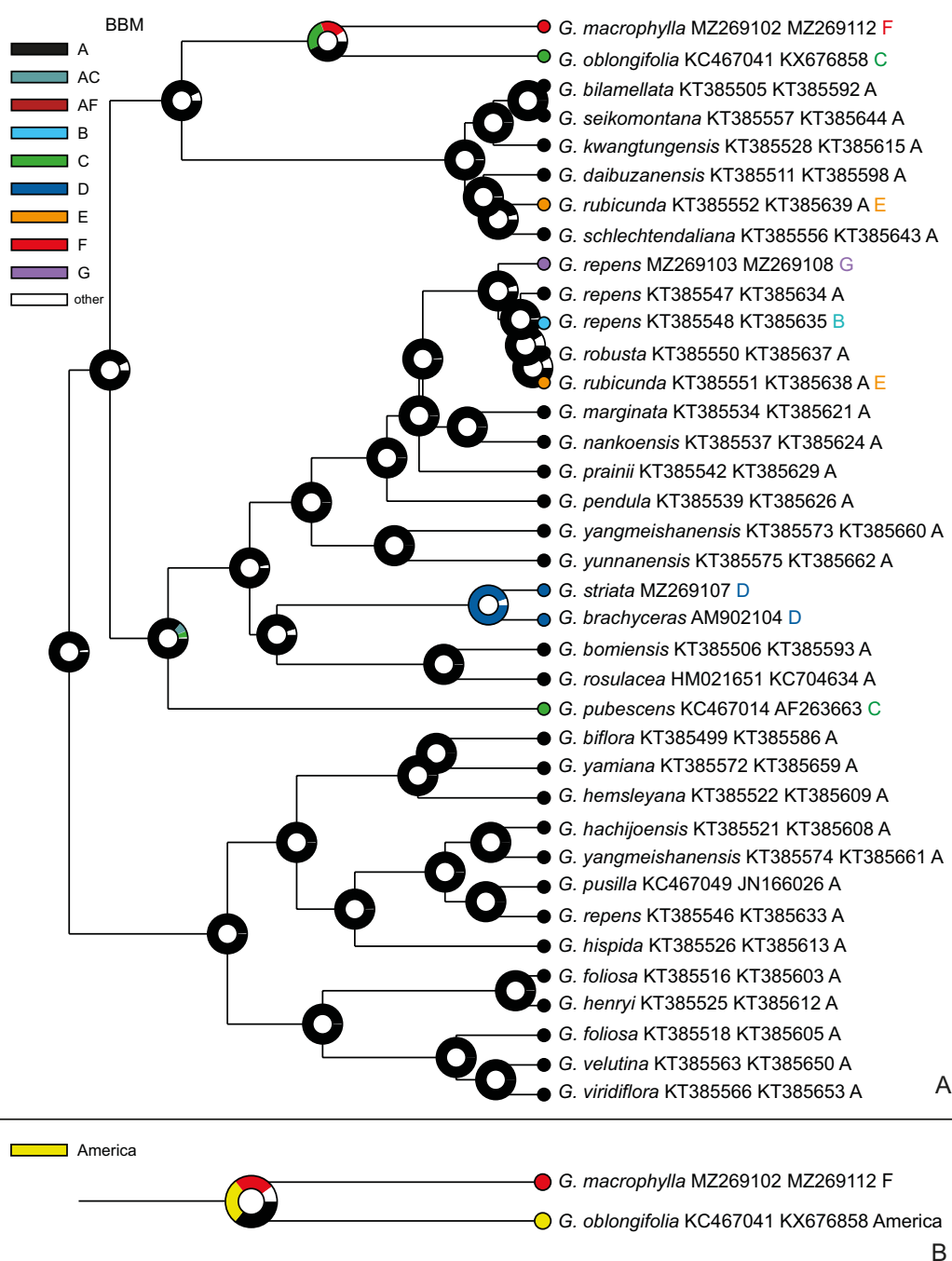
appears relatively likely that they may form at least partly geographically defined clades. Therefore, we cannot rule out that *G. macrophylla* is closest related to other Meso-American *Goodyera* species which were not included in our analysis. Among such taxa accepted by POWO (2019) are *G. bradeorum* Schltr., *G. corniculata* (Rchb.f.) Ackerman, *G. dolabripetala* (Ames) Schltr., *G. erosa* (Ames &

C.Schweinf.) Ames, F.T.Hubb. & C.Schweinf., *G. fimbrilabia* Ormerod, *G. hispaniolae* Dod, *G. major* Ames & Correll, *G. micrantha* Schltr., *G. modesta* Schltr., *G. ovatilabia* Schltr., *G. polyphylla* Ormerod, *G. purpusii* Ormerod, *G. turialbae* Schltr., *G. venusta* Schltr., *G. zacuapanensis* Ormerod. This supports Pace's (2020) view that more phylogenetic work on *Goodyera* is needed.

Geographic origin

A close putative phylogenetic relationship of *Goodyera macrophylla* to American taxa prompts the question on the origin of this Macaronesian laurel forest nemoral element. Most biogeographic analyses yielded North and or Central America as the source area for *G. macrophylla*. Given the fact that Madeira is of volcanic origin (Geldmacher & al. 2006) which requires biotic dispersal from outside of the island at a certain point, colonisation from America to Madeira seems plausible in this case. The small, light seeds of Orchidaceae are easily dispersed by wind. Another orchid illustrating the dispersal ability from North America to Atlantic islands is *Spiranthes romanzoffiana* Cham. which is found in large parts of North America and in Ireland and Great Britain. Dueck & al. (2014) discussed wind transport and exozoochory by birds as possible vectors explaining this disjunction. References to biogeographical links from America to Macaronesia are rather scarce. Kondraskov & al. (2015) found only 6% of laurel forest elements originating from America. Accordingly, *G. macrophylla* is in line with other taxa showing Macaronesian-American links like *Persea indica*, *Arbutus canariensis* Veill. ex Duhamel (Hileman & al. 2001; Kondraskov & al. 2015), *Clethra arborea* Aiton (Fior & al. 2003) and possibly *Pericallis* D. Don. (Panero & al. 1999; but see Swenson & Manns 2003). Among other orchids occurring on Madeira are the two endemics, *Orchis mascula* subsp. *scopulorum* (Summerh.) H.Sund. ex H.Kretzschmar, Eccarius & H.Dietr. and *Dactylorhiza foliosa* (Rchb.f.) Soó and the Mediterranean *Neotinea maculata* (Desf.) Stearn as well as *Gennaria diphylla* (Link) Parl. (Press & Short 1994). Phylogenetic analyses indicate affinities with the European/Mediterranean flora for the first three of these four Madeiran species (the relationships of *Gennaria diphylla* are not resolved; Bateman et al. 2003), stressing the exceptional biogeographic pattern of *Goodyera macrophylla*.

Estimated age

Our divergence time estimations yielded ca. 4 (1.3-7.2) my and 6.3 (2.45-10.63) my for the stem node of *Goodyera macrophylla*, respectively in the ITS and cpDNA datasets. This discrepancy is likely due to the different phylogenetic topologies which are discussed above. Still, these molecular clock calculations suggest that *G. macrophylla* earliest originated in the Upper Pliocene or Lower Miocene.

These dates could still be adjusted if any of the so far unsampled *Goodyera* species would appear as the closest relative of the Madeiran endemic. These cases could lead to a younger stem node age of *G. macrophylla*. Other MLF elements of presumably similar stem node age are *Heberdenia excelsa* (Aiton) DC., *Picconia excelsa* (Aiton) DC., *Bystropogon* L'Hér. sect. *Canariense* La-Serna, and the *Pericallis hansenii* (G.Kunkel) Sunding clade (Kondraskov &

al. 2015; Schüßler 2020). Still, the majority of investigated MLF plants is younger than *Goodyera*. Using a different interpretation of divergence times, e.g., favouring crown nodes ages as indicator for colonisation times (García-Verdugo & al. 2019), a much younger colonisation in the Pleistocene indicated by the crown node of *G. macrophylla* (0.04–1.85 my) would indeed be possible. In the end, stem and crown node ages represent the temporal span in which island colonisation happened. In our case this includes the Upper Miocene, Pliocene and Pleistocene. Fossils of the São Jorge flora on Madeira (Góis-Marques & al. 2017) show that suitable conditions for laurel forests, in special the stink-laurel temperate forest association sensu Capelo & al. (2005) existed at least since 1.8 my ago. The variation of infraspecific cpDNA is in line with crown node ages, suggesting that *G. macrophylla* diversified on Madeira since the Pleistocene. This is also of interest for conservation purposes. Still, the level of clonality within populations is still unknown and under current investigation (Gouveia & al. in prep.).

Considering its sister group relationship to American taxa, it seems obvious that the Madeiran species does not fulfil the criteria for the relict hypothesis of Macaronesian laurel forests (Kondraskov & al. 2015). Originally, this hypothesis relates to Central European and Mediterranean laurel forests, which went extinct at the latest by the end of the Pliocene (Kondraskov & al. 2015). A sister group relationship to American taxa contradicts this assumption. Theoretically, the relict hypothesis could only be upheld, if extinction of more closely related continental European *Goodyera* taxa is assumed. However, since there is no evidence for this, we refrain from such explanation. Frey & Pickering's (1975) hypothesis that *G. macrophylla* is a relict species of a former Atlantic vegetation, is not supported by our data. Under this scenario, closest relatives of *G. macrophylla* should still be present in laurel forests of the Canary Islands or the Azores, which is not the case. Only the assumption of extinction events in the Atlantic archipelagos could then uphold this hypothesis. This is theoretically possible, but in our view rather unlikely. Based on the available data, we hypothesise that *G. macrophylla* colonised the Madeiran laurel forest earliest in the lower Miocene/upper Pliocene from America.

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Appendix 1. Genbank accessions of sequences of *Goodyera* and related lineages included in the phylogenetic analysis. Items are separated by semicolon in this order for each taxon: taxon name; voucher; ITS; *trnL-F*; and *matK* Genbank accession/s numbers. Voucher information is only given for the newly generated sequences. Missing data: –. An asterisk indicates sequences used in the combined analysis; a superindex indicates the accession of the taxon in figure 3 (analysis of combined data sets).

Anoectochilus montanus Schltr.; –; FJ473344, JQ045488*; FJ571295*; AJ310061*. *Aspidogyne querceticola* (Lindl.) Meneguzzo; –; FJ473336*; FJ571288*; AY368386*. *Erythrodes blumei* (Lindl.) Schltr.; –; KT343982*; MK451777*; MK451798*. *Erythrodes latifolia* Blume; –; KT343983; –; –. *Gonatostylis vieillardii* (Rchb.f.) Schltr; –; FJ473325*; FJ571278*; GQ917045*. *Goodyera bifida* (Blume) Blume; –; KC191725, KC191730; –; –. *Goodyera biflora* (Lindl.) Hook.f.; –; KT343989*; KT385499 *; KT385586*. *Goodyera bilamellata* Hayata; –; KT343991*; KT385505*; KT385592*. *Goodyera bomiensis* K.Y.Lang; –; KT343995*; KT385506*; KT385593*. *Goodyera brachyceras* (A.Rich. & Galeotti) Garay & G.A.Romero; –; AM778169*; –; AM902104*. *Goodyera carnea* A.Rich.; –; KC191738; –; –. *Goodyera clavata* N.Pearce & P.J.Cribb; –; KC191723; –; –. *Goodyera daibuzanensis* Yamam.; –; KT344000*; KT385511*; KT385598*. *Goodyera foliosa* (Lindl.) Benth. Ex C.B.Clarke; –; KT344001*; KT385516*, KT385518; KT385603*, KT385605 . *Goodyera fumata* Thwaites; –; KT344010*; KT385519*; KT385606*. *Goodyera hachijoensis* Yatabe; –; HM141001, KT344013*; KT385521*; KT385608*. *Goodyera hemsleyana* King & Pantl.; –; KT344014*; KT385522*; KT385609*. *Goodyera henryi* Rolfe; –; HM021590*; KT385525*; KT385612*. *Goodyera hispida* Lindl.; –; KC205160*; KT385526*; KT385613*. *Goodyera kwangtungensis* C.L.Tso; –; KT344024, KT344025*; KT385528*; KT385615*. *Goodyera macrophylla* Lowe; Portugal, Madeira, Sequeira 9073 (UMAD); MZ269118*[1]; MZ269106*[1]; MZ269109*[1]. *Goodyera macrophylla* Lowe; Portugal, Madeira, Sequeira 9074 (UMAD); MZ269113*[2]; MZ269104*[2]; MZ269111*[2]. *Goodyera macrophylla* Lowe; Portugal, Madeira, Sequeira 9114 (UMAD); MZ269114*[3]; MZ269105*[3]; MZ269110*[3]. *Goodyera macrophylla* Lowe; Portugal, Madeira, Sequeira 10600 (UMAD); MZ269115*[4]; MZ269102*[4]; MZ269112*[4]. *Goodyera malipoensis* Q.X.Guan & S.P.Chen; –; KM593691; –; –. *Goodyera marginata* Lindl.; –; KT344030*; KT385534*; KT385621*. *Goodyera nankoensis* Fukuy.; –; KT344034*; KT385537*; KT385624*. *Goodyera oblongifolia* Raf.; –; HM141005, KC205153*, MG216082; KC467041*; KX676858*. *Goodyera pendula* Maxim.; –; HM141002, KT344040*; KT385539*; KT385626*. *Goodyera polygonoides* F.Muell.; –; KC205156; –; –. *Goodyera prainii* Hook.f.; –; KT344042*; KT385542*; KT385629*. *Goodyera procera* (Ker Gawl.) Hook.; –; KT344043*; KT385543*; KT385630*. *Goodyera pubescens* (Willd.) R.Br.; –; AJ539519*, FJ473326, MG216197; KC467014*; AF263663*. *Goodyera pusilla* Blume; –; KT344047*; KC467049*; JN166026*. *Goodyera repens* (L.) R.Br.;

Germany, Baden-Württemberg, Eglingen, leg. Thiv 6213 (STU); MZ269116*[1]; MZ269103*[1]; MZ269108*[1]. ***Goodyera repens*** (L.) R.Br.; –; AF366896, FJ473327, JN114519*[2], KM593693*[3], KT344049*[4]; KT385546*[2], KT385547*[3], KT385548*[4]; KT385633*[2], KT385634*[3], KT385635*[4]. ***Goodyera robusta*** Hook.f.; –; KT344053; KT385550; KT385637. ***Goodyera rosulacea*** Y.N.Lee; –; HM021557; HM021651; KC704634. ***Goodyera rubicunda*** (Blume) Lindl.; –; KT344055*; KT385551*, KT385552, KT385553; KT385638*, KT385639, KT385640. ***Goodyera schlechtendaliana*** Rchb.f.; –; KT344064*; KT385554*, KT385556; KT385641*, KT385643. ***Goodyera seikoomontana*** Yamam.; –; KY966602*; KT385557*; KT385644*. ***Goodyera striata*** Rchb.f.; Mexico, leg. Carrada García 127 (P) MNHN-P-P01019179; MZ269117*; –; MZ269107*. ***Goodyera tesselata*** Lodd.; –; HM141006; –; –. ***Goodyera thailandica*** Seidenf.; –; KT344070*; KT385559*; KT385646*. ***Goodyera velutina*** Maxim. ex Regel; –; HM140991, KT338712*; KT385563*; KT385650*. ***Goodyera viridiflora*** (Blume) Blume; –; KT344083*; KT385566*, KT385569; KT385653*, KT385656. ***Goodyera vittata*** (Lindl.) Benth. ex Hook.f.; –; KT344086*; KT385570*; KT385657*. ***Goodyera wolongensis*** K.Y.Lang; –; KT344087*; KT385571*; KT385658*. ***Goodyera yamiana*** Fukuy.; –; KT344088*; KT385572*; KT385659*. ***Goodyera yunnanensis*** Schltr.; –; KT344090*; KT385575*; KT385662*. ***Goodyera x chejuensis*** S.Kim ex C.S.Lee & N.S.Lee; –; KT338714; –; –. ***Goodyera x tamnaensis*** C.S.Lee, S.H.Yeau, K.S.Lee, N.S.Lee; –; HM021598, HM021600; –; –. ***Kreodanthus simplex*** (C.Schweinf.) Garay; –; FJ473328*; FJ571281*; –. ***Ludisia discolor*** (Ker Gawl.) A.Rich.; –; KT344102*; KT385580*; MK451807*. ***Macodes dendrophila*** Schltr.; –; KT344103; –; –. ***Macodes petola*** (Blume) Lindl.; –; KT344104; –; –. ***Pristiglottis elongata*** (Blume) Cretz. & J.J.Sm.; –; MT872160*; –; –. ***Pristiglottis*** spec.; –; –; KC467051; –. ***Pterostylis longifolia*** R.Br.; –; –; AJ409445*; AJ310062*. ***Pterostylis vittata*** Lindl.; –; AY134657*; –; –. ***Zeuxine affinis*** (Lindl.) Benth. ex Hook.f.; –; KT344114*; –; –. ***Zeuxine agyokuana*** Fukuy.; –; HM140997; –; –. ***Zeuxine odorata*** Fukuy.; –; KT344115; –; –. ***Zeuxine*** sp.; –; –; KT385581*; KT385664*.