

BIOGEOGRAPHIC RECONSTRUCTION OF THE GENUS FERULA INFERRED FROM ANALYSES OF nrDNA AND cpDNA SEQUENCES

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Received 2019. 05. 22; accepted for publication 2019. 11. 20

Panahi, M., 2019. 12. 30: Biogeographic reconstruction of the genus *Ferula* inferred from analyses of nrDNA and cpDNA sequences. -*Iran. J. Bot.* 25 (2): 79-94. Tehran.

The divergence time of the largely Asian element, the genus *Ferula* L. (subtribe Ferulinae, tribe Scandiceae, Apiaceae) was initially analyzed using nrDNA, internal transcribed spacer (ITS) sequence data followed with three regions of cpDNA (*rps16* and *rpoC1* introns and *rpoB-trnC* intergenic spacers) from 141 representatives of subtribe Ferulinae (*Ferula*, *Dorema*, *Leutea*) and relatives. Further analyses of the biogeographical history of the *Ferula* group were carried out using BEAST, S-DIVA and BBM in RASP on all datasets. The results suggested that the initial split within Ferulinae occurred during Early Pliocene about 4.8 Ma and earlier ancestor of *Ferula* group has been originated mostly in Armeno-Iranian province. One descendent giving rises to the *Ferula* clade and another descendent subsequently underwent further divergence to account for *Leutea* lineages about 1.8-2 Ma. The rapid diversification within Ferulinae clade has occurred since the Middle Pliocene. Subsequent diversification of the *Ferula* clade intensified in the Irano-Turanian region during the late Pliocene in the Central Asian zone and toward west in Mediterranean zone during early Pleistocene. The ancestor of Chinese group of *Ferula* spread from Central Asia to eastern Asia in the Pliocene (2.2-4 Ma). The ancestor of the Mediterranean group of *Ferula* occurred in the West Mediterranean with high marginal probability. These diversifications likely corresponded with the Himalayas-Tibetan plateau uplift-driven climatic changes and aridification of Central Asia and also after refilling the Mediterranean basin and Zanclean flood during the Pliocene respectively.

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Key words: Biogeography; *Ferula*; Apiaceae; molecular phylogeny; cpDNA; nrDNA

بیورژوگرافی جنس فرولا (*Ferula* L.) از تیره چتریان برگرفته از آنالیز توالی‌های ناحیه ژنومی هسته و کلروپلاست مهرانوش پناهی: استادیار پژوهشی، مؤسسه تحقیقات جنگلها و مراتع کشور، سازمان تحقیقات، آموزش و ترویج کشاورزی، تهران، ایران در این مطالعه، زمان انشقاق عنصر بزرگ آسیایی *Ferula* L. متعلق به زیرطایفه Ferulinae، طایفه Scandiceae، و تیره چتریان (Apiaceae) توسط توالی‌های ناحیه ITS (Internal transcribed spacer) ژنوم هسته و سه ناحیه کلروپلاستی غیرکدکننده (اینترون *rps16* و اینترون *rpoC1* و ناحیه بین ژنی *rpoB-trnC*) تخمین زده شد. داده‌های حاصل از ۱۴۱ تاکسون زیرطایفه Ferulinae (*Ferula*, *Dorema*, *Leutea*) به همراه گونه‌های خویشاوندشان، توسط آنالیزهای BEAST، S-DIVA و BBM اجرا شده در برنامه RASP مورد ارزیابی قرار گرفتند. نتایج بدست آمده مشخص نمود که شکاف اولیه درون Ferulinae در طول اوایل پلیوسن (حدود ۴/۸ میلیون سال قبل) رخ داده و تاکسون‌های دودمان اولیه این زیرطایفه عمدتاً از پروانس ارمنستان-ایران منشاء گرفته‌اند. بنابراین یک انشعاب اجدادی به اعضای گروه *Ferula* منجر شده و متعاقباً انشعاب دیگر تحت انشقاق بیشتر اعضای دودمان *Leutea* را (حدود ۱/۸ الی ۲ میلیون سال قبل) پدید آورده است. تنوع‌یابی درون گروه *Ferula*، در ناحیه ایرانو-تورانی صورت گرفته به طوری که در اواخر پلیوسن در ناحیه آسیای مرکزی و در ابتدای پلیستوسن به سمت غرب در ناحیه مدیترانه‌ای تشدید

شده است. اجداد گونه‌های شرق دور *Ferula*، در دوره پلیوسن (۲/۲-۴ میلیون سال قبل) از آسیای مرکزی به سمت آسیای شرقی گسترش یافته و اجداد گروه مدیترانه‌ای در ناحیه مدیترانه غربی با ضریب احتمالی بالا تنوع یافته‌اند. احتمالاً این انشقاق‌ها به ترتیب با بالا آمدن صفحه هیمالیا-تبت، تغییرات اقلیمی و خشک شدن حوزه آسیای مرکزی، و همچنین دوباره پرشدن حوزه مدیترانه‌ای و جریان Zanclean در پلیوسن ارتباط دارد.

INTRODUCTION

Historical biogeography is concerned with dispersal, extinction and vicariance (speciation events), all combined with the geological history of the Earth in order to explain the present and past distribution patterns of biological diversity and their underlying environmental and historical causes (Riddle & al. 2008). Proponents of vicariance explanations defend that distribution patterns can mainly be explained by geological, tectonic-isolating events and dispersal pattern are largely the result of recent migration events (Sanmartin 2012). Since the advent of molecular phylogenetics, some novel methods have been developed in order to reconstruct the biogeographic history of a taxon or a group of taxa. This approach used to find the evolutionary relationships of organisms in form of a tree to infer their biogeographic history.

Within past decades, methods for estimating divergence times including Bayesian and likelihood methods have been developed and modified (Drummond & al. 2006; Thorne, Kishino & Painter 1998; Lamm & Redelings 2009) and a variety of models and methods already exist and compare in historical biogeography that some allow for different kinds of cladogenetic events occur along branches (Matzke 2014; Ali, & al. 2012; Buerki & al. 2011). Model selection by Zhang & al. (2017) highlight a great need for comprehensive model exploration and statistically mediated data-to-model fit optimization in support of reliable historical biogeographic inferences.

Based on recent suggestions for the ancestor of Apiales that originated in the Australasian tropics before migrating into the northern temperate areas, where many members of subfamily Apioideae are found today; apioids may have originated in the southern Africa and migrated northward to Eurasia, where they have experienced a rapid radiation (Plunkett, Soltis & Soltis 1996; Chandler & Plunkett 2004; Calviño, Martínez & Downie 2008). The southern African origin of subfamily Apioideae was inferred by Calviño & al. (2006); these authors also investigated the evolution of life form in the subfamily and concluded that although a number of southern African protoapioid taxa have a woody habit, the ancestral condition for this subfamily is likely to be herbaceous.

DNA sequence data have proven to be a promising

and powerful tool for elucidating relationships within Apiaceae that were difficult to resolve with morphological data alone (Downie & al. 2001). Molecular data are also useful for reconstructing infrageneric relationships and for inferring the biogeographic histories of Apiaceae genera (Wen & al. 2002). The last Phylogenetic findings revealed the taxonomic position of *Ferula* L. within tribe Scandiceae Spreng. subtribe Ferulinae Engl. in companion with *Dorema* D. Don and *Leutea* Pimenov (Kurzyna-Młynik & al. 2008). Subsequent phylogenetic analyses (using nrDNA ITS and three cpDNA noncoding sequences) showed that *Leutea* is sister to *Ferula*, while *Dorema* is nested within it (Panahi & al. 2015) and recently the major clades within Ferulinae have been proposed (Panahi & al. 2018). Most members of *Ferula* comprising 170 species (Pimenov & Leonov 1993) are distributed in Central Asia or Irano-Turanian Floristic Region, but some members occur in adjacent areas to the east (China) or to the west in the Mediterranean region reaching as far as the Canary Islands, e.g. *F. linkii* Webb (Korovin 1947; Chamberlain & Rechinger 1987; She & al. 2005). The genus includes both narrow endemics and broadly distributed species with overlapping distributions. Therefore, proposing an obvious radiation and diversification pattern for this genus would be necessary allowing us to study the biogeographical connections between the distributed regions and its pattern. Different geological and climatic events are likely to have played a role in the origin and diversification of *Ferula* in the Irano-Turanian and Mediterranean floristic regions. Korovin (1940) regarded ecological conditions, particularly the availability of water, as major factor affecting the diversification of *Ferula*. Based on the climatic conditions in the Irano-Turanian Region, he suggested that the ancestor of *Ferula* was a monocarpic mesophyte with broad leaf lobes, whereas polycarpy and narrow leaf lobes appeared as adaptations to more arid conditions. Analyzing the evolution of life history strategies against a molecular phylogeny, Kurzyna-Młynik & al. (2008) confirmed Korovin's hypothesis that monocarpy is ancestral condition for *Ferula*.

Here, it is generated a dated phylogeny evolution of the genus to address the following questions: (1) When and where did *Ferula* originate? (2) When did the

descendent of *Ferula* in different area originated and along which route did they colonize in those regions?

MATERIALS AND METHODS

Floristic regions

A distribution map of *Ferula* was generated and

divided it into floristic regions according to the designations of Takhtajan (1986). The geographic range of *Ferula* was divided into 14 areas, based on the extant distribution of the genus and the floristic regions it covers was defined on the basis of Takhtajan's classification (table 1).

Table 1: Geographic area obtained from Takhtajan (1986) classification using in Biogeography analyses.

Symbol	Province List
A	Mesopotamian Prov. [Iraq, NE Syria, SE Turkey, SW Iran]
B	Central Anatolia Prov.
C	Armeno-Iranian Prov.
D	Hyrcanian Prov.
E	Turanian or Aralo-Caspian Prov.
F	Turkestan Prov.
G	Northern Baluchistan Prov.
H	Central Tian-Shan Prov.
I	Dzungaria and Tian Shan Prov.
J	South Mediterranean
K	West Mediterranean (Iberian Prov.)
L	East Mediterranean
M	South Iranian Prov. (Sudano Zambezi region, Paleotrop. King)
N	Sikang Yunnan Prov. (Eastern Asiatic region, Boreal Subking.)

Sampling and phylogenetic analyses

The plant materials (dried leaves) used for molecular studies have been collected from herbarium specimens that were indicated in the last phylogenetic studies (Panahi & al. 2018). Phylogenetic relationships were reconstructed using combined DNA sequences from nuclear ribosomal DNA (ITS regions) and three loci of the chloroplast genome (cpDNA): the *rps16* intron, the *rpoC1* intron and *rpoB-trnC* intergenic spacer. The final data set comprised a total of 3499 characters from 141 accessions: 136 from the ingroup, representing Ferulinae members in 126 species of *Ferula* (the former genus *Dorema* that is now subsumed under *Ferula* (Panahi & al. 2015) is represented by 6 species) and 8 species of *Leutea*. The outgroups included two species of the *Glaucosciadium* clade (*G. cordifolium* (Boiss.) B. L. Burtt & P. H. Davis and *Mozaffariania insignis* Pimenov & Maassoumi) and 3 species of *Thapsia* L. from subtribe Daucinae that comprised a sister group to subtribe Ferulinae, were examined for four chosen markers (nrDNA and 3 cpDNA targets). Voucher information, references and GenBank accession numbers were represented in previous studies (Panahi & al. 2015; Banasiak & al. 2016; Panahi & al. 2018).

Molecular dating analyses

In order to obtain calibrated trees that may serve for the reconstruction of biogeographical history of Ferulinae, a series of analyses in BEAST version 1.10.4 (Suchard & al. 2018) was performed. The core algorithm implemented in BEAST v. 1.10.4 is Metropolis-Hastings MCMC (Drummond & Rambaut 2007). The target is to provide a posterior distribution (PP) on a set of molecular sequences with focus on calibration point for phylogenetic trees incorporating a time scale. This is achieved by explicitly modeling the rate of molecular evolution on each branch in the tree. One of the most used models is *lognormal relaxed molecular clock* model that does not assume a constant rate across lineages. The molecular clock is one of the useful tools to investigate the timing of phylogenetic events, to date the origin of taxonomic groups, to estimate rates of speciation and extinction and to determine the timing of dispersal events (Weir & Schluter 2008; Thorne, Kishino & Painter 1998).

Totally, 141 taxa were used in the combined cpDNA and nrDNA data matrix that manually aligned using Mesquite 3.6 (Maddison and Maddison 2017), then the matrix was used for the Bayesian analyses using BEAST and the tree was used as a starting tree.

(Drummond & Rambaut 2007). To estimate divergences time, through Bayesian analyses using BEAST, the data were imported in BEAUti program (v.1.10.4, Suchard & al. 2018). The GTR substitution model of sequence evolution with Gamma distribution and proportion of invariable sites (GTR + G + I) best fitted the data. An uncorrelated lognormal model was used to describe the relaxed clock (Drummond & al. 2006; Brown & Yang 2010) and a pure birth branching process (Yule model) was chosen as prior. Due to a lack of Ferulinae or Scandiceae fossils that might serve for tree calibration, it was used a secondary calibration point placed at the crown node of Ferulinae based on the estimation on divergence time of the main lineages of Apioideae done by Banasiak & al. (2013). The estimated median age of the crown node of Ferulinae was equal to 5.7997; this was subsequently taken as a mean of normal distribution with standard deviation equal to 1.312, which reflects 97.5 % HPD confidence interval ranges for that estimate. All gaps were removed from the matrix because they can pose difficulty for model-based phylogenetic estimation (Yang 2006).

50 million generations of the MCMC chains were run with sampling every 1000 generations. Convergence to the stationary distribution was checked by visual examination of the plotted posterior estimates using Tracer ver. 1.7.1 (Rambaut & al. 2018) and the ESS scores were satisfactory (more than 200). After discarding the first 5,000 trees as burn-in, the samples were summarized on the maximum clade credibility tree using TreeAnnotator v.1.10.4 (Suchard & al. 2018) with the posterior probability (PP) limit set to 0.5 and median node heights. The results were visualized using FigTree ver. 1.4.4 (Suchard & al. 2018).

Biogeographical analyses

Due to the increasing availability of molecular phylogenetic data, historical biogeography has much developed for last two decades. It was used the recently developed statistical dispersal-vicariance analysis (S-DIVA) and Bayesian binary MCMC analysis (BBM) implemented in RASP version 4.0 Beta (Yu & al. 2015) to reconstruct the possible ancestral ranges of the genus on our phylogenetic tree. S-DIVA uses the collection of trees from a Bayesian analysis and can handle optimization uncertainty in reconstructing biogeographic histories (Yu & al. 2010). The latter method averages the frequencies of an ancestral range at every node over all input trees. BBM, unlike S-DIVA, offers a statistical procedure for inferring states at ancestral nodes using a full hierarchical Bayesian approach (Nylander & al. 2008; Ronquist & Huelsenbeck 2003).

It was used a set of 45,000 resultant Bayesian trees after discarding first 5,000 trees as a burn-in (from MCMC output of combined data) to carry out S-DIVA analyses. The maximum clade credibility tree was used as a target tree to be annotated. All the parameters were set to default including the option of the maximum number of individual unit areas that was set to four and the ancestral reconstructions option was selected.

To perform BBM analyses, MCMC chains were run in RASP for 5 million generations using the model Fixed JC + G (Jukes-Cantor + Gamma) for state frequencies. In BBM 'null range' could be allowed to analysis option which means that the ancestral range contains none of the unit areas. We have performed the analyses with null distribution and wide distribution. Finally, the possible ancestral ranges at each node on a selected tree were obtained. The states were sampled every 100 generations. Ten simultaneous MCMC chains were run and the temperature was adjusted to 0.1 in order to keep an appropriate heat range. To compare the accuracy of results, we have done S-DIVA and also BBM analyses on the trees inferred.

RESULTS

Biogeographic inference using S-DIVA analysis

The species of *Ferula* are distributed mostly in Central Asia except from clade C (subgenus *Ferula* refer to panahi & al. 2018) that is distributed in the Mediterranean area and clade J (subgenus *Sinoferula*) that comprises species from west China. As explained in the Materials and Methods section, the biogeographic divisions used in the RASP analyses refer to Takhtajan's (1986) phytogeographical classification.

For S-DIVA analysis, 45,000 Bayesian trees were analyzed in RASP after discarding 5,000 trees as burn-in. The resulting reconstruction suggests a complex biogeographical history, in which dispersal and vicariance events have been vital in shaping of current distribution pattern of Ferulinae. S-DIVA postulates 105 dispersals, 48 vicariances and 0 extinctions; majority of these events are located on the backbone of the tree (fig. 1).

For common ancestor between Ferulinae members and *Leutea* clade, S-DIVA postulates the event route of node 276 (letter *i*, fig. 1) as CF->CFN->C|FN, i.e., the ancestor and its descendants originated in the Armeno-Iranian region and dispersed to the Turkestan and Eastern Asiatic regions followed by one vicariance and one dispersal, but that occurred with poor marginal probability (0.08). The pie chart of node 276 presents a combination of different area distributions (CF= 17.95 %, CN= 17.95 %, CFN= 17.95 % and etc. (less than 8%)) and the most likely area occurred in

reconstructions is the Armeno-Iranian and Turkestan regions. However, the ancestral area reconstruction at this node was generally ambiguous and the black part of the pie chart denotes reconstructions with probabilities lower than a threshold. The same problem is apparent for the basal node (281 that the black part is ca. 83%, letters *ii*, fig. 1) connecting Ferulinae and Daucinae with the outgroups. *Leutea* group (node 275) have been derived from the common ancestor of Ferulinae and originated in the Armeno-Iranian region (91 % of occurrence) and dispersed to the Hyrcanian province and adjacent areas ca. 1.8-2 Ma with high marginal probability (0.91).

S-DIVA suggests FN->N|F as event route for Ferulinae at node 268 (letters *iii*, fig. 1) i.e., the ancestors occurring in the Turkestan region and dispersed to the Eastern Asiatic region and that event was followed by vicariance. The probability for this event route was, however, low (0.28). The pie chart at this node presents a mixture of different area distributions and the most frequent area is FN with 50%; therefore, the estimation of ancestral area is ambiguous.

When examining the biogeographic reconstructions for *Ferula* clades, one may notice that for some clades, reconstructions are not ambiguous and with high

probability support. For instance, node 257 represents the ancestor of the Mediterranean group of *Ferula* with the event route as K->K^K->K|K, i.e., the ancestors and its descendants occurred in the area K or West Mediterranean, that received high marginal probability (0.96). Another groups of *Ferula* that are supported with high (100%) marginal probability include: J (node 267), G (node 180), L (node 195), I (node 183), and K (node 188) and their ancestral distributions comprise Eastern Asiatic region (clade J), Turkestan region (clade G), Central Tian-Shan and Turkestan regions (clade L), Turkestan region (clade I), and Turkestan and Central Tian-Shan (clade K) (fig. 1). Clade D except another member, *F. kosopoljanskyi* Korovin which has a basal position in node 261 with high marginal probability occurred in Turkestan region.

The other major clades of *Ferula* including clades A (node 248), H (node 163), B (node 249) and E (node 194) did not receive a strong probability support value for any particular reconstructions (all these nodes have been marked with letters *iv* in fig. 1).

S-DIVA analysis presents the most dispersal between and within the areas have occurred in Armeno-Iranian and Turkestan regions (table 2) with the most speciation rate within these areas as 31 and 46, respectively (table 3).

Table 2: Dispersal table that represents the speciation range of within areas that calculated in both analyses S-DIVA and BBM (in bold).

Area	From S-DIVA / BBM	To S-DIVA / BBM	Within S-DIVA / BBM
A	0.00 / 3.00	3.00 / 3.00	0 / 0
B	0.00 / 0.00	0.00 / 1.00	0 / 0
C	30.83 / 27.00	16.00 / 12.00	31 / 35
D	1.00 / 1.00	5.00 / 4.00	2 / 2
E	3.50 / 4.50	10.00 / 8.00	1 / 2
F	32.83 / 31.50	14.00 / 6.00	46 / 53
G	2.33 / 2.00	10.00 / 11.00	1 / 0
H	10.00 / 4.00	15.00 / 15.00	7 / 6
I	0.00 / 0.00	2.00 / 2.00	0 / 0
J	0.50 / 1.50	3.00 / 1.00	0 / 2
K	6.50 / 1.50	1.00 / 2.00	6 / 6
L	0.00 / 0.00	4.00 / 5.00	0 / 0
M	1.00 / 0.00	5.00 / 5.00	0 / 0
N	0.50 / 0.00	1.00 / 1.00	2 / 2

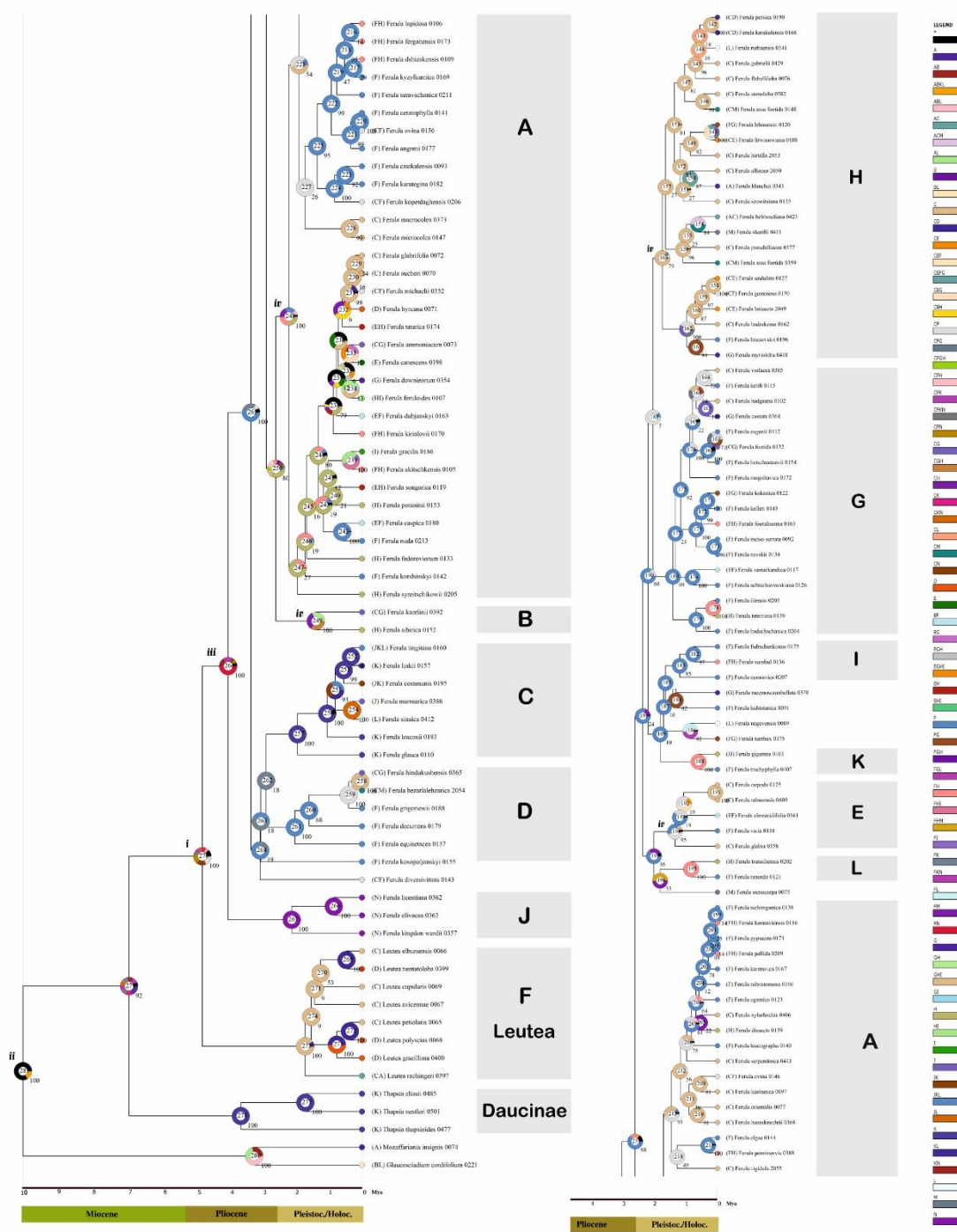


Fig. 1. Graphical output from S-DIVA (RASP). Graphical results of ancestral distributions at each node of the phylogeny of subtribe Ferulinae obtained by S-DIVA. Bayesian credibility values (PP) are indicated beside the pie chart of each node. Indicated letters in each node were discussed in concerned subchapter. Color key to possible ancestral ranges at different nodes were indicated here; black with an asterisk represents other ancestral ranges and other areas according to indicated legend (see table 1).

Table 3: Dispersal between areas that calculated in S-DIVA and BBM analyses.

S-DIVA	BBM
C->A: 2.5	A->B:1
C->D: 5	A->C:1
C->E: 4.33	A->L:1
C->F: 8.5	C->A:3
C->G: 4.5	C->D:4
C->H: 1	C->E:2
C->L: 1	C->F:5.5
C->M: 4	C->G:4
D->C: 1	C->H:2
E->C: 1.5	C->K:1
E->F: 1	C->L:1
E->H: 0.5	C->M:4
E->I: 0.5	C->N:0.5
F->C: 9.5	D->C:1
F->E: 3.33	E->C:1
F->G: 4.5	E->F:0.5
F->H: 12.5	E->G:2
F->K: 1	E->H:1
F->L: 1	F->C:8
F->M: 1	F->E:3.5
G->C: 1	F->G:5
G->E: 0.33	F->H:11
G->H: 0.5	F->I:0.5
G->I: 0.5	F->K:1
H->C: 2	F->L:1
H->E: 2	F->M:1
H->F: 4	F->N:0.5
H->G: 1	G->H:1
H->I: 1	G->I:1
J->L: 0.5	H->C:1
K->C: 1	H->E:2.5
K->J: 3	H->I:0.5
K->L: 1.5	J->L:1.5
K->N: 1	K->J:1
M->A: 0.5	K->L:0.5
M->H: 0.5	
N->F: 0.5	

Biogeographic inference using BBM analysis

Bayesian Binary Mapping analyses (BBM) in RASP were performed on a set of 45,000 Bayesian trees after discarding 5000 trees as burn-in (trees obtained from MCMC output of combined data in BEAST). The wide root distribution option (fig. 2) and also null distribution were tested and the reconstructed distributions at nodes were generally similar with the exception of the basal nodes 280 and 281, in which the most area probabilities assume for none of the unit areas as white part of the pie chart in the nodes (fig.3).

BBM analysis suggests slightly different ancestral ranges at basal nodes and postulates 151 dispersals, 42 vicariations and any extinction event, with the majority of these events located on the backbone of the tree. The reconstructed biogeographic tree inferred from BBM analysis is represented in fig. 2. The analysis suggests the most frequent ancestral ranges for node 279 (letter *i*, fig. 2), a common ancestor of Daucinae and

Ferulinae, is Armeno-Iranian region with highest percent occurrence (33.25 %). The presented event route for this node is C->CK->K|C, i.e., the ancestor and its descendants occurred in the Armeno-Iranian region and dispersed to the West Mediterranean region by one vicariance and two dispersals. However, this scenario received low marginal probability (0.17).

The ancestral area reconstruction for node 276 (letters *ii*, fig. 2) mostly comprised the Armeno-Iranian region, that the ancestors of *Ferula* and *Leutea* occurred in this region and then dispersed to Turkestan region (with 0.1 marginal probability). Node 275 represents the ancestor of *Leutea*. The most favored ancestral range at this node is C (Armeno-Iranian) region with subsequent dispersal to the Hyrcanian Province and dispersal division of the range. Such a scenario is supported with relatively acceptable marginal probability (0.55). It seems that the initial split within Ferulinae occurred during early Pliocene (4.8-5

Ma, letters *ii*, fig. 2). One descendant stayed there, giving rise to the *Ferula* clade (node 268) and another descendant subsequently underwent further divergence to account for *Leutea* lineages. The ancestor lineage of *Leutea* has originated 1.8-2 Ma.

In the node 268 (letters *iii*, fig. 2) representing the ancestor of *Ferula*, the most probable event route was CF->CFN->N|CF, i.e., dispersal from the Armeno-Iranian and Turkestan regions to the Eastern Asiatic region followed by one vicariance and one dispersal. This node received high Bayesian support value PP= 1 but the reconstruction has a low marginal probability, 0.23. However, it seems that the Armeno-Iranian region was the main center of early diversification of Ferulinae. The rapid diversification within Ferulinae clade have occurred since the Middle Pliocene (4.2 Ma, letters *iii*, fig. 2) and ca. 2.2 Ma led to the divergence between the Chinese species and the remaining members of *Ferula*.

Node 265 (letters *iv*) marks the split between two groups of *Ferula*, node 264 that comprise the Mediterranean species (Clade C) with clade D and *Ferula diversivittata* Regel & Schmalh. (received low Bayesian support) and the main clade of *Ferula* (node 251) that comprises mostly species distributed in Central Asia (PP = 68), this divergence took place ca. 3.4 Ma.

The ancestor of those two clades C and D (node 262) originated in the Turkestan region and dispersed to the West Mediterranean province; two dispersals and one vicariance followed (event route: F->FK->F|K). This scenario is supported with high marginal probability 0.82 but *F. kosopoljanskyi* has a basal position instead of accompanying by congeners (clade D). The ancestor of the Mediterranean group diverged ca. 2.2 Ma resulting in two lineages, *F. glauca* L. and the remaining members of the clade. Another ancestor for other Ferulinae members, derived ca. 3.2 Ma from node 251 that comprised the most species of *Ferula* located within Central Asia (node 250, marked by letter *v*, fig. 2). The reconstruction at node 250, a common ancestor of clade B and a large clade of *Ferula* (clade A), suggests Turkestan and Armeno-Iranian regions as ancestral range.

Area reconstructions for node 248, an ancestor of clade A, are ambiguous. Its subclades comprising members of last genus *Dorema* and species of *Ferula* that are mostly distributed in west Siberia and Aralo-Caspian region (node 247, letters *vi*, in fig. 2) originated in Central Tian-Shan and Turkestan regions ca. 2.2 Ma

(probability 0.23). For the second subgroup (node 227, letters *vii*), the following event route was reconstructed with probability of 0.44: CF->CF^C->C|CF, i.e., origin in the Turkestan region, dispersal to Armeno-Iranian region and subsequent vicariance event ca. 1.8 Ma. The third subgroup (node 215, letters *x*) most probably originated in area CF = 73.95 % (Armeno-Iranian + Turkestan) but this reconstruction received low probability (0.26). Clade B (node 249) of *Ferula* includes two species that distributed in northern part of Irano-Turanian region and southern provinces of the Circumboreal region and has a common ancestor with group A members. Its ancestor has been diversified about 1.8 Ma that the route event reconstructed as C->CGH->CG|H, i.e., origin in Armeno-Iranian and dispersed in Northern Baluchistan and Central Tian-shan areas, following by three dispersal and one vicariance, although it received low marginal probability (0.49).

The other members of *Ferula* clades derived from node 251 including clades L, E, K, I, G and H (fig. 2). Clades L (node 195), K (node 188), I (node 183), and G (node 180) originated mostly in the Turkestan province that received high marginal probability 0.84, 0.89, 0.82, and 0.70 respectively. However, clade E (node 194) shows low probability (0.27) with the combination of two regions, Armeno-Iranian and Turkestan. Based on BBM analysis, an earlier ancestor of another large group of *Ferula*, clade H (node 163) that has been originated mostly in Armeno-Iranian province ca. 2.1 Ma, received 0.36 marginal probability. The ancestor of two clades E and L that *F. stenocarpa* Boiss. & Hausskn. ex Boiss. has been joined to them, originated from the ancient ancestor of node 197 ca. 2.3 Ma and diversified through one dispersal event. Probably the ancestor of group L had a continental dispersal toward Tian-Shan area that led to forming its descendants.

In general, BBM tends to suggest single distribution areas for ancestral nodes more often than S-DIVA and S-DIVA favors a more widespread ancestral distribution consisting of all distribution areas of descendent branches (table 3, figs. 1 and 3). In the BBM analysis the amount of most dispersal between and within the areas have been occurred higher than those postulated by S-DIVA in the Armeno-Iranian (35) and Turkestan (53) regions (table 2). Also, the rate of speciation within these two regions are much more than the other regions, that confirm the diversification of *Ferula* mostly have been occurred in these areas.

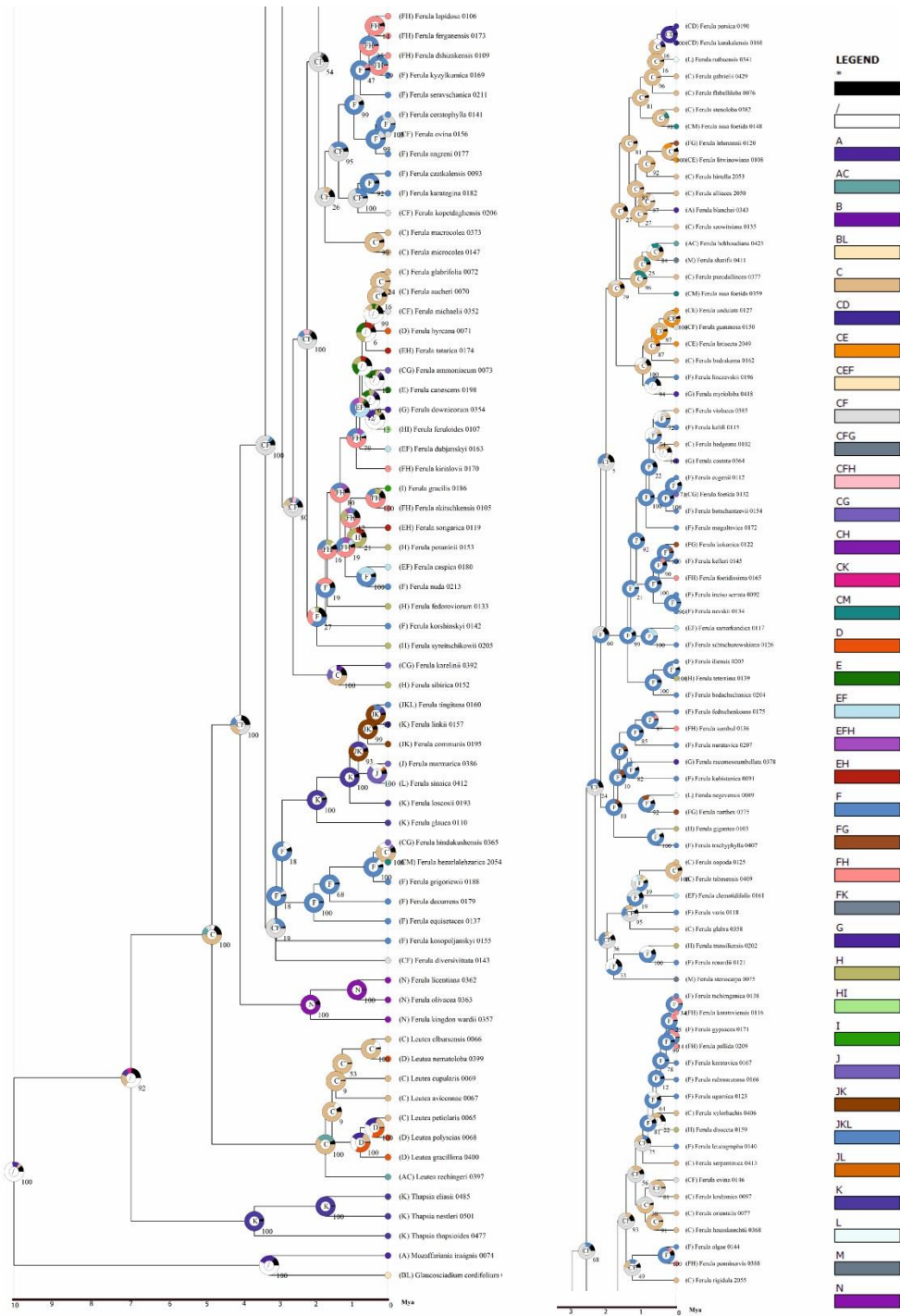


Fig. 3. Graphical output from BBM analysis with null distribution option as the most likely states tree. Pie charts at each node show the most occupied area by individuals. White part of pie charts indicated assuming none of the unit areas occupied.

DISCUSSION

Divergence time estimation

Due to a lack of fossil evidence, it has been difficult to reconcile the biogeography and divergence times in Apioideae. Spalik & al. (2010) estimated divergence times of the main lineages of Apioideae using fossil pollen of basal Apioideae as calibration points through a Bayesian approach (BEAST) based on ITS sequence data and these estimates were congruent with those inferred previously based on a strict molecular clock in Asteraceae (Sang & al. 1994). In the next study, using three calibration points from fossil pollen of euapioid Umbelliferae (Bupleureae, Pleurospermeae and a common ancestor of Scandiceae, Smyrnieae, Aciphyllae and Acronema clade), Banasiak & al. (2013) estimated divergence times of Apioideae based on ITS sequence data. This study suggested that Scandiceae originated in the mid-Miocene (20-26 Ma) while the separation between Ferulinae and its closest subtribes Daucinae and Scandicinae occurred ca. 18 Ma (Banasiak & al. 2013).

Geographical characteristics of the endemism centers of Ferulinae

Ferula has a broad distribution in Central Asia (Turkmenistan, Tajikistan, Uzbekistan, Kyrgyzstan, Kazakhstan), Iran, Afghanistan and Pakistan, reaching its eastern boundary in northwestern India to western China (East Turkmenistan) and extending in western direction to the Mediterranean region, southwestern Europe and North Africa. Floristically, this broad region comprises the central and eastern parts of the Irano-Turanian region, Mediterranean region and northern part of the Sahara region (Madjnoonian, 1999).

The most important center of endemism of Ferulinae is Irano-Turanian region that was first identified by De Candolle (1820) and then by Boissier (1867) as the "Oriental region". This area is bordered by the Black and Caspian Seas and covers the area of Iran and Turan (Central Asia) was delimited in different ways by various authors (e.g. Zohary, 1973; Takhtajan, 1986; White & Léonard, 1991; Davis & al., 1994). The Irano-Turanian region is climatically distinct from neighboring regions (Mediterranean, Euro-Siberian, Saharo-Sindian, and Central Asiatic). It has been suggested that the Irano-Turanian flora was less affected by late Quaternary glaciations compared to the Mediterranean and Euro-Siberian regions and its floristic boundaries shifted during the Quaternary to the recipient neighboring Saharo-Sindian and Mediterranean regions (Djamali & al., 2012). At present, Irano-Turanian floristic elements are well represented on eastern Mediterranean islands and even in the western Mediterranean region. This disjunctive

distribution pattern suggests that the Irano-Turanian flora might have been much more widespread during the Quaternary glacial periods and probably in the Pliocene. Pérez-Collazos & al. (2009) speculated that steppe plant populations in the western Mediterranean are derived from western Asian-eastern Mediterranean lineages that migrated across landmasses and potential land bridges around and across the Mediterranean Sea during the driest periods of the Messinian salinity crisis. Accordingly, this migration route may have been taken by the ancestor of the Mediterranean group of *Ferula* in Pliocene (fig. 4, blue arrow).

Within the area traditionally covered by the *Flora Iranica*, some areas have similar properties to the Mediterranean region in being biodiversity hotspots and diversification centers (Madjnoonian, 1999). One of these areas is the Hyrcanian Province of the Euro-Siberian phytogeographical region that covers the southern Caspian coastal plain and the northern slopes of the Alborz Mountains in northern Iran and southeastern Azerbaijan. Hyrcanian Province may have been a refugial area for some species of *Leutea* such as *L. nematoloba* (Rech.f.) Pimenov that are taxonomically and geographically separated from other species (fig. 2).

The Mediterranean basin is one of the world's major biodiversity hotspots and an outstanding biogeographical crossroad for the European, Saharan and Irano-Turanian regions, i.e. terrestrial connections separating the Tethys and Paratethys Seas during the Miocene (Jolivet & al. 2006; Rögl, 1999) might have allowed for biotic exchanges between eastern and western Mediterranean. The recent studies suggest that Mediterranean refugia have been less affected by past environmental change than European refugia and therefore, have conserved considerable genetic and species diversity (Médail & Daidema 2009). The Mediterranean group of *Ferula* is an example of a group that originated in the Irano-Turanian region and diversified in the Mediterranean region (fig. 4).

Recognizing biogeographic pathways for Ferulinae

According to both S-DIVA and BBM reconstructions, the common ancestor of Ferulinae and *Leutea* originated in the Armeno-Iranian region. The initial split within Ferulinae occurred in Armeno-Iranian province during the late Miocene and early Pliocene (4.8 Ma). Thereafter, the *Ferula* group split into an eastern Central Asian lineage and an Asian-Mediterranean lineage ca. 4.2 Ma (fig. 4). The Chinese lineage originated ca. 2.3 Ma following by vicariance event between eastern Asia and Irano-Turanian region. Eastern Central Asian lineage of *Ferula* spread from Central Asia to eastern Asia in the Pliocene (4-3 Ma, fig. 4, red arrow).



Fig. 4. Biogeographic pathways of *Ferulinae* originated from Armeno-Iranian region to adjacent areas (Central Asia to East presented by red arrow and toward Mediterranean region in blue arrow).

The extent and height of Himalayas-Tibetan Plateau have a profound effect on the climate of Asia; the uplift of the Tibetan plateau is likely to have started during or shortly after the beginning of the collision of the Indian plate with Eurasia (about 45 to 40 Ma; Favre, & al. 2015) and the development of the Asian monsoon system is closely linked to this uplift history and adjacent mountain ranges (Wen, & al. 2014). It is notable that until the late Miocene (about 6.2-5.0 Ma) Central Asia and the Mongolian Plateau were not as arid as they are today. These regions, therefore could act as migratory routs for plant exchanges between the eastern Palearctic and eastern Europe (Guo, & al. 2004). For example, aridification of central Asia coincided with the enhanced uplift of the Himalayas-Tibetan Plateau in the late Pliocene (3.6 Ma) caused the rapid radiation within the *Angelica sensu stricto* clade (Liao & al. 2013). However, the uplift of the Himalayas-Tibetan Plateau in the late Pliocene and the aridification may have precluded the migration of *Ferula* species to the Asiatic Far East except from a few species that occur in China. Also, some eastern Asian species, such as *Ferula groessingii* Riedl & Riedl-Dorn and *F. bungeana* Kitag., show close relationship with species occurring in Armeno-Iranian and Turkestanian provinces, for instance, *F. rigidula* Fisch. ex DC. (based on phylogenetic analyses of ITS sequences, unpublished data). So, the species adapted to colder climate of higher altitudes of Tian Shan are derived from migrants from warmer western areas (fig. 4, red arrow). Similar scenario was suggested for *Bupleurum* L.; the genus migrated from North Africa or western

Mediterranean region eastwards through the Middle East and the Caucasus reaching finally the Hengduan Mountains (Wang & al. 2008).

According to BBM reconstruction, Central Asian lineage of *Ferula* split ca. 3.5 Ma (letters *iv*, fig. 2) forming two lineages: western Asian-Mediterranean and Central Asiatic; that divergence times of these *Ferula* lineages would be the early to mid-Pliocene after refilling the Mediterranean Sea and Zanclean flood. Divergence estimation time for Central Asiatic *Ferula* lineages (clades A, G, H) are between 2-3 Ma. However, since the species boundaries in *Ferula* are not clear, one may also suspect that the high number of species, particularly of the Central Asiatic endemics, may have resulted from taxonomic splitting rather than rapid radiation of the genus in this region and that those closely related species constitute part of some more widely distributed and diversified polymorphic species.

Two scenarios may be proposed to explain the presence of *Ferula* in the Mediterranean region. Those scenarios suggest a pre-Quaternary migration from the eastern enclaves of Central Asia to the west during the Pliocene (ca. 3.8-2 Ma), either across the northern border of the Mediterranean basin as marked by the present distribution of *F. sadleriana* Ledeb. in Hungary and Romania (Cannon 1968) or through a northern African migration route as evidenced by the presence of members of *Ferula* in Sinai, Palestine, Egypt and Libya (*F. sinaica* Boiss., *F. marmarica* Asch. & Taub. ex Asch. & Schweif., *F. vesceritensis* Coss. & Durieu ex Batt. & Trab.) (fig. 4). These migration pathways were probably facilitated by the fluctuating desiccation

and re-fill of the Mediterranean basin that modified the climate toward a continental dryer-cooler ambient and united the Asian and European continent. This would have made possible the expansion of the migrating populations (Pérez-Collazos & Catalán 2007).

The patterns of glaciations have left traces on distribution and richness of species in those parts of temperate Europe that made important refugia for plant vegetation during the glacial periods of the Pleistocene (Médail & Daidema 2009). Willis (1996) reviewed the available evidence and concluded that only three peninsulas of southern Europe—Iberian, Italian and Balkan—constituted refugial area where temperate flora survived after several glacial and interglacial periods. However, she also recommended that these periods of isolation did have an effect upon individual populations especially promoting speciation. Each refugial area was effectively an isolated community likewise the populations within them. Southern Europe is still considerably much richer floristically than northern Europe and the Mediterranean region is a regional center of endemism (Davis & al. 1994). Most species of *Ferula* are distributed in central and western Asia, a few are scattered in eastern Mediterranean and north-western Africa and only three, including *F. communis* L., *F. tingitana* L. and *F. loscosii* (Lange) Willk. grow in the Iberian Peninsula (Pérez-Collazos & al. 2009). Recent phylogenetic studies established the derived position of the Mediterranean lineage from Asian ancestors (Kurzyńska-Młynik & al. 2008). Pérez-Collazos & al. (2009) hypothesized that *F. loscosii* originated in the Ebro valley region from relict Miocene ancestors and subsequently expanded southwards. Immigration route for *F. loscosii* populations within Iberian Peninsula have followed a northern colonization route with subsequent southwards migration (Pérez-Collazos & Catalán 2007; Pérez-Collazos & al. 2009).

The evolutionary studies on several Mediterranean terrestrial animal lineages show that those species reached the Iberian Peninsula and the Mediterranean basin through northern African pathway and then dispersed northwards; this pattern confirms the potential glacial refugia for steppe taxa in this region (Pérez-Collazos & al. 2009). *Ferula communis* and *F. tingitana* with distribution range throughout the Mediterranean region are grouped with the Canary Islands endemic *F. linkii* and this group is sister rather to the eastern Mediterranean *F. sinaica* and *F. marmarica* than to geographically closer Iberian *F. loscosii*. One may speculate that the common ancestor of this entire clade originated ca. 1.2 Ma in the Iberian Peninsula, which is one of the three most important glacial refugia in Europe (Willis 1996) and spread

across the Mediterranean in interglacial periods. Subsequent vicariance events resulted in descendant species.

Conclusions

In recent studies, non-coding parts of the organelle genomes are frequently used to infer intergeneric and interspecific relationships (Kadereit & al. 2008) to study infraspecific variation at the population level (Pleines, Jakob & Blattner 2009) and to evaluate the evolutionary processes accompanying dispersal and vicariance events (Li & al. 2010). The non-coding loci that were used in current study resolved to some extent the taxonomic position of some taxa (Panahi & al. 2018) and allowed the biogeographical inference. Vicariance and migration from Irano-Turanian region to adjacent areas have contributed to the biogeographic pattern of this group. The diversification of Ferulinae and its dispersion to the Mediterranean region and to eastern Asia occurred in the Pliocene.

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