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Phylogenetics, phylogeography and vicariance of polyphyletic *Grammosciadium* (Apiaceae: Careae) in Anatolia

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Detailed phylogenetic relationships, evolutionary histories and phylogeographical hypotheses are still quite rare for the many genera of Apiaceae. One of the reasons for this is that traditional generic circumscriptions based on morphology and anatomy largely failed to recognize the group as monophyletic. This is also true for apioid members of Apiaceae with *c.* 400 genera. Here we focus on *Grammosciadium s.l.* centred in Turkey and unravel its evolutionary history. Based on three loci from the plastid genome and nuclear-encoded internal transcribed spacer regions 1 and 2 from the nuclear encoded ribosomal RNA operon, we show that *Grammosciadium* in its current circumscription is not monophyletic and represents an assemblage of species from four genera (*Carum*, *Chamaesciadium*, *Fuernrohria*, *Grammosciadium*). Diversification of this group started *c*. 7 Mya in the late Miocene. Most present-day species arose during the Pliocene with most of the intra-species diversification occurring during the Pleistocene. Plastome type variation does not resolve any of the main clades and there is little spatial structure of the distribution of high plastid genetic variation. The majority of species of pre-Quarternary origin are found east of the Anatolian Diagonal, running diagonally across central and eastern Turkey. This might indicate that the Diagonal acted as a barrier to gene flow and migration during the Pliocene and that eastern Anatolia may have served as a cradle for Pleistocene diversification and speciation processes in Turkey. We also provide a key to the species of the revised genus and introduce several taxonomic changes.

ADDITIONAL KEYWORDS: Anatolian Diagonal – *Carum* – evolutionary history –*Fuernrohria* – *Grammosciadium* – internal transcribed spacers 1 and 2 – plastid DNA – Turkey.

INTRODUCTION

Grammosciadium DC. is a taxonomically difficult genus of 11 taxa in tribe Careae (Apiaceae) (Spalik, Wojewodzka & Downie, 2001; Spalik & Downie 2007; Ajani *et al.*, 2008; Zakharova, Degtjareva & Pimenov, 2012; Bani & Koch, 2015; Bani *et al.*, 2016a;, Bani, Karakaya & Ceter, 2016b). It falls in the 'apioid superclade', which is the largest clade in the subfamily Apioideae with its *c.* 400 genera and 2900 species (Spalik & Downie, 2007; Banasiak *et al.*, 2013).

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Grammosciadium is closely related to *Fuernrohria* K.Koch (Downie *et al.*, 2010; Zakharova *et al.*, 2012; Terentieva *et al.*, 2015). *Grammosciadium* and *Fuernrohria* were usually placed in tribes Scandieae and Coriandreae, respectively, but this view is not generally accepted (e.g. Vinogradova, 1995) and various preliminary molecular analyses have supported a close relationship between *Grammosciadium* and *Fuernrohria* (Katz-Downie *et al.*, 1999; Downie *et al.*, 2000a, b, 2001; Valiejo-Roman *et al.*, 2006; Terentieva *et al.*, 2008). *Carum* L. has recently been shown to be polyphyletic, with its members being found in different tribes of subfamily Apioideae (Zakharova *et al.*, 2012). A close relationship between *Grammosciadium*

and *Carum carvi* L., the type species of *Carum*, has been demonstrated. However, mostly because of limited sampling, the monophyly of *Grammosciadium* has remained unresolved (Papini, Banci & Nardi, 2007; Zakharova *et al.*, 2012). Other studies included only a few representatives of Careae, making firm conclusions about the monophyly of the genera problematic (e.g. Ajani *et al.*, 2008; Banasiak *et al.*, 2013). The stem group age of Careae is *c.* 22 Mya and the tribe has a centre of origin in the Irano-Turanian region (Banasiak *et al.*, 2013; Calviño *et al.*, 2016). The onset of diversification in the group of taxa studied here, comprising *Grammosciadium* and *Fuernrohria*, can be roughly estimated to 5–6 Mya based on a small taxon sampling of Careae (Banasiak *et al.*, 2013). In general, because morphological characters often display high levels of homoplasy and phylogenetic inference is scarce (e.g. Downie *et al.*, 2010; Zakharova *et al.*, 2012), the taxonomy and systematics of these genera are still unresolved.

Grammosciadium has been split into two subgenera and six sections, reflecting previous taxonomic concepts (Table 1). Comprehensive morphometric analyses have built upon this concept and differentiated significantly between the four taxa from section *Stenodiptera* (Koso-Pol.) Tamamsch. & V.M.Vinogr. [in subgenus *Caropodium* (Stapf & Wettst.) Tamamsch. & V.M.Vinogr.] and placed *G. schischkinii* (V.M.Vinogr. & Tamamsch.) V.M.Vinogr. and *Caropodium armenum* (Bordz.) Schischkin in synonymy of *G. pterocarpum* Boiss. Two new subspecies have been described in *G. pterocarpum* (Bani *et al.*, 2016a). Similarly, one new subspecies was described in *G. macrodon* Boiss. of subgenus *Grammosciadium* (Bani & Koch, 2015).

With the exception of these studies, there are no reliable quantitative morphometric studies that allow definition of species boundaries in *Grammosciadium* and comparison with taxa from related genera. Although Zakharova *et al.* (2012) were not able to prove para- or polyphyly of *Grammosciadium*, they proposed a 'clade A' with '*Carum carvi* and its allies'. Besides *C. carvi*, this clade was shown to include four other *Carum* species, one species of *Chamaesciadium* C.A.Mey. from the Caucasus and adjacent regions, *Grammosciadium* and *Fuernrohria*. Of the five investigated *Grammosciadium* spp. four clustered together, namely *G. scabridum* Boiss., *G. macrodon*, *G. daucoides* DC. and *G. platycarpum* Boiss. & Hausskn. *Grammosciadium pterocarpum* was placed in a basal polytomy with *Fuernrohria* and *C. carvi*. However, we will show later that in their analysis, *G. platycarpum* was wrongly determined by Zakharova *et al.* (2012; AF073551) and actually represents *G. macrodon*. Close affinities between *Chaemaesciadium* and *Carum* were proposed earlier (e.g. Koso-Poljansky, 1914, 1916; Rechinger, 1987) and are indeed supported by morphological characters. *Fuernrohria* and *Grammosciadium* are also similar to each other in leaf and flower characters (Vinogradova, 1995). However, phylogenetic placement of these two genera with '*Carum carvi* and its allies' was commented upon as follows: '… the inferred affinity … with *Carum* is surprising and they could by no means be united with the latter' (Zakharova *et al.*, 2012).

The Irano-Turanian phytogeographical region (IT region) is well known for its floristic species richness (Takhtajan, 1986; Manafzadeh, Staedler & Conti, 2017) and is characterized by levels of endemism exceeding 25% (e.g. Davis, 1971; Zohary, 1971; Takhtajan, 1986; Koch, Karl & German, 2017). Takhtajan (1986) concluded that even the number of endemic genera from the apioid super clade is > 60, therefore representing 16% of all IT endemic genera, with *Grammosciadium* among them. *Grammosciadium* is distributed across Inner Anatolia, the East Anatolian mountains, the mountains of Transcaucasica and the Elburz and Zagros ranges in Iran and Iraq, respectively (Vinogradova, 1970). In addition, *G. scabridum*, which was thought to be restricted to Iran and Iraq, was recently documented in Turkey (Behçet, Kaval & Rüstemoğlu, 2012). However, critical investigations of the specimens collected from this locality (Behçet *et al.*, 2012) showed that they have young fruits only, and the diagnostic character (winged fruits) used to distinguish between *G. scabridum* and *G. platycarpum* could not be scored. Therefore, we conclude that

Table 1. Secondary calibration points (stem group ages) used in BEAST analyses of tribe Careae based on a phylogenetic analyses of 1194 apioid Apiaceae (Banasiak *et al.*, 2013); the shape and log-scale parameters of the lognormal distribution were set to the maximum-likelihood estimates inferred from the posterior distribution of corresponding node ages obtained by Banasiak *et al.* (2013)

Lineage	Median age (Mya)	Shape parameter, μ	Log-scale parameter, σ
Careae	16.68	2.8105663248	0.1405047315
Pyramidoptereae	17.93	2.8096345498	0.1404215425
Selineae	23.67	3.1623673515	0.0848250429
Oenantheae	25.66	3.2448813202	0.0838900841

G. scabridum is still not unambiguously shown to be distributed in Turkey.

Thus, the genus in its traditional circumscription is a western IT element restricted to the Anatolian Plateau. As such, *Grammosciadium* is highly suited for study of the floristically defined division of Anatolia, the so-called Anatolian 'Diagonal' (Davis, 1971; Ekim & Güner, 1986; Bilgin, 2011), in a spatio-temporal context. This diagonal region runs from the north-eastern corner of the Mediterranean Sea to the south-eastern corner of the Black Sea and it divides the Anatolian phytogeographical subregion into western and eastern Anatolia. This pattern was first studied in detail by Davis (1971) and, of 550 species considered, 61% of these followed a western versus eastern distribution pattern. The Diagonal itself represents an important refuge area for many endemic species. Attempts to review and classify spatio-temporal patterns for this divide at intra-specific levels have been made (Bilgin, 2011), but no general pattern was observed, the authors concluding that isolation processes during Pleistocene climatic fluctuations and subsequent genetic differentiation is the most over-arching process of intraspecific evolution in Anatolia. It was also concluded, however, that there is evidence for Anatolia being a large general hotspot of genetic diversity from the continental perspective (Bilgin, 2011). Accordingly, in studies of animals and plants including age calculations, intraspecific diversification processes are placed in the Pliocene or Pleistocene with a 1:1 ratio (referring to reviewed studies cited in Bilgin, 2011).

Here, we analyse a comprehensive sampling of *Grammosciadium* spp. and accessions across the distribution range in Anatolia (western IT region) and sequenced the nuclear encoded internal transcribed spacer (ITS) regions 1 and 2 of ribosomal DNA for subsequent phylogenetic analyses. The results were used to test for monophyly of *Grammosciadium*, to elaborate on a temporal evolutionary scenario and to estimate divergence times within the framework of previously published studies (Banasiak *et al.*, 2013). Three DNA regions from the plastid genome were sequenced to compare maternally inherited genetic variation with results from ITS-based phylogenetic analysis to infer spatial patterns of genetic variation and to develop a first phylogeographical scenario and introducing Anatolia as a cradle of species biodiversity.

MATERIAL AND METHODS

Plant material and taxon sampling

This study takes advantage of two former phylogenetic, phylogeographical studies. A comprehensive molecular–systematic analysis of the polyphyletic genus *Carum* was performed recently (Zakharova *et al.*, 2012) and included *Grammosciadium* spp. Phylogenetic results obtained in their contribution are congruent with recent biogeographical findings presented for Apiaceae (Banasiak *et al.*, 2013). Consequently, we used all accessions and species with available DNA sequence information for ITS (internal transcribed spacers 1 and 2 of nuclear ribosomal RNA) included by Zakharova *et al.* (2012) for phylogenetic reconstructions and calibrated phylogenetic trees using information provided by Banasiak *et al.* (2013). During our analyses we identified two potentially erroneously determined accessions from GenBank: AH008898 was published as '*G. scabridum*' and misidentified. There are two labels on the same voucher sheet. It was initially identified as *G. platycarpum*, and later as *G. scabridum*. The specimen has immature fruits, and there are no petals. Thus it is impossible to conclude that it is *G. scabridum*. However, the specimen resembles perfectly *G. macrodon* subsp. *macrodon* or *G. cornutum* (Nábělek) C.C.Townsend. The second accession, AH008896, clearly resembles *G. macrodon*. We checked the respective voucher and there is no doubt about the identity of this taxon.

In addition, a representative sampling of the various species and subspecies of *Grammosciadium* was collected in the wild over the past few years and was analysed here (Fig. 1). We also sequenced representatives of newly sampled *C. carvi*, *Falcaria vulgaris* Bernh. and *Fuernrohria setifolia* K.Koch to compare newly obtained DNA sequence variation with already existing database entries (AF077878 and JQ792211, U78378 and U78438, AF008633 and AF009221, respectively) and to check for our data quality. In summary, 88 individuals from 56 populations were analysed. Most of the accessions from *Grammosciadium* have been studied earlier morphologically (Bani & Koch, 2015; Bani *et al.*, 2016a, b). Detailed accession information is provided in Supporting Information Table S1. All *Grammosciadium* vouchers have been deposited at ANK under the indicated collection numbers.

DNA extraction, amplification and sequencing

Total DNA was extracted using the Invisorb Spin Plant Mini Kit (Stratec Biomedical AG, Birkenfeld, Germany). PCR amplification of the markers used (nuclear ITS, plastid *trnL* intron and *trnL-trnF* intergenic spacer) was performed in a volume of 25 μL, using 10 μM each primer, respectively, 2.0 mM $MgCl_2$ and 0.5 U MangoTaq polymerase (Bioline, Luckenwalde, Germany). The primers used for ITS amplification were originally designed by White *et al.* (1990) with some modifications (18F: 5′-GAAAGGAGAAGTGCTAACAAGA-3′,

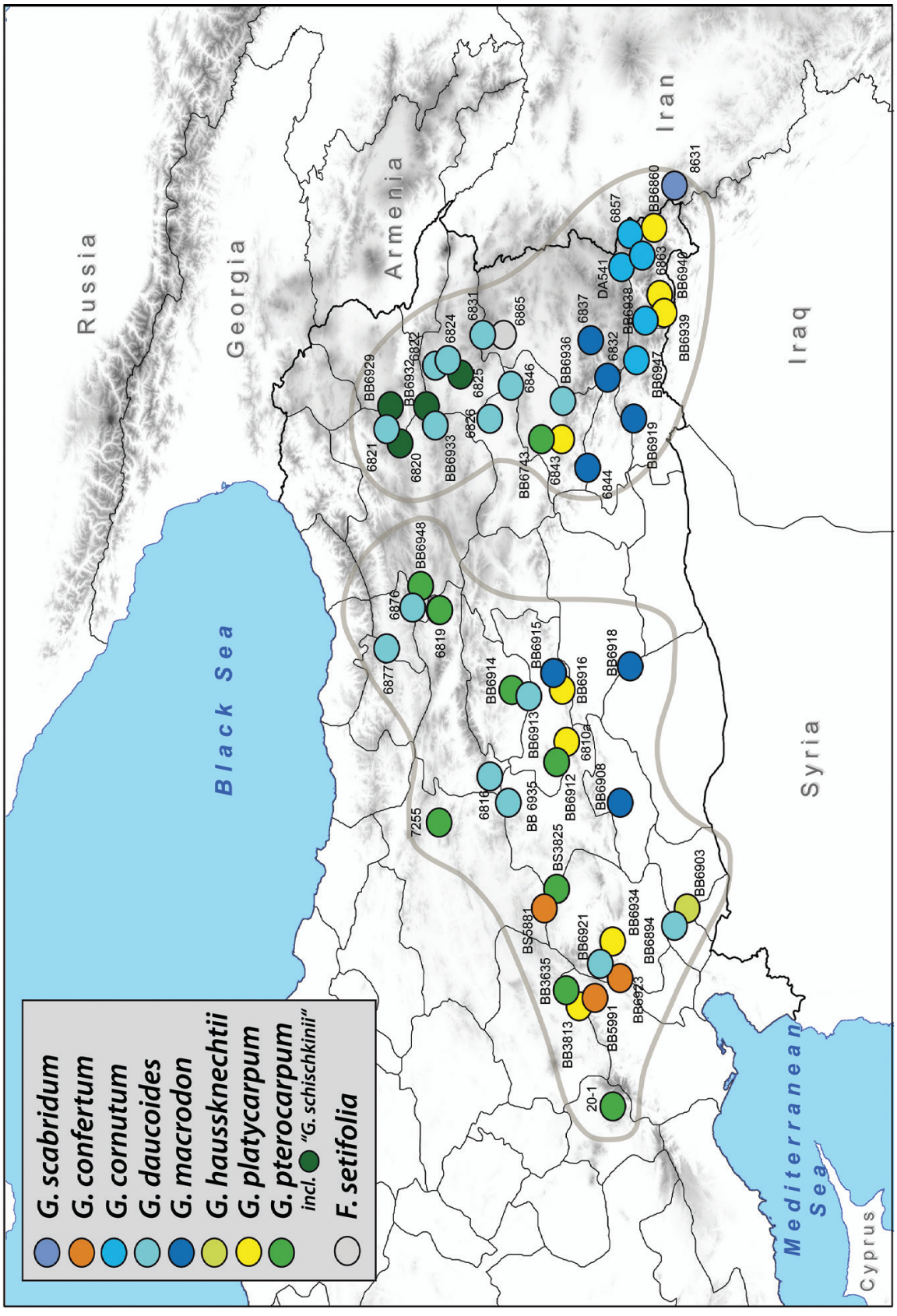


Figure 1. Distribution of analysed *Grammosciadium* and *Fuernrohria* accessions from Turkey and the Near East. Accession codes are indicated and correspond to Supporting Information Table S1. Two distribution ranges are indicated. The western range is partly congruent with the Anatolian Diagonal, which runs diagonally across central and eastern Turkey from the north-eastern corner of the Mediterranean Sea to the south-eastern corner of the Black Sea.

25R: 5′-GGGTAATCCCGCCTGACCTGG-3′). For some fragments, PCR quality was further increased to amplify ITS1 and ITS2 separately using internal primers ITS2a (5′-GCTGCGTTCTTCATCGATGC-3′) and ITS3 (5′-GCATCGATGAAGAACGTAGC-3′), respectively. Amplification of the *trnL* intron and the *trnL-trnF* intergenic spacer was performed using primers c, d and e of Taberlet *et al.* (1991) (c: 5′ -CGAAATCGGTAGACGCTACG-3′, d: 5′ -GGGGATAGAGGGACTTGAAC-3′, and e: 5′ -GGTTCAAGTCCCTCTATCATCCC-3′) and a primer designed by Dobeš, Mitchell-Olds & Koch (2004) (5′ -GATTTTCAGTCCTCTGCTCTAC-3′). The plastid *rpl16* intron was amplified using primers rpl16-rev (5′-TCTTCCTCTATGTTGACG-3′) and rpl17-for (5′-AATAATCGCTATGCTTAGTG-3′). All primers were extended by the M13 sequence for subsequent sequencing using M13 universal sequencing primers. The amplifications were run on a PTC 200 Peltier thermal cycler (MJ Research, Waltham, MA, USA) under the following conditions for ITS: 3 min initial denaturation at 95 °C; 30 cycles of amplification with 30 s at 95 °C, 30 s at 44 °C and 1 min at 72 °C; and 5 min of final elongation at 72 °C. For all three plastid loci annealing temperature was increased to 50 °C. PCR success was checked with electrophoresis in a 1% agarose gel in TAE-buffer. PCR product clean-up was executed using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Custom Sanger-sequencing was performed at GATC Biotech (Konstanz, Germany). The electropherograms were checked and trimmed to the borders of the analysed markers using the program SeqMan DNA-Star Lasergene software package (DNASTAR, Madison, WI, USA).

Chromosome number evaluation

The seeds which were used for karyological studies were selected from following the accessions: *G. daucoides* (*B. Bani 6982*), *G. macrodon* subsp. *macrodon* (*B.Bani 6887*), *G. macrodon* subsp. *nezaketiae* B.Bani (*B.Bani 6868*), *G. cornutum* (*B.Bani 6863*), *G. confertum* Hub.-Mor. & Lamond (*B.Bani 6890*), *G. haussknechtii* Boiss. (*B.Bani 6903*), *G. platycarpum* (*B.Bani 6951*) and *G. pterocarpum* subsp. *pterocarpum* (*B.Bani 6885*, *B.Bani 6966*, *B.Bani 6872*). Seeds were obtained from as many individuals as possible to prepare bulked seed samples. Seeds were germinated at 4 °C (up to 60 days for germination). Root tips were harvested after the night phase under optimal growth conditions with low starch content and incubated in 8-hydroxyquinoline (0.2 g/L) for 4 h at room temperature and washed afterwards with distilled water. Fixation performed with a 3:1 mixture of absolute ethylalcohol/glacial acetic acid at room temperature for 24 h. After washing with distilled water, root tips were transferred into 1 M HCl solution at 60 °C for 10–12 min and washed again with distilled water. Finally root tips were stained with 2% acetic orcein for 1 h with subsequent washing with water for 5 min. Prepared root tips were squashed, prepared for microscopical inspection (embedding in Entellan), and analysed using a Leica DFC295 camera attached to a Leica DM3000 microscope. At least five metaphase plates were measured for each prepared sample. Analysis of chromosome number evolution was performed with the program ChromEvol vers. 2.0 (Mayrose, Barker & Otto, 2010) and using an unconstrained maximum-likelihood tree of an ITS dataset comprising taxa from Careae (see also paragraph on phylogenetic analyses). Published chromosome numbers were taken from the following records (Retina & Pimenov, 1977; Löve & Löve in Löve, 1982; Pimenov & Vasilieva, 1983; Davlianidze, 1985; Daushkevich, Alexeeva & Pimenov, 1991, 1995; Vasilieva, Alexeeva & Pimenov, 1994; Pimenov *et al.*, 1996; Pimenov, Alexeeva & Kljuykov, 1998; Kiehn *et al.*, 2000; Nazarova & Ghukasyan, 2004; Shner, 2004; Shner *et al.*, 2004).

Editing and sequence alignment

New ITS DNA sequence data were added to a large and tribal-wide dataset (Zakharova *et al.*, 2012). The alignment was prepared by MAFFT (Katoh & Standley, 2013) using L-INS-I strategy and further manually adjusted in PhyDE (http://www.phyde.de). The final alignment consists of 503 bp (5.8S rDNA excluded) from 225 accessions and is available with the online material and includes GenBank accession codes for already published data (Supporting Information Table S2). For the BEAST analysis the alignment has been reduced and all identical sequences have been removed, resulting in a dataset comprising 173 accessions (ribotypes). Plastid DNA sequences from the *trnL* intron, *trnL-trnF* intergenic spacer and *rpl16* intron from *Grammosciadium* and its closest relatives were aligned separately using PhyDE and finally combined into a single concatenated alignment of 1943 bp (finally reduced to 1742 bp because of exclusion of ambiguous or missing sequence data). Alignment lengths of the *trnL* intron, *trnL-trnF* intergenic spacer and *rpl16* intron were 622 bp (reduced to 566 bp), 396 bp (reduced to 366 bp) and 925 bp (reduced to 808 bp), respectively. The nexus input file is provided with Supporting Information Table S3.

Phylogenetic analyses and tree calibration

For ITS phylogenetic analyses, we performed maximum-likelihood (ML) and Bayesian inference (BI) analyses. The best-fitting nucleotide substitution

model (GTR+I+G) was selected using MrModeltest 2.3 (Nylander, 2004), according to the Akaike information criterion (AIC). ML analyses were performed in RAxML (Stamatakis, 2014) implemented in raxmlGUI (Silvestro & Michalak, 2012), with the search strategy set to rapid bootstrapping. Clade support was evaluated by bootstrap analysis of 1000 replicates. In the Bayesian analyses using MrBayes v. 3.2.6 (Ronquist *et al.*, 2012), four simultaneous runs with four chains each were run for 20 million generations, sampling every 1000 trees. The first 25% of these trees were discarded as burn-in when computing the consensus tree (50% majority rule). For efficient swapping of the chains, the temperature was set to 0.01. Sufficient mixing of the chains was considered to be reached when the average standard deviation of split frequencies was below 0.01. Stationarity of the Markov chains was also confirmed in Tracer (http://tree.bio.ed.ac.uk/software/tracer/) and reliable effective sample size values (> 200) were ensured.

From the plastid DNA alignment 1943 bp in length we excluded positions 1–56, 993–1022 and 1831–1947 from subsequent analyses, because of missing sequence information from several accessions and, more importantly, because of numerous polyT-stretches and an accumulation of indels in these regions. ML and Bayesian analyses have been performed as described above using *Falcaria vulgaris* as an outgroup (a taxon found in an early-branching clade of Careae). However, these analyses did not produce any strongly supported phylogenetic trees (data not shown) and consequently here we show results from a SplitsTree analysis using SplitsTree4 vers. 4.14.4 (Huson & Bryant, 2006). Gaps were treated as missing characters and were not coded separately. A NeighborNet was calculated using uncorrected *p*-distances. Since *F. vulgaris* plastid DNA sequence information was distant compared to the other taxa, the analysis was repeated without *F. vulgaris* and was restricted to *C. carvi*, *F. setifolia* and all *Grammosciadium* plastid DNA sequence types. However, the topology of the networks did not change. Bootstrap analysis was performed running 1000 iterations.

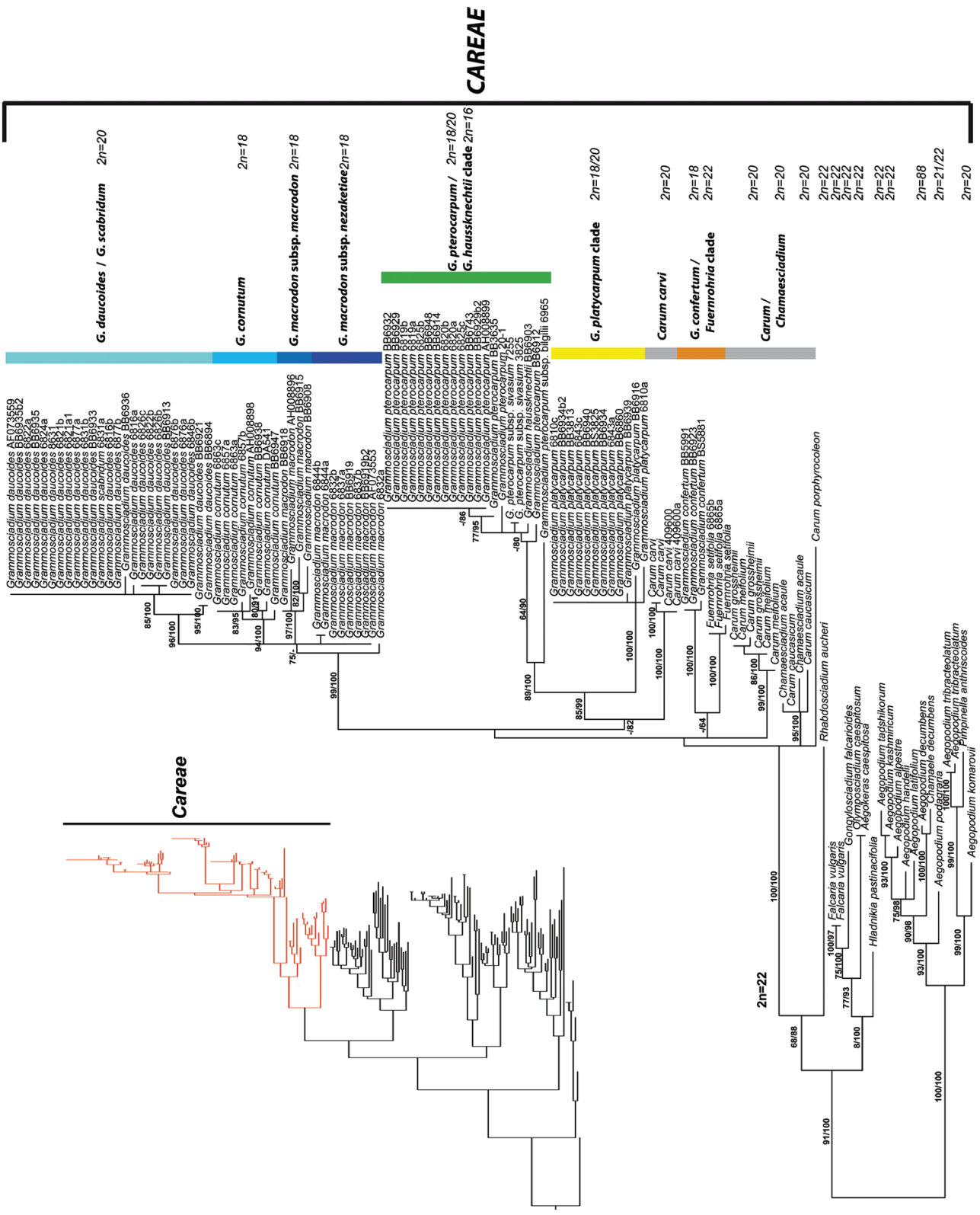
Reconstruction of a dated tree is problematic because macrofossil evidence for primary node calibration is missing. This issue has been intensively discussed for Apiaceae by Banasiak *et al.* (2013). Here we followed their approach using remnants of microfossil pollen for tree calibration. Pollen attributed to Pleurospermeae was documented from the Priabonian (38.0–33.9 Mya; Gruas-Cavagnetto & Cerceau-Larrival, 1984). Therefore, the first calibration point was placed at the stem node of Pleurospermeae and constrained to a log-normal distribution with a lower bound (offset) of 33.9 Mya. The upper bound was set to 55.8 Mya using the confidence intervals and posterior density shown by Banasiak *et al.* (2013). Secondary calibration points (median) were set at the stem nodes of Careae of 16.68 Mya, Pyramidoptereae of 17.93 Mya, Pimpinelleae/Selineae of 23.67 Mya and Oenantheae (excluding *Trocdaris* Raf.) of 25.66 Mya (Table 1). The standard deviation of the lognormal distribution was set to 0.5. The shape and log-scale parameters of the lognormal distribution were set to the ML estimates inferred from the posterior distribution of ages of corresponding nodes obtained by Banasiak *et al.* (2013). The results presented by Banasiak *et al.* (2013) are also based on ITS analysis, and we compared all posterior densities for the calibration points between the studies, which makes it a good control for estimating divergence times in general. The shape and log-scale parameters of these distributions were inferred through fitting to the posterior distributions for the chosen nodes obtained in the previous analyses (Banasiak *et al.*, 2013). These calculations were performed using SPSS vers. 22 (Released 2013, IBM SPSS Statistics for Windows, Version 22.0; IBM Corp., Armonk, NY, USA).

To test the significance of monophyly of *Grammsciadium* we ran an ML analysis with a subset of the ITS alignment (Careae) with and without constraining *Grammosciadium* into monophyly and compared respective analyses using CONSEL software (Shimodaira & Hasegawa, 2001).

RESULTS

Taxon identity and monophyletic groups in Careae

Phylogenetic analyses of the family-wide ITS-based datasets on ML and BI are fully congruent with each other (Supporting Information Figs S1, S2) and are also fully in congruence with previous phylogenetic studies (e.g. Zakharova *et al.*, 2012; Banasiak *et al.*, 2013). The redrawn results from ML analysis (based on Supporting Information Fig. S1) are shown in Figure 2 with both ML bootstrap and Bayesian posterior values indicated. In Careae, we were not able to resolve any deeper nodes significantly, but the monophyly of *Grammosciadium* was not supported. *Grammosciadium confertum* grouped consistently with *Fuernrohria setifolia*, and *Carum carvi* was nested between the two other major *Grammosciadium* clades. In ML analyses of Careae only and comparing results from constrained (H2) (monophyly of *Grammsciadium*) and unconstrained (H1) analyses the topology of the unconstrained phylogenetic tree is identical to the family-wide ML tree. In the constrained (monophyly of *Grammosciadium*) ML tree the entire structure of relationships among *Grammosciadium* spp., *C. carvi*, *Fuernrohria* and other *Carum* spp. disappeared, and we revealed a large and increased polytomy; generally bootstrap values are *c.* 30% or less. The tree structure of the unconstrained analysis (H1) has a higher likelihood (–lnL = 3004.176) than that of the constrained analysis (H2) (–lnL = 3007.533). This result



is consistent with an approximate Bayesian posterior probability test (H1/H2: $P = 0.967/0.033$), which also rejects the hypothesis of H2 with $P < 0.05$.

In all phylogenetic trees one clade consists of *G. daucoides* (including *G. scabridum*), *G. macrodon* and *G. cornutum*. ITS is able to separate the recently described subspecies *G. macrodon* subsp. *nezaketiae* along a basal polytomy from *G. macrodon* subsp. *macrodon* (Bani & Koch, 2015) and this might indicate that these can be also treated at the species level. BEAST analysis even recognizes *G. macrodon* subsp. *nezaketiae* as a monophyletic group (Fig. 4), but the Bayesian posterior value is < 50%.

The second clade consists of *G. platycarpum* and a group of taxa combining *G. pterocarpum*, *G. haussknechtii* and *G. schischkinii* (accessions *6820*, *6925*, *6929* and *6932*; Fig. 1), which has been shown to be largely indistinguishable from *G. pterocarpum* and has been synonymized accordingly (Bani *et al.*, 2016a). Accessions of *G. pterocarpum* subsp. *sivasicum* (accession *7255*) analysed here have shared ITS and plastid types, supporting previous morphological results. Also *G. pterocarpum* subsp. *bilgilii* (one accession analysed, accession *6965*) has a unique ITS type, therefore supporting morphological evidence. Genetic variation among accessions within the various species or subspecies is extremely low and ITS was the only marker which could serve as a suitable DNA barcode of high efficiency. This is congruent with an Apiaceae-wide evaluation of DNA barcodes showing a 73.3% identification efficiency of ITS (Liu *et al.*, 2014). In agreement with morphological data, ITS data fail to differentiate between *G. schischkinii* and *G. pterocarpum*; *G. scabridum* (accession *8631*) shares identical ITS sequences with some *G. daucoides* accessions (Fig. 2).

Plastid DNA sequence data support some of the results from the ITS analysis (Fig. 3). *Fuernrohria setifolia* is placed closest to *G. confertum*, and *C. carvi* is nested in *Grammosciadium* closer to *G. platycarpum* and the *G. cornutum/daucoides/macrodon* accessions. Although *G. schischkinii* is morphologically challenging (considering its type specimen), it is not distinct from *G. pterocarpum* as a whole (Bani *et al.*, 2016a). We found that some populations with haplotypes E2–E6 and E8 (Fig. 3) are separated from other plastid types of *G. pterocarpum* accessions. These haplotypes show a strong biogeographical signal (Fig. 3, north-eastern group) and are from the same region as the type specimen of *G. schischkinii* (39°31′11″N, 42°48′15″E). *Grammosciadium pterocarpum* subsp. *sivasicum* has its own distinct haplotype, but all remaining plastid types cannot be attributed significantly to certain species or subspecies except for single accessions analysed here (*G. haussknechtii* with unique haplotype F17). Consistent with the ITS analysis, *G. scabridum* (accession 8631) shares a plastid haplotype with *G. daucoides*. However, the entire DNA sequence data set (ITS and plastid data) contains also conflicting results and all of them affect *G. pterocarpum/G. schischkinii*. *Grammosciadium schischkinii* (accession *6929*) is placed in the plastid DNA network with *G. platycarpum* (although with its ITS it is placed with other *G. schischkinii*) and *G. pterocarpum* accession *6914* carries another unique plastid type (A5; unrelated to *G. pterocarpum/G. schischkinii* plastid types). This supports the conclusion that in its previous circumscription *G. schischkinii* does not exist as a distinct taxon. However, we also do not have any further significant evidence, for example for introgression and hybridization among different species, that might account for this finding and future detailed population-based genetic studies might unravel these aspects.

The genetic data do not show any obvious geographical patterns (data not shown), and there is only limited biogeographical substructure following genetically defined taxonomic groups (Fig. 1). *G. macrodon* subsp. *nezaketiae* is restricted to the eastern range (largely congruent to the Anatolian Diagonal). In addition, a genetically confirmed subclade of *G. pterocarpum* accessions (plastid types) is found here. Endemic taxa for the eastern range are *Fuernrohria setifolia* and *G. scabridum*. In the western range, we found only genetically defined *G. confertum* and the endemic *G. haussknechtii*. The most meaningful phylogeographical splits are between (1) *G. confertum* (West) versus its sister-species *Fuernrohria setifolia* (East), and (2) *G. macrodon* subsp. *macrodon* (West) versus *G. macrodon* subsp. *nezaketiae* (East), which might not be sister taxa. The remaining taxa, *G. daucoides* and *G. platycarpum*, show a wide distribution, but differing from each other, because *G. platycarpum* is the genetically better defined taxon (ITS and chloroplast DNA).

Divergence time estimates

Our divergence time estimates are fully congruent with those estimated earlier at the family level (Banasiak *et al.*, 2013). Divergence time estimates of tribe Careae are presented in Figure 4 (full information is shown with Supporting Information Fig. S3); and we also show the

Figure 2. Phylogenetic tree based on ITS data of tribe Careae. Redrawn from the apioid-wide ML analysis shown with Supporting Information Figure S1 (indicated in red with the cartoon in the upper left). ML bootstrap (left) and Bayesian posterior values (right) are provided if > 75%. Chromosome numbers have been collected from the literature and are indicated for respective taxa. Colour codes of taxa follow Figure 1.

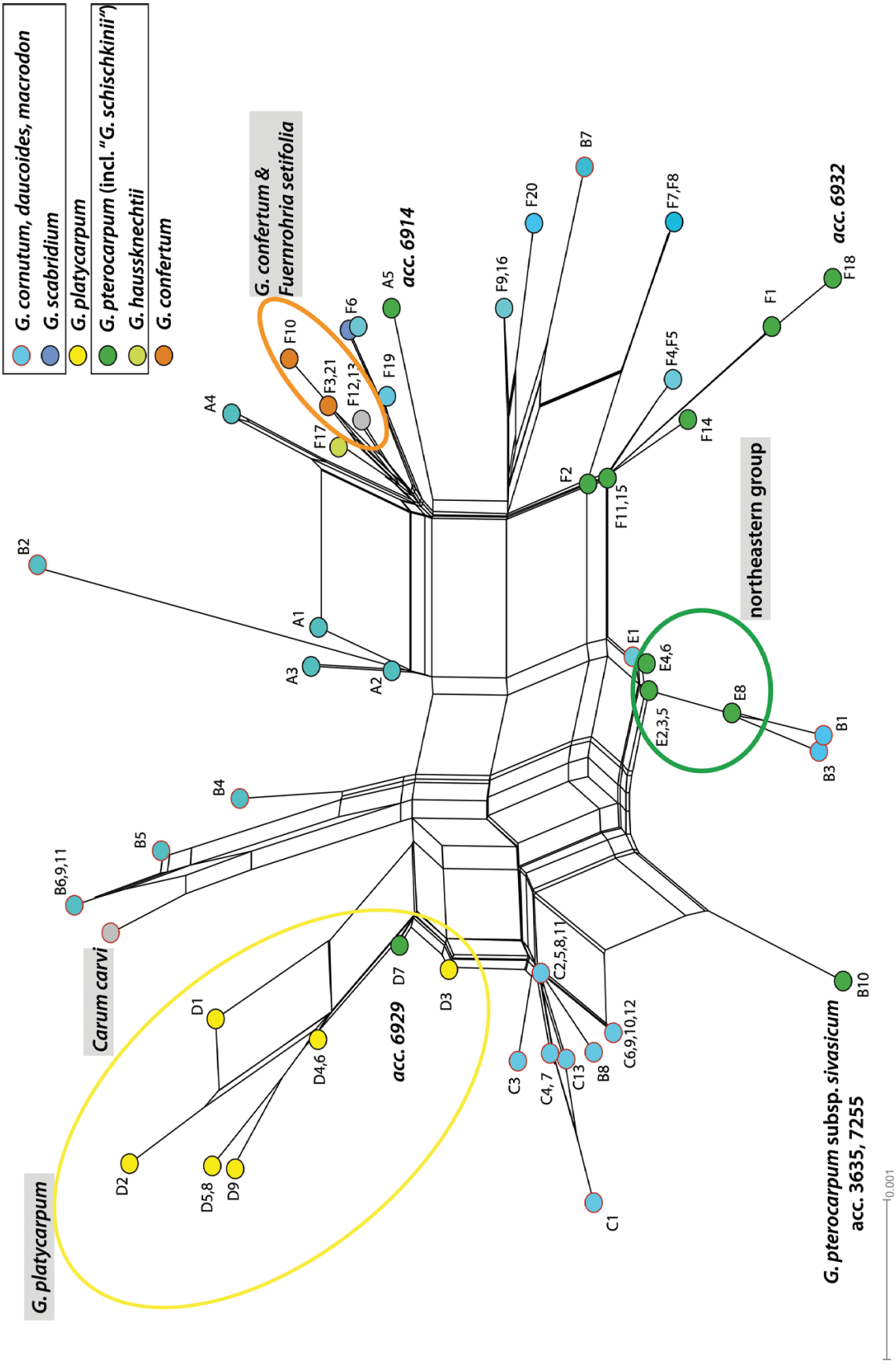
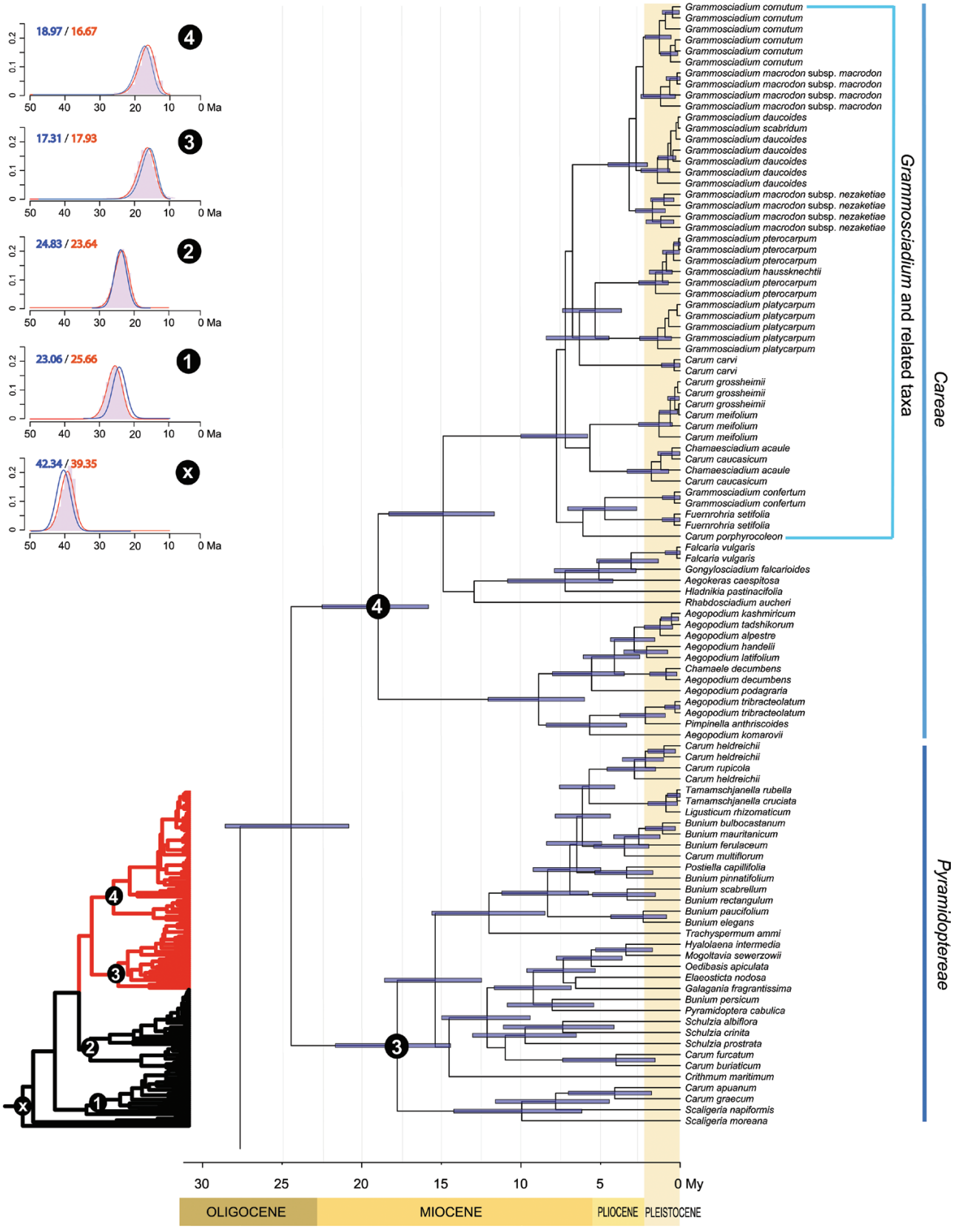


Figure 3. Parsimony SplitsTree graph for plastid DNA variation in *Grammosciadium*, *Fuernrohria* and *Carum carvi*. Colour code of accessions follows Figure 1. Bootstrap values from 1000 replicates are all < 75% and are not shown.



graphs comparing posterior densities obtained in this study for secondary calibration points (blue bars) plotted against the posterior densities obtained in the original large-scale and fossil-calibrated data (red bars; Banasiak *et al.*, 2013). The approach we use has been adopted from Spalik *et al.* (2014). Comparison of the analyses indicates not only nearly identical median split time values, but also that the distribution of posterior densities is congruent. We take this as evidence that divergence time estimates presented with our study for *Grammosciadium* diversification are consistent with previous studies. The phylogenetic hypothesis based on BEAST analysis is also congruent with ML and Bayesian analysis with one exception, and the deepest split among the various *Grammosciadum* clades/groups is calculated to 7.77 Mya. Stem group (split between Careae and Pyramidoptereae) and crown group ages of Careae are 24.43 and 18.97 Mya, respectively. Stem group ages of the *Grammosciadium* clades (1) *G. daucoides/scabridum* (2.77 Mya), (2) *G. cornutum* (2.32 Mya), (3) *G. macrodon* (3.21 Mya), (4) *G. platycarpum* (5.33 Mya), (5) *G. pterocarpum* (5.33 Mya) and (6) *G. confertum* (4.73 Mya) are all in the Pliocene. Among these splits the geographically meaningful phylogeographical splits of (i) *G. confertum* (west) versus *Fuernrohria setifolia* (east), and (ii) *G. macrodon* subsp. *macrodon* (west) versus *G. macrodon* subsp. *nezaketiae* (east) are at *c*. 4.73 and 3.21 Mya, respectively. Consequently all splits between species/subspecies date back to the Pliocene, and all further splits dated to the Pleistocene are found within species-specific ITS lineages. The aforementioned difference in phylogenetic signal among BEAST and ML/BI indicates that the *Fuernrohria/G. confertum* clade is placed sister to *Grammosciadium* including *C. carvi*, some other *Carum* spp. and *Chamaesciadium*.

Chromosome number variation

Chromosome number reports are scarce among Careae. However, there is convincing evidence that the group of taxa analysed here (*Carum*, *Chamaesciadium*, *Fuernrohria*, *Grammosciadium*) that started to radiate (crown group age) *c*. 19 Mya is characterized by an ancestral shift from $2n = 22$ to $2n = 20$ chromosomes (Fig. 2) (results from ChromEvol analysis, new chromosome counts provided with Supporting Information Table S1). It is only *Fuernrohria* that has been reported with $2n = 22$ chromosomes, but with good evidence for a sister-relationship with *G. confertum*. The results of the BEAST analysis are congruent with the chromosome number data, because *Fuernrohria/G. confertum* is placed as sister to all $2n = 20$ taxa (Fig. 4). All remaining taxa of Careae show the ancestral karyotype of Careae with $2n = 22$ with two known exceptions, *Aegopodium komarovii* (Karjagin) Pimenov & Zakharova and *A. decumbens* (Thunb. ex J.A.Murray) Pimenov & Zakharova. (Fig. 2). The results from our own ongoing chromosome number counts differ from the literature for *G. platycarpum* and *G. pterocarpum* (Table 2) and indicate parallel chromosome number reduction even below $2n = 20$ in the majority of *Grammosciadium* spp.

DISCUSSION

Phylogenetics, taxonomy and morphology

Although the phylogenetic trees presented here do not significantly resolve any deeper nodes among *Grammosciadium* evolutionary lineages (Figs 2, 4), we can conclude that there are three strongly supported lineages comprising (i) *G. daucoides*, *G. scabridum*, *G. cornutum* and *G. macrodon*, (ii) *G. pterocarpum*, *G. haussknechtii* and *G. platycarpum*, with *C. carvi* less significantly joined with this second clade, and (iii) *G. confertum* and *Fuernrohria*. This makes *Grammosciadium* a polyphyletic group and indicates the need for taxonomic changes rendering *Grammosciadium* monophyletic. Since *C. carvi* is the type of *Carum* and since *Fuernrohria* is a monotypic and morphologically defined genus, we suggest considering lineage (i) as a newly circumscribed *Grammosciadium s.s*. *Grammosciadium* taxa from lineage (ii) have been previously grouped into subgenus *Caropodium* (Table 2), the name initially introduced as a genus long ago (Stapf, 1886) with some binominals already available (*C. haussknechtii*, *C. platycarpum*, *C. pterocarpum*). To reflect the sister-relationship of *Fuernrohria* and *G. confertum* in lineage (iii), *G. confertum* has to be newly defined generically and we thus propose the genus *Vinogradovia* based on *Grammosciadium* section *Heterocarpum*. The name *Heterocarpum* has been used earlier for the sectional classification of this single species (Vinogradova, 1995) but it cannot be used as a name of a genus for a nomenclatural reason (homonymy). The details are given in the 'Taxonomic conclusions' paragraph below.

Figure 4. Maximum clade credibility tree resulting from BEAST analysis under an uncorrelated lognormal molecular clock. The figure shows part of the tree as indicated in red lines with the cartoon in the lower left (entire tree is presented in Supporting Information Fig. S3). Node ages are represented by median heights and the 95% posterior density interval is shown. Circles with numbers (1–4) denote secondary calibration points, and circle X denotes the microfossil pollen used as the primary calibration. Posterior densities of calibration points obtained in this study (blue line) are plotted against posterior densities obtained in the original calibration using this and additional fossil data (red bars and red line; Banasiak *et al.*, 2013).

Table 2. Traditional taxonomy and systematics of *Grammosciadium s.l.* and *Fuernrohria*; diploid chromosome numbers are indicated with literature data given in parentheses

Taxon			Chromosome number
Grammsciadium DC.			
Subgenus	*Grammosciadium*		
	Section	*Grammosciadium*	
		G. daucoides DC.	20 (20)
		G. scabridum Boiss.	
	Section	*Macrodon* Koso-Pol.	
		G. macrodon Boiss. subsp. *macrodon*	18
		G. macrodon subsp. *nezaketiae* Bani	18
	Section	*Ceratodon* Tamamsch. & V.M.Vinogr.	
		G. cornutum (Nábělek) C.C.Towns.	18
	Section	*Heterocarpum* V.M.Vinogr.	
		G. confertum Hub.-Mor. & Lamond	18
Subgenus	*Caropodium* (Stapf & Wettst.) Tamamsch. & V.M.Vinogr.		
	Section	*Caropodium*	
		G. platycarpum Boiss. & Hausskn.	18 (20)
	Section	*Stenodiptera* (Koso-Pol.) Tamamsch. & V.M.Vinogr.	
		G. haussknechtii Boiss.	16
		G. pterocarpum Boiss. subsp. *pterocarpum* [including *Caropodium armenum* (Bordz.) Schischkin, *G. schischkinii* (V.M.Vinogr. & Tamamsch) V.M.Vinogr.]	18 (20)
		G. pterocarpum subsp. *bilgilii* Bani	
		G. pterocarpum subsp. *sivasicum* Bani	18
Fuernrohria K.Koch			
		F. setifolia K.Koch	22

In light of this new generic classification, it is worth discussing cytological, morphological and anatomical characters. Chromosome numbers do not provide any congruent result. Respective members of *Caropodium* have diploid chromosome numbers of 16, 18 and 20 (considering also data from the literature). The newly circumscribed genus *Grammosciadium* in this study comprises representatives containing $2n = 18$ and 20. Also, *Vinogradovia* (*G. confertum*) with $2n = 18$ differs from its sister-species *Fuernrohria* with $2n = 22$. The most diagnostic characters for distinguishing taxa of *Grammosciadium* and *Fuernrohria* concern fruit characters (morphological and anatomical) and flower characteristics. These characters are summarized in Supporting Information Table S4. The close relationship between *G. confertum* and *Fuernrohria* is supported by an anatomical character, namely topology and arrangement of vascular bundles in transverse sections of fruit mericarps. In contrast to all other species, the bundles are continuous and hard to separate from each other (arranged as a ring: Vinogradova, 1995; Bani, Mavi & Adigüzel, 2011; Bani & Koch, 2015). The phylogenetic hypotheses derived from ML/Bayesian and BEAST analyses provide, with little support, two alternative relationships for *C. carvi* with respect to *Grammosciadium* and *Caropodium*. ML/Bayesian analyses suggest a relationship with (*Grammosciadium*(*Carum carvi*, *Caropodium*)) (scenario 1), whereas the BEAST analysis indicates a relationship with (*Carum carvi*(*Grammosciadium*, *Caropodium*)) (scenario 2). The second scenario is more consistent with traditional classifications, but based on morphological characters used for delimitation of the various species of *Grammosciadium* (fully supported by molecular analysis), neither of the hypotheses is more intuitive. Discrete characters such as number of vascular bundles in fruit mericarps (except *G. cornutum*), mericarp surface structure and occurrence of winged fruits and continuous characters such as fruit width and presence of sepals distinguish *Grammosciadium* and *Caropodium* (e.g. Bani & Adigüzel, 2010; Bani & Koch, 2015; Bani *et al.*, 2016a, b), but they do not allow us to make any firm conclusion about the phylogenetic–systematic position of *Carum carvi*.

Additional morphological characters such as ray number and length, sepal length, stylopodium length, style length and fruit dimensions (Bani *et al.*, 2016a, b) and leaf and bract characters (e.g. Bani & Koch, 2015) have been used successfully in morphometric analyses

to differentiate the various taxa introduced here at species and subspecies rank, but these characters do not differentiate taxa at higher taxonomic levels.

Phylogeographical implications

The crown group age of Careae was assumed to be *c.* 18 Mya. This estimate is largely congruent with previous studies (e.g. Banasiak *et al.*, 2013; Calviño, Teruel & Downie, 2016). With a number of other tribes such as Selineae or Pimpinelleae, Careae belong to a clade confined to the IT floristic region as an ancestral area since the Oligocene 31 Mya (Banasiak *et al.*, 2013). This long-term persistence in the IT region might explain the extraordinarily high number of genera and endemic taxa of Apioideae found there. The onset of diversification in *Grammosciadium* and related taxa was placed in the Miocene *c.* 7.5 Mya and gave rise to the various species of *Carum*, *Chamaesciadium*, *Fuernrhoria* and *Grammosciadium* until the end of the Oligocene. In Apioideae as a whole, the increase of evolutionary lineages over 40 My is relatively constant (log-scale) (Banasiak *et al.*, 2013), and consequently diversification events in Careae cannot be attributed to any hypothetical shift in speciation rates in Apioideae. With our data the majority of species or groups of species are of pre-Quarternary origin and are restricted to defined regions in Anatolia (Fig. 5). Most of them are found east of the Anatolian Diagonal, except *G. confertum* west of the Diagonal. This might indicate that the Diagonal acted as barrier to gene flow and migration during the Pliocene, but demonstrating high permeability throughout the Pleistocene. The three widespread Anatolian species (Fig. 5) evolved (stem group age) either earlier (*G. platycarpum*, *G. pterocarpum*; late Miocene) or later during the Pleistocene (*G. daucoides*). This might suggest that the Pleistocene, with its cycling environmental conditions, drastically affected humidity and, thereby, the distribution and extent of open steppe vegetation, providing a period in time for new diversification. In this regard, *G. pterocarpum* might be one such example. This taxon further differentiated during the Pleistocene and shows a distribution pattern (Figs 3, 5) with defined maternally inherited plastid types at the north-eastern corner of its distribution range. Similarly, these distribution patterns at the margin of the total distribution range of putative ancestral species are found with *G. pterocarpum* subsp. *bilgilii*, *G. haussknechtii* and *G. pterocarpum* subsp. *sivasicum* (Fig. 5b) and *G. scabridum* (Fig. 5f). Similar spatio-temporal diversification patterns in Anatolia have been also observed in other plant species such as *Arabis alpina* L. and its relatives (Koch *et al.*, 2006; Ansell *et al.*, 2011; Karl *et al.*, 2012) and *Aubrieta* Adans. (Koch *et al.*, 2017), indicating that the Pleistocene was an important epoch for speciation processes in Anatolia. There are only a few examples studying vicariance patterns characterized by the Anatolian Diagonal and unravelling a temporal evolutionary scenario. Bilgin (2011) provided a review of studies of intraspecific genetic diversity in Anatolia as a whole with particular reference to divergence times. However, among the discussed studies there is only one from plants (Bittkau *et al.*, 2005) and this study focuses on Aegean Island diversity also established during the Pleistocene (*Nigella arvensis* L. alliance). All other dated results reviewed by Bilgin (2011) are from animals and exemplify a diverse and complex amalgamation of spatiotemporal patterns, and it was concluded that the multiple chains of mountains including the Anatolian Diagonal do not seem to have been impermeable boundaries for post- (or peri-)glacial expansion (e.g. Ciplak, Demirsoy & Bozcuk, 1993; Demirsoy, 1996). This is fully supported by our results from spatial plastid DNA type variation. It is not possible to structure the identified haplotypes as indicated with the network analysis (Fig. 3) in a spatial context and to define geographically characterized groups (details not shown), except the example of *G. pterocarpum*. Plastid DNA-based gene diversity calculations also support this result, and in most of our cases high haplotype diversity (h) and moderate to high nucleotide diversity (π) are found in those species that originated prior to the Pleistocene (Table 3). High h and high π can be considered as an expected signature for long-term stable populations with large long-term effective population sizes. Alternatively, such a pattern could also be the result from admixture of individuals from historically sundered populations, but for this scenario our data do not provide further evidence. There are only a few studies in Apiaceae providing plastid DNA-based genetic diversity statistics on the species level. In *Pastinaca sativa* L. haplotype diversity (h) and nucleotide diversity (π) estimated from 114 individuals were 0.55–0.94 and 0.0067–0.0139, respectively (Jogesh *et al.*, 2015). In *Mulinum spinosum* Pers. high h and π were estimated (0.757 and 0.00245, respectively), on the basis of 71 individuals (Sede *et al.*, 2012). In both cases intraspecies differentiation processes were placed also into the Pleistocene.

Taxonomic conclusions

The first attempt to group the members of *Grammosciadium s.l.* was made by Boissier (1872). Schischkin (1923) properly classified the winged members in a separate genus by resurrecting *Caropodium*, which was described in 1886

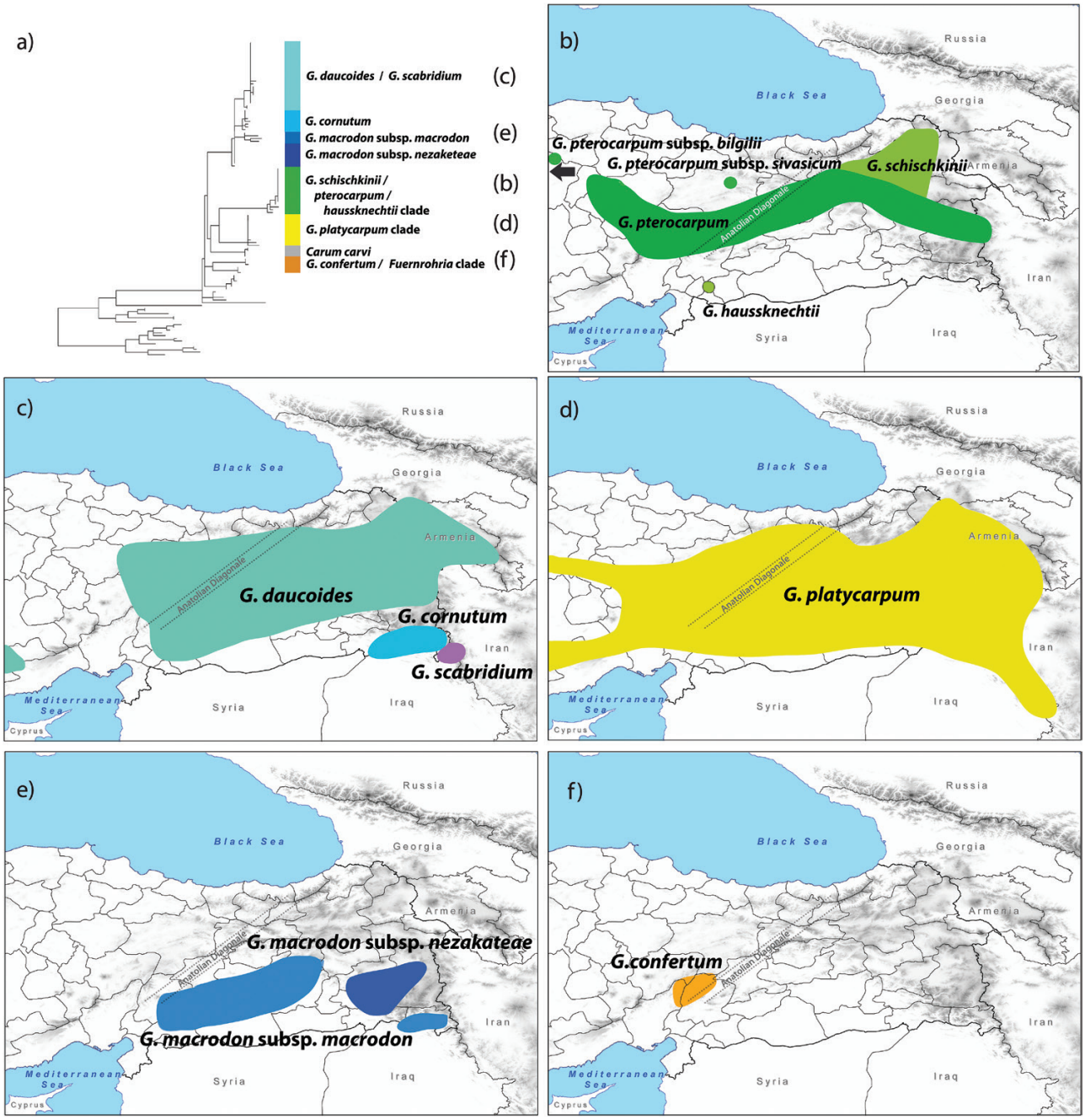


Figure 5. Cartoons of the distribution of the various *Grammosciadium* taxa and *Fuernrohria* (b–f). The schematic phylogenetic tree (a) has been redrawn from Figure 2. Colour codes correspond to Figures 1–3.

(Stapf, 1886). Finally Tamamschian & Vinogradova (1969b, 1970) reduced *Caropodium* to the rank of subgenus. However, our molecular phylogenetic hypothesis strongly supports the concept of Schischkin (1923). We designate the clades of *G. pterocarpum*/*G. haussknechtii* and *G. platycarpum* as a genus, *Caropodium*. Vinogradova (1995) discussed the possibility of recognizing *G. confertum* as a separate genus based on peculiar fruit morphology and anatomy, but favoured placing it into a monotypic section *Heterocarpum* of *Grammosciadium* subgenus *Grammosciadium sensu* Tamamschian & Vinogradova (1969b, 1970). Here we raise this section to the genus level. The new genus *Vinogradovia* has unique characters including strongly conferted, thickened and erect rays, sessile central umbels, strongly conferted fruits and heteromorphic mericarps, as discussed by Vinogradova (1995). Another

Table 3. Haplotype (h) and nucleotide (π) diversity calculations for the phylogenetically defined taxa of *Grammosciadium*; analyses were performed with DnaSP version 5.10.1 (Librado & Rozas, 2009), and standard deviations are given in parentheses

Taxon	Number of sequences	Number of haplotypes	h	π
G. pterocarpum	17	10	0.868 (0.070)	0.0034 (0.0005)
G. platycarpum	10	6	0.778 (0.137)	0.0021 (0.0006)
G. daucoides	26	12	0.911 (0.035)	0.0039 (0.0003)
G. macrodon	5	4	0.900 (0.161)	0.0024 (0.0011)
G. macrodon subsp. *nezaketiae*	5	4	0.900 (0.161)	0.0025 (0.0006)
G. cornutum	7	4	0.810 (0.130)	0.0045 (0.0009)
G. confertum	3	1	0.000	0.0000

character (reticulate-striate mericarp ornamentation), which supports the distinctness of the genus, was presented in a recent study (Bani *et al.* 2016b). Summarizing, we divide the polyphyletic genus *Grammosciadium s.l.* into three genera: *Grammosciadium s.s.*, the restored *Caropodium* and a newly described *Vinogradovia*.

The morpho-anatomical characters which support the molecular phylogenetic hypothesis are presented here. In addition to fruit wing characters, the genera of *Grammosciadium s.s.* and *Caropodium* can be distinguished traditionally by calyx length (Vinogradova 1995; Bani *et al.*, 2015, 2016b). However, detailed morphological examinations have shown that shorter calyces were also observed in samples of *G. daucoides* (0.12–1.70 mm). Thus, this character is no longer diagnostic. Bani *et al.* (2016b) indicated that fruit surface characters are useful for discrimination of these genera (rugose-favulariate and tuberculate-striate in *Grammosciadium s.s.*; reticulate-tuberculate and ribbed-striate in *Caropodium*). The presence of sclereids (stone cells) in transverse sections of the roots was found to be a good character defining the genus *Caropodium* (Ulusoy *et al.*, 2016). We observed irregularly arranged sclerenchyma tissue (not stone cells) in transverse sections of roots in *Grammosciadium s.s.* and *Vinogradovia*. The number of vascular bundles in transverse sections of mericarps of *Grammosciadium s.s.* is five (except for *G. cornutum* with nine vascular bundles) as in all members of *Caropodium*. *Grammosciadium cornutum* has an intermediate position between these genera; *Vinogradovia* and *Fuernrohria* have continuous vascular bundles in transverse sections of mericarps.

Grammosciadium s.s.

This genus comprises four species with five taxa in total. The most discriminative characters are from fruits and flowers. However, form and shape of oil-ducts in valleculae divide these five taxa into two groups. The first group contains *G. daucoides* and *G. scabridum*, which have large and elliptical vallecular oil-ducts in transverse sections of the mericarps. The second group consists of *G. macrodon* subspp. *macrodon* and *nezaketiae* and *G. cornutum*, which share the character of small and orbicular oil-ducts in vallecular regions of the mericarps. The sister-relationship of *G. macrodon* subspp. *macrodon* and *nezaketiae* remains unresolved, but at least BEAST analysis indicate that *G. macrodon* subsp. *nezaketiae* might be treated in future as a separate species with more significant phylogenetic information at hand.

Oil-ducts in the petals of *G. daucoides* are characteristically short and confined to a notch. All other taxa have long and linear oil-ducts in the petals. The number of vascular bundles also clearly separates *G. cornutum* (nine bundles) from the other species (five bundles), which is also the case for the presence of sclerenchymatous tissue in valleculae in *G. cornutum*. Large and elliptical vascular bundles in transverse sections of the mericarps and also dorsally compressed mericarps are characteristic for *G. macrodon* subspp. *macrodon* and *nezaketiae*. All other species have nearly isodiametric and relatively small vascular bundles and laterally compressed mericarps. Fruit primary ridges are generally prominent. One exception is found with *G. macrodon* subsp. *macrodon*, which has obscure primary fruit ridges. Long and recurved calyx teeth were observed in *G. cornutum* and *G. macrodon* subsp. *nezaketiae* (> 1.9 mm), clearly separating these two taxa from *G. daucoides* and *G. scabridum*. (calyx teeth straight and < 1.6 mm); *G. macrodon* subsp. *macrodon* has an intermediate position in terms of calyx teeth characteristics (straight and 1.6–6.0 mm).

Caropodium

As a result of the phylogenetic analysis presented herein, *G. platycarpum*, *G. haussknechtii*,

G. pterocarpum subspp. *pterocarpum*, *sivasicum* (accessions *BS3825*, *7255*) and *bilgilii* (*6965*) are transferred from *Grammosciadium s.l.* to *Caropodium*, which now comprises five taxa. Morpho-anatomical affinities and important characteristics of *Caropodium* were previously discussed in detail (Bani *et al.*, 2016a). Long pedicellate stipular segments are characteristic for *C. platycarpum*. All other taxa in this genus have nearly sessile stipular segments (Bani *et al.*, 2016a). Spreading fruits, and thickened and straight umbel rays (in fruiting stage) separate *C. platycarpum* and *C. haussknechtii* from the *C. pterocarpum* complex. Width of fruit wings was previously used as one of the most discriminative characters (Boissier, 1872; Bordzilowski 1915; Tamamschian and Vinogradova, 1969a, b, 1970; Vinogradova, 1995). The *C. pterocarpum* complex, in particular, shows high morphological variation as indicated earlier (Bani *et al.*, 2016a). Width of fruit wings gradually increases from west to east. Recently, an isolated population in western Anatolia has been discovered and described as a new subspecies (subsp. *bilgilii*) based on narrower wings and shorter fruits (Bani *et al.*, 2016a). Although the widest winged populations were observed in areas adjacent to the type locality of previously synonymized species *G. schischkinii* (north-east of the distribution range of *G. pterocarpum*, Fig. 5), it was not possible to find any character to support the distinctness of these populations when considering all populations within the distribution area of the *C. pterocarpum* complex, with continuous characters always overlapping.

We provide below identification keys to the genera and infrageneric taxa, followed by a description of *Grammosciadium*, *Vinogradovia* and *Caropodium* including infrageneric classifications. However, infrageneric classification is presented here only to avoid future confusion with earlier taxonomic concepts. Generally, we do not see any necessity for further subgeneric or sectional classification.

Key to *Carum*, *Caropodium*, *Fuenrrohria*, *Grammosciadium s.s.* and *Vinogradovia*

1. Fruits less than three times longer than broad
2. Bracteoles present……*Fuernrohria* (*F. setifolia*)

*2. Bracteoles absent……*Carum* (*C. carvi*)

*1. Fruits more than three times longer than broad
3. Lateral ridges of mericarps unwinged
4. Central umbels sessile; fruiting rays conferted……*Vinogradovia*

*4. Central umbels pedicellate, fruiting rays not conferted……*Grammosciadium*

*3. Lateral ridges of mericarps winged……*Caropodium*

Grammosciadium, *key to the taxa*

1. Petal oil-duct short, confined to notch……*G. daucoides*

*1. Petal oil-duct linear
2. Nine vascular bundles in each mericarp……*G. cornutum*

*2. Five vascular bundles in each mericarp
3. Wing-like stria present on dorsal side of primary ridges of mericarps; sepals recurved ……*G. macrodon* subsp. *nezaketiae*

*3. Wing-like stria absent on dorsal side of primary ridges of mericarps; sepals erect or patent
4. Vallecular vittae smaller and orbicular; vascular bundles elliptical in each mericarp ……*G. macrodon* subsp. *macrodon*

*4. Vallecular vittae larger and elliptical; vascular bundles isodiametric in each mericarp ……*G. scabridum*

Caropodium, *key to the taxa*

1. Fruiting rays thickened; fruits spreading
2. Stipular segments of cauline leaves long pedicellate; two funicular oil-ducts present in each mericarp……*C. platycarpum*

*2. Stipular segments of cauline leaves nearly sessile; funicular oil-duct absent in each mericarp……*C. haussknechtii*

*1. Fruiting rays not thickened; fruits erect
3. Two funicular oil-ducts present in each mericarp……*C. pterocarpum* subsp. *sivasicum*

*3. Funicular oil-duct absent in each mericarp
4. Wings of mericarp > 1 mm broad……*C. pterocarpum* subsp. *pterocarpum*

*4. Wings of mericarp < 1 mm broad……*C. pterocarpum* subsp. *bilgilii*

Synopses of Grammosciadium, Carodium *and* Vinogradovia

Grammosciadium *DC., Coll. Mem. 5: 62 (1829)*
Type [LT, designated by Pimenov & Tikhomirov, Novosti Sist. Vyssh. Rast. 16: 163 (1979)]: *G. daucoides* DC.

Perennial plants with stout tap roots crowned with a fibrous collar. Leaves three- to five-pinnatisect; segments linear and mucronate. Cauline leaves with leaf-like stipules. Bracts and bracteoles present. Umbels polygamous. Sepals present. Petals white with central oil-duct, outer petals somewhat radiate. Mericarps glabrous, ± terete, linear to oblong, vittate, primary ridges five. Vascular bundles five or nine in each mericarp.

Grammosciadium *section* Grammosciadium

1. ***G. daucoides* DC., Coll. Mem. 5: 62 (1829).**
   ≡*Prionitis daucoides* (DC.) Koso-Pol. in Bull. Soc. Imp. Naturalistes Moscou 29: 140 (1916).
   =*G. szovitsii* Boiss. in Ann. Sci. Nat. ser. 3(2): 67 (1844).
   =*G. aucheri* Boiss. in Ann. Sci. Nat. ser. 3(2): 67 (1844).
   ≡*P. aucheri* (Boiss.) Koso-Pol. in Bull. Soc. Imp. Naturalistes Moscou 29: 140 (1916).
   =*G. aucheri* subsp. *pauciradiatum* Freyn & Sint. in Oesterr. Bot. Z. 42: 128 (1892).
2. *G. scabridum* Boiss. in Ann. Sci. Nat. ser. 3(2): 66 (1844).
   ≡*Prionitis scabrida* (Boiss.) Koso-Pol. in Bull. Soc. Imp. Naturalistes Moscou 29: 140 (1916).
   = *G. longilobum* Boiss. & Hausskn. in Boiss., Fl. Orient. 2: 900 (1872).
   ≡*Prionitis longiloba* (Boiss.) Koso-Pol. in Bull. Soc. Imp. Naturalistes Moscou 29: 140 (1916).

Grammosciadium *section* Macrodon *Koso-Pol. in Journ, Russ. Bot. 1915 (1–2): 12 (1915).*

3. *G. macrodon* Boiss. in Ann. Sci. Nat. ser. 3(2): 67 (1844).

3a. *G. macrodon* subsp. *macrodon*
   ≡*Prionitis macrodon* (Boiss.) Koso-Pol. in Bull. Soc. Imp. Naturalistes Moscou 29: 137 (1916).

3b. *G. macrodon* subsp. *nezaketiae* Bani in Phytotaxa 224 (3): 271 (2015).

***Grammosciadium* section *Ceratodon* Tamamsch. & V.M.Vinogr.** [in Vinogradova, Monogr. Review of *Grammosciadium* DC.: abstract of PhD thesis. 14 (1971), nom. inval.], **sect. nov.** Mericarps with nine vascular bundles in transverse section developed both in primary ribs and valleculae (vs. five in primary ribs only in other *Grammosciadium* spp.).

Type: *G. cornutum* (Nábělek) C.C.Towns.

The name of this section first appeared in the abstract of a PhD thesis (Vinogradova, 1971) with neither a description nor diagnosis and was therefore not effectively or validly published. Similarly, it is also a *nomen nudum* in Vinogradova (1995), apparently the only paper where it was subsequently used. As long as one of the assumed authors repeatedly used this name in a given sense clearly demonstrating the same intention, it is validated here with original authorship.

5. *G. cornutum* (Nábělek) C.C.Towns. in Kew Bull. 20: 83 (1966).
   ≡*G. macrodon* var. *cornutum* Nábělek in Publ. Fac. Sci. Univ. Masaryk, Brno 35: 124 (1923).

Vinogradovia *Bani, D.A.German & M.A.Koch, nom. et stat. nov.*

Type: *V. conferta* (Hub.-Mor. & Lamond) Bani, D.A.German & M.A.Koch

Based on: *Grammosciadium* subgenus *Caropodium* section *Heterocarpum* V.M.Vinogr. in Bot. Zhurn. 80 (1): 94 (1995).

Biennial plants with fusiform roots without a fibrous collar. Leaves three- (four-)pinnatisect; segments linear and mucronate. Cauline leaves with leaf-like stipules. Rays strongly thickened and conferted. Bracts and bracteoles present. Umbels polygamous, central umbel sessile. Sepals present. Petals white with central oil-duct, outer petals somewhat radiate. Mericarps glabrous, ± terete, heteromorphic, linear to oblong, vittate, primary ridges five. Vascular bundles continuous as a ring in each mericarp.

The new generic name is introduced in order to avoid creation of '*Heterocarpum*', which apparently would be treated as a later homonym of a name of the lichen genus *Heterocarpon* Müll. Arg. (1885). *Vinogradovia* commemorates Vera Mikhaylovna Vinogradova (1937–2008) who contributed greatly to the systematics of *Grammosciadium s.l.* and, in particular, emphasized the distinctiveness of *G. confertum* by placing it in a separate section of *Grammosciadium*.

1. *V. conferta* (Hub.-Mor. & Lamond) Bani, D.A.German & M.A.Koch comb. nov.

Basionym: *G. confertum* Hub.-Mor. & Lamond in Notes Roy. Bot. Gard. Edinburgh 31: 75 (1971).

Caropodium Stapf et Wettst. in Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 51: 317 (1886). Type: *Caropodium meoides* Stapf & Wettst.
=*Stenodiptera* Koso-Pol. in Journ. Russ. Bot. 1915 (1–2): 12 (1915).

Perennial plants with stout tap roots crowned with a fibrous collar. Leaves three- to six-pinnatisect; segments linear and mucronate. Cauline leaves with leaf-like stipules. Bracts and bracteoles present. Umbels polygamous. Sepals present. Petals white with central oil-duct, outer petals somewhat radiate. Mericarps glabrous, ± terete, linear to oblong, vittate, primary ridges five, laterals winged. Vascular bundles nine in each mericarp.

Caropodium *section* Caropodium

1. *C. platycarpum* (Boiss. & Hausskn.) Schischkin in Not. Syst. Herb. Hort. Bot. Petrop. 4: 30 (1923).

Basionym: *G. platycarpum* Boiss. & Hausskn. in Boiss., Fl. Orient. 2: 901 (1872). ≡*Stenodiptera platycarpa* (Boiss. & Hausskn.) Koso-Pol. in Journ. Russ. Bot. 1915 (1–2): 13 (1915).
=*Caropodium meodies* Stapf et Wettst. in Denkschr. Kaiserl. Akad. Wiss., Wien. Math.-Naturwiss. Kl. 51: 317 (1886).

Caropodium *section* Stenodiptera *(Koso-Pol.) Bani & M.A.Koch comb. nov.*

Type [LT designated here]: *Caropodium pterocarpum* (Boiss.) Schischkin

Basionym: *Stenodiptera* Koso-Pol. in Journ. Russ. Bot. 1915 (1–2): 12 (1915).

2. *C. haussknechtii* (Boiss.) Schischkin in Not. Syst. Herb. Hort. Bot. Petrop. 4: 30 (1923).
Basionym: *Grammosciadium haussknechtii* Boiss., Fl. Orient. 2: 901 (1872).
≡*Stenodiptera haussknechtii* (Boiss.) Koso-Pol. in Journ. Russ. Bot. 1915 (1–2): 13 (1915).
3. *C. pterocarpum* (Boiss.) Schischkin in Not. Syst. Herb. Hort. Bot. Petrop. 4: 30 (1923).

3a. *C. pterocarpum* subsp. *pterocarpum*
Basionym: *Grammosciadium pterocarpum* Boiss. in Ann. Sci. Nat. ser. 3(2): 68 (1844).
≡*Stenodiptera pterocarpa* (Boiss.) Koso-Pol. in Journ. Russ. Bot. 1915 (1–2): 13 (1915).
=*Grammosciadium pterocarpum* subsp. *longipes* Freyn in Bull. Herb. Boiss. sér. 2, 1: 269 (1901).
=*Stenodiptera armena* Bordz. in Mem. Soc. Nat. Kiev 25 (1): 96 (1915).
=*Caropodium pterocarpum* var. *schischkinii* V.M.Vinogr. & Tamamsch. in Notes Roy. Bot. Gard. Edinburgh 28: 203 (1968).
≡*Grammosciadium schischkinii* (V.M.Vinogr. & Tamamsch.) V.M.Vinogr. in Bot. Zhurn. 80 (1): 94 (1995).

3b. *C. pterocarpum* subsp. *bilgilii* (Bani) Bani & M.A.Koch comb. nov.
Basionym: *Grammosciadium pterocarpum* subsp. *bilgilii* Bani in PhytoKeys 68: 80 (2016).

3c. *C. pterocarpum* subsp. *sivasicum* (Bani) Bani & M.A.Koch comb. nov.
Basionym: *Grammosciadium pterocarpum* subsp. *sivasicum* Bani in PhytoKeys 68: 81 (2016).

CONCLUSIONS

Grammosciadium has been shown to be paraphyletic in its traditional circumscription. Morphological and anatomical characters are not able to unravel the relationships with *Carum* and *Fuernrohria* unambiguously, but in light of molecular data, morphological characters can be interpreted accordingly to present a new generic concept. Phylogenetic analyses demonstrate that most species started to evolve during the late Miocene and Pliocene in Anatolia, highlighting the importance of this region as a cradle of biodiversity of the western IT region.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Detailed accession list and GenBank accession numbers.
Table S2. Nexus file of ITS sequence data.
Table S3. Nexus file of plastid sequence data.
Table S4. Summary of diagnostic morphological and anatomical characters.
Figure S1. Phylogenetic tree based on nuclear ITS data (maximum-likelihood inference of tribal alignment).
Figure S2. Phylogenetic tree based on nuclear ITS data (Bayesian inference of tribal alignment).
Figure S3. Results from BEAST analysis based on nuclear ITS data (*.tre input file).