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Review Article

Perspectives in taxonomy and phylogeny of the genus *Astragalus* (Fabaceae): a review

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Abstract

The genus *Astragalus* L. (Fabaceae) is reviewed from both phyloenetic and taxonomic points of view. As the largest genus of flowering plants it has attracted many researchers, but much work remains to be done. A short taxonomic history with special focus on infrageneric classification of the genus, a list of phylogenetic studies including the applied markers and sampling strategies as well as a short discussion on evolution of morphological characters are presented.

Keywords: *Astragalus*, Fabaceae, Taxonomy, Phylogeny

Introduction

With some 2900 species distributed in both Old- (1; ca. 2400 spp.) and New World (2; ca. 500 spp.), *Astragalus* L. is by far the largest genus of flowering plants, following by *Bulbophyllum* Thouars of Orchidaceae with approximately 2032 species and *Psychotria* L. of Rubiaceae comprising about 1951 species (1). The plants vary from short living annual herbs (ca. 80 spp.) to perennial rhizomatous or hemicryptophytic herbs (ca. 2500 spp.) and to cushion forming spiny shrubs (ca. 300 species) in habit (Fig. 1). Most species grow in semi-arid and arid areas throughout the world, but a few species prefer humid habitats (e.g. *A. glycyphyllos* L.), or are known as weeds. The plants show the typical papilionaceous flowers and are characterized by any unique morphological synapomorphy. As a result of this fact, the delimitation of the genus is sometimes very difficult and the assignment of some species (such as *A. annularis* Forssk.) to this genus is doubtful and not supported by phylogenetic studies (2-4). According to recent investigations, the genus is currently placed in the well supported Astrgalean clade of tribe *Galegeae* s. l. close to genera *Oxytropis* DC., *Phyllolobium* Fisch., *Colutea* L., *Lessertia* DC., *Swainsona* Salisb., *Carmichaelia* R.Br. and few small genera (5). The taxonomy and phylogeny of *Astragalus* are very challenging. In the present paper we will review the background of taxonomic and phylogenetic studies on *Astragalus*, the sources of complexity in the taxonomy and phylogeny of the genus, and future perspectives in studies on this genus.

Taxonomic background

Since its description in volume 2 of '*Species Plantarum*' (6), *Astraglus* has been subjected to many taxonomic studies aiming mostly to achieve a natural subgeneric classification. Among these systems, Bunge's (7) classification of the genus in 1868 into eight subgenera and 105 sections, has been widely used until recently (8, 9). Based on detailed morphological studies with emphasis on indumentum type (focusing on hair attachment: basifixed vs. medifixed), Podlech (10) explained the convergent nature of many of the morphological characters used in the delimitation of subgenera as proposed by Bunge (7) and reduced the number of recognized subgenera to three: subgen. *Astragalus*, subgen. *Cercidothrix* Bunge and subgen. *Trimeniaeus* Bunge. He also considered a unique position for tragacanthic

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Figure 1. The variation of habit in *Astragalus*. **(A)** *A. sieversianus* Pall. (sect. *Astragalus*; clade A), **(B)** *A. aegobromus* Boiss. & Hohen. (sect. *Caprini*; clade A), **(C)** *A. kirrindicus* Boiss. & Noë (sect. *Alopecuroidei*, clade A), (**D**) *A. siliquosus* Boiss. (sect. *Theiochrus*; clade B), **(E)** *A. schmalhausenii* Bunge (sect. *Oxyglottis*; clade B), F**:** *A. hamosus* L. (sect. *Bucerates*, clade C), **(G)** *A. fridae* Rech.f. (sect. *Incani*, clade H), **(H)** *A. muschianus* Kotschy & Boiss. (sect. *Rhacophorus*, clade G), (**I**) *A. semnanensis* Bornm. & Rech.f. (sect. *Semnanenses*, clade G), **(J)** *A. megalocystis* Bunge (sect. *Anthylloidei*, clade G), **(K)** *A. bakaliensis* Bunge (sect. *Hispiduli*; clade F), **(L)** *A. spachianus* Boiss. & Buhse (sect. *Malacothrix*; clade F).

species of *Astragalus* (subgen. *Tragacantha* Bunge) suggesting an evolutionary lineage with a basal position for them. He elevated this group to generic rank later giving them the formal name *Astracantha* Podlech (11). However, due to presence of several intermediate species that were not carefully examined at that time (e.g. members of sect. *Adiaspastus* Bunge) with relatively long peduncles (members of *Astracantha* were characterized by sessile inflorescences), *Astracantha* was reduced to synonymy under *Astragalus* again (12). Even the monophyly of this group of species has been doubted in a cladistic analysis, because they nested within a clade that included most spiny members of *Astragalus* representing various sections (13). At the same time, an exact diagnostic key to all 16 sections of spiny *Astragalus* was presented (13). In Flora Iranica, the area of highest diversity of the genus, 955 species were assigned to sections, but no subgenus was implied. The same system is applied in the most recent monograph of the genus in the Old World (14). A summary of infrageneric classification systems of *Astragalus* focusing on those applied widely is presented in Table 1.

Another challenging subject in the taxonomy of *Astragalus* concerns with the delimitation of this genus from a few small genera. Recently, the monotypic genus *Podlechiella* Maassoumi & Kaz. Osaloo has been described (3) and the genus *Phyllolobium* Fisch. has been resurrected (15) based on both morphological and sequence data. Furthermore, the genera *Barnebyella* Podlech and *Ophiocarpus* (Bunge) Ikonn. were included in *Astragalus* again, while the placement of some species such as *A. epiglottis* L. and *A. annularis* in *Astragalus* is put strongly under question (3).

Due to the large size of the genus, its circumscription will remain uncertain until the majority of known morphological lineages, especially the morphologically aberrant species and groups, are analyzed for adequate numbers of nuclear and plastid markers. Moreover, most sections and subgenera described in the genus are defined based on only one or few (mostly variable) morphological characters depending upon the botanist's subjective hypotheses on evolutionary trends in the group.

Table 1. History of infrageneric classification of *Astragalus*.

	De Candolle (1802)	Bunge (1869)	Boissier (1872)	Barneby (1964)	Podlech (1982)	Podlech and Zarre (2013)
No. of recognized subgenera or units at first infrageneric informal ranks	4	9	10	7 (phalanxes)	3	-
No. of recognized sections	18	105	91	93	-	2398
No. of recognized species	244	964	758	368	-	136
Geographic area of taxonomic treatment	World	World	Asia and Africa	North America	Old World	Old World

Background of phylogenetic studies

Before the era of molecular systematics, the preliminary phylogenetic studies in *Astragalus* were focused on certain groups of species, as for example on North American species (16) and spiny ones (13), using morphological data and chromosome counts. The first molecular phylogenetic study that included a reasonable number of species was conducted by Liston in 1992 (17) who surveyed the restriction site map of chloroplast DNA (cpDNA) *rpoC1* and *rpoC2* genes focusing on selected North American species of the genus. This study was followed by application of Restriction Fragment Length Polymorphism (RFLP) to the whole cpDNA (18). Both studies reconstruct a supported clade that includes North American species characterized by aneuploid sets of chromosomes. The basic chromosome number (x) of Old World *Astragalus* had been reported to be 8 (19) while in North and South America the majority of species shows x = 11-15 (20). The same result was obtained using nuclear DNA (nrDNA) ITS and cpDNA *trnL-trnF* sequences of 115 species of *Astragalus* from various selected groups of the genus (2). Although this clade of American species of *Astragalus* (the so-called 'Neo-Astragalus') is well-supported, it also forms a larger clade together with some Old World Mediterranean species (e.g. *A. echinatus* Murr.) intergrading some basal and some derived clades. The age of *Astragalus* node has been estimated 12-16 Mya using cpDNA *matK* sequences (21). Compared to ITS, the *matK* fragment also provided stronger support for the main known clades in *Astragalus*. Another important study focused on the origin of South American species of 'Neo-Astragalus' using two new plastid markers, namely *trnD-trnT* and *trnfM-trnS1*, along with ITS (22). In the same study based on a molecular clock analysis, it was concluded that 'Neo-Astragalus' belongs to the group of plants showing recent rapid radiation.

The main phylogenetic studies concerning the Old World species of *Astragalus* was undertaken by Kazempour Osaloo and co-workers (3, 4); their studies included representative species of various known formal sections of the genus. They applied only ITS sequences as the basic datasets (with 124 and 212 taxa, respectively). These studies confirmed the monophyly of the genus and its close relationship to *Oxytropis* DC. and the members of subtribe Coluteinae (sensu (23)). However, ITS was not sufficiently informative to provide adequate resolution in many clades of the genus. More recent studies have been focused on certain internal clades of *Astragalus* using various markers (24-26). These studies showed that a combined approach of different markers would increase the resolution and the supports of the clades in *Astragalus*. Table 2 lists the main molecular phylogenetic studies conducted on *Astragalus* indicating the molecular markers applied and some important information.

Table 2. DNA markers used in previous molecular phylogenetic studies on *Astragalus*.

	Marker	Reference	No. Taxa analyzed	Size of fragment (bp)	No. parsimony informative characters	% informative characters
Nuclear DNA	ITS	(5)	212	654	211	32.3%
		(2)	115	679	202	30%
	ETS	(25)	43	543	-	31%
Plastid DNA	*trnY-trnT*	(25)	43	549	-	24%
	trnD-trnT	(22)	48	1001	28	2.8%
	trnfM-trnS1	(22)	48	1066	21	1.96%
	trnL	(2)	34	638	50	8%
	ndhF	(4)	36	2103	163	7.75%
	trnL-trnF	(24)	52	1097	129	11.75%
	trnS-trnG	(25)	43	370	-	28%
	mat K	(31)	235	1674	1042	73%
	psbA-trnH	(25)	35	193	-	9%

The fertilization of embryo sac occurs very early in *Astragalus* and when the flower buds are still very small in size (27, 28). So, the rate of allogamy and hybridization in the genus seems to be very low. It is also assumed that nuclear ribosomal sequences though present in multiple copies, have been subject to concerted evolution (3). Thus reconstruction of phylogeny using such markers, especially ITS due to its higher evolutionary rate, has been popular, and as a result most of available sequences of *Astragalus* are ITS sequences (http://www.ncbi.nlm.nih.gov/nuccore/?term=Astragalus). A Bayesian 50% majority rule tree gained from analysis of ITS sequences for 388 species of *Astragalus* and allied genera is summarized in Figure 2. The final data matrix is comprised a total of 664 bp aligned characters of which 273 positions (41%) were parsimony informative. The model of molecular substitution in this analysis was set as GTR+G+I.

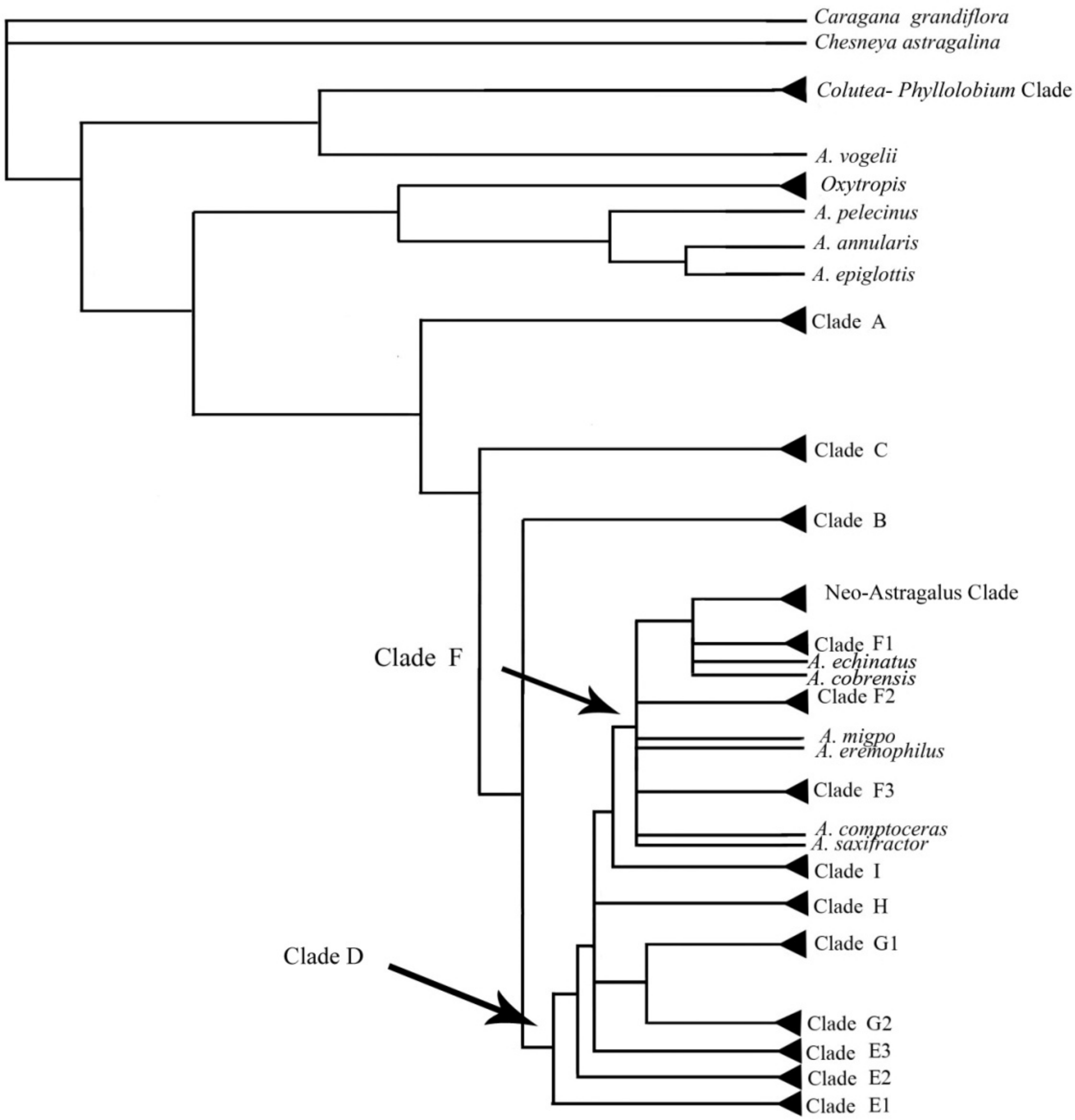

Figure 2. Summarized 50% majority rule tree of Bayesian analysis of 388 ITS sequences available in the GenBank for *Astragalus* and its allies. Clades A-I are labeled after Kazempour Osaloo and co-workers (4).

Evolution of morphological characters

Classical taxonomic studies weighted some morphological studies more than others and used them for characterizing sections or even subgenera. Some of the most important features used were: presence of medifixed (or forked) indumentums vs. basifixed one, annual vs. perennial herbaceous vs. spiny habit, presence of pollen brush vs. its absence, tubular or campanulate vs. inflated vs. turbinate (or infandibular calyx), and bilocular vs. unilocular fruits. However, in light of molecular phylogenetic studies, it became clear that all these characters are homoplasious and have originated several times and independently in separate clades. However, considering the developmental process behind each of these characters or examining the ultra-structure features, might increase the value of such characters in delimitation of natural formal infrageneric taxa in the genus. Some examples are given below:

1- Embryological studies - The embryo in members of sect. *Incani* DC. (clade H, Fig. 2) are characterized by a narrow suspensor composed of two rows of cells. Furthermore, the first leaves of seedlings among the species of this section are unifoliolate (27, 28).

2- Hair micro-morphological studies - Among the annual species of *Astragalus*, the pods of the members of sect. *Platyglottis* Bunge and some other annual species are covered by hairs attached to a tubercle at the base. This feature is also observed in some perennial herbaceous species of other sections such as sect. *Malacothrix* Bunge which is placed in the same clade (clade F) as the former (29).

3- Spine anatomy - The arrangement of vascular bundles and the thickness of sclerenchymatous bundles show similar patterns in tragacanthic *Astragalus* (equal to *Astracantha*) as in other spiny species of *Astragalus* mainly of subgen. *Calycophysa* Bunge (clade G), suggesting a close relationship between them. In this group of species, spine anatomy provides a tool for separation of taxa at species level rather than at higher ranks (30).

Perspectives

The number of studies conducted on the genus *Astragalus* is still too few considering the huge size of the genus. The genus is in urgent need of comprehensive phylogenetic studies covering all its lineages and distribution area using several plastid and nuclear markers. The divergence time is still not surveyed for many clades within *Astragalus* and the biogeographical patterns are not studied for most members of the genus. Beside the molecular aspects, micro-morphology and embryological studies might also be helpful in definition of reliable synapomorphies for the main clades corresponding to formal infrageneric taxa in the genus.

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