



Phylogeny, biogeography, and character evolution in the genus *Scilla* s.l. and its close relatives *Chionodoxa*, *Gemicia*, *Puschkinia*, and *Prospero* (Asparagaceae)

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Abstract

Scilla s.l. is taxonomically one of the most problematic genera of the Asparagaceae. Within the last few decades, several genera were segregated from this genus; however, there is still no consensus on the taxonomic status of *Scilla* segregates. Although some previous studies indicated a polyphyletic origin for the genus *Scilla*, there has been no comprehensive phylogenetic study focusing on the entire complex including *Chionodoxa*, *Prospero*, *Puschkinia*, and the recently described *Gemicia*. To achieve this, we evaluated three plastid regions, namely *rbcL*, *trnL-F*, and *matK*, for 79 accessions. The monophyly of *Puschkinia* and *Prospero* was supported by our phylogenetic analyses; however, *Chionodoxa* and *Gemicia* were placed into a clade with representatives of the *S. bifolia* species group. While our divergence time estimation analysis indicated a Miocene origin for all studied genera of polyphyletic *Scilla* s.l., the results of our ancestral area and ancestral state analyses showed that *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia*, together with *Brimeura* Salisb., *Bellevalia*, *Hyacinthella*, and *Arawia*, probably had a most recent common ancestor without a perianth tube and a corona, and with a non-papillate testa surface, which evolved about 36 Ma in the Mediterranean region.

Keywords Hyacinthaceae · Phylogeny · *Prospero* · *Puschkinia* · *Scilla* · Systematics

Introduction

Chionodoxa Boiss., *Scilla* L., *Prospero* Salisb., and *Puschkinia* Adams are closely related genera traditionally subsumed within Liliaceae s.l. All of these genera have been segregated from Liliaceae s.l. and examined under Hyacinthaceae by many authors (Mathew 1987, Dashwood and Mathew 2005; Speta 1998a; Pfosser and Speta 1999).

However, similar to many other angiosperm taxa, the taxonomic statuses of these genera were changed recently and they were placed into the subfamily Scilloideae of Asparagaceae (Chase et al. 2009; Angiosperm Phylogeny Group 2009; Reveal and Chase 2011).

Among these genera, the genus *Puschkinia* is clearly distinguishable from its relatives by having a floral corona, perigone tube, and seeds with sarcotesta (Yıldırım 2014) (Fig. 1a–b). The genus comprises three species (*P. scilloides* Adams, *P. peshmenii* Rix & Mathew, and *P. bilgineri* Yıldırım) distributed from Dagestan and Beshtau in the northern Caucasus and eastern Turkey to Lebanon and northwestern Iran (Rix and Mathew 2007; Yıldırım 2014). Although Rukšāns recently described a new species, *P. kurdistanica* (Rukšāns 2019), its taxonomic status is still controversial.

Scilla vardaria Yıldırım & Gemicici was first described from Turkey within the genus *Scilla* taking into account the results of unpublished phylogenetic analyses of chloroplast DNA data, although its morphology represents a transition between *Scilla*, *Chionodoxa*, and *Puschkinia* (Yıldırım et al. 2013). Yıldırım and Altıoğlu (2021)

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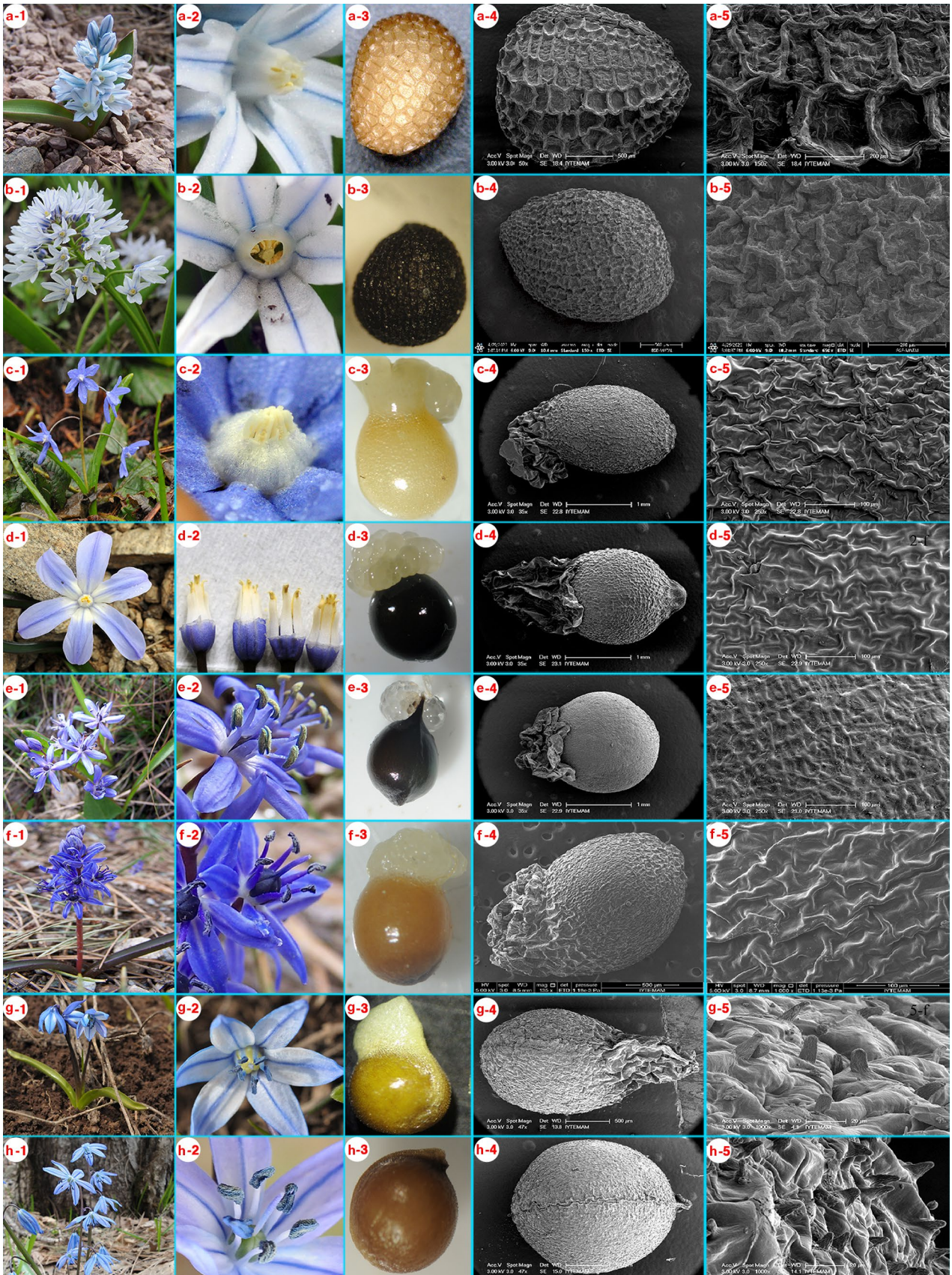


Fig. 1 Habit (1), corona with lobes (2), seed (3) and SEM photographs of seed and seed surface (4, 5): **a** *Puschkinia scilloides*, **b** *P. bilgineri*, **c** *Gemicia vardariana*, **d** *Chionodoxa luciliae*, **e** *Scilla bifolia*, **f** *S. albinerve*, **g** *S. siberica* subsp. *armena*, **h** *S. ingridae*

subsequently changed the status of the species considering its distinct morphology and the polyphyly of the genus *Scilla*, describing a monospecific genus named *Gemicia* Yıldırım (Fig. 1c).

Chionodoxa has long been treated as a distinct genus due to several unique characters, such as having a distinct perigone tube and filaments that are well developed and flattened and form a central cone at the top of the perigone tube (Yıldırım et al. 2013; Yıldırım and Altoğlu 2021) (Fig. 1d). Despite these morphological differences, *Chionodoxa* was listed as a synonym of *Scilla* based on cytotaxonomy, seed morphology, and molecular data as well as its ability to form hybrids with *S. bifolia* L. (Speta 1971, 1976, 1981; Pfosser and Speta 1999; Trávníček et al. 2009). However, considering the polyphyletic framework of *Scilla* s.l., *Chionodoxa* was resurrected by Yıldırım and Altoğlu (2021) recently, although its species have close phylogenetic affinities with *S. bifolia* (Fig. 1e).

The genus *Scilla* s.l. consists of 91 species distributed in Europe, Africa, and western Asia (Govaerts 2022). The generic limits of *Scilla* have long been controversial and it was segregated into many smaller genera by Speta (1998a), with only the *S. bifolia* (i.e., the generic type of the genus) species group including the species of *Chionodoxa* retained in *Scilla*. However, this taxonomic treatment has not been adopted by many other authors (e.g., Govaerts 2022).

The genus *Prospero* comprises about 25 species and is distributed through southern England and the Mediterranean basin to the Caucasus and northern Iraq (Speta 1982, 1998b). With the exception of the recently described spring-flowering *Prospero cudidaghense* Firat & Yıldırım, the members of this genus bloom in autumn and are characterized by their lack of bracts and prophylls and their hysteranthous leaves, which appear in autumn and wither in spring, except for *P. seisumsiana* (Rukšāns & Zetterl) Yıldırım., whose leaves appear in spring (Yıldırım 2014; Firat and Yıldırım 2016). Until recently, all members of *Prospero* were long treated under the generic name *Scilla* by many authors; however, detailed molecular, morphological, and karyological studies have indicated the segregation of this genus from *Scilla* (Speta 1982, 1998a; Yıldırım 2014 Govaerts 2022).

The current study presents the first extensive sampling of the molecular phylogeny of the genera *Chionodoxa*, *Gemicia*, *Scilla*, *Prospero*, and *Puschkinia* based on analyses of *matK*, *rbcL*, and *trnL-F* sequences of plastid DNA.

Our sampling reflects the full morphological variation of these genera. The main goals of this study are: (1) to test the monophyly and infrageneric classification of *Scilla* s.l. and closely related genera in light of molecular data, (2) to show the evolution of the important morphological characters using for the classification of these genera, (3) to estimate the divergence time of the crown node of the main lineages, and (4) to reconstruct the biogeographic history of *Scilla* s.l.

Materials and methods

Taxon sampling

The plant materials used in this study were collected from their natural habitats in different regions of Turkey from 2007 to 2017. Voucher specimens were deposited in the herbaria of Ege University (EGE) and Hacettepe University (HUB).

The present study included 75 samples from genera of the Hyacinthaceae belonging to the ingroup (Appendix). Of these, 48 *Scilla* samples (including one *xChionoscilla* and five *Chionodoxa* samples), seven *Puschkinia*, four *Prospero*, two *Hyacinthella* Schur, one *Bellevalia* Lapeyr., and one *Gemicia* were newly sequenced for the current study, while 12 sequences were downloaded from GenBank (please note that the same voucher number sequences were included). We also included four outgroups, namely *Lachenalia* Jacq. ex Murray, *Ledebouria* Roth, *Drimia* Jacq., and *Ornithogalum* L. from the tribes Hyacintheae, Urgineae, and Ornithogaleae of the subfamily Scilloideae (Appendix). Appendix includes the GenBank numbers for new and previously published DNA sequences.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from herbarium and silica gel-dried materials using the QIAGEN DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Three chloroplast DNA regions, namely *rbcL*, *trnL-F*, and *matK*, as well as the entire nuclear internal transcribed spacer (ITS) were sequenced. Polymerase chain reaction (PCR) amplifications were conducted using previously published primers (Table 1). PCR mixtures of 50 μ L consisted of 25 μ L of PCR Master Mix (Thermo Fisher, Waltham, MA, USA), 23.5 μ L of distilled water, 0.5 μ L of each primer, and 1 μ L of template DNA. After the DNA fragments amplified by PCR were electrophoresed into a

Table 1 Primers used and PCR profiles for amplifying and sequencing

Region	Name of the primer	Primer sequence	Source	
ITS	ITS1 (F)	TCC GTA GGT GAA CCT GCG G	White et al. 1990	
	ITS 4 (R)	TCC TCC GCT TAT TGA TAT GC		
<i>trnL-trnF</i>	<i>trnL</i>	CGA AAT CGG TAG ACG CTA CG	Taberlet et al. 1991	
	<i>trnF</i>	ATT TGA ACT GGT GAC ACG AG		
<i>matK</i>	XF	TAA TTT ACG ATC AAT TCA TTC	Ford et al. 2009	
	5R	GTT CTA GCA CAA GAA AGT CG		
<i>rbcL</i>	F	ATG TCA CCA CAA ACA GAG ACT AAA GC	CBOL Plant working group, 2009	
	R	GTA AAA TCA AGT CCA CCR CG		
PCR profile	Region			
	ITS	<i>trnL-trnF</i>	<i>matK</i>	<i>rbcL</i>
Initial denaturation	95 °C for 3'	94 °C for 4'	94 °C for 3'	98 °C for 30''
Denaturation	96 °C for 30''	94 °C for 1'	94 °C for 30''	98 °C for 10''
Annealing	58 °C for 30''	50 °C for 1'	52 °C for 30''	53 °C for 40''
Extension	72 °C for 2'	72 °C for 1'	72 °C for 45''	72 °C for 1'
Final extension	72 °C for 8'	72 °C for 7'	72 °C for 10'	72 °C for 5'
Number of cycles	30 cycles	28 cycles	35 cycles	30 cycles
Source	Jang et al. 2013	Martínez-Azorín et al. 2011	Li et al. 2011	CBOL Plant Working Group, 2009

1% agarose gel, products were sent to MedSanTek (İstanbul, Turkey) for purification and sequencing.

The ITS region could not be effectively amplified despite several attempts in the current study. It is noteworthy that this region was also not included in any previous studies (e.g., Stedje 1998; Pfosser and Speta 1999; Buerki et al. 2012; Chen et al. 2013). Furthermore, while only one entire ITS (ITS1 + 5.8 + ITS2) sequence was deposited in GenBank for the genus *Scilla* (i.e., *Scilla siberica* Haw., KT898137.1), for several *Scilla scilloides* (Lindl.) Druce (syn. *Barnardia japonica* (Thunb.) Schult. & Schult.f.) samples, only short (\approx 200 bp) ITS1 regions were deposited. The remaining sequences are clearly associated with *Scilla*-related fungi. Therefore, it is obvious that, instead of *Scilla* ITS regions,

fungal DNA associated with the leaves of *Scilla* are amplified during PCR (e.g., Miranda et al. 2010).

Including the outgroups, the *trnL-F*, *matK*, and *rbcL* data matrices comprised 66, 68, and 78 sequences, respectively. The number of individuals, aligned ingroup lengths, total variable characters, and number of parsimony-informative sites for the *trnL-F*, *matK*, and *rbcL* regions are given in Table 2.

Alignment, model selection, and phylogenetic analyses

Sequences were assembled and aligned using Geneious Pro 4.8.4 software (Kearse et al. 2012) and were subsequently edited manually. Details of these alignments are presented

Table 2 Total number of individuals, aligned ingroup lengths, total variable characters, and number of parsimony-informative sites

Region	Total individuals	Aligned ingroup length (bp)	Total variable characters	Parsimony-informative characters (%)
<i>trnL-F</i>	66	1085	244	113 (10.4)
<i>matK</i>	68	838	218	114 (13.6)
<i>rbcL</i>	78	513	52	26 (5.1)
Total evidence tree	79	2436	514	253 (10.4)
“Total evidence tree” with no missing information	61	2401	499	238 (9.9)

in Table 2. Five different markers were used in analyses: *matK*, *rbcL*, *trnL-F*, *rbcL + trnL-F + matK*, and *rbcL + trnL-F + matK* (no missing data). Besides maximum likelihood (ML) and Bayesian inference (BI) analyses for individual markers (i.e., *matK*, *rbcL*, and *trnL-F*) and combined markers (i.e., *rbcL + trnL-F + matK +*) (Table 3), “no missing data (i.e., all loci present in all taxa)” analysis was also conducted with the total evidence dataset to see the effect of missing data. The “no missing data” dataset included 61 sequences, with four outgroups and 57 ingroup taxa. For each DNA region, the substitution models were evaluated using the Akaike information criterion in jModelTest v2.1.6 (Guindon and Gascuel 2003; Darriba et al. 2012) as implemented in the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>).

The ML analyses were performed using RAxML version 8.2.12 (Stamatakis 2014) as implemented in the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>). Other than the “let RAxML halt bootstrapping automatically” option and the GTRGAMMA model, default ML search options were employed.

Bayesian analyses were conducted using MrBayes 3.2.7a (Ronquist et al. 2012) via the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>) with four parallel Monte Carlo Markov chain (MCMC) runs (one cold and

three heated) for ten million generations, sampling every 100 generations. Burn-in was set to 25% (the first 25,000 trees), and maximum credibility trees were constructed from the remaining 75,000 trees. This was repeated twice and the convergence of the chains was ensured. To produce the phylogenetic tree images, Figtree v.1.4.4 (Rambaut 2012) was used.

Divergence time estimation

Node ages for the most recent common ancestor (MRCA) of *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia* were estimated using BEAST v.1.8.1 (Drummond and Rambaut 2007; Drummond et al. 2012), implemented in the CIPRES Science Gateway (Miller et al. 2010; <http://www.phylo.org/>). The dataset was imported into BEAUti v.1.8.1 (Drummond et al. 2012) to generate XML input files. The Yule speciation process was selected as a prior with a randomly generated starting tree, and a normal prior distribution model was applied (please note that there are no reliable fossils assigned to Hyacinthaceae) (Forest 2009). As substitution models, the models described below were applied for each of the individual marker analyses. MCMC chains were run for 2×10^7 generations, with sampling every 1000th generation. The log files of the two independent runs were combined

Table 3 Summary of the results of the maximum likelihood (ML) and Bayesian inference (BI) analyses of *matK*, *rbcL*, and *trnL-F*. X indicates that the genus/species was not monophyletic and a check mark indicates a monophyletic genus/species. Bootstrap (BS) and

posterior probability (PP) values are, respectively, indicated within parentheses. Empty cells indicate that data were not available (i.e., fewer than two or no samples were included)

Name of the clade	<i>matK</i>	<i>rbcL</i>	<i>trnL-F</i>	<i>matK + rbcL + trnL-F</i>	<i>matK + rbcL + trnL-F</i> “no missing information”
<i>Chionodoxa</i>	X	X	X	X	X
<i>Prospero</i>	✓(98%, 1.00)	X	✓(96%, 1.00)	✓(99%, 1.00)	✓(100%, 1.00)
<i>Puschkinia</i>	✓(94%, 1.00)	✓(74%, 1.00)	✓(98%, 1.00)	✓(90%, 1.00)	✓(100%, 1.00)
<i>Hyacinthella</i>	✓(100%, 1.00)	✓(64%, X)	✓(84%, 1.00)	✓(99%, 1.00)	✓(100%, 1.00)
<i>Bellevalia</i>	✓(96%, 0.99)	X	✓(99%, 1.00)	✓(99%, 1.00)	✓(100%, 1.00)
<i>Scilla</i>	X	X	X	X	X
<i>S. alinihatiana</i>	✓(96%, 1.00)	✓(82%, 0.94)	✓(99%, 1.00)	✓(100%, 1.00)	✓(100%, 1.00)
<i>S. bifolia</i>	X	X	X	X	X
<i>S. bithynica</i>	✓(97%, 1.00)	✓(90%, 1.00)	✓(91%, 1.00)	✓(100%, 1.00)	✓(100%, 1.00)
<i>S. hyacinthoides</i>	✓(100%, 1.00)	✓(71%, X)		✓(100%, 1.00)	
<i>S. ingridae</i>	✓(97%, 1.00)	X	✓(91%, 1.00)	X	✓(99%, 1.00)
<i>S. leepii</i>	X	X	✓(61%, 1.00)	✓(63%, 0.99)	✓(62%, 0.99)
<i>S. melaina</i>	✓(97%, 0.96)	X	✓(84%, 0.96)	✓(89%, 1.00)	✓(100%, 1.00)
<i>S. persica</i>	✓(100%, 1.00)	✓(87%, 0.80)	✓(95%, 0.98)	✓(100%, 0.97)	✓(100%, 1.00)
<i>S. siberica</i>	X	X	X	X	X

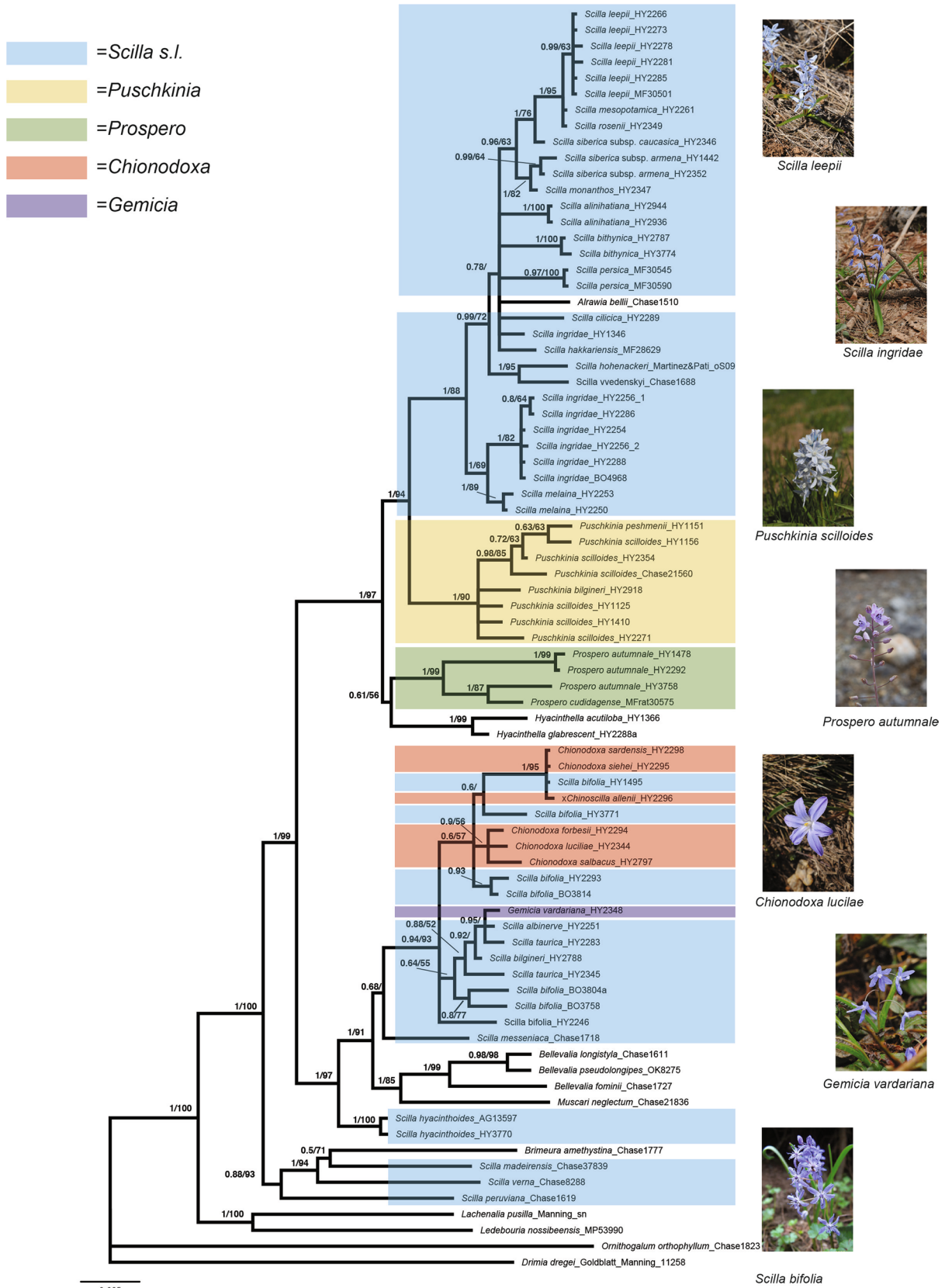


Fig. 2 Bayesian inference phylogram of Hyacinthaceae based on *rbcL*+*trnL-F*+*matK* dataset. Bootstrap (BS) values are provided for the key nodes after the posterior probability (PP). Only PP values of >0.5 and BS values of >50% are shown. *Scilla* s.l. and related genera considered in the current study are highlighted by color. Photographs of representatives of some important lineages are given on the right side of the phylogenetic tree. Photos by Hasan Yıldırım

with LogCombiner v.1.8.2 (Rambaut and Drummond 2015). Tracer v.1.6 (Rambaut et al. 2015) was used to visualize the convergence statistics and TreeAnnotator v1.8.1 (Rambaut and Drummond 2007) was used to generate the maximum clade credibility tree. For the calibration points, since no fossils have been assigned to the genera mentioned above, we used two calibration points from previous studies: (i) the age of the family Hyacinthaceae was set to 70 ± 0.1 Ma and (ii) the age of the Mediterranean Hyacinthoideae was set to 43.44 Ma (36.50–50.69) (Anderson and Janssen 2009; Buerki et al. 2012).

Ancestral area reconstructions

To estimate the ancestral ranges of *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia* and detect possible dispersal routes, we used the Bayesian binary MCMC (BBM) option (Ronquist and Huelsenbeck 2003) of RASP v.4.2 (Reconstruct Ancestral State in Phylogenies; Yu et al. 2015), with default settings to calculate probabilities of the most likely ancestral areas for each clade, except the “maximum number of areas” option, which was set to 3, and the model of evolution. Analyses were run for 1×10^6 generations with sampling every 100th generation. Burn-in was set to the first 2500 iterations.

For biogeographical analysis, we used the output MCMC tree obtained from our BEAST analysis. We defined the following three phytogeographical regions to take into account the geographical distributions of the studied taxa: (A) Circumboreal, (B) Irano-Turanian, and (C) Mediterranean, with the remaining phytogeographical regions coded as (D). For the monophyletic species (i.e., *Scilla alinihatiana* Aslan & Yıldırım, *S. bithynica* Boiss., *S. hyacinthoides* L., *S. leepii* Speta, *S. melaina* Speta, *S. persica* Hausskn., *S. siberica* subsp. *armena* (Grossh.) Mordak, and *Puschkinia scilloides* Adams), we used the geographic information for only one taxon (Online Resource 1).

Ancestral state reconstructions

We traced three morphological traits that are important for the identification of *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia* based on the long-term experience of Hasan Yıldırım (the fourth author of this study) with this entire

complex: presence or absence of a perianth tube (A = YES, B = NO), presence or absence of corona (A = YES, B = NO), and testa surface papillate or not (A = YES, B = NO) (Supplementary material S1). Similar to the ancestral area analysis, we used the BBM (Ronquist and Huelsenbeck 2003) option of RASP v.4.2 (Reconstruct Ancestral State in Phylogenies; Yu et al. 2015) for our ancestral character analyses. We employed the same settings as in the ancestral area analyses, except for the “maximum number of areas” option, which was set to 1.

Results

The GTR+G model of molecular evolution had the highest score for both the *matK* and *trnL-F* regions and GTR+I+G was the most suitable model for the *rbcL* region. While the *trnL-F* region yielded the highest number of parsimony-informative characters as the most successful plastid region in terms of sequencing, *rbcL* yielded the fewest parsimony-informative characters (Table 2). It was also noteworthy that the support values were higher for both ML and BI “*matK*+*rbcL*+*trnL-F* no missing information” analysis. Alignment details are given in Table 2 and a summary of the results of *rbcL*, *trnL-F*, and *matK* ML and BI analyses is provided in Table 3.

Phylogenetic analyses

Similar to previous studies (e.g., Buerki et al. 2012), *Scilla* was polyphyletic in all ML and BI analyses based on *rbcL*, *trnL-F*, *matK*, and *rbcL*+*trnL-F*+*matK* datasets (Table 3, Fig. 2). *Prospero* (96–100% bootstrap support (BS) and 1.00 posterior probability (PP)) and *Puschkinia*.

(74–100% BS and 1.00 PP) were strongly supported as monophyletic within *Scilla* in all analyses except the *rbcL* phylogenetic tree. However, *Chionodoxa* was not monophyletic in any of the analyses.

Among the *Scilla* species, *S. alinihatiana* (82–100% BS, 0.94–1.00 PP), *S. bithynica* (90–100% BS, 1.00 PP), *S. melaina* (84–100% BS, 0.96–1.00 PP), *S. persica* (87–100% BS, 0.80–1.00 PP), and *S. hyacinthoides* (71–100% BS, 1.00 PP) (please note that we could not sequence *S. hyacinthoides* for all regions, and we had only one sequence for both *S. albinerve* Yıldırım & Gemici and *S. monanthos* K.Koch) were monophyletic in *matK*, *trnL-F*, *rbcL*+*trnL-F*+*matK*, and *rbcL*+*trnL-F*+*matK* “no missing data” ML and BI analyses, while *S. siberica* and *S. bifolia* were not monophyletic in any of the analyses (Table 3, Fig. 2). On the other

hand, while *S. leepii* was not monophyletic in the *matK* and *rbcL* analyses, monophyly was supported for this species with low bootstrap support (61–63%) and high posterior probability (0.99–1.00). Similarly, *S. ingridae* Speta was monophyletic in *matK*, *trnL-F*, and *rbcL + trnL-F + matK* “no missing data” ML and BI analyses (91–99% BS and 1.00 PP), but due to one sample (HY1346), this species was not monophyletic in the *rbcL + trnL-F + matK* analyses.

It is noteworthy that two main clades were strongly supported in the *rbcL + trnL-F + matK* ML and BI trees (Fig. 2). The first clade was the *S. bifolia* clade, with BS of 97% and PP of 1.00, comprising *S. bifolia*, *S. albinerve*, *S. bilgineri*, *S. messeniaca* Boiss., *S. hyacinthoides*, *S. taurica*, *Bellevalia*, *Muscari neglectum* Guss. ex Ten., *Gemicia vardariana*, *Chionoscilla allenii*, *Chionodoxa sardensis* Whittall ex Barr & Sugden, and *C. salbacus* Yıldırım, together with *C. siehei* Stapf., *C. luciliae* Boiss., and *C. forbesii* Baker, which were previously included in the genus *Chionodoxa*. It is also significant that *S. bifolia* was polyphyletic and scattered within subclades. The second clade (97% BS and 1.00 PP) included the remaining *Scilla* species and the genera *Alrawia* (Wendelbo) Perss. & Wendelbo, *Fessia* Speta, *Puschkinia*, *Prospero*, and *Hyacinthella*, which were accepted as separate genera by previous researchers (e.g., Pfosser and Speta 1999) (Fig. 2). In addition, monophyletic *Bellevalia* Lapeyr. (85% BS and 0.99–1.00 PP) was sister to one *Muscari* Mill. sample (85% BS and 1.00 PP); however, this clade was also embedded in *Scilla* samples (Fig. 1). Similarly, one *Alrawia* sample and one *Brimeura* sample were embedded in *Scilla* (Fig. 1). The morphologically similar *S. ingridae* (excluding the HY1346 sample of *S. ingridae*) and *S. melaina* were furthermore retrieved as sisters to each other (69% BS and 1.00 PP) in the total evidence phylogenetic analyses.

Divergence time estimate analysis

Our molecular dating analysis largely confirmed the findings of previous studies (e.g., Buerki et al. 2012). According to our results, the crown age of Mediterranean Hyacinthoideae was estimated to be at least 36.3 Ma (95% highest posterior density (HPD) interval: 31.8–42.0), the crown age of *Prospero* as 15.1 Ma (95% HPD: 10.62–19.17), and the crown node of *Puschkinia* as 10.22 Ma (95% HPD: 6.81–14.29) (Fig. 3). While the *Chionodoxa* species were accepted in the genus *Scilla* in the previous (e.g., Speta, 1998a, b) study, the crown age of the ancestor of *C. forbesii*, *C. siehei*, and *C. luciliae* Boiss was estimated as 7.64 Ma (95% HPD: 5.13–10.32).

The recently described species *Prospero cudidaghense* (Firat and Yıldırım 2016) diverged from *P. autumnale* (L.) Speta 7.34 Ma (95% HPD: 2.57–12.47).

On the other hand, for the two main clades of naturally growing Anatolian *Scilla* s.l., namely the *S. bifolia* clade and the second clade, which included the remaining *Scilla* species and the genera described as separate genera by previous researchers (e.g., *Fessia*, *Alrawia*), the crown ages were estimated as 12.02 Ma (95% HPD: 8.42–15.63) and 13.23 (95% HPD: 10.46–16.66), respectively. Additionally, for the second clade (e.g., *S. leepii*, *S. ingridae*), a closer look showed that the diversification of lineages within this clade occurred during the Pleistocene (0–2.5 Ma).

Ancestral area reconstructions

The BBM biogeography analysis indicated a possible Mediterranean + Irano-Turanian origin for the Mediterranean Hyacinthaceae (node 121) (Fig. 4; Online Resource 2). Additionally, the analysis supported an Irano-Turanian origin for the genus *Puschkinia* (node 109; 95.1% B) and Circumboreal + Irano-Turanian + Mediterranean origin for *Prospero* (node 114; 88.1% ABC). A possible Mediterranean origin was also suggested by the analysis for the two *Chionodoxa* lineages (nodes 69 and 73; 62.8% C and 76.6% C, respectively).

Ancestral character reconstructions

Ancestral state analysis based on the existence of a perianth tube revealed that the MRCA of the Mediterranean Hyacinthoideae and the family Hyacinthaceae most probably had no perianth tube (nodes 125 and 121; 100% B for both), and this character evolved several times independently within the family (Fig. 5; Online Resource 3). According to the results of the ancestral character analysis of existence of a corona, the ancestral form of both the family Hyacinthaceae and the Mediterranean Hyacinthoideae had no corolla, either (100% B for both), and this character evolved two times independently within the family, namely at the origins of *Puschkinia* and *Gemicia vardariana* (Yıldırım & Y.Gemici) Yıldırım (Online Resources 4–5). Lastly, for ancestral state analysis of the testa surface being papillate or not, our results indicated that the MRCA of both the family Hyacinthaceae (node 125; 98% B) and the Mediterranean Hyacinthoideae (node 121; 100% B) had a non-papillate testa surface (Online Resource 6). This character evolved at least twice within the family, namely at the origin of the clade that included *S. siberica* and its relatives and that of *S. albinerve* (Online Resource 7).

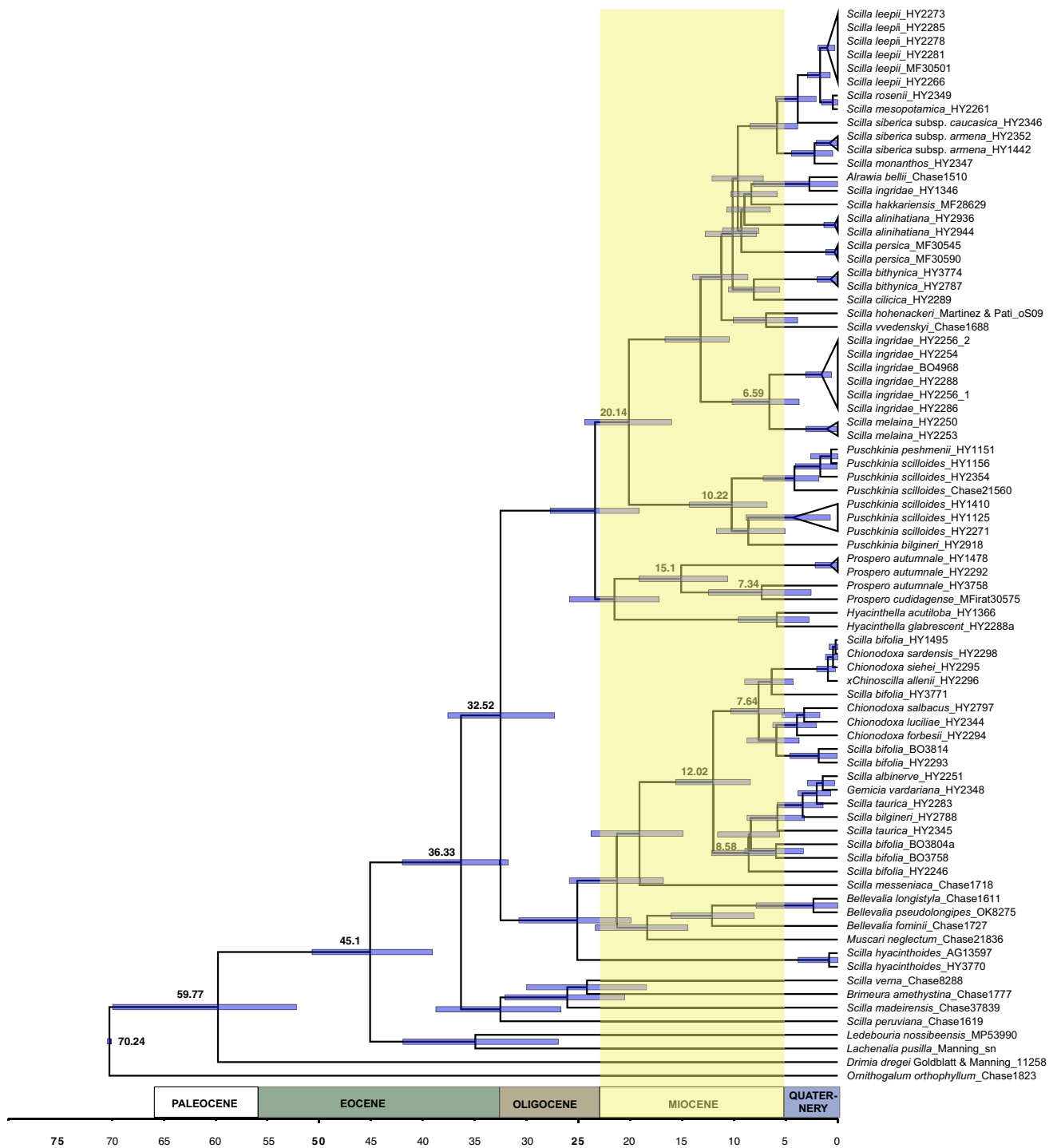


Fig. 3 Summary of divergence time analysis of *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia*. Posterior probabilities for the key nodes are indicated above the branches. Bars at each node represent the 95% HPD interval for divergence time estimates. Scale bar in million years

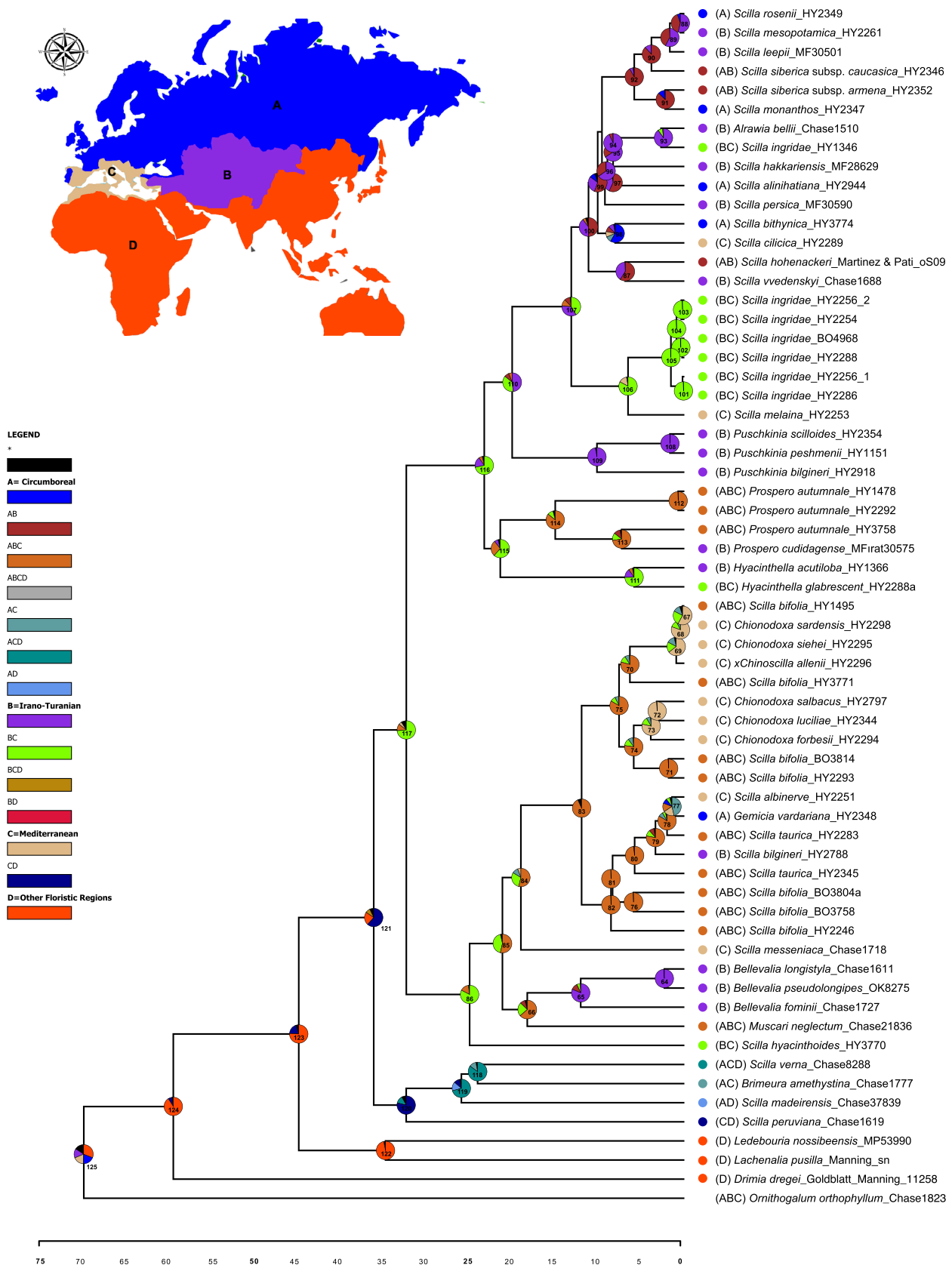


Fig. 4 Ancestral range estimates of *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia*. Inferred ancestral range probabilities are illustrated in pie charts for each node. Color-coded geographic regions are indicated on the world map following Takhtajan (1986). Scale bar in million years



Flowers of *Puschkinia scilloides* with corona



Flowers of *Scilla leepii* without corona

LEGEND

A= Corona present



A= Corona absent

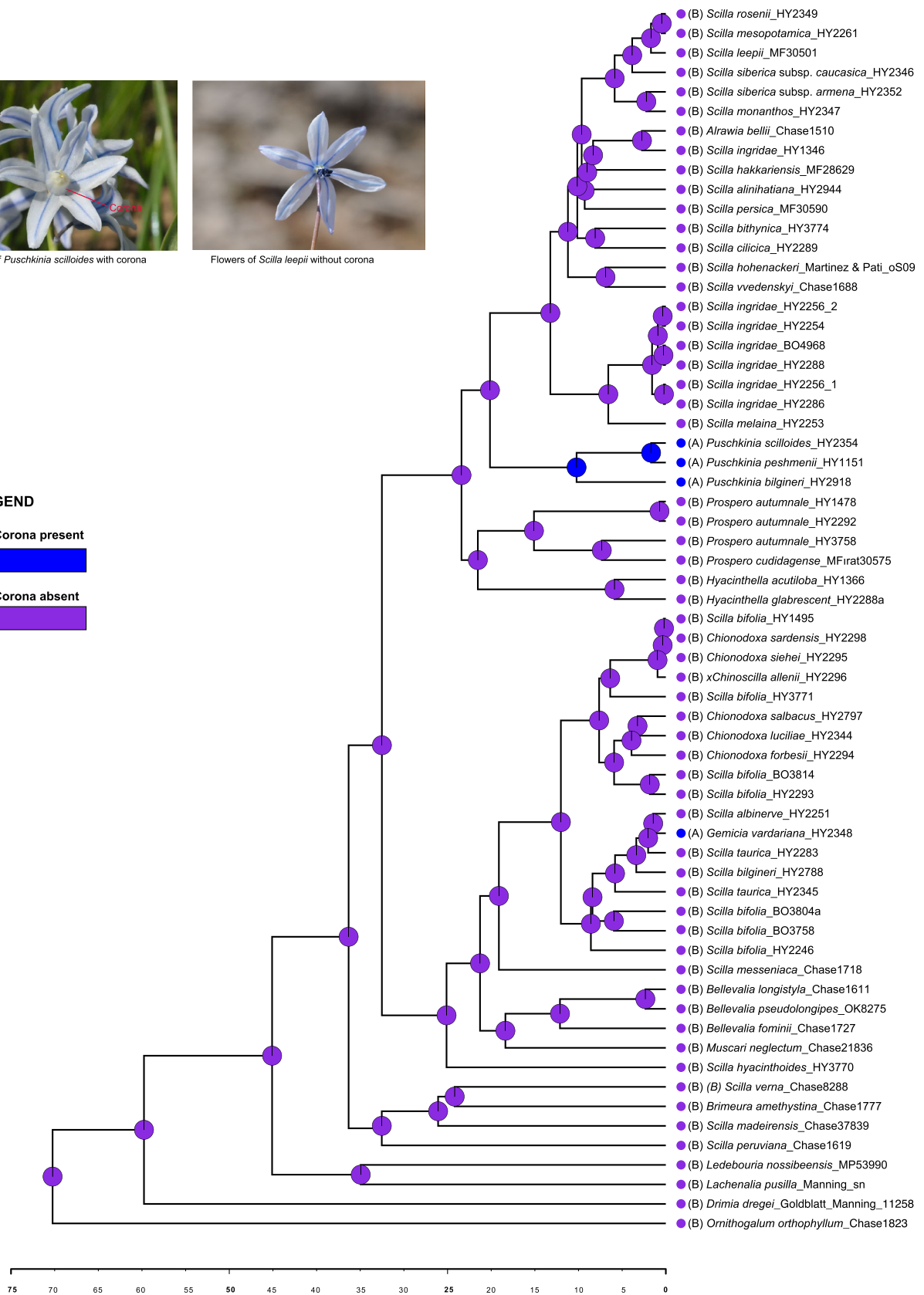


Fig. 5 Results of the “presence or absence of perigone tube” ancestral state reconstruction. Pie charts at each node represent the ancestral state probabilities. Scale bar in million years

Discussion

This study has provided new plastid DNA sequences of *Scilla* s.l. and the closely related genera *Chionodoxa*, *Gemicia*, *Prospero*, and *Puschkinia*, constituting the first comprehensive molecular phylogenetic study of the entire complex. Our findings have indicated the polyphyletic origin of *Scilla* s.l. as declared in previous studies based on plastid DNA sequences (Pfosser and Speta 1999; Wetschnig and Pfosser 2003; Ali et al. 2012). The remaining four genera, namely *Chionodoxa*, *Gemicia* (sequenced here for the first time), *Prospero*, and *Puschkinia*, along with the other *Scilla* segregates like *Zagrosia* and *Fessia* (please note that these were regarded as *Scilla* on the phylogenetic trees), fell into the polyphyletic *Scilla* clade. Among them, *Puschkinia* and *Prospero* were clearly monophyletic, whereas *Chionodoxa* and the recently described *Gemicia* shared a clade with the representatives of the *S. bifolia* species group (Fig. 2). It became clear that the morphological characters used for delimiting these genera were not congruent with phylogenetic patterns as claimed in previous phylogenetic studies (Pfosser and Speta 1999; Wetschnig and Pfosser 2003; Ali et al. 2012).

Therefore, we agree with the position accepting the morphological delimitation of these genera (*Chionodoxa*, *Gemicia*, *Prospero*, and *Scilla* s.l.), at least for now, as our study and all previous studies relied on the results of plastid DNA phylogenies and the monophyly of *Scilla* s.l. and related genera has never been tested via nuclear markers (please see the reasons for using chloroplast DNA in the Materials and Methods section). In future, wider genome analysis for this complex group could give a more certain answer.

The presence of a floral corona has been regarded as a unique character by many authors for the genus *Puschkinia* among the genera that have been studied here before the genus *Gemicia* was described (Yıldırım et al. 2013; Yıldırım 2021). This character was clearly derived from flowers without corona and independently evolved in *Puschkinia* and *Gemicia* as seen in Fig. 4. Although bearing a corona, *Gemicia* could easily be separated from *Puschkinia* with several morphological characters including its anthers at the top of the corona (vs. inserted at the middle of corona), petals without midrib (vs. with distinct midrib), seeds without sarcotesta (vs. sarcotesta), etc. (Fig. 1a–c).

Gemicia vardariana has some morphological similarities with *Scilla* species, especially *S. bifolia*, and these similarities were supported by phylogenetic analyses. However, the presence of the floral corona and its pronounced seed morphology support keeping *Gemicia* as a separate genus until new data are obtained.

A large population group of *S. bifolia* distributed throughout Europe, the Aegean Islands, and the western

and inner parts of Anatolia is characterized by mostly blackish (rarely yellowish) seeds having free elaiosomes and white scaled leaves in their bulbs (Fig. 1e). The remaining populations of the *S. bifolia* group plus *S. albinerve*, *S. bilgineri*, and *S. taurica* Fuss. distributed in the southeast, northeast, and east of Anatolia are characterized by yellowish seeds having elaiosomes adherent to the testa and red scaled leaves in their bulbs. The morphological and geographical differences between the two groups were moderately supported by our phylogenetic analyses (Fig. 2). Whereas the first clade comprised the western populations of *S. bifolia* including *Chionodoxa* and \times *Chionoscilla allenii*, the second clade included *S. taurica*, *S. bilgineri*, *S. albinerve*, and *Gemicia vardariana* with 0.88 PP and 52% BS (Fig. 2).

The unexpected phylogenetic position of *G. vardariana* seems to be strange at first glance; however, this is also congruent with the geographical and morphological characters discussed above. The eastern populations of the *S. bifolia* species group (including *G. vardariana*) show some morphological affinities to the *S. siberica* species group (*S. siberica*, *S. monanthos*, *S. leepii*), especially in terms of seed base. This species group is isolated from the western group by the Taurus and Anatolian Diagonal mountain ranges, also known as the Taurus Way (Çıplak 2008; Gür 2016; Kaya and Çıplak 2017; Özüdođru and Mummenhoff 2020; Özüdođru et al. 2020, 2022). Considering that the uplift of eastern Anatolia along with the Alborz, Zagros, and Koppeh-Dagh Mountains started to accelerate with the collision of the Arabian plate with the Eurasian plate during the Miocene (Manafzadeh et al. 2017; Eslami-Farouji et al. 2021), the time to the MRCA of the eastern *S. bifolia* species group with a median value of 8.58 mya (Late Miocene, from 11.63 to 5.33 mya) seems to be congruent with the geological history of the region (Fig. 3).

The late Miocene origin of the genus *Puschkinia* was supported by our divergence time estimation analyses with a median value of 10.22 mya (95% HPD: 6.81–14.29). The distribution of the members of this genus is restricted to the Irano-Turanian phytogeographic region (Rix and Mathew 2007; Yıldırım 2014), and this region seems to be a center of origin of *Puschkinia* based on our biogeographical analyses (Fig. 4). Therefore, our estimations are fully congruent with the geological history of the region where the *Puschkinia* species currently occur, as in the case of the eastern *S. bifolia* species group.

Although Ali et al. (2012) suggested a Mediterranean origin for the clade that included *Scilla* and relatives, those results do not support the findings of our analyses, which suggested the Mediterranean and Irano-Turanian regions together as the ancestral area.

In summary, the results of our molecular dating, ancestral area analyses, and ancestral state analyses have shown

that *Scilla*, *Prospero*, *Chionodoxa*, and *Puschkinia*, including *Brimeura*, *Bellevalia*, *Hyacinthella*, and *Alrawia*, probably had a MRCA without a perianth tube (100%), without a corona (100%), and with a non-papillate testa surface (98%), which evolved about 36 Ma in the Mediterranean region (Fig. 4). The monophyly of *Puschkinia* and *Prospero* was shown by our phylogenetic analyses; however, *Chionodoxa* and *Gemicia* were placed into a clade with the representatives of the *S. bifolia* species group. Thus, it is clear that further studies are needed to reveal the precise phylogenetic placement of these genera using nuclear markers.

Appendix

Taxon sampling of the current study based on *matK*, *trnL-F*, and *rbcL* plastid regions. Information is presented in the following order: taxon name and voucher number; locality; GenBank accession numbers for *matK*, *trnL-F*, and *rbcL*. A dash (-) indicates that the region could not be sampled.

Hyacinthaceae. *Scilla* L.: × *Chionoscilla allenii* HY2296; İzmir, Nif Mountain; OM972799, OM994077, OM972798. ***Chionodoxa forbesii*** Baker HY2294; Muğla, Fethiye, Babadağ; OM972800, OM994028, OM972737. ***C. luciliae*** Boiss. HY2344 İzmir, Ödemiş, Bozdağ; OM972801, OM994029, OM972738. ***C. salbacus*** Yıldırım HY2797; Denizli, Babadağ, at the bottom of Karababa Hill; OM972850, OM994030, OM972739. ***C. sardensis*** Whittall ex Barr & Sugden HY2298; İzmir, Mahmut Mountain; OM972802, OM994031, OM972740. ***C. siehei*** Stapf. HY2295; İzmir, Nif Mountain; OM972803, OM994032, OM972741. ***Scilla albinerve*** Yıldırım & Gemici HY2251; Hatay, Arsuz, Amanos Mountains; OM972806, OM994044, OM972758. ***S. alinihatiana*** Aslan & Yıldırım HY2944; Rize, İkizdere, from Sivrikayalar to highland; OM972804, OM994042, OM972756. ***S. alinihatiana*** HY2936; Artvin, Yusufeli, Marsis Mountain; OM972805, OM994043, OM972757. ***S. bifolia*** L. BO3758; Çankırı, Ilgaz, Şeyh Yunus village; OM972807, OM994047, OM972765. ***S. bifolia*** BO3804a; Isparta, Dedegöl Mountain; OM972808, OM994046, OM972764. ***S. bifolia*** BO3814; Muğla, Fethiye, Tuzlabeli; OM972809, -, OM972763. ***S. bifolia*** HY2293; Muğla, Köyceğiz, Sandras Mountain; OM972811, -, OM972761. ***S. bifolia*** HY2246; İzmir, Bozdağ; -, -, OM972759. ***S. bifolia*** HY3771; Belgrad Forest; OM972813, OM994049, OM972767. ***S. bifolia*** HY1495; Nif Mountain; OM972814, OM994048, OM972766. ***S. bilgineri*** Yıldırım HY2788; Adıyaman, Gölbaşı; -, OM994050, OM972768. ***S. bithynica*** Boiss. HY3774; Adapazarı, Geveye, Doğançay village; OM972815, OM994052, OM972770. ***S. bithynica*** HY2787; Bartın, Karagözü

Hill, Arıtolu; OM972816, OM994051, OM972769. ***S. cilicica*** Siehe HY2289; Mersin, Erdemli, Lomes Canyon; OM972817, OM994053, OM972771. ***S. hakka-riensis*** Firat & Yıldırım MF28629; Derecik; OM972818, OM994069, OM972790. ***S. hyacinthoides*** L. HY3770; Muğla, Datça, Yakaköy; OM972819, -, OM972773. ***S. hyacinthoides*** AG13597; Gaziantep, İslahiye, Yesemek Open Air Museum; OM972820, -, OM972772. ***S. ingridae*** Speta HY2288; Adana, Pozantı, Akçatekir Highland; OM972821, OM994058, OM972779. ***S. ingridae*** HY1346; Adana, Tufanbeyli; -, -, OM972774. ***S. ingridae*** HY2286; Kahramanmaraş, Andırın; OM972822, OM994057, OM972778. ***S. ingridae*** HY2254; Adana, Aladağlar, Maden Pass; OM972823, OM994054, OM972775. ***S. ingridae*** BO4968; Kayseri, Dededağı; OM972824, OM994059, OM972780. ***S. ingridae*** HY2256_1; Gaziantep, Nurdağı Pass; OM972825, OM994055, OM972776. ***S. ingridae*** HY2256_2; Gaziantep, Nurdağı Pass; -, OM994056, OM972777. ***S. leepii*** Speta HY2266; Malatya, Doğanşehir; OM972826, OM994060, OM972781. ***S. leepii*** MF30501; Çatak; OM972827, OM994065, OM972786. ***S. leepii*** HY2285; Van, Çatak Canyon; OM972828, OM994064, OM972785. ***S. leepii*** HY2278; Elazığ, on the Maden-Ergani road; OM972829, OM994062, OM972783. ***S. leepii*** HY2281; Siirt, Şirvan, on the Nallıkaya road; OM972830, OM994063, OM972784. ***S. leepii*** HY2273; Adıyaman, Nemrut Mountain; OM972831, OM994061, OM972782. ***S. madeirensis*** Menezes Chase 37,839; Madeira; JX090401, -, JX090589. ***S. melaina*** Speta HY2250; Hatay, Dörtöl, Kuzuculu; OM972832, OM994067, OM972788. ***S. melaina*** HY2253; Hatay, Atik Plateau; OM972833, OM994066, OM972787. ***S. mesopotamica*** Speta HY2261; Şanlıurfa, Halfeti; OM972834, OM994068, OM972789. ***S. messeniaca*** Boiss. Chase 1718; Greece; JX090402, JX090480, JX090590. ***S. monanthos*** K.Koch HY2347; Rize, Çamlıhemşin, Ayder Highland; -, OM994070, OM972791. ***S. persica*** Hausskn. MF30545; Siirt, Şirvan district, Herzane Hill; OM972835, OM994071, OM972792. ***S. persica*** MF30590; Şırnak, Besta Dereler; OM972836, OM994072, OM972793. ***S. peruviana*** L. Chase 1619; Tunisia; AJ581423, JX090481, JX090591. ***S. rosenii*** K.Koch HY2349; Ardahan, Yalnızçam Mountains; OM972837, OM994073, OM972794. ***S. siberica*** subsp. ***armena*** (Grossh.) Mordak HY2352; Kars, Sarıkamış, Çamyazı village; OM972838, OM994075, OM972796. ***S. siberica*** subsp. ***armena*** HY1442; Erzurum, Şenkaya; -, OM994074, OM972795. ***S. siberica*** subsp. ***caucasica*** (Misch.) Mordak HY2346; Trabzon, Şalpaazarı, Sıdağı; OM972839, OM994076, OM972797. ***S. taurica*** Fuss. HY2345; Trabzon, Şalpaazarı, Sıdağı; OM972810, OM994045, OM972762. ***S. taurica*** HY2283; Siirt, Şirvan, Gökbudak, Belenoluk; OM972812, -, OM972760. ***S. verna*** Huds. Chase 8288; England; JX090403, JX090482, JX090592. ***Puschkinia***

Adams., *P. bilgineri* Yıldırım HY2918; Van, Karabet Pass; OM972840, OM994039, OM972749. *P. peshmenii* Rix & B.Mathew HY1151; Hakkari, Yüksekova; -, -, OM972750. *P. scilloides* Adams HY2271; Malatya, Pötürge, on the Nemrut Mountain road; OM972841, OM994040, OM972754. *P. scilloides* HY2354; Van, Gevaş, Artos Mountain; OM972842, OM994041, OM972755. *P. scilloides* HY1125; Van, Deveboynu Peninsula; -, -, OM972751. *P. scilloides* HY1156; Van, Güzelsu, Güzeldere; -, -, OM972752. *P. scilloides* HY1410; Van, Oruçlu; -, -, OM972753. *P. scilloides* Chase 21,560; Lebanon; JX090397, JX090476, JX090585. *Gemicia* Yıldırım.: *G. vardariana* Yıldırım & Gemici HY2348; Rize, Çamlıhemşin, Ayder Highland; OM972843, OM994033, OM972742. *Prospero* Salisb.: *P. autumnale* (L.) Speta HY3758; Muğla, Marmaris, Taşlıca village; OM972844, OM994038, OM972747. *P. autumnale* HY1478; Denizli, Servergazi; OM972845, OM994036, OM972745. *P. autumnale* HY2292; Muğla, Köyceğiz, Sandras Mountain, Beyobası; OM972846, OM994037, OM972746. *P. cudidaghense* Fırat & Yıldırım M.Fırat 30575; Şırnak, Silopi, Cudi Mountain; OM972847, -, OM972748. *Hyacinthella* Schur.: *H. acutiloba* K.Perss. & Wendelbo HY1366; Malatya, Darende, Akçababalı Mountain; OM972848, OM994034, OM972743. *H. glabrescent* (Boiss.) K.Perss. & Wendelbo HY2288a; Adana, Pozantı, Akçatekir Highland; OM972849, OM994035, OM972744. *Bellevatia* Lapeyr.: *B. fominii* Woronow Chase 1727; Turkey; JX090318, JX090493, JX090415. *B. longistyla* (Miscz.) Grossh. Chase 1611; Turkey; JX090320, JX090495, JX090417. *B. pseudolongipes* Karabacak & Yıldırım OK8275; Siirt, Siirt University, Kezer Campus; -, -, OM972736. *Fessia* Speta.: *F. vvedenskyi* (Pazij) Speta Chase 1688; Tajikistan; JX090361, JX090540, JX090444. *F. hohenackeri* (Fisch. & C.A.Mey.) Speta Mario Martínez & Julio Pati_o S09; Spain; JX090360, JX090539, JX090443. *Alrawia* (Wendelbo) Perss. & Wendelbo.: *A. bellii* (Baker) Perss. & Wendelbo Chase 1510; Iran; JX090315, AM902384/AM902479, AM902332. *Muscari* Mill.: *M. neglectum* Guss. ex Ten. Chase 21,836; Turkey; JX090381, JX090562, Z77278. *Brimeura* Salisb.: *B. amethystina* (L.) Chouard. Chase 1777; Spain; JX090326, JX090501, JX090422. **OUT-GROUPS.** *Lachenalia pusilla* Jacq. Manning s.n.; South Africa; JX090375, JX090452, JX090554. *Ledebouria nossibeensis* (H.Perrier) J.C.Manning & Goldblatt MP53990; Madagascar; JX090376, JX090453, AM909711. (Baker) J.C.Manning & Goldblatt 11,258; South Africa; JX090348, JX090434, JX090522. *Ornithogalum orthophyllum* Ten. Chase 1823; Greece; JX090391, JX090467, JX090574.

Information on Electronic Supplementary Material

Online Resource 1. Input matrix for the ancestral area and state reconstructions.

Online Resource 2. Lagrange results of ancestral area reconstruction.

Online Resource 3. Results of character reconstruction for presence or absence of perigone tubeancestral.

Online Resource 4. Results of character reconstruction for presence or absence of corona.

Online Resource 5. Ancestral state reconstruction of the “presence or absence of corona”.

Online Resource 6. Results of character reconstruction for testa surface papillate or not.

Online Resource 7. Ancestral state reconstruction of the “testa surface”.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00606-022-01835-x>.

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Data availability The GenBank accession numbers for all successfully sequenced trnL-F, rbcL, and matK DNA barcodes can be found in “Appendix”.

Declarations

Conflict of interest The authors declare that they do have no conflict of interest.

References

- Ali SS, Yu Y, Pfosser M, Wetschnig W (2012) Inferences of biogeographical histories within subfamily Hyacinthoideae using S-DIVA and Bayesian binary MCMC analysis implemented in RASP (Reconstruct Ancestral State in Phylogenies). *Ann Bot (Oxford)* 109:95–107. <https://doi.org/10.1093/aob/mcr274>
- Anderson CL, Janssen T (2009) Monocots. In: Hedges SB, Kumar S (eds) *The timetree of life*. Oxford University Press, Oxford
- Angiosperm Phylogeny Group (2009) An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. *Bot J Linn Soc* 161:105–121
- Buerki S, Jose S, Yadav SR et al (2012) Contrasting biogeographic and diversification patterns in two Mediterranean-type ecosystems. *PLoS ONE* 7:e39377. <https://doi.org/10.1371/journal.pone.0039377>

- CBOL Plant Working Group (2009) A DNA barcode for land plants. *Proc Natl Acad Sci USA* 106:12794–12797
- Chase MW, Reveal JL, Fay MF (2009) A subfamilial classification for the expanded asparagalean families Amaryllidaceae, Asparagaceae and Xanthorrhoeaceae. *Bot J Linn Soc* 161:132–136. <https://doi.org/10.1111/j.1095-8339.2009.00999.x>
- Chen S, Kim D-K, Chase MW, Kim J-H (2013) Networks in a large-scale phylogenetic analysis: reconstructing evolutionary history of Asparagales (Liliana) based on four plastid genes. *PLoS ONE* 8:e59472. <https://doi.org/10.1371/journal.pone.0059472>
- Çıplak B (2008) The analogy between glacial cycles and global warming for the glacial relicts in a refugium: a biogeographic perspective for conservation of Anatolian Orthoptera. *Insect Ecol Conserv* 5:135–163
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Meth* 9:772
- Dashwood M, Mathew B (2005) Hyacinthaceae-little blue bulbs. RHS Plant Trials and Awards, Bulletin Number 11. Royal Horticultural Society, Wisley
- de Miranda VFO, Martins VG, Furlan A, Bacci M Jr (2010) Plant or fungal sequences? An alternative optimized PCR protocol to avoid ITS (nrDNA) misamplification. *Brazil Arch Biol Technol* 53:141–152
- Drummond AJ, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol Biol* 7:214. <https://doi.org/10.1186/1471-2148-7-214>
- Drummond AJ, Suchard MA, Xie D, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molec Biol Evol* 29:1969–1973. <https://doi.org/10.1093/molbev/mss075>
- Eslami-Farouji A, Khodayari H, Assadi M et al (2021) Phylogeny and biogeography of the genus *Hesperis* (Brassicaceae, tribe Hesperideae) inferred from nuclear ribosomal DNA sequence data. *Pl Syst Evol* 307:17. <https://doi.org/10.1007/s00606-020-01727-y>
- Firat M, Yıldırım H (2016) *Prospero cudidaghense* sp. nov. (Asparagaceae): a new species from southeastern Anatolia, Turk J Bot 40:388–393. <https://doi.org/10.3906/bot-1508-3>
- Ford CS, Ayres KL, Toomey N et al (2009) Selection of candidate coding DNA barcoding regions for use on land plants. *Bot J Linn Soc* 159:1–11. <https://doi.org/10.1111/j.1095-8339.2008.00938.x>
- Forest F (2009) Calibrating the Tree of Life: fossils, molecules and evolutionary timescales. *Ann Bot (Oxford)* 104:789–794. <https://doi.org/10.1093/aob/mcp192>
- Goaverts R (2022) World checklist of Asparagaceae. Royal Botanic Gardens, Kew. Available at: <http://wcp.science.kew.org/>. Accessed 11 Mar 2022
- Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704. <https://doi.org/10.1080/10635150390235520>
- Gür H (2016) The Anatolian diagonal revisited: Testing the ecological basis of a biogeographic boundary. *Zool Middle East* 62:189–199. <https://doi.org/10.1080/09397140.2016.1226544>
- Jang T-S, Emadzade K, Parker J et al (2013) Chromosomal diversification and karyotype evolution of diploids in the cytologically diverse genus *Prospero* (Hyacinthaceae). *BMC Evol Biol* 13:136. <https://doi.org/10.1186/1471-2148-13-136>
- Kaya S, Çıplak B (2017) Phylogeography of *Psorodonotuscaucasicus* group: independent double invasion of Balkans from Transcaucasia. *Syst Entomol* 42:118–133
- Kearse M, Moir R, Wilson A et al (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649. <https://doi.org/10.1093/bioinformatics/bts199>
- Li Y, Gao L-M, Poudel RC et al (2011) High universality of matK primers for barcoding gymnosperms. *J Syst Evol* 49:169–175. <https://doi.org/10.1111/j.1759-6831.2011.00128.x>
- Manafzadeh S, Staedler YM, Conti E (2017) Visions of the past and dreams of the future in the Orient: the Irano-Turanian region from classical botany to evolutionary studies. *Biol Rev Cambridge Philos Soc* 92:1365–1388. <https://doi.org/10.1111/brv.12287>
- Martínez-Azorín M, Crespo MB, Juan A, Fay MF (2011) Molecular phylogenetics of subfamily Ornithogaloideae (Hyacinthaceae) based on nuclear and plastid DNA regions, including a new taxonomic arrangement. *Ann Bot (Oxford)* 107:1–37
- Mathew B (1987) The smaller bulbs 145. Batsford, London
- Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Gateway Computing Environments Workshop (GCE)*. IEEE, New Orleans, pp 1–8
- Özüdoğru B, Mummenhoff K (2020) Phylogenetic and biogeographical history confirm the Anatolian origin of *Bornmuellera* (Brassicaceae) and clade divergence between Anatolia and the Balkans in the Plio-Pleistocene transition. *Turk J Bot* 44:593–603
- Özüdoğru B, Özgüşi K, Perктаş U, Gür H, (2020) The Quaternary range dynamics of *Noccaea iberidea* (Brassicaceae), a typical representative of subalpine/alpine steppe communities of Anatolian mountains. *Biol J Linn Soc* 131:986–1001
- Özüdoğru B, Karacaoğlu Ç, Akaydın G et al (2022) Ecological specialization promotes diversity and diversification in the East Mediterranean genus *Ricottia* (Brassicaceae). *J Syst Evol* 60:331–343. <https://doi.org/10.1111/jse.12749>
- Pföster M, Speta F (1999) Phylogenetics of Hyacinthaceae based on plastid DNA sequences. *Ann Missouri Bot Gard* 86:852–875. <https://doi.org/10.2307/2666172>
- Rambaut A, Drummond AJ (2007) TreeAnnotator. Available at: <http://beastbio.ed.ac.uk/TreeAnnotator>
- Rambaut A, Drummond AJ (2015) LogCombiner v1.8.2. Available at: <http://evolve.zoo.ox.ac.uk/beast/>
- Rambaut A, Suchard MA, Xie D, Drummond AJ (2015) Tracer v1.6
- Rambaut A (2012) FigTree v1.4. tree figure drawing tool version 1.4.4. Available at: <http://tree.bio.ed.ac.uk/software/figtree>
- Reveal JL, Chase MW (2011) APG III: Bibliographical information and synonymy of magnoliidae. *Phytotaxa* 19:71. <https://doi.org/10.11646/phytotaxa.19.1.4>
- Rix M, Mathew B (2007) 582 *Puschkinia peshmenii*: Hyacinthaceae. *Curtis's Bot Mag* 24:54–57
- Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572–1574. <https://doi.org/10.1093/bioinformatics/btg180>
- Ronquist F, Teslenko M, van der Mark P et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol* 61:539–542. <https://doi.org/10.1093/sysbio/sys029>
- Ruksäns J (2019) *Puschkinia kurdistanica* (Asparagaceae) - a new species from the shores of Lake Van in Turkey. *Int Rock Gardener* 116:3–18
- Speta F (1971) Beitrag zur Systematik von *Scilla* L. subgen. *Scilla* (inklusive *Chionodoxa* Boiss.). *Oesterr Bot Z* 119:6–18. <https://doi.org/10.1007/bf01373105>
- Speta F (1976) Über *Chionodoxa* Boiss., ihre Gliederung und Zugehörigkeit zu *Scilla* L. *Naturk Jahrb Stadt Linz* 21:9–79
- Speta F (1981) Bemerkungen über die Familie Hyacinthaceae. *J Agardh Linzer Biol Beitr* 13:79–80
- Speta F (1982) Die Gattungen *Scilla* L. s. str. und *Prospero* Salisb. im Pannonischen Raum. *Veröff Intern Arbeitsgem, Clusius-Forschung Güssing* 5:1–19

- Speta F (1998a) Systematische Analyse der Gattung *Scilla* L. s. l. (Hyacinthaceae). *Phyton* (Horn) 38:1–141
- Speta F (1998b) Hyacinthaceae. In: Kubitzki K (ed) *Flowering Plants, Monocotyledons: Liliaceae (except Orchidaceae)*. Springer, Berlin, Heidelberg, pp 261–285
- Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313. <https://doi.org/10.1093/bioinformatics/btu033>
- Stedje B (1998) Phylogenetic relationships and generic delimitation of sub-Saharan *Scilla* (Hyacinthaceae) and allied African genera as inferred from morphological and DNA sequence data. *Pl Syst Evol* 211:1–11. <https://doi.org/10.1007/BF00984908>
- Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol Biol* 17:1105–1109. <https://doi.org/10.1007/BF00037152>
- Takhtajan A (1986) *Floristic regions of the world*. University of California Press, Berkeley
- Trávníček B, Duchoslav MA, Šarhanová P, Šafářová L (2009) Squills (*Scilla* s.lat., Hyacinthaceae) in the flora of the Czech Republic with taxonomical notes on Central-European squill populations. *Acta Mus Mor Sci Biol* 94:157–205
- Wetschnig W, Pfosser M (2003) The *Scilla plumbea* puzzle—present status of the genus *Scilla* sensu lato in southern Africa and description of *Spetaea lachenaliiflora*, a new genus and species of Massonieae (Hyacinthaceae). *Taxon* 52:75–92
- White TJ, Bruns T, Lee S et al (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protoc Guide Method Appl* 18:315–322
- Yıldırım H (2014) *Puschkinia bilgineri* Asparagaceae alt familia Scilloideae : Dođu Anadolu Bölgesi'nden yeni bir Karsümbülü *Puschkinia* Adams türü. *Bađbahçe Bilim Dergisi* 1:1–10
- Yıldırım H, Altođlu Y (2021) *Chionodoxa* Boiss. (Asparagaceae) Cinsinin Taksonomik Statüsünün Sürdürülmesi, Türkiye'de Yayılış Gösteren Taksonlarının Morfolojik Revizyonu ve Yeni Bir Türü. *Bađbahçe Bilim Dergisi* 8:51–95
- Yıldırım H, Gemici Y, Wilkin P (2013) *Scilla vardaria* (Asparagaceae subfamily Scilloideae): a threatened new species of *Scilla* L. from Northeast Turkey with a floral corona. *Phytotaxa* 91:50–60
- Yıldırım H (2021) *Gemicia* Yıldırım, Kuşkonmazgiller (Asparagaceae)'den, Kuzeydođu Anadolu/Türkiye'den Monotipik Yeni Bir Cins. *Bađbahçe Bilim Dergisi* 8:38–50. <https://doi.org/10.35163/bagbahce.892396>
- Yu Y, Harris AJ, Blair C, He X (2015) RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Mol Phylogen Evol* 87:46–49. <https://doi.org/10.1016/j.ympev.2015.03.008>

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