

Research Article

Morphological cladistic analysis of some bifurcate hairy sections of *Astragalus* (Fabaceae) in Iran

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Abstract: The phylogenetic relationships among some bifurcate hairy sections of *Astragalus* L. were reconstructed using 38 vegetative and reproductive morphological characters. A data matrix comprising 36 ingroups and 2 outgroups was analysed using the maximum parsimony method. The present results revealed that sections *Onobrychoidei* DC. and *Ornithopodium* Bunge are interrelated taxa and form a single monophyletic group. The current status of section *Dissitiflori* DC. is a nonmonophyletic group. Its members form several subclades and unresolved branches across the tree. Sections *Erioceras* Bunge and *Cytisodes* Bunge as well as *Corethrum* Bunge are sister taxa and form weakly to highly supported monophyletic groups, respectively. *Astragalus pravitzii* Podlech, which was recently transferred to section *Ornithopodium* from section *Dissitiflori*, has no affinity with that section.

Key words: Astragalus, cladistic, Dissitiflori, Erioceras, Ornithopodium, Onobrychoidei, phylogeny

Introduction

Astragalus L. (Fabaceae) is probably the largest genus of flowering plants on earth and contains an estimated 2500 annual and perennial species and about 250 sections worldwide (Lock & Simpson, 1991; Podlech, 1998). The majority of species are found in the temperate semiarid and arid continental regions of south-western and central Asia, the Sino-Himalayan region, western North America, and along the Andes in South America (Lock & Simpson, 1991; Yakovlev et al., 1996; Maassoumi, 1998). Moreover, many

Astragalus species are distributed in Mediterranean climatic regions along the Pacific coasts of North and South America and in southern Europe and northern Africa (Maassoumi, 1998). South-western and central Asia is the centre of diversity for *Astragalus*, and there may be more than 800 species (belonging to more than 60 sections) in Iran, which has a high endemism rate of 65% (Podlech, 1986, 1998, 1999; Maassoumi, 1998, 2003, 2005). The earlier classification of *Astragalus* was carried out by De Candolle (1825) at the sectional level. After him,

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Bunge (1868-69) recognised 10 subgenera for the Old World Astragalus, and 9 of these were accepted by Gontscharov et al. (1946) for the flora of the former USSR. Podlech (1982) further reduced the number of subgenera by recognising only 2 groups of perennials, subgenera Astragalus and Cercidothrix Bunge, solely on the basis of type of pubescence; annual species were placed within subgenus Trimeniaeus Bunge irrespective of hair characteristics (Podlech, 1994). Later on, Maassoumi (1998) reduced Bunge's subgenera to 8 by transferring the species of 2 subgenera, Caprinus Bunge and Pogonophace Bunge, into others. Various molecular studies on the genus showed that none of these subgenera are monophyletic groups (Wojciechowski et al., 1999; Kazempour Osaloo et al., 2003, 2005). As a result, Maassoumi (2003) did not accept any subgenera for the genus while revising Astragalus for Flora of Iran. The only inclusive molecular phylogenetic analyses of the Old World Astragalus using nrDNA ITS and, in part, plastid gene ndhF sequences demonstrated 8 large monophyletic groups (so-called A to H; Kazempour Osaloo et al., 2003, 2005), as none of these overlap with those subgeneric classifications. Among the many species-rich sections analysed in these studies, only sections Ammodendron Bunge, Cenanthrum Koch, Chronopus Bunge, Laxiflori Agerer-Kirchhoff, Lotidium Bunge, and Incani DC. are monophyletic. Based on the work of Kazempour Osaloo et al. (2003, 2005), sections with basifixed hairs are basal, and some of these are intermixed with medifixed sections in the upper part of the cladograms. However, with these results, the phylogenetic relationships among many sections remained largely unresolved, requiring complementary molecular and morphological studies using more taxon sampling of each section. On the basis of these molecular analyses (Kazempour Osaloo et al., 2003, 2005), some bifurcate hairy species belonging to sections such as Dissitiflori DC., Erioceras Bunge, Onobrychoidei DC., and Ornithopodium Bunge form a large polytomic assemblage (clade F; Kazempour Osaloo et al., 2003, 2005) that shows some affinity; however, relationships among them remained unresolved. Although the above mentioned sections have been studied taxonomically in Iran or adjacent regions by various authors (Ghahreman et al., 1996; Maassoumi et al., 2000; Ranjbar & Karamian, 2002; Ranjbar, 2004;

Ghahremani-nejad, 2004; Maassoumi, 2005; Ekici et al., 2011), no previous molecular or morphological phylogenetic study has focused on these exclusively.

The objectives of the present study were to test the phylogenetic status of sections *Dissitiflori*, *Erioceras*, *Onobrychoidei*, *Ornithopodium*, *Corethrum* Bunge, and *Cytisodes* Bunge in Iran on the basis of morphological features and to explore the position of some debatable and newly introduced species from Iran in relation to these sections.

Materials and methods

Taxon sampling

A total of 34 species of *Astragalus* representing some related sections (*Dissitiflori*, *Erioceras*, *Corethrum*, *Onobrychoidei*, and *Ornithopodium*) plus 2 species belonging to the section *Cytisodes* were included as ingroup taxa in the analyses. Based on previous phylogenetic studies of the various sections of *Astragalus* (Kazempour Osaloo et al., 2003, 2005), 2 species in sections *Incani* DC. and *Caraganella* Bunge were selected as outgroups.

Characters and character states

Characters used in the cladistic analyses were obtained through examination of fresh materials in the field and herbarium specimens deposited at the Central Herbarium of Iran (TARI), the herbarium at Shahid Beheshti University (S. Beheshti Univ. Hb.), and the herbarium of Ferdowsi University (FUMH). Voucher specimen information is given in Table 1. The 38 characters and their relevant states used in the present analyses are given in Table 2. The data matrix is given in Table 3. Character state transformations were selected as unordered. The polarity of characters was determined using the outgroup method (Maddison et al., 1984).

Cladistic analyses

Analyses were performed on the data matrix using maximum parsimony (MP) as implemented in version 4.0b10 of PAUP* (Swofford, 2002). Multiple tree searches were conducted using heuristic search options that included random addition sequences (1000 replicates) holding 5 trees per replicate, and tree bisection-reconnection (TBR) branch swapping with retention of multiple parsimonious trees (maximum

Species	Voucher	Section
A. argyroides Beck	31795 (TARI); 31777 (TARI); 15323 (TARI)	Dissitiflori
A. eburneus Bornm. & Gauba	44936 (TARI); 43050 (TARI); 42868 (TARI)	Dissitiflori
A. juladakensis Maassoumi	50898 (TARI); 90551 (S. Beheshti Univ. Hb.)	Dissitiflori
A. juratzkanus Freyn & Sint.	72351 (TARI); 39457 (TARI); 92530 (S. Beheshti Univ. Hb.)	Dissitiflori
A. melanocalyx Boiss. & Buhse	5860 (TARI); 15263 (TARI); 17976 (TARI)	Dissitiflori
A. nigrolineatus Sirj. & Rech. f.	29042 (FUMH); 11988 (FUMH); 22773 (FUMH)	Dissitiflori
A. pravitzii Podlech	2118 (TARI); 7362 (TARI); 2607 (TARI)	Dissitiflori
A. saadatabadensis Podlech	15784 (TARI); 92531 (S. Beheshti Univ. Hb.); 92532 (S. Beheshti Univ. Hb.)	Dissitiflori
A. sitiens Bunge	26633 (TARI); 11270 (TARI); 28806 (TARI)	Dissitiflori
A. ruscifolius Boiss.	92535 (S. Beheshti Univ. Hb.); 28640 (TARI); 15798 (TARI)	Dissitiflori
A. xiphidium Bunge	72780 (TARI); 4425 (TARI); 228 (TARI)	Dissitiflori
A. brachyodontus Boiss.	92533 (S. Beheshti Univ. Hb.); 27666 (TARI); 13735 (TARI)	Ornithopodium
A. glochideus Boiss.	56952 (TARI); 55145 (TARI); 82606 (TARI)	Ornithopodium
A. jodostachys Boiss. & Buhse	56871 (TARI); 45496 (TARI); 80113 (TARI)	Ornithopodium
A. lunatus Pall.	12396 (TARI); 82421 (TARI); 75469 (TARI)	Ornithopodium
A. ornithopodioides Lam.	27860 (TARI); 28015 (TARI); 80129 (TARI)	Ornithopodium
A. stevenianus DC.	30128 (TARI); 41104 (TARI); 30067 (TARI)	Ornithopodium
A. goktschaicus Grossh.	68845 (TARI); 70521 (TARI); 13756 (TARI)	Onobrychoidei
A. lilacinus Boiss.	33157 (TARI); 83516 (TARI); 40424 (TARI)	Onobrychoidei
A. tehranicus Boiss.	47890 (TARI); 28824 (TARI); 15814 (TARI)	Onobrychoidei
A. alamliensis Rech. f.	84461 (TARI); 83358 (TARI); 14535 (TARI)	Erioceras
A. anacamptus Bunge	15932 (FUMH); 92532 (S. Beheshti Univ. Hb.); 50423 (TARI)	Erioceras
A. catacamptus Bunge	5328 (TARI); 82720 (TARI); 50423 (TARI)	Erioceras
A. djenarensis Sirj. & Rech. f.	40602 (FUMH); 16786 (TARI); 92555 (S. Beheshti Univ. Hb.)	Erioceras
A. keredjensis Podlech	82404 (TARI); 15449 (TARI); 23515 (TARI)	Erioceras
A. neosytinii Ranjbar	84571 (TARI); 77446 (TARI); 1250 (TARI)	Erioceras
A. nubicola Podlech	11165 (TARI); 2707 (TARI); 92544 (S. Beheshti Univ. Hb.)	Erioceras
<i>A. pakravaniae</i> Podlech & Maassoumi	92563 (S. Beheshti Univ. Hb.); 55534 (TARI); 55472 (TARI)	Erioceras
A. pentanthus Boiss.	1917 (TARI); 82459 (TARI); 16875 (TARI)	Erioceras
A. sympileicarpus Rech. f.	83362 (TARI); 91333 (S. Beheshti Univ. Hb.); 17121 (FUMH)	Erioceras
A. versipilus Rech. f. & Koeie	34272 (FMUH); 84615 (TARI); 25303 (FUMH)	Erioceras
A. zoshkensis Ghahremani-nejad	77059 (TARI); 48819 (TARI)	Cytisodes
A. gigantirostratus Maassoumi	72339 (TARI); 34860 (TARI); 53087 (TARI)	Cytisodes
A. aestimabilis Podlech	38523 (TARI); 1939 (TARI)	Corethron
A. dendroproselius Rech. f.	2832 (TARI); 30231 (TARI); 1152 (TARI)	Corethron
A. viridis Bunge	36132 (TARI); 30231 (TARI); 7329 (TARI)	Corethron
A. supervises (Kuntze) Sheld.	80104 (TARI); 82344 (TARI); 11473 (TARI)	Incani
A. stocksii Benth. ex Bunge	22325 (TARI); 23347 (TARI); 70132 (TARI)	Caraganella

Table 1. Voucher information for specimens used in present cladistic analysis.

Table 2. Characters and character states used in cladistic analysis.

- 1- Habit: spiny lignified (0); nonspiny lignified (1); herbaceous (2).
- 2- Plant height: ≤10 cm (0); 10-50 cm (1); >50 cm (2).
- 3- Shoot branching: low (0); high (1).
- 4- Stem: absent (0); stem with long internodes (1); stem with short internodes (2).
- 5- Stem status: standing (0); prostrate (1).
- 6- Stem hair compression: dispersed (0); dense (1).
- 7- Stem hair status: appressed (0); standing (1).
- 8- Black hair on stem: absent (0); present (1).
- 9- Stipule length: $\leq 2 \text{ mm } (0)$; > 2 mm (1).
- 10- Stipule colour: greenish (0); membranous white (1).
- 11- Stipule hair compression: dispersed (0); dense (1).
- 12- Stipule hair colour: only white (0); white mixed with black (1).
- 13- Leaf type: paripinnate (0); imparipinnate (1); single leaflet (2).
- 14- Leaf length: $\leq 2 \text{ cm } (0)$; 2-7 cm (1); >7 cm (2).
- 15- Leaflet pairs number: ≤3 (0); 3-10 (1); >10 (2).
- 16- Leaflet L/W ratio: ≤1.5 (0); >1.5 (1).
- 17- Leaflet shape: linear (0); oblong elliptic (1); elliptic (2); obovate (3).
- 18- Leaflet hair type: both sides densely covered (0); both sides dispersedly covered (1); one side densely and other dispersedly covered (2).
- 19- Black hair on peduncle: absent (0); present (1).
- 20- Inflorescence: sparse raceme (0); dense raceme (1).
- 21- Calyx type: campanulate (0); tubular (1); gibbose tubular (2).
- 22- Calyx hair status: appressed hair (0); standing hair (1).
- 23- Calyx hair symmetry: symmetrical (0); asymmetrical (1).
- 24- Calyx length: ≤5 mm (0); 5-15 mm (1); >15 mm (2).
- 25- Calyx teeth type: equal (0); unequal (1).
- 26- Calyx teeth internal surface hair: absent (0); present (1).
- 27- Corolla colour: yellow (0); purple (1); blue (2).
- 28- Standard L/W ratio: ≤2.5 (0); >2.5 (1).
- 29- Standard shape: elliptic (0); obovate (1); rhomboid (2).
- 30- Standard tip: obtuse (0); acute (1); emarginated (2).
- 31- Ovary stalk: absent (0); present (1).
- 32- Style hair: absent (0); present (1).
- 33- Pod shape: linear (0); oblong elliptic (1); beaded linear (2).
- 34- Pod cross section: orbicular (0); triangular (1).
- 35- Pod hair: absent (0); present (1).
- 36- Pod hair type: long and asymmetrical (0); short and symmetrical (1).
- 37- Hair compression on pod: dispersed (0); dense (1).
- 38- Black hair on pod: absent (0); present (1).

Character no. Species	111111111222222222333333333 12345678901234567890123456789012345678	
A. supervisus	1110?100100022003011200101001210001110	
A. stoksii	021101010000010030100?0010001010200100	
A. argyroides	11010100101011013010200111011200001010	
A. eburneus	11110101110011110000200101011200001001	
A. juratzkanus	11110101110011111010200101010110001001	
A. melanocalyx	11110100100011113010200101111210001000	
A. nigrolineatus	11010100111011110000200211011000001010	
A. pravitzii	11011100111011113000200100000210001000	
A. ruscifolius	11110101101011003010200101011200001000	
A. saadatabadensis	11011100010011013000200100001210001000	
A. sitiens	11010100110011010000200101001000001011	
A. xiphidium	11110100110011110010200101010201001010	
A. aestimabilis	21110001111011110011211101201000101111	
A. dendroproselius	211101011100111111112111012101001011111	
A. viridis	21110101111012111101211101211000101111	
A. zoshkensis	11120101110012111210211101000101101111	
A. gigantirostratus	1112011101001111101011110000000101110	
A. juladakensis	12110101110012110010201101?11001101111	
A. alamliensis	10021100100111111011211101011200111110	
A. anacamptus	10021110100010111011111101001000111110	
A. catacamptus	11021100110111112001211111001200111110	
A. djenarensis	11021100100011111011211101011200111110	
A. keredjensis	11021100110011112011211101111200110???	
A. neosytini	11021100100111111011211101012000111110	
A. nubicola	10121100110010111011111101002001??????	
A. pakravaniae	11021110100010112111211201012200111010	
A. pentanthus	10021100110111112011111101112200111110	
A. sympileicarpus	10021110100111111011111201010000111110	
A. versipilus	10021110100111112011211101111200111110	
A. brachyodontus	11110001111011111010100111202200201000	
A. glochideus	11110100111111211110100111201100201011	
A. ornithopodioides	11110100101011213011000101202200201010	
A. jodostachys	11010100101012211110100110201100201011	
A. lunatus	111101001110112111001001011110100201001	
A. stevenianus	1111010011111111011100111211110201001	
A. goktschaicus	11110100101011213011000101202200201010	
A. lilacinus	11110000101011110011100111202100201010	
A. tehranicus	1111010011111111011100111211110201001	

Table 3. Data matrix of morphological characters used in cladistic analyses. ?: character states were unavailable.

trees = 15,000). Analyses were then conducted using a successive reweighting strategy (Farris, 1989) in order to improve the tree indices and decrease the effect of characters showing high homoplasy on tree topologies. Weights were assigned to characters using the "reweight characters" option based on the rescaled consistency (RC) index (Farris, 1989) with a base weight of 1. After 4 rounds of reweighing, no change in tree indices was observed, and a strict consensus tree of this analysis was computed. Supports for clades were evaluated by bootstrapping (Felsenstein, 1985) using 1000 replicates with the heuristic search option, random addition sequence, and TBR branch swapping.

Results

The phylogenetic analysis based upon equally weighted characters yielded 327 most-parsimonious trees of 39 steps in length, with a consistency index (CI) of 0.464 and retention index (RI) of 0.821 (Figure 1). All characters used in the analyses were parsimony-informative. Parsimony analysis of equally weighted characters resulted in a phylogenetic tree comprising 2 major clades (Figure 1): 1 clade is composed of 3 species of section Onobrychoidei plus 6 species of Ornithopodium, and the second is a larger assemblage that comprises members of the remaining sections analysed here. Within this assemblage, 1 subclade is solely composed of section Erioceras, weakly supported (bootstrap percentage (BP) = 51%), and the second subclade contains the species of section Corethrum (BP = 70%). The relationships within these sections were not properly resolved. All members of section Dissitiflori did not form a clade.

Successive reweighting analyses generated 90 most-parsimonious trees of 26.85 steps in length, CI = 0.654, and RI = 0.870. The strict consensus tree of these trees is shown in Figure 2. The general topology of the tree was the same as in Figure 1, except that species relationships were resolved and statistically improved. Within the larger clade, *A. xiphidium* Bunge and *A. juladakensis* Maassoumi were sister taxa (BP = 64%), as were *A. saadatabadensis* Podlech and *A. pravitzii* Podlech. In addition, section *Corethrum* (Maassoumi, 2005) was sister to the sections *Cytisodes* and *Erioceras* (Figure 2). Again, all members of section *Dissitiflori* did not form a clade.

Discussion

In previous phylogenetic studies based on nrDNA ITS sequences, Kazempour Osaloo et al. (2003, 2005) demonstrated that many sections of *Astragalus* did not form a monophyletic group, as currently circumscribed, and should be revised following additional molecular and morphological studies.

Cladistic analysis of the morphological data revealed that members of sections *Onobrychoidei* and *Ornithopodium* are intermixed and nested in the moderately supported single clade (Figures 1 and 2).

In the phylogenetic tree based on nrDNA ITS sequences, sections *Onobrychoidei* and *Ornithopodium*, plus a few members of *Malacothrix* Bunge, are sister taxa and formed their own subclade within clade F. Within this subclade, *A. ornithopodioides* (sect. *Ornithopodium*) united with *A. tehranicus* Boiss. & Hohen. (sect. *Onobrychoidei*), suggesting that these 2 sections are closely related phylogenetically (Kazempour Osaloo et al., 2003). On the other hand, Ghahremani-nejad (2004) postulated that these 2 sections are gross morphologically interrelated. Our results are also in agreement with the findings of a recent work in which the members of the 2 sections were intermixed on the basis of seed morphology and micromorphology (Vural et al., 2008).

Recently, *Astragalus pravitzii*, endemic to Iran, was separated from section *Dissitiflori* and placed in section *Ornithopodium* by Podlech and Sytin (2010). Our results verified, however, the position of *A. pravitzii* within the section *Dissitiflori*, and it is allied with another member of the section, *A. saadatabadensis* (Figures 1 and 2).

As noted above in Results, members of sections *Cytisodes*, *Corethrum*, *Dissitiflori*, and *Erioceras* were well nested within a single clade (Figure 1).

Section *Cytisodes* is distinguished among bifurcate hairy sections with a stem of short internodes, a calyx with standing and asymmetrical hairs, and long pods (Bunge, 1868-1869). This section was introduced for *Flora of Iran* after the discovery of a remarkable new species (*A. gigantirostratus* Maassoumi, Ghahr., Ghahremani & Matin) from the eastern Elburz Mountains by Maassoumi et al. (1999). Later on, Maassoumi (2005) moved the newly established species, *A. zoshkensis* F.Ghahremani. (Ghahremaninejad, 2003), from section *Dissitiflori* to *Cytisodes* based on calyx hairs and pod features (see Podlech, Morphological cladistic analysis of some bifurcate hairy sections of Astragalus (Fabaceae) in Iran

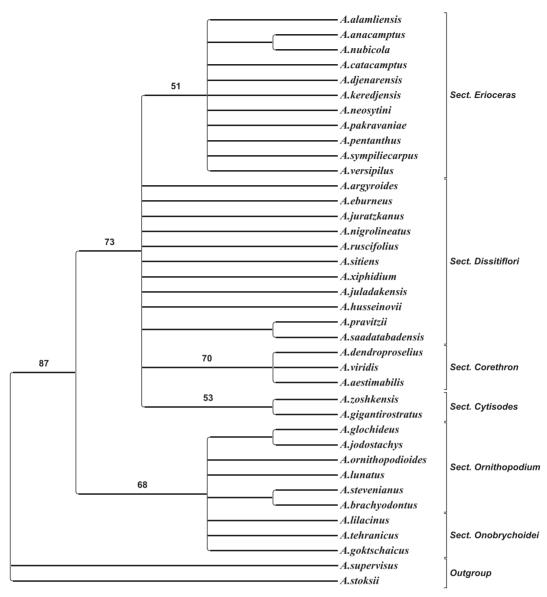


Figure 1. Strict consensus tree of 327 most-parsimonious trees obtained from an equally weighted morphological cladistic analysis. Bootstrap values greater than 50% are shown above the branches.

2010). On the basis of our results, *A. zoshkensis* and *A. gigantirostratus* are sister taxa and allied with the members of *Erioceras* (Figure 1). This result is in agreement with a recent molecular study in which the only sampled species from *Cytisodes* (*A. gigantirostratus*) was nested in a subclade along with members of *Erioceras* (Kazempour Osaloo et al., 2003, 2005).

A recent taxonomic work assumed that *A. viridis* Bunge and *A. dendroproselius* Rech. f. (plus *A. kharvanensis* Ranjbar, not analysed here) are closely related to each other, in the so-called viridis group, within section *Dissitiflori* (Ranjbar, 2004). This is consistent with our morphology-based cladistic analysis, which found that the first 2 species plus *A. aestimabilis* Podlech are closely related (Figures 1 and 2). On the other hand, these 3 species were separated from section *Dissitiflori* and moved to section *Corethrum* based on their elliptic pods and asymmetrical standing indumentum on the calyx (Maassoumi, 2005). However, the present study revealed that these taxa are related to section

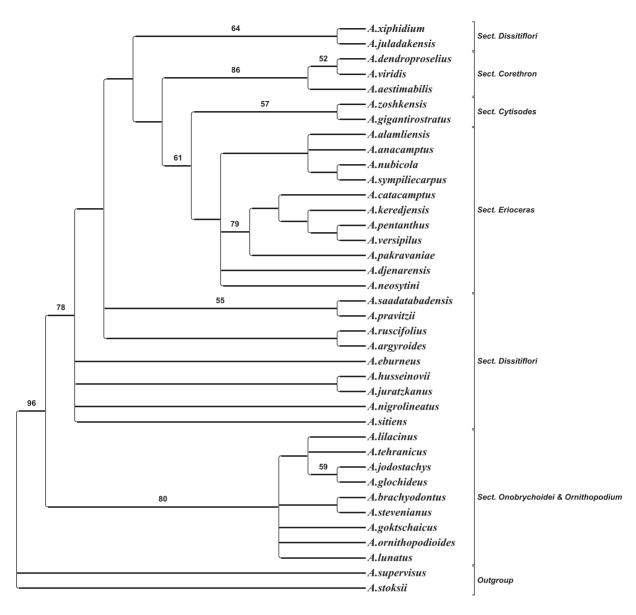


Figure 2. Strict consensus tree of 90 most-parsimonious trees obtained from morphological cladistic analysis after successive reweighting. Bootstrap values greater than 50% are shown above the branches.

Dissitiflori, nesting together in a subclade beside members of *Dissitiflori* and *Erioceras* (Figures 1 and 2). According to Kazempour Osaloo et al., using nrDNA ITS data, *A. viridis* (as a representative of section *Corethrum* in Iran) nested with other species of section *Dissitiflori*. It seems that the viridis group, as circumscribed in section *Corethrum* (Maassoumi, 2005), should be returned to section *Dissitiflori*. Indeed, the group was treated within section *Dissitiflori* in a recent work by Podlech (2010). With this in mind, the section *Dissitiflori* is still not monophyletic. Its members form several subclades and unresolved branches across the larger clade (Figures 1 and 2). It is distinguished from the section *Erioceras*, based on symmetrical short hairs on the calyx and pod (vs. asymmetrical long hairs) and a crescent, linear pod (vs. oblong elliptic linear) (Maassoumi, 2005; Podlech et al., 2010). *Erioceras* formed a weakly supported subclade allied with *Cytisodes*, and at least 2 subclades of 4 and 5 species can be found within it (Figure 2). The sister group relationships among these sections are consistent with the molecular phylogenetic work of Kazempour Osaloo et al. (2003, 2005).

In short, more taxon sampling from these sections, especially from the huge section *Dissitiflori*,

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and DNA sequences (nuclear and chloroplast DNAs) are definitely needed to evaluate the phylogenetic status of these sections explicitly. Indeed, our studies of these sections are ongoing.

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