

Phylogenetic and biogeographical history confirm the Anatolian origin of *Bornmuellera* (Brassicaceae) and clade divergence between Anatolia and the Balkans in the Plio-Pleistocene transition

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Abstract: Understanding disjunct distribution patterns in the Balkan Peninsula and Anatolia is important in order to reconstruct robust biogeographical hypotheses. This is instrumental in understanding the recolonization patterns of Europe during the Quaternary glaciation/interglaciation periods and the potential role of Anatolia as a refugium. Unfortunately, only a few studies have been conducted to uncover such processes. Here, we used all eight species of the genus *Bornmuellera* (Brassicaceae) with a scattered distribution in the Balkans and Anatolia to reconstruct its biogeographic history. We applied nuclear internal transcribed spacer (ITS) and plastid *trnL-F* regions and showed that 1) *Bornmuellera* is monophyletic and, 2) It is originated in the Pliocene in Anatolia (3.88 million years ago (mya), 3) Anatolian species are not monophyletic and, 4) Divergence between the representatives of one Anatolian clade (*B. cappadocica* and *B. kiyakii*) and the Balkan clade coincided with the Plio-Pleistocene transition (3.2–2.6 mya).

Key words: Anatolia, Balkan Peninsula, *Bornmuellera*, Brassicaceae, Taurus Way

1. Introduction

The Balkan Peninsula is considered to be one of the most important biodiversity hotspots for the European Flora and also a source for postglacial colonization of middle and northern Europe in the Quaternary (Hewitt, 1996; Nieto Feliner, 2014; Caković et al., 2015). It is well known that this region was less affected by glaciation during the Quaternary and represented a refugium for temperate species as was the Italian and Iberian peninsulas (Hewitt, 2004). Besides these refugia, some studies also suggest Anatolia as a glacial refugium for temperate species and the source area for colonization of temperate Europe after the Quaternary glacial period (Hewitt, 1996; Çıplak, 2008; Ansell et al., 2011; Korkmaz et al., 2014). It is well known that Anatolia has never experienced pronounced glaciation events during the Quaternary compared to the northern Eurasian latitudes (Ansell et al., 2011). However, the high mountain peaks of Anatolia, including the Kaçkar Mountains, Aladağlar, etc., were covered by glaciers during this period, whereas lower altitudes were covered by forest and steppe communities (Atalay, 1996; Ansell et al., 2011; Şenkul and Doğan, 2013; Gür, 2017). Additionally, the Anatolian Mountains are characterized by a high level of topographic and climatic heterogeneity,

and thus representing potential microrefugia (Ansell et al., 2011; Şekercioğlu et al., 2011).

Due to its geographical location between the temperate and subtropical regions, unique tectonic history, together with its heterogeneous topography and climate, Anatolia possesses a very rich flora and fauna (Şekercioğlu et al., 2011). Despite its biodiversity, both the spatio-temporal processes and species distribution patterns in Anatolia are not clear (Bilgin, 2011; Özüdoğru et al., 2015; Perktaş et al., 2015a). Specifically, the contribution of Anatolia to European biodiversity through the Balkans and Thrace is largely unknown, although there are some zoological studies unraveling the historical relationships between the Balkans and the Anatolian biotas (Çıplak, 2008; Chobanov et al., 2016; Kaya and Çıplak., 2017). For Brassicaceae, some species-level phylogeographic studies have been recently carried out. These studies comprise widespread species, such as *Arabis alpina* L., *Microthlaspi erraticum* (Jord.) T. Ali & Thines, *M. perfoliatum* (L.) F.K.Mey., and either Anatolia was shown as the source area for the recolonization of Europe during interglacial periods and/or the diversity center for related species (Ansell et al., 2011; Ali et al., 2016; 2019)

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Although it has long been known that the Balkans, Anatolia, and Caucasus mountains share related species complexes (Nagy et al., 2003), the importance of the Anatolian Mountains as a dispersal corridor between these regions (e.g. the role of these Mountains in the range expansions of species from one region to another, exchanges among these regions, etc.) has only recently been recognized (Kaya and Çıplak 2017). The term “Taurus Way” was first described by Çıplak (2008) and was later elaborated by Kaya and Çıplak, (2017) as a dispersal corridor for the connection between the biotas of the Caucasus and Balkans via the Anatolian Diagonal Mountains (eastern Anatolia), the southern Taurus chains, and the Aegean highlands (Figure 1). Such a disjunct distribution pattern was observed for several taxa (e.g. *Amphoricarpus* Vis. (Asteraceae) (Caković et al., 2015), *Digitalis* L. sect. *Globiflorae* Benth. (Plantaginaceae), (Eker et al., 2016) but phylogenetically tested only for some grasshoppers (*Psorodonatus caucasicus* species complex) (Kaya and Çıplak, 2017). To the best of our knowledge, no plant group has yet been subjected to such a study.

The genus *Bornmuellera* was traditionally considered to encompass seven Balkan and Anatolian species. *B. baldaccii* (Degen) Heywood, *B. dieckii* Degen and *B. tymphaea* (Hausskn.) Haussknecht are confined to the Balkan Peninsula, whereas *B. angustifolia* (Hausskn. ex Bornm.) Cullen & T.R. Dudley, *B. cappadocica* (Willd.) Cullen & T.R. Dudley, *B. glabrescent* (Boiss. & Balansa) Cullen &

T.R. Dudley and *B. kiyakii* Aytaç & Aksoy are endemic to Anatolia. As for Anatolian species, *B. cappadocica* was recently recorded in a region close to the Turkish border in Iran (Sajedi and Amini Rad, 2013). A recent comprehensive phylogenetic study of the tribe Alysseae by Rešetnik et al. (2013) showed that monotypic genera *Leptoplax* Schulz and *Physocardamum* Hedge were phylogenetically nested within *Bornmuellera*. Based on these molecular findings and some morphological characters (i.e., perennial life form, presence of malpighiaceae trichomes, non-saccate sepals, white petals, inflated and glabrous siliculae), *Leptoplax emarginata* (Boiss.) O.E. Schulz and *Physocardamum davisii* Hedge were transferred to *Bornmuellera* as *B. emarginata* (Boiss.) Rešetnik and *B. davisii* (Hedge) Rešetnik, respectively (Rešetnik et al., 2013; 2014). Additionally, Kandemir et al. (2014) noticed that *B. angustifolia* and *P. davisii* are conspecific. Subsequently, they treated the taxon as *Physocardamum angustifolium* (Hausskn. ex Bornm.) Kandemir due to differences in fruit morphology between *Bornmuellera* and *Physocardamum*. This treatment was rejected by relevant databases including BrassiBase (<https://brassibase.cos.uni-heidelberg.de/>; Kiefer et al., 2014) and AlyBase (Španiel et al., 2015), and we recognize this taxon as *B. angustifolia* (including *B. davisii*). Therefore, the genus *Bornmuellera* comprises eight Anatolian and Balkan species.

The present study is the first phylogenetic study of the genus *Bornmuellera* based on all eight species and

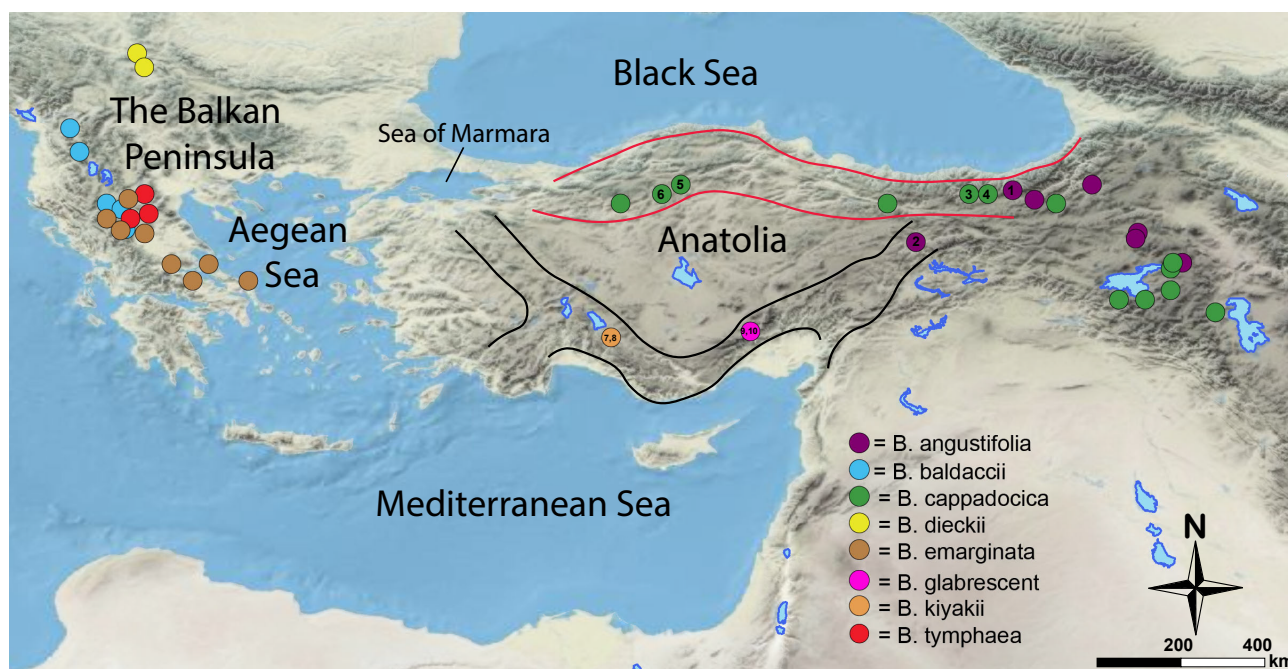


Figure 1. The distribution of *Bornmuellera* species in the Balkans and Anatolia/Turkey. Black lines on the map indicate the “Taurus Way” dispersal corridor connecting the Balkans and Anatolia according to Kaya and Çıplak (2017). Red lines indicate North Anatolian Mountains. The location of the new sequences is shown by numbers (see Table 1) embedded into the relevant circles.

nuclear (ITS 1 and 2) and chloroplast markers (*trnL-F*) and was designated to (1) study the monophyly and origin of the genus *Bornmuellera*, unravel (2) the phylogenetic relationships of the species, and (3) the biogeographic history of the genus with special reference to the Taurus Way dispersal corridor.

2. Materials and methods

Leaf material of Anatolian *Bornmuellera* species were obtained from silica dried collections and herbarium vouchers. Ten *Bornmuellera* specimens were used for the first time in this study. Voucher information and GenBank accession numbers of the newly sequenced samples are presented in Table 1. ITS and *trnL-F* sequences of the remaining *Bornmuellera* species from the Balkan Peninsula were obtained from GenBank (Table 1). As seen in Table 1, *B. baldaccii* and *B. emarginata* are represented by only one individual in the *trnL-F* dataset. Based on the results of Rešetnik et al. (2013), *Aurinia saxatilis* (L.) Desv., *Alyssum dasycarpum* Stephan ex Willd., *Alyssum murale* Waldst. & Kit and *Phyllolepidium rupestre* (Sweet) Trinajstić were used as outgroups.

2.1. DNA extraction, amplification, and sequencing.

DNA from silica-dried leaf material and herbarium specimens were isolated using NucleoSpin Plant II Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. ITS1, ITS2, and 5.8s rDNA were amplified using forward primer 5'-TCCGTAGGTGAACCTGCGG-3' and reverse primer 5'-TCCTCCGCTTATGATATGC-3'. These primers correspond to ITS1 and ITS4 of White et al. (1990). The *trnL-F* was amplified using primers C and F of Taberlet et al. (1991). Amplification of ITS and *trnL-F* and sequencing followed the protocols given in Bowman et al. (1999) and Mummenhoff et al. (2001), respectively.

2.2. Phylogenetic analyses and divergence time estimation

The raw ITS and *trnL-F* sequences were edited with Codon code aligner (CodonCode Corporation, Centerville, MA, USA) and combined with the sequences obtained from GenBank. Data sets were aligned by MUSCLE v.3.6 (Edgar 2004). The best-fitting nucleotide substitution models were chosen using MEGA X (Kumar et al. 2018). The GTR+G model was selected by MEGA for both data sets as the best substitution model. The individual ITS and *trnL-F* data sets were analyzed with maximum likelihood (ML) and coalescent based Bayesian analyses. ML analyses were performed with RAxML (Stamatakis, 2006) choosing the GTRGAMMA model for 1000 bootstrap iterations. Coalescent based species tree reconstruction and dating analyses were performed in BEAST v.2.6.1 (Bouckaert et al., 2014). Since our data set contains multiple individuals per species, these analyses were conducted in *BEAST

(STAR-BEAST), which uses the multispecies coalescent model to get separate estimates of the gene trees and a species tree (Heled and Drummond, 2010). In this analysis gene ploidy values of ITS and *trnL-F* data sets were set to 2.0 and 0.5, respectively.

Following the time estimations of Rešetnik et al., (2013) the age of the tribe Alysseae was constrained to 17.1 ± 1 million years ago (ma) with a normal distribution. Three independent Markov Chain Monte Carlo (MCMC) runs were conducted with 10 million generations and sampled every 1000 generations. The log and trees files obtained from independent runs were combined using Logcombiner. The first 10% of the sampled generations were discarded as burn-in. To obtain a maximum clade credibility tree, the software TREEANNOTATER v.2.6.0 (<https://www.beast2.org/treeannotator/>) was used for both species and gene trees. Finally, a species tree and two gene trees (ITS and *trnL-F*) were visualized using FigTree v 1.4.4 (Rambaut, 2009).

2.3. Historical Biogeography

Species occurrence data were obtained from field and Herbarium studies (ANK, B, DUOF, E, GAZI, HUB, K, VANF). Additional data were taken from the Global Biodiversity Facility (GBIF; <http://www.gbif.org>).

For biogeographical analyses of the genus *Bornmuellera*, two geographical areas (Anatolia and Balkan Peninsula) were defined. To test whether *Bornmuellera* originated in Anatolia or Balkan Peninsula, we used R package BioGeoBEARS (Matzke, 2013; R Core Team, 2013) in RASP (Yu et al., 2015). This R-package uses a likelihood-based framework and allows for testing six biogeographical models (DEC, DEC+J, DIVALIKE, DIVALIKE+J, BayArea, BayArea+J). In this analysis, the Maximum clade credibility (MCC) tree obtained from *BEAST analysis was used. The MCC tree was pruned to include *Bornmuellera* species and *Phyllolepidium rupestre* in RASP. Due to the distribution of outgroups that may pose limitations to the historical biogeographic analysis, more distantly related outgroups were eliminated from the MCC tree in the software RASP and only *Prupestre* used as outgroup.

3. Results

Characteristics of the ITS and *trnL-F* regions are summarized in Table 2.

The monophyly of the genus *Bornmuellera* was strongly supported by ML analyses of ITS and *trnL-F* data sets with 100 bootstrap support (BS) (Figure 2). While the Balkan *Bornmuellera* species were monophyletic in the ITS data set (BS = 97), this relationship was not supported by *trnL-F* data (Figure 2). The consensus species and gene (ITS and *trnL-F*) trees from the three independent runs of the Bayesian MCMC analysis are given in Figures 3 and 4.

Table 1. GenBank accession numbers for the sequences (ITS + *trnL*-F region including *trnL*(UAA) gene, *trnL*-F spacer) used in the phylogenetic analyses. Voucher information (collector and collection number, country, district, and herbarium acronyms) are given for specimens sequenced for this study and are marked with an asterisk (*). A dash (-) indicates missing data. Numbers in parenthesis after species names refer to the locations in Figure 1.

Species	Location information	GeneBank number ITS/ <i>trnL</i> -F
<i>B. angustifolia</i> (1)	Turkey: Bayburt: Karakaya Mountain, alpine meadow, 3000 m, 20.07.2015, <i>B. Özüdođru</i> 3697 (HUB)	MT271843*/MT274505*
<i>B. angustifolia</i> (2)	Turkey, Erzincan, Kemaliye, Sırakonaklar Village, 17.05.2006, <i>H. Altmözlü</i> 5317(HUB)	MT271844*/MT274506*
<i>B. cappadocica</i> (3)	Turkey, Gümüşhane, Şiran, between Tersun Pass and Gülaçar Village, 19.07.2015, <i>B. Özüdođru</i> 3682(HUB)	MT271845*/MT274507*
<i>B. cappadocica</i> (4)	Turkey, Gümüşhane, Torul, above Artabel Village, <i>B. Özüdođru</i> 3685 (HUB)	MT271846*/MT274508*
<i>B. cappadocica</i> (5)	Turkey, Çankırı, Şabanözü, Tülü hill, 16.04.2016, <i>B. Özüdođru</i> 3959 (HUB)	MT271847*/MT274509*
<i>B. cappadocica</i> (6)	Turkey, Çankırı, between Orta and Şabanözü, Bulduk pass, 16.04.2016, <i>B. Özüdođru</i> 3967 (HUB)	MT271848*/MT274510*
<i>B. kiyakii</i> (7)	Turkey, Konya: Derebucak; Çamlık Village, Kızıldağ, 1340 m, 30.03.2016, <i>B.Özüdođru</i> bk1	MT271849*/MT274511*
<i>B. kiyakii</i> (8)	Turkey, Konya: Derebucak; Çamlık Village, Kızıldağ, 1340 m, 30.03.2016, <i>B.Özüdođru</i> bk2	MT271850*/MT274512*
<i>B. glabrescent</i> (9)	Turkey, Adana, Pozantı, Çetinlik Hill, 28.06.2015, <i>B. Özüdođru</i> 3614 (HUB)	MT271851*/MT274513*
<i>B. glabrescent</i> (10)	Turkey, Adana, Karsantı, above Tahtalı Plateu, 24.06.1973, <i>E. Yurdakulol</i> 1301 (ANK)	MT271852*/MT274514*
<i>B. baldaccii</i>	Greece, Trikala	EF514635/KF022818
<i>B. baldaccii</i>	Macedonia	EF514636/-
<i>B. dieckii</i>	Macedonia, Šar Planina	KF022637/KF022820
<i>B. dieckii</i>	Macedonia, Šara, Ostrovica	KF022638/KF022821
<i>B. emarginata</i>	Greece, Stera Ellas, Evvia	KF022666/-
<i>B. emarginata</i>	Greece, Pindos, 4 km E Katára-Pass	KF022666/KF022845
<i>B. tymphaea</i>	Greece, Pindos, between Katára pass and Métsovo	EF514639/KF022823
<i>B. tymphaea</i>	Greece, Pindos, between Panagía and Koridallós	EF514640/KF022822
<i>Alyssum dasycarpum</i>	Jordan, Shaubak	KF022532/KF022735
<i>Alyssum dasycarpum</i>	Turkey, Eskişehir	KF022531/KF022734
<i>Alyssum murale</i>	Croatia, Mosor	KF022567/KF022764
<i>Alyssum murale</i>	Georgia, Caucasus, Dzume valley	KF022569/KF022766
<i>Aurinia saxatilis</i>	Romania, Podişul Dobrogei, Cheia, Rez. Cheia	KF022626/KF022810
<i>Aurinia saxatilis</i>	Slovakia, Stupava	KF022625/KF022809
<i>Phyllolepidium rupestre</i>	Greece, Pindos, Kakorráhi	KF022669/KF022848
<i>Phyllolepidium rupestre</i>	Kosovo, Šar planina	KF022670/KF022849

Both species and gene trees are concordant and support monophyly of *Bornmuellera* with 1.00 posterior probability as in the ML analyses. Additionally, all these trees confirm three monophyletic clades within *Bornmuellera*. The first clade (Clade I) consists of two Anatolian species (*B. angustifolia* and *B. glabrescent*) and is supported by 1.00

pp (Figures 3 and 4). The second Anatolian clade (Clade II) includes *B. cappadocica* and *B. kiyakii*, whereas the third clade consists of Balkan species. Topologies of ITS and *trnL*-F gene trees are fully concordant except for the position of *B. emarginata*: Two accessions are placed in the ITS tree, sister to the clade consisting of *B. tymphaea*,

Table 2. Summary statistics of ITS and *trnL*-F region (*trnL*(UAA) gene and *trnL*-F spacer.

	ITS	<i>trnL</i> -F
Number of sequences	26	24
Alignment length	652	469
Variable sites	163	73
Parsimony-informative characters	149	70
Consistency index	0.817021	0.821053
Retention index	0.905702	0.907104

B. baldaccii and *B. dieckii*, whereas in the cpDNA tree the one *B. emarginata* accession studied is sister to only *B. baldaccii* and *B. dieckii*, however, with weak support (0.42 pp, Figure 4).

According to time estimation analyses, the origin of the *Bornmuellera* dates back to 3.88 million years ago (mya) (2.49–5.37, 95% highest probability density (HPD) interval). The main phylogeographical break between Anatolian and Balkan species was dated to 2.95 mya (1.78–

4.1, 95% HPD interval) in late Pliocene / early Pleistocene (Figure 4).

The model test performed in BioGeoBEARS suggests that the most suitable model for our data set is DIVALIKE without J parameters (AICc_{wt} value = 0.46, Table 3). Reconstruction of the ancestral area based on the DIVALIKE model indicates that *Bornmuellera* most likely originated in Anatolia (Figure 4, node 2, 93.40%) and the current distribution of the Anatolian and Balkan species was shaped by two dispersals and one vicariance event (Figure 5). Four and three speciation events were further suggested by DIVALIKE analysis within Anatolia and the Balkan Peninsula, respectively. For the most recent common ancestor of the Balkan and the second Anatolian clade, the analysis suggested that these two regions, i.e., Anatolia and Balkan Peninsula (region AB), are characterized by equivocal probability (node 4, 100 %) and one vicariance event at this node which resulted in the divergence of these two clades.

4. Discussion

The generic circumscription of *Bornmuellera* has been expanded recently with the inclusion of the genera

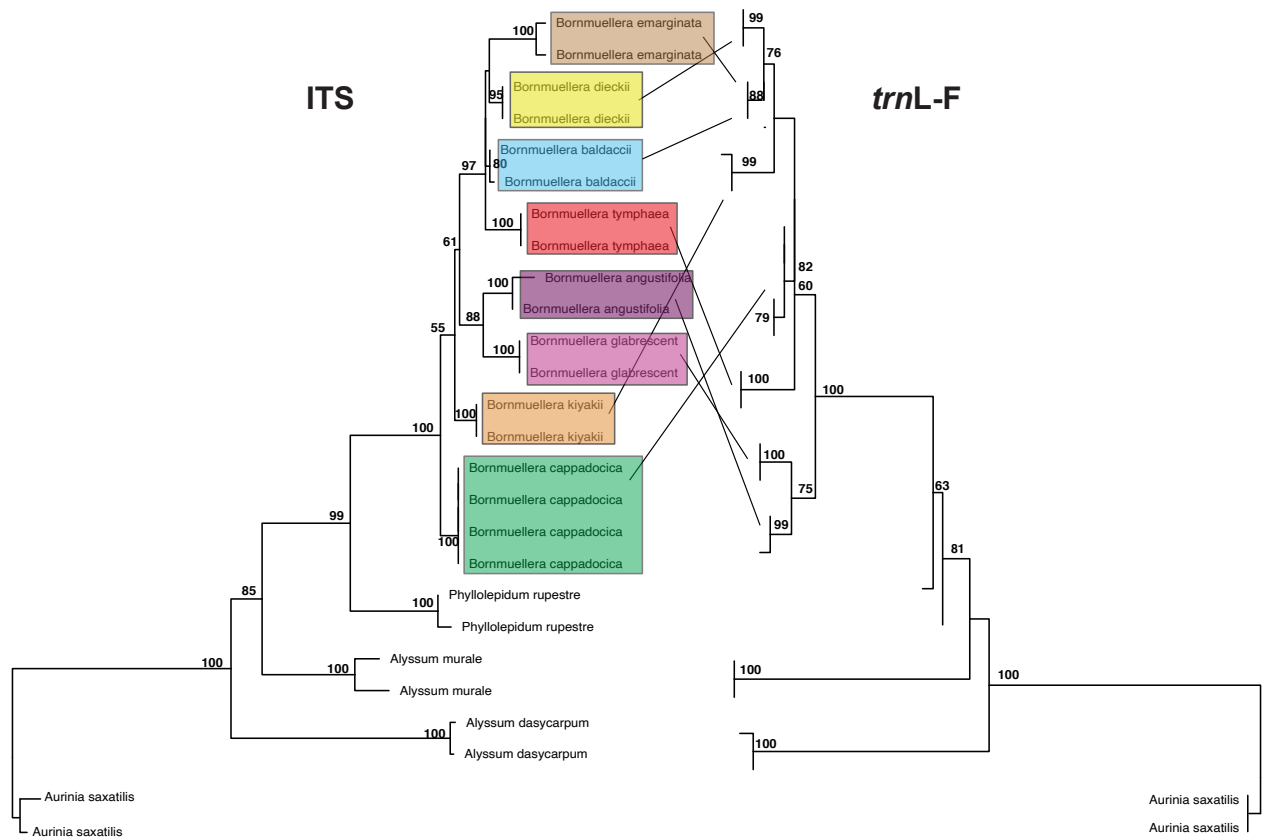


Figure 2. ITS and *trnL*-F gene trees obtained from RAxML analyses. All *Bornmuellera* species are highlighted by colored rectangles. Different positions of species in ITS and *trnL*-F trees are indicated by black lines. Bootstrap values (BS) values >50 are given at nodes.

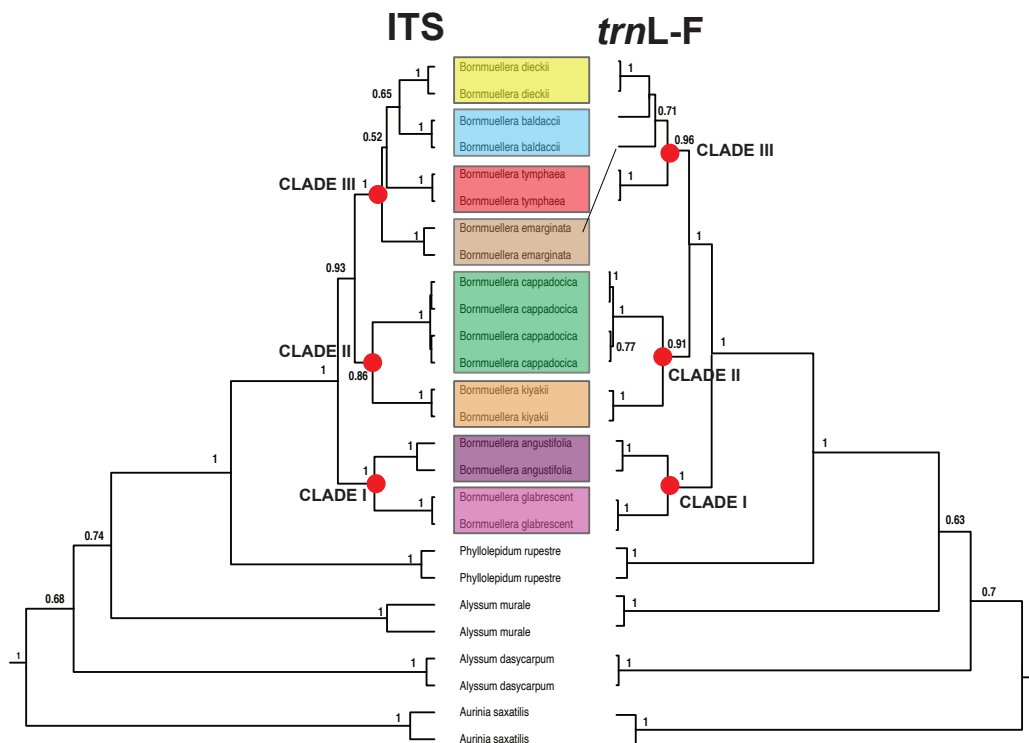


Figure 3. ITS and *trnL-F* gene trees obtained from *BEAST analysis. All *Bornmuellera* species are highlighted by colored rectangles. Clades obtained from species tree analysis (see Figure 4) are indicated by red circles. The different position of *B. emarginata* between ITS and *trnL-F* trees is indicated by a black line. Posterior probabilities (PP) values >0.5 are given at nodes.

Physocardamum and *Leptoplax* (Rešetnik et al., 2014). This broader generic delimitation is clearly supported by the current study based on the analysis of all known *Bornmuellera* species. Both ML and Bayesian (including species and gene trees) analyses confirmed (i) *Bornmuellera* as a monophyletic genus in the tribe Alyseae, (ii) Balkan species represent a clade (but not in ML analysis of *trnL-F* data) whereas (iii) Anatolian species are not monophyletic. Surprisingly, eastern Anatolian *Bornmuellera angustifolia* (previously *Physocardamum davisii*) with its angustiseptate and membranous-inflated fruits is sister to southern Anatolian local endemic *B. glabrescent* in all analyses. The generic concept suggested by Rešetnik et al., (2014) was not followed by Kandemir et al. (2014) who treated *B. angustifolia* as monotypic *Physocardamum angustifolium*. The main argument provided by Kandemir et al. (2014) refers to the differences in fruit morphology between *Bornmuellera* and *Physocardamum*. The latter taxon has angustiseptate (vs. latiseptate), bigger membranous-inflated (vs. not membranous and inflated) and reflexed (vs. not reflexed) fruits. However, substantial homoplasy and convergent evolution of fruit characters and many other morphological traits have been well documented in Brassicaceae (Franzke et al., 2011; Huang et al., 2016)

and especially fruits do not represent reliable phylogenetic characters.

The second Anatolian clade (Clade II) in the species tree consists of relatively widespread *B. cappadocica* and the local endemic *B. kiyakii* (Figure 4). In their original publication, the authors of the latter species (Aytaç and Aksoy, 2000) noted that *B. kiyakii* is closely related to *B. angustifolia* as both species share very characteristic tiny leaves. However, this close relationship could not be supported by the current phylogenetic analyses. Thus, this leaf character appears to be homoplastic, as was recently shown for other Brassicaceae leaf characters (Kiefer et al., 2019).

The remaining *Bornmuellera* clade (Clade III) includes Balkan species and represents, in agreement with Rešetnik et al., (2013), a monophyletic group (except ML results of *trnL-F* data). However, as nodes are not strongly supported, phylogenetic relationships within this clade should be further studied by additional single-copy markers or high throughput genomic analysis. Topological differences between ITS and *trnL-F* data in ML analyses might indicate incomplete lineage sorting, historical hybridization events or gene duplication/loss as shown in many studies (e.g. Smith et al., 2015; Vargas et al., 2017)

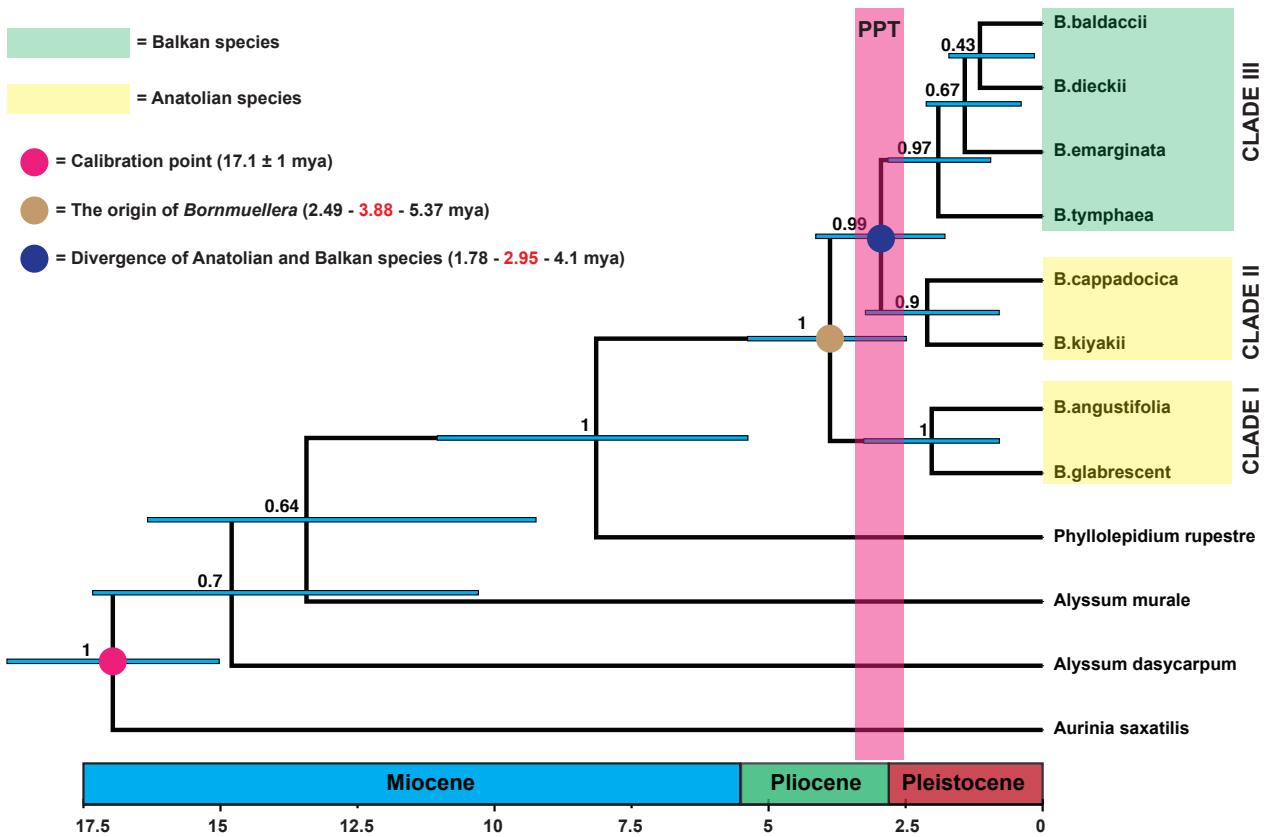


Figure 4 Time calibrated species tree of *Bornmuellera* obtained from *BEAST analysis of ITS and *trnL*-F data. The secondary calibration point, the origin of *Bornmuellera*, and divergence of Anatolian and Balkan species are indicated on the tree by pink, light brown, and blue solid circles, respectively. The light blue bars on the nodes indicate 95% highest probability density (HPD) intervals of time to the most recent ancestor (tmrca). The vertical pink bar shows the time interval of Plio-Pleistocene transition (3.2–2.6 mya). Posterior probabilities (PP) of nodes are given above the nodes.

Table 3. Results of the BioGeoBEARS analyses. LnL: loglikelihood, np: number of parameters, d: dispersal rate, e: extinction rate, j: jump dispersal rate, AICc: Akaike information criterion corrected, AICc_wt: Akaike weight.

Model	LnL	np	d	e	j	AICc	AICc_wt
DEC	-6.2	2	0.05	1.00E-12	0	18.39	0.33
DEC+J	-5.04	3	0.02	1.00E-12	0.09	20.87	0.1
DIVALIKE	-5.87	2	0.07	2.00E-09	0	17.74	0.46
DIVALIKE+J	-5.37	3	0.04	1.00E-12	0.07	21.55	0.07
BAYAREALIKE	-9.76	2	2.00E09	0.09	0	25.53	0.01
BAYAREALIKE+J	-6.04	3	0.03	1.00E-07	0.09	22.87	0.04

and comprehensive analyses (additional markers and accessions) are needed to elucidate this complex pattern. However, our biogeographical results based on species tree estimation are not influenced by conflicting nuclear and chloroplast data since *BEAST analyses estimate a species tree by taking into account the variation among different gene trees (Heled and Drummond, 2010).

The potential role of Anatolia during the Pleistocene recolonization of Europe has already been suggested in the seminal work of Hewitt (2004). Although this hypothesis has been tested and the refugium role of Anatolia proved for some organisms, including *Chorthippus parallelus* and *Microthlaspi erraticum*, (Korkmaz et al., 2014; Ali et al., 2016), the interpretation of Anatolia as representing

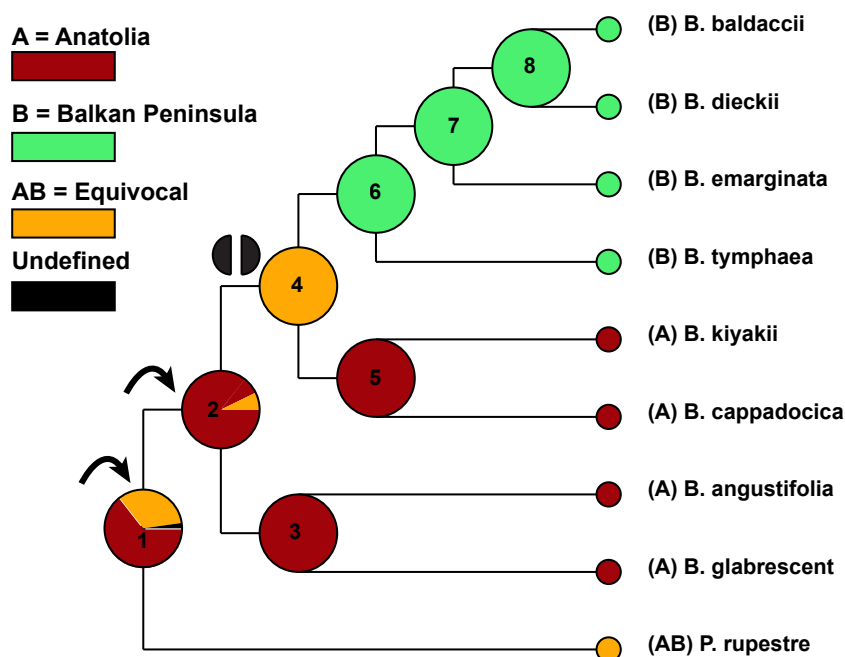


Figure 5. Graphical output of ancestral area reconstruction analysis based on DIVALIKE model. The color code defines extant and potential ancestral ranges and specific symbols refer to vicariance (two black semi-circles) and dispersal (black arrow) events, respectively. Ancestral ranges of nodes are shown in pie charts (for percentage of nodes see results).

a single refugium is an underestimation (Bilgin, 2011). The *refugia within refugia* model was first proposed for the Iberian Peninsula. This model suggests multiple smaller areas (refugia) within one big refugium and can be implemented for Anatolia as well as the Balkan Peninsula (Gómez and Lunt, 2007; Nieto Feliner, 2014; Perктаş et al., 2015b). 52 putative refugia were described by Médail and Diadema (2009) throughout the Mediterranean basin and six of them (including western, central and eastern Taurus Mountains) can be assigned to Anatolia and they are concordant with the “refugia within refugia” model. Considering the disjunct distribution pattern of *Bornmuellera* species both in Anatolia and the Balkan Peninsula, our study provides evidence enabling us to understand the historical connections between these areas. Similar disjunct distribution patterns can also be observed for members of the genus *Amphoricarpos*/Asteraceae (Balkans, Anatolia, and Georgia; (Caković et al., 2015), or *Digitalis* sect. *Globiflorae*/Plantaginaceae (Balkans, North Anatolia / Caucasus, South Anatolia; Eker et al., 2016).

However, historical connections between the flora of the Balkans and Anatolia via the Taurus Mountains (Taurus Way) or North Anatolian Mountains are still unexplored. Considering the basal position of the Anatolian species and the current distribution of species in both the Taurus Way (Figures 1 and 3) and North Anatolian Mountains,

our study suggests two alternative dispersal corridors for colonizing the Balkan Peninsula from an ancestral Anatolian stock.

Due to the complex tectonic history of the Mediterranean Basin, this region is floristically rich and harbors a high amount of endemic species (Nieto Feliner, 2014). The most remarkable geological event in the history of the Mediterranean Basin is the Messinian Salinity Crisis (MSC, 5.96-5.33 mya). Due to the desiccation of the Mediterranean Sea during this period, connections between the Balkans and Anatolia through land bridges most likely existed during MSC until the Plio-Pliostecene transition (PPT, 3.2-2.6 mya) allowing the migration and exchange of species until the establishment of a straight through the Sakarya River Basin (Hsü, 1972; Elmas, 2003; Nieto Feliner, 2014; Hill et al., 2017; Kaya and Çıplak, 2017).

Our results of both time estimations and DIVALIKE based historical biogeographical analyses are fully congruent with this geological history. *Bornmuellera* most likely originated in the Pliocene in Anatolia (3.88 mya, 2.49-5.37 95 % HPD) and the Balkan Peninsula was colonized from this ancestral stock via the Taurus Way or North Anatolian Mountains (Figures 1, 2 and 4). The reason for the ambiguity in the dispersal route is that the second Anatolian clade (Clade II) consists of two species,

one of which (*B. cappadocia*) is distributed in eastern and northern Anatolia and the other (*B. kiyakii*) occurs locally in southern Anatolia. Our results further support a vicariance event in the Pliocene-Pliostocene transition and this scenario is concordant with the time of disconnection of the Balkans and Anatolia via the Sakarya River Basin straight as explained above.

Our study represents the first attempt at understanding processes explaining the distribution patterns of Anatolian/Balkan plant lineages, which seem to be well documented for some animal groups (Çıplak, 2008; Çıplak et al., 2015; Chobanov et al., 2016; Kaya and Çıplak, 2017). Additionally, the current study provides some evidence for our understanding of dispersal corridors (e.g. Taurus Way, or Anatolian Diagonal) for plant species between the

Balkans and Anatolia and throughout Anatolia. However, more studies are needed to understand biogeographic patterns within Anatolia and the role of Anatolia for the recolonization of Europe during the Quaternary interglacial periods.

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