Phylogenetic Affinities of Wild and Cultivated Ornithogaloideae Based on ITS and *trnL–F* DNA Sequences by Extended Sampling from Iran

K. Riahi Rad[1], A. Babaei[1*], V. Mozaffarian[2], and D. Potter[3]

ABSTRACT

The taxonomic classification of subfamily Ornithogaloideae has been a subject of considerable controversy in recent decades. *Ornithogalum* is a relatively large genus in Ornithogaloideae including valuable ornamental and medicinal plants. These wild ornamentals, which are introduced into agriculture recently, are becoming increasingly popular as cut flowers, pot plants, and for gardening. This is the first molecular phylogenetic study that includes 10 of the 13 *Ornithogalum* species native to Iran. The aims of the present study were to use ITS and *trnL-F* sequences to explore phylogenetic relationships and to evaluate genetic resources of Ornithogaloideae naturally occurring in Iran, with an increased sampling of species to be compared to previous phylogenetic studies. In the present study, the combined tree resulted in the best-resolved phylogenetic relationships at the generic level. The results of Maximum Likelihood and Bayesian analysis of molecular data were compared to those from hierarchical cluster analysis of morphological characters. Based on the results, all specimens collected in Iran across all previously recognized taxonomic genera in Ornithogaloideae were placed in *Ornithogalum sensu stricto* and *Loncomelos*, which is in line with the morphological analysis. Divergent placements of multiple specimens of a single species in *L. brachystachys*, *O. orthophyllum,* and *O. sintenisii* are attributed to the possibility of past hybridization events, although incomplete lineage sorting and ITS paralogy cannot be overlooked. Increased understanding of naturally occurring variation among wild *Ornithogalum* populations of Iran and the phylogenetic relationships of wild and cultivated species of Ornithogaloideae could contribute to important opportunities to introduce new ornamentals and improve the agricultural performance of ornamental varieties.

Keywords: Molecular phylogenetics, Nuclear and Plastid DNA sequences, *Ornithogalum*, *Loncomelos*, *Melomphis*, Ornamental plant.

INTRODUCTION

Ornithogalum L. belongs to subfamily Ornithogaloideae of Hyacinthaceae, a family of about 700-900 species of bulbous plants mainly distributed through Africa, Europe, and south-west Asia, with one small genus found in South America (Oziroe¨) (APG II 2003). The taxonomic classification of this subfamily remains a subject for speculation in recent years (Martínez-Azorín *et al.*, 2011). Manning Forest *et al*. (2009) extensively studied the possible classifications within this subfamily based on plastid molecular sequences, and finally proposed three tribes (*Dipcadieae*, *Ornithogaleae,* and *Albuceae*), with only four genera, *Albuca* L., *Dipcadi* Medik., *Ornithogalum* L. and *Pseudogaltonia* Kuntze. They divided genus *Ornithogalum* into four subgenera i.e. *Galtonia* Decne., *Aspasia* Salisb., *Avonsera* Speta., and *Ornithogalum*. Although

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[1]Department of Horticulture Science, Tarbiat Modares University, Tehran, Islamic Republic of Iran.

[2]Research Institute of Forests and Rangelands, Tehran, Islamic Republic of Iran.

[3]Department of Plants Science, University of California Davis, Davis, USA.

*Corresponding author; e-mail: arbabaei@modares.ac.ir

Galtonia, *Avonsera,* and *Aspasia* were homogeneous in morphology, *Ornithogalum* (with seven sections) was extremely heterogeneous. Moreover, in order to make *O.* section *Ornithogalum* monophyletic, Manning *et al.* (2009) included *Loncomelos* Raf. [*O.* subgenus *Beryllis* (Salisb.) Baker] within this genus, therefore, constructing a morphologically heterogeneous aggregate which is difficult to define with regard to other sections. In the most recent classification on the basis of the phylogenetic analyses based on plastidial and nuclear DNA regions by Martínez-Azorín *et al.* (2011), 19 monophyletic genera are accepted within Ornithogaloideae: *Albuca*, *Avonsera*, *Battandiera* Maire., *Cathissa* Salisb., *Coilonox* Raf., *Dipcadi*, *Eliokarmos* Raf., *Elsiea* F.M.Leight., *Ethesia* Raf., *Galtonia*, *Honorius* Gray, *Loncomelos*, *Melomphis* Raf., *Neopatersonia* Schonland, *Nicipe* Raf., *Ornithogalum*, *Pseudogaltonia*, *Stellarioides* Medik., and *Trimelopter* Raf. In their study, the *Loncomelos* clade was sister to the monophyletic *Ornithogalum s. str.*, which provides easy morphological circumscription for both groups. As pointed out by Müller Doblies and Müller Doblies (1996) dividing genus *Ornithogalum* based on the DNA molecular sequences seems to be by far the better taxonomic solution than including *Albuca* and other genera into *Ornithogalum*, which makes a huge taxonomic 'dustbin'.

Several species of Ornithogaloideae such as *Eliokarmos thyrsoides* (Jacq.) Raf. and *E. dubius* (Houtt.) Mart.-Azorín, M.B.Crespo & Juan have been developed as cut flowers (Luria *et al.*, 2000) and pot plants (Anderson 2006). Also, *Ornithogalum Umbellatum* L., *Loncomelos pyramidale* (L.) Raf. and *Honorius nutans* (L.) Gray. are used in gardens and *Melomphis arabica* (L.) Raf. and *Galtonia saundersiae* (Baker) Mart.-Azorín, M. B. Crespo & Juan as cut flowers (Plančić *et al.*, 2015). On the other hand, many plants from this subfamily have been used for various medicinal purposes, while several species are implicated in livestock poisoning (Watt and Breyer-Brandwijk 1932, Botha *et al.*, 2000). For example, *O. cuspidatum* Bertol. is used in Iranian traditional medicine to treat respiratory and inflammatory diseases (Samavati *et al.*, 2010). Furthermore, it has been used as an anti-irritant and relaxant and as a food additive (Nazifi *et al.*, 2010). Recently, some studies suggested Ornithogaloideae species contain compounds that exhibit cytotoxic properties against a variety of malignant tumor cells (Asadi *et al.*, 2014). Despite this potential importance, the genetic resources available within this subfamily are still largely unexploited (Anderson, 2006).

Iran is recognized as one of the 34 hotspots of biodiversity in the world (http://www.biodiversityhotspots.org/xp/Hotspots). It is also a country with high diversity of bulbous plants. More than 200 species of bulbous plants from different families grow in Iran (Farahmand and Nazari, 2015), including 15 species of *Ornithogalum* (Rechinger, 1995; Maroufi, 2010; Heidaryan, 2011; Bidarlord and Ghahremaninejad, 2016). Baker (1872) traditionally placed these species in 3 subgenera: *O.* subgen. *Beryllis* (Salisb) Baker (= *Loncomelos*), *O.* subgen. *Ornithogalum* (Syn.: Subgen *Heliocharmos* Baker) (= *Ornithogalum s. str.*), and *O.* subgen. *Caruelia* (Parl) Baker (= *Melomphis* p. p.) (Rechinger, 1995).

Although there are several published studies about the phylogenetic and morphological characterization of Ornithogaloideae around the world, to date there are no published studies of phylogenetic affinities for *Ornithogaloideae* species native to Iran. Placing Iranian species in a phylogenetic context should improve our understanding of the extent and distribution of their biodiversity, which, in turn, should have important implications for germplasm selection in breeding programs aimed at development of these species as ornamental and medicinal plants.

In the present study, we aimed to use nuclear (ITS) DNA and plastid (trnL–F) sequences to: (a) Explore phylogenetic relationship of the cultivated and wild Ornithogaloideae species, with increased sampling of species native to Iran compared to previous studies; (b) Evaluate generic and infrageneric taxonomy of 3 genera of Ornithogaloideae from Iran; and (c) Evaluate genetic resources of this genus in Iran in order to provide important information for plant breeders interested in generating new Ornithogaloideae cultivars for medicinal and ornamental purposes.

MATERIALS AND METHODS

Plant Material

Young leaves of 50 specimens representing 10 species of *Ornithogalum, Loncomelos* and *Melomphis* were collected through 50 localities in Iran (Table 1). The samples were labeled and immediately placed in liquid nitrogen. The coordinates of the readings were determined by the Global Positioning System (GPS). The map for geographical distribution of collected samples was generated from GPS data using ArcGIS Desktop (ESRI, Inc.) (Figure 1a-b). Samples were stored at -80°C till DNA extraction. DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, USA) according to the manufacturer's recommendations. The identification of taxa was made by an expert botanist [Mozafarian (PhD)], Research Institute of Forests and Rangelands, Tehran, Iran) and deposited in the herbarium of Iranian Biological Research Center. Voucher numbers are shown in Table S2.

Morphological Characters

All plants chosen for DNA extraction were morphologically described and 26 morphological characters were used for a hierarchical cluster analysis. Of these, 11 were continuous (including plant, scape and inflorescence length; leaves and flower number; leaves, perigone and bulb length and width) while the remaining 15 were discrete

(Table 2). The datasets were subjected to a hierarchical cluster analysis using Ward's method and squared Euclidean distances (Version 9.3.1; SAS Institute Inc, Cary, NC).

Sequence Generation

The *trnL* intron and *trnL–F* spacer (the trnL–F region; hereafter trnL–F) were amplified as a single fragment using primers c and f (Taberlet *et al*., 1991). ITS was amplified using ITS6 and ITS9 primers (Potter *et al*., 2007). A total volume of 50 µL was used in the PCR amplification process as follows: 2 µL dNTP, 5 µL 10X PCR buffer (Qiagen, USA), 1 µL (10 µM) of forward and reverse primers (each), 1 µL of $MgCl_2$ (25 mM), 0.5 µL of Taq DNA Polymerase, 38.5 µL deionized water and 1 µL of DNA template plus 1 µL DMSO for ITS amplification. PCR amplifications were performed with an initial 5 min at 95 °C, followed by 35 cycles of 1 minute at 95°C, 1 minute at 50°C, 1 minute at 72°C, followed by a final 7 minutes at 72°C. PCR products were subjected to gel electrophoresis and were cleaned up using a QIAquick Gel Extraction Kit (Qiagen, USA). Purified PCR products were directly sequenced in both forward and reverse directions, using the corresponding primers. The sequencing process was performed using ABI 3730 Capillary Electrophoresis Genetic Analyzers. Sequencer 5.4 (Gene Codes Corporation, Ann Arbor, MI, USA) was used to assemble complementary strands. The sequences' identities were confirmed by comparison with various published sequences

Table 1. Populations included in the phylogenetic analyses of the genus *Ornithogalum* L. collected in Iran.

| Subfamily	Genus	Species name	Samples number
Ornithogaloideae	*Loncomelos*	*Loncomelos kurdicum* Bornm.	1
		Loncomelos arcuatum (Stev.) Speta	4
		Loncomelos brachystachys (C. Koch.) Hort. Gorenk. ex Schult. f.	12
		Loncomelos bungei Boiss.	1
	Ornithogalum	*Ornithogalum oligophyllum* E. D. Clarke.	1
		Ornithogalum orthophyllum Ten.	5
		Ornithogalum sintenisii Freyn.	11
		Ornithogalum neurostegium Boiss. & C. I. Blanche ex Boiss.	3
		Ornithogalum cuspidatum (Bertol.) Griseb.	9
	Melomphis	*Melomphis persica* (Hausskn. ex Bornm.) Mart.-Azorín, M. B. Crespo & Juan	3

(a)

(b)
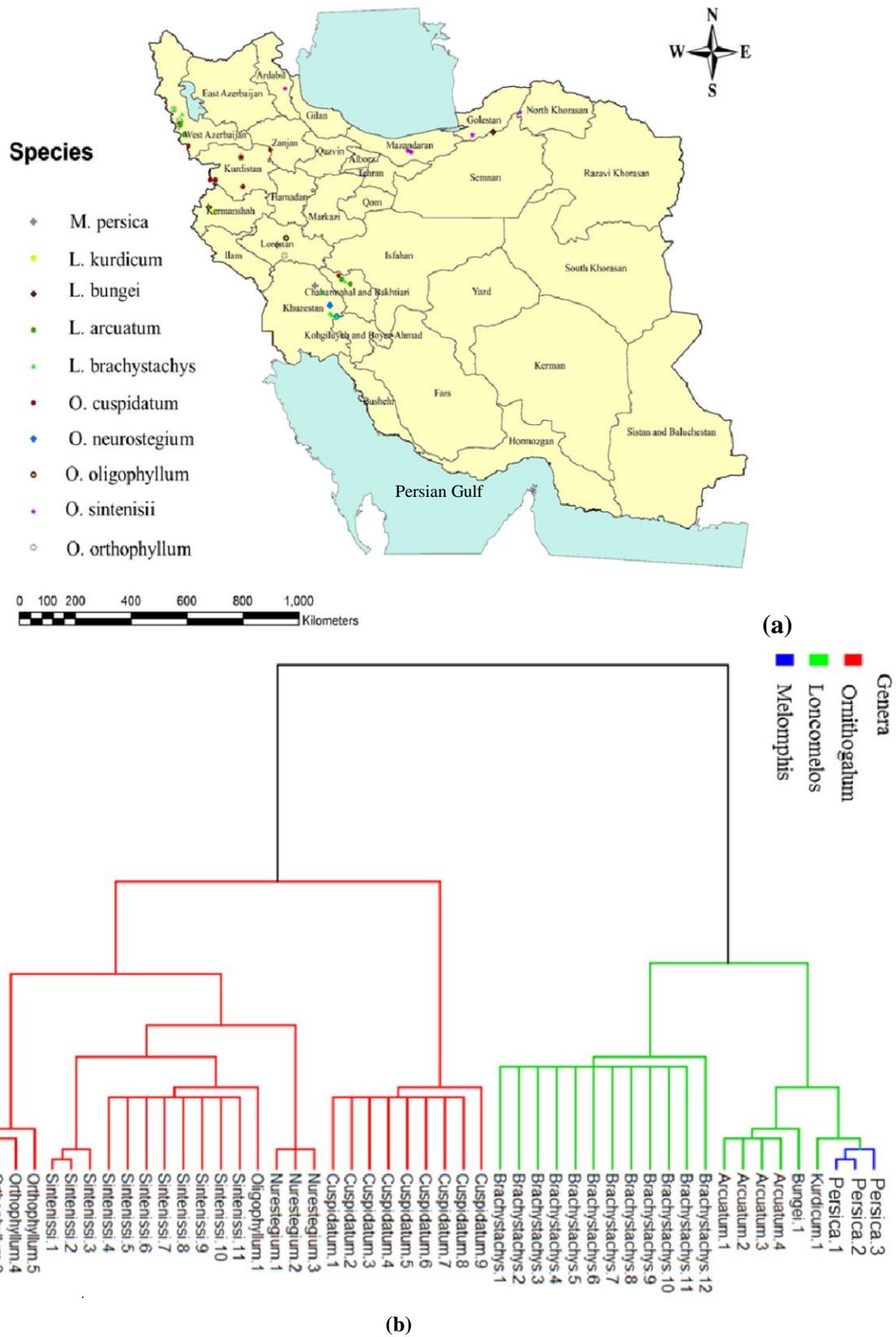
Figure 1. Geographical distribution of collected samples (a) and hierarchical cluster analysis of morphological characters (b) using Ward's method.

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Table 2. Discrete morphological characters included in the analysis of genus *Ornithogalum* in Iran.

Characters	Descriptor state		Characters	Descriptor state	
1. Leaves color	Chartreuse	1	9. Petal stripe color	Without strip	0
	Electric lime	2		Dandelion	1
	Bright green	3		Dark yellow	2
	Bitter lemon	4		Fluorescent yellow	3
	Green	5		Green	4
	Dark olive green	6		Deep green	5
	Deep green	7	10. Petal stripe width	Without strip	0
2. Leaves tip shape	Semi _lanceolate	0		Narrow	1
	lanceolate	1		Semi_wide	2
3. Leaves position	Erect	0		Wide	3
	Non erect	1		Wider	4
4. Margin of leaves	Non_wavy	1	11. Ovary color	Dandelion	1
	Slightly wavy	2		Dark yellow	2
	Wavy	3		Brown	3
5. Leaves shape	Wide linear	1		Dark brown	4
	Semi wide linear	2		Dark chocolate	5
	Linear	3	12. Anther color	Creamy	1
	Narrowly linear	4		Yellow	2
	Very narrowly linear	5		Deep lemon	3
6. Leaves longer than inflorescence	No	0		Dark yellow	4
	Equal	1			
	Yes	3	13. Bulb color	Creamy	1
7. Inflorescence compaction	Non_compact	1		Eggshell	2
	Compact	2		Canary	3
	Compressed and compact	3		Corn	4
				Citrine	5
8. Perianth color	Snow	1	14. Scale style	Semi_ contiguous	1
	Conditioner	2		Semi_free	2
	Milk	3		Free	3
	Cream	4	15. Erect scape	Yes	1
	Blond	5		No	0

available in GenBank. Consensus sequences for each specimen were deposited in GenBank; accession numbers are listed in supplementary data (Table S2).

Phylogenetic Reconstruction

Bowiea volubilis Harv. ex Hook. f. was selected as the out-group based on Martínez-Azorín *et al.* (2011). The Ornithogaloideae was used as a query to search the non-redundant (nr) dataset of GenBank in order to obtain additional ingroup sequences (Searched on 1st of May, 2017)

(Table S2). The majority of available *trnL–F* sequences in Genbank were short (300-500 bp); in order to avoid large proportions of missing data, we did not include the sequences under 908 bases in length in our *trnL–F* and combined data analyses.

In combination with sequences generated by this study, 112 sequences from 93 taxa for ITS, and 70 sequences from 44 taxa for *trnL–F* were included in the following analyses. The collected sequences were first aligned using MUSCLE (Edgar, 2004) through MEGA7 (Kumar *et al.*, 2016) followed by manual adjustments. Aligned sequences were then analyzed using jModeltest 2

v2.1.7 (Darriba *et al.*, 2012) to estimate the best substitution model based on AIC, cAIC, and BIC. The estimated model was then passed on to RAxML v8.2.8 (Stamatakis, 2014) under default algorithm (Option: -fd) to generate a maximum likelihood tree. Model parameters and equilibrium base frequencies were estimated by RAxML. Four parallel searches were conducted in order to avoid selecting a tree lodged on a local optimum. Bootstrap values were calculated from 100 replicates, and corresponding coefficients were indicated above each branch.

The Bayesian analysis was conducted with MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2005). The Markov Chain Monte Carlo chains were run for 20×10^6 for ITS and 10×10^6 generations for *trnL–F* and combined trees and setting the 'burnin' at 50,000 for ITS and 25,000 for *trnL–F* and combined trees. We used the Markov Chain Monte Carlo method within a Bayesian framework to estimate the posterior probabilities of the phylogenetic trees (Larget and Simon, 1999) using the 50% majority rule.

RESULTS

Morphological Characters

On the basis of the morphological analysis, including 26 characters, the species of *Loncomelos* [= *O.* subgen. *Beryllis* (Salisb) Baker] and *Melomphis* [= *O.* subgen. *Caruelia* (Parl) Baker] were grouped together in one clade. *Melomphis persica* (Hausskn. ex Bornm.) Mart.-Azorín, M. B. Crespo & Juan, the sole member of *Melomphis* (= *O.* subgenus *Caruelia*) included in our study, was placed as sister to *Loncomelos kurdicum* Bornm.. *Ornithogalum* s. str. [= *O.* subgen. *Ornithogalum* (Syn.: Subgen *Heliocharmos* Baker)] was placed as sister to *Loncomelos* and *Melomphis*. In the morphological character tree, multiple individuals of a single species clustered together.

3.2. Phylogenetic Relationships amongst Ornithogaloideae Species Based on DNA Sequences

Despite difficulties in amplification and sequencing, PCR amplicons were successfully obtained for all the samples, and for most examined taxa yielded data useful for phylogenetic evaluation. Six of these sequences when aligned to others were missing a part of the 5.8S rDNA region; thus, we determined these as pseudogenes (Hřibová *et al.*, 2011). After the exclusion of these pseudogene sequences, 26 sequences for ITS and 33 sequences for *trnL–F* regions remained from the original 50 genotypes.

For ITS, the resulting alignment contained 691 positions (with 448 variable and 316 parsimony-informative sites). For *trnL–F*, the resulting alignment contained 1,089 positions (with 169 variable and 97 parsimony-informative sites). For the combined analysis, the resulting alignment contained 1682 positions (with 481 variable and 267 parsimony-informative sites). Moreover, jModeltest2 (Darriba *et al.*, 2012) found GTR+I+G for ITS, GTR+G for *trnL–F* and GTR+G for combined tree to be the best model based on AIC, cAIC, and BIC. In Maximum Likelihood analysis, RAxML with estimated parameters ($\alpha = 1.450162$ and $I = 0.272202$ for ITS, $\alpha = 0.309291$ for trnL–F and $\alpha = 0.299892$ for combined tree) generated the most likely phylogeny with the likelihood score of -6203.766527 for ITS, -3006.911030 for *trnL–F* and -8064.615813 for combined tree. In all three of our trees, all of the Iranian native species appeared in a single large clade. In the ITS and combined trees, the clade was strongly supported (1.00 PP; 100% BS), while in the *trnL–F* tree, the clade was weakly supported (69 PP; 50% BS) (Figures 2, 3, and 4).

ITS tree

The ITS tree includes two main clades. The first one comprises *Ornithogalum*, *Honorius*, *Loncomelos*, *Cathissa*, *Melomphis*, *Nicipe* and *Elsiea* (Clade A), which includes two main sub-clades. The first sub-clade contains *Ornithogalum* and *Honorius* and the second one includes *Loncomelos* plus 3 specimens of *M. persica*. The Iranian species are nested in clade A within genera *Ornithogalum*, *Honorius* and *Loncomelos*. The genus *Nicipe* forms a monophyletic clade sister to the clade comprising *Ornithogalum*, *Honorius* and *Loncomelos*. The species of *Cathissa*, *Melomphis* and *Elsiea* do not form monophyletic clades.
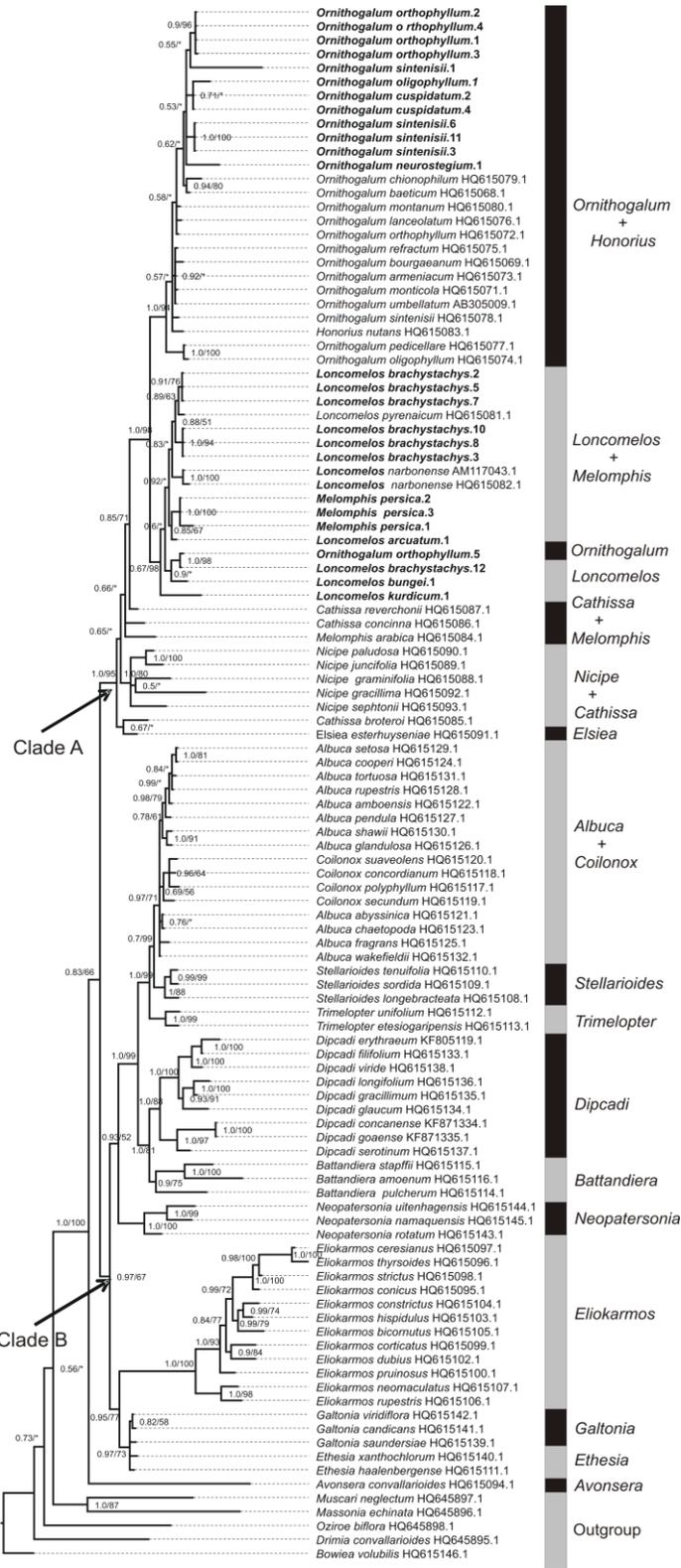
Figure 2. Bayesian inference analysis obtained from ITS sequences in the phylogenetic analysis of Ornithogaloideae. Sequences specified with bold words are generated by this study. The new proposed generic circumscription of the present study for Iranian species is shown on the right side. Posterior probabilities and bootstrap percentages are shown next to nodes. The generic treatment by Martínez-Azorín *et al*. (2011) is followed for the studied samples.
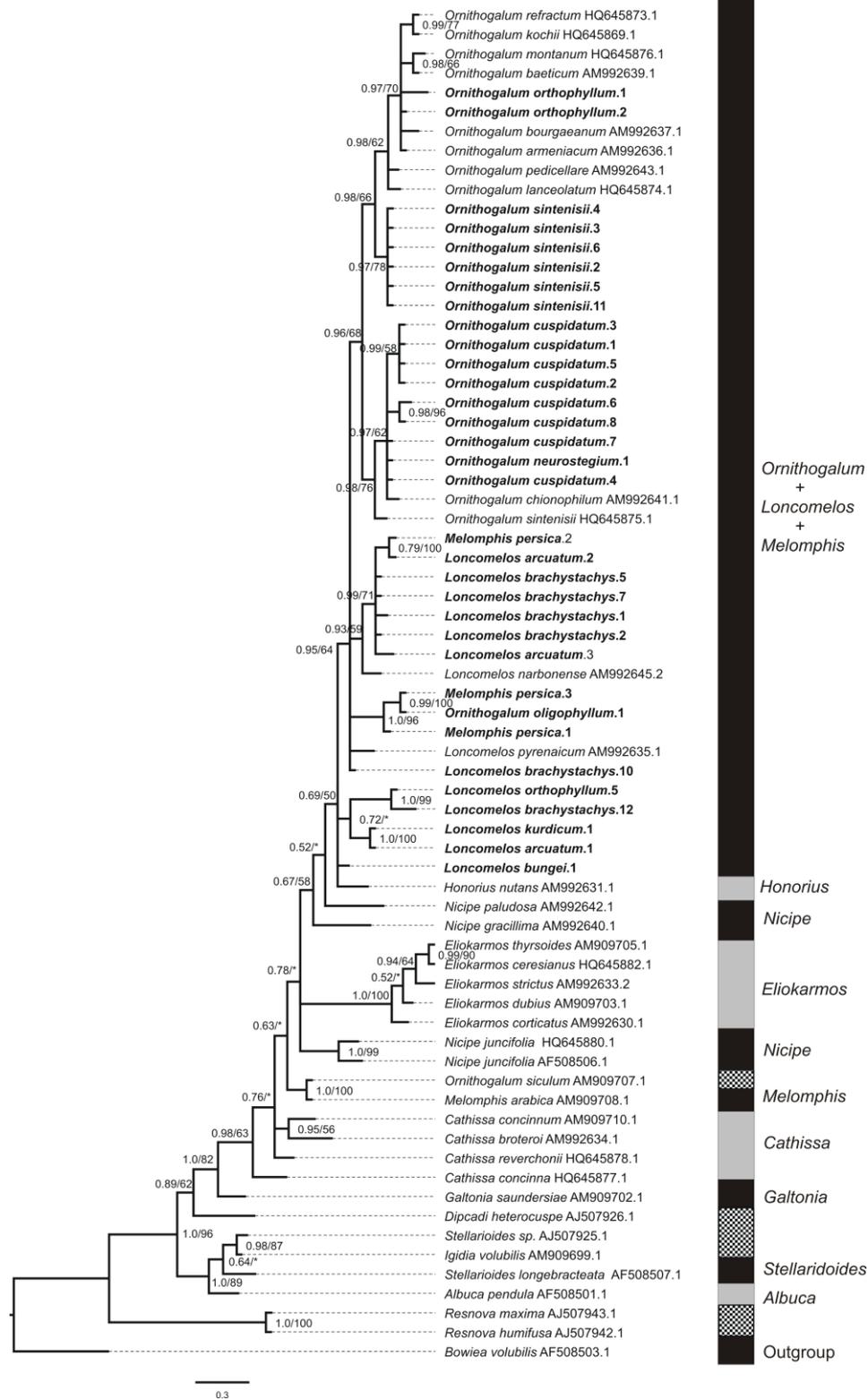
Figure 3. Bayesian inference analysis obtained from *trnL-F* sequences in the phylogenetic analysis of Ornithogaloideae. Sequences specified with bold words are generated by this study. The new proposed generic circumscription of the present study for Iranian species is shown on the right side. Posterior probabilities and bootstrap percentages are shown next to nodes. The mentioned genera in this tree are accepted by Martínez-Azorín, Crespo *et al*. (2011). The species not studied by Martínez-Azorín *et al*. (2011) are shown in checkered.
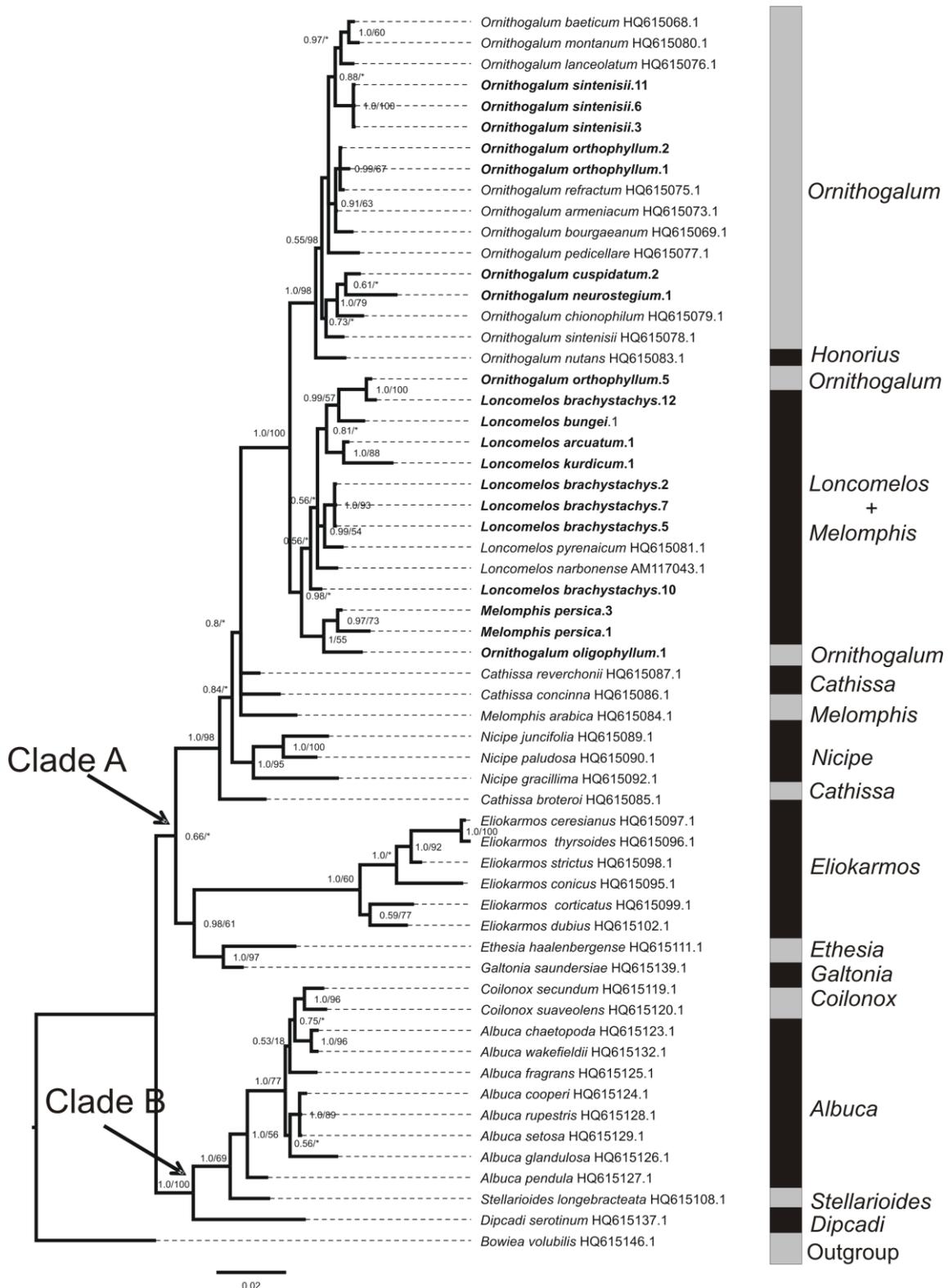
Figure 4. Bayesian inference analysis obtained from the combination of ITS and *trnL–F* sequences in the phylogenetic analysis of Ornithogaloideae. Sequences specified with bold words are generated by this study. The new proposed generic circumscription of the present study for Iranian species is shown on the right side. Posterior probabilities and bootstrap percentages are shown next to nodes. The mentioned genera in this tree are accepted by Martínez-Azorín *et al*. (2011).

The first sub-clade in clade A includes *Ornithogalum* and *Honorius*. In this sub-clade, *O. sintenisii* specimens do not form a monophyletic group and are placed in three different sub-clusters; one includes 3 specimens and the other one includes *O. sintenisii*.1. The sequence of the specimen obtained from GenBank, which was also collected in Iran (Martínez-Azorín *et al.*, 2011), and the specimens collected in this study are resolved into two distant clades. The four specimens of *O. orthophyllum* Ten. collected in Iran form a sub-cluster and are placed distant from the specimen obtained from GenBank, which is based on a collection from Italy (Martínez-Azorín *et al.*, 2011). *O. neurostegium* Boiss. & C. I. Blanche ex Boiss. is placed as sister to the rest of species in *Ornithogalum*. Two specimens of *O. cuspidatum* formed a sub-cluster sister to *O. oligophyllum* E. D. Clarke. (Figure 2).

The second sub-clade in clade A includes *Loncomelos* plus 3 specimens of *Melomphis. persica*. Seven specimens of *L. brachystachys* are also placed in this sub-clade, but in different sub-clusters; three specimens of *L. brachystachys* including 2, 5, and 7, formed one sub-cluster, and three other specimens of *L. brachystachys* including 8, 3, and 10, formed another sub-cluster. *L. brachystachys*.12 plus *O. orthophyllum*.5 are placed on a well-supported sub-cluster (1.0 PP; 98% BP) which is sister to *L. bungei* Boiss.1. All 3 specimens of *M. persica* formed a small well-supported (1.0 PP; 100% BP) sub-cluster (Figure 2) which is sister to *L. arcuatum* Steven. (85 PP; 67% BP). Finally, *L. kurdicum* is sister to all of these taxa (67 PP; 98% BP).

Clade B includes *Albuca*, *Coilonox*, *Stellarioides*, *Trimelopter*, *Dipcadi*, *Battandiera*, *Neopatersonia*, *Eliokarmos*, *Galtonia*, *Ethesia* and *Avonsera*. This clade is further divided into two main sub-clades. The first one comprises genera *Albuca* plus *Coilonox,* which are sisters to *Stellarioides,* and finally *Trimelopter* falls as sister to this clade. Also, *Dipcadi* and *Battandiera* form sister clades and *Neopatersonia* falls as sister to the latter. The second sub-clade includes *Eliokarmos* as monophyletic. *Galtonia* and *Ethesia* are sister to *Eliokarmos*.

trnL–F Tree

In the *trnL–F* tree one main clade can be distinguished and successively sister groups appear in that large clade. According to our results, the *trnL–F* analysis reveals a large clade (0.69 PP; 50% BS) for the Iranian native species included in this study. The *trnL–F* tree follows the ITS tree arrangement where *Ornithogalum*, *Honorius* and *Loncomelos* are placed in one clade. In this tree, *Ornithogalum* and *Loncomelos* cannot be identified as monophyletic. Also, in this tree, different specimens of each species do not form monophyletic groups. All members of *Ornithogalum* s. str., except *O. oligophyllum*.1 and *O. orthophyllum*.5, grouped in one clade. The specimens of *L. brachystachys* are placed in different sister clades as are 3 specimens of *M. persica*. The other sister clade shows a close relationship between *L. kurdicum* and *L. arcuatum* which form a well-supported cluster (1.00 PP; 100% BS). Furthermore, the *trnL–F* tree reveals the same relationship as ITS tree between *L. brachystachys*.12 and *O. orthophyllum*.5 specimens (1.00 PP; 99% BS). Different specimens of *O. sintenisii* form a monophyletic group (95 PP; 78% BS) and different specimens of *O. cuspidatum* are placed in sister clades. *Eliokarmos* is recovered as monophyletic (1.0 PP; 100% BS) and sister to the rest of genera in the main clade. The other genera are placed as sister clades to this main clade.

Combined Tree

The combined tree, including 59 taxa, reveals that the specimens included in this study are clearly divided into 2 main clades. The first main clade in the combined tree, Clade A, includes *Ornithogalum*, *Honorius*, *Loncomelos*, *Cathissa*, *Melomphis*, *Nicipe*, *Eliokarmos, Ethesia* and *Galtonia*. There is a well-supported main sub-clade (1.0 PP; 100% BP) comprising *Ornithogalum*, *Honorius* and *Loncomelos.* In this clade, these three genera are divided in two main sub-clades which include all Iranian species. The second sub-clade in clade A includes *Eliokarmos*, *Ethesia* and *Galtonia*. Clade B, the

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second main clade, comprises *Coilonox*, *Albuca*, *Stellarioides* and *Dipcadi*.

Within clade A, specimens of *M. persica* form a moderately supported sub-cluster (97 PP; 73% BP) which is found as sister to *O. oligophyllum*. *Lonomelos brachytachys*.12 and *O. orthophyllum*.5 are found in the same position as in the *trnL–F* and ITS trees within a well-supported sub-cluster (1.0 PP; 100% BP). The phylogenetic relationships among the rest of the species and specimens in the combined tree are generally similar to those in the ITS tree (Figure 2).

DISCUSSION

The New Proposed Generic Circumscription for Iranian Species within Ornithogaloideae:

As traditionally treated by Baker (1872), this subfamily is considered as one genus and consists of 9 subgenera, 3 of which occur in Iran: *O.* subgen. *Beryllis* (Salisb) Baker, *O.* subgen. *Ornithogalum* (Syn.: subgen *Heliocharmos* Baker), and *O.* subgen. *Caruelia* (Parl) Baker (Rechinger, 1995). According to the most recent classification (Martínez-Azorín *et al.*, 2011), 19 monophyletic genera are accepted within subfamily Ornithogaloideae. These 19 genera are well characterized morphologically, and are circumscribed in such a way that allows easy definition and makes easier subsequent attribution of a taxon to a given genus. In the new arrangement, Martínez-Azorín *et al.* (2011) treated the species traditionally included in *O.* subgenus *Beryllis* in 4 different genera including *Loncomelos*, *Nicipe*, *Stellarioides,* and *Trimelopter*, the species traditionally included in *O.* subgenus *Ornithogalum* in genus *Ornithogalum* and the species traditionally included in *O.* subgenus *Caruelia* in genera *Melomphis* and *Eliokarmos*.

The species traditionally classified by Baker (1872) in *O.* subgenus *Ornithogalum* and *O.* subgenus *Beryllis* include Eurasian and North African species. In our morphological analysis, each of these subgenera formed a monophyletic group. As Martínez-Azorín *et al.* (2011) noted, these two subgenera are clearly differentiated by the morphology of inflorescences, capsules, and

seeds as *Ornithogalum* and *Loncomelos*, respectively. Furthermore, *O.* subgenus *Caruelia*, represented by *M. persica* in this study, formed a small cluster and was placed in a nested position within *Loncomelos*.

The nested position of *Honorius* within *Ornithogalum* (1.0 PP; 94% BS) and also the sister relationship of these two genera with *Loncomelos* clade (1 PP; 98% BP) in our ITS tree (Figure 2) is similar to that in the ITS tree reported by Martínez-Azorín *et al.*, (2011). In our ITS tree, all sampled taxa of *Ornithogalum* from Iran fell in the clades corresponding to *Ornithogalum s. str.* and *Honorius*, which together formed a strongly supported clade (1.0 PP; 94% BS). Furthermore, the Iranian *Loncomelos* species were placed in a clade, similar to the results of Martínez-Azorín *et al.* (2011). *Melomphis persica* appeared as an independent clade and nested within *Loncomelos* in our ITS tree. In contrast, the *trnL–F* tree generated in this study revealed the nested position of *Ornithogalum* within *Loncomelos* with a sister relationship to *Honorius* (0.62 PP; 50% BS) (Figure 3) and justified segregation of the latter genus from *Ornithogalum*. The phylogenetic study by Manning *et al.* (2009) revealed the same relationship. Like ITS tree, the *trnL–F* tree included *M. persica* in *Loncomelos*. The combined tree (Figure 4) justified segregation of *Ornithogalum*, *Loncomelos* and *Honorius* and placed them in three different clades. In our combined tree, *M. persica* again was placed within the *Loncomelos* clade. *Melomphis persica* is clearly differentiated from *Loncomelos* by the morphology of the inflorescences, tepals and gynoecium, which are more similar to *M. arabica*; on the other hand, it is similar to *Loncomelos* in some morphological characters like plant height, leaf number, leaf length, flower number, perigone length and width, and bulb length, width, and color. The phylogenetic placement of *M. persica* and its morphological characters could reflect a possible hybrid origin from a cross between *M. arabica* and some eastern *Loncomelos* species. However, a wider sampling of related taxa is needed to improve our knowledge of phylogenetic relationships within these genera. The topology of our combined tree is similar to that obtained by Martínez-Azorín *et al.* (2011).

Our results support the classification of all Iranian species of Ornithogaloideae in *Ornithogalum s. str.* and *Loncomelos*. The ITS, *trnL–F* and combined trees reveal that Iranian species were placed in clades corresponding to *Ornithogalum*, *Loncomelos*, and *Honorius* clades and clearly are divided into 2 main clades in the ITS and combined trees (Figures 2 and 4) which is in line with the morphological analysis (Figure 1). Genus *Honorius*, represented by *H. nutans*, segregated in different ways from these two genera in different trees. In the *trnL–F* and combined trees *Loncomelos* clade did not form a monophyletic group where *O. oligophyllum* was placed within this genus. This conflict suggests the possibility of past hybridization events in *O. oligophyllum*. However, further studies are needed to confirm its final position. Despite this misplacing, which may reflect hybridization event in this species, we consider the combined tree as the most informative phylogenetic tree in this study (Figure 4) and it justifies segregation of *Ornithogalum*, *Loncomelos* and *Honorius*. Furthermore, combination of ITS and *trnL–F* sequences resulted in a phylogenetic tree that supports the monophyly of genera previously described on the basis of molecular data and morphological characters as shown by Martínez-Azorín *et al.* (2011). *Honorius*, *Ornithogalum*, *Loncomelos*, *Melomphis*, *Nicipe*, *Elikarmos*, *Ethesia*, *Galtonia*, *Coilonox*, *Albuca*, *Stellarioides*, *Dipcadi* are segregated as proper genera on the basis of last classification by Martínez-Azorín *et al.* (2011). The phylogenetic relationships of genus *Cathissa* are not fully resolved in this tree, maybe because of the lack of *trnL–F* sequences in this tree for the related taxa and genera. The most important cultivated horticultural species include *E. thyrsoides*, *E. dubius*, *O. umbellatum*, *H. nutans*, *M. arabica* and *G. saundersiae*. Similar to the most recent study by Martínez-Azorín *et al.* (2011), our phylogenetic tree based on ITS tree placed *Melomphis*, *Ornithogalum*, *Honorius* in the first main clade (clade A) and *Eliokarmos* and *Galtonia* in the second main clade (clade B). Also, our combined tree derived from ITS and *trnL–F* sequences is similar to the combined tree derived from ITS, *trnL–F*, *matK* and *rbcL* sequences in Martínez-Azorín *et al.* (2011) study, both of which placed all five of these genera in clade A.

Relationships at the Species Level for Iranian Species

Divergent placement of multiple specimens of a single species is found in *L. brachystachys*, *O. orthophyllum* and *O. sintenisii*. In the ITS tree, seven specimens of *L. brachystachys* were placed in three different sub-clusters. In this regard, the bio-geographical distribution of each group has a peculiar pattern. Specimens from northwestern Iran (*L. brachystachys*.2, 5, 7) formed the first sub-cluster and specimens from southwestern Iran formed the second sub-cluster (*L. brachystachys*. 8, 3, 10). The third sub-cluster included *L.brachystachys*.12 and *O. orthophyllum*.5. Based on morphological data, the first and second sub-clusters grouped together but are morphologically diagnosable by scape length, which is longer in *L. brachystachys*.2, 5, 7. Karyotypic differences among the species and ecotypes of *Ornithogalum* have been observed (Anderson, 2006). Five cytotypes were documented among *L. brachystachys* specimens collected in five different locations of Iran, with the chromosome numbers 2n= 12, 2n= 16, 2n=22, 2n= 24 and 2n=30 (Heidaryan, 2011). However, we do not have the cytotypes for the specimens we sampled.

Within *Ornithogalum*, considerable divergence of specimens of a single species was found for *O. sintenisii*. The specimens of this species were placed in quite different positions; 4 specimens which are collected for this study were placed in the same sub-clade of clade A in the ITS tree but in different positions. *O. sintenisii*.4, *O. sintenisii*.6 and *O. sintenisii*.11 formed one sub-cluster distant from *O. sintenisii*.1. However, this relationship reflects the clear morphological divergence in morphology. In the morphological analysis, different specimens of *O. sintenisii* were placed in two sister clades. *O. sintenisii*.1 was placed in a clade sister to *O. sintenisii*.4, *O. sintenisii*.6 and *O. sintenisii*.11 clade (Figure 1). *O. sintenisii*.1 is defined morphologically by smaller plant size and more numerous flowers in comparison with *O. sintenisii*.4, *O. sintenisii*.6 and *O. sintenisii*.11. The specimen obtained from GenBank, which was also collected in Iran (Martínez-Azorín *et al.*, 2011), was placed far from these 4 specimens. Potential conflict may arise within a single dataset as a result of

extended paralogy (Catalán *et al*., 2004). Pseudogene copies of ITS sequences, accumulating quite a few mutations in *O. sintenisii*.1, and misidentification of the specimen used from the GenBank are all valid on theoretical grounds to cause divergent placements of the *O. sintenisii* sequences. Thus, this divergence remains a subject for speculation and requires more studies.

In the ITS tree, the four specimens of *O. orthophyllum* collected in Iran formed a sub-cluster and placed distant from the specimen obtained from GenBank, which is based on a collection from Italy (Martínez-Azorín *et al*., 2011). *O. orthophyllum* is a poorly-known floristic species and was treated in different ways as a synonym for other species or defined as other species (Herrmann, 2002). This is a native species to Italy and at the latest checklist of the vascular flora native to Italy it is recognized a different species from the previous statements (Bartolucci *et al*., 2018). Unfortunately, we did not have access to *trnL-F* sequences of the specimen collected in Italy. Further studies are required to distinguish final position of these specimens. In both the ITS and *trnL–F* trees as well as the combined tree, *L. brachystachys*.12 and *O. orthophyllum*.5 formed a well-supported sub-clade and were placed in the same position in the *Loncomelos* clade, apart from the remaining specimens of related species. Morphological clustering does not explain the close relationship between these 2 specimens in the phylogenetic trees based on sequence data. In morphological clustering, *L. brachystachys*.12 falls as sister to the rest of *L. brachystachys* specimens and *O. orthophyllum*.5 falls as sister to the rest of *O. orthophyllum* specimens (Figure 1). On the other hand, both of these specimens are characterized by their pale color in comparison with the rest of related specimens. Their special color in morphological characters could provide evidence to consider them as natural mutants. The conflict between morphological and genetic results for these two specimens may be due to interspecific hybridization, which is reported in this genus (van Raamsdonk, 1985; Griesbach *et al*., 1993; Joung and Roh, 2004). We have compared ITS and *trnL–F* trees to identify topological incongruences that may indicate hybridization events involved in the origin of *L.*

brachystachys.12 and *O. orthophyllum*.5. If they were hybrids between *L. brachystachys* and *O. orthophyllum* species, we would expect phylogenetic trees based on their cpDNA sequences to place them in a clade with their maternal parent, while trees based on nuclear ITS sequences might place both species in a clade with the paternal parent. Thus, if they are hybrids, the maternal parent must be a third species, presumably one not sampled in this study. A final possibility would be to consider each of them as a new species. However, before taking such a step, further investigations are required to understand their identities.

However, conflict between the *trnL–F*, ITS and combined trees in the placement of the different specimens of each species may reflect the possibility of past hybridization events (Catalán *et al*., 2004), although incomplete lineage sorting and ITS paralogy cannot be overlooked (Catalán *et al*., 2004). Also, pseudogene copies of ITS sequences and chromosome duplication events may have an impact on the nuclear ITS sequences used in the phylogenetic reconstruction leading to the divergent placements of different members of the species observed here (Marinho *et al*., 2014). A final possible explanation would be the misidentification of the specimens used to generate the sequences we obtained from Genbank or collected samples for this study.

Distribution of Wild and Cultivated Species of Ornithogaloideae

Ornithogaloideae are distributed in Africa, Asia-Temperate (which includes Iran), and Europe (Eastern, Middle Northern, Southeastern, Southwestern). The natural habitats of the wild species of *Ornithogalum* and *Loncomelos* range from dry semi-desert areas to marshy wetlands and riverbanks, to mountaintops (Anderson, 2006). Species of these genera have also been introduced and naturalized in Africa (northwest coast of Africa), Asia-Tropical, Europe (Eastern and Northern), Australia (South Australia, Western Australia), and North America (Manning *et al*., 2003; Manning *et al*., 2009; eMonocot, 2017; Royal Botanic Gardens, 2017; The Global Biodiversity Information Facility, 2017). Our results followed those of Martínez-

Azorín *et al.* (2011), where the first main clade in ITS and combined trees includes all north African and Eurasian taxa of *Ornithogalum*, *Honorius*, *Loncomelos*, *Melomphis* and *Cathissa* plus the eastern and southern African species that correspond to *Elsiea* and *Nicipe*. The second main clade comprises *Albuca*, *Coilonox*, *Stellarioides*, *Trimelopter*, *Dipcadi*, *Battandiera*, *Neopatersonia*, *Eliokarmos*, *Galtonia*, *Ethesia* and *Avonsera*, which mainly occur in Africa.

CONCLUSIONS

This is the first phylogenetic study employing nuclear ITS and plastid *trnL–F* sequences to examine the phylogenetic diversity of several Ornithogaloideae species collected from a broad range of climatic conditions of Iran with the goals of improving our understanding of relationships among these species within Ornithogaloideae, and comparing molecular diversity of these populations in Iran. Following the concept of Martínez-Azorín *et al.* (2011) classification, we put the species of *O.* subgenus *Ornithogalum* collected in Iran in *Ornithogalum* and the species of *O.* subgenus *Beryllis* collected in Iran in *Loncomelos* on the basis of phylogenetic and morphological evidence. Additionally, the inclusion of the only member of *Melomphis* included in this study, *M. persica*, within genus *Loncomelos* in the morphological clustering and trees based on phylogenetic analysis of molecular data requires a wider sampling of related taxa to improve the status of our knowledge of phylogenetic relationships among these genera. Better understanding of the complex phylogenetic relationships that exist within Ornithogaloideae species contributes important information about their evolutionary history and helps to improve our knowledge for developing effective conservation, utilization, and management practices. Improved understanding of naturally occurring variation among wild *Ornithogalum* populations of Iran and the phylogenetic relationships among wild and cultivated species, as revealed in this study, could provide important opportunities to introduce new ornamental plants or improve the agricultural performance of modern ornamental varieties. Also, these results offer the opportunity to guide future breeding initiatives involving *Ornithogalum*.

REFERENCES

1. Anderson, N. O. 2006. Flower Breeding and Genetics: Issues, Challenges and Opportunities for the 21st Century, SSBM.
2. APG II, A. 2003. "An Update of the Angiosperm Phylogeny Group Classification for the Orders and Families of Flowering Plants: APG II." *Bot. J. Linn. Soc.,* **141(4):** 399-436.
3. Asadi, H., Orangi, M., Shanehbandi, D., Babaloo, Z., Delazar, A., Mohammadnejad, L., Shahneh, F.Z., Valiyari, S. and Baradaran, B. 2014. "Methanolic Fractions of Ornithogalum Cuspidatum Induce Apoptosis in PC-3 Prostate Cancer Cell Line and WEHI-164 Fibrosarcoma Cancer Cell Line." *Adv. Pharma. Bull.,* **4(Suppl. 1)**: 455-458.
4. Baker, J. G. 1872. "Revision of the Genera and Species of Scilleæ and Chlorogaleæ." *Bot. J. Linn. Soc.,* **13(68)**: 209-266.
5. Bartolucci, F., Peruzzi, L., Galasso, G., Albano, A., Alessandrini, A., Ardenghi, N., Astuti, G., Bacchetta, G., Ballelli, S. and Banfi, E. 2018. "An Updated Checklist of the Vascular Flora Native to Italy." *Pl. Biosyst.,* **152(2):** 179-303.
6. Bidarlord, M. and Ghahremaninejad, F. 2016. *Ornithogalum boissieri* (Asparagaceae), a New Species from the Talesh Mountains, Iran. *Ann. Bot. Fenn. BioOne,* **53 (1–2)**: 69-72.

7. Botha, C., Schultz, R., Van der Lugt, J. and Archer, C. 2000. "A Krimpsiekte-Like Syndrome in Small Stock Poisoned by *Ornithogalum toxicarium* Archer & Archer." *J. S. Afr. Vet. Assoc.,* **71(1):** 6-9.

8. Catalán, P., Torrecilla, P., Rodrıguez, J. A. L. and Olmstead, R. G. 2004. Phylogeny of the Festucoid Grasses of Subtribe Loliinae and Allies (Poeae, Pooideae) Inferred from ITS and *trnL–F* Sequences. *Mol. Phylogenet. Evol.,* **31(2)**: 517-541.

9. Darriba, D., Taboada, G. L., Doallo, R. and Posada D. 2012. jModelTest 2: More Models, New Heuristics and Parallel Computing. *Nat. Methods,* **9(8):** 772-772.

10. Edgar, R. C. 2004. MUSCLE: Multiple Sequence Alignment with High Accuracy and High throughput. *Nucl. Acids Res.,* **32(5)**: 1792-1797.

11. eMonocot. 2017. *EMonocot: An Online Resource for Monocot Plants, Ornithogalum L.*. From: http://e-monocot.org/taxon/urn:kew.org:wcs:taxon:282 876.

12. Farahmand, H. and Nazari, F. 2015. Environmental and Anthropogenic Pressures on Geophytes of Iran and the Possible Protection Strategies: A Review. *J. Hortic. Sci. Technol.,* **2(2):** 111-132.

13. Griesbach, R., Meyer, F. and Koopowitz, H. 1993. Creation of New Flower Colors in *Ornithogalum* via Interspecific Hybridization. *J. Am. Soc. Hortic. Sci.,* **118(3):** 409-414.

14. Heidaryan, F. 2011. Biosystematic Study of Genus *Ornithogalum* in Iran. PhD. Islamic Azad University. (in Persion)

15. Herrmann, N. 2002. Biological Flora of Central Europe: *Ornithogalum angustifolium*" nom. prov., Syn. pp *O. orthophyllum* ssp. kochii= *O. kochii* Parl., *O. gussonei* Ten. Flora. **197(6):** 409-428.

16. Hřibová, E., Čížková, J., Christelová, P., Taudien, S., de Langhe, E. and Doležel, J. 2011. The ITS1-5.8 S-ITS2 Sequence Region in the Musaceae: Structure, Diversity and Use in Molecular Phylogeny. *PLoS One,* **6(3)**: e17863.

17. Huelsenbeck, J. and Ronquist, F. 2005. *MrBayes, Version 3.1. 2*. Available from the Authors: http://morphbank. ebc. uu. se/mrbayes3/info. php.

18. Joung, Y. H. and Roh, M. 2004. Paternity Determination of *Ornithogalum* Seedlings Using DNA Markers. *J. Hortic. Sci. Biotechnol.,* **79(2):** 316-321.

19. Kumar, S., Stecher, G. and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.,* **33 (7)**: 1870–1874.

20. Larget, B. and Simon, D. L. 1999. Markov Chain Monte Carlo Algorithms for the Bayesian Analysis of Phylogenetic Trees. *Mol. Biol. Evol.,* **16(6):** 750-759.

21. Luria, G., Watad, A., Cohen-Zhedek, Y. and Borochov, A. 2000. Growth and Flowering of *Ornithogalum dubium. VIII International Symposium on Flower Bulbs 570*, Cape Town, South Africa. 12.

22. Manning, J. C., Forest, F., Devey, D. S., Fay, M. F. and Goldblatt, P. 2009. A molecular Phylogeny and a Revised Classification of *Ornithogaloideae* (Hyacinthaceae) Based on an Analysis of Four Plastid DNA Regions. *Taxon.,* **58(1):** 77-107.

23. Manning, J. C., Goldblatt, P. and Fay, M. F. 2003. A Revised Generic Synopsis of Hyacinthaceae in Sub-Saharan Africa, Based on Molecular Evidence, Including New Combinations and the New Tribe Pseudoprospereae. *Edinburgh J. Bot.,* **60(03):** 533-568.

24. Marinho, R. C., Mendes-Rodrigues, C., Balao, F., Ortiz, P. L., Yamagishi-Costa, J., Bonetti, A. M. and Oliveira, P. E. 2014. Do Chromosome Numbers Reflect Phylogeny? New Counts for Bombacoideae and a Review of Malvaceae sl. *Am. J. Bot.,* **101(9):** 1456-1465.

25. Maroufi, H. 2010. Two New Plant Species from Kurdistan Province, West of Iran. *J. Bot.,* **16(1)**: 76-80.

26. Martínez-Azorín, M., Crespo, M. B., Juan, A. and Fay, M. F. 2011. Molecular Phylogenetics of Subfamily Ornithogaloideae (Hyacinthaceae) Based on Nuclear and Plastid DNA Regions, Including a New Taxonomic Arrangement. *Ann. Bot.,* **107(1)**: 1-37.

27. Müller Doblies, U. and Müller Doblies, D. 1996. Revisionula Incompleta Ornithogalorum Austro-Africanorum (Hyacinthaceae). *Feddes Repert.,* **107(5-6)**: 361-548.

28. Nazifi, E., Movafeghi, A., Nazemiyeh, H., Asnaashari, S., Moghadam, S. B. and Delazar, A. 2010. Phytochemical Analysis of Essential Oils from Different Plant Parts of *Ornithogalum cuspidatum* Bertol. *Pharma. Sci.,* **16(1):** 37-46.

29. Plančić, M., Božin, B., Kladar, N., Rat, M. and Srđenović, B. 2015. Phytochemical Profile and Biological Activities of the Genus

Ornithogalum L. (Hyacinthaceae). *Biol. Serb.,* **36(1-2)**.

30. Potter, D., Still, S. M., Grebenc, T., Ballian, D., Božič, G., Franjiæ, J. and Kraigher, H. 2007. Phylogenetic Relationships in Tribe Spiraeeae (Rosaceae) Inferred from Nucleotide Sequence Data. *Plant Syst. Evol.,* **266(1-2):** 105-118.

31. Rechinger, K. 1995. *Ornithogalum* in KH Rechinger Flora Iranica. *Flora des iranischen Hochlandes und der umrahmenden Gebirge, Graz, Austria,* **165:** 119-132.

32. Royal Botanic Gardens, K. 2017. *Ornithogalum* L., Sp. Pl.. **306(1753)**. from http://apps.kew.org/wcsp/.

33. Samavati, M., Babaloo, Z., Delazar, A., Baradaran, B., Nazifee, E., Mohammadi, A. and Movasahgpour, A. 2010. Cytotoxic and Apoptotic Effects of *Ornithogalum cuspidatum* Methanolic Extract on WEHI-164 Fibrosarcoma Cancer Cell Line. *Pharma. Sci.,* **16(3)**: 149-156.

34. Stamatakis, A. 2014. RAxML Version 8: A Tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics,* **30(9):** 1312-1313.

35. Taberlet, P., Gielly, L., Pautou, G. and Bouvet, J. 1991. Universal Primers for Amplification of Three Non-Coding Regions of Chloroplast DNA. *Plant. Mol. Biol.,* **17(5):** 1105-1109.

36. The Global Biodiversity Information Facility, G. 2017. *Ornithogalum* L. From: http://data.gbif.org.

37. van Raamsdonk, L. 1985. Crossing and Selfing Experiments in the *Ornithogalum umbellatum/augustifolium* Complex. *Plant Syst. Evol.,* **150(3-4)**: 179-190.

38. Watt, J. M. and Breyer-Brandwijk, M. G. 1932. The Medicinal and Poisonous Plants of Southern Africa. The American Journal of Tropical Medicine and Hygiene **1(5)**: 544-545.

مطالعه روابط فیلوژنی گونه‌های وحشی و اهلی زیرخانواده Ornithogaloideae بر اساس توالی‌های ITS و *trnL–F* با توسعه نمونه برداری از ایران

ک. ریاحی راد، ع. بابایی، و. مظفریان، و د. پتر

چکیده

طبقه‌بندی زیرخانواده Ornithogaloideae در طی دهه‌های اخیر به مساله مهمّی در علم تاکسونومی تبدیل شده است.*Ornithogalum* یک جنس بزرگ در زیرخانواده Ornithogaloideae می-باشد؛ و حاوی گیاهانی با ارزش زینتی و دارویی می‌باشد. این گیاهانِ زینتیِ وحشی، که اخیرا به صنعت گیاهان زینتی معرفی شده‌اند، به گیاهان زینتی محبوبی به عنوان گل‌های شاخه بریده، گیاهان گلدانی و جهت کشت و کار در باغ‌ها تبدیل شده‌اند. مطالعه حاضر اولین مطالعه فیلوژنتیکی جهتِ مطالعه ۱۰ گونه از گیاهانی که تحت عنوان جنس Ornithogalum در ایران معرفی شده‌اند می‌باشد. کاربرد توالی‌های ITS و *trnL_F* جهت درک روابط فیلوژنتیکی گونه‌های مختلف این جنس، ارزیابی منابع ژنتیکی این زیرخانواده با گسترشِ نمونه‌برداری از ایران در مقایسه با مطالعاتِ قبلی از اهداف این مطالعه می‌باشد. در مطالعه حاضر، بهترین روابط فیلوژنتیکی در سطح جنس از درختِ ترکیبی قابل استنباط است. نتایج حاصل از آنالیزهای فیلوژنتیکی به روش حداکثر درست‌نمایی و روش بیزین با نتایج حاصل از آنالیز

خوشهای ویژگیهای ریختی مورد مقایسه قرار گرفت. بر اساس نتایجِ حاصل از مطالعه حاضر، همه ژنوتیپهایِ جمعآوری شده از ایران، در میان آخرین جنسهایِ معرفی شده در زیرخانواده Ornithogaloideae، در دو جنس *Ornithogalum* و *Loncomelos* طبقهبندی شدند؛ این طبقهبندی با نتایج حاصل از آنالیزهای ریختی همراستا میباشد. پراکنش ژنوتیپهای مختلف یک گونه در درخت فیلوژنتیکی در گونههای *O. brachystachys, O. orthophyllum* و *.O sintenisii* مشاهده شد؛ که ممکن است به علت رخ دادن دورگگگیری، تفرق ناقص آللها و ژنهای پارالوگ ITS باشد. تنوّعِ طبیعیِ موجود میان جمعیتهای مختلفِ این گیاه در ایران، و روابطِ فیلوژنتیک میان گونههای وحشی و کشت شده میتواند فرصت مهمی جهت معرفی گیاهان زینتی جدید به صنعت گیاهان زینتی و یا بهبود صفات باغبانی ارقام تجاری موجود باشد.