Phylogeny and Biogeography of *Artemisia* subgenus *Seriphidium* (Asteraceae, Anthemideae)

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Abstract Subgenus *Seriphidium* is one of the largest groups within *Artemisia*, encompassing more than one hundred species, some of them having considerable ecological and economical importance. However, the evolution of subg. *Seriphidium* has received less attention in comparison to other subgenera of *Artemisia*, probably, apart from the difficulty of sampling throughout its very large distribution area, because of the low molecular and morphological variability observed in previous studies. Here, we use thorough taxonomic sampling within both *Artemisia* and subg. *Seriphidium* to reconstruct the evolutionary history of the subgenus, employing nuclear and plastid DNA sequences as well as various phylogenetic, biogeographic and diversification dynamics tools to analyse the data. Our results show that subg. *Seriphidium* is not monophyletic, but segregated into two main clades: one large monophyletic group corresponding to the formerly recognised sect. *Seriphidium* and a second, small clade, phylogenetically distant from the first. Biogeographic and diversification analyses indicate that a rapid radiation of species within sect. *Seriphidium* occurred in Central Asia during the Miocene-Pliocene transition. The results of our biogeographic analysis suggest that this diversification process started around the Tian-Shan, Pamir and Hindu Kush mountain ranges,

subsequently expanding into the Eurasian continent. Finally, we uncovered numerous incongruences between taxonomic and genetic information in several *Seriphidium* species, which could be explained by morphological uniformity, hybridisation and/or incomplete lineage sorting processes.

Keywords biogeography; Compositae; cpDNA; diversification; molecular phylogenetics; nrDNA.

Short title Phylogeny and biogeography of *Artemisia* subgenus *Seriphidium*

Introduction

The genus *Artemisia* L. is the largest of its subtribe (Artemisiinae Less.) and tribe (Anthemideae Cass.), as well as one of the largest in the Asteraceae, which in turn is the largest family of plants (Funk & al., 2009). There is not yet a universal agreement on the number of taxa *Artemisia* contains: 300-500 specific and infraspecific taxa (Bremer & Humphries, 1993; Vallès & McArthur, 2001; Vallès & Garnatje, 2005), 346 species and 69 varieties (Ling, 1994), 388 species (Boulos, 2002), 400 species (Mabberley, 2008) or 522 species (Oberprieler & al., 2009). The genus contains all life forms except trees: annual, biennial, perennial herbs, subshrubs and shrubs, some large in stature. It is also well known for the relevant antimalarial artemisinin and other pharmacological and economic aspects (Wright, 2002; Ferreira & al., 2005; Vallès & al., 2011).

Artemisia is present, with many landscape-dominating species, on every continent except Antarctica, where no representatives of Asteraceae grow (Funk & al., 2005; Vallès & al., 2011). Its predominant distribution area comprises the Northern Hemisphere, mainly the Old World, with a great centre of diversification in temperate Asia, also reaching the New World, and with rare occurrences in the Southern Hemisphere (Bremer, 1994; Vallès & McArthur, 2001; Vallès & al., 2011). The current distribution of this genus, most diverse in China and surrounding areas followed by Russia and adjacent states, Europe, USA and North Africa (Poljakov, 1961b; Tutin & al., 1976; Ling, 1991; Bremer & Humphries, 1993; McArthur & Sanderson, 1999), is the result of periods of glaciation, long and short distance dispersal and vicariance (e.g. Tkach & al. 2008a;

Hobbs & Baldwin, 2013). The oldest fossil pollen records belonging to *Artemisia* are from the end of the Eocene and Late Oligocene (Zaklinskaja, 1957; Zhu & al., 1985; Wang, 2004; Miao & al., 2011), and its early Miocene expansion in Central Asia (Wang, 2004). Colonisation processes in the late Miocene and Pliocene and differentiation in the Pleistocene (Sanz & al., 2011) led to the current situation of two major centres (Eurasia and North America) of diversity (Riggins & Seigler, 2012).

The relevance of *Artemisia* both in terms of species diversity and economic importance contributed to the broad scientific interest in the genus (Vallès & al., 2011 and references therein). However, the gaps that remain in our knowledge of the genus and of its taxonomic complexity still call for further research. In the case of infrageneric classification, rearrangements have been frequently made to untangle the existing taxonomic knot, resulting in the present day structure of main groups of *Artemisia*. Even though there are some conflicts between classical and molecular datasets, *Artemisia* has been classically divided into the generally accepted five subgenera *Artemisia*, *Absinthium* (Miller) Less., *Dracunculus* (Besser) Rydb., *Seriphidium* Besser ex Less. and *Tridentatae* (Rydb.) McArthur (Torrell & al., 1999, and references therein), to which one more, *Pacifica* Hobbs & Baldwin, has been added recently (Hobbs & Baldwin, 2013).

Subgenus *Seriphidium* constitutes one of the most diverse groups of *Artemisia* from a morphological perspective. Considering floral (disc and ray flower occurrence and fertility) and receptacle (presence of hairs) characters, Besser (1829, 1832, 1834, 1835) grouped all homogamous species of *Artemisia* (comprising Old World and New World species) in sect. *Seriphidium* Besser, raised to subgenus rank by Rouy (1903). The species-poor (around 13 species) North American *Seriphidium*, first considered sect. *Tridentatae* Rydb. in the subgenus (Rydberg, 1916), were raised to an independent subgenus considering their geographical distribution, growth habit, and karyotypic and chemotaxonomic attributes (McArthur & al., 1981). The homogamous *Artemisia* species in Eurasia were segregated from the rest of the genus by Poljakov (1961a), who established the new genus *Seriphidium* (Besser) Poljakov. Nevertheless, the same author did not follow his own proposal in a flora published the same year (Poljakov, 1961b), in which *Seriphidium* appears within *Artemisia*. Several authors, among them Ling (1982, 1995), Bremer & Humphries (1993), Bremer (1994), Dobignard (1997) and Ling & al. (2006), followed Poljakov (1961a), additionally including the members of *Tridentatae* in

the genus *Seriphidium*. Various authors had suggested, based on morphological, karyological or chemical characters, the independence and the convergent evolution of *Seriphidium* and *Tridentatate* and even a closer relationship between *Tridentatae* and subg. *Artemisia* than between *Tridentatae* and subg. *Seriphidium* (McArthur & Plummer 1978; McArthur & Pope, 1979; McArthur & al., 1981, 1998; Seaman, 1982; Shultz, 1986; Jeffrey, 1995). Later, molecular data confirmed the monophyly of subg. *Tridentatae* (McArthur & al., 1998; Kornkven & al., 1998, 1999; Torrell & al., 1999, Tkach & al. 2008a, b; Garcia & al., 2011) and clearly supported the independence of the North American complex of *Tridentatae* from *Seriphidium*. Concerning the generic or subgeneric status of *Seriphidium*, molecular work (Torrell & al., 1999; Watson & al. 2002; Vallès & al., 2003; Watson, 2005; Tkach & al., 2008a, b; Sanz & al., 2008, 2011; Shultz, 2009; Garcia & al., 2011 and Riggins & Seigler, 2012) unequivocally showed the inclusion of *Seriphidium* within *Artemisia*. Two comprehensive studies, seminal for Asteraceae (Kubitzki, 2007; Funk & al., 2009), have accredited *Seriphidium* as a subgenus within *Artemisia*, even if some authors (Dobignard & Chatelain, 2011; Haghighi & al., 2014) still persist in presenting *Seriphidium* as an independent genus, a solution not supported by the current knowledge..

Subgenus *Seriphidium* comprises approximately 130 species with 30 infraspecific taxa worldwide (Bremer & Humphries, 1993; Ling, 1994), distributed mainly in arid regions of Central, Southwest and western Asia, the Middle East, North Africa and Europe. Species of the subgenus are shrubs, subshrubs or perennial (very rarely annual, such as *A. leucodes* Schrenk) herbs, growing mostly in temperate climates usually in arid or semi-arid habitats, where they often dominate the landscape across wide areas. The taxonomic complexity of the subgenus has led to the description of many taxa considered synonyms of existing species by some authors (see The Plant List, http://www.theplantlist.org, where 173 *Seriphidium* names are listed, with, e.g., 15 synonyms for *A. santonicum* L.). The taxonomic subdivision of subg. *Seriphidium* illustrates the taxonomic complexity. Poljakov (1961b), Filatova (1986) and Ling (1991) recognised two, six and three sections, respectively (see Table 1). In addition, *A. annua* L. (an annual species classically placed in subg. *Artemisia*) appears, along with *A. persica* Boiss. (belonging to subg. *Absinthium*), as the sister group of subg. *Seriphidium* in various molecular studies (Watson & al., 2002; Sanz & al., 2008), whereas the annual *A. leucodes*

(belonging to subg. *Seriphidium*) appears outside the subgenus in molecular phylogenies (Vallès & al., 2003).

Diverse and comprehensive molecular studies have been conducted on *Artemisia*, some of them concerning the whole genus (and related genera) and some others focused on particular subgenera, *Absinthium*, *Dracunculus* and *Tridentatae* (Vallès & al., 2011 and references therein; Riggins & Seigler, 2012). Conversely, subg. *Seriphidium* has received comparatively little attention in this respect, probably due to the strong homogeneity of DNA sequences in the regions studied to date, which limited the resolution of phylogenetic reconstructions (Vallès & al., 2011). Indeed, previous studies indicated that subg. *Seriphidium* is the youngest assemblage in *Artemisia*, which explains the morphological, karyological and DNA sequence homogeneity of the subgenus (Torrell & al., 2003; Sanz & al., 2008; 2011). Due to its very large distribution area, taxonomic sampling of *Seriphidium* in earlier molecular studies has also been considerably restricted, additionally limiting the possibility of unravelling the evolutionary history of this important subgenus.

In the present study, we aim to gain deeper insight into the evolution of *Seriphidium* by conducting molecular systematic and biogeographic analyses covering a substantial number of taxa of the subgenus. The main objectives of the present study are: 1) To provide a more robust molecular phylogenetic reconstruction, applying the latest methods to reconstruct biogeographic and diversification patterns in *Artemisia*, with particular attention to subg. *Seriphidium*, 2) to use different chloroplast and nuclear regions to study the systematic structure within *Seriphidium*, evaluating the monophyly of the subgenus, and 3) to test whether the large diversity of *Seriphidium* is the result of a shift in the diversification rate of the group, investigating also the biogeographic context that could be related to this radiation process.

Materials and Methods

Taxon sampling.– In order to establish a solid evolutionary framework in which to circumscribe subg. *Seriphidium*, we performed the most comprehensive phylogenetic reconstruction of *Artemisia* to date. Specifically, previously published ITS and 3′-ETS sequences of 269 taxa representing all subgenera of *Artemisia* were retrieved from

Genbank. Information on subgeneric assignment, GenBank accession numbers and the original publication source of these sequences can be found in the Appendix 1 of the Electronic Supplement. Furthermore, nine additional *Seriphidium* species were newly sequenced for ITS and 3'-ETS regions, making a total of 45 species of the subgenus with sequences for those nuclear markers. Overall, the *Artemisia* dataset based on nrDNA comprised 263 species (277 taxa) of the genus plus three outgroup species [i.e. *Brachanthemum titovii* Krasch.; *Lepidolopsis turkestanica* (Regel & Schmalh.) Poljakov; *Tanacetum parthenium* (L.) Sch.Bip.]. These outgroup species were selected because their close systematic affinities to *Artemisia* (Funk & al. 2009), as well as the availability of sequences in Genbank.

To study the evolutionary relationships within subg. *Seriphidium* more deeply, 92 species (97 taxa) of S*eriphidium* representing the entire geographical distribution of this group were sequenced for two fast-evolving chloroplast DNA regions. For several taxa with large distribution areas, multiple samples from distant locations were included. In total, we analysed 123 specimens for subg. *Seriphidium*, plus 12 additional *Artemisia* species representing the major systematic subdivisions of the genus according to previous phylogenetic studies (e.g. Vallès & al., 2011; Riggins & Seigler, 2012). Despite the main aim of the cpDNA dataset was to study the evolution within subg. *Seriphidium*, these further representatives of *Artemisia* were included to explore as well the phylogenetic relationship between subg. *Seriphidium* and the rest of the genus, based on data from an alternative genome compartment (i.e. nuclear vs plastid DNA data). *Chrysanthemum indicum* L. was employed here as outgroup species because of the availability of plastid DNA sequences in Genbank and its close phylogenetic relationship to *Artemisia* (Funk & al., 2009). Most of the samples of subg. *Seriphidium* were obtained from the herbaria of BCN, BC, LE, W and E, and several were collected on field trips in Sardinia (Italy), Spain, Pakistan, and Switzerland. The voucher specimens collected from Pakistan were deposited in the herbarium of the Quaid-I-Azam University, Islamabad (ISL), while Sardinian, Spanish and Swiss specimens were deposited in the herbarium of the Centre de Documentació de Biodiversitat Vegetal, Universitat de Barcelona (BCN). The plastid sequences of *Artemisia* representatives were sequenced from voucher specimens deposited in BCN herbarium (i.e. *A. annua* and *A. absinthium* L.) or obtained from Genbank (i.e. the rest of them). Further details of the *Seriphidium* and *Artemisia* samples employed for the

analyses based on cpDNA data, including taxonomic determination, voucher codes, and collection information can be found in Appendix 2 (Electr. Suppl.).

DNA extraction, PCR amplification, and sequencing.– The CTAB method (Doyle & Doyle, 1987) with modifications (Soltis & al., 1991; Cullings, 1992) was used to extract total genomic DNA from silica-dried material derived from fresh plant tissue and herbarium samples. Amplification of ITS and ETS for the newly added *Seriphidium* samples was performed as described in Pellicer & al. (2011). Considering the low variability among species of the subgenus observed in previous studies, a pilot study comprising 11 fast-evolving nuclear and plastid markers was carried out (see Table S1 in the Electronic Supplement for additional information). The most variable regions within *Seriphidium*, the chloroplast intergenic regions *rpl*32-*trn*L and *ndh*C-*trn*V, were chosen for further sequencing for the analysis of relationships within the subgenus. For old herbarium material, internal primers for selected plastid regions were designed *de novo* to deal with degraded DNA (see Table S1). Direct sequencing of the amplified DNA segments was performed with Big Dye Terminator Cycle Sequencing v3.1 (PE Biosystems, Foster City, California, USA) at the Unitat de Genòmica, Centres Científics i Tecnològics, Universitat de Barcelona (CCiTUB) on an ABI PRISM 3700 DNA analyser (PE Biosystems, Foster City, California, USA). The sequencing primers used were the same as the amplification primers. Sequences were edited, assembled and aligned manually using Chromas Lite v.2.01 (Technelysium PTy, Tewantin, Queensland, Australia) and Bioedit v.7.0.9 (Ibis Biosciences, Carlsbad, California, USA). Genbank accession numbers are given in Appendices 1 and 2 of the Electronic Supplement.

Phylogenetic analyses. *Artemisia* ITS (including ITS1 and ITS2) and 3'ETS were first analysed separately, using Bayesian inference to assess congruence among these nuclear markers. Then, the sequences from the two regions were concatenated in a final aligned matrix of 869 characters (Table 2). This combined nrDNA dataset was analysed using both Bayesian inference (BI) and maximum likelihood (ML) to assess the circumscription of subg. *Seriphidium* within *Artemisia*. The best nucleotide substitution model was determined with jModelTest v.2.1 (Posada, 2008), independently for ITS and ETS sequences, according to the Akaike information criterion (AIC). BI analyses was conducted with MrBayes v.3.2.1 (Ronquist & al., 2012), with ITS and ETS substitution

parameters estimated in different partitions for the combined analysis. Two independent Markov chain Monte Carlo (MCMC) analyses with four Metropolis-coupled chains each were run for 5,000,000 generations, sampling every 100 generations. The first 25% of the trees were discarded as 'burn-in', after confirming that the average standard deviation of the split frequencies was < 0.01, and the potential scale reduction factor approached 1.0 for all parameters. The remaining samples were pooled to construct 50% majority rule consensus trees that approximate the posterior distribution of the phylogenetic reconstructions, and to obtain clade posterior probabilities. ML analysis was performed with RAxML-HPC v.8 (Stamatakis, 2014), partitioning the combined nrDNA dataset in two different ITS and ETS regions as in the BI analysis. We employed the GTRCAT nucleotide substitution model for both partitions, with the default settings for the optimisation of individual per-site substitution rates. The best-scoring ML tree with clade support values was obtained from 10 independent runs, with 1,000 rapid bootstrap replicates each run. All these analyses were performed within the CIPRES Science Gateway (Miller & al., 2010), and the resulting summary trees were visualised in Figtree v.1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).

The *Seriphidium* dataset comprises newly generated sequences of *rpl*32-*trn*L and *ndh*C-*trn*V plastid regions for 123 specimens (92 species; 97 taxa) of the subgenus plus 12 additional *Artemisia* species and one outgroup. Before being combined in a final alignment of 2,040 characters (Table 2), the two plastid markers were analysed separately using BI to confirm congruence among them (results not shown; available upon request from corresponding author). The cpDNA dataset was not concatenated with nrDNA sequences of *Seriphidium* because only 25 specimens of the subgenus had been sequenced for both the nuclear and plastid regions. Furthermore, previous phylogenetic studies employing nrDNA (e.g. Torrell & al., 2003; Sanz & al., 2008) as well as our own preliminary results based on ITS and ETS data indicated a complete lack of resolution within subg. *Seriphidium*. The combined cpDNA matrix for the study of relationships within subg. *Seriphidium* was analysed using BI and ML, employing the same methods as in the phylogenetic analyses of the *Artemisia* nrDNA dataset (see above). To increase the information contributed by plastid data, gaps in the sequences were coded in a binary matrix (0: absence / 1: presence), following the simple InDel coding method (Simmons & Ochoterena, 2000) implemented in FastGap v.1.2 (Borchsenius, 2009). This binary matrix

was incorporated as a partition in the combined cpDNA dataset and analysed using the F81-like restriction site model in an additional MrBayes run.

Molecular dating and diversification analyses.– We estimated species divergence times in *Artemisia* by analysing the nrDNA dataset with BEAST v.1.8.2 (Drummond & al., 2012). Choice of model priors was based on the Path Sampling (PS) and Stepping Stone (SS) sampling methods (Baele & al., 2013), selecting the birth-death model as the tree prior and the uncorrelated lognormal rate variation among branches as the clock prior. A broad uniform distribution (10^{-1}-10^{-6}) for the mean substitution rate and a default exponential prior for the standard deviation parameter were specified. The substitution models for the ITS and ETS partitions were those used for phylogenetic inference. Calibration was based on biostratigraphic and palaeomagnetic studies from Central Asia, setting the first occurrence of *Artemisia* pollen in deposits from the early Oligocene, around 30–32 million years ago (Ma) (Zhu & al., 1985; Song & al., 2004; Wang, 2004). In accordance with these data, and given the incompleteness of the fossil record and the fact that fossils indicate minimum ages, we constrained the stem age of the most recent common ancestor of *Artemisia* with a lognormal prior, an offset of 30 million years, a mean of 1.0, and a standard deviation of 1.5. The 95% HPD probability distribution for this node calibration includes the onset of the Eocene (*c.* 56 Ma), the age of the earliest Asteraceae fossil discovered in Asia (Wang, 2004), where tribe Anthemideae likely originated (Funk & al., 2009). Many younger fossils of *Artemisia*-like pollen exist, but the morphological uniformity of this pollen type impedes using them to calibrate more internal nodes of the tree (Tkach & al., 2008a). There is also a reliable first report of *Artemisia*-like pollen from North America (Graham, 1996), but given the multiple origins of New World *Artemisia* (Riggins & Seigler, 2012), this fossil cannot be attributed to a particular node. Alternatively, we employed this first North American *Artemisia* fossil as an independent test to evaluate the accuracy of our time-calibrated phylogeny by comparing the age of this fossil with the divergence time inferred for the first unequivocal North American lineage in our phylogenetic reconstruction (see Tkach & al., 2008a; Hobbs & Baldwin, 2013). The Markov chain was run for 2×10^8 generations, sampling every 10,000th generation. Tracer v.1.6 (Rambaut & al., 2014) was used first to check the

convergence and mixing of each parameter, and then to confirm that the effective sample size (ESS) of each parameter was sufficient to provide reasonable estimates of the variance in model parameters (i.e. ESS values > 200, after excluding a burn-in fraction of 10%). Trees were summarised in a maximum clade credibility (MCC) tree obtained in TreeAnnotator v.1.8.2 (distributed as a part of the BEAST package) and visualised in FigTree v.1.4.2.

We modelled the macroevolutionary dynamics of diversification across the *Artemisia* phylogenetic tree with BAMM v.2.5 (Bayesian Analysis of Macroevolutionary Mixtures: Rabosky & al., 2013) in order to quantify speciation and extinction rates and to identify different diversification regimes throughout the evolutionary history of the genus. All analyses were conducted on a pruned version of the BEAST chronogram, excluding the outgroups, and limiting sampling within *Artemisia* to a single tip per species. To account for incomplete taxon sampling, we specified the taxonomic coverage of *Artemisia* species in our study considering the number of species in the genus recognised by Funk & al. (2009) and Kubitzki (2007). BAMM was run for 5 million MCMC generations, sampling every 1,000 generations. BAMM tools v.2.1.5 (Rabosky & al., 2014), CODA (Plummer & al., 2006) and 'ape' (Popescu & al., 2012) packages were used to set the priors for BAMM, assess MCMC convergence and visualise the output.

Ancestral area reconstruction was conducted with an ML approach using the recently developed program BioGeoBEARS (Matzke, 2013), which allows comparison among multiple models of range evolution. All analyses were performed on the same pruned MCC tree employed for the diversification analysis. Taxon occurrence data were taken from the Global Biodiversity Facility (GBIF; http://www.gbif.org), inspected based on national and regional floras and coded as present or absent in nine large-scale/global geographic areas. The areas were delimited according to their biogeographic significance for *Artemisia* based on spatial concentrations of high-species richness and endemism (e.g. Manafzadeh & al., 2015; Favre & al., 2016), specifying different dispersal probabilities among the nine areas. We also included time-stratified dispersal probability matrices in the model to account for the changing distances between the regions over geological time (Table S2). These probabilities were estimated as 0.01 for distant areas in different continents, 0.5 for geographically close but non-contiguous regions and 1.0 for contiguous

areas. Four time slices were considered: before 50 Ma, 50–20 Ma, 20–10 Ma, and 10 Ma to present. The first 50 Ma strata defines the time before the Indian-Asian collision (Favre & al., 2015, and references therein); the break at 20 Ma corresponds to the Arabia-Eurasia collision (Manafzadeh & al., 2015); and the 10 Ma threshold approximates the start of the orogeny of the Himalaya (Renner, 2016). The maximum number of combined areas in the analysis was set to four, because the native distribution of *Artemisia* species was never found to comprise more than four areas.

As an alternative method to assess the start of the diversification of *Seriphidium*, we also estimated the divergence time among the species of the subgenus with a strict clock model using BEAST v.1.8.2. In this case, taking into account the multi-specimen sampling per species of the cpDNA dataset employed for this analysis, we chose the coalescent model as the tree prior. Given that a taxon specific substitution rate had not been previously calibrated for non-coding cpDNA regions of *Seriphidium*, we assumed a mean substitution rate of 1.52×10^{9} s/s/y (1.0×10^{9} s/s/y as the lower limit and 3.0×10^{9} s/s/y as the upper limit) based on previously estimated (synonymous) substitution rates for cpDNA in angiosperms (Wolfe & al., 1987; Richardson & al., 2001). The substitution model was chosen according to jModeltest. The Markov chain was run for 2×10^{8} generations, sampling every 10,000th generation. Tracer v.1.6 was used to check convergence, mixing and effective sample size of each parameter. As explained above, trees were summarised in a maximum clade credibility (MCC) tree obtained in Tree Annotator v.1.8.2 and visualised in FigTree v.1.4.2.

Results

Phylogenetic, biogeographic and diversification analyses in *Artemisia*.– The summary of numerical data from nrDNA sequences for all *Artemisia* samples is presented in Table 2. The topologies of the trees obtained from independent Bayesian analyses for ITS and ETS regions showed no conflicts between significantly supported (PP > 0.95) clades (Fig. S1). Only a few discordances between clades with low support (PP < 0.95) were observed, which we considered soft incongruences, and the two nrDNA regions were concatenated. The Bayesian and the maximum likelihood approaches employed to analyse the combined

nrDNA dataset showed similar phylogenetic reconstructions. As the tree based on BI showed slightly better resolution than the tree obtained with ML, only the consensus tree resulting from BI is presented in Fig. 1. The best-scoring tree derived from the ML analysis is available as Electronic Supplement (Fig. S2). The monophyly of *Artemisia*, including *Seriphidium*, was strongly supported (PP=1.00; BS=84%), with the outgroup taxa forming an unresolved node. Most of the backbone nodes of the trees showed strong support (PP>0.90; BS>70%), except for a few lineages which also resulted in poorly resolved nodes in previous studies specifically devoted to the study of their evolutionary history (e.g. Tkach & al., 2008a; b; Pellicer & al., 2011; Riggins & Seigler, 2012; Hobbs & al., 2013). In the present study, all clades which comprise *Seriphidium* species were fully supported. All species – excepting *A. deserti* Krash. and *A. leucodes* – assigned to sect. *Seriphidium sensu* Poljakov (1961b) and Ling (1994), comprising most of the species of the subgenus, were grouped in a monophyletic clade (PP=1.00; BS=81%). This group was sister to a clade formed by four annual *Artemisia* species (*A. annua*, *A. anethoides* Mattf., *A. anethifolia* Weber ex Stechm. and *A. apiacea* Hance). The resolution within this core clade of *Seriphidium* species was poor in both the BI and the ML phylogenetic reconstructions based on ITS-ETS; only one small group composed of *A. quettensis* Podlech and *A. ciniformis* Krasch. & Popov ex Poljakov showed significant statistical support (see Fig. 1). The rest of subg. *Seriphidium* species assigned to sect. *Junceum* Poljakov (*A. juncea* Kar. & Kir. and *A. leucodes*) plus *A. deserti* – which was assigned to sect. *Seriphidium* according to Poljakov (1961b) – grouped together in a different supported clade (PP=1.00; BS=100%), phylogenetically distant from the clade encompassing sect *Seriphidium* species. The monophyletic group comprising sect. *Junceum* species (*sensu* Poljakov) plus *A. deserti* was placed within a major clade mainly composed of taxa from subg. *Absinthium*.

The chronogram obtained from the BEAST analysis of nrDNA sequences of *Artemisia* is presented in Fig. S3 (Elect. Suppl.). The topology of this MCC tree showed overall congruence with Bayesian and ML phylogenetic inference. This time-calibrated phylogenetic reconstruction placed the start of diversification of *Artemisia* at about 30.38 Ma. The branch with sect. *Seriphidium* splits from its sister group *c*. 9.12 Ma, while diversification in the section started approximately 5.42 Ma (95% HPD 2.86-8.42 Ma). The clade composed of the species of sect. *Junceum* splits from its sister clade *c*. 15.25

Ma, but its diversification did not start until *c*. 5.63 Ma. The BAMM phylorate plot of diversification showed significant rate heterogeneity across *Artemisia* (Fig. S4). The regime of diversification shifts with the maximum *a posteriori* probability indicated two accelerations of speciation rates during the evolutionary history of the genus (Fig. 1; Fig. S4). One diversification rate shift occurred on the branch leading to the clade composed of the species of sect. *Seriphidium*. The other diversification rate shift identified was located within subg. *Dracunculus*, occurring at a branch within the core clade of species defined by Pellicer & al. (2011). The marginal probability of a shift on those particular branches was 0.66 and 0.48, respectively, while the cumulative shift probability in both cases is above 0.90. The mean speciation rate of those clades experiencing diversification shifts was considerably higher (0.506 species Myr^{-1} for the sect. *Seriphidium* clade; 0.531 species Myr^{-1} for subg. *Dracunculus*) than the background rate of the genus (0.307 species Myr^{-1}).

In BioGeoBEARS, likelihood ratio tests favoured DEC models, showing significantly lower AIC scores than DIVALIKE and BAYAREALIKE models (Table S3). Furthermore, the DEC model including cladogenetic dispersal by founder events (+J) always fitted the data better than the alternative model without founder events, so the results of DEC+J are reported here (Fig. S5). Our estimations placed the origin of early diverging lineages of *Artemisia* in Central Asia, with subsequent and independent dispersals mainly to America, Africa and other regions of Eurasia, where the genus continued its diversification process. The origin of the species radiations that occurred in sect. *Seriphidium* and subg. *Dracunculus* were both placed in Central Asia, with further diversification mainly taking place in other areas within the Eurasian continent. The other models calculated resulted in the same ancestral range reconstructions (i.e. Central Asia origin) for those two clades that experienced diversification rate shifts (results not shown; output files available upon request from the corresponding author).

Phylogenetic and diversification analyses within subgenus *Seriphidium*.– The summary statistics from the cpDNA dataset of *Seriphidium* samples can be found in Table 2. Because BI resulted in better resolved trees than ML, only the results of BI are shown in Figure S6 and Figure 2. The phylogenetic analyses including and excluding gap information resulted in fully congruent trees, but support of certain nodes varied between

the two approaches. According to both analyses, the two clades of *Seriphidium* species already observed in the phylogenetic inference of *Artemisia* derived from the nrDNA dataset were recovered again (PP=1.00 in all cases). As in the nrDNA analyses, *A. annua* is sister species of sect. *Seriphidium*, while sect. *Junceum* appears in an unresolved position with *A. absinthium* and *A. frigida* Willd. (subg. *Absithium*) plus *A. arctica* (Besser) Leonova (a species assigned to the paraphyletic subg. *Artemisia*). Within sect. *Seriphidium*, *A. densiflora* Viv. from Sardinia and *A. ramosa* C.Sm. ex Link from the Canary Islands emerged as early-diverging species in relation to the rest of the species of the group, with unresolved relationships between them. The rest of the species of sect. *Seriphidium* formed a monophyletic group (PP = 0.93 and 0.95, excluding and including the gaps, respectively). Several supported clades (e.g. haplogroups B, D, F and G, among other smaller ones) as well as a few non-monophyletic groups (e.g. haplogroups A, C and E) of species within sect. *Seriphidium* were inferred (Fig. 2). Most of the samples belonging to the same species were placed within the same clade, but there were also a few cases of multiple accessions of one species appearing in different supported groups (e.g. *A. maritima* L.; *A. serotina* Bunge; *A. santonica* L.).

The cpDNA chronogram of sect. *Seriphidium* generated by BEAST was congruent with the topology of MrBayes trees based on the same markers (Fig. S7). The age of the crown diversification of sect. *Seriphidium* was estimated at 2.15 Ma (95% HPD = 0.84-3.92) according to the strict clock model. The estimated age for this clade is younger than, but partially overlapping with, the dating derived from the nrDNA *Artemisia* dataset.

Discussion

Circumscription of subgenus *Seriphidium*.– Our reconstruction of the evolutionary history of *Artemisia* showed overall congruence with previous phylogenetic studies conducted on this large and diverse genus (Sanz & al., 2008; Garcia & al., 2011; Hayat, 2011; Pellicer & al., 2011; Riggins & Seigler, 2012; Hobbs & Baldwin, 2013). The vast majority of lineages identified in those earlier studies have also been recovered in our study, which encompasses the largest taxonomic sampling available to date in *Artemisia*, particularly for subg. *Seriphidium*.

Our results confirm the findings of earlier molecular analyses of *Artemisia* (e.g. Kornkven & al., 1998, 1999; Torrell & al., 1999), that the inclusion of *Seriphidium* in *Artemisia* is strongly supported. Consequently, there are no arguments to consider *Seriphidium* an independent genus as done by Bremer & Humphries (1993) based on morphological evidence. Currently, this situation is largely accepted (as in the two main comprehensive modern revisions of family Asteraceae: Kubitzki, 2007, and Funk & al., 2009), although some authors still maintain the subgenus as a generic entity (Wu & al., 2011).

Old World *Seriphidium* species were traditionally considered monophyletic according to previous systematic studies of *Artemisia* (e.g. Kornkven & al., 1999; Torrell & al., 1999; Riggins & al., 2012). However, in our phylogenetic reconstruction based on nuclear DNA markers (Fig. 1), *Seriphidium* species belong to two monophyletic clades. Most of the taxa typically defined as *Seriphidium* constitute a large, supported clade sister to a group of annual species (i.e. *A. annua*, *A. apiacea*, *A. anethoides* and *A. anethifolia*). However, a few other species also classically assigned to subg. *Seriphidium* (i.e. *A. juncea*, *A. leucodes* and *A. deserti*) cluster in a separate monophyletic clade, nested within a clade with species of subg. *Absinthium* (Fig. 1). *Artemisia leucodes,* the only annual species within this group (Pellicer & al., 2014), shows a much longer branch than the other two species. The same segregation of *Seriphidium* species in two groups evolutionarily distantly related is obtained from the cpDNA dataset, primarily employed to study evolutionary relationships within the subgenus (Fig. S6). This disintegration of *Seriphidium* is somewhat in line with the taxonomy –based on morphological characters– proposed by Poljakov (1961a; b), who recognised two sections: sect. *Seriphidium* and sect. *Junceum*. The first section comprised most of the species of subg. *Seriphidium*, subdivided into 11 series. Section *Junceum* included only two species, *A. juncea* and *A. leucodes,* split into two independent series. The same author placed *A. deserti* in sect. *Seriphidium* but segregated this taxon in the monospecific series *Tridentatae*. According to our phylogenetic reconstruction, *A. leucodes* and *A. juncea* are evolutionarily closer to each other, with *A. deserti* as their sister species. Similarly, Filatova (1986) grouped *A. juncea*, *A. leucodes* and *A. deserti* in subsect. *Junceae* within sect. *Junceum*, while Ling (1991) separated *A. leucodes* and *A. deserti* in two series within sect. *Seriphidium,* segregating *A. juncea* into its own monospecific section. Therefore, Poljakov, Filatova and Ling quite accurately pointed out relationships among *A. leucodes*, *A. deserti* and *A.*

juncea, but failed to realise that their evolutionary differentiation extended beyond the circumscription of the subgenus. According to our results, these three species should be excluded from *Seriphidium* to obtain a monophyletic subgenus. Further studies are needed to investigate the systematic position of *A. minchunensis* Ling & Y.R.Ling, a species endemic to the Gansu region in China that Ling (1991) segregated into the monospecific sect. *Minchunensia* Y.R.Ling.

Historical biogeography and diversification of *Artemisia*.–

The backbone topology of our time-calibrated tree obtained from BEAST is largely congruent with the phylogenetic inferences we obtained from Bayesian and ML analyses. The divergence time estimates for the main lineages of *Artemisia* are comparable – i.e. confidence intervals overlap – although generally older than in previous time-calibrated phylogenies of the genus (Tkach & al., 2008a; Sanz & al., 2011; Hobbs & Baldwin, 2013). The best independent test proposed to evaluate the accuracy of divergence time inferences within the genus (Tkach & al., 2008a; Hobbs & Baldwin, 2013) is the comparison with the earliest North American fossil of *Artemisia* [*c.* 23 Ma (Graham, 1996)]. Our time-calibrated phylogenetic reconstruction (Fig. S3) estimated the stem age of the first unequivocal North American lineage at 18.6 Ma (95% HPD: 12.5-25.7 Ma), overlapping with the fossil record [as reported in Hobbs & Baldwin (2013)], whereas the estimates from Tkach & al. (13.5 Ma; 95% HPD: 10-17 Ma) or Sanz & al. (average = 10.8 Ma; SD = 1.5) were slightly younger. These results are also consistent with the spatial and temporal evolutionary history of *Artemisia* inferred from pollen data in China (Miao & al., 2011), suggesting an origin of the genus in the arid-semiarid middle latitudes of Asia in the late Eocene, and an early expansion west and east during the Oligocene and Miocene.

Our biogeographic and divergence time analyses indicate that the core clade of subg. *Seriphidium* originated in Central Asia around the Late Miocene – Early Pliocene when the lineage experienced a major diversification shift. This trace of rapid diversification could explain the lack of phylogenetic resolution– evidenced by the presence of a large polytomy in the trees based on nrDNA data– obtained within this group (Withfield & Lockart, 2007). However, as will be discussed below, we cannot discard other possibilities, such as hybridisation among the species of the subgenus. Despite the fact that the species sample in our study is quite representative at both clade and geographical

levels, unbalanced taxon sampling could also be biasing the results of diversification and biogeographic analyses. However, the relative sampling of *Seriphidium* species included in the diversification and biogeographic analyses [45% of the described species of the subgenus according to Bremer & Humphries (1993)] is slightly below the sampling for the whole genus [50% of described species of *Artemisia* according to Funk & al. (2009)]. Therefore, more balanced sampling would result in more species included in the *Seriphidium* clade, likely resulting in even higher diversification rates for the subgenus. Similarly, the geographical distribution of *Seriphidium* taxa included in the nrDNA dataset employed for biogeographic analyses – mostly obtained from previously published Genbank sequences – is mainly biased towards species from western countries. As can be observed in our cpDNA dataset with an extended *Seriphidium* sampling, incorporating more taxa of this subgenus would mean adding more species from Central Asia, which supports the robustness of our biogeographic results.

Strikingly, the evolutionary history of subg. *Seriphidium* is very similar to that of a large lineage of species of subg. *Dracunculus*. First, the branch leading to this group of *Dracunculus* species contains the other diversification shift inferred by BAMM (Fig. 1; Fig. S4). Second, as in *Seriphidium*, the resolution within this subclade of *Dracunculus* is extremely low, a result already obtained by Pellicer & al. (2011) in a phylogenetic study devoted to the subgenus. Finally, the origin of this lineage of *Dracunculus* species is placed in the same area (i.e. Central Asia) and in the same period (Miocene-Pliocene transition) as the diversification of *Seriphidium*. These results agree with space-time reconstructions of the distribution of *Artemisia* based on pollen data from Central Asia (Miao & al., 2011), showing a late expansion of the genus evidenced by the large increase of *Artemisia* fossil records in the region between the Miocene and Pliocene.

Recent diversification and radiation seem to be a common pattern for many groups of plants that show the greatest diversity of species in the Qinghai–Tibet Plateau and adjacent areas (see Favre & al., 2015; Renner, 2016, and references therein). From a climatic history perspectives, pronounced cold and aridity appear to have begun in Central Asia by the late Oligocene or early Miocene, potentially caused by the effect of ongoing global cooling and/or Paratethys Sea regression (Guo & al., 2002; Sun & al., 2010). These circumstances resulted in the evolution of a well-developed dryland flora in particular

areas of inner Asia (Takhtajan, 1969; Grubov, 1999), whereas in other regions of the world the climate still showed significantly more tropical characteristics (Wulff, 1944; 1950; Thompson, 2005). When aridity increased across Eurasia in connection with global cooling during the Miocene and Pliocene (Sun & al., 2010; Miao & al., 2012), certain elements of the Central Asian flora which were preadapted to cold and arid conditions could thrive (e.g. *Rheum* (Polygonaceae): Sun & al., 2012; *Haplophyllum* (Rutaceae): Manafzadeh & al., 2014; *Dontostemon* and *Clausia* (Brassicaceae): Friesen & al., 2015; *Nitraria* (Nitrariaceae): Zhang & al., 2015a). Indeed, Central Asia has been proposed to be the main source and centre of diversity of the current xerophytes of Eurasia, the Mediterranean Basin and North Africa (Manafzadeh & al., 2016 and references therein). In this context, the species of subgenera *Dracunculus* and *Seriphidium* represent major examples of pre-adapted lineages which originated in Central Asia and radiated explosively – both locally and distantly – in response to global climate cooling and drying that started in the Miocene-Pliocene transition.

Limitations and incongruences within *Seriphidium*.– The plastid DNA dataset, comprising samples of 97 *Seriphidium* taxa – the majority of them sequenced for the first time – allowed the identification of some major clades in the subgenus. However, our phylogenetic inference is far from resolving evolutionary relationships among most of the species of this group. The major clade of *Seriphidium* showed unexpected early-diverging taxa. Although with only weak statistical support, two species, one from the Mediterranean island Sardinia (*A. densiflora*) and the other from Tenerife in the Canary Islands (*A. ramosa*), form a trichotomy with the rest of *Seriphidium* species.

These two species belong to two morphological groups of subg. *Seriphidium* with which they do not group in the phylogenetic reconstruction. Particularly, *A. densiflora*, described as a Corsican-Sardinian endemic, has been considered a subspecies of a species with which it shares morphological, ecological (apart from the insularity) and karyological characters (*A. caerulescens* L. ssp. *densiflora* (Viv.) Gamisans ex Kerguélen & Lambinon); but this is not reflected in the DNA sequence analysis, where *A. caerulescens* appears in a derived clade with *A. herba-alba* Asso, *A. barrelieri* Besser, and *A. oranensis* (Deb.) Filatova. In turn, *A. ramosa* has been considered endemic to the Canary Islands,

but has also been reported from Morocco (Charpin & Vallès, 1985) and is part of the taxonomically intricate *A. herba-alba* group (see comments on this complex in the "Biogeographic patterns within *Seriphidium*" subheading).

According to the cpDNA data, the average sequence divergence of *A. densiflora* and *A. ramosa* from the rest of the species of the subgenus (0.72% and 0.29%, respectively) is larger than the mean divergence of the other species in relation to the rest of the members of the subgenus (0.22%). In the absence of selection, the amount of time required for a mutation to be fixed through random drift in a population –or a lineage– is a function of its effective population size (Ne) (Kimura & Ohta, 1969). Island endemics are likely to have undergone both a severe population bottleneck during the initial colonisation of the island and subsequent long-term reduction in census population size due to range restriction, leading to a reduction in Ne relative to mainland species (e.g. Frankham, 1997). As a result, island endemic species have proven to show an increased fixation of nearly neutral mutations compared to continental congeners (Woolfit & Bromham, 2005). This process likely explains the larger sequence divergence observed in the island *Seriphidium* species, causing phylogenetic confusion and biogeographic incongruence.

Apart from the insular species occupying those equivocal early-diverging positions, the cpDNA tree shows several large groups with poor internal resolution. These groups are not in agreement with the classical taxonomic treatments mainly based on morphological features of leaves and inflorescences by Poljakov (1961a; b) and Ling & al. (2006). These authors defined 11 and 13 series, respectively, in sect. *Seriphidium*, which are mostly congruent: most of the series appear in both systems and contain the same taxa, except some species described after Poljakov (1961a; b). However, these series are scattered across our phylogenetic reconstruction derived from plastid data (Fig. 2). The same applies to the infrageneric system proposed by Filatova (1986), even if this author's system, in her own words, differs strongly from Poljakov (1961b). She defined several subsections within six sections – based on morphological traits of leaves, capitula or stems, apart from life form and growth habit – that do not agree with the phylogroups derived from the molecular analyses (Fig. 2). The incongruence between morphological and molecular data could suggest that the morphological characters that have been used taxonomically are not reliable for indicating true evolutionary relationships (e.g. Hilpold

& al., 2014). Indeed, this lack of congruence among morphology and our phylogenetic inference extends to the circumscription of species. Those species that are represent with more than one individual in our sampling (e.g. *A. maritima* L., *A. santonica* L., *A. nitrosa* Weber ex Stechm.) were not always placed in the same evolutionary group. Some morphological traits may have developed convergently in several species and lineages, and high plasticity of morphological characters is also likely.

Another partial explanation for discordance between morphological and molecular data may be the extreme morphological uniformity of subg. *Seriphidium*, which could be a product of the recent differentiation of the subgenus as discussed above. This is, in addition, complicated by the fact that these taxa have a late flowering time as compared to most plants and even with other *Artemisia* groups (Podlech (2013). This resulted in many specimens present in herbaria (collected on floristic expeditions, often performed when most plants – but not *Artemisia* and even less so *Seriphidium* – are in flower) to be incomplete: they lack floral structures, which are relevant for taxonomic purposes, as already noted by Podlech (2013). Moreover, after having consulted hundreds of herbarium sheets of the genus for more than 30 years (unpubl. res.), we have the definite impression that too many species have been described, particularly in subg. *Seriphidium*, based on very slight morphometric differences. As said in the introduction, 15 synonyms are attributed to *A. santonicum*, supporting this statement. These problems have led to misidentifications even by specialists for the genus, which may have led to erroneous interpretations that can be reflected in phylogenetic analyses. One of the best experts, author of the robust monograph of *Artemisia* in Flora Iranica (Podlech, 1986), where subg. *Seriphidium* is particularly abundant, has amended the identification of some taxa provided by himself in other studies (Podlech, 2013), thus admitting and showing the difficulties described. Accordingly, a global taxonomic revision of the subgenus based on both morphological and molecular evidence would now be needed.

Morphological uniformity and the derived difficulty for plant identification, however, may not be the only cause for the incongruence between molecular data and taxonomy. Other factors that could contribute to the observed patterns are incomplete lineage sorting (ILS) and/or hybridisation. The most important preconditions for ILS are large population sizes in the ancestral populations and few generations between speciation events (Pamilo &

Nei, 1988). Our diversification analyses have indeed inferred a significant speciation burst in *Seriphidium* (Fig. S4). In addition, the species of this group can be important components of the landscape (Ling & al., 2006; Vallès & al., 2011), with populations of many thousands of individuals. Therefore, although our data are insufficient to perform a robust test of the role of ILS, the available evidence indicates that the retention of ancestral polymorphisms in our study group cannot be rejected. Similarly, *Seriphidium* species have some features that make them prone to hybridisation in their evolutionary history. Hybridisation is expected to occur mainly where two different lineages meet in the same geographical area (Abbott & al., 2013), a situation that is currently common among several species of the subgenus (Poljakov, 1961a; b; Ling & al., 2006). Moreover, since pollen and seed dispersal in *Artemisia* is predominantly by wind (Vallès & al., 2011), gene flow over reasonably long distances is comparatively easier than in other groups. Finally, the recent differentiation among *Seriphidium* species –as inferred from the divergence time estimates derived from both nuclear and plastid markers– strongly facilitates interspecific gene flow (Mallet, 2005). Consequently, several cases of hybridisation between different *Seriphidium* taxa in nature have indeed been described (Ling & al., 2006). In summary, ILS and hybridisation could affect part of the results of our phylogenetic analyses, but further studies are necessary to unravel the precise role of these processes in the evolutionary history of *Seriphidium*.

Biogeographic patterns within *Seriphidium*.– Even though our molecular data are not sufficient to fully resolve all phylogenetic relationships within *Seriphidium*, they nonetheless provide some insights into biogeographic processes that took place during the evolutionary history of this group (Fig. 2). The species ascribed to the early-diverging groups in our phylogenetic reconstruction of the subgenus (haplogroups A and B) show a restricted distribution in Central Asia in the mountainous regions of Afghanistan, Pakistan, Tajikistan and Kyrgyzstan (i.e. western Tian-Shan, Pamir and the Hindu Kush ranges) (Fig. 2). The more derived haplogroups (C to G) have a wider distribution and are predominant in other, generally distant areas (e.g. Africa, Europe and northern Asia). This geographical structure in our data suggests that the origin of the diversification within *Seriphidium* could be located in this orographically complex area of Central Asia (i.e.

western Tian-Shan, Pamir and Hindu Kush), subsequently dispersing and colonising other regions of the Eurasian continent and North Africa. The particular role played by these mountain chains as cradle and source for the rich xerophytic flora of Central Asia has already been hypothesized (e.g. Manafzadeh & al., 2016 and references therein). Indeed, the Tian-Shan, Pamir, and Hindu Kush chains have been proposed to be a global biodiversity hotspot and to have served as a Tertiary refugium for many plant lineages currently showing wider geographical distribution (e.g. Agakhanjanz & Breckle, 1995; Kadereit & al., 2008; Giam & al., 2010; Meng & al., 2015; Zhang & al., 2015b). In *Seriphidium*, the significant concentration of diversity found in these mountain ranges supports their role as refugia as well. Further studies would be necessary to unravel the precise biogeographic role of these mountain ranges for the flora of the region.

The analyses of the plastid DNA dataset also provided biogeographic results for the *Artemisia maritima* L. complex, an ecologically important and evolutionarily intricate group of taxa widely distributed in Europe and North Asia (Persson, 1974). All European samples determined as *A. maritima* (i.e. corresponding to *A. maritima* L. ssp. *maritima*, *A. maritima* L. ssp. *salina* (Willd.) Nyman, and *A. maritima* L. ssp. *humifusa* (Fries ex Hartman) K.Persson) are found in the same haplogroup E (Fig. 2). In contrast, the samples from northern Asia, determined as *A. maritima* L. var. *incana* (Kell.) Krasch. and *A. maritima* L. ssp. *gmeliniana* (Besser) Krasch., are positioned in a different genetic group (haplogroup C). Other species classically assigned to the *A. maritima* complex were also recovered in haplogroup E (e.g. *A. taurica* Willd., *A. vallesiaca* All., *A. dzevanovskyi* Leonova) or haplogroup C (e.g. *A. gracilescens* Krasch. & Iljin, *A. nutans* Willd.), casting doubts on the suggested evolutionary affinities among the members of the complex.

Our results also recovered other groups which can be discussed from a biogeographic perspective. A number of samples from the western Mediterranean region assigned to different species (i.e. *A. caerulescens* L., *A. herba-alba* Asso, *A. barrelieri* Besser, and *A. oranensis* (Deb.) Filatova) are grouped in a supported monophyletic clade (haplogroup F). On the basis of morphological and biogeographic affinities, some of these species (i.e. *A. herba-alba*, *A. barrelieri* and *A. oranensis*) had already been proposed to form a taxonomic group – the *A. herba-alba* complex – according to different authors (Ouyahya & Viano, 1988; Torrell & Vallès, 1995; Blondel & al., 2010; Podlech, 2013; Zahra & al.,

2014; Bougoutaia & al., 2016). In contrast, some species previously related to *A. herba-alba*, such as *A. inculta* Delile, *A. oliveriana* J.Gay ex Besser or *A. sieberi* Besser, are not included in the same clade. This *A. herba-alba* complex seems to be evolutionarily close to haplogroup E, dominant across central Europe. Indeed, *A. caerulescens* is unequivocally included within the Mediterranean lineage but has been related to some Central European taxa such as *A. vallesiaca* and *A. maritima* in classic systematics studies (e.g. Persson, 1974). These results suggest a close evolutionary relationship among the *A. herba-alba* complex and haplogroup E, from which the Mediterranean lineage could have derived.

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Table 1. Taxonomic treatment of *Seriphidium* (at sectional, subgeneric or generic level) and its subdivision into sections.

Besser (1834)	Rouy (1903)	Rydberg (1916)	Poljakov (1961b)	Filatova (1986)	Ling (1991, 1995)
Sect. *Seriphidium* Besser	Subg. *Seriphidium* (Besser) Rouy	Subg. *Seriphidium* (Besser) Rouy Sect. *Seriphidium* *Sect. *Tridentatae* Rydb.	Subg. *Seriphidium* (Besser) Rouy Sect. *Seriphidium* **Sect. *Junceum* Poljakov	Subg. *Seriphidium* (Besser) Rouy Sect. *Calciphilum* Filatova Sect. *Leucophyton* Filatova Sect. *Sclerophyllum* Filatova Sect. *Halophilum* Filatova Sect. *Pycnanthum* Filatova **Sect. *Junceum* Poljakov	Genus *Seriphidium* (Besser) Fourr. **Sect. *Seriphidium* Sect. *Juncea* (Poljakov) Y.R.Ling & Humphries Sect. *Minchunensia* Y.R.Ling

*Sect. *Tridentatae* is placed outside subg. *Seriphidium* according to recent phylogenetic studies (e.g. Garcia & al. 2011).

**These sections contain one species (i.e. *A. leucodes* Schrenk) that is placed outside subg. *Seriphidium* according to recent phylogenetic studies (e.g. Vallès & al. 2003).

Table 2. Summary statistics from the nrDNA dataset of genus *Artemisia* and the cpDNA dataset of subg. *Seriphidium*. The numbers in brackets indicate the results obtained for the ingroup of *Artemisia* nrDNA dataset and for sect. *Seriphidium* of cpDNA dataset.

nrDNA dataset	ITS	ETS	ITS+ETS
Number of taxa	280 (277)	280 (277)	280 (277)
Total characters	487	381	869
Number of informative characters	173 (165)	120 (117)	293 (282)
cpDNA dataset	*rpl*32-*trn*L	*ndh*C-*trn*V	*rpl*32-*trn*L + *ndh*C-*trn*V
Number of taxa	109 (97)	109 (97)	109 (97)
Total characters	924	1116	2040
Number of informative characters	39 (12)	37 (11)	76 (23)
Number of informative indels	19(11)	12(8)	31(19)

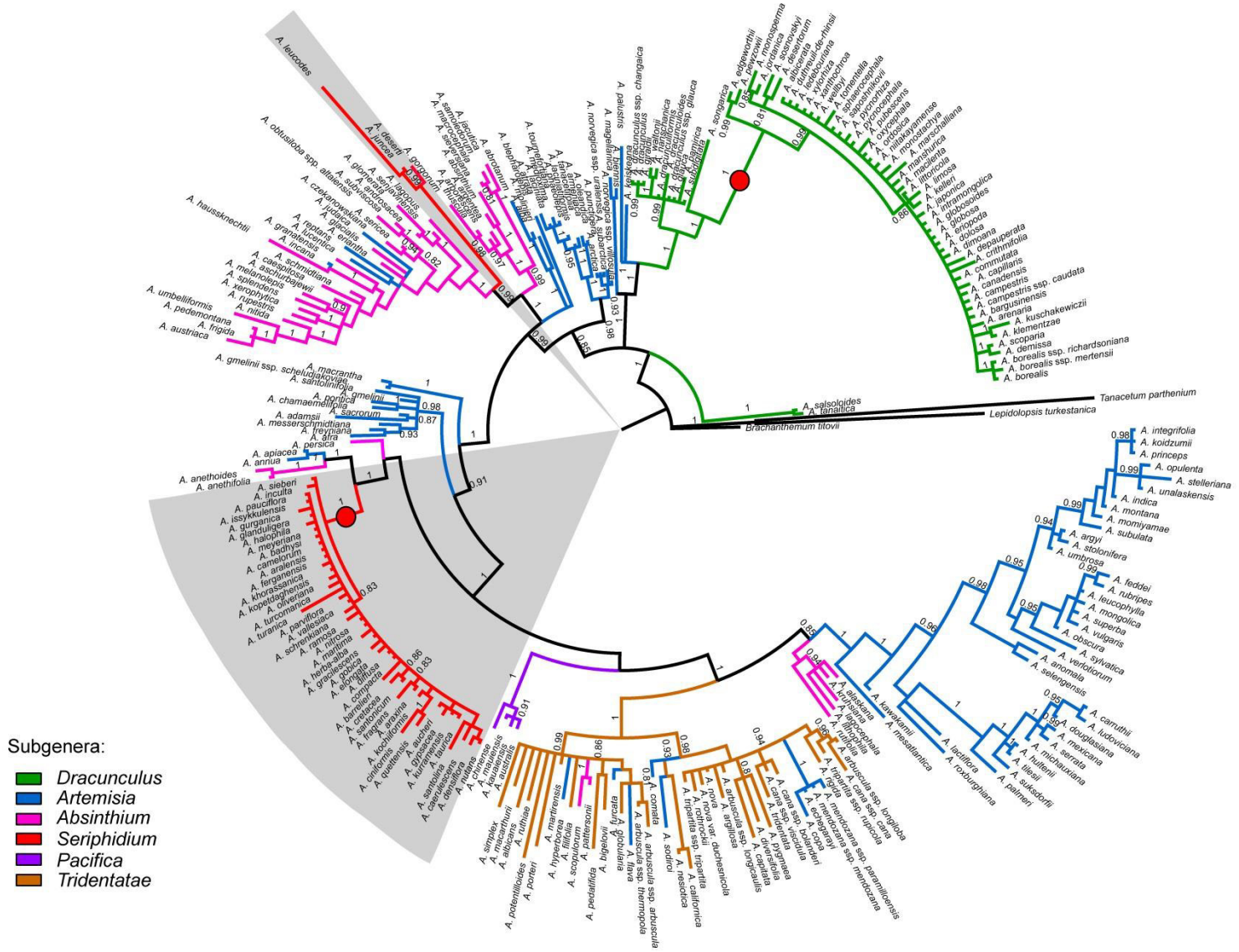

Fig. 1. Consensus tree (50% majority-rule) from Bayesian inference of the combined ITS + 3'ETS dataset of *Artemisia*. Posterior probability values (PP) are indicated along branches (values below 0.80 are not shown). The styles of the strokes that draw the branches indicate the traditional subgeneric classification of *Artemisia*. Circles show diversification shifts identified in the BAMM analysis.

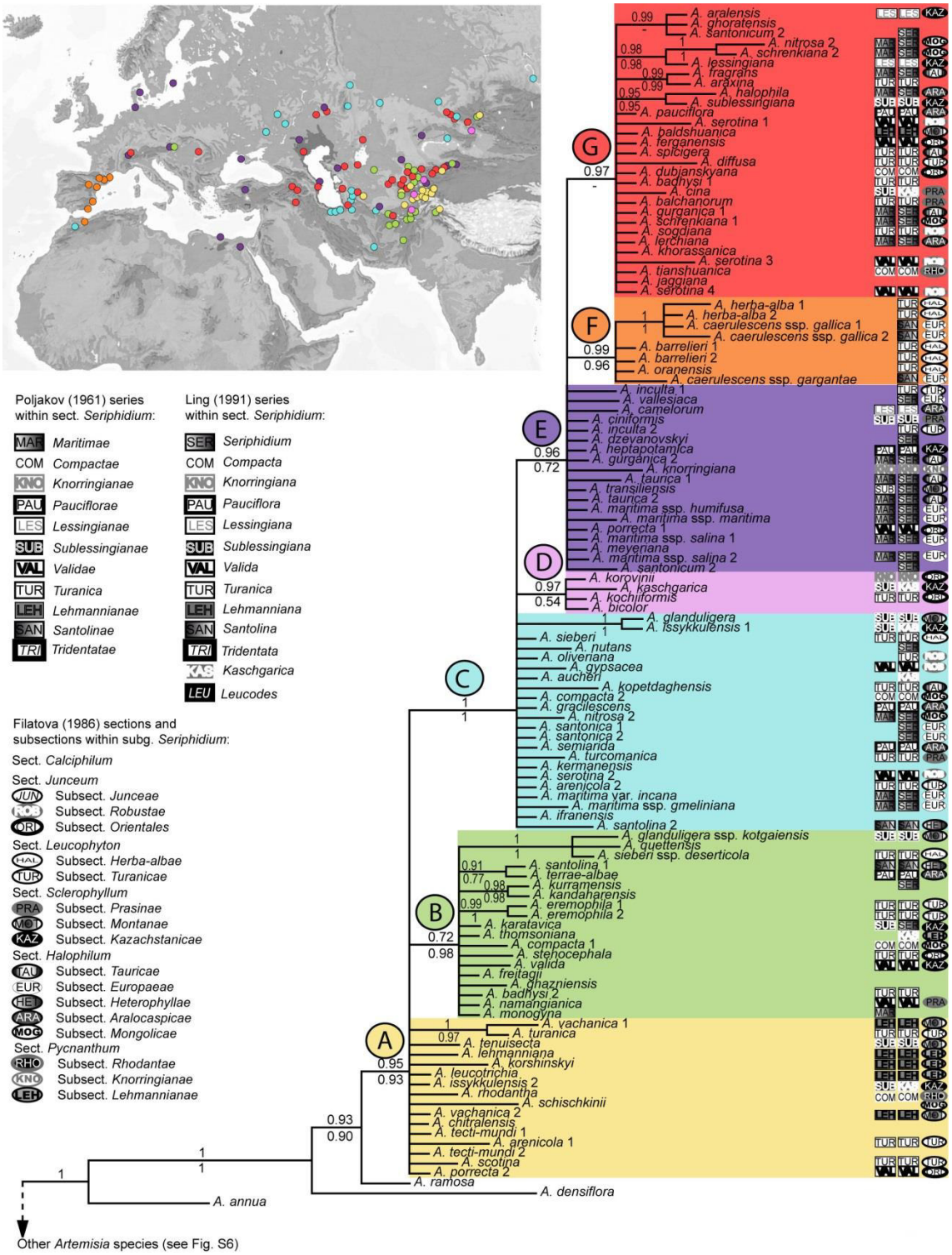

Fig. 2. *Artemisia* subg. *Seriphidium* sect. *Seriphidium* consensus tree based on Bayesian inference of the combined plastid (*rpl32-trnL*, *ndhCtrnV*) regions. Posterior probability (PP) values obtained from the analyses excluding and including indels are shown above and below branches, respectively. The infrageneric classification of species of subg. *Seriphidium* in series according to Poljakov (1961b) and Ling (1991) and in subsections according to Filatova (1986) are indicated after species names. Large letters inside geometric shapes (A–G) indicate haplogroups identified in the Bayesian analysis. Geographical distribution of samples with haplogroup correspondence is shown in the map (obtained from the Spanish Geographical Institute [©Instituto Geográfico Nacional]).