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Taxonomic relationships in *Astragalus* sections *Hololeuce* and *Synochreati* (Fabaceae): evidence from RAPD-PCR and SDS-PAGE of seed proteins

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Taxonomic status of the Turkish members of the sections *Hololeuce* and *Synochreati* of *Astragalus* (Fabaceae) was analysed using variation of morphological features, seed proteins and RAPD markers. Dendrograms based on Nei's genetic distance method UPGMA were used to compare the species. The results of cluster analysis were in broad agreement with morphological classifications of these species, and they were slightly different to the arrangement of these species in *Flora of Turkey*. Based on RAPD, seed protein and morphological analyses, *A. vuralii* H. Duman & Aytaç is synonymized with *A. andrasovszkyi* Bornm., *A. cylindraceus* DC. with *A. globosus* Vahl, *A. alindanus* Boiss. with *A. hirsutus* Vahl, *A. paecilanthus* Boiss. & Heldr. with *A. sibthorpianus* Boiss., *A. squalidus* Boiss. & Noë with *A. amoenus* Fenzl, *A. kangalicus* Kit Tan & Sorger with *A. fragrans* Willd., and *A. seydishehiricus* Kit Tan & Ocakverdi, *A. isparticus* Kit Tan & Sorger and *A. ocakverdii* Kit Tan & Sorger with *A. acmonotricus* Fenzl. In addition to these, *A. karputanus* Boiss. & Noë should be treated as a subspecies of *A. bicolor* Lam.

Key words: *Astragalus*, RAPD-PCR, SDS-PAGE, taxonomy

Introduction

Astragalus is probably one of the largest genera of vascular plants in Eurasia, with an estimated number of 2500–3000 species. Many species are narrow endemics, while relatively few are widespread, distributed mainly in the Northern Hemisphere, Central Asia, and western North America (Podlech 1986, Maassoumi 1998). It is also the largest genus in Turkey, where it is represented by ca. 470 species in 62 sections (Chamberlain & Matthews 1970, Davis *et al*. 1988, Ekim *et al*. 1991, Özhatay *et al*. 1994, 1999, Adıgüzel 1999, Podlech 1999, Aytaç 2001, Aytaç *et al*. 2001, Ekici & Aytaç 2001, Aytaç & Ekici 2002, Göktürk *et al*. 2003).

In the *Flora of Turkey*, many taxonomical problems in some genera and sections were mentioned, but not solved, because of the limited time and material. The editors suggested that further revision should be carried out to solve these problems (Chamberlain & Matthews 1970,

Davis & Hedge 1975). *Astragalus* is an example with many unresolved taxonomical problems, for example in sections such as *Onobrychium* and *Hololeuce*. The classical identification of *Astragalus* of course depends on morphological characteristics. These characteristics are often affected by environmental and/or developmental factors during plant growth (Cai *et al*. 1999). The advent of molecular taxonomy techniques offered a solution to many problems, which were for a long time out of reach of classical taxonomic methods and approaches. Currently, the methods of construction of phylogenetic trees on the basis of molecular data are widely used in systematics. Due to the tremendous developments in the field of molecular biology, a variety of different molecular techniques to analyze genetic variation has emerged in recent decades.

Some types of molecular markers used today are RFLP, AFLPs, RAPD and seed protein profiles. RFLPs (Tanksley *et al*. 1989) and RAPD (Williams *et al*. 1990) are quite stable and highly reproducible. RFLP markers are less polymorphic, more expensive and laborious compared with RAPD. Seed protein profiles are a powerful tool to ascertain genetic homology at the molecular level and to resolve taxonomic and phylogenetic problems (Singh *et al*. 1994). Analysis of seed protein profiles by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) has been used succesfully for identification of some plant species (Ladizinsky & Hymowitz 1979, Cooke 1984, Saraswati *et al*. 1993). To our knowledge, there is no published information on the use of DNA and protein markers for the characterization of genetic diversity in the *Astragalus* species we study in this contribution.

It was our objective to clarify the systematic status of two sections of the genus *Astragalus* by studying RAPD-PCR and seed protein profiles in addition to morphological analysis.

Materials and methods

Morphological observations were carried out from dried specimens conserved at a number of herbaria (AEF, ANK, ATA, B, BM, CUM, E, EGE, GAZI, HUB, ISTF, K, KNYA, M, MSB, P, W) and fresh materials collected in the wild. Information on taxa used in the present investigation is summarized in Table 1. The author names follow Brummitt and Powell (2001).

A set of fifty 10-mer oligonucleotides from Operon Technologies were used for RAPD amplification. The conditions reported by Williams *et al*. (1990) were used for RAPD-PCR. Amplification was achieved in a Techne (UK) Progene thermocycler. After the cycling was completed, 15 μl of the reaction products were analysed alongside small molecular weight markers on a 2% agarose gel in the presence of ethidium bromide and photographed under UV light.

For seed protein analysis protein extraction was performed according to Saraswati *et al*. (1993). Electrophoresis was carried out following the Laemmli (1970) method. Each run included marker proteins, of which molecular weights were known (BioRad). Proteins on the gel were fixed and stained overnight with Coomassie Brilliant Blue G-250 according to Demiralp *et al*. (2000). Molecular weights of protein bands were estimated by their relative mobilities.

In the data analysis, bands on RAPD and SDS-PAGE gels were scored as either present (1) or absent (0) for all species studied. Common band analysis was conducted using the computer programme POPGEN to determine the genetic distance values between them. The figures for genetic distance were then used as input data for cluster analysis to generate dendrograms.

Results

Astragalus specimens were collected in Turkey in 1995–1999 and studied morphologically (Chamberlain *et al*. 1988, Davis *et al*. 1988, Ekim *et al*. 1991).

For the RAPD-PCR analysis, 40 random primers were tested in the amplification reactions with *Astragalus* species. Among them, 10 primers were chosen for further analysis. Bands were ranging from 200 bp to 1500 bp in size. Some of the bands were monomorphic, while some of them showed at least one polymorphism. RAPD results clearly differentiated between each of the twenty-seven species of *Astragalus*: *A. incertus* Ledeb., *A. hyalolepis* Bunge, *A. bicolor* Lam.,

A. vuralii H. Duman & Aytaç, *A. karputanus* Boiss. & Noë, *A. caudiculosus* Boiss. & Huett, *A. andrasovszkyi* Bornm., *A. alyssoides* Lam., *A. cylindraceus* DC., *A. alindanus* Boiss., *A. hirsutus* Vahl, *A. cataonicus* Bunge, *A. dumanii* M. Ekici & Aytaç, *A. globosus* Vahl, *A. sibthorpianus* Boiss., *A. paecilanthus* Boiss. & Heldr., *A. stenosemius* Boiss. & Noë, *A. stenosemioides* Bornm. *ex* Chamb. & Matthews, *A. amoneus* Fenzl, *A. squalidus* Boiss. & Noë, *A. gaeobotrys* Boiss. & Balansa, *A. fragrans* Willd., *A. kangalicus* Kit Tan & Sorger, *A. acmonotrichus* Fenzl, *A. seydishehiricus* Kit Tan & Ocakverdi, *A. isparticus* Kit Tan & Sorger, and *A. ocakverdii* Kit Tan & Sorger.

The analysis of seed proteins showed that all studied genotypes had a specific protein pattern except the species, which were taxonomic synonyms based on morphological data. The seed protein composition determined by SDS-PAGE for members of the sections *Hololeuce* and *Synochreati* are presented in Figs. 1 and 2, respectively. Based on the DNA electrophorograms and protein profiles the indices of similarity were calculated (Tables 2–3). The dendrogram for section *Hololeuce* (Fig. 3) allows two main groups to be distinguished. The upper group was again subdivided into three subgroups having genetic distances between 0% and 37%. The upper cluster contains *A. amoneus*, *A. squalidus*, *A. vuralii*, *A. andrasovskyi*, *A. karputanus*, *A. bicolor*, *A. cataonicus*, *A. dumanii*, *A. paecilanthus* and *A. sibthorpianus*. Among these section members, *A. amoneus* differs from *A. squalidus* by a genetic distance of 37%. *Astragalus vuralii* differs from *A. andrasovszkyi* by a genetic distance of 33%. *Astragalus karputanus* shows no differences from *A. bicolor* (genetic distance 0%), *A. cataonicus* shows no differences from *A. dumanii* (genetic distance 0%), and *A. paecilanthus* shows no differences from *A. sibthorpianus* (genetic distance 0%).

Table 1. Specimens used in this study.

Species	Collection site	Altitude (m)	Habitat	Collectors
Sect. *Hololeuce*				
A. incertus	A8 Trabzon: pass Soğanlı	2600	meadows	*M. Ekici 2138 et al.*
A. hyalolepis	B8 Erzurum: Palandöken mt.	2900	meadows	*M. Ekici 2016 et al.*
A. bicolor	A8 Erzurum: Erzurum	2000	steppe	*M. Ekici 1990 et al.*
A. karputanus	B5 Nevşehir: Göreme	1100	steppe	*M. Ekici 2102 et al.*
A. caudiculosus	B8 Erzurum: Palandöken mt.	2750	meadows	*M. Ekici 2027 et al.*
A. andrasovszkyi	B4 Konya: Cihanbeyli	1000	steppe	*M. Ekici 2066 et al.*
A. vuralii	B4 Ankara: Polatlı	850	steppe	*M. Ekici 2061 et al.*
A. alyssoides	B8 Erzurum: Palandöken mt.	2700	meadows	*M. Ekici 2014 et al.*
A. cataonicus	C3 Isparta: Dedegöl mt.	2400	screen	*M. Ekici 2038 et al.*
A. dumanii	B6 K.Maraş: Berit mt.	2300	screen	*M. Ekici 2057 et al.*
A. hirsutus	C3 Konya: Beyşehir	1400	screen	*M. Ekici 1877 et al.*
A. alindanus	C2 Antalya: Elmalı.	2100	screen	*M. Ekici 1938 et al.*
A. globosus	B8 Erzurum: Palandöken mt.	2400	meadows	*M. Ekici 2012 et al.*
A. cylindarceus	B5 Kayseri: Ali Dağ	1750	steppe	*M. Ekici 1910 et al.*
A. sibthorpianus	A2 Bursa: Uludağ	2100	steppe	*M. Ekici 2113 et al.*
A. paecilanthus	C3 Isparta: Çiçekdağ	1600	*Quercus* forest	*M. Ekici 2086 et al.*
A. amoenus	C3 Konya: Seydişehir	1600	*Pinus* forest	*M. Ekici 1889 et al.*
A. squalidus	B4 Ankara: Ahlatlıbel	1200	steppe	*M. Ekici 2109 et al.*
Sect. *Synochreati*				
A. gaeobotrys	B2 Uşak: Uşak	950	*Pinus* forest	*M. Ekici 1897 et al.*
A. fragrans	A8 Erzurum: Narman	2400	meadows	*M. Ekici 1997 et al.*
A. kangalicus	A6 Sivas: Kangal	2000	steppe	*M. Ekici 1923 et al.*
A. acmonotrichus	B2 Kütahya: Murat Dağı	1500	screen	*M. Ekici 2059 et al.*
A. ocakverdii	C4 Konya: Seydişehir	1600	*Pinus* forest	*M. Ekici 2062 et al.*
A. isparticus	C3 Isparta: Çiçekdağı	1700	forest	*M. Ekici 2034 et al.*
A. seydishehiricus	C4 Konya: Seydişehir	1400	open *Quercus*	*M. Ekici 1956 et al.*

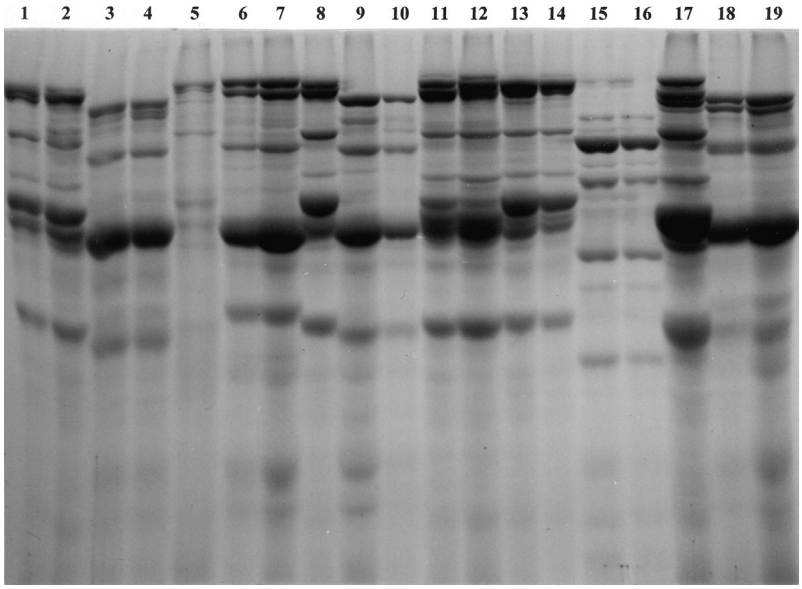

Fig. 1. Electrophoretic pattern of seed proteins in *Astragalus* sect. *Hololeuce*. (1) *A. incertus*, (2) *A. hyalolepis*, (3) *A. bicolor*, (4) *A. karputanus*, (5) *A. caudiculosus*, (6) *A. andrasovszkyi*, (7) *A. vuralii*, (8) *A. alyssoides*, (9) *A. cataonicus*, (10) *A. dumanii*, (11) *A. hirsutus*, (12) *A. alindanus*, (13) *A. globosus*, (14) *A. cylindraceus*, (15) *A. sibthorpianus*, (16) *A. paecilanthus*, (17) *A. stenosemius*, (18) *A. amoenus*, (19) *A. squalidus*.

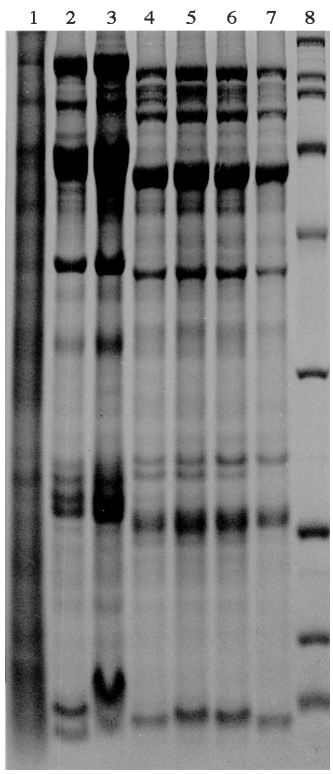

Fig. 2. Electrophoretic pattern of seed proteins in *Astragalus* sect. *Synochreati*. (1) *A. gaeobotrys*, (2) *A. fragrans*, (3) *A. kangalicus*, (4) *A. seydishehiricus*, (5) *A. isparticus*, (6) *A. ocakverdii*, (7) *A. acmonotrichus*, (8) Marker (myosin 200.000, β-galactosidase 116.250, phosphorylase *b* 97.400, serum albumin 66.200, ovalbumin 45.000, carbonic anhydrase 31.000, trypsin inhibitor 21.500, lysozyme 14.400, apotinin 6.500 Da).

The lower group was also subdivided into two subgroups having genetic distances between 0% and 63%. The second group contains *A. globosus*, *A. cylindraceus*, *A. hyalolepis*, *A. incertus*, *A. alyssoides*, *A. stenosemius*, *A. stenosemioides*, *A. caudiculosus*, *A. hirsutus* and *A. alindanus*. Among these, *A. globosus* differs from *A. cylindraceus* by a genetic distance of 0.3%, *A. hyalolepis* differs from *A. incertus* and *A. alyssoides* by a genetic distance of 0.3% and 28% respectively, *A. stenosemius* differs from *A. stenosemioides* by a genetic distance of 37%, and *A. caudiculosus* differs from *A. hirsutus* by a genetic distance of 63%. *Astragalus hirsutus* shows no genetic differences from *A. alindanus* (genetic distance 0%).

The dendrogram for section *Synochreati* (Fig. 4) allows two groups to be distinguished. The upper cluster contains only *A. gaeobotrys* having genetic distances to all other members of the section between 47% and 98%. The lower cluster was subdivided into two groups having genetic distance between 0% and 28%. *Astragalus fragrans* and *A. kangalicus* grouped in the first cluster, and the second cluster consisted of *A. isparticus*, *A. acmonotrichus, A. seydishehiricus* and *A. ocakverdii*. Among these species, *A. gaeobotrys* differs from *A. fragrans* and *A. kangalicus* by genetic distances of 69% and 47% respectively, *A. fragrans* differs from *A. kangalicus* by a genetic distance of 13%, and

Table 2. Genetic distance values based on RAPD results and seed proteins of *Astragalus* sect. *Hololeuce*. (1) *A. amoenus*, (2) *A. squalidus*, (3) *A. paecilanthus*, (4) *A. sibthorpianus*, (5) *A. globosus*, (6) *A. cylindraceus*, (7) *A. vuralii*, (8) *A. andrasovszkyi*, (9) *A. hirsutus*, (10) *A. alindanus*, (11) *A. karputanus*, (12) *A. bicolor*, (13) *A. cataonicus*, (14) *A. dumanii*, (15) *A. incertus*, (16) *A. alyssoides*, (17) *A. hyalolepis*, (18) *A. caudiculosus*, (19) *A. stenosemius*, (20) *A. stenosemioides*.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	–																			
2	0.0317	–																		
3	0.4212	0.3747	–																	
4	0.4212	0.3747	0.0000	–																
5	0.5213	0.5754	0.8267	0.8267	–															
6	0.5213	0.5754	0.8267	0.8267	0.0000	–														
7	0.2877	0.3302	0.5213	0.5213	0.3302	0.3302	–													
8	0.2877	0.3302	0.5213	0.5213	0.3302	0.3302	0.0000	–												
9	0.6325	0.6931	0.6931	0.6931	0.2877	0.2877	0.4212	0.4212	–											
10	0.6325	0.6931	0.6931	0.6931	0.2877	0.2877	0.4212	0.4212	0.0000	–										
11	0.2877	0.3302	0.5213	0.5213	0.6325	0.6325	0.2877	0.2877	0.4212	0.4212	–									
12	0.2877	0.3302	0.5213	0.5213	0.6325	0.6325	0.2877	0.2877	0.4212	0.4212	0.0000	–								
13	0.3302	0.3747	0.5754	0.5754	0.5754	0.5754	0.4212	0.4212	0.3747	0.3747	0.0984	0.0984	–							
14	0.3747	0.3302	0.5213	0.5213	0.6325	0.6325	0.4700	0.4700	0.4212	0.4212	0.1335	0.1335	0.0317	–						
15	0.5754	0.6325	0.9008	0.9008	0.1699	0.1699	0.5754	0.5754	0.3302	0.3302	0.5754	0.5754	0.5213	0.5754	–					
16	0.5754	0.6325	0.9008	0.9008	0.1699	0.1699	0.5754	0.5754	0.3302	0.3302	0.5754	0.5754	0.5213	0.5754	0.0000	–				
17	0.5754	0.6325	0.9008	0.9008	0.1699	0.1699	0.5754	0.5754	0.3302	0.3302	0.5754	0.5754	0.5213	0.5754	0.0000	0.0000	–			
18	0.4700	0.5213	0.6325	0.6325	0.3302	0.3302	0.4700	0.4700	0.3302	0.3302	0.5754	0.5754	0.5213	0.5754	0.3747	0.3747	0.3747	–		
19	0.5213	0.4700	0.8267	0.8267	0.2877	0.2877	0.6325	0.6325	0.4700	0.4700	0.6325	0.6325	0.5754	0.5213	0.2469	0.2469	0.2469	0.3302	–	
20	0.4700	0.4212	0.6325	0.6325	0.2469	0.2469	0.5754	0.5754	0.5213	0.5213	0.5754	0.5754	0.5213	0.4700	0.2877	0.2877	0.2877	0.2877	0.1699	–

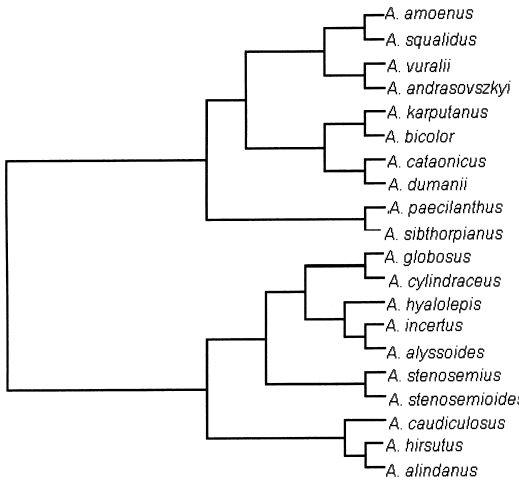

Fig. 3. Dendrogram of *Astragalus* sect. *Hololeuce*.

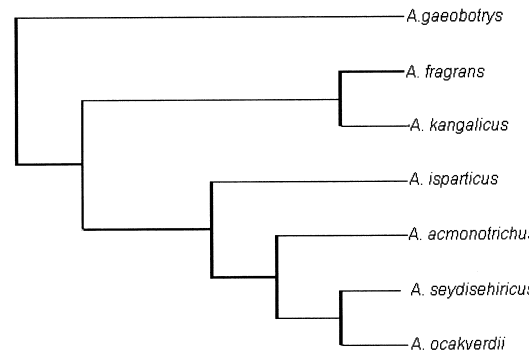

Fig. 4. Dendrogram of *Astragalus* sect. *Synochreati*.

A. isparticus differs from *A. acmonotrichus* by a genetic distance of 28%. *Astragalus seydishehiricus* shows no differences from *A. ocakverdii* (genetic distance 0%).

Discussion

Astragalus amoenus and *A. squalidus* grouped in the first cluster. Different populations of these two species show a similar protein banding pattern. We have collected these two "species" in different localities and suggest *A. squalidus* to be a synonym of *A. amoenus*, a conclusion supported by the results of seed protein analysis and RAPD analysis.

Another member of the first cluster, *A. cataonicus*, is related to *A. dumanii*, but can be distinguished by characteristics such as bracts, calyx, calyx-teeth and stipule (Appendix). *Astragalus karputanus* only differs from *A. bicolor* in the leaflet number, that being 5–8 and 8–15 respectively. We therefore reduce *A. karputanus* to a subspecies of *A. bicolor*. The protein analysis data provided evidence that these two taxa are very closely related.

Also in the first cluster, *A. andrasovszkyi* was recorded as a new species in 1915 from type collection only, and it was not collected again until 1993 (but the collections were published as a new species *A. vuralii* by Duman and Aytaç (1993). We collected the taxon from type locality in 1995 and, after close morphological examination, we regard *A. vuralii* as a synonym of *A. andrasovszkyi* (Appendix). The last members of the first cluster, *A. paecilanthus* and *A. sibthorpianus* were treated as separate species in *Flora of Turkey*, only differing in the length of calyx and standard. However, after a thorough morphological study (Appendix), it is clear that *A. paecilanthus* is a taxonomic synonym of *A. sibthorpianus*. In seed protein analysis the banding patterns of the two are similar.

Table 3. Genetic distance values based on RAPD results and seed proteins of *Astragalus* sect. *Synochreati*. (1) *A. fragrans*, (2) *A. kangalicus*, (3) *A. gaeobotrys*, (4) *A. acmonotrichus*, (5) *A. seydishehiricus*, (6) *A. isparticus*, (7) *A. ocakverdii*.

	1	2	3	4	5	6	7
A. fragrans	–						
A. kangalicus	0.1335	–					
A. gaeobotrys	0.6931	0.4700	–				
A. acmonotrichus	0.4700	0.2877	0.9808	–			
A. seydishehiricus	0.4700	0.2877	0.4700	0.2877	–		
A. isparticus	0.4700	0.2877	0.4700	0.2877	0.0000	–	
A. ocakverdii	0.4700	0.2877	0.4700	0.2877	0.0000	0.0000	–

The first members of the second large cluster are *A. globosus* and *A. cylindraceus*. These two species are very similar morphologically. In the *Flora of Turkey*, *A. cylindraceus* keyed out in error as having two bracteoles; this conflicts with the description, where bracteoles are described as being absent or up to 5 mm. We have found that several specimens are morphologically intermediate between *A. globosus* and *A. cylindraceus* and such specimens cannot be placed with certainity in either species. The intermediates, as well as the protein pattern, support our conclusion to reduce the latter name into synonymy with the former.

In the second group, the protein banding patterns of *A. hyalolepis*, *A. incertus* and *A. alyssoides* differ from each other. *Astragalus incertus* has sub-bifurcate hairs on the leaflets, which renders it easily distinguishable from the two other species. *Astragalus stenosemioides* and *A. stenosemius* also grouped in the second cluster. Both are endemic to Turkey and are closely related morphologically and have similar protein patterns. The remaining members of the second cluster are *A. alindanus* and *A. hirsutus*, which were regarded as closely related in the *Flora of Turkey*, and our molecular results support that notion.

Morphologically, *A. gaeobotrys* was distinguished from the others by fruit shape. *Astragalus fragrans* differs from *A. kangalicus* in the leaflets, peduncule and calyx length. According to morphology and genetic distances, we consider *A. fragrans* a taxonomic synonym of *A. kangalicus*. Seed protein profiles of *A. isparticus*, *A. ocakverdii*, *A. seydishehiricus* and *A. acmonotrichus* were very similar and the genetic distances among these four range between 0% and 28%; they are also very similar morphologically. *Astragalus ocakverdii* is identical with and thus a taxonomic synonym of *A. acmonotrichus* in section *Synochreati*. Similarly, the morphological features of *A. isparticus* and *A. seydishehiricus* fall within the range found in *A. acmonotrichus*.

As a result of molecular studies in conjunction with morphological data and field observations, we conclude that the number of species recognised in the sections *Hololeuce* and *Synochreati* is less than previously thought. Although *A. ocakverdii* is usually included in section *Hololeuce* (Davis 1988), a morphological analysis indicates that it should be transferred to section *Synochreati*. The accepted names in the list are in bold and their taxonomic synonyms in italics but not in bold.

1. *Astragalus* sect. *Hololeuce* Bunge

Mém. Acad. Imp. Sci. Saint Pétersbourg 11 (16): 104. 1868. — LECTOTYPE (Podlech 1990): *A. hololeucus* Boiss. & Buhse.

Astragalus alyssoides Lam.

Encycl. Méth. Bot. 1: 317. 1785. — LECTOTYPE (Podlech 1998): Armenia. '*Astragalus orientalis, argenteus angustifolius, flore leucophaeao*', *J.P. de Tournefort* 29 (P-LA: photo MSB!; isolectotypes BM; P-TRF nr. 3634!: photo MSB!).

Astragalus amoenus Fenzl

Pug. Pl. Nov. Syr.: 4. 1842. — *Tragacantha amoena* (Fenzl) Kuntze, Revis. Gen. 2: 943. 1891. — LECTOTYPE (Podlech 1999): Turkey. In alpibus Tauri occid., aestate 1836 *Th. Kotschy 124* (W!; isolectotypes B!, BM, G, G-BOIS, K!, LE, M!, MSB!, OXF, P!, W!).

Astragalus squalidus Boiss. & Noë

Diagn. pl. orient., ser. 2, 2: 29. 1856, *syn. nov*. — HOLOTYPE: Turkey. Prope Sivas, VI.1842 *Noë 1084* (G-BOIS; iso B!)

Astragalus andrasovszkyi Bornm.

Magyar Bot. Lapok 14: 54. 1915. — TYPE: Turkey. Lycaonia, Anatolia centr., ad pag. Karabagh, 17.V.1911 *Andrasovszky 442* (holotype B!; isotypes: BP, JE)

Astragalus vuralii H. Duman & Aytaç

Thaiszia 1: 19. 1991, *syn. nov*. — HOLOTYPE: Turkey. B3 Ankara, 18 km W of Polatlı, Acıkır district, 840 m, 25.V.1990 *H. Duman & Z. Aytaç 3032* (GAZI!; isotypes ANK!, HUB!).

Astragalus bicolor Lam.

Encycl. Méth. Bot. 1: 317. 1785.

subsp. ***bicolor***

Tragacantha bicolor (Lam.) Kuntze, Revis. Gen. 2: 943. 1891. — LECTOTYPE (Podlech 1998): Armenia. '*Astragalus armeniacus incanus, angustifolius, fl. in eod. capitulo candid. et purpureo*', *J.P. de Tournefort* (P-LA; isolectotypes B-W 14082, BM, M!, P-JUSS 15253, P-TRF nr. 3629: photo MSB!).

subsp. ***karputanus*** (Boiss. & Noë) Ponert

Feddes Repert. 83: 631. 1973. — *Astragalus karputanus* Boiss. & Noë, Diagn. pl. orient., ser. 2, 2: 26. 1856. — *Tragacantha karputana* ("Boiss." Boiss. & Noë) Kuntze, Revis. Gen. 2: 945. 1891. — HOLOTYPE: Armenia. Prope Karput Armeniae mer., VI.1852 *F.W. Noë 963* (G-BOIS).

Astragalus cataonicus Bunge

Mém. Acad. Imp. Sci. Saint Pétersbourg 11 (16): 107. 1868. — *Tragacantha cataonica* (Bunge) Kuntze, Revis. Gen. 2: 943. 1891. — SYNTYPES: Turkey. In Tauro cilicico [in summo jugus inter Gisyl deppe et Koschan, 2350 m], *Th. Kotschy 158* (K!); in Tauro cilicico, alpe 'Bulgar Dagh', in rupestribus montis Gisyl Deppe, 8000´, 1853 *Th. Kotschy 169*.

Astragalus caudiculosus Boiss. & A. Huet

Diagn. pl. orient., ser. 2, 2: 25. 1856. — *Tragacanatha caudiculosa* ("Boiss." Boiss. & A. Huet) Kuntze, Revis. Gen. 2: 943. 1891. — *Astragalus xerophilus* Ledeb. subsp. *caudiculosus* (Boiss. & A. Huet) Ponert, Feddes Repert. 83: 631. 1973. — HOLOTYPE: Turkey. Tech Dagh prope Erzeroum Armeniae, 9000–10000´, VII.1853 *A. Huet du Pavillon* (G-BOIS; isotypes BM, G, GOET, K!, P: photo MSB!).

Astragalus dumanii M. Ekici & Aytaç

Ann. Bot. Fennici 38: 171–174. 2001. — HOLOTYPE: Turkey, prov. Maraş, [C6] Göksun, Berit Dağı, 2300–2400 m, 8.9.1997, *M. Ekici 2057 & Aytaç* (GAZI!; isotype MSB!).

Astragalus globosus Vahl

Symb. Bot. 1: 60. 1790. — LECTOTYPE (Podlech 1998): '*A. orientalis, foliis viciae argenteis, caule nudo, erecto floribus luteis*', *J.P. de Tournefort* (P-TRF 3644: photo MSB!; isolectotypes B-W 14081, M!)

Astragalus cylindraceus DC.

Astragalogia: 223. 1802, *syn. nov.* — TYPE: '*A. orientalis foliis viciae incanis, caule nudo erecto, floribus luteis*', *J.P. de Tournefort* (*vidi olim in hb. Desfontaines*; holotype P-TRF 3621, photo MSB!).

Astragalus hirsutus Vahl

Symb. Bot. 1: 59. 1790. — LECTOTYPE (Podlech 1998): '*A. orientalis, candidissimus tomentosus*', *J.P. de Tournefort* (P: hb. Vaillant: photo MSB!; isolectotype B-W 14080).

Astragalus alindanus Boiss.

Diagn. pl. orient., ser. 1, 2: 85. 1843, *syn. nov.* — HOLOTYPE: Turkey. In Caria prope Moglah, Alindam veterum, *P.M.R. Aucher-Eloy 1326* (G-BOIS; isotypes G, P!)

Astragalus hyalolepis Bunge

Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 105. 1868. — SYNTYPES: In Iberia, Alvar, *Wilhelms*; prope Trialethi, *E. Wiedemann* (LE, P: photo MSB!); ad lacum Goktscha ad pedem montis Tschitschaglu, 2000 m, *A. P. Owerin* (P: photo MSB!); *ibid. N. von Seidlitz* (P: photo MSB!).

Astragalus incertus Ledeb.

Fl. Ross. 1: 647. 1843. — HOLOTYPE: Turkey. Mt. Ararat, *Hehn* (LE).

Astragalus sibthorpianus Boiss.

Diagn. pl. orient., ser. 1, 2: 84. 1843. — *Tragacantha sibthorpiana* (Boiss.) Kuntze, Revis. Gen. 2: 948. 1891. — SYNTYPES: In Olympo Bithyno, *J. Sibthorp*; *ibid.*, *P.M.R. Aucher-Eloy 1348* (BM!, G-BOIS, K!, LE, MSB!, OXF, P); *ibid.*, VII.1842, *E. Boissier*.

Astragalus paecilanthus Boiss. & Heldr.

Diagn. pl. orient., ser. 2, 6: 54. 1859, *syn. nov.* — SYNTYPES: Turkey. Davros Dagh Pisidiae, 1530 m, 26.V. (fl.) (K!, OXF); *ibid.*, 6.6. (fr.) 1845 *T.H.H. von Heldreich* (G-BOIS, K!, P, W!); Ouchak [Usak] Phrygiae, alt. 3000´, 26.V. & 6.VI.1857, *B. Balansa 1127* (B!, BM, G, JE, K!, LE, MSB!, OXF, P!, W!); Davros Dagh Pisidiae, 5000–6000´, 28.V.1848, V.1848 *T.H.H. von Heldreich*.

Astragalus stenosemioides D.F. Chamb. & V.A. Matthews

Notes Roy. Bot. Gard. Edinburgh 29: 303. 1969. — HOLOTYPE: Turkey, B5 Kayseri, Cappadocia, mt. Argaei (Erciyas Da.), 2700–3000 m, 18.VI.1890 *J.F.N. Bornmüller 1637* (BM; isotypes: B!, BR, K!, W!).

Astragalus stenosemius Boiss.

Diagn. pl. orient., ser. 2, 2: 27. 1856. — *Tragacantha stenosemia* (Boiss.) Kuntze, Revis. Gen. 2: 948. 1891. — HOLOTYPE: Turkey. Prope Bakker Maaden (Ardana) Armeniae meridionalis, VI.1852 *F.W. Noë 813* (G-BOIS; isotype W!).

2. *Astragalus* section *Synochreati* DC.

Prodr. 2: 291. 1825. — LECTOTYPE (Podlech 1990): *Astragalus fragrans* Willd.

Astragalus acmonotrichus Fenzl

Pug. Pl. Nov. Syr.: 5. 1842. — HOLOTYPE: Turkey. Taurus occ., *Th. Kotschy 121* (W!; isotypes K!, LE, P!)

Astragalus ocakverdii Kit Tan & Sorger

Pl. Syst. Evol. 154: 115. 1986, *syn. nov.* — HOLOTYPE: Turkey. C4, prov. Konya, Seydisehir, Tınaz Dağı, 1600 m, 11.VI.1981 *H. Ocakverdi 1348* (KNYA!; isotypes E!: photo MSB!)

Astragalus seydishehiricus Kit Tan & Ocakv.

Notes Roy. Bot. Garden Edinburgh 44: 157. 1986, *syn. nov.* — HOLOTYPE: Turkey. C4 Konya, Seydişehir, north side of Kuyucak Dağ, c. 1500 m, 28.VI.1982 *H. Ocakverdi 1628* (E!: photo MSB!)

Astragalus isparticus Kit Tan & Sorger

Aliso, 11: 626. 1987, *syn. nov.* — HOLOTYPE: Turkey. C3 Isparta, Çiçekdağ, 1800 m, 17.VI.1967 *F. Sorger 67-6-15* (W!; isotype: E!)

Astragalus fragrans Willd.

Sp. Pl. 3: 1294. 1802. — HOLOTYPE: '*A. orientalis minimus foliis viciae, flore ex viridi-flavescente odoratissimo*', *Tournefort* cor. 28 (B-Willd. 14049; isotypes BM!, P-TRF 3620).

Astragalus kangalicus Kit Tan & Sorger

Aliso 11: 626. 1987, *syn. nov.* — HOLOTYPE: Turkey. B6

Sivas, 40 km E Kangal, 2000 m, 29.VI.1970 *F. Sorger 70-22-62* (W!)

Astragalus gaeobotrys Boiss. & Balansa

Diagn. pl. orient., ser. 2, 2: 29. 1859. — HOLOTYPE: Turkey. In Phrygiae monte Almadagh, au nord d'Ouchak, 10.VI.1857 *B.Balansa 325* (G; isotype P!)

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Appendix. Morphological characters of species after revision.

	A. incertus	*A. hyalolepis*	*A. bicolor* subsp. *bicolor*	*A. bicolor* subsp. *karputanus*	*A. caudiculosus*	*A. andrasovszkyi* syn. *A. vuralii*
Stipules	free	free	free	free	free	free
• length	6–8(–12) mm	5–10 mm	4–8 mm	4–8 mm	3–6 mm	4–8 mm
• shape	narrowly triangular to triangular	ovate to narrowly ovate	triangular	triangular	ovate-triangular	ovate
Leaves	2–10 cm	4–10(–15) cm	2.5–12 cm	2.5–12 cm	1.5–5 cm	1–12 cm
Leaflets						
• length	2–9 mm	5–13(–17) mm	2.5–12 cm	2.5–12 cm	3–10 mm	4–15(–20) mm
• paired	10–18(–20)	4–8	8–15	5–8	5–11	3–8
• shape	narrowly elliptic to elliptic or ovate	narrowly elliptic to elliptic	oblong to narrowly elliptic	oblong to narrowly elliptic	narrowly elliptic to elliptic	narrowly elliptic to oblong or narrowly obovate
• indumentum	sub-bifurcate sericeous	sparsely hairs to glabrescent on upper side, densely with (–) hairs on underside	silvery hairs, sometimes on upper side more sparsely hairs than on unerside	silvery hairs, sometimes on upper side more sparsely hairs than on unerside	(–) bifurcate sericeous	silvery hairs
Peduncle	1.5–14 cm	5–12(–22) cm	(2–)5–23 cm	(2–)5–23 cm	1–7 cm	2–13 cm
Infloroscence	10–15-flowered	10–20-flowered	5–25-flowered	5–25-flowered	5–10-flowered	3–10-flowered
• size	1–1.5 × 1.3 cm	2.5–4 × 2–3 cm	1.5–3 × 1.5–2.5 cm	1.5–3 × 1.5–2.5 cm	2–2.5 × 1.5–2 cm	2–3 × 1.5–2 cm
• shape	globose, ovoid or shortly oblong	ovoid to oblong	globose to ovoid	globose to ovoid	globose	ovoid to shortly oblong
Bracts						
• length	4–10 mm	3–7 mm	5–11 mm	5–11 mm	2–4 mm	2–6 mm
• shape	narrowly triangular	ovate to narrowly ovate	narrowly triangular	narrowly triangular	narrowly ovate	narrowly triangular
Bracteoles	absent	absent	absent	absent	absent	absent
Calyx						
• length	5–8 mm	8–12 mm	6–10 mm	6–10 mm	6–9 mm	7–10 mm
• shape	tubular	tubular	tubular-campanulate	tubular-campanulate	tubular	tubular-campanulate
• indumentum	long sub-bifurcate white and with shorter bifurcate black hairs	(–) white and black hairs, often predominantly black hairs	densely long (l) sub-bifurcate white hairs and with short (–) white and some black hairs	densely long (l) sub-bifurcate white hairs and with short (–) white and some black hairs	(–) black and white bifurcate hairy	(–) long white and short black hairs
Calyx teeth						
• length	2–3 mm	2–4 mm	3–5 mm	3–5 mm	0.5–1.5 mm	2–3 mm
• shape	subulate	narrowly triangular to linear	subulate	subulate	linear-subulate	linear to subulate

Corolla						
• color	violet purplish-red or lilac	violet, lilac or purple	whitish, lavender, bluish, violet or yellow	whitish, lavender, bluish, violet or yellow	purple or violet	white, cream or lilac
• length (standard)	12–18 mm	22–28 mm	15–22 mm	15–22 mm	12–18 mm	18–21 mm
• shape (standard)	oblong to elliptic	oblong to narrowly elliptic	elliptic to ovate	elliptic to ovate	narrowly rhombic-elliptic to rhombic	elliptic
Legume						
• size	8–13 × 3.5 mm	7–14 × 3–5 mm	8–11 × 3–4 mm	8–11 × 3–4 mm	5–9 × 3–4 mm	7–10 × 4–6 mm
• shape	narrowly ovate	ovoid-oblong	ovoid to oblong	ovoid to oblong	ovoid-oblong	ovoid
• indumentum	(l) long sub-bifurcate hairs	(l) white and black hairs	densely long (l) bifurcate white hairs	densely long (l) bifurcate white hairs	(l) sub-bifurcate long white hairs and a few (–) short black hairs	densely long (l) sub-bifurcate white hairs
	A. alyssoides	*A. cataonicus*	*A. dumanii*	*A. hirsutus* (syn. *A. alindanus*)	*A. globosus* (syn. *A. cylindraceus*)	*A. sibthorpianus* (syn. *A. paecilanthus*)
Stipules	free	free	free	adnate	adnate	adnate
• length	2–6 mm	5–7 mm	5–8 mm	7–11 mm	6–12 mm	4–10 mm
• shape	narrowly triangular to triangular	narrowly triangular to ovate	ovate to narrowly ovate	narrowly triangular	narrowly triangular to triangular	ovate to narrowly triangular
Leaves	2–6 cm	1–4 cm	1–3 cm	1–8 cm	3–17 cm	(1–)3–10 cm
Leaflets						
• length	4–7 mm	4–7 mm	5–14 mm	4–12 mm	6–13 mm	3–10 mm
• paired	5–9	4–8	3–7	3–10	7–18	7–13
• shape	narrowly elliptic to narrowly oblong	obovate to elliptic	linear to linear-ellitic	oblong or narrowly elliptic to narrowly obovate	narrowly elliptic to elliptic	narrowly elliptic to narrowly obovate or elliptic
• indumentum	silvery hairy	silvery hairy	densely (–) white hairs	silvery pilose	the younger ones densely (–) white hairs, soon ± glabrescent especially on the upper side	on upper side sparsely hairs or glabrous, on underside densely white hairs
Peduncle	2–12 cm	0.5–2 cm	0.5–1.5 cm	1–5 cm	5–24 cm	0.5–10(–14) cm
Infloroscence	10–30-flowered	20–40-flowered	10–25-flowered	(7–)10–20-flowered	15–40-flowered	15–40-flowered
• size	1–4.5 × 1–2 cm	1.5–2.5 × 2–2.5 cm	1–2 × 1–2 cm	1–2.5 × 1–2.5 cm	1.5–3.5 × 2–2.5 cm	1.5–2 × 1–2 cm
• shape	globose to shortly cylindric	globose	globose to ovoid	globose to ovoid	globose to oblong	globose to ovoid
Bracts						
• length	2–4 mm	6–12 mm	4–6 mm	6–10 mm	6–10 mm	5–8 mm
• shape	narrowly triangular	narrowly triangular	narrowly triangular	linear	narrowly ovate	narrowly triangular
Bracteoles	absent	absent	absent	absent or 1–2, 2–4 mm	absent or 2, 2–3 mm	absent or 1, 3–4 mm

Continues

Appendix. Continued.

Calyx						
• length	4–7 mm	10–14 mm	7–10 mm	6–10 mm	7–12 mm	7–12 mm
• shape	tubular	tubular	tubular	tubular-campanulate	tubular	tubular-campanulate to tubular
• indumentum	densely (–) black and white bifurcate-pilose	densely villous with long (l) sub-bifurcate and short (–) white hairs, a few (–) black hairs	(–) black and white hairs	densely (l) long sub-bifurcate white hairs and with short black and white hairs	densely long sub-bifurcate white hairs and few (–) short black hairs	(l) white bifurcate-pilose, sometimes with additional black hairs
Calyx teeth						
• length	1–2 mm	5–7 mm	2–4 mm	2–3 mm	3–5 mm	3–5 mm
• shape	narrowly trianglar to linear	linear	linear	linear	linear	subulate
Corolla						
• color	white or lilac	lilac or purple	lilac	yellow	yellow	red or whitish yellow
• length (standard)	9–12 mm	13–18 mm	15–18 mm	14–18 mm	15–20 mm	12–18(–20) mm
• shape (standard)	elliptic to rhombic-elliptic	rhombic-obovate	elliptic	obovate or oblong-elliptic	oblong-obovate	oblong to oblong-elliptic
Legume						
• size	6–8 × 3–4 mm	14–16 × 6–8 mm	13–15 × 5–6 mm	7–9 × 2–3 mm	7–10 × 3 mm	6–10 × 2–3 mm
• shape	ovoid	ovoid	narrowly ovate	oblong to ovoid	oblong to narrowly ovoid	narrowly ovoid
• indumentum	(–) short black and white hairs and (l) long sub-bifurcate white hairs	long sub-bifurcate white hairs	long (l) sub-bifurcate white hairs	densely (l) long white sub-bifurcate hairs	long (l) bifurcate white hairs	densely with crispate (–) white hairs
	A. stenosemius	*A. stenosemioides*	*A. amoenus* (syn. *A. squalidus*)	*A. gaeobotrys*	*A. fragrans* (syn. *A. kangalicus*)	*A. acmonotrichus* (syn. *A. seydishehiricus*) (syn. *A. isparticus*) (syn. *A. ocakverdii*)
Stipules	Adnate	adnate	adnate			
• length	9–12 mm	5–10 mm	5–7 mm	5–12 mm	4–15 mm	4–11 mm
• shape	narrowly triangular	narrowly triangular to triangular	ovate to triangular	broadly ovate	ovate to narrowly ovate	ovate-narrowly ovate
Leaves	1–6 cm	1–3 cm	1–5 cm	6–10 cm	1.5–17 cm	2–14 cm
Leaflets						
• length	4–10 mm	3–6 mm	2–8 mm	2–12 mm	8–14 mm	(2–)5–12 mm
• paired	4–10	5–8	4–10	10–18	7–20	8–20

• shape	narrowly elliptic to elliptic	narrowly elliptic or narrowly obovate to elliptic	narrowly elliptic to narrowly obovate, rarely oblong	elliptic narrowly ovate	oblong-narrowly elliptic or sometimes linear	elliptic to obovate
• indumentum	densely (–) short white hairs	bifurcate sericeous	on upper side sparsely hairy to glabrous, on underside densely (–) shortly white hairs	(–) bifurcate pilose	on upperside sparsely hairy to glabrous, on underside sparsely to loosely (–) hairy	glabrescent to densely bifurcate sericeous
Peduncle	2.5–10 cm	1–3 cm	0.5–8 cm	2–6 cm	0.5–10 cm	1–7 cm
Infloroscence	20–40-flowered	15–25-flowered	10–20-flowered	7–15-flowered	10–25-flowered	10–30-flowered
• Size	1.5–4 × 1–1.5 cm	1.5–2 × 1–1.5 cm	1–2.5 × 1–2 cm	2.5 × 2–2.5 cm	2–4 × 2.5–3 cm	
• shape	ovoid or shortly cylindrical	globose to shortly oblong	globose to oblong	globose	globose to ovoid	globose-ovoid
Bracts						
• length	5–10 mm	5–8 mm	2–4 mm	4–7 mm	(2–)4–6 mm	3–6 mm
• shape	narrowly ovate to often keeled	narrowly triangular	narrowly triangular	narrowly triangular	narrowly triangular-linear	linear to narrowly triangular
Bracteoles	absent	absent or 2, ca. 2 mm	absent or 1, 0.5–1 mm	absent	absent or 2	absent or 2
Calyx						
• length	7–10 mm	6–7 mm	6–9 mm	6–9 mm	7–10(–11) mm	6–11 mm
• shape	tubular	tubular	tubular-campanulate	tubular	tubular	tubular
• indumentum	densely long (l) white hairs	densely (–) black and white or sometimes only white hairs	sparsely with (l) predominantly white hairs or sometimes with predominantly black hairs	predominantly sparsely to densely (–) black bifurcate-villous	sparsely (–) black or black and white bifurcate hairy	sparsely-densely long black and white bifurcate pilose
Calyx teeth						
• length	4–5 mm	3–4 mm	2–4 mm	1–3 mm	(1–)2–4 mm	1–3 mm
• shape	linear	linear	Subulate	narrowly triangular	narrowly triangular	triangular-linear
Corolla						
• color	purple to crimson	purplish-pink	yellow or purple	purplish-yellowish	yellow	purple or purplish-yellow
• length (standard)	10–14 mm	9–12 mm	12–15(–17) mm	13–16 mm	14–17 mm	14–18 mm
• shape (standard)	narrowly pandurate-oblong	oblong-pandurate	Ovate	broadly elliptic	ovate elliptic	broadly ovate
Legume		unknown				
• Size	6–10 × 3–4 mm		7–10 × 3 mm	8–15 × 5–6 mm	10–18 × 3–4 mm	17–30 × 2–4 mm
• Shape	narrowly ovoid		narrowly ovoid to oblong	oblong, straight or slightly curved	falcate	narrowly cylindrical and falcate
• indumentum	densely white hairs		densely white bifurcate-pilose to villous	sparsely to densely white (–) simple-pilose	sparsely white (–) bifurcate-pilose	(–) bifurcate white hairy

(l) spreading, (–) adpressed