

Molecular systematics of the genus *Astragalus* L. (Fabaceae): Phylogenetic analyses of nuclear ribosomal DNA internal transcribed spacers and chloroplast gene *ndhF* sequences

S. Kazempour Osaloo¹, A. A. Maassoumi², and N. Murakami³

¹Department of Plant Biology, Faculty of Basic Sciences, Tarbiat Modaress University, Tehran, Iran

²Department of Botany, Research Institute of Forests and Rangelands, Tehran, Iran

³Department of Botany, Graduate School of Science, Kyoto University, Kyoto, Japan

Received March 14, 2001; accepted February 23, 2003

Published online: November 20, 2003

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Abstract. Comparative sequencing of internal transcribed spacers (ITS) and 5.8S gene of nuclear ribosomal DNA was carried out to examine phylogenetic relationships among subgenera and sections of Old World *Astragalus* as well as the recent segregate genera *Barnebyella* and *Ophiocarpus*. For a subset of these taxa (43 accessions), the nrDNA ITS data were supplemented by sequences from the chloroplast *ndhF* gene. Phylogenetic trees resulting from separate analyses of the nrDNA ITS and *ndhF* sequences were in conflict mainly on the position and relationships of *Ophiocarpus aitchisonii*, *Astragalus hemsleyi*, *A. grammocalyx*, *A. coelicolor*, *A. capito*, *A. epiglottis* and *A. annularis*. Excluding these taxa, phylogenetic analysis of a combined nrDNA ITS-*ndhF* data matrix was also conducted, so that in the resulting tree, most clades were more resolved and better statistically supported than those were in the separate analyses. Our results indicate that the monotypic segregate genera *Barnebyella* (= *A. migpo*), *Ophiocarpus* (= *A. ophiocarpus*) and morphologically isolated annual species *A. dipelta* (= *Didymopelta turkestanica*), *A. schmalhauseni* (= *Sewerzowia turkestanica*) and *A. vicarius* (= *S. vicaria*) are clearly nested within *Astragalus*. Our results confirm earlier studies that shows *A. vogelii* is allied with the genera *Colutea* and *Oxytropis* rather than with

any *Astragalus* species. It is therefore excluded from *Astragalus* and elevated to the new generic rank and named as *Podlechiella* Maassoumi and Kazempour Osaloo. None of the eight traditionally recognized *Astragalus* subgenera *Epiglottis*, *Trimeniaeus*, *Phaca*, *Hypoglottis*, *Calycophysa*, *Tragacantha*, *Cercidothrix* and *Calycocystis* are monophyletic. Similarly, the monophyly of Podlech's new subgenera *Trimeniaeus*, *Astragalus* and *Cercidothrix* is not supported. Among the many species-rich sections analyzed here, only *Cenanthrum*, *Chronopus*, *Laxiflori*, *Lotidium*, *Incani* and *Amodendron* are monophyletic.

Key words: *Astragalus*, *Barnebyella*, Chloroplast *ndhF* gene, Fabaceae, Molecular systematics, nrDNA ITS, *Ophiocarpus*, Phylogeny, *Podlechiella*.

Astragalus L. (Fabaceae), as the largest genus of vascular plants on earth, contains an estimated number 3000 annual and perennial species and 245 taxonomic sections (Lock and Simpson 1991, Maassoumi 1998, Podlech 1998). This genus is a remarkable example of adaptive radiation on a global scale, distributed primarily around the northern hemisphere and South America. The greatest

number of species is found in the cool temperate semiarid and arid continental regions of southwest and central Asia (ca. 1500 spp.), the Sino-Himalayan region (500 spp.), western North America (ca. 400–450 spp.) and along the Andes in South America (ca. 100 species). In addition, 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. *Astragalus* is a member of the tribe Galegeae (Polhill 1981a) and belongs to a large clade of 45 papilionoid genera, referred to as the “IR-lacking clade”, or “IRLC”, which is marked by loss of the 25 kbp inverted repeat in the chloroplast genome (Lavin et al. 1990, Liston 1995). Within the IRLC, *Astragalus* together with *Biserrula* L., *Oxytropis* DC., all of subtribe Coluteinae and the New Zealand endemic tribe Carmichaelieae comprises a well supported monophyletic group (“Astragalean clade”: Sanderson and Liston 1995, Sanderson and Wojciechowski 1996). More recently, Wojciechowski et al. (1999) using nrDNA ITS sequence data for 115 *Astragalus* species (77 New World aneuploid species plus 38 Old World euploid and aneuploid representatives) demonstrated that all but five species are united in a highly supported single clade, so-called *Astragalus* s. str. Within this assemblage, Old World (including North American euploids) species form a basal grade that is sister to the “Neo-*Astragalus* clade” (New World aneuploids plus the Mediterranean *A. echinatus*).

For the purpose of this paper, we devote our argument only to the classification history of the genus in the Old World. Bunge (1868, 1869, 1880) recognized 10 subgenera for the Old World *Astragalus*. In the Flora of former USSR, Gontscharov et al. (1946) accepted nine subgenera of Bunge except heterogeneous *Pogonophace*. Species of this subgenus were transferred to subgenera *Phaca* and *Trimeniaeus*. Podlech (1982) dramatically reduced the number of these subgenera by recognizing only two groups of perennials (excluding subgenus *Tragacantha*) solely on the basis of type of

pubescence, namely subgenus *Astragalus* with basifixed hairs, and subgenus *Cercidothrix* with medifixed hairs. He (Podlech 1983) removed many species of subgenus *Tragacantha* to a new segregate genus, *Astracantha* (see below). Later, Podlech (1994) placed all annual species, except *A. vogelii* (*Cercidothrix*) with medifixed hairs, in the subgenus *Trimeniaeus* without paying attention to the type of pubescence. Maassoumi (1998) modified Bunge’s system, combining his ten subgenera into eight by transferring species of *Caprinus* and *Pogonophace* to *Phaca*, *Trimeniaeus* or *Cercidothrix*. The remaining subgenera sensu Maassoumi (1998) are *Epiglottis*, *Hypoglottis*, *Calycophysa*, *Tragacantha* and *Calycocystis*. Wojciechowski et al. (1999) commented on that their results were not consistent with the monophyly of subgenera *Epiglottis*, *Trimeniaeus*, *Hypoglottis*, *Phaca* and *Cercithothrix* (except for subgenus *Tragacantha*) in the context of the monophyly of *Astragalus* and large clades within. However, the monophyly of the two subgenera *Calycophysa* and *Calycocystis* has not yet been examined. These eight subgenera altogether cover >150 sections in the Old World. The monophyly of the proposed sections of *Astragalus* has not been tested and relationships among them remain problematic.

Astragalus is morphologically diverse, especially in vegetative morphology and the structure of the pod. Several segregate genera have been recently proposed in the Old World, one of which is the Eurasian segregate *Astracantha* Podl. (= subgenus *Tragacantha* sensu Bunge 1868, 1869; Maassoumi 1998) with 214 species (Podlech 1983). *Astracantha* was recognized based upon the striking thorny cushion-forming habit, reduced pods and inflorescences and gum production. This characteristics are also found in species of several sections (e.g. *Hymenostegis*, *Anthylloidei*, *Poterion*, *Acidoes*) retained in *Astragalus*. Engel (1991) provided anatomical evidence for the distinction of the *Astracantha* from the thorny cushion-forming *Astragalus*. Subsequently, Zarre and Podlech (1997) with critical analysis of morphological and anatomical features, concluded that this

generic delimitation was untenable and synonymized it with *Astragalus* under the subgenus *Astragalus* (sensu Podlech 1982). The recent molecular study based upon nrDNA ITS and chloroplast *trnL* intron sequence data also clearly rejected the recognition of *Astracantha* as separate from *Astragalus* (Wojciechowski et al. 1999). However, its relationship with other thorny cushion-forming species of *Astragalus* has not been examined by molecular data. The other notable segregates are the southwestern Asian annuals *Barnebyella* Podl. (= *Dorycnium calycinum* Stocks, *Astragalus migpo* Kamelin, Podlech 1994), *Ophiocarpus* (Bunge) Ikonn. (= *A. ophiocarpus* Bunge, Ikonnikov 1977) and *Thlaspidium* Rassulova (= *A. thlaspi* Lipisky, Rassulova 1978 cited in Podlech 1994). These species have been segregated as monotypic genera mainly because of unusual pod morphologies. In addition to these, some species like *A. dipelta*, *A. schmalhausonii*, *A. vicarius* and *A. compositus* have been treated under their old generic names (*Didymopelta turkestanica*, *Sewerzowia turkestanica*, *S. vicaria* and *S. composita* respectively) by some authors (e.g. Hutchinson 1964, Rassulova 1978 cited in Podlech 1994). Hitherto, the phylogenetic status and relationships of these taxa have not been evaluated by molecular data.

In this study, nrDNA ITS and chloroplast gene *ndhF* were sequenced for phylogenetic reconstructions. In the last ten years, sequencing of the ITS regions has been the main source of nuclear DNA characters for inferring intra- and intergeneric evolutionary relationships in plants (e.g. Baldwin 1992; Baldwin et al. 1995; Wojciechowski et al. 1993, 1999; Kazempour Osaloo and Kawano 1999). The *ndhF* gene, which is located in the small single-copy region of the chloroplast genome close to the junction with the inverted repeat, is approximately 2220 base pairs in length and codes for a subunit of a putative NADH dehydrogenase involved in chloroplast photorespiration (Sugiura 1992). Several studies have demonstrated the great potential of *ndhF* sequence data for resolving relationships

at a range of taxonomic levels, from closely related species (Bohs and Olmstead 1997, Schnabel and Wendel 1998), to the generic, and familial level (e.g. Olmstead and Sweere 1994, Scotland et al. 1995, Catalán et al. 1997, Olmstead et al. 2000). Our molecular phylogenetic study, based on both *ndhF* and in particular nrDNA ITS sequences, is the first survey at the broad level for the Old World *Astragalus*, mostly from Iran, as it is one of the most important centers of biodiversity of the genus.

The objectives of this study, therefore, were to:

- (1) reconstruct nrDNA ITS and *ndhF* phylogenies separately and in combination for the Old World *Astragalus* and related genera;
- (2) compare the nrDNA ITS phylogeny with the *ndhF* phylogeny;
- (3) explore the relationships and phylogenetic status of several segregate annual genera (*Barnebyella*, *Ophiocarpus*, *Didymopelta* and *Sewerzowia*); and
- (4) evaluate the taxonomic classification of subgenera and sections of the Old World *Astragalus* in the light of our molecular phylogenetic results.

Materials and methods

Taxon sampling. A total of 124 Old World *Astragalus* species/subspecies (including newly segregate genera *Barnebyella* and *Ophiocarpus*) plus aneuploid *A. oophorus* of the New World, was sequenced for nr DNA ITS+5.8S (Appendix 1). Sequences of another 11 *Astragalus* species (including the New World aneuploid *A. arizonicus*) and *Biserrula pelecinus* L. (= *A. pelecinus* (L.) Barneby) determined by Wojciechowski et al. (1993, 1999) were obtained from Genbank. Our sampling of *Astragalus* endemic to Eurasia and Africa included annual and perennial species/subspecies from 75 sections of all eight Old World subgenera (sensu Maassoumi 1998). This represents nearly half of the Old World sections recognized by Podlech (1986) and Maassoumi (1998). *Astragalus oophorus* and *A. arizonicus* were used as placeholders for the “Neo-*Astragalus* clade” which is nested within the Old World *Astragalus* based on previous studies

(Wojciechowski et al. 1993, 1999). We have also sequenced the chloroplast gene *ndhF* for a subset of 37 species of *Astragalus* and the two segregate genera sampled for nrDNA ITS. In addition to *Astragalus*, three related genera from the “Astragalean clade” (Sanderson and Liston 1995, Sanderson and Wojciechowski 1996) including *Biserrula*, *Oxytropis* and *Colutea*, were included in the nrDNA ITS data set (Appendix 1). With the exception of *Biserrula*, the remaining two genera were also sequenced for the *ndhF* gene. *Caragana grandiflora* and *Chesneya astragalina* were chosen as outgroups in both data sets according to previous studies (Sanderson and Wojciechowski 1996, Wojciechowski et al. 1999).

DNA extraction. Leaf materials were sampled from herbarium specimens deposited in the Herbarium of the Research Institute of Forests and Rangelands (TARI), Tehran, Iran, or in the Herbarium of Kyoto University (KYO), Kyoto, Japan. But, in the case of *Astragalus sinicus* L., fresh leaves were taken from plants growing in Kyoto, Japan. Its voucher specimen was deposited at TARI. Total genomic DNA was extracted following the modified CTAB method of Doyle and Doyle (1987).

Polymerase chain reaction (PCR). The complete nrDNA ITS + 5.8S region was amplified using primers ITS4 and ITS5 of White et al. (1990). The chloroplast gene *ndhF* was amplified using primers 16F (Schnabel and Wendel 1998) and 2110R (Olmstead and Sweere 1994). These double-stranded DNA amplifications were performed in a 50 µl volume containing 36 µl of sterile water, 5 µl of 10 × GeneTaq universal buffer (Wako Nippon Gene), 5 µl of 2.5 mM dNTP mixture (Wako Nippon Gene), 1 µl of each primer (10 pmol/µl), 0.8 µl (4 units) of Taq polymerase (Wako Nippon Gene), and 2 µl of genomic DNA template (20–40 ng). Amplification was done in a DNA Thermal Cycler (Perkin Elmer Cetus, model PJ2000) for 35 cycles (Kazempour Osaloo and Kawano 1999). Each set of reactions was monitored by the inclusion of a negative (no template) control. To remove unused amplifying primers and dNTPs, the PCR product was electrophoresed in a 1% agarose gel (using 1 × TAE as the gel buffer) stained with ethidium bromide, and then excised under UV light with a scalpel. The gel slice containing the DNA fragment was transferred to a 1.5 ml microcentrifuge tube and the DNA was

recovered from the agarose gel using the Gene Clean III Kit (Bio 101, Inc., USA) according to the manufacturer’s instruction. The purified DNA was resuspended in 20 µl of sterile water.

DNA sequencing. Purified double-stranded DNAs were then used in cycle sequencing reactions that were conducted using the Big DyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA). The same nrDNA ITS primers ITS4 and ITS5 were used for cycle sequencing reactions. For the *ndhF* gene, the sequencing primers 16F (Schnabel and Wendel 1998), 536F, 972F, 1603F, 2110R, 1318R and 536R (Olmstead and Sweere 1994) were employed. The reactions were purified using the Ethanol Precipitation Protocol 1 (according to the Perkin Elmer Corporation’s instruction protocol, revision A, August 1995) to remove unincorporated dye terminators and then completely dried in a vacuum. The reaction pellets were resuspended in 6 µl of loading buffer and analyzed in an ABI PrismTM 377 DNA Sequencer.

Phylogenetic analyses. Alignment of the nrDNA ITS sequences required the introduction of numerous single and multibase insertion/deletion events (indel). For the *ndhF* data, alignment was trivial as only three indel events of 6–9 base pairs needed to be postulated. In both nrDNA ITS and *ndhF* analyses, indel positions were treated as missing data and only in the former, a small subset of these were included as additional characters, cases wherein alignment was absolutely unambiguous. Individual sequences from taxa included in the present study have been deposited in the DNA Data Bank of Japan (DDBJ) (see Appendix 1 for accession numbers). The aligned nrDNA ITS and *ndhF* data sets are available upon request from the corresponding author. Phylogenetic analyses were performed on the aligned data matrices both separately and in combination (i.e. for those 43 taxa where both sequenced regions were available). Initially, phylogenies were inferred from all data matrices using maximum parsimony method (MP) as implemented in the version 4.0b4a (Macintosh PPC) of PAUP* (Swofford 2000). Due to the large number of taxa (142) in the nrDNA ITS data set, we could not ascertain the number of equally most parsimonious trees. As a result, the following heuristic search strategies (according to Catalán et al. 1997, Downie et al. 1998) were

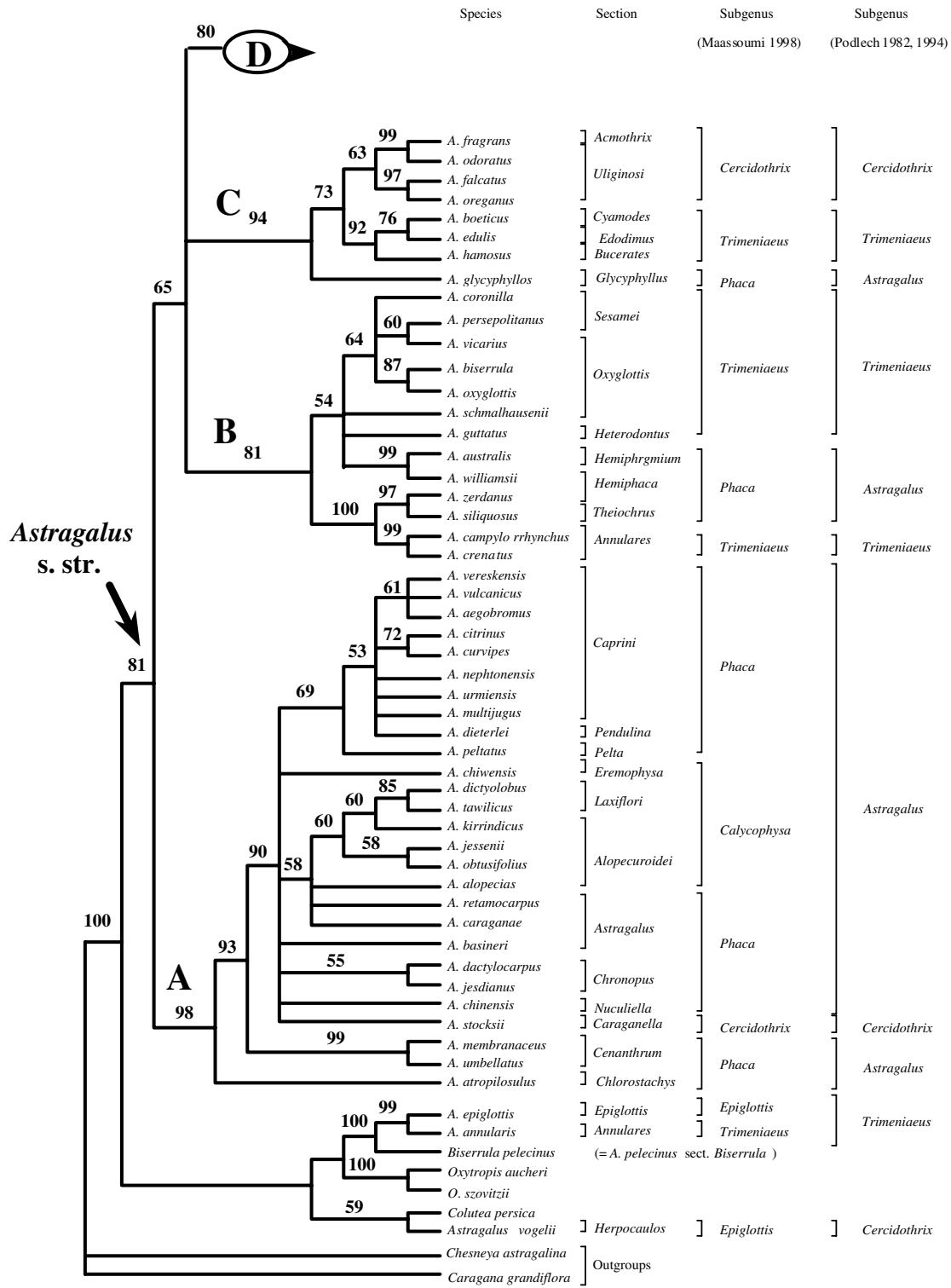
employed: One thousand replications of random addition sequence with tree bisection-reconnection (TBR) branch swapping were initiated, but no more than five of the shortest trees from each replication were saved. These equally most parsimonious trees were then used as starting trees for TBR branch swapping (with MulTrees and Steepest Descent selected). In all analyses, the maximum number of trees saved was set at 10000 and these trees were permitted to swap to completion. The strict consensus of these 10000 shortest trees was subsequently used as a topological constraint in another round of 1000 replications of random addition sequence. But, in this analysis, only those trees that did not fit the constraint tree were saved. No additional trees were found at the length of the initial 10000 trees, which suggests that the strict consensus tree adequately summarizes the available evidence, even though the exact number of trees at that length is not known. To obtain confidence limits for various clades, a bootstrap analysis (Felsenstein 1985) was conducted. Bootstrap values were calculated from 1000 replicate analyses, simple-addition sequence and TBR branch swapping with a set MAXTREES limit of 100 trees per bootstrap replicate. For both the *ndhF* and combined nrDNA ITS-*ndhF* data sets as well as for a reduced nrDNA ITS data set to 43 taxa, a finite number of shortest trees was obtained separately using the heuristic search option involving 100 replications of random addition sequence and TBR branch swapping (with MulTrees and Steepest Descent selected). Bootstrap values with 1000 replications were calculated using the heuristic search option, simple addition sequence and TBR branch swapping. Distance trees for both the nrDNA ITS and *ndhF* data sets were obtained using the neighbor-joining method (NJ; Saitou and Nei 1987) in PAUP*, using the two-parameter method of Kimura (1980). One thousand bootstrap replicates for each data set were completed.

Combinability of the nrDNA ITS and *ndhF* data sets was assessed using the permutation tail probability (PTP) test (Faith and Cranston 1991) to test for the presence or absence of phylogenetic signal and the partition homogeneity test (the incongruence length difference test of Farris et al. 1994) to test for incongruence between the data sets. These two tests were applied using the PAUP* version 4.0b4a. The partition homogeneity test was implemented with invariant characters excluded (Cunningham 1997) using TBR branch swapping with 1000 replicates. The maximum number of trees held in memory (MAXTREES) option was set to 100 to allow the test to proceed to completion.

Results

nrDNA ITS sequence data. The alignment of nrDNA ITS sequences for 136 *Astragalus* species/subspecies (including new segregates *Barnebyella* and *Ophiocarpus*) and for four species of 3 Astragalean genera plus two outgroups produced a matrix of 648 base pairs (bp) in length. Despite the presence of numerous indels which ranged in size from one to 34 nucleotides, we conservatively included only seven of these as additional characters in the nrDNA ITS data set (numbers 649–655 in the data set). There were 189 parsimony informative characters out of 655 total characters. The length of the nrDNA ITS region ranged from 567 bp in *Astragalus macrobotrys* and *A. squarrosus* (both of section *Ammodendron*) to 613 bp in *Chesneya astragalina* (an outgroup species). Pairwise sequence divergence values were generally less than 8% substitution per site across *Astragalus* as a whole. Divergence values between *Astragalus* and other Astragalean genera as well as outgroup taxa were typically under 13% substitution per site. The nrDNA ITS

Fig. 1. Strict consensus of 10000 most parsimonious trees resulting from phylogenetic analysis of 142 complete nrDNA ITS sequences for *Astragalus* and related genera (Length = 804 steps; CI = 0.547; RI = 0.831). Numbers above branches are bootstrap values for 1000 replicate analyses (with a set maxtrees limit of 100 trees per replicate); values < 50% are not indicated. Clades within *Astragalus* s. str. identified by letters (A–H) are discussed in the text. New World aneuploid *Astragalus* taxa (species, section and subgenus) are indicated by asterisk



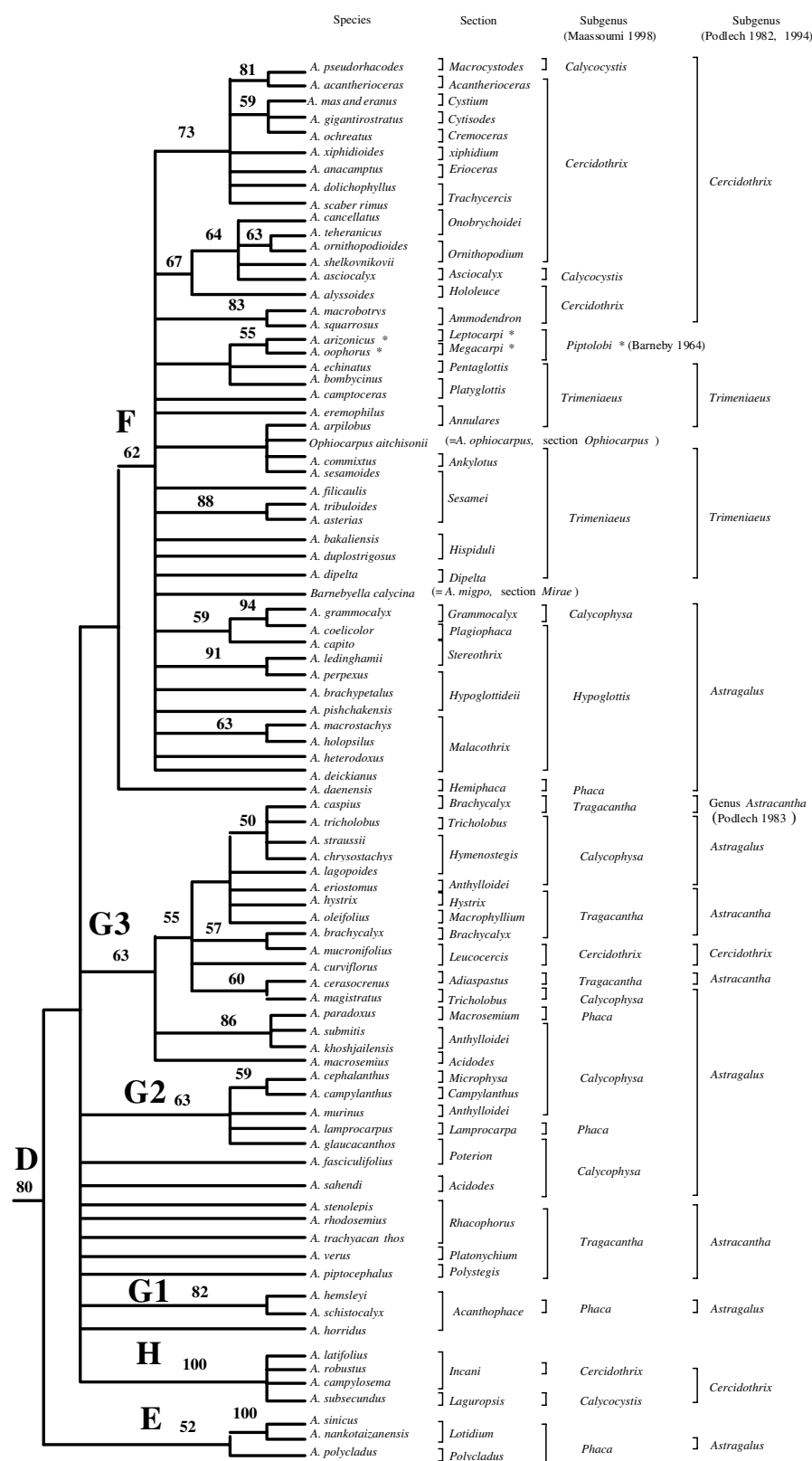


Fig. 1 (continued)

region was resequenced for the following taxa, in which, their sequences have minor differences relative to the published ones (Wojciechowski et al. 1999). *Oxytropis szovitsii*, *Astragalus vogelii*, *A. epiglottis*, *A. atropilosulus*, *A. alopecias*, *A. crenatus* (= *A. corrugatus*), *A. siliquosus*, *A. hamosus*, *A. boeticus*, *A. asterias*, *A. tribuloides*, and *A. echinatus*. These differences may be due to technical errors or to the existence of nucleotide variations within species analyzed. Nevertheless, we included these resequenced taxa in the data matrix rather than the published ones. Likewise, the nrDNA ITS sequences of *A. sinicus* and *A. cerasocrenus* were very different from those of the already published ones (Wojciechowski et al. 1999).

Maximum parsimony analysis of the full nrDNA ITS data set resulted in many thousands of equally parsimonious topologies. The strict consensus of 10000 of these trees, with accompanying bootstrap values, is presented in Fig. 1. These trees have a length of 804 steps, a consistency index (CI) of 0.547, and a retention index (RI) of 0.831. The neighbor-joining distance tree (Fig. 2) rendered, with few exceptions, a similar topology for phylogenetic relationships within and between *Astragalus* and its allies as the most parsimonious tree. Phylogenetic analyses of the nrDNA ITS data set by the two methods demonstrated that all *Astragalus* species sampled except three annuals, *A. vogelii*, *A. epiglottis* and *A. annularis*, belong to a well supported monophyletic group (with bootstrap values of 81 or 90%), so-called, *Astragalus* s. str. as recognized in previous studies (Sanderson and Wojciechowski 1996, Wojciechowski et al. 1999). Besides these species, three close relatives of the genus including *Oxytropis* (*O. szovitsii* and *O. aucheri*), *Colutea persica* and *Biserrula pelecinus* are located at the base of the trees as sister taxa to *Astragalus* s. str. *Biserrula pelecinus* together with two closely related species, *A. epiglottis* (sect. *Epiglottis*) and *A. annularis* (sect. *Annulares*) form a highly supported monophyletic group with bootstrap values of 99 or 100%. Likewise, *A. vogelii* (sect. *Herpocaulos*) forms a clade with *C. persica* (59 or 62% bootstrap).

Both MP strict consensus and NJ trees (Figs. 1 and 2) reveal that the *Astragalus* s. str. is composed of two large clades. One clade (hereafter called the clade “A”) is strongly supported (97 or 98% bootstrap) and contains representatives of perennial sections *Chlorostachys*, *Cenanthrum*, *Caraganella*, *Nuculiella*, *Chronopus*, *Astragalus*, *Alopecuroidei*, *Laxiflori*, *Eremophysa*, *Pelta*, *Pendulina* and *Caprini*. Section *Chlorostachys*, represented by east African *A. atropilosulus*, forms the most basal branch of this group. Next is section *Cenanthrum*, represented by *A. umbellatus* and *A. membranaceus*, which is in turn sister to the large core of the clade “A” (supported by bootstrap values of 82 or 90%).

The second clade is a larger assemblage that, in turn, comprises three well supported clades, namely, clade “B” (75 or 81%), clade “C” (90 or 94%), and clade “D” (73 or 80% bootstrap). The NJ analysis shows the clade “B” is followed, in order, by clades “C” and “D”. But, in the MP tree, the sister relationship among these clades collapsed into a trichotomy.

Clade “B” comprises representatives of annual sections *Annulares*, *Heterodonthus*, *Oxyglottis*, and *Sesamei* and of perennial sections *Theiochrus*, *Hemiphaca* and *Hemiphragmium*. Next is clade “C”, in which, section *Glycyphyllus*, represented by *A. glycyphyllus*, is sister to a highly supported small core clade (73 or 86% bootstrap). This core clade comprises annual sections *Bucerates*, *Cyamodes* and *Edodimus* as well as perennial sections *Uliginosi* and *Acmothrix*.

Clade “D” forms the largest core of *Astragalus* s. str., in which several smaller clades with weak to high bootstrap supports are apparent (labeled E–H in Figs. 1 and 2). Clade “E” represents a weakly (< 53% bootstrap) supported group of two east Asian sections *Lotidium* and *Polycladus* that is sister to all other members of clade “D”. Clade “F” is a weakly supported (62% bootstrap in MP) large group which is composed of weakly/well-supported subclades and sections/species belonging to the unsupported subclades

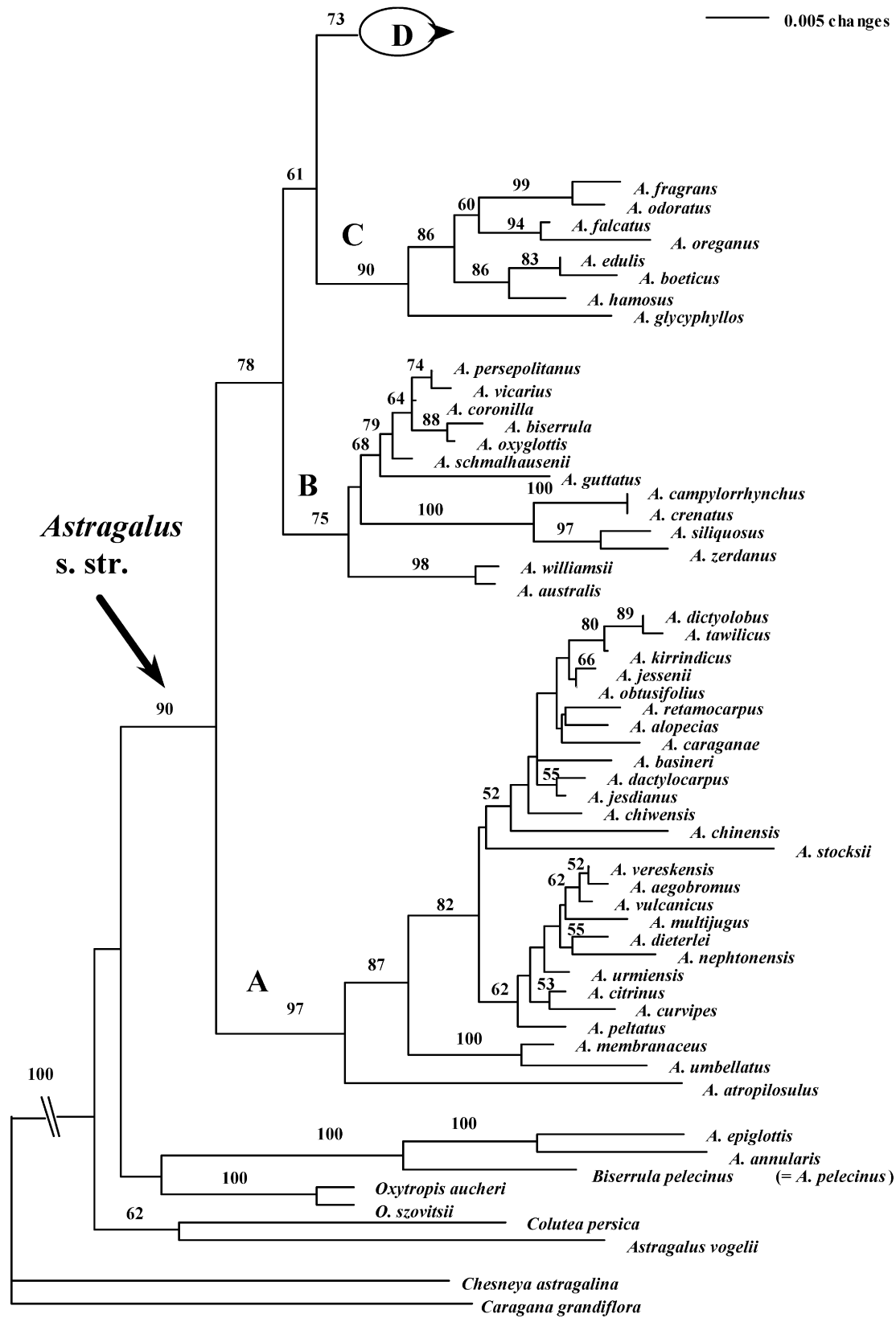
(< 50% bootstrap) or unresolved branches. The group “F” is also marked by a 7 bp deletion (except that the members of section *Ammodendron* have a long deletion of 34 bp) in ITS1. In both MP and NJ analyses, *A. daenensis* of section *Hemiphaca* is allied very weakly with this group (< 50% bootstrap support). In the MP tree, unlike in the NJ one, thorny cushion-forming taxa plus non-thorny perennial *A. paradoxus* altogether are not gathered in a single clade (“G”). Instead, they form several smaller clades (labeled G1–G3) and branches. Clade “H” is a strongly (100% bootstrap) supported group comprising sections of *Incani* and *Laguropsis* (*A. subsecundus*). This clade “H” is allied with all cushion-forming taxa (clade “G”) only in the NJ tree but unsupported (< 50% bootstrap).

***ndhF* sequence data.** Our *ndhF* sequences produced a matrix of 2103 bp in length. Three indels of 6 or 9 nucleotides were detected and accounted for minor length variation in our sequences. These indels were not used to construct the phylogenetic trees. There were 163 parsimony informative characters out of 2103 total characters in the *ndhF* data matrix. Pairwise sequence divergence values were generally less than 3% substitution per site across *Astragalus* as a whole. Divergence values between *Astragalus* and other Astragalean genera were typically lower than 5% substitution per site. MP analysis of the *ndhF* sequence data for 43 *Astragalus* and related taxa resulted in 180 most parsimonious trees. The strict consensus of these trees, each 503 steps in length, with a CI of 0.819 and an RI of 0.842, is shown in Fig. 3, with accompanying bootstrap values. With one exception, the NJ tree (not shown) was topologically identical to the MP strict consensus tree. The difference included the sister relationship of *A. horridus*-

A. schistocalyx clade with the remaining cushion-forming species (clade “G”). The *ndhF* data show all *Astragalus* species except *A. vogelii* are united in a single large clade that is weakly supported (with bootstrap value of 57%). *Colutea persica* and *Oxytropis aucheri* together with *A. vogelii* are placed at the base of the tree. This large assemblage of *Astragalus* comprises the following successive clades. The first one is a clade representing a sister relationship between *Ophiocarpus aitchisonii* and cushion-forming *A. hemsleyi* and three closely related species, *A. capito*, *A. coelicolor* and *A. grammocalyx*. This clade (called hereinafter *Ophiocarpus*-*A. grammocalyx* clade) is strongly supported (89% bootstrap), as is each of the respective subclades. The second is the *A. epiglottis*-*A. annularis* clade which is highly supported (100% bootstrap) and is sister to the large core of *Astragalus*. This large core forms a well supported monophyletic group (93% bootstrap) which comprises, in turn, two large clades. One large clade is composed of clade “A” (sections *Caraganella*, *Caprini*, *Alopecuroidei* and *Astragalus*) and clade “B” (sections *Theiochrus*, *Annulares* and *Oxyglottis*). Next large one is clade “D” which contains several polytomies including clades “C” (sections *Bucerates* and *Uliginosi*), “E” (section *Lotidium*), “F” (minus *A. echinatus* and *A. oophorus*), “G” (cushion-forming taxa) and “H” (section *Laguropsis*). The Mediterranean *A. echinatus* and the New World aneuploid *A. oophorus* unite as sister taxa (a highly supported clade) which in turn ally strongly with clade “G” (94% bootstrap support).

Combined nrDNA ITS-*ndhF* data. The PTP test was run on the full nrDNA ITS, the reduced ITS and *ndhF* data sets, and indicated that each data set had a significant phylogenetic structure ($P = 0.001$ for all data sets). The

Fig. 2. Neighbor-joining distance tree resulting from phylogenetic analysis of 142 complete nrDNA ITS sequences for *Astragalus* and related genera. Branch lengths are proportional to distances estimated from Kimura’s (1980) two-parameter method (note scale bar). Numbers above branches are bootstrap values for 1000 replicate analyses; values < 50% are not indicated. Clades within *Astragalus* s. str. identified by letters (A–H) are discussed in the text



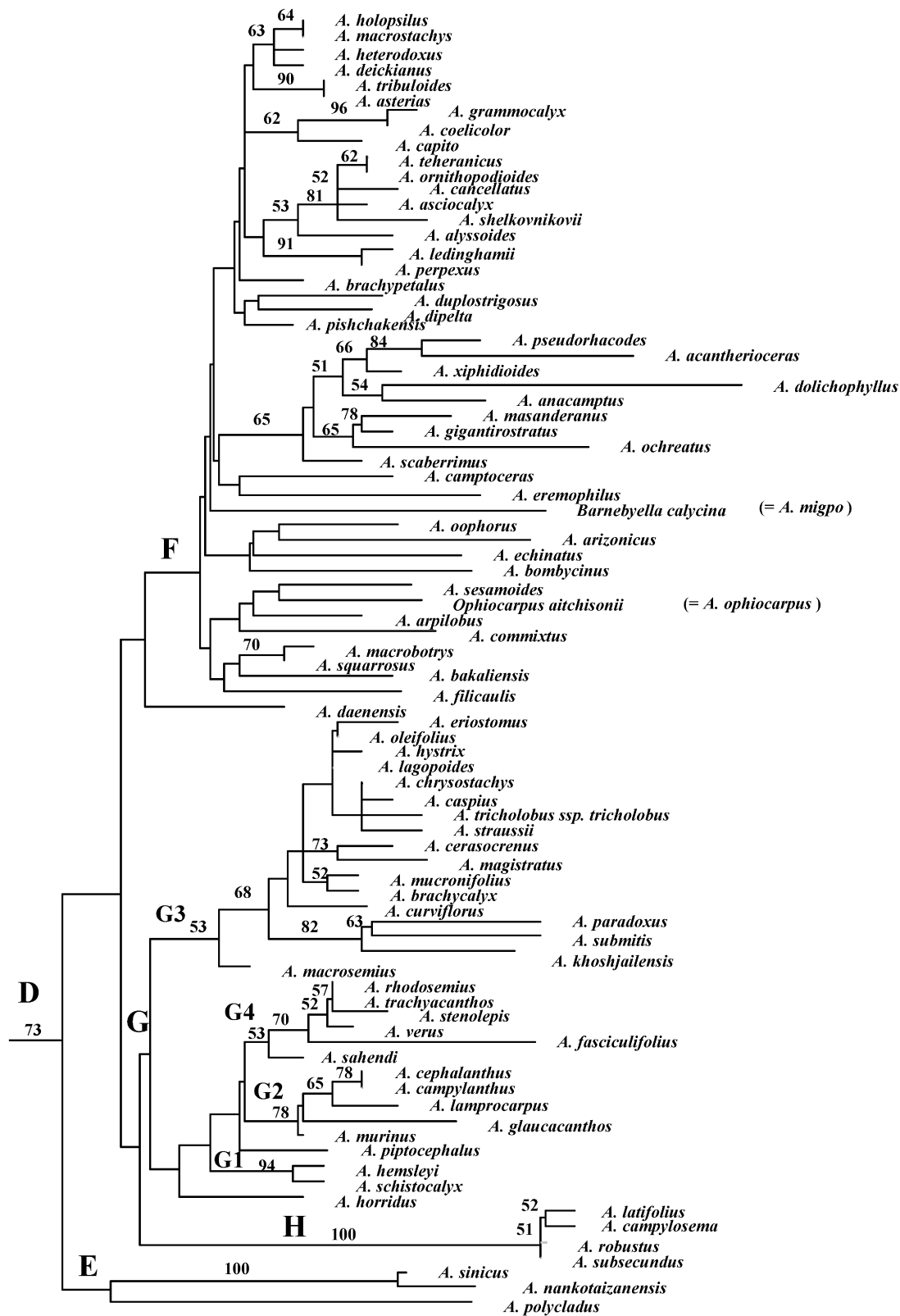


Fig. 2 (continued)

partition homogeneity test showed that the null hypothesis that the two data partitions (the reduced ITS and *ndhF* data sets) were homogeneous (not incongruent) was rejected ($P=0.001$). This result indicates statistically significant incongruence between the data sets (the reduced ITS and *ndhF* data sets). A comparison of Fig. 4A and Fig. 4B indicates substantial incongruence in the placements of some taxa, such as *Ophiocarpus aitchisonii*, *Astragalus hemsleyi*, *A. capito*, *A. coelicolor* and *A. grammocalyx* as well as *A. epiglottis* and *A. annularis*, but not wholesale conflict in all clades. Therefore, these taxa were not included in the analysis of combined nrDNA ITS-*ndhF* data set.

MP analysis of the aligned combined nrDNA ITS-*ndhF* sequences (2739 nucleotide positions) for 36 taxa of *Astragalus* and related genera resulted in 44 most parsimonious trees, each of 794 steps (CI=0.775; RI=0.780; Fig. 5). Once again, progressing upwards from the base, *Oxytropis* plus *Colutea* and *A. vogelii* are the sister group to all remaining *Astragalus* which corresponds to *Astragalus* s. str. in the nrDNA ITS tree (Figs. 1 and 2). In this large core, clade “A” (sections *Caraganella*, *Caprini*, *Alopecuroidei* and *Astragalus*) is followed successively by clade “B” (sections *Oxyglottis*, *Theiochrus* and *Annulares*), clade “C” (sections *Bucerates* and *Uliginosi*) and the large clade “D” [circumscribed by group “E” (section *Lotidium*) through group “H” (section *Lagupopsis*)].

Discussion

Discrepancy between nrDNA ITS and *ndhF* phylogenies. The molecular trees obtained in this study, based on nrDNA ITS and *ndhF* sequences, are topologically discordant. It has been known that a molecular tree does not necessarily agree completely with the actual evolutionary pathways of the taxa under study (Doyle 1992, 1997; Maddison 1997; Soltis and Kuzoff 1995). The most significant difference between the nrDNA ITS and *ndhF* phylogenies is the placement of *Ophiocarpus aitchisonii*, *A. hemsleyi*, *A. capito*, *A. coelicolor* and

A. grammocalyx. These taxa belong to three different clades derived in the nrDNA ITS phylogeny (Figs. 1, 2 and 4A) while they form the most basal clade (*Ophiocarpus*-*A. grammocalyx* clade) of *Astragalus* in the *ndhF* phylogeny (Figs. 3 and 4B). There are many biological processes, such as hybridization/introgression, lineage sorting, unequal rates of molecular evolution and long branch attraction that can lead to fallacious phylogenies (Rieseberg and Soltis 1991, Soltis and Kuzoff 1995, Wendel and Doyle 1998). This discrepancy between nuclear- and chloroplast-DNA based trees regarding the placement of these taxa can be the result of hybridization/introgression event and subsequent chloroplast capture long time ago. Hybridization and introgression are thought, however, to be rare to nonexistent among *Astragalus* species (Liston 1992, Sanderson and Doyle 1993, Judd et al. 1999). Parallelism in long branches (Felsenstein 1978) may afford a more plausible explanation for this discrepancy. The *ndhF* tree (Fig. 4B), unlike the nrDNA ITS one (Fig. 4A), obviously shows that *Ophiocarpus*-*A. grammocalyx* clade and each of its subclades have long branches possibly due to parallel nucleotide substitutions mistakenly identified as actual synapomorphy (Felsenstein 1978). Sequence data from nrDNA ITS put *Ophiocarpus* in a small unresolved clade with three annual species *A. arpilobus*, *A. commixtus* and *A. sesamoides* within the large clade “F” (Figs. 1 and 2). *ndhF* sequence data, however, ally *Ophiocarpus* strongly with *A. hemsleyi* of section *Acanthophace*. This *ndhF* result is not supported by morphological evidence. *Ophiocarpus* differs from *A. hemsleyi* in possessing: annual rather than woody perennial habit; longitudinally grooved rather than smooth stems; imparipinnate rather than paripinnate leaves; multiflorous (3–5 flowers) racemes rather than a laterally solitary flower; and linear/curved, unilocular rather than ovoid, bilocular pods (Maassoumi 1986, 1989; Podlech 1994). According to these features, *Ophiocarpus* is much closer to the annual species, as indicated by nrDNA ITS data

(Figs. 1 and 2), than to perennial *A. hemsleyi*. Interestingly, in both phylogenies, *A. grammocalyx*, *A. coelicolor* and *A. capito* are very closely related species.

Another substantial difference between the nrDNA ITS and *ndhF* phylogenies is the placement of a *A. epiglottis* and *A. annularis*. They are placed along with *Biserrula pelecinus* and *Oxytropis* (*O. aucheri* and *O. szovitsii*) outside the *Astragalus* s. str. clade in the nrDNA ITS tree (Figs. 1 and 2), whereas form the second deepest clade within *Astragalus* assemblage in the *ndhF* tree (Fig. 3). Previous studies (Liston and Wheeler 1994, Wojciechowski et al. 1999) both show *B. pelecinus* and *A. epiglottis* (*A. annularis* was not analyzed) on long branches near base of *Astragalus* clade.

The third conflict between the two gene trees is on the relative position of a pair species *A. echinatus* and *A. oophorus*. These two species, which are relatively distantly separated from thorny cushion-forming taxa (clade “G”) in the nrDNA ITS tree (Fig. 4A), become the sister group to them in the *ndhF* tree (Fig. 4B). Phylogenetic analyses of the two other chloroplast fragments, *rpoC* genes (Liston and Wheeler 1994) and *trnL* intron (Wojciechowski et al. 1999) indicated the same relationship as of the *ndhF*. At present, we can not judge which one of the above mentioned processes have caused these discrepancies. Additional nuclear genes are necessary to resolve all of these problems precisely. Excluding the most incompatible taxa (the five species from *Ophiocarpus* through *A. grammocalyx* plus *A. epiglottis* and *A. annularis*), the analysis of combined nrDNA ITS-*ndhF* data set provided a tree topology, which is more resolved than either of the separate analyses and, in general, greater bootstrap support for the most clades (see Fig. 5). With keeping these problems in mind, we present below the phylogenetic implications for *Astragalus* based upon both nrDNA ITS and *ndhF* as well as the combined sequence data.

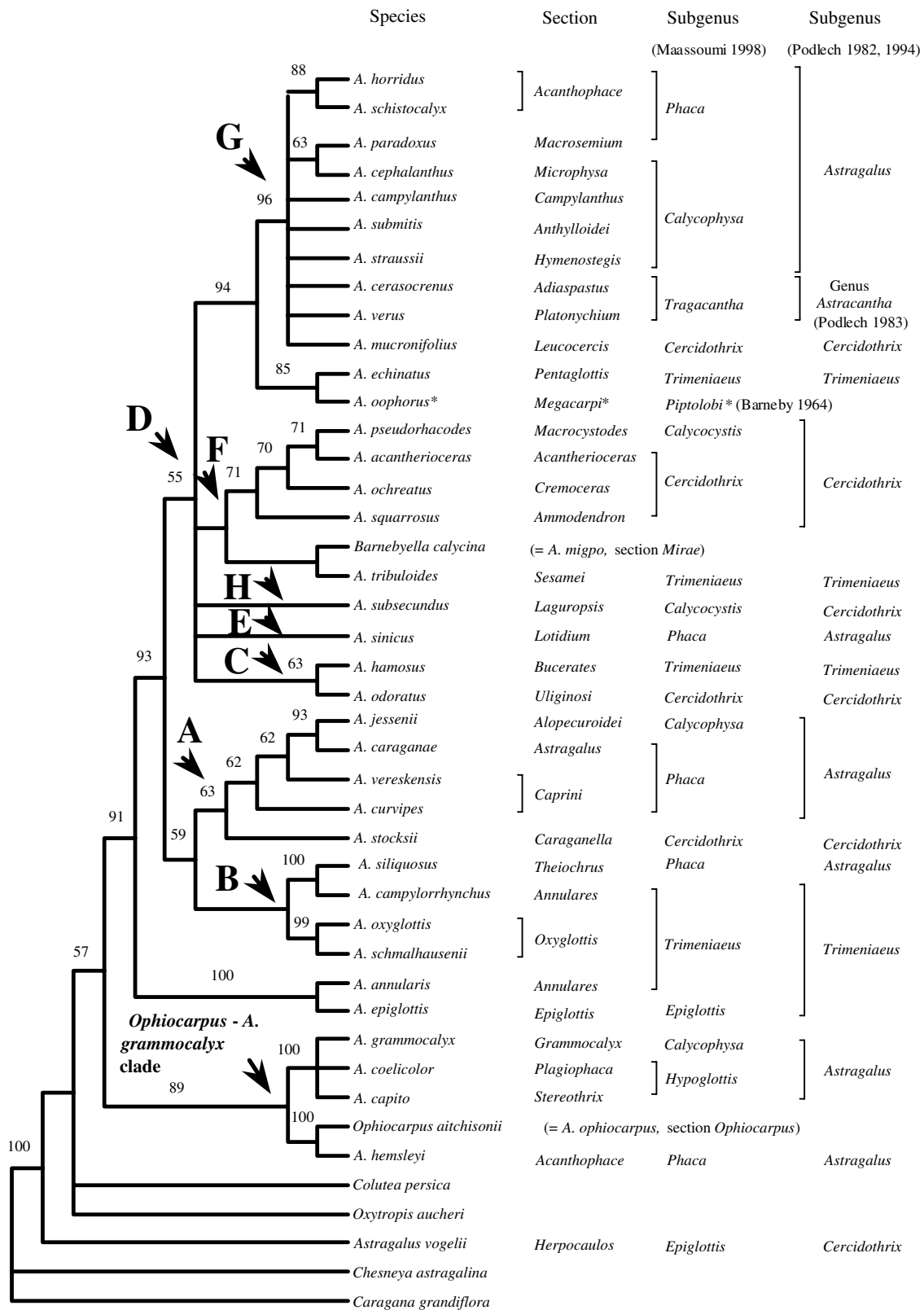
The phylogenetic position of problematic annual genera. As reviewed in the introduc-

tion, a number of monotypic and polytypic annual genera have been considered allied to, or part of *Astragalus*. Most notable of these are *Barnebyella*, *Ophiocarpus*, *Didymopelta* and *Sewerzowia*.

More recently, southwest Asian *A. migpo* (= *Dorycnium calycinum*) was moved to the new separate genus *Barnebyella* based on its elongated calyx in fruiting time and fruit features that are subglobular, laterally compressed, unilocular and one seeded (Podlech 1994). The genus *Dorycnium* was in fact treated in the tribe Loteae (Polhill 1981b). On the basis of these odd features, Podlech (1994) concluded that this species (*Barnebyella calycina*) is not related to *Astragalus* nor to *Dorycnium*. The latter taxon is more closely related to the genus *Lotus* (Allan and Porter 2000) which, in turn, has no close affinity with *Astragalus* (Hu et al. 2000). Our molecular analyses indicate that *B. calycina* is nested within *Astragalus*, and allied weakly with *A. tribuloides* (*ndhF* data), rejecting the treatment and idea of Podlech (1994).

The monotypic *Ophiocarpus* (*O. aitchisonii*) is another annual species widely distributed in southwest Asia. It is distinguished from all of *Astragalus* by its odd fruit morphology. The fruit is sessile, linear or arcuately curved, unilocular and constricted between the seeds. Based on these features, Podlech (1994) provisionally accepted the segregate genus *Ophiocarpus*, but stated that it required further study, such as using DNA analyses, to determine its affinities. As noted in previous part, both nrDNA ITS and *ndhF* sequence data place *Ophiocarpus* within *Astragalus*. Consequently, based on these molecular data, both *Barnebyella* and *Ophiocarpus* are suggested to be returned into *Astragalus* again.

Astragalus dipelta (= *Didymopelta turkestanica*) is an annual species distributed widely in central Asia through Afghanistan and Iran. This taxon is distinguished from other *Astragalus* species by a 2-seeded didymous pod on a filiform stipe. More recent authors (e.g. Hutchinson 1964) have nonetheless resurrected *Didymopelta* as a distinct genus. Our nrDNA ITS



phylogeny shows that this species like former segregates is nested among other *Astragalus* species within the large clade “F” (Figs. 1 and 2). It is allied with annual *A. duplostrigosus* of section *Hispiduli* only in the NJ tree.

Some recent authors (e.g. Hutchinson 1964, Rassulova 1978 cited in Podlech 1994) treated the three annual species *A. schmalhausonii*, *A. vicarius* and *A. compositus* as members of the segregate genus *Sewerzowia*. These species are characterized by particular fruit morphology including boat-shaped valves with spinous margins. Most recently, Podlech (1991, 1994) has placed these species in *Astragalus* section *Oxyglottis*. Significantly, our molecular data not only place two of which sampled here, *A. schmalhausonii* and *A. vicarius*, within *Astragalus*, but closely ally them with other relatives in sections *Oxyglottis* and *Sesamei*. *Astragalus schmalhausonii* and *A. vicarius* are morphologically very similar to each other, but in the nrDNA ITS tree, they did not unite as sister taxa (Figs. 1 and 2). The former species is allied moderately with *A. persepolitani* of section *Sesamei* within a clade containing *A. biserrula*, *A. oxyglottis* and *A. coronilla*, while *A. schmalhausonii* is sister to all of them (NJ tree, Fig. 2). An independent DNA fragment, such as the *ndhF* gene, is needed to evaluate these relationships.

Infrageneric relationships and classification. Relationships among the subgenera and sections of the Old World *Astragalus*, like that of the New World ones (Wojciechowski et al. 1999), are highly problematic. It is noteworthy, therefore, that results of our study, while with much greater sampling of the Old World taxa than previous studies (Wojciechowski et al. 1993, Wojciechowski et al. 1999), essentially came to the same conclusions as Wojciechowski et al. (1999) that the vast majority of

Astragalus species belong to one monophyletic group-*Astragalus* s. str. These phylogenies show that none of the traditionally recognized subgenera (Maassoumi 1998) in the genus are monophyletic. Our data convincingly demonstrate the absurdity of Podlech’s (1982, 1991, 1994) one-character taxonomy approach to the subgeneric classification (recognizing only three subgenera) in *Astragalus*-annual versus perennial, and basifixed versus medifixed hairs, and annuals evolving separate from and earlier than perennials (see Figs. 1, 2 and 3). In order to avoid confusion, below we will discuss our results only in the context of Maassoumi’s (1998) subgeneric classification.

I. subgenus Epiglottis

This is the smallest subgenus of *Astragalus* with only the two monotypic sections *Epiglottis* (*A. epiglottis*) and *Herpocaulos* (*A. vogelii*). Both nrDNA ITS and *ndhF* sequence data were obtained for these two species, and they did not emerge as sister taxa in all analyses. Instead, *A. epiglottis* is strongly supported (99/100% bootstrap) as sister to *A. annularis* of section *Annulares* (subgenus *Trimeniaeus*) along with *Biserrula pelecinus*. They are placed outside the *Astragalus* s. str. clade in nrDNA ITS tree (consistent with Wojciechowski et al. 1999), whereas form the second deepest clade within *Astragalus* assemblage in the *ndhF* tree (*B. pelecinus* is not sampled for *ndhF*). Previous studies (Liston and Wheeler 1994, Wojciechowski et al. 1999) both show *B. pelecinus* and *A. epiglottis* (*A. annularis* was not analyzed) on long branches near base of *Astragalus* clade. It would be premature to remove *A. epiglottis* and *A. annularis* from *Astragalus* before additional nuclear or chloroplast genes are sequenced. *Biserrula pelecinus* and these

Fig. 3. Strict consensus of 180 most parsimonious trees resulting from phylogenetic analysis of 43 *ndhF* sequences for *Astragalus* and related genera (Length = 503 steps; CI = 0.819; RI = 0.842). Numbers above branches are bootstrap values for 1000 replicate analyses; values < 50% are not indicated. Clades/branches identified by letters (A–H) are comparable with those outlined in Figs. 1 and 2. New World aneuploid *Astragalus* taxa (species, section and subgenus) are indicated by asterisk

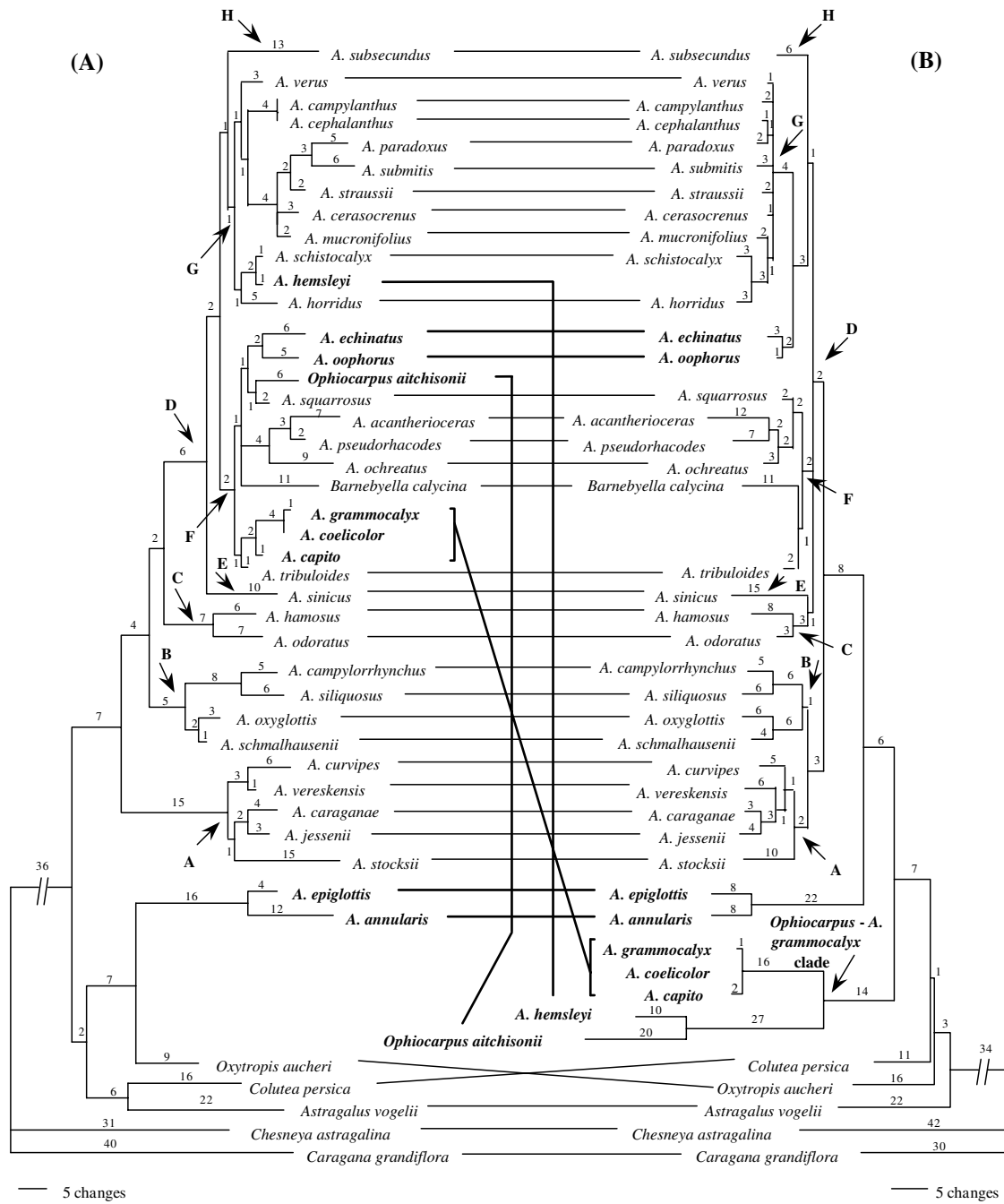


Fig. 4. Comparison of nrDNA ITS tree (A) with *ndhF* one (B) resulting from parsimony analysis of 43 sequences from each data set for *Astragalus* and related genera. The same taxa are connected by lines between the two trees. Taxa that are incongruent between the trees are marked by boldface and boldline. In each tree, branch lengths are proportional to the number of nucleotide substitutions (note scale bar). Clades/branches identified by letters (A–H) are comparable with those outlined in Figs. 1, 2 and 3

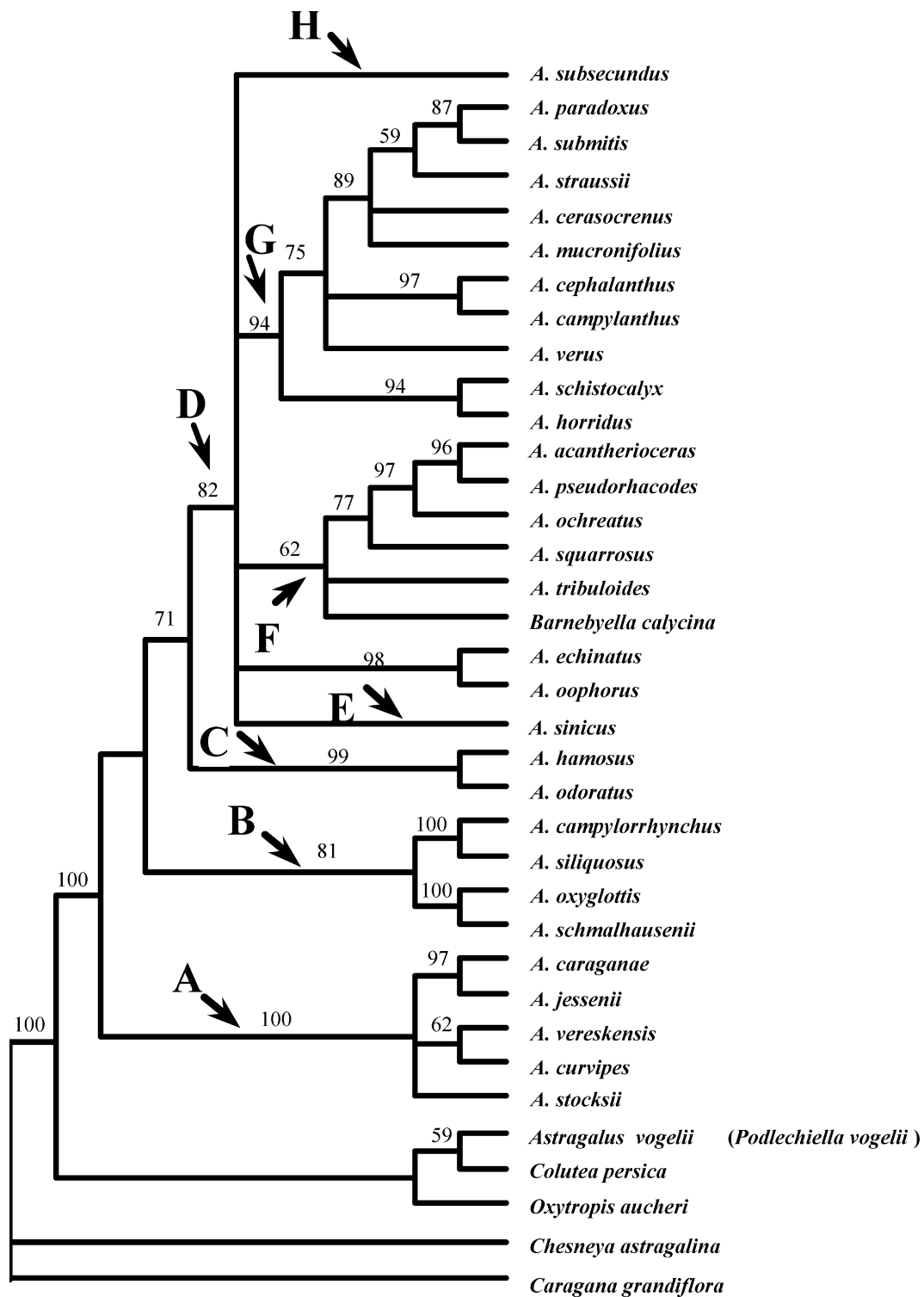


Fig. 5. Strict consensus of 44 most parsimonious trees resulting from phylogenetic analysis of 36 combined nrDNA ITS-*ndhF* sequences for *Astragalus* and related genera (Length = 794 steps; CI = 0.775; RI = 0.780). Numbers above branches are bootstrap values for 1000 replicate analyses; values < 50% are not indicated. Clades/branches identified by letters (A–H) are comparable with those outlined in Figs. 1, 2 and 3

two *Astragalus* species share several morphological features, including annual habit, dorsiventrally flattened pods and flowers with only five fertile anthers. Despite the great affinity, each of the two *Astragalus* species display substantial autapomorphic nucleotide substitutions (see Fig. 4), indicating that they diverged from a common ancestor long time ago. This pattern is correlated with morphology so that *A. epiglottis* is different from *A. annularis* in possessing several features including, asymmetrically medifixed hairs, dense capitate racemes and trigonose pods.

Astragalus vogelii, the other presumed member of subgenus *Epiglottis*, is allied with the genera *Colutea* and *Oxytropis* in a clade that forms the sister relationship to the remaining *Astragalus*. This situation has also been already detected by other workers (Liston and Wheeler 1994, Wojciechowski et al. 1999). Podlech (1984, 1991, 1999) has repeatedly suggested that *A. vogelii* has evolved from perennial *Astragalus* more recently. But, his idea is not supported by the molecular data. *Astragalus vogelii* is morphologically and ecologically isolated among the other annual species. It possesses a unique combination of medifixed hairs and unilocular fruits, and is the only *Astragalus* widely distributed in the subtropical deserts of north Africa and southwest Asia (Podlech 1984, 1999). Thus, on the basis of these morphological features and supported by the molecular data, we exclude this peculiar taxon from *Astragalus* and elevate it to a new generic rank named as *Podlechiella* Maassoumi and Kazempour Osaloo (see taxonomic treatment).

II. subgenus *Trimeniaeus*

This subgenus is also not monophyletic as currently circumscribed. It contains all remaining annual species (including *Biserrula pelecinus* = *A. pelecinus*) divided into 14 currently recognized sections. Twenty six representatives from 12 sections of the subgenus were included in nrDNA ITS study (see Figs. 1 and 2). Seven species from 5 sections of the subgenus were also analyzed for the *ndhF* gene. The results

show that these species are all scattered throughout the trees. Liston and Wheeler (1994) and Wojciechowski et al. (1993, 1999) using chloroplast *rpoC* gene restriction site data and nrDNA ITS sequences, respectively, also came to the same conclusion, although these authors sampled a more limited number (four to six) of species in this subgenus. Moreover, nrDNA ITS data shows that its species-rich sections such as *Annulares*, *Oxyglottis* and *Sesamoides* are not each monophyletic.

After a careful morphological examination of all Old World annual sections of *Astragalus*, Podlech (1991, 1998, 1999) has claimed that all except the monotypic sections *Herpocaulos* (*A. vogelii*) and *Cyamodes* (*A. boeticus*) are closely related and that they have a common origin. He postulated that they may have evolved very early from perennial ancestors, before the latter's separation within the Old World into groups with basifixed and medifixed hairs. However, all available molecular data clearly show that the annual *Astragali* form a very heterogeneous group and have evolved independently from various groups of *Astragalus* several times (see also Podlech 1982).

According to Podlech (1991), the monotypic section *Cyamodes* appears to be closely related to species of the perennial section *Glycyphyllus* (subgenus *Phaca*) rather than to any other annual sections. *Astragalus boeticus* shares several features with section *Glycyphyllus*, including a robust habit, free leafy stipules, loose racemes and linear leathery pods (Podlech 1991, 1999). In contrast, sequence data from nrDNA ITS indicate that *Cyamodes* and *Glycyphyllus* are not closest relatives, suggesting that these morphological features may be symplesiomorphic or homoplastic. Instead, *Cyamodes* is allied with the annual monotypic section *Edodimus* (*A. edulis*), and altogether in turn sister to *A. hamosus* of section *Bucerates*. Section *Glycyphyllus*, represented by *A. glycyphyllos*, as a member of clade "C", is sister to a core subclade containing these three annual sections plus the two perennial sections *Uliginosi* and *Acmothrix* (of subgenus *Cercidothrix*) (Figs. 1 and 2).

III. subgenus *Phaca*

The largest subgenus, *Phaca* (45 sections and 725 species), as currently circumscribed, is not a monophyletic group. This conclusion was first reached by Wojciechowski et al. (1999) and those authors went to great lengths in that article to discuss the relationship of *Phaca*, as originally circumscribed, to *Astragalus* and how clearly its' continued recognition is not longer tenable. Some of its members are highly derived and others basal within *Astragalus*. Several sections of the subgenus such as *Astragalus*, *Caprini*, *Pendulina*, *Pelta*, *Chronopus*, *Glycyphyllus*, *Acanthoplace*, *Lamprocarpa* and *Macrosemium* were analyzed for the first time in the present work. Of which, sections *Astragalus*, *Caprini*, *Pendulina*, *Pelta* and *Chronopus* are part of the large clade "A" that is sister to the rest of *Astragalus* s. str. (nrDNA ITS data). As discussed above, section *Glycyphyllus* is a member of clade "C". Thorny cushion-forming sections *Acanthoplace* and *Lamprocarpa* as well as nonthorny herbaceous section *Macrosemium* are members of the large clade "G" (NJ tree, Fig. 2).

The largest section, *Caprini*, is paraphyletic due to the inclusion of the trifoliolate *A. dieterlei* of section *Pendulina*. These two sections are morphologically similar to each other. Section *Caprini* comprises ca. 280 species divided into 4 subsections: *Caprini*, *Erionotus*, *Purpurascens* and *Gontscharoviella* (Podlech 1988, 1999). Although more extensive taxon sampling is needed to address intrasectional relationships of *Caprini*, it is noteworthy, that nrDNA ITS sequence data support the monophyly of subsection *Erionotus* (including *A. citrinus* and *A. curvipes*). Podlech (1986, 1988) has claimed that the huge section *Caprini* has close affinities to the sections *Astragalus*, *Chronopus* and *Aegacantha*. However, our nrDNA ITS data show *Caprini* together with *Pendulina* is closely related to the section *Pelta* (*A. peltatus*) rather than to loosely resolved sections *Astragalus* and *Chronopus*.

Acanthoplace, a small section of six species (Deml 1972), three of which were included

herein, is not monophyletic. In the nrDNA ITS tree, two species of the section, *A. hemsleyi* and *A. schistocalyx* are sister taxa, whereas *A. horridus* is not allied with them. And in the *ndhF* tree, *A. schistocalyx* and *A. horridus* are sister taxa, while, as discussed above, *A. hemsleyi* is allied with *Ophiocarpus aitchisonii* in the most basal clade of *Astragalus* assemblage. This is an unexpected result in that *Acanthoplace* is a small and morphologically uniform group whose monophyly has not been questioned (Deml 1972). The combined nrDNA ITS-*ndhF* data reveal that *A. horridus* and *A. schistocalyx* are allied strongly with the rest of thorny cushion-forming sections (clade "G" in Fig. 5). Among the thorny cushion-forming sections, only *Acanthoplace* and *Aegacantha* (not analyzed here) are characterized by semibilocular/bilocular, over two-seeded pods (Deml 1972, Maassoumi 1989). The present molecular phylogeny might suggest that this fruit type is a plesiomorphic character state for this section. A more likely scenario would be that section *Acanthoplace* have evolved earlier than any other thorny cushion-forming sections from their common ancestor.

The newly established section *Lamprocarpa* (*A. lamprocarpus*) is monotypic (Maassoumi 1994). This species is gross morphologically similar to members of *Acanthoplace* except in possessing ovoid inflated and unilocular pods. However, nrDNA ITS data put it in a clade ("G2") containing *A. glaucacanthos*, *A. murinus*, *A. cephalanthus* and *A. campylanthus*.

Section *Macrosemium* is small (two species), but is represented herein by only a single species, *A. paradoxus*; hence, the monophyly of it cannot be addressed. A phylogenetic relationship of section *Macrosemium* with the thorny cushion-forming sections and in particular with section *Anthylloidei* within the large clade "G" is unexpected. But it shares a unique feature with most of these taxa, including attachment of the claws of wing and keel petals to the staminal tube (Chamberlin and Matthews 1970, Maassoumi 1989).

Two representatives (*A. sinicus* and *A. nankotaizanensis*) of another section (*Lotidium*) from the subgenus *Phaca* were included in our molecular study. Previous *rpoC* and nrDNA ITS studies (Liston and Wheeler 1994, Sander-son and Wojciechowski 1996, Wojciechowski et al. 1999) revealed that *A. sinicus* was allied strongly with *A. complanatus* (section *Phyllolobium*) and placed outside the *Astragalus* s. str. clade. In contrast, our own nrDNA ITS and *ndhF* sequence data not only place *A. sinicus* within the *Astragalus* s. str. clade, but closely ally (in nrDNA ITS tree) it with *A. nankotaizanensis*. Luo et al. (2000), based on the RAPD markers, also indicated the genetic affinity of *A. sinicus* with two Taiwanese endemic *A. nankotaizenensis* and *A. nokoensis*. Therefore, the present results show that the voucher specimen for *A. sinicus*, from which DNA was extracted, might be misidentified by Liston and Wheeler (1994).

IV. subgenus *Tragacantha*

Subgenus *Tragacantha* (= genus *Astracantha* sensu Podlech 1983) contains exclusively thorny cushion-forming sections (11 sections and over 280 species) distributed primarily throughout southwest and south-central Asia (Podlech 1983; Maassoumi 1998, 2000). Zarre and Podlech (1997) following critical analysis of morphological features, concluded that this group is not monophyletic and many species of which have close relatives within other thorny sections of *Astragalus* (but see Zarre-Mobarakeh 2000). In a recent analysis of nrDNA ITS sequences for 115 *Astragalus* species, subgenus *Tragacantha* appeared monophyletic (Wojciechowski et al. 1999). However, they included only three species of the subgenus from two sections, *Adiaspastus* and *Rhachophorus*. Our broader analysis of nrDNA ITS sequences from the species representing 7 of 11 tragacanthic sections reveals that species of *Tragacantha* are scattered among the thorny cushion-forming species of subgenera *Calycophysa*, *Phaca* and *Cercidonthrix* in the large clade “G” (see Figs. 1 and 2). The analyses of both *ndhF* and combined nrDNA ITS-*ndhF* sequences data, even at the

limited taxon sampling, also show that these tragacanthic species do not form a clade (Figs. 3 and 5). Thus, our analyses clearly indicate that the subgenus *Tragacantha* is paraphyletic, supporting the finding of Zarre and Podlech (1997).

V. subgenus *Calycophysa*

The present molecular results also suggest that subgenus *Calycophysa* is polyphyletic. Its thorny cushion-forming and non-thorny herbaceous sections are nested respectively in the clade “G”, and clades “A” and “F” that are well differentiated and well separated phylogenetically. This finding has not been suggested by previous workers. The non-thorny herbaceous sections are *Alopecuroidei*, *Laxiflori*, *Eremophysa* and *Grammocalyx*. nrDNA ITS data suggest that the first three sections are nested in the large clade “A”. But *Grammocalyx*, represented here by *A. grammocalyx*, is allied with its closest taxa, *A. coelicolor* (of section *Plagiophaca*) and *A. capito* (of section *Stereothrix*) (both of subgenus *Hypoglottis*, see below) within the large clade “F”. As noted earlier, *ndhF* data also suggest that these three species are very closely related, but along with *Ophiocarpus aitchisonii* and *A. hemsleyi*, they form the most basal clade of *Astragalus* species.

Sections *Alopecuroidei*, and *Laxiflori* are paraphyletic and monophyletic respectively, but closely related taxa that are allied weakly with unresolved section *Astragalus* of subgenus *Phaca*. In contrast, monophyly of section *Eremophysa*, represented here by *A. chiwensis*, can not be addressed.

Thorny members of subgenus *Calycophysa* nested in the large clade “G”, are sections *Anthylloidei*, *Hymenostegis*, *Tricholobus*, *Acidodes*, *Poterion*, *Campylanthus* and *Microphysa*. Of which, none of the first five sections appear to be monophyletic in the nrDNA ITS tree. Section *Campylanthus* and *Microphysa* are sister taxa, but are represented herein by only a single species; hence, the monophyly of which cannot be evaluated.

In short, all the cushion-forming taxa are characterized by a suite of correlated morpho-

logical features with this habit, including paripinnate leaves, persistent spiny rachis, nearly sessile inflated calyces and ovoid unilocular, 1–2 seeded pods (except section *Acanthophaea*) (Podlech 1982, 1983; Chamberlin and Matthews 1970; Deml 1972; Maassoumi 1989, 1995, 2000). The molecular results, however, do not show that these features represent synapomorphies for cushion-forming taxa nested in clade “G”. First, there is no consistent and well supported clade in the figures that corresponds to clade “G” except in figure 5; second, all these taxa are not a clade themselves- due to the inclusion of the noncushion-forming herbaceous section *Macrosemium*.

VI. subgenus *Hypoglottis*

Ten species of subgenus *Hypoglottis* (sections *Plagiophaca*, *Malacothrix*, *Hypoglottidei* and *Stereothrix*.) were included in the present nrDNA ITS analysis, and do not form a single clade. Wojciechowski et al. (1999), including only three species from two sections of the subgenus, also suggested that this subgenus may not be monophyletic. Section *Plagiophaca* is monotypic. Only NJ analysis of nrDNA ITS data demonstrates that the section *Malacothrix* is a weakly (63%) supported monophyletic group which is allied with *A. asterias*-*A. tribuloides* clade. The nrDNA ITS data do not support monophyly of sections *Hypoglottidei* and *Stereothrix*. Of three species analyzed from the former section, *A. perpexus* is allied strongly with *A. ledinghamii* of section *Stereothrix*. The two other ones are unrelated taxa. The nrDNA ITS data suggest that delimitation of these sections, like many others, based upon morphological characters (Maassoumi 1989, Podlech 1986) is artificial and needs re-classification based on molecular phylogenetic results.

VII and VIII. subgenera *Cercidothrix* and *Calycocystis*

Podlech (1998) postulated that species with medifixed hairs (subgenera *Cercidothrix* and *Calycocystis* or Podlech’s (1982) new subgenus

Cercidothrix) form a natural group that was derived from species with basifixed hairs (subgenus *Astragalus* sensu Podlech 1982). However, both nrDNA ITS and *ndhF* phylogenies (see also Wojciechowski et al. 1999) indicate that subgenera *Cercidothrix* and *Calycocystis*, are polyphyletic. Members of the former subgenus form several distinct monophyletic groups or single branches throughout the molecular trees.

The species-rich section *Ammodendron* is monophyletic in the nrDNA ITS tree. The large section *Incarni* also appears to be a monophyletic group (see NJ tree in Fig. 2). Section *Uliginosi* is a paraphyletic group due to the inclusion of *A. fragrans* (of section *Acmothrix*). Within this group, the Eurasian *A. falcatus* unites with North American *A. oregonus* (and *A. canadensis* of section *Uliginosi*) rather than with Eurasian *A. odoratus*. Our finding is fully concordant with Barneby’s hypothesis (1964) that *A. falcatus* is not closely related to the other members of the Old World section *Uliginosi*.

Sections *Onobrychoidei* and *Ornithopodium* plus section *Asciocalyx* (of subgenus *Calycocystis*) form one clade in the nrDNA ITS tree, but species from the sections are intermixed. Sections *Trachycercis* and *Leucocercis* appear not to be monophyletic. Our molecular data in agreement with Boissier’s idea (1843) based on the importance of rachis thorns in grouping species, clearly indicate that section *Leucocercis* is related with other thorny cushion-forming taxa nested in clade “G” rather than with any medifixed hair herbaceous sections.

The remaining sections are small or large (ranging from three species for *Acantherioceras* and *Cremoceras* to 147 species for *Xiphidium*), but are represented herein by only a single species; hence, the monophyly of these sections can not be evaluated. Section *Caraganella* (subgenus *Cercidothrix*), represented here by *A. stocksii*, is morphologically (spiny shrub, short bell-shape calyx and long stipitate pods) similar to the relatives of *Astragalus* such as *Caragana*, *Chesneya* and *Lessertia*. It has been considered that the

section is a very ancient palaeoxeromorphic and isolated taxon which has no close relative within *Astragalus* (Podlech 1975, 1998). Both nrDNA ITS and in particular *ndhF* and the combined data show that *A. stocksii* is allied, however, strongly with sections *Caprini*, *Astragalus* (both of subgenus *Phaca*) and *Alopecuroidei* (subgenus *Calycophysa*) in clade "A".

Significantly, subgenus *Calycocyttis*, represented here with three species *A. pseudorhacodes*, *A. asciocalyx* and *A. subsecundus* (belonging to sections *Macrocystodes*, *Asciocalyx* and *Laguopsis* respectively) is well allied in three different clades with only members of subgenus *Cercidothrix*, indicating that members of these subgenera characterized by medifixed hairs are morphologically and genetically related.

In summary, based on the results of the phylogenetic analyses of molecular data presented here, the traditional subgenera of *Astragalus* recognized by Bunge (1868, 1869, 1880) and later modified by Maassoumi (1998), are clearly not monophyletic. Likewise, as mentioned above, our nrDNA ITS phylogeny clearly shows that many of the Old World *Astragalus* sections analyzed here are not monophyletic. Therefore, circumscription of those sections needs to be carried out in the light of the resulting molecular phylogenies and future works.

Taxonomic treatment

Podlechiella Maassoumi et Kazempour Osaloo, Gen. Nov.

Syn.: *Astragalus* sect. *Herpocaulos* Bunge, Mem. Acad. Imp. Sc. Petersb. 11 (16): 9 (1868).

Typus: *P. vogelii* (Webb) Maassoumi et Kazempour Osaloo, Genus monotypicum.

Diagnosis:

Planta annua, pilis asymmetricis medifixis vel partim subbasifixis mixtis. Legumen oblongum, valvis tenui-membranaceis, brevissime sub-asymmetricis medifixis et longe subbasifixis pilis vestitum, uniloculare.

Podlechiella vogelii (Webb) Maassoumi et Kazempour Osaloo, Comb. Nov.

Basionym: *Phaca vogelii* Webb, in Hooker, Niger Fl.: 123 (1848) et Icon. Plant. Tab. 763. (1848).

= *Astragalus vogelii* (Webb) Bornm., Beih. Bot. Centralbl. 33 (2): 233 (1915). Lectotypus: Maritime rocks, St. Antonio, one of the Cape de Verde Islands, *Vogel* 46 (K, non vidi). Lectotypification: D. Podlech, Mitt. Bot. Staatssamml. München 20: 444 [1984].

= *A. prolixus* Bunge, Mem. Acad. Imp. Sc. Petersb. 11 (16): 9 (1868) in clave et. L.c. 15 (1): 6 (1869). *Tragacantha prolixa* (Bunge) O. Kuntze, Revis. Gen. 947 (1891). *A. vogelii* subsp. *prolixus* (Bunge) Maire, Mem. Soc. Hist. Nat. Afr. Nord. 3: 126 (1933). Lectotypus: Aegypten, Wadi Gamuhs, *Sieber* (P, non vidi; iso-: FI, G, K, M, WU).

= *A. gautieri* Bat. & Trabut, Bull. Soc. Bot. France 53: 26 (1907). Typus: Oued silet, *Chudeau* (non vidi).

P. vogelii* subsp. *fatimensis (Chiov.) Maassoumi et Kazempour Osaloo, Comb. Nov.

Basionym: *Astragalus fatimensis* Chiov., Ann. Reale Ist. Bot. di. Roma 8:95 (1903).

= *A. arabicus* Bunge, Mem. Acad. Imp. Sc. Petersb. 11 (16): 9 (1868), in clave et. L.c. 15 (1): 6 (1869), nom illeg. non Kotschy (1866). *A. vogelii* subsp. *fatimensis* Maire, Mem. Soc. Hist. Nat. Afr. Nord. 3: 126 (1933). Lectotypus: Arabia, El Gidon, Jan. 1825, *Ehrenberg* (P, non vidi; iso-: K).

Note. We could not observe the type specimen of *Phaca vogelii* Webb (= *Astragalus vogelii* (Webb) Bornm. L.). Instead, we saw the description and illustration of this type specimen in both Hooker's Niger Flora: 123 (1848) and Icones Plantarum Tab. 763 (1848). In addition, the type of the species and its subspecies as well as their synonyms were carefully examined by Podlech (1984, 1999). Therefore, we are sure that all specimens belong to *Podlechiella vogelii*. This new genus was named in the honour of Prof. Dr. D. Podlech, who contributed greatly to the taxonomy of the Old World *Astragali*.

Appreciation is gratefully extended to Drs. H. Tobe, M. Hakki and H. Nagamasu for providing

Appendix 1. Taxa included in nrDNA ITS and chloroplast gene *ndhF* phylogenetic analyses

Species	Section ^a	Subgenus/ Phalanx ^a	Origin, Voucher accession and herbarium ^b	DDBJ accession numbers ITS	<i>ndhF</i>
<i>Astragalus epiglottis</i> L.	<i>Epiglottis</i>	<i>Epiglottis</i>	Morocco; Podlech 45851 (TARI)	AB051910	AB052042
<i>A. vogelii</i> (Webb.) Bornm.	<i>Herpocaulos</i>	<i>Epiglottis</i>	Iran; Mozaf. et al. 39103 (TARI)	AB051911	AB052041
<i>A. commixtus</i> Bunge	<i>Ankylotus</i>	<i>Trimeniaeus</i>	Iran; Assadi & Maass. 55701 (TARI)	AB051925	
<i>A. annularis</i> Forsskal	<i>Annulares</i>	<i>Trimeniaeus</i>	Iran; Maass. & Abou. 51921 (TARI)	AB051912	AB052043
<i>A. arpilobus</i> Kar. & Kir.	<i>Annulares</i>	<i>Trimeniaeus</i>	Iran; Freitag & Mozaf. 28435 (TARI)	AB051913	
<i>A. campylorrhynchus</i> Fischer	<i>Annulares</i>	<i>Trimeniaeus</i>	Iran; Maass. 47561 (TARI)	AB051914	AB052048
<i>A. crenatus</i> Schultes	<i>Annulares</i>	<i>Trimeniaeus</i>	Iran; Foroughi 55 (TARI)	AB051915	
<i>A. eremophilus</i> Boiss.	<i>Annulares</i>	<i>Trimeniaeus</i>	Iran; Maass. & Abou. 52028 (TARI)	AB051916	
<i>A. edulis</i> Dur. ex Bunge	<i>Edodimus</i>	<i>Trimeniaeus</i>	Israel; USDA 244273	AF121677 ^c	
<i>A. hamosus</i> L.	<i>Bucerates</i>	<i>Trimeniaeus</i>	Iran; Maass. 47586 (TARI)	AB051936	AB052055
<i>A. boeticus</i> L.	<i>Cyanodes</i>	<i>Trimeniaeus</i>	Iran; Maass. & Abou. 51949 (TARI)	AB051937	
<i>A. dipelta</i> Bunge	<i>Dipelta</i>	<i>Trimeniaeus</i>	Iran; Assadi & Maass. 50172 (TARI)	AB051926	
(syn. <i>Didymopelta turkestanica</i> Regel & Schmalh.)					
<i>A. guttatus</i> Banks & Soland	<i>Heterodontus</i>	<i>Trimeniaeus</i>	Iran; Maass. & Abou. 56984 (TARI)	AB051935	
<i>A. bakaliensis</i> Bunge	<i>Hispiduli</i>	<i>Trimeniaeus</i>	Iran; Bonvan 9922 (TARI)	AB051924	
<i>A. duplostrigosus</i> Post & Beauverd	<i>Hispiduli</i>	<i>Trimeniaeus</i>	Iran; Bokhari & Wendelbo 113 (TARI)	AB051923	
<i>A. biserrula</i> Bunge	<i>Oxyglottis</i>	<i>Trimeniaeus</i>	Iran; Wendelbo & Assadi 28021 (TARI)	AB051931	
<i>A. oxyglottis</i> Bieb.	<i>Oxyglottis</i>	<i>Trimeniaeus</i>	Iran; Maass. & Abou. 52079 (TARI)	AB051932	AB052045
<i>A. schmalhauseni</i> Bunge (syn. <i>Sewerzowia turkestanica</i> Regel & Schmalh.)	<i>Oxyglottis</i>	<i>Trimeniaeus</i>	Iran; Maass. 55146 (TARI)	AB051933	AB052046

Appendix 1 (continued)

Species	Section ^a	Subgenus/ Phalanx ^a	Origin, Voucher accession and herbarium ^b	DDBJ accession numbers ITS	<i>ndhF</i>
<i>A. vicarius</i> Lipsky (syn. <i>Sewerzowia</i> <i>vicaria</i> (Lipsky) Rassulova)	<i>Oxyglottis</i>	<i>Trimeniaeus</i>	Iran; Maass. 47570 (TARI)	AB051934	
<i>A. echinatus</i> Murray	<i>Pentaglottis</i>	<i>Trimeniaeus</i>	Morocco; Podlech 46718 (TARI)	AB051938	AB052062
<i>A. bombycinus</i> Boiss.	<i>Platyglottis</i>	<i>Trimeniaeus</i>	Iran; Babakhanlou & Amin 15422 (TARI)	AB051929	
<i>A. camptoceras</i> Bunge	<i>Platyglottis</i>	<i>Trimeniaeus</i>	Iran; Maass. 47576 (TARI)	AB051930	
<i>A. asterias</i> Hohen.	<i>Sesamei</i>	<i>Trimeniaeus</i>	Iran; Runemark & Mozaf. 30957 (TARI)	AB051917	
<i>A. coronilla</i> Bunge	<i>Sesamei</i>	<i>Trimeniaeus</i>	Iran; Assadi & Maass. 55895 (TARI)	AB051918	
<i>A. filiculis</i> Kar. & Kir.	<i>Sesamei</i>	<i>Trimeniaeus</i>	Iran; Assadi & Maass. 50762 (TARI)	AB051919	
<i>A. persepoltanus</i> Boiss.	<i>Sesamei</i>	<i>Trimeniaeus</i>	Iran; Foroughi & Assadi 17897 (TARI)	AB051920	
<i>A. sesamoides</i> Boiss.	<i>Sesamei</i>	<i>Trimeniaeus</i>	Iran; Assadi & Maass. 50670 (TARI)	AB051921	
<i>A. tribuloides</i> Delile	<i>Sesamei</i>	<i>Trimeniaeus</i>	Iran; Maass. & Abou. 52003 (TARI)	AB051922	AB052057
<i>A. brachypetalus</i> Trautv.	<i>Hypoglottidei</i>	<i>Hypoglottis</i>	Iran; Wendelbo & Foroughi 12655 (TARI)	AB051999	
<i>A. perpexus</i> Maassoumi	<i>Hypoglottidei</i>	<i>Hypoglottis</i>	Iran; Mozaf. 59969 (TARI)	AB051998	
<i>A. pishchakensis</i> Maassoumi	<i>Hypoglottidei</i>	<i>Hypoglottis</i>	Iran; Mozaf. 27388 (TARI)	AB052000	
<i>A. deickianus</i> Bornm.	<i>Malacothrix</i>	<i>Hypoglottis</i>	Iran; Maass. & Mirhosseini 59381 (TARI)	AB051992	
<i>A. heterodoxus</i> Bunge	<i>Malacothrix</i>	<i>Hypoglottis</i>	Iran; Assadi & Bazgosha 56102 (TARI)	AB051991	
<i>A. holopsilus</i> Bunge	<i>Malacothrix</i>	<i>Hypoglottis</i>	Iran; Mozaf. 54347 (TARI)	AB051989	
<i>A. macrostachys</i> DC.	<i>Malacothrix</i>	<i>Hypoglottis</i>	Iran; Maass. & Abou. 56992 (TARI)	AB051990	
<i>A. coelicolor</i> Sirj. & Rech. f.	<i>Plagiophaca</i>	<i>Hypoglottis</i>	Iran; Wendelbo & Assadi 29725 (TARI)	AB051995	AB052076
<i>A. capito</i> Boiss.	<i>Stereothix</i>	<i>Hypoglottis</i>	Iran; Foroughi 2913 (TARI)	AB051996	AB052075
<i>A. ledinghamii</i> Barneby	<i>Stereothix</i>	<i>Hypoglottis</i>	Iran; Mozaf. 44676 (TARI)	AB051997	
<i>A. hensleyi</i> Aitch. & Baker	<i>Acanthophaea</i>	<i>Phaca</i>	Iran; Zarre 69578 (TARI)	AB052003	AB052064
<i>A. horridus</i> Boiss.	<i>Acanthophaea</i>	<i>Phaca</i>	Iran; Mozaf. 54874 (TARI)	AB052002	AB052065
<i>A. schistocalyx</i> Bunge	<i>Acanthophaea</i>	<i>Phaca</i>	Iran; Assadi & Maass. 21256 (TARI)	AB052004	AB052066
<i>A. basineri</i> Trautv.	<i>Astragalus</i>	<i>Phaca</i>	Iran; Assadi & Maass. 50259 (TARI)	AB051943	
<i>A. caraganae</i> Fisch. & Mey.	<i>Astragalus</i>	<i>Phaca</i>	Iran; Mozaf. & Maass. 48076 (TARI)	AB051942	AB052052
<i>A. retamocarpus</i> Boiss. & Hohen.	<i>Astragalus</i>	<i>Phaca</i>	Iran; Maass. 55136 (TARI)	AB051944	

Appendix 1 (continued)

<i>A. aegobromus</i> Boiss. & Hohen.	Caprini	<i>Phaca</i>	Iran; Maass. 55116 (TARI)	AB051953
<i>A. citrinus</i> Bunge	Caprini	<i>Phaca</i>	Iran; Maass. 47586 (TARI)	AB051954
<i>A. curvipes</i> Trautv.	Caprini	<i>Phaca</i>	Iran; Maass. 47553 (TARI)	AB051955
<i>A. multijugus</i> DC.	Caprini	<i>Phaca</i>	Iran; Mozaf. & Maass. 47957 (TARI)	AB051956
<i>A. neptonensis</i> Freyn	Caprini	<i>Phaca</i>	Iran; Maass. 55006 (TARI)	AB051957
<i>A. urniensis</i> Bunge	Caprini	<i>Phaca</i>	Iran; Maass. 55137 (TARI)	AB051958
<i>A. vereskensis</i> Maassoumi & Podl.	Caprini	<i>Phaca</i>	Iran; Maass. 55016 (TARI)	AB051959
<i>A. vulcanicus</i> Bornm.	Caprini	<i>Phaca</i>	Iran; Maass. 55134 (TARI)	AB051960
<i>A. membranaceus</i> Bunge	Cenanthrum	<i>Phaca</i>	China; Hu 1131	AF121675 ^c
<i>A. umbellatus</i> Bunge	Cenanthrum	<i>Phaca</i>	USA; Parker 88-78	AF121683 ^c
<i>A. atropilosulus</i> (Hochst.) Bunge	Chlorostachys	<i>Phaca</i>	Ethiopia; Yamashita et al. 1068 (KYO)	AB051939
<i>A. dactylocarpus</i> Boiss.	Chronopus	<i>Phaca</i>	Iran; Freitag & Mozaf. 28506 (TARI)	AB051945
<i>A. jesdianus</i> Boiss. & Buhse	Chronopus	<i>Phaca</i>	Iran; Assadi 23236 (TARI)	AB051946
<i>A. glycyphyllos</i> L.	Glycyphyllos	<i>Phaca</i>	Iran; Assadi & Sardabi 24090 (TARI)	AB051941
<i>A. daenensis</i> Boiss.	Hemiphaca	<i>Phaca</i>	Iran; Foroughi & Assadi 18019 (TARI)	AB051963
<i>A. williamsii</i> Rydb.	Hemiphaca	<i>Phaca</i>	Canada; Calder & Gillett 25825	AF121685 ^c
<i>A. zerdanus</i> Boiss.	Hemiphaca	<i>Phaca</i>	Iran; Assadi & Abou. 46167 (TARI)	AB051964
<i>A. australis</i> (L.) Lam.	Hemiphragmium	<i>Phaca</i>	USA; Tiehm & Williams 11985	AF121686 ^c
<i>A. lamprocarpus</i> Maassoumi	Lamprocarpa	<i>Phaca</i>	Iran; Runemark & Lazari 26506 (TARI)	AB052015
<i>A. sinicus</i> L.	Lotidium	<i>Phaca</i>	Japan; Kazempour Osaloo 1999-01 (TARI)	AB051965
<i>A. nankotaiensis</i> Sasaki	Lotidium	<i>Phaca</i>	Taiwan; Hu 1062	AF121680 ^c
<i>A. paradoxis</i> Bunge	Macrosemium	<i>Phaca</i>	Iran; Wendelbo & Assadi 19281 (TARI)	AB052001
<i>A. chinensis</i> L.	Nuculiella	<i>Phaca</i>	Switzerland; USDA 415802	AF121681 ^c
<i>A. peltatus</i> Podl. & Deml	Pelta	<i>Phaca</i>	Afghanistan; Rechinger 37517 (TARI)	AB052034
<i>A. dieterlei</i> Podl.	Pendulina	<i>Phaca</i>	Iran; Mirtajaddini 19500b (TARI)	AB051961
<i>A. polycladus</i> Bureau & Franchet	Polycladus	<i>Phaca</i>	China; Donoghue 094 (1996)	AF121676 ^c
				AB052050
				AB052074
				AB052053

Appendix 1 (continued)

Species	Section ^a	Subgenus/ Phalanx ^a	Origin, Voucher accession and herbarium ^b	DDBJ accession numbers ITS	ndhF
<i>A. siliquosus</i> Boiss.	<i>Theiochrus</i>	<i>Phaca</i>	Iran; Assadi & Maass. 50465 (TARI)	AB051940	AB052047
<i>A. macrosemius</i> Boiss & Hohen.	<i>Acidodes</i>	<i>Calycophysa</i>	Iran; Wendelbo & Cobham 14495 (TARI)	AB052030	
<i>A. sahendi</i> Buhse	<i>Acidodes</i>	<i>Calycophysa</i>	Iran; Mozaf. 69854 (TARI)	AB052029	
<i>A. alopecias</i> Pallas	<i>Alopecuroidei</i>	<i>Calycophysa</i>	Iran; Assadi & Mozaf. 35930 (TARI)	AB051949	
<i>A. jessenii</i> Bunge	<i>Alopecuroidei</i>	<i>Calycophysa</i>	Iran; Mozaf. & Maass. 48062 (TARI)	AB051950	AB052051
<i>A. kirrindicus</i> Boiss.	<i>Alopecuroidei</i>	<i>Calycophysa</i>	Iran; Maass. 55130 (TARI)	AB051951	
<i>A. obtusifolius</i> DC.	<i>Alopecuroidei</i>	<i>Calycophysa</i>	Iran; Maass. & Abou. 52015 (TARI)	AB051952	
<i>A. eriostomus</i> Bornm.	<i>Anthylloidei</i>	<i>Calycophysa</i>	Iran; Mozaf. 63794 (TARI)	AB052007	
<i>A. khoshjailensis</i> Sirj. & Rech. f.	<i>Anthylloidei</i>	<i>Calycophysa</i>	Iran; Maass. 47580 (TARI)	AB052010	
<i>A. murinus</i> Boiss.	<i>Anthylloidei</i>	<i>Calycophysa</i>	Iran; Assadi & Abou. 46094 (TARI)	AB052008	
<i>A. submits</i> Boiss. & Hohen.	<i>Anthylloidei</i>	<i>Calycophysa</i>	Iran; Mozaf. & Maass. 47960 (TARI)	AB052009	AB052068
<i>A. campylanthus</i> Boiss.	<i>Campylanthus</i>	<i>Calycophysa</i>	Iran; Mozaf. & Maass. 47790 (TARI)	AB052028	AB052069
<i>A. chiwensis</i> Bunge	<i>Erenophysa</i>	<i>Calycophysa</i>	Iran; Freitag & Jadidi 29007 (TARI)	AB051962	
<i>A. grammocalyx</i> Boiss & Hohen.	<i>Grammocalyx</i>	<i>Calycophysa</i>	Iran; Maass. 55123 (TARI)	AB051994	AB052077
<i>A. chrysostachys</i> Boiss.	<i>Hymenostegis</i>	<i>Calycophysa</i>	Iran; Mozaf. & Nowruzi 34108 (TARI)	AB052011	
<i>A. lagopoides</i> Lam.	<i>Hymenostegis</i>	<i>Calycophysa</i>	Iran; Assadi & Olfat 68825 (TARI)	AB052013	
<i>A. straussii</i> Bornm.	<i>Hymenostegis</i>	<i>Calycophysa</i>	Iran; Mozaf. & Maass. 47793 (TARI)	AB052012	AB052072
<i>A. dictyolobus</i> Bunge	<i>Laxiflori</i>	<i>Calycophysa</i>	Iran; Mozaf. 69963 (TARI)	AB051947	
<i>A. tawilicus</i> Townsend	<i>Laxiflori</i>	<i>Calycophysa</i>	Iran; Maass. 59351 (TARI)	AB051948	
<i>A. cephalanthus</i> DC.	<i>Microphysa</i>	<i>Calycophysa</i>	Iran; Mozaf. & Maass. 47788 (TARI)	AB052027	AB052070
<i>A. fasciculifolius</i> Boiss.	<i>Poterion</i>	<i>Calycophysa</i>	Iran; Mozaf. 49867 (TARI)	AB052016	
<i>A. glaucacanthos</i> Fischer	<i>Poterion</i>	<i>Calycophysa</i>	Iran; Assadi et al. 33356 (TARI)	AB052017	
<i>A. magistratus</i> Maass. et al.	<i>Tricholobus</i>	<i>Calycophysa</i>	Iran; Assadi & Mozaf. 35244 (TARI)	AB052032	
<i>A. tricholobus</i> ssp. <i>tricholobus</i> emend. Tietz	<i>Tricholobus</i>	<i>Calycophysa</i>	Iran; Mozaf. & Nowroozi 34005 (TARI)	AB052031	
<i>A. cerasocrenus</i> Bunge (syn. <i>Astracantha</i> <i>cerasocrena</i> (Bge.) Podl.)	<i>Adiaspastus</i>	<i>Tragacantha</i>	Iran; Assadi & Maass. 50846 (TARI)	AB052022	AB052071

Appendix 1 (continued)

<i>A. brachycalyx</i> Fischer (syn. <i>Astracantha brachycalyx</i> (Fisch.) Podl.)	<i>Brachycalyx</i>	<i>Tragacantha</i>	Iran; Assadi & Mozaf. 37096 (TARI)	AB052026
<i>A. caspius</i> Bieb. (syn. <i>Astracantha</i> <i>caspius</i> (Bieb.) Podl.)	<i>Brachycalyx</i>	<i>Tragacantha</i>	Iran; Mozaf. & Maass. 48081 (TARI)	AB052025
<i>A. hystrix</i> Bunge	<i>Hystrix</i>	<i>Tragacantha</i>	Iran; Maass. & Mozaf. 78604 (TARI)	AB052014
<i>A. oleifolius</i> DC. (syn. <i>Astracantha oleifolia</i> (DC.) Podl.)	<i>Macrophyllum</i>	<i>Tragacantha</i>	Iran; Maass. & Mozaf. 79612 (TARI)	AB052019
<i>A. verus</i> Olivier	<i>Platonychium</i>	<i>Tragacantha</i>	Iran; Mozaf. & Maass. 47797 (TARI)	AB052023
<i>A. ptiotocephalus</i> Boiss.	<i>Polystegis</i>	<i>Tragacantha</i>	Iran; Maass. & Mozaf. 76763 (TARI)	AB052018
<i>A. stenolepis</i> Fischer	<i>Rhacophorus</i>	<i>Tragacantha</i>	Iran; Maass. 55128 (TARI)	AB052021
<i>A. trachyacanthos</i> Fischer	<i>Rhacophorus</i>	<i>Tragacantha</i>	Iran; Mozaf. & Maass. 47962 (TARI)	AB052024
<i>A. rhodosemius</i> Boiss. & Hausskn.	<i>Rhacophorus</i>	<i>Tragacantha</i>	Iran; Maass. & Mozaf. 78702 (TARI)	AB052020
<i>A. acantherioceras</i> Rech. f. & Koie	<i>Acantherioceras</i>	<i>Cercidothrix</i>	Iran; Mozaf. 48627 (TARI)	AB051977
<i>A. fragrans</i> Willd.	<i>Acnothrix</i>	<i>Cercidothrix</i>	Iran; Maass. & Abou. 56916 (TARI)	AB051967
<i>A. macrobotrys</i> Bunge	<i>Anmodendron</i>	<i>Cercidothrix</i>	Iran; Assadi & Mozaf. 35654 (TARI)	AB051986
<i>A. squarrosus</i> Bunge	<i>Anmodendron</i>	<i>Cercidothrix</i>	Iran; Maass. & Abou. 52026 (TARI)	AB051987
<i>A. stocksii</i> Benth. ex Bunge	<i>Caraganella</i>	<i>Cercidothrix</i>	Iran; Foroughi 10802 (TARI)	AB051966
<i>A. ochreateus</i> Bunge	<i>Cremoceras</i>	<i>Cercidothrix</i>	Iran; Assadi & Maass. 55568 (TARI)	AB051981
<i>A. masanderanus</i> Bunge	<i>Cystium</i>	<i>Cercidothrix</i>	Iran; Maass. 55127 (TARI)	AB051969
<i>A. giganthrostratus</i> Maassoumi et al.	<i>Cytisodes</i>	<i>Cercidothrix</i>	Iran; Maass. et al. 72339 (TARI)	AB052033
<i>A. anacamptus</i> Bunge	<i>Erioceras</i>	<i>Cercidothrix</i>	Iran; Assadi & Mozaf. 35835 (TARI)	AB051978
<i>A. alyssoides</i> Lam.	<i>Hololeuce</i>	<i>Cercidothrix</i>	Iran; Maass. 64819 (TARI)	AB051970
<i>A. campylosema</i> Boiss.	<i>Incani</i>	<i>Cercidothrix</i>	Iran; Mozaf. & Nowroozi 34384 (TARI)	AB051984
<i>A. latifolius</i> Lam.	<i>Incani</i>	<i>Cercidothrix</i>	Iran; Assadi & Mozaf. 30428 (TARI)	AB051982
<i>A. robustus</i> Bunge	<i>Incani</i>	<i>Cercidothrix</i>	Iran; Maass. 64906 (TARI)	AB051983
<i>A. curviflorus</i> Boiss.	<i>Leucocercis</i>	<i>Cercidothrix</i>	Iran; Zehzad et al. 66937 (TARI)	AB052005
<i>A. mucronifolius</i> Boiss.	<i>Leucocercis</i>	<i>Cercidothrix</i>	Iran; Riazi 5905 (TARI)	AB052006
<i>A. cancellatus</i> Bunge	<i>Onobrychoidei</i>	<i>Cercidothrix</i>	Iran; Foroughi 6259 (TARI)	AB051972
<i>A. teheranicus</i> Boiss.	<i>Onobrychoidei</i>	<i>Cercidothrix</i>	Iran; Emami 31838 (TARI)	AB051973

Appendix 1 (continued)

Species	Section ^a	Subgenus/ Phalanx ^a	Origin, Voucher accession and herbarium ^b	DDBJ accession numbers ITS	<i>ndhF</i>
<i>A. ornithopodioides</i> Lam.	<i>Ornithopodium</i>	<i>Cercidothrix</i>	Iran; Mozaf. & Nowroozi 34629 (TARI)	AB051975	
<i>A. shelkovnikovii</i> Grossh.	<i>Ornithopodium</i>	<i>Cercidothrix</i>	Iran; Foroughi 6032 (TARI)	AB051971	
<i>A. dolichophyllus</i> Pallas	<i>Trachycercis</i>	<i>Cercidothrix</i>	Iran; Assadi & Maass. 20244 (TARI)	AB051980	
<i>A. scaberrimus</i> Bunge	<i>Trachycercis</i>	<i>Cercidothrix</i>	China; Wang et al. 17 (KYO)	AB051988	
<i>A. falcatus</i> Lam.	<i>Uliginosi</i>	<i>Cercidothrix</i>	USA; Weber 15359	U50488–9 ^c	
<i>A. odoratus</i> Lam.	<i>Uliginosi</i>	<i>Cercidothrix</i>	Iran; Mozaf. 64537 (TARI)	AB051968	AB052054
<i>A. oreganus</i> Nutt. ex T. & G.	<i>Uliginosi</i>	<i>Cercidothrix</i>	USA; McCarthy 107	AF121687 ^c	
<i>A. xiphidioides</i> Freyn & Sint.	<i>Xiphidium</i>	<i>Cercidothrix</i>	Iran; Mozaf. 67591 (TARI)	AB051976	
<i>A. ascioalalyx</i> Bunge	<i>Asciocalyx</i>	<i>Calycocystis</i>	Iran; Assadi & Maass. 50328 (TARI)	AB051974	
<i>A. subsecundus</i> Boiss.	<i>Laguroopsis</i>	<i>Calycocystis</i>	Iran; Maass. 55105 (TARI)	AB051985	AB052056
<i>A. pseudorhacodes</i> Gontsch.	<i>Macrocystodes</i>	<i>Calycocystis</i>	Iran; Assadi & Mozaf. 35472 (TARI)	AB051979	AB052061
<i>A. arizonicus</i> A. Gray	<i>Leptocarpi</i>	<i>Piptolobi</i>	USA; Sanderson 968	AF121690 ^c	
<i>A. oophorus</i> Wats.	<i>Megacarpi</i>	<i>Piptolobi</i>	USA; Tiehm 12045 (KYO)	AB051993	AB052063
<i>Barnebyella calycina</i> (Stocks) Podl. (syn. <i>Astragalus migpo</i> R. Kam.)	<i>Mirae</i>	<i>Trimeniaeus</i>	Iran; Rechinger 51029 (TARI)	AB051928	AB052039
<i>Biserrulla pelecinus</i> L. (syn. <i>Astragalus</i> <i>pelecinus</i> (L.) Barneby)	<i>Biserrula</i>	<i>Trimeniaeus</i>	Australia (adventive); USDA186284	U50518–9 ^c	
<i>Ophiocarpus aitchisonii</i> (Baker) Podl. (syn. <i>Astragalus</i> <i>ophiocarpus</i> Bunge)	<i>Ophiocarpus</i>	<i>Trimeniaeus</i>	Iran; Maass. 55143 (TARI)	AB051927	AB052040
<i>Caragana grandiflora</i> (M. B.) DC. <i>Chesneya astragalina</i> Jaub. & Spach. <i>Colutea persica</i> Boiss.			Iran; Assadi & Shahsavari 65834 (TARI)	AB051905	AB052035
			Iran; Assadi & Maass. 55503 (TARI)	AB051906	AB052036
			Iran; Foroughi 17434 (TARI)	AB051907	AB052037

Appendix 1 (continued)

<i>Oxytropis aucheri</i> Boiss.	Iran; Maass. 55104 (TARI)	AB051908	AB052038
<i>O. szovitsii</i> Boiss. & Buhse	Iran; Maass. 55090 (TARI)	AB051909	

^a Section and subgenus/phalanx names for *Astragalus* species follow the treatments of Maassoumi (1998, 2000) and Podlech (1991) for the Old World, and Barneby (1964) for North America

^b Abbreviations used in plant accession information: Abou., Abouhamzeh; Maass., Maassoumi; Mozaf., Mozaffarian; KYO, Kyoto University Herbarium, Kyoto, Japan; TARI, Herbarium of the Research Institute of Forests and Rangelands, Tehran, Iran; USDA, U.S. Department of Agriculture Plant Introduction Station

^c nrDNA ITS sequences for these taxa obtained from DNA Data Bank of Japan (DDBJ)

copies of some taxonomic literatures of *Astragalus* from Germany and Japan. The first author thanks Mrs. R. Janamoi for help in collecting *A. sinicus* in field. This research was supported by the JSPS Postdoctoral Fellowship to S. Kazempour Osaloo.

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Addresses of the authors: Dr. Shahrokh Kazempour Osaloo (e-mail: skosaloo@modares.ac.ir), Department of Plant Biology, Faculty of Basic Sciences, Tarbiat Modares University, Tehran, 14115-175, Iran. Dr. Ali Asghar Maassoumi, Department of Botany, Research Institute of Forests and Rangelands, Tehran, 13185-116, Iran. Dr. Noriaki Murakami (e-mail: k53870@sakura.kudpc.kyoto-u.ac.jp; corresponding author), Department of Botany, Graduate School of Science, Kyoto University, Kyoto, 606-8502, Japan.