Phylogeny of *Astragalus* sect. *Alopecuroidei* based on the combined nrDNA ITS and morphology

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Abstract

A phylogenetic analysis of *Astragalus* sect. *Alopecuroidei* based on nrDNA ITS as well as morphological character data is presented. A total of fifty informative morphological characters were analyzed to reconstruct phylogenetic relationships for 23 taxa of the sect. *Alopecuroidei* and two species of sect. *Laxiflori* plus *A. sieversianus* and *A. caryolobus* as outgroups. The present analysis revealed that the *Alopecuroidei* with the inclusion of sect. *Laxiflori* is monophyletic. This is well consistent with the previous works even with the small taxon sampling. *Astragalus alopecias* was positioned at the base of the resulting trees. The present study indicates that all six/four informal species groups, except the Kirrindicus (=Obtusifolius) group and Turbinatus group, recognized within the *Alopecuroidei* were not monophyletic. nrDNA and morphology-based phylogenies were conflicting regarding the position of *A. saetiger*, *A. turbinatus* and *A. neoassadianus*.

Keywords: *Alopecuroidei*, *Astragalus*, *Fabaceae*, cladistic analyses, nrDNA ITS, morphology

فیلوژنی بخش *Alopecuroidei* از جنس گون براساس داده‌های ترکیبی nrDNA ITS و ریخت‌شناسی*

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خلاصه

آنالیز فیلوژنتیکی بخش *Alopecuroidei* از جنس گون براساس داده‌های nrDNA ITS و ریخت‌شناسی ارایه می‌شود. در مجموع، ۵۰ صفت ریخت‌شناسی اطلاعاتی، به منظور بازسازی روابط فیلوژنتیکی ۲۳ آرایه از بخش *Alopecuroidei* و دو گونه از بخش *Laxiflori* همراه با گونه‌های *A. sieversianus* و *A. caryolobus* به عنوان برون گروه، مورد مطالعه قرار گرفتند. طبق تحقیق حاضر، بخش *Alopecuroidei* به همراه بخش *Laxiflori* یک گروه تک‌نیا تشکیل می‌دهد که نتایج به دست آمده با کارهای قبلی، به خوبی مطابقت دارد. در مطالعه حاضر، *A. alopecias* در قاعده درخت‌های نتیجه شده قرار گرفته، شش/ چهار گروه گونه‌ای غیررسمی *Alopecuroidei*، تک‌نیا نیستند. اما گروه‌های Kirrindicus (=Obtusifolius) و Turbinatus تک‌نیا بودند. درخت فیلوژنتیکی ارایه شده براساس اطلاعات DNA ریبوزومی هسته با درخت فیلوژنتیکی ارایه شده براساس اطلاعات ریخت‌شناسی در محل قرارگیری *A. saetiger*، *A. turbinatus* و *A. neoassadianus* با یکدیگر مغایرت دارند.

واژه‌های کلیدی: *Alopecuroidei*، آنالیز کلادیستیک، تیره حبوبات، ریبوزومی هسته‌ای، مورفولوژی، ناحیه فاصله‌گر رونویسی شونده درونی توالی

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Introduction

Astragalus L. (*Fabaceae*) is the richest genus of vascular plants on earth, represented by over 2500 species (Lock & Simpson 1991, Maassoumi 1998, Lewis *et al.* 2005, Mabberley 2008). *Astragalus* phylogenetically belongs to a large group of herbaceous and temperate papilionoid legumes that lack the chloroplast-DNA inverted repeat, which is called the Inverted Repeat Lacking Clad (IRLC) and includes important crop genera (Lavin *et al.* 1990, Wojciechowski *et al.* 1999, 2000, 2004, Wojciechowski 2005, Kazempour Osaloo 2007). *Alopecuroidei* DC. is one of the largest sections of the Old World *Astragalus* distributed mainly in Iran, Turkey and the former USSR (Podlech 1999, Maassoumi 1998). The section was established by De Candolle (1825), and was placed in the artificial subgenus *Calycophysa* by Bunge (1868–69). This section was taxonomically revised by Becht (1978) and then was treated for the Flora Iranica by Podlech (1999) and for Flora of Iran by Maassoumi (2003), respectively. Section *Alopecuroidei*, with over 50 species, is morphologically characterized by the robust habit, leaf with free stipules, spherical/cylindrical inflorescence with yellow and dens flowers, bilocular legumes and inflated calyx at fruiting time. Moreover, in this section inflorescence and bracts covered with mixed black and white hairs and pods splitting the calyx at fruiting time (Podlech 1999, Maassoumi 1989, 2003). Members of the section possess tricolporate subprolate or prolate spheroidal pollen grains with a microreticulate exine ornamentation and semi-angular amb (Akan *et al.* 2005). The sect. *Alopecuroidei* were represented by 27 taxa in Iran of which 16 are endemic (Podlech 1999, Maassoumi 2003, Ranjbar & Karamian 2003).

Morphological studies on *Alopecuroidei* in Iran have been performed by Maassoumi (1995, 2003) and Ranjbar *et al.* (2002). On the basis of morphological characters, the Iranian representatives of the section has been subdivided into six or four informal species groups by Maassoumi (1995) and Ranjbar *et al.* (2002), respectively. The delimitation of these groups was mainly based on combination of vegetative and reproductive characters. According to Maassoumi (1995), the following informal six groups have been recognized: (1) Obtusifolius group, with bracteolate calyx, (2) Macrocephalus group, with glabrous stem, rachis and peduncle, (3) Maaboudii group, with appressed hair on the stem, rachis and peduncle, (4) Alopecias group, with small pod and sessile/short peduncle, (5) Turbinatus group, with hairy standard, based on a single species, and (6) Megalotropis group, with long patent hair on the stem, rachis and peduncle. Later on, Ranjbar *et al.* (2002) modified and reduced these six species groups to four groups including, Kirrindicus group (=Obtusifolius group), Macrocephalus group (=Maaboudii and Turbinatus groups), Alopecurus group (=Alopecias group) and Megalotropis group.

Astragalus dictyolobus Bunge, *A. tawilicus* Townsend and *A. phlomoides* Boiss. along with four other species that have been, previously, classified into the sect. *Alopecuroidei* (= sect. *Alopecias*), were transferred to the sect. *Laxiflori* (lectotype: *A. dictyolobus*) by Agerer-Kirchhoff & Agerer (1977) and followed by Podlech (1999) in the Flora Iranica. The main difference between two sections is in the inflorescence density. *A. phlomoides* was, then, transferred from the latter section to the *Alopecuroidei* by Ranjbar *et al.* (2002) and treated as a member of this section by Maassoumi (2003).

Molecular systematic analyses of *Astragalus* using nrDNA ITS sequences demonstrated that *Alopecuroidei* with fewer exemplar plus *Laxiflori* nested in an unresolved assemblage within clad “A” (Kazempour Osaloo *et al.* 2003, 2005). A subsequent molecular phylogenetic work using multiple DNA sequence data on the sect. *Caprini* DC. and its allies revealed that *Alopecuroidei* plus *Laxiflori* were placed in a clade along with sect. *Astragalus* and *Chronopus* Bunge as being sister to sections *Caprini* and *Caraganella* Bunge (Riahi *et al.* 2011).

Hitherto, no detail phylogenetic analysis using both morphological and DNA sequence data has been conducted to evaluate monophyly and relationships

within *Alopecuroidei*. In the present study, using a combination of nrDNA ITS and morphology, we attempt to achieve the following goals:
(1) to evaluate phylogenetic relationship among sect. *Alopecuroidei* and sect. *Laxiflori*, and (2) to examine the evolutionary relationships within it with paying attention to the proposed informal species group.

Materials and Methods

- Taxon sampling

Twenty three taxa belonging to the sect. *Alopecuroidei* and two species of sect. *Laxiflori*, as the closest sect. to *Alopecuroidei* were included in the analysis. *A. sieversianus* and *A. caryolobus* belonging to sect. *Astragalus* were selected as outgroups following to (Riahi *et al.* 2011). A total of 27 *Astragalus* species sampled in this study are listed in Table 1.

- DNA isolation, PCR and sequencing

Genomic DNA was extracted from fresh or dried leaf material using the modified cetyle trimethyammonium bromide (CTAB) method of Doyle and Doyle (1987). The nrDNA ITS region was amplified as a sharp single fragment using the primers ITS5m of Sang *et al.* (1995) and ITS4 of White *et al*. (1990). The PCR reaction was performed a 20 µl volume for each products containing 8.0 µl deionized water, 10 µl of the 2 Taq DNA polymerase master mix Red (Amplicon, Cat. No. 180301), 0.5 µl of each primer (5 pmol/µl), and 1 µl of template DNA (20 ng/µl). PCR procedures was as follows: predenaturation at 94º C for 3 min followed by 25 cycles: denaturation at 94º C for 50 sec, annealing at a temperature 55º C for 40 sec and elongation at 72º C for 55–75 sec and terminal elongation of 7 min at 72º C. Each region was sequenced using the 'Big dye terminator cycle sequencing ready reaction kit' with the appropriate primers in an ABI Prism 3730xl DNA Analyzer (Applied Biosystems, USA).

- Sequence alignment

Sequences were edited using BioEdit ver. 7.0.90 (Hall 1999). The dataset was aligned using ClustalX (Larkin *et al.* 2007) and alignment errors were edited manually. The alignment of the dataset required introduction of some single base indels. Positions of indels were treated as missing data.

- Morphological characters and character states

The study of morphological characters in the genus was performed from fresh or preserved material collected during field trips and herbarium collections of TARI or adopted from appropriate references (Maassoumi 1995, 2003, Podlech 1999, Ranjbar *et al.* 2002, Ranjbar & Karamian 2003). Fifty informative characters with the relevant character states used in the present analysis were given in Table 2. The polarity of characters was determined using the outgroup method (Maddison *et al.* 1984). Data matrix of taxa and coded characters were given in Table 3.

- Phylogenetic analyses

Parsimony method: Phylogenetic analyses were performed on the aligned nrDNA ITS and morphological data matrix (Table 2) separately and in combination. Parsimony analyses were conducted using PAUP* version 4.0b10 (Swofford 2002). The heuristic searches were performed with 1000 replicates of random addition sequence with ACCTRAN optimization, tree-bisection-reconnection (TBR) branch-swapping with MulTrees on and steppest descent off installed in a Macintosh computer. In both analyses, branch support values were calculated using a full heuristic search with 1000 bootstrap replicates (Felsenstein 1985) each with simple addition sequence. Combinability of these two datasets was assessed using the partition homogeneity test (the incongruence length difference (ILD) test of Farris *et al.* (1995) as implemented in PAUP*. The test was conducted with exclusion of invariant characters (Cunningham 1997) using the heuristic search option involving simple addition sequence and TBR branch swapping with 100 homogeneity replicates.

Bayesian method: Model of sequence evolution were selected using the program MrModeltest (Nylander 2004) based on the Akaike information criterion (AIC) (Posada and Buckley 2004). nrDNA ITS dataset was analyzed with K80 model (Lset nst = 2 rates = equal) using the program MrBayes version 3.1.2 (Ronquist & Huelsenbeck 2003). Morphological characters were included as a separate partition along with nrDNA ITS sequences and a standard (morphology) discrete state model [l set coding = variable, (nst = 1)+G] was applied to this partition. Posteriors on the model parameters were estimated from the data, using the default priors. The analysis was done with 2 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 (Page 2001).

Table 1. Taxa included in the nrDNA ITS and morphological analyses

Species	DNA source (location, voucher)	GenBank accession number
Astragalus jessenii Bung	Iran, Khorasan, Assadi & Maassoumi 50860 (TARI)	AB741281
A. echinops Boiss.	Iran, Azarbayejan, Kazempour 2009 (TMUH.)	AB741278
A. hamadanus Boiss.	Iran, Hamadan, Safikhani *et al*. 2991 (TARI)	AB741280
A. hymenocalyx Boiss.	Iran, Hamadan, Assadi & Mozaffarian 36880 (TARI)	AB741623
A. saetiger Becht	Iran, Hamadan, Maassoumi & Shahsavari 80690 (TARI)	AB741297
A. foliosus Podlech, Maassoumi & Ranjbar	Iran, Zanjan, Mozafarian & Maassoumi 78528 (TARI)	AB741279
A. phlomoides Boiss.	Iran, Esfahan, Mozafarian 1721 (TARI)	AB741294
A. alopecias Pallas	Iran, Khorasan, Assadi & Maassoumi 50860 (TARI)	AB741272
A. alopecurus Pallas	Iran, Azarbayejan, Assadi & Sardabi 24013 (TARI)	AB741273
A. ponticus Pallas	Iran, Azarbayejan, Amini 1721 (TARI)	AB741296
A. turbinatus Bunge	Iran, Khorasan, Mozaffarian 67570 (TARI)	AB741302
A. zarjabadensis Ranjbar	Iran, Azarbayejan, Maassoumi 80125 (TARI)	AB741304
A. speciosus Boiss. & Hohen.	Iran, Tehran, Assadi & Maassoumi 51720 (TARI)	AB741301
A. meridionalis Bunge	Iran, Hormozgan, Mozaffarian 63564 (TARI)	AB741291
A. obtusifolius DC.	Iran, Kermanshah, Kashkooli & Soltani 12392 (TARI)	AB741293
A. kirrindicus Boiss.	Iran, Yazd, Mozaffarian 77484 (TARI)	AB741282
A. schahrudensis Bunge	Iran, Gorgan, Wendelbo & Forooghi 12702 (TARI)	AB741298
A. maaboudii Ranjbar	Iran, Kordestan, Wendejbo *et al*. 11927 (TARI)	AB741283
A. megalotropis Bunge	Iran, Azarbayajan, Mozaffarian & Nowrozi 34519 (TARI)	AB741288
A. macrocephalus ssp. *macrocephalus* Willd	Iran, Azarbayejan, Assadi & Shahsavari 65442 (TARI)	AB741287
A. macrocephalus ssp. *finitimus* (Bunge) Chamberlain	Iran, Fars, Mozaffarian 71490 (TARI)	AB741285
A. neoassadianus Ranjbar	Iran, Khorasan, Roohchamani 6025 (TARI)	AB741292
A. ajubensis Bunge	Iran, Kerman (TARI)	AB741271
A. tawilicus Townsend	Iran, Hamadan, Mozaffarian 64407 (TARI)	AB051948
A. dictyolobus Bunge	Iran, Azarbayejan, Assadi & Shahsavari 65570 (TARI)	AB741277
A. sieversianus Pallas	Iran, Khorasan, Assadi & Maassoumi 50669 (TARI)	AB741299
A. caryolobus Bunge	Iran, Hamadan, Mozaffarian 64409 (TARI)	AB741276

Abbreviation used in a accession information: TARI, Herbarium of the Research Institute of Forests and Rangelands, Tehran. TMUH, Tarbiat Modares University Herbarium, Tehran (Iran).

Table 2. The morphological characters and character states used in the cladistic analysis

Character	Character states
Height of plant (cm)	≤50(0) >50(1)
Hair density on stem	Dense(0), lax(1), glabrous(2)
Hair status on stem	Patent(0), appressed(1), glabrous(2)
Stipule shape	Lanceolate/filiform(0), triangular(1)
Stipule texture	Herbaceous(0), membranous(1)
Stipule surface	Glabrous(0), hairy(1)
Stipule margin	Ciliate(0), glabrous(1)
Stipule length (mm)	≤15(0) >15(1)
Stipule width (mm)	≤7(0) >7(1)
Leaf length (cm)	≥15(0) <15(1)
Leaflet number (pairs)	>17(0) 17–11(1) <11(2)
Leaflet shape	Ovate/elliptic/obcordate(0), oblong(1), lanceolate(2)
Leaflet folding	Flat(0), folded(1)
Leaflet apex	Obtuse(0), acute(1), emarginate(2)
Hair density on leaflet upper surface	Glabrous(0), lax(1), dense(2)
Hair density on leaflet lower surface	Dense(0), lax(1), glabrous(2)
Leaflet length (mm)	<15(0) 15–25(1) >25(2)
Leaflet width (mm)	≤10(0) >10(1)
Petiolule length (mm)	>1(0) ≤1(1)
Inflorescence shape	Cylindrical(0), spherical/ovate(1)
Inflorescence density	Lax(0), dense(1)
Inflorescence length (cm)	<4(0) 4–6(1) >6(2)
Inflorescence diameter (cm)	≤4(0) >4(1)
Hair density on peduncle	Aabsent(0), dense(1), lax(2)
Hair status on peduncle	Aabsent(0), appressed(1), patent(2)
Peduncle length	Sessile(0) >2 cm(1) ≤2 cm(2)
Bract length (mm)	≤10(0) >10(1)
Presence of bracteol	Aabsent(0), present(1)
Calyx shape	Tubular(0), campanulate(1)
Calyx at fruiting time	Non-inflate(0), inflate(1)
Calyx length (mm)	≤14(0) >14(1)
Calyx teeth shape	Subulate/linear/filiform(0), deltoid(1)
Calyx teeth length (mm)	≤7(0) >7(1)
Standard shape	Oblong/obovate(0), elliptic/ovate/obcordate(1)
Standard length (mm)	>18(0) ≤18(1)
Standard width (mm)	>10(0) ≤10(1)
Standard claw length (mm)	≤9(0) >9(1)
Wing length (mm)	≥22(0) <22(1)
Wing limb length (mm)	<12(0) ≥12(1)
Wing limb width (mm)	>4(0) ≤4(1)
Auricle of wing length (mm)	>3(0) ≤3(1)
Keel length (mm)	≤22(0) >22(1)
Auricle of keel shape	Rounded(0), triangular(1)
Auricle of keel length (mm)	≤1(0) >1(1)
Stamen length (mm)	>22(0) ≤22(1)
Ovary shape	Spindle(0), ovate(1)
Ovary length (mm)	≤25(0) >25(1)
Hair density on pod	Dense(0), lax(1)
Pod length (mm)	>8(0) ≤8(1)
Pod at fruiting time	Exerting (splitting) from the calyx(0), included in the calyx(1)

Table 3. Data matrix of morphological characters used in cladistic analysis. Missing data are coded as "?" a={01} b={02} c={12}

Species	Character code
Astragalus caryolobus	0cb000000000100a000000?00000000000000000101010000
A. sieversianus	10000001002011002100000?0001000101000000000100000000
A. obtusifolius	0000100000200bca011111112111a110110001111001100010
A. meridionalis	1000100000100a100011111121011100010000100001110010
A. kirrindicus	00001100002000200111111121010100011001111000110000
A. maaboudii	00110111001b01002011010112100111010000111010001??0
A. macrocephalus ssp. *macrocephalus*	1cb1011110100101200111122110a110110010101111011100
A. macrocephalus ssp. *finitimus*	0221011101200001210111022110a110110100111111011100
A. ajubensis	122000001012010210011002c1001100010001011011110110
A. alopecias	100010000000000101000120000000111111101011000110110
A. echinops	101011000010002210011101c111a100011101111000110000
A. hamadanus	00000000011102000011110222001110111101011000100010
A. megalotropis	0a011001001111a01011100c22101110100010111010010000
A. ponticus	0220100111101101101a10022210011a001101011000100000
A. schahrudensis	1221000110200a022101111c22100111110000110111011100
A. speciosus	0110010000100b0021011001c2000100011001110000110000
A. jessenii	0cb1000000221101101110022210a110110001011010110100
A. hymenocalyx	0cc00000010100a01011110222000101011101011000100??0
A. saetiger	00a001000100100a00111000000001111011101011000110100
A. foliosus	11010000000101a0101111022200a111011101011000100??0
A. turbinatus	00a0110000000001010111101c110a110101110111000010100
A. phlomoides	0cb101100000002020011a2112210011a110110101101011100
A. zarjabadensis	11110001101b01012111111121210011a010010111101011??0
A. neoassadianus	000a0100012a112210111112221001101101000111??010??0
A. alopecurus	10111000100c11012000101222101111100111011000100010
A. dictyolobus	00000100000a0b001010020221000110100111111010000101
A. tawilicus	0000011000000b0000100201?100a110100100111010000101

Results

- Molecular analysis

The nrDNA ITS dataset consist of 27 taxa with 602 aligned nucleotide sites, of which 21 sites are parsimony informative. The length of the nrDNA ITS varies from 601 bp to 602 bp in the studied *Astragalus* species. Parsimony analysis of the dataset resulted in a single shortest tree having length of 22 steps and consistency index (CI) = 0.955 and a retention index (RI) = 0.981 (excluding uninformative characters). A 50% majority-rule consensus tree obtained from Bayesian analysis with posterior probabilities and bootstrap values is presented in Fig. 1. This tree is, topologically, the same as the most parsimonious tree resulting from the parsimony method (the tree not shown). In Bayesian tree, *A. alopecias* Pallas plus *A. turbinatus* Bunge and *A. neoassadianus* Ranjbar positioned as sisters to a large clade of the remaining species. This clade is, in turn, composed of two well supported subclades and one branch (*A. echinops* Boiss.). One subclade comprises 12 taxa of sect. *Alopecuroidei* only and the second one contains seven species of the section plus *A. dictyolobus* and *A. tawilicus* of sect. *Laxiflori*.

- Morphological analyses

The parsimony analysis based on equally weighted characters generated a single most-parsimonious tree of 238 steps with CI = 0.252 and RI = 0.523. The tree along with bootstrap values was shown in Figure 2. In this tree, all clades with the exception of two subclades have bootstrap values of less than 50%. *A. alopecias* is placed at the base of the tree followed by *A. saetiger* Becht as sister to the remaining species studied. *A. dictyolobus* and *A. tawilicus* of sect. *Laxiflori* are sister taxa (BS = 72), being weakly sister to *A. maaboudii* Ranjbar, positioned within the sect. *Alopecuroidei*.

- The combined data analysis

The partition homogeneity test suggested that the nrDNA ITS and morphology were congruent (P=0.94). Parsimony analysis of the combined dataset resulted in 33 shortest trees of length 285 steps, CI = 0.305, RI =

0.542. A 50% majority-rule consensus tree from Bayesian analysis of the combined dataset is presented in Figure 3. This Bayesian tree is well resolved and supported than the strict consensus tree of 33 most parsimonious trees does (not shown). Moreover, the combined nrDNA ITS-morphology tree is topologically almost similar to the morphology-based tree and statistically to the nrDNA ITS tree. The main difference of the combined tree with nrDNA ITS tree is regarding to the position of the *A. saetiger* near the base of the tree and the derived position of closely related species *A. turbinatus* and *A. neoassadianus* within it. Again, *A. alopecias* formed the first-diverging lineage followed by *A. saetiger* being sister to the large assemblage of the remainder species. This larger clade is composed of three clades. One clade, "A", comprises five analyzed species (from *A. hymenocalyx* through *A. foliosus*). The second clade, "B", composed of five species, of which *A. speciosus* Boiss. & Hohen. is sister to a well-supported subclade of four closely related species (*A. echinops*, *A. obtusifolius* DC., *A. meridionalis* Bunge and *A. kirrindicus* Boiss.). The third clade, "C", comprises three successive subclades, "C1", "C2" and "C3". The two species of *Laxiflori* constituted subclades "C1" with the high support (PP = 1, BS = 92%). The next is the subclade "C2" containing *A. turbinatus* and *A. neoassadianus* (PP=1), being sister to the subclade "C3". This is, in turn, composed of nine taxa divided into two subclades. The first one contains *A. megalotropis* Bunge plus *A. jessenii* Bunge and *A. ajubensis* Bunge (PP = 0.93). The second one was represented by *A. maaboudii*, *A. phlomoides*, two subspecies of *A. macrocephalus* Willd., *A. schahrudensis* Bunge and *A. arjabadensis* Ranjbar (PP = 0.82).

Discussion

- Evaluation of phylogenetic relationship among sect. *Alopecuroidei* and sect. *Laxiflori*

The present study represents phylogenetic analysis of *Astragalus* sect. *Alopecuroidei* based on nrDNA ITS as well as morphological character data separately and in combination. Similar to individual datasets, analysis of the combined dataset indicated that *Alopecuroidei* with inclusion of *Laxiflori* form a well-supported monophyletic group (PP = 1, BS = 100%). The members of sect. *Laxiflori* (*Astragalus dictyolobus* and *A. tawilicus*), were placed among the species of sect. *Alopecuroidei*, a finding consistent with results of Kazempour Osaloo *et al.* (2003, 2005) and Riahi *et al.* (2011). Our molecular analysis based upon chloroplast DNAs, *trn*T-*trn*Y, *trn*H-*psb*A and *mat*K sequences is also consistent with this finding (Javanmardi *et al.* unpublished data). As noted above, *A. dictyolobus* and *A. tawilicus*, because of possessing lax inflorescence, have been placed in the sect. *Laxiflori* (Podlech 1999, Maassoumi 1995, 2003). *Alopecuroidei* and *Laxiflori* do share many morphological features, including roubust stem, yellowish corolla, inflated calyx at fruiting time and bilocular pod. Hence, *Laxiflori* should be reduced as a synonymy of *Alopecuroidei.* To delimit the boundary of this section explicitly, more taxon sampling especially from the closely related sect. *Astragalus* is definitely needed. Indeed, our ongoing research on these sections is in progress, and we will do the classification of them upon the completion of our analyses.

- Relationships within the sect. *Alopecuroidei*

The present study indicates that all six/four informal species groups except the Kirrindicus (=Obtusifolius) group and Turbinatus group, recognized within the *Alopecuroidei* (Maassoumi 1995, Ranjbar *et al.* 2002) are not monophyletic (Figs 1 & 3). This species group, comprising *A. echinops*, *A. kirrindicus*, *A. obtusifolius* and *A. meridionalis* (plus *A. sarzehensis* Ranjbar and *A. stepporum* Podl., not analyzed herein), is characterized by the bracteolate calyx, as a unique synapomorphy. Unlike nrDNA ITS phylogeny, both morphology and the combined phylogenies showed that *A. speciosus* was weakly allied with this group, the clade "B" (Figs 1 & 3). This species was classified, however, under the Alopecurus group (Ranjbar *et al.* 2002). It seems nrDNA ITS data put truly the species within the latter group (Figs 1–3).

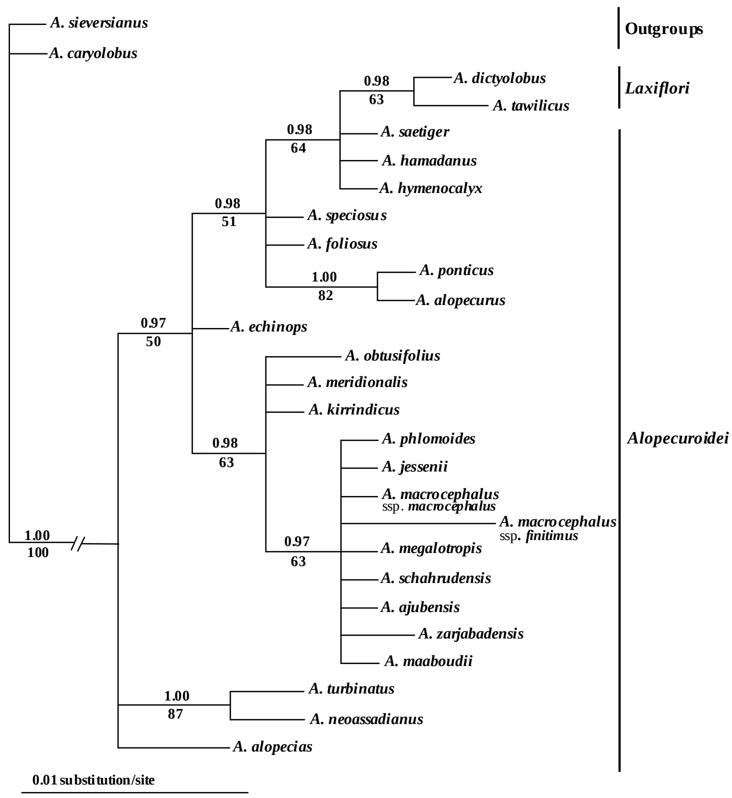

Fig. 1. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the nrDNA ITS data set. Numbers above branches are posterior probabilities and the numbers below them indicate MP bootstrap values. Values < 50% were not shown.

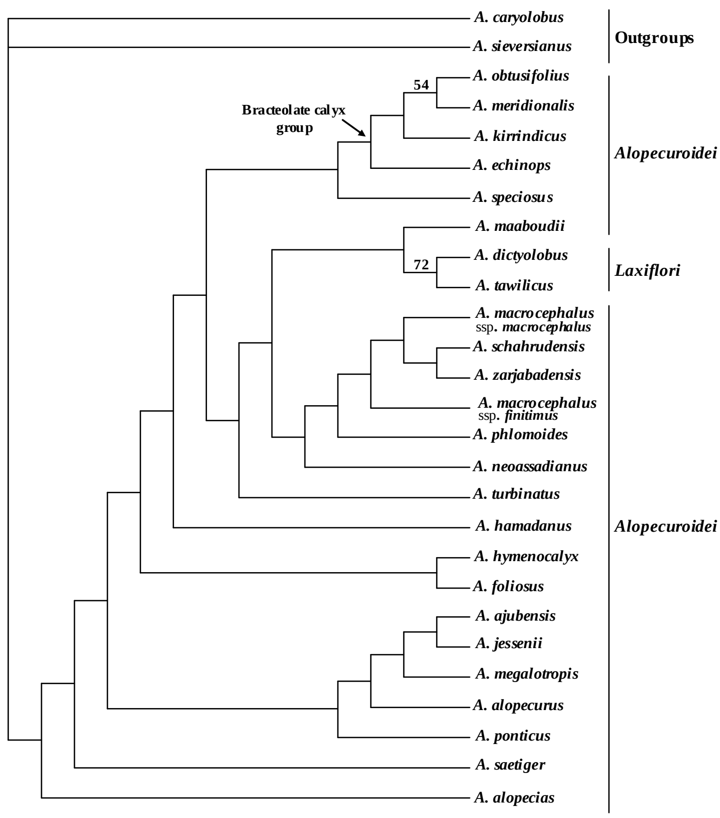

Fig. 2. The most parsimonious tree obtained from a morphological cladistic analysis with equal weighting. (Length=238, CI=0.252, RI=0.523). Bootstrap values greater than 50% were shown above the branches.

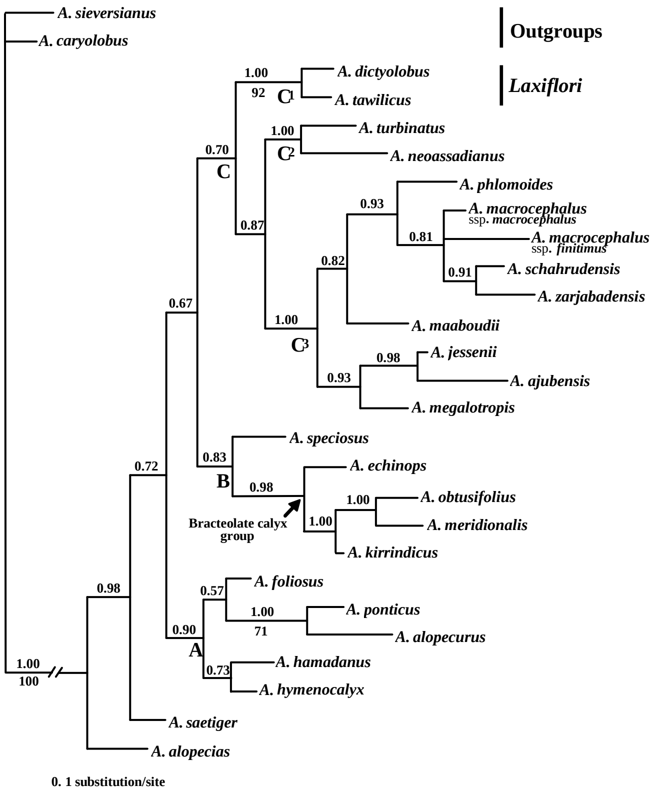

Fig. 3. Fifty percent majority rule consensus tree resulting from Bayesian analysis of the combined nrDNA ITS and morphology data set. Numbers above branches are posterior probabilities and the numbers below them indicate MP bootstrap values. Values < 50% were not shown.

The clade “A” consists of *A. alopecurus* Pallas, *A. foliosus* Podlech, Maassoumi & Ranjbar, *A. ponticus* Pallas, *A. hymenocalyx* Boiss and *A. hamadanus* Boiss (Alopecias group of Maassoumi, 1995). *A. alopecias* were assumed to be related with these species (Maassoumi 1995, Ranjbar *et al.* 2002). Whereas, all of our analyses clearly demonstrated that this species positioned at the base of the trees, distantly related with them (Figs 1–3). The position of *A. saetiger* is different between our trees. Its affinity with the other members of Alopecurus (= Alopecias) group is well consistent with our nrDNA ITS phylogeny than to both morphology-based and the combined phylogenies. On the other hand, because of larger keel petals, *A. hamadanus* and *A. megalotropis* were treated as members of Megalotropis group (Ranjbar *et al.* 2002). However, the position of *A. hamadanus* within the clade “A” along with *A. hymenocalyx*, both from western Iran, is well corroborated with Maassoumi’s (1995) hypothesis. Overall, this clade, is well correspond to Alopecurus (=Alopecias) group with excluding at least *A. alopecias* (Fig. 3). The members of this group can be distinguished by the leaflet number over 15 pairs and lax patent hairs on the peduncle. All members of Macrocephalus group (sensu Ranjbar *et al.* 2002) along with *A. megalotropis* of Megalotropis group minus *A. turbinatus* and *A. neoassadianus* formed a well supported monophyletic group, “C3” (Figs 1–3). Position of these two species is fluid in the different trees. They are moderately allied with those species of the clade “C3” in the combined tree, but positioned near the base of nrDNA ITS tree. These data support Maassoumi’s (1995) idea to keep these two species in Turbinatus group. Nevertheless, they do not share any clear synapomorphy.

- Conclusion

The present work demonstrated that the sect. *Alopecuroidei* with the inclusion members of sect. *Laxiflori* is a monophyletic group. Both nrDNA ITS and morphological data is well congruent. Although, the two data set is conflicting on the position of *A. saetiger*, *A. neoassadianus* and *A. turbibatus*. Overall, nrDNA ITS and the combined nrDNA ITS-morphology represent a better picture of relationships than the morphological data among the taxa studied. To circumscribe the sect. *Alopecuroidei* clrearly, more taxon sampling from the related sections such as sections *Astragalus* and *Eremophysa* as well as more DNA fragments are absolutely necessary.

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