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Anatomical study of *Astragalus* sect. *Caprini* (Leguminosae) from NE Iran

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Abstract

This study has examined petiole and leaflet anatomy characteristics of 20 species of the *Astragalus* sect. *Caprini* from Khorassan Razavi Province (NE Iran) using light microscopy. For this, cross section of the fresh or dried mature petiole and leaflet were prepared and stained by differential staining. Some differences were noticed in the outline shape of petioles cross section, the shape of pith, the number of collenchymatous layers and vessels, the type of sclerenchymatous tissue above the phloem and the shape of midrib and lamina. Here is suggested this study on the other species of sect. *Caprini* around the world. Maybe stem anatomy results can show some differences among the species.

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Introduction

Astragalus L. sect. *Caprini* de Candolle of the family Leguminosae comprises about 280 species of a total of 2850 in the genus. This section is widely distributed throughout North Eurasia, North Africa, West Europe, and Central Asia (Podlech, 1986 a; b; 1988; 1999; Maassoumi, 1998; Podlech and Maassoumi, 1987a; b; Podlech and Zarre, 2013). There are 115 species of sect. *Caprini* in Iran of which 28 species are found in NE Iran (Massoumi, 1998). In this research, the cross sections of petioles and leaflets of 20 species of *Astragalus* sect. *Caprini* in NE Iran were studied. Anatomical studies have previously been made of the variation in petiole anatomy of the European spiny species of *Astragalus* L. (Leguminosae- Papilionoideae- Galgeae) (Haddad and Barnett, 1989), evolution of rachis thorn in *Astragalus* and *Astracantha* Podlech (Leguminosae) and the systematic applicability of thorn anatomy (Engle, 1991), Spine anatomy and its systematic application in *Astragalus* sect. *Rhacophorus* Bunge. (Fabaceae) in Iran (Pirani *et al.*, 2006), Petiole anatomy in *Astragalus* sect. *Incani* DC. (Fabaceae) in Iran (Mehrabian *et al.*, 2007), Two new species of *Astragalus* sect. *Anthylloideae* DC. (Fabaceae) in which petiole anatomical characteristics was assessed (Sabaii *et al.*, 2007). Despite above studies, leaflet and petiole internal structure of *Astragalus* sect. *Caprini* has not been reported. So the present survey was carried out to evaluate the variation of anatomical characteristics in the both organs.

Materials and methods

The fresh or dried mature leaflet and petiole of 20 *Astragalus* species from NE Iran were selected and fixed in FAA solution (Table. 1). Manual cross sections were made from the middle part of petioles and leaflets, after decolourization, stained by green methyl and carmine then, photographed in different magnification of light microscopy ZEISS model 1.25X, CONTAX camera model 167MT. Some anatomical characteristics were assessed such as the outline shape of petioles and pith cross sections, the type of sclerenchymatous cells above the phloem, the number of vascular bundles and collenchymatous layers in petiole, the shape of midrib, lamina and the type of mesophyll.

Results and discussion

The results showed variation in the outline shape of petiole cross section and pith, the number of vascular bundles, the type of sclerenchymatous tissue above the phloem and the number of collenchymatous layers and vascular bundles. In leaflet, the mesophyll was isolateral and, the shape of lamina and midrib in abaxial and adaxial sides, the type of sclerenchymatous tissue above the phloem and the number of collenchymatous layer were variable. The anatomical characteristics of the petiole and leaflet are summarized in Table 2, 3 and Fig.s 1A-X, 2A-T.

Table 1. Studied specimens of *Astragalus* sect. *Caprini*.

Species	Locality
<i>Astragalus assadii</i> Podlech and Maassoumi	Chenaran, Frizi, DahaneJaji village, 1900 m, Saghi 9186 (IAUM); Dahane Akhلامad, 1600 m, <i>Aiatolahi & Rezaei 12714</i> (FUMH); 9 km south of Quchan, Nowrouzi 1600 m, <i>Ghoreishalhosseini 949G</i> (FUMH); Chenaran, Frizi, Dahane Jaji village, Ghourghi 1500 m, <i>Faghihnia & Mehrvar 24111</i> (FUMH).
<i>A. callainus</i> Podlech	South of Kalat Naderi, Jarf village, Sareh Meidan, 1950 m, <i>Rafeei and Zangouei 27415</i> (FUMH); 15 km Kashmar to Neyshabour road, 1500 m, <i>Faghihnia&Zangouei 18439</i> (FUMH); Taiebad, north west of

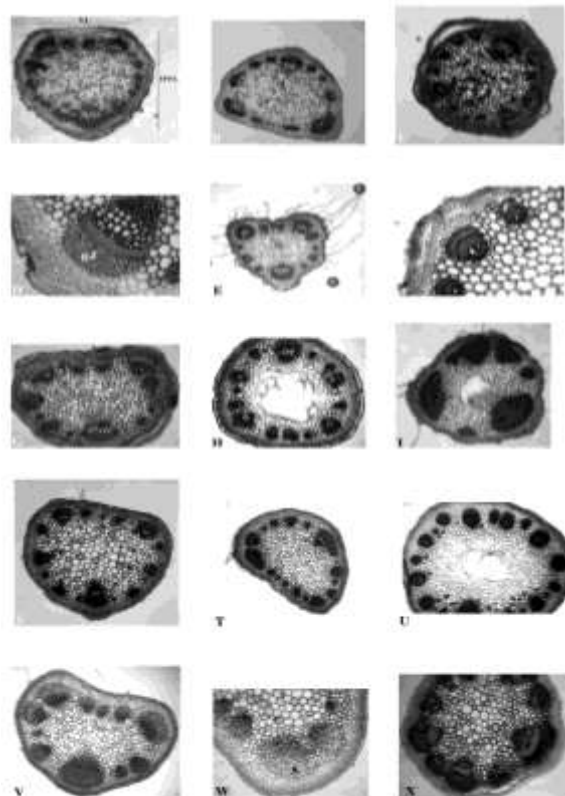
Species	Locality
	Torbate Jam, Kordian, 1600 m, <i>Aiatolahi&Zangouei 12497</i> (FUMH); East of NeyshabourKharvOlia, 1650 m, <i>Joharchi&Zangouei 18568</i> (FUMH).
<i>controversus</i> Maassoumi and Podlech	Torbate Heidarieh, Kadkan, Begh mount, 1900 m, <i>Faghihnia & Zangouei 17577</i> (FUMH).
<i>A. erubescens</i> Podlech	Chenaran, Frizi, Binaloud mount, Cheshmeh Parishan, 2400 m, <i>Faghihnia & Zangouei 23614</i> (FUMH); Sarakhs, Mazdavand, 1000 m, <i>Aiatolahi & Rezaei 12012</i> (FUMH); 110 km Sarakhs to Mashhad, 900 m, <i>Faghihnia & Zangouei 19499</i> (FUMH); Kashmar, south west of Rivash, Band Ghora, 1800 m, <i>Faghihnia & Zangouei 25383</i> (FUMH).
<i>A. esferayenicus</i> Podlech and Maassoumi	Chenaran, Golmakan, Cheshmeh Sabz, <i>Aiatolahi & Zangouei 13273</i> (FUMH); Mashhad, Mej, Martalkh, 2300 m, <i>Joharchi & Zangouei 22028</i> (FUMH); South of Mashhad, Moghan mount, 2500 m, <i>Joharchi 33656</i> (FUMH); 12 km south of Zoshk, 2100- 2500 m, <i>Joharchi & Zangouei 22028</i> (FUMH).
<i>A. gompholobium</i> Bunge	Kashmar, Kouhsorkh, 1600 m, <i>Saghi, 9203</i> (IAUM); Chenaran, Golmakan, Esjil river, <i>Joharchi 12552</i> (FUMH); South of Sabzevar, Halakabad mount, 1300 m, <i>Joharchi & Zangouei</i> (FUMH); South west of Torbat Heidarieh, Piriahoo, 1300 m, <i>Faghihnia & Zangouei 23803</i> (FUMH).
<i>A. jarmolenkoi</i> Gontsch.	65 km Quchan to Dargaz, 2050 m, <i>Saghi 9204</i> (IAUM); Dargaz, Tandoureh, between Shekarab and Chehelmir, 1000 m, <i>Joharchi&Zangouei 20477</i> (FUMH).
<i>kashmarensis</i> Maassoumi and Podlech	50 km Kashmar to Neyshabour, 1800 m, <i>Saghi 9205</i> (IAUM); 4 km Ghadamgah to Neyshabour, Eshaghabad village, 1250 m, <i>Faghihnia and Zangouei 17495</i> (FUMH); Kashmar to Neyshabour, 50 km Kashmar, 1600 m, <i>Faghihnia and Zangouei 18455</i> (FUMH).
<i>l. macropelmatus</i> Bunge. subsp. <i>pseudobuchtormensis</i> Podlech	East of Quchan, SombolTappéh, between Goukan and Kachlanlou, <i>Joharchi & Zangouei 14420</i> (FUMH); 80 km Gonabad, Lout Omrani, 1100 m, <i>Faghihnia & Zangouei 19727</i> (FUMH); Between Torbat Heydarieh and Kameh, 1050 m, <i>Joharchi & Zangouei 12859</i> (IAUM); West north of Kashmar, Kalate Teimour mount, 1800 m, <i>Faghihnia & Zangouei 24731</i> (FUMH); 5 km Taibad to Torbat Jam, 750 m, <i>Faghihnia & Zangouei 18283</i> (FUMH).
<i>A. nephtonensis</i> Freyn	Quchan, Kabkan, 1550 m, <i>Joharchi & Zangouei 16302</i> (FUMH); Chenaran, between Abghad and Ferizi, 1480 m, <i>Memariani & Zangouei 35851</i> (FUMH); Kashmar, west of Rivash, between Torogh and Kalate Teimour, 1500 m, <i>Hojat & Zangouei 32596</i> (FUMH); Dargaz, between Shekarab and Tandoureh, 1650-2000 m, <i>Rafei & Zangouei 29078</i> (FUMH); 60 km Sarakhs to Mashhad, 800 m, <i>Faghihnia & Zangouei 21401</i> (FUMH).

Species	Locality
<i>A. orbiculatus</i> Ledeb.	Ghaen to Gonabad, near Khezri, Pirmardashah, <i>Aiatolahi & Zangouei 14386</i> (FUMH); Between Kashmar and Neyshabour, Chalpo, <i>Zangouei 38848</i> (FUMH); Chalpo, Sargodarili, 1857 m, <i>Memariani & Zangouei 38920</i> (FUMH).
<i>A. pellitus</i> Bunge	Mashhad to Sarakhs road, Chahal hills, <i>Joharchi & Zangouei 14530</i> (FUMH); Torbat Heydarieh, Kameh Olia village, 1780 m, <i>Saghi 9206</i> (IAUM); 10 km Quchan to Dargaz, 1500 m, <i>Saghi 9208</i> (IAUM); 4 km Ghadamgah to Neyshabour, Eshaghabad village, 1250 m, <i>Faghihnia & Zangouei 17494</i> (FUMH); East south of Torbat Jam, Ghaleh Hamam, 800 m, <i>Joharchi & Zangouei 19679</i> (FUMH).
<i>pseudobrachystachys</i> Sirj and Rech.	35 km Gonabad to Tobat Heidarieh, 850 m, <i>Rafei & Zangouei 25126</i> (FUMH); 5 km Taibad to Torbat Jam, 750 m, <i>Faghihnia & Zangouei 19727</i> (FUMH).
<i>pseudoindurascens</i> Sirj and Rech.	Mashhad, Zoshk valley, Abdolah river, 2200 m, <i>Saghi 9209</i> (IAUM); Mashhad, Kang mount, 2100 m, <i>Faghihnia & Zangouei 27601</i> (FUMH); Chenaran, Frizi to Abghad, 1600 m, <i>Emadzadeh & Memariani 36476</i> (FUMH).
<i>Pseudokurrumensis</i> Sirj. and Rech.	Hezar Masjed mount, 2700 m, <i>Saghi 9210</i> (IAUM).
<i>A. renzianus</i> Podlech	Mashhad, Pivehgen mount, 1900 m, <i>Zangouei & Faghihnia 22334</i> (FUMH); Taiebad, Abghad, Kordian, 1500 m, <i>Aiatolahi 12496</i> (FUMH); Fariman to Torbat Jam, RekhnehGorg village, 1250 m, <i>Zangouei & Faghihnia 26767</i> (FUMH); Neyshabour, Boujan, <i>Rashed & Aiatolahi, 13439</i> (FUMH); South of Sabzevar, Halakabad mount, 1300 m, <i>Joharchi & Zangouei 16185</i> (FUMH).
<i>A. reticulate-versus</i> Maassoumi and Podlech	South west of Sarakhs, Maadan Agh Darbandmout, 1500 m, <i>Hojat & Zangouei 31922</i> (FUMH); South east of Torbat Heidarieh, Piriahou, 1300 m, <i>Faghihnia & Zangouei 23804</i> (FUMH); south west of Torbat Jam, Miansara mount, 1300 m, <i>Joharchi 34037</i> (FUMH).
<i>A. schmidii</i> Podlech	North west of Torbat Heydarieh, 7 km Fahdieh to Roud Maajan, 1610 m, <i>Rafei & Zangouei 26808</i> (FUMH); South east of Kalat Naderi, Khanezou stream to Cheshmeh, 1450 m, <i>Rafei & Zangouei 27331</i> (FUMH); 10 km Mashhad to Sarakhs, <i>Joharchi & Zangouei 21921</i> (FUMH).
<i>subrosulariformis</i> Sirj. and Rech.	Mashhad, Zoshk valley, Abdolah stream, north slope of Binaloud mount, 2500 m, <i>Saghi 9211</i> (IAUM); Quchan to Dargaz, Tivan Kenar pass, 2220 m, <i>Saghi 9212</i> (IAUM); South of Kalat Naderi, Jarf village, Sareh Meidan, 1950 m, <i>Rafei & Zangouei 27415</i> (FUMH).
<i>touranicus</i> Freitag and Podlech	Chenaran to Golmakan, Esjil stream, <i>Joharchi 12550</i> (FUMH); Maadan Agh Darband mount, 1500 m, <i>Hojat & Zangouei 31931</i> (FUMH)

Table 2. Petiole characteristics of studied *Astragalus* sect. *Caprini* specie.

Species	VL/DVL	The shape of petiole outline	The shape of pith	LVB/TVB	The type of sclerenchymous cell	SD/VBD	The number of collenchymous cell
<i>A. assadii</i>	0.63	Cordate	Circular	5/14	Gelatinous fiber	2.5	4
<i>A. callainus</i>	1	D shaped	Circular	5/14	Gelatinous fiber	2.5	3
<i>A. Controversus</i>	1	Circular	Circular	3/14	Gelatinous fiber	2.3	3
<i>A. erubescens</i>	1.07	Cordate	rectangular	3/10	Fiber	0.9	3
<i>A. Esferayensis</i>	0.91	D shaped	Elliptic	5/13	Gelatinous fiber	3	2
<i>A. gompholobium</i>	0.92	Circular	Circular	5/14	Sclerid- fiber	0.28	3
<i>A. jarmolenkoi</i>	0.8	Cordate	Elliptic	3/9	Gelatinous fiber	2.5	3
<i>A. kashmarensis</i>	0.57	D shaped	Star shaped	4/11	Gelatinous fiber	1.25	2
<i>A. macropelmatus</i> subsp. <i>pseudobuchtormensis</i>	0.7	Elliptic	elliptic	3/12	Fiber	1	3
<i>A. nephtonensis</i>	0.87	Elliptic	elliptic	3/11	Gelatinous fiber	2.5	3
<i>A. orbiculus</i>	0.61	Cordate	elliptic	3/8	Fiber	1	3
<i>A. pellitus</i>	0.57	Circular	Star shaped	4/7	Gelatinous fiber	4.5	2
<i>A. pseudobrachystachys</i>	1.18	Circular	circular	5/21	Fiber	1	3
<i>A. pseudoindurascens</i>	0.83	D shaped	elliptic	7/23	Gelatinous fiber	1.75	4
<i>A. pseudokurramensi</i>	0.63	Circular	circular	3/13	Sclerid	1.5	2
<i>A. renzianus</i>	0.82	D shaped	circular	5/13	Gelatinous fiber	2.5	2
<i>A. reticulate-versus</i>	1.21	D shaped	D shaped	8/18	Sclerid- fiber	2.5	2
<i>A. schmidii</i>	1.22	Elliptic	Elliptic	8/20	Sclerid- fiber	1.33	5
<i>a. subrossulariformis</i>	1.08	Cordate	Elliptic	6/11	Sclerid	1.5	3
<i>A. touranicus</i>	0.93	Circular	Star shaped	5/11	Sclerid- fiber	3	3

VL/DVL: ventral axis length/dordi- ventral axis length, LVB/TVB: large vascular bundle number/total vascular bundles number,SD/VBD: The diameter of sclerenchymatous above the phloem/the diameter of phloem.



40. I) *A. pellitus* × 40. J) *A. jarmolenkoi* × 40. K) *A. kashmarensis* × 40. L) *A. macropelmatus* subsp. *pseudobuchtormensis* × 40. M) *A. nephtonensis* × 40. N) *A. orbiculus* × 40. O) *A. pseudobrachystachys* × 40. P) *A. pseudoindurascens* × 40. Q) *A. pseudokurramensis* × 40. R) *A. renzianus* × 40. S) *A. reticulate-venosus* × 40. T) *A. schmidii* × 40 U, V) *A. subrossularis* × 40, 100. W,X) *A. touranicus* × 40, 100. f: fiber, s: sclerid, g.f: gelatinous fiber.

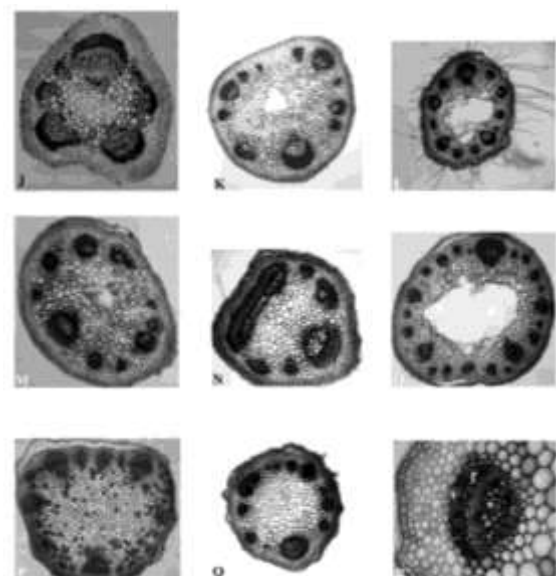


Fig. 1. Micrographs of petiole cross section: A) *A. assadii* × 40. B) *A. callainus* × 40. C, D) *A. controversus* × 40, 100. E, F) *A. erubescens* × 40, 100. G) *A. esferyanicus* × 40. H) *A. gompholobium* ×

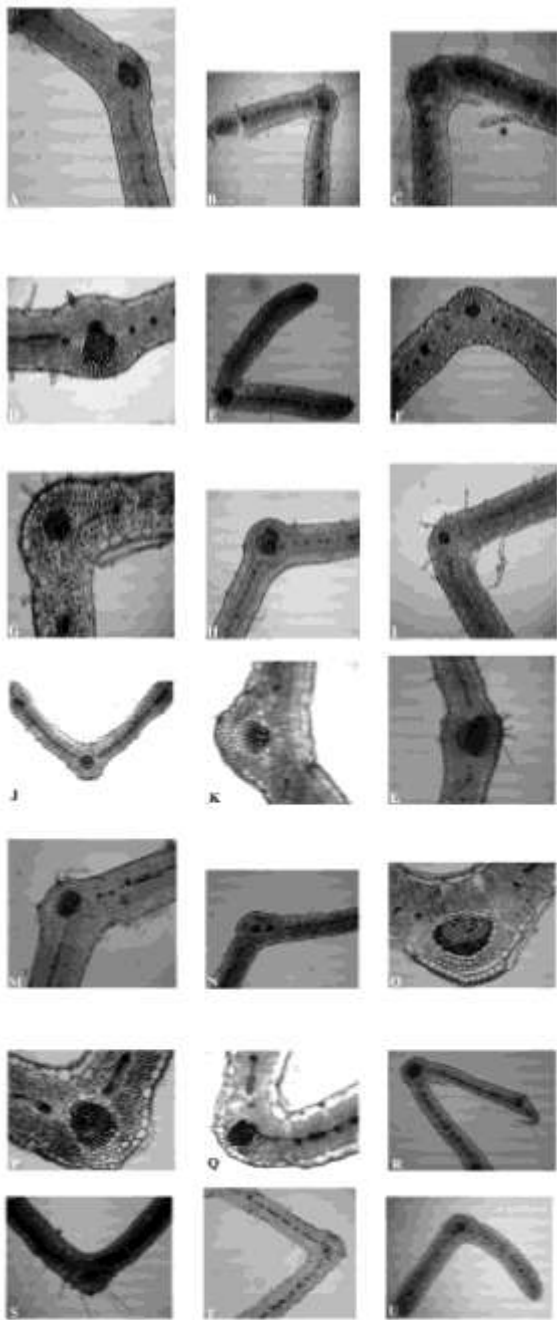


Fig. 2. Micrographs of leaflet cross section: A) *A. assadii* × 40. B) *A. callainus* × 40. C) *A. controversus* × 40. D) *A. erubescens* × 100. E) *A. esferyanicus* × 40. F) *A. gompholobium* × 40. G) *A. jarmolenkoi* × 100. H) *A. kashmarensis* × 40. I) *A. macropelmatus* subsp. *pseudobuchtormensis* × 40. J) *A. nephtonensis* × 40. K) *A. pellitus* × 100. L) *A. orbiculatus* × 40. M) *A. pseudobrachystachys* × 40. N) *A. pseudoindurascens* × 40 O) *A. pseudokurruensis*. × 100. P) *A. renzianus* × 100. Q) *A. reticulate-venosus* × 40. R) *A. schmidii* × 40. S) *A. subrussolaris* × 40. T) *A. touranicus* × 40.

In this research, four main outline shape of petiole cross section (D-shaped, circular, elliptic and cordate) and five petiole pith shape (circular, elliptic, rectangular, star shaped and D-shaped) were observed. The minimum and maximum VL/DVL (ventral axis length/dorsi ventral axis length) were in *A. pellitus* Bunge, *A. kashmarensis* Maassoumi and Podlech (0.57) and *A. schmidii* Podlech (1.22) respectively. Also, the number of large vascular bundles/total number of vascular bundles changed among the species. The minimum and maximum number of vascular bundles were in *A. pellitus* (7) and *A. pseudoindurascens* (Sirj. and Rech. f.) (23). Also, four types of sclenchymatous cells were noticed in strands above the phloem (i.e. fiber, gelatinous fiber, sclerid and fiber-sclerid). In all species, SD/VBD (sclerenchymatous strand above the phloem diameter/ phloem diameter) was more than 1 except *A. erubescens* Podlech and *A. gompholobium* Bunge less than 1. This ratio was 1 in *A. macroplematus*, *A. orbiculatus* (Ledeb.) and *A. pseudobrachystachys* Sirj. & Rech.f. Based on Engle's report, SD/VBD > 1 is a derived character state so, all studied specimens except above species are advanced. The leaflet anatomical results displayed four main lamina shape (open V- shaped, close V- shaped, U-shaped and with angle 180°). Some variation was observed in the shape of midrib in adaxial and abaxial sides (flat, depressed and prominent) and the number of parenchyma and collenchyma layers. The maximum number of vascular bundles was noticed in *A. pseudoindurascens* while the minimum number was found in *A. controversus* Maassoumi and Podlech, *A. erubescens*, *A. jarmolenkoi* Gontcharov, *A. macroplematus*, *A. nephtonensis* Freyn, *A. orbiculatus*, *A. pseudokurruensis* Sirj. and Rech. f. In previous studies Haddad and Barnett showed variation in shape of petiole; number of cortical layers; number of vascular bundles; amount of collenchyma tissue and shape and abundance of pith cells in petiole transverse section from the 14 species of European spiny *Astragalus* (Haddad and Barnett, 1989). Sabaii *et al.* 2007 reported, that the petiole anatomy showed significant differences between *A. veiskaramii* Zarre, Podlech

and Sabaii and *A. halicacabus* Lam. in sect. *Anthylloidei*. The petioles of *A. veiskaramii* are larger in diameter, the main vascular bundles are larger and the pith cells are in 9-11 rows (compared with 12-14 cells rows in *A. halicacabus*)” (Sabaii *et al.*, 2007). Mehrabian *et al.*, 2007 assessed the number of parenchymatous cell layer in pith, number of vascular bundles, length of ventral and dorsi-ventral axis, diameter of ventral lateral vascular bundle (VLB) and diameter of dorsal median bundle (DMB) in cross sections of petioles in 24 species of *Astragalus* sect. *Incani*. They believe that the anatomical characters are not useful for circumscribing section but are suitable evidences for taxonomical differentiation in species in this section”. Pirani *et al.* 2006 in cross sections of 35 species of *Astragalus* mainly belonging to sect. *Rhacophorus*, mentioned number of parenchymatous cell layers in pith, diameter of median sclerenchymatous vascular bundle sheat and diameter of the cross section as whole can be informative.

In conclusion, based on the results of the present research, the outline shape of petiole cross section, type of cells in sclerenchymatous tissue above the phloem, lamina shape indicated variation but are not useful for circumscription and identification of *Astragalus* sect. *Caprini* species.

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