Molecular Phylogeny of the Genus *Lathyrus* (*Fabaceae-Fabeae*) Based on cpDNA *matK* Sequence in Iran

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Background: More than 60 species of the genus *Lathyrus* are distributed in Southwest Asia. It is the second largest genus of the tribe Fabeae, after *Vicia*, in the region (and in Iran with 23 species). In the regional Flora (Flora of Turkey, Flora Iranica and flora of Iran), the genus has been divided into 9-10 sections. Here we analyzed the phylogeny of *Lathyrus* and its relationship with *Pisum* based on plastid gene *matK* sequences.
Objectives: The present study utilized several approaches including maximum parsimony, Bayesian and maximum likelihood methods to evaluate the monophyly and relationship within the genus *Lathyrus*, both at the sectional level and species level, mainly based on the taxa growing in Iran.
Materials and Methods: A total of 52 accessions, representing 38 species of *Lathyrus*, three species of *Pisum* and four species of *Vicia* and *Lens* as out-groups, were analyzed for reconstructing the phylogenetic relationship using chloroplast gene *matK* sequences. Maximum parsimony, Bayesian and maximum likelihood methods were used to construct phylogenetic trees.
Results: The present study indicated that *Pisum* was nested among *Lathyrus* species. Two members of the *Lathyrus* section, *Clymenum* (*Lathyrus ochrus* and *L. Clymenum*) with *Pisum*, formed a weakly supported clade as sister to the larger polytomy comprising the remainder of the *Lathyrus* species. Several sections of *Lathyrus* including *Lathyrostylis*, *Lathyrus* and *Clymenum* were monophyletic. *Lathyrus roseus* (of the monotypic section *Orobon*) were nested among the members of section *Lathyrus*. The newly taxon described species *L. alamutensis*, endemic to Iran, were nested among other species of *Lathyrostylis*. *Linearicarpus*, *Orobus* and *Pratensis* were not monophyletic sections. *Pratensis* and the monotypic section *Aphaca* were the closest taxa. In our analysis, *L. Pratensis* formed a sister group relationship with the *Aphaca* clade, not its own section.
Conclusions: Shimodaira-Hasegawa (SH) test of the *matK* data set showed that all of the analyzed *Lathyrus* species formed their own clade and *Pisum* was sister to them. Furthermore, when we removed the two above-mentioned *Lathyrus* species, the analysis retrieved *Pisum*, as a well-supported clade being sister to the *Lathyrus* calde.

Keywords: Fabaceae; Lathyrus; Tribe Fabeae

1. Background

The tribe Fabeae (= Vicieae) contains four genera, of which *Lathyrus* L. and *Vicia* L. with ca 160 species are the largest (1, 2). Lens Mill. with 4-6 species and *Pisum* L. with 4 species (*Vavilovia formosa* (Stev.) Fed. of the monotypic genus *Vavilovia* Fed. was treated as a part of *Pisum* as *P. formosum* (Stev.) Alef. (3) are small genera. *Vicia* is paraphyletic and along with *Lens*, as a monophyletic taxon, formed a sister group relationship with a clade of *Lathyrus* and *Pisum* (2-5). Species of *Lathrys* are distributed throughout the Northern Hemisphere, tropical East Africa and temperate South America. Its main center of diversity is in the Mediterranean and Irano-Turanian regions, with smaller centers in North and South America (2, 6-8). Many species of *Lathyrus* are used extensively as cover crops, for fodder (*L. cicera* L., *L. hirsutus* L. and *L. sativus* L.), as ornamentals (*L. odoratus* L. [sweet pea], *L. latifolius* L. [everlasting pea] and *L. sylvestris* L.) and as human food (*L. sativus* [grass pea, Indian pea, chuckling vetch], *L. ochrus* (L.) DC. and *L. montanus* Bernh. (with edible root tubers) and also for erosion control, as green manure and for medicine; toxins are present in some species, causing *Lathyrism* (2).

More than 60 species of the genus have been distributed in the Southwest Asia (2). In the regional Flora including Flora of Turkey (9), Flora Iranica (10) and Flora of Iran (11), the genus was divided into 9-10 sections. Almost three decades ago, Kupicha (6) based on morphological

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characters, by assessing the older classifications of the genus, proposed her sectional system encompassing 13 sections, of which three were newly established: *Orobus* (L.) Gordon, *Lathyrostylis* (Griseb). Bassler, *Pratensis* Bassler, Neurolobus Bassler, *Orobon* Tamamshjan, *Orobastrum* Boiss, Viciopsis Kupicha, *Linearicarpus* Kupicha, *Lathyrus*, Aphaca (Miller) Dumort. Nissolia (Miller) Dumort, *Clymenum* (Miller) DC. and Noto*Lathyrus* Kupicha. She divided section *Orobastrum* into three monotypic sections *Orobastrum*, *Linearicarpus* and Viciopsis. She included section *Cicercula* (Medic.) Gren. and Godr. in section *Lathyrus* and added a new section of South American species, *Notho-Lathyrus*. We followed her system of classification in the present study.

Dogan et al. (12) conducted a numerical taxonomic study on 52 species of Turkish *Lathyrus* using 40 external vegetative and floral morphological characters. They divided the genus into nine sections, of which section *Gorgonia* Dogan was described as a new taxon. Leht (13) based on cladistic analysis of 210 morphological characters suggest that all species of section *Pratensis* Bassler should belong to section *Aphaca*. He placed the monotypic section *Orobon* (with species *L. roseus Steven*) within section *Orobus* (13). Hitherto, several works based on molecular data have been conducted on the phylogeny of *Lathyrus*. Asmussen and Liston (7) sampled 42 species of *Lathyrus* based on cpDNA restriction site characters and their study included representatives of all sections but *Viciopsis*. They generally agreed with the sectional classification of Kupicha (6), yet they suggested that *Orobon* and *Orobastrum* should be transferred to the section *Lathyrus*, and *NothoLathyrus* to *Orobus*. Croft et al. (14) used Random Amplified Polymorphic DNA (RAPD) analysis on eight accessions of *L. sativus* and some allies species. Badr et al. (15) utilized Amplified-Fragment Length Polymorphism

(AFLP) data to examine the systematic relationship in section *Lathyrus*. Ben Brahim et al. (16) studied isozyme variation and phylogenetic relationships between ten species of the genus. Kenicer et al. (8) studied 53 species of the genus based on nrDNA ITS, cpDNA *trn*L-F and *trn*S-G sequence data (except members of two Kupicha's sections, *Orobon* and *Viciopsis*). Their results generally supported the monophyly of sections *Lathyrus* and *Lathyrostylis*, but questioned monophyly of the larger section *Orobus*.

2. Objectives

In this study, we examined the phylogenetic relationships among 38 species of the genus *Lathyrus* based on chloroplast gene *matK*. All species of the genus growing in Iran and some species from Turkey were included. Unlike some previous studies (7, 8) the present study employed several approaches including maximum parsimony, Bayesian and maximum likelihood methods to evaluate the monophyly and relationship within *Lathyrus*, both at the sectional level and species level.

3. Materials and Methods

3.1. Taxon Sampling

Fifty-two accessions for matK were analyzed. A total of 38 species representing eleven sections of *Lathyrus* (6) and three species of *Pisum* (including *Vavilovia formosa*, see 3) were included in the phylogenetic analyses using each of the separate datasets as well as the combined dataset. The two species of Lens and Vicia were chosen as outgroups according to previous studies (4, 5, 7, 8). List of the analyzed species along with their voucher information and GenBank accession numbers are presented in (Table 1).

Table 1. Sampled Taxa Used in This Study With Their GenBank Accession Number (*mat*K) [o]

Species	DNA Source (Location, Voucher)	GenBank Accession No
Section Aphaca	-	-
***Lathyrusaphaca* L**[b]	Iran: B. Alizadeh 1005 (WARCNH)	AB935057
***Lathyrusaphaca* L.**[a]	USDA 2865272	AF522084
***Lathyrusaphaca* L.**[a]	Portugal: Schaefer, H	HM851115
Section *Clymenum*	-	-
***LathyrusClymenum* L.** [a]	Portugal: Schaefer, H	HM851116
***Lathyrusochrus* (L.) DC.** [b]	Turkey: H. Sagban 3137 (GAZI)	AB935075
***Lathyrusochrus* (L.) DC.** [a]	Portugal: Schaefer, H	HM851120
Section Lathyrostylis	-	-
***Lathyrusalamutensis* Mozaffarian et al.**[b]	Iran: Mozaffarian, Ahvazi & Charkhchian 88388 (TARI)	AB935055
***Lathyrusarmenus* (Boiss. & Huet) Celak.** [b]	Turkey: Aytaç 8317 (GAZI)	AB935058
***Lathyrusboissieri* Sirj.**[b]	Iran: Runemark & Mozaffarian 29308 (TARI)	AB935060
***Lathyrusbrachypterus* Celak.** [b]	Turkey: Aytaç & A. Duman 5441 (GAZI)	AB935061
***Lathyruscyaneus*(Steven) K. Koch.** [b]	Iran, Azerbayjan: Mozaffarian 70010 (TARI)	AB935065
***Lathyrusdigitatus* (M. Bieb) Fiori**[b]	Turkey: M. Vural 4033 (GAZI)	AB935067

Lathyruselongatus(Bornm.) Širj.[b]	Turkey: F. Tezcan (GAZI)	AB935068
Lathyruskarsianus Davis[b]	Turkey: G. Akgül 1700 (GAZI)	AB935073
Lathyrustukhtensis Czecz. [b]	Turkey: A. Güner 5798 (GAZI)	AB935084
Lathyrusvariabilis (Boiss. & Kotschy) Celak. [b]	Turkey: Z. Aytaç & H. Duman 4599 (GAZI)	AB935085
Section Lathyrus	-	-
Lathyrusannuus L.[b]	Iran: Hewer 3676 (TARI)	AB935056
Lathyruscassius Boiss. [b]	Iran: Neamati & Ghaderi 4581 (TARI)	AB935062
Lathyruschloranthus Boiss. [b]	Iran: Kazempour 2008-5 (TMUH)	AB935063
*Lathyruscicera*L.[b]	Iran: Joharchi & Zangooei 23122 (FUMH)	AB935064
Lathyrushirsutus L.[b]	Iran: Naqinezhad 27738 (TUH)	AB935069
Lathyrushirsutus L.[a]	Portugal: Schaefer, H	HM851117
*Lathyruslatifolius*L.[a]	Portugal: Schaefer, H	HM851118
Lathyrus pseudo-cicera Pamp. [b]	Turkey: N. Adiguzell 2238 (GAZI)	AB935077
Lathyrusrotundifolius Willd. [b]	Iran: Sonboli 496 (TMUH)	AB935080
Lathyrussativus L.[b]	Iran: Alizadeh & Zangooei 15589 (FUMH)	AB935081
Lathyrussativus L.[a]	USDA 283562	AF522086
Lathyrustingitanus L.[a]	USDA 451858	AF522087
Lathyrustuberosus L.[b]	Iran: Alizadeh et al., 5118 (WARCNH)	AB935083
Section Linearicarpus	-	-
Lathyrusinconspicuus L.[b]	Iran: Faghihnia & Zangooei 34027 (FUMH)	AB935071
Lathyrussphaericus Retz. [b]	Iran: Joharchi & Zangooei 33235 (FUMH)	AB935082
Lathyrusvinealis Boiss. & Noe. [b]	Iran: Wendelbo & Assadi 16788 (TARI)	AB935087
Lathyrusworonowii Bornn. [b]	Turkey: Z. Aytaç 8693 (GAZI)	AB935088
Section Orobon	-	-
Lathyrusroseus Stev. [b]	Turkey: Aytaç 8157 (GAZI)	AB935079
Section Orobus	-	-
Lathyrus aureus (Steven) Brandza[b]	Turkey: O. Eyüboÿlu 1661 (GAZI)	AB935059
Lathyrus humilis (Ser.) Sprengel[b]	USSR: Ivanovsky 2894b (TARI)	AB935070
Lathyrusincurvus(Roth) Willd. [b]	Iran: Kazempour 2008-4 (TMUH)	AB935072
Lathyrusjaponicus Willd[a]	Portugal: Schaefer, H	HM851119
quinquenervis (Miq) Litv[b]	USSR: Sukatschew & Poplawska 2898 (TARI)	AB935078
Lathyrusvernus(L.) Bernh. [b]	Iran: Runemark & Mozaffarian 28061 (TARI)	AB935086
Section Ratensis	-	-
Lathyrusczeczottianus Bassler [b]	Turkey: O. Eyüboÿlu 1308 (GAZI)	AB935066
Lathyruslaxiflorus(Desf.) Kuntze[b]	Faghihnia, Rafeiee & Zangooei 25516(FUMH)	AB935074
LathyrusPratensis L. [b]	Iran: Heidari et al. 1803 (WARCNH)	AB935076
Pisum formosum (Stev.) Alef.[b]	Iran: Mozaffarian 88389 (TARI)	AB935089
Pisumformosum (Stev.) Alef. [b]	Turkey: M. Vural & N. Adigüzel s.n. (GAZI)	AB935090
Pisumfulvum Sibth & Sm. [b]	Turkey: Adiguzel & Aytaç 1896 (GAZI)	AB935091
Pisumsativum [b]	Iran: Alizadeh et al. 1225 (WARCNH)	AB935092
Lens cyanea(Boiss & Hohen.) Alef. [b]	Iran: Joharchi 34755-a (FUMH)	AB935093
Lens ervoides (Brign.) Grande[b]	Turkey : R. M. Nesbitt & D. Samuel 2297 (GAZI)	AB935094
Lens ervoides (Brign.) Grande[a]	USDA 572330	AF522090
Vicia ervilia (L.) Willd. [b]	Iran: Emadzadeh, Memariani & Zangooei 36169 (FUMH)	AB935095
Vicia hyrcanica Fisch. &. Mey. [b]	Iran: Memariani & Zangooei 38986 (FUMH)	AB935096

[o]Abbreviations: WARCNH, West Azerbaijan Research Center of Natural Resources and Agriculture Herbarium; FUMH, Ferdowsi University of Mashhad Herbarium, Mashhad, Iran; GAZI, Gazi University Herbarium, Ankara, Turkey; TMUH, Tarbiat Modares University Herbarium, Tehran, Iran; TUH, Tehran University Herbarium, Tehran, Iran; TARI, Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran; USDA, seeds accessions obtained from US Department of Agriculture Plant Introduction program. With the exception of TMUH and WARCNH, herbarium acronyms are according to Holmgren and Holmgren (17).
[a] Sequences of matK region for those taxa were retrieved from GenBank
[b] Sequences of matK region for those taxa were determined in this study

3.2. DNA Isolation, PCR and Sequencing

Total genomic DNA was isolated from leaf material (stipules of *Lathyrus aphaca L.*) using the modified Cetyltrimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (18). The chloroplast gene *mat*K was used. The partial *mat*K gene was amplified using primers matK384F (designed in this study) and trnK2R (5). Total volume of amplification reactions was 20 μL, which contained 8.2 μL deionized water, 10 μL of the 2 × Taq DNA polymerase master mix Red (Amplicon, Cat. No. 180301, 150 mM Tris-HCL pH 8.5, 40 mM (NH$_4$)2SO$_4$, 3.0 mM MgCl$_2$, 0.4 mM dNTPs, 0.05 units.μL^{-1} Amplicon Taq DNA polymerase, inert red dye and a stabilizer) 0.5 μL of each primer (5 pmol.μL^{-1}) and 1 μL of template DNA (20 ng.μL^{-1}). The PCR cycles consisted of predenaturation at 95°C for 2.30 minutes followed by 35 cycles: denaturation at 94°C for 45 seconds, annealing at a temperature depending on the region, at 51°C for matK and elongation at 72°C for 1.20 minutes. A final elongation step of seven minutes at 72°C was performed. Each region was sequenced using the 'Big dye terminator cycle sequencing ready reaction kit' with appropriate primers in an ABI Prism 3730XL DNA analyzer.

3.3. Sequence Alignment

The dataset was aligned using CLUSTAL (19) and adjusted manually. Positions of insertions and deletions were treated as missing data.

3.4. Phylogenetic Analyses

3.4.1. The Maximum Parsimony Method

Maximum Parsimony (MP) analyses were conducted using the PAUP* version 4.0b10 (20) for phylogenetic analysis. The heuristic search option was employed for the dataset, using tree bisection-reconnection (TBR) branch swapping, with 1000 replications of random addition sequence and an automatic increase in the maximum number of trees. Uninformative characters were excluded from the analysis. Branch support values were calculated using a full heuristic search with 1000 bootstrap (BS) replicates (21) each with simple addition sequence.

3.4.2. Bayesian Method

Model of sequence evolution for the dataset was selected using the program MrModeltest version 2.3 (22) as implemented in MrMTgui (23) based on the Akaike information criterion (AIC) (24). The matK dataset was analyzed using the GTR + I + G model. The program MrBayes (25) was used for the Bayesian phylogenetic analysis. Posteriors on the model parameters were estimated from the data, using the default priors. The analysis was done with four million generations, using Markov chain Monte Carlo search. MrBayes performed two simultaneous analyses starting from different random trees (Nruns = 2) each with four Markov chains and trees sampled at every 100 generations. The first 25% of trees were discarded as the burn-in. The remaining trees were then used to build a 50% majority rule consensus tree accompanied with posterior probability (PP) values. Tree visualization was carried out using Tree View version 1.6.6.

3.4.3. Maximum Likelihood

Maximum likelihood (ML) analyses were performed for the datasets in the program Genetic Algorithm for Rapid Likelihood Inference (GARLI) (26). The model of evolution employed for each data set was the same as that of Bayesian analyses.

Parametric bootstrap values for ML were calculated in GARLI, based on 100 replicates with one search replicate per bootstrap replicate.

3.4.4. Shimodaira-Hasegawa Test

Shimodaira-Hasegawa (SH) test (27) in PAUP*, based on maximum likelihood analyses was used to test the hypothesis that *Lathyrus* is monophyletic and *Pisum* is the sister group for *Lathyrus*. To accept that the hypothesis probability value (P-value) should be more than 0.05.

4. Results

4.1. Sequence Dataset

The aligned *mat*K dataset was 1077 nucleotide sites long, of which 186 sites were potentially parsimony informative. The length of *mat*K varies from 623 BP in *Lathyrus aphaca, L. ochrus, L. Clymenum L., L. tingitanus L.* and *L. latifolius* (the smaller length of *mat*K gene for these species is due to incomplete sequencing, which was retrieved from GenBank) to 1065 in *L. alamutensis* Mozaffarian, Ahvazi & Charkhchian. Parsimony analysis of the dataset resulted in 10000 trees of length (L) = 468 steps, Consistency Index (CI) = 0.519 and Retention Index (RI) = 0.798. The 50% majority rule consensus tree resulting from the Bayesian analysis of the *mat*K dataset with posterior probabilities and bootstrap values is shown in Figure 1.

In the present study, for all trees derived from MP, Bayesian and ML methods, *Pisum* was nested among *Lathyrus* species. Two members of the section *Clymenum* (*Lathyrus ochrus* and *L. Clymenum*) with *Pisum* subclade formed a weakly supported clade as sister to larger polytomy comprising the remainder *Lathyrus* species.

Here, we described the relationship between the taxa based upon the tree from Bayesian analysis (Figure 1). All multi-specific sections of *Lathyrus* (*Lathyrostylis, Lathyrus* and *Clymenum*), with the exception of *Linearicarpus, Orobus* and *Pratensis* were well-supported monophyletic groups. *Pratensis* and the monotypic section Aphaca were the closest taxa. Results of the SH test based upon the dataset showed that *Lathyrus* is a monophyletic taxon and *Pisum* is sister to this taxon (P > 0.05).

Figure 1. 50% Majority Rule Consensus Tree Resulting from Bayesian Analyses of the matK Data Set. Numbers above branches are maximum parsimony (MP) bootstrap/maximum likelihood (ML) bootstrap/posterior probabilities (pp), values < 50% are not shown. *matK genes was newly sequenced for these taxa

5. Discussion

5.1. Phylogenetic Status of the Genus Lathyrus

Kupicha (6) and Lock & Maxted (2) stated that *Lathyrus* is a monophyletic genus. In studies of Steele et al. (4) and Kenicer et al. (8), *Pisum sativum L.* along with species of *Lathyrus* formed a clade with bootstrap value of 100%. In the latter work, *Lathyrus* species, with the inclusion of sections *Nissolia*, *Clymenum* and *Neurolobus*, formed a weakly supported clade (bootstrap value of < 50%). In the present study, at the base of the tree, the *Pisum* clade was weakly allied with *L. ochrus* and *L. clymenum* (sect. *Clymenum*) and

thus, *Lathyrus* was not monophyletic (Figure 1). SH test (p > 0.05) of the dataset showed that all analyzed *Lathyrus* species formed their own clade and *Pisum* was sister to them. Furthermore, when we removed these two *Lathyrus* species, the analysis retrieved *Pisum* as a well-supported clade being sister to *Lathyrus* calde (tree not shown). *Lathyrus* and *Pisum* are similar in some morphological characters, including presence of pollen brush on the adaxial side of the style; non-brochiododromous leaflet venation (i.e. veins reaching the margins) and accumulate pisatin, a phytoalexin that is not found in other members of Fabeae. In addition, both genera lack canavanine (4, 8, 28-31). The two genera, however, differ in some diagnostic char-

acters as conduplicate or folded leaflet ptyxis and folded longitudinal style in *Pisum* (except *P. formosum* for the former character), but super volute or rolled ptyxis and non-folded longitudinal style in *Lathyrus* (1, 4). Moreover, the wider hybridization experiments between *Lathyrus* and *Pisum* species have shown cross-incompatibility (32).

5.2. Relationships within Lathyrus

The tree resulting from the dataset composed of several clades. Some of the clades had also appeared in previous molecular studies (7, 8); albeit the relationships were well resolved and supported in our tree. Below we examine some of these lineages.

5.3. Section Orobus

Kupicha (6) accepted the heterogeneous section *Orobus* with 54 species having a perennial habit, multijugate leaves with or without tendrils. As she noted, "the section contains a diversity of vegetative and floral forms and many of its members appear to be rather distantly related".

Our matK analysis did not retrieve section *Orobus* as a monophyletic group. This is in agreement with the conclusion made by Kenicer et al. (8). *Lathyrus vernus* (L.) Bernh, *L. incurvus* (Roth) Willd and *L. aureus* (Steven) Brandza, are successive sisters to a subclade of three species *L. quinquenervus* (Miq.) Litv. *L. japonicus* Willd. and *L. humilis* (Ser.) Sprengel (all of sect. *Orobus*) (BS=81%, 89% and pp = 1.00) and *sect. Linearicarpus* (*L. woronowii* Bornm, *L. inconspicuus L.* and *L. sphaericus Retz.*). Kupicha (6) claimed that a few species of section *Orobus* (e.g. *L. quinquenervus*, *L. alpestris* (Waldst. & Kit.)) are closely related to members of *section Lathyrostylis*, whereas our analysis did not show a direct relationship between these two sections.

5.4. Sections Aphaca and Pratensis

The present study is concordant with previous reports (6-8) regarding the close relationship of sections *Aphaca* and *Pratensis*. They do share large hastate stipules, a unique pattern of nodal anatomy and distinctive wing petal architecture. *Aphaca* is a ditypic section and *Pratensis* is a small section comprising six species (6). The former section is represented by a single species (*L. aphaca*) with three accessions, and the second one by three species with three accessions (*L. Pratensis L.*, *L. laxiflorus* (Desf.) Kuntze and *L. czeczottianus* Bassler). In our analysis, *L. Pratensis* formed a sister group relationship with the *Aphaca* clade not its own section.

5.5. Section Lathyrus

In our results, all members of the section *Lathyrus* with inclusion of *L. roseus* Steven (of the monotypic section *Orobon*) formed a well-supported clade (BS = 71%, 77% and pp = 0.97). Within this calde, *L. tingitanus* is well allied with the remainder species. The remainder species

made up two subclades. One subclade included only annual species, *L. cicera*, *L. pseudocicera* Pampan. and *L. sativus* (BS = 99%, 98% and pp = 1.00), which were previously classified under either section Cicercula (Medic.) Godr. (9, 10, 33) or section *Clymenum* (11). These species are characterized by a canaliculate style. The second subclade comprised of both annual and perennial species (BS = 97%, 95% and pp = 1.00). Members of this subclade were already placed in sections *Cicercula* (annuals), *Lathyrus* (perennials) and the monotypic *Orobon* (*L. roseus*) (9, 10, 33). Kupicha (6) questioned the monophyly of section *Cicercula* and synonymized it with sect *Lathyrus*. This treatment was confirmed and followed by Kenicer et al. (8) and the present study. However, Kupicha maintained section *Orobon* (*L. roseus*) by suggesting that a bushy habit and isodiametric and strongly wavy-walled epidermal cells distinguishes this group from the species of section *Lathyrus*. Yet, the flower and fruit characteristics agree entirely with those of section *Lathyrus* (6). She claimed that this species is related to the delicate perennials (e.g. *L. tuberosus* L.) of the section *Lathyrus*. Indeed, in the present study and Asmussen and Liston's (7) analyses, this species was allied with *L. tuberosus*. Hence, we are in agreement with Asmussen and Liston (7) in that the section *Orobon* should be combined with section *Lathyrus*.

5.6. Section Lathyrostylis

Lathyrostylis (= Platystylis) is a relatively uniform section comprising 21 species (6, 12), of which 10 species were included. Our matK as well as the combined (nrDNA ITS, *trn*L-F and trnS-G) sequence data of Kenicer et al. (8) indicated that this section formed a well-supported clade (BS = 94%, 65%, PP = 1.00). Czefranova (33) placed species of this section within section *Orobus*. However, Bassler (34) and later Kupicha (6) suggested that the two sections are distinct. The current study and previous molecular studies clearly showed that members of *section Lathyrostylis* are not related to section *Orobus*, supporting the recent morphology-based treatments (6, 34). It is important to note that the section is represented by three species *L. cyaneus* (Stev.) C. Koch, L. boissieri Sirj. and *L. alamutensis* in Iran only, of which, the latter was recently established as a new species from western Alborz mountain ranges. This new species has very specific characteristics with two flap-like appendages above the claw of standard (35).

Linearicarpus is a small section with seven annual species of which four were analyzed here (6). Analysis of *mat*K sequence data revealed that the section at the current status is not monophyletic. Its three species *L. inconspicuus*, *L. sphaericus* and *L. woronowii* are closely related taxa and allied with some members of section *Orobus*. Yet, *L. vinealis Boiss. & Noe* (fourth species), falls distantly from them in a trichotomy. Previous morphology (12) and molecular-based (7, 8, 15) analyses questioned

the monophyly of this section. It should be mentioned that in Flora of Turkey (9) and Flora Iranica (10), *L. inconspicuus*, *L. sphaericus*, *L. vinealis* and *L. woronowii* plus some others were placed in section *Orobastrum*, whereas in Flora of Iran (11), they along with *L. nissolia* were classified under sect. Nissolia. Kupicha (6) combined section *Orobastrum* with *sect. Linearicarpus* and recognized Nissolia as a monotypic section.

5.7. Section Clymenum

The section *Clymenum* is composed of only three annual species, *L. gloeospermus Warb. & Eig.*, *L. Clymenum* and *L. ochrus*. Previous researchers (7, 8) by analyzing all these three species questioned the monophyly of this section. They all reached the same conclusion as ours, claiming that *L. Clymenum* and *L. ochrus* are closely related taxa. Although, *L. gloeospermus* was well separated from the remainder of the section. In contrast to the latter species, *L. Clymenum* and *L. ochrus* do share several floral features including hollow, finger-like pouches on the standard, an unusual type of style (spathulate with apex ended to sterile fleshy mucro or arista which divides the stigma into two halves) and pods with winged sutures (6).

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Authors' Contribution

All authors participated equally in the study

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