

PETIOLE ANATOMY IN ASTRAGALUS SECT. INCANI DC. (FABACEAE) IN IRAN (A PHYLOGENETICAL APPROACH)

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Cross sections of petioles in 24 species of *Astragalus* Sect. *Incani* has been studied for a comparison of anatomical characters based on phylogeny in this section. The characters which employed in this study are as follows: Numbers of parenchymatous cell layers in pith, number of bundles, length of ventral axis, length of dorsiventral axis, diameter of ventral lateral vascular bundle (VLB) and diameter of dorsal median bundle (DMB). Furthermore the evolutionary direction in anatomical characters (character polarity) is studied. The results reveal that anatomical characters are not useful for circumscribing section but are suitable evidences for taxonomical differentiation in species in this section and can be use for delimiting small natural groups. Moreover, they are helpful characters for determining some complexes in this section.

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آناتومی دمبرگ جنس گون، بخش *Incani* در ایران یک رویکرد فیلوژنتیکی

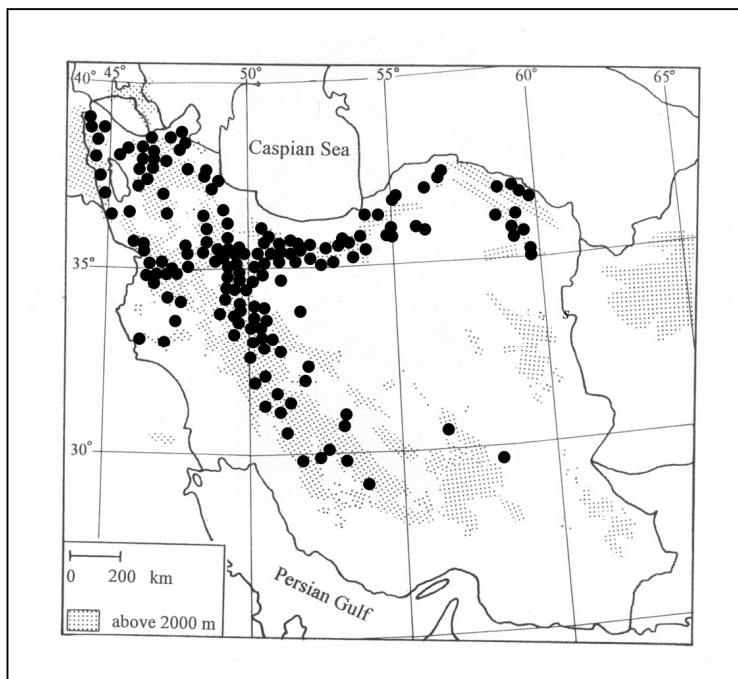
احمدرضا محرابیان، دکتر شاهین زارع، دکتر دینا عزیزیان و دکتر دیترش پودلش

برش‌های عرضی 24 گونه از جنس *Astragalus* متعلق به بخش *Incani* برای مقایسه صفات تشریحی مبتنی بر فیلوژنی مورد بررسی قرار گرفت. صفات مورد استفاده در این مطالعه عبارتند از: تعداد لایه‌های سلول پارانشیمی در مغز، تعداد دستجات آوندی، طول محور شکمی، طول محور پشتی-شکمی، قطر دسته آوندی شکمی جانبی و قطر دسته آوندی پشتی-میانی. به علاوه جهت گیری تکاملی (قطبیت صفات) در ساختارهای تشریحی مورد بررسی قرار گرفت. این مطالعه نشان می‌دهد که صفات تشریحی در تعیین محدوده بخش مناسب نیستند، اما در تمایز تاکسونومیک در سطح گونه مناسب بوده و می‌توانند در محدود نمودن گروه‌های طبیعی کوچک بکار روند. به علاوه در تشخیص برخی پیچیدگی‌های تاکسونومیک در این بخش مفید می‌باشند.

Introduction

Astragalus L. (*Fabaceae*) is generally considered as the largest genus of vascular plants with an estimated 2500-3000 species (Podlech 1986; Lock & Simonsen 1991). The centre of development seems to be in the arid and semi-arid mountain parts of northern hemisphere (Polhill 1981) with about 2500-3000 species. Sect. *Incani* DC. is one of the most important and species richest sections among medifixed hairy

Astragalus species which includes about 135 species worldwide. Iran with 73 species (see map1) have 57 endemic taxa (Rechinger & Dufler 1969; Ghahremani-nejad 2004; Podlech & Maassoumi 2003; Ranjbar et al. 2003; Zarre et al. 2005a, 2005b), Turkey with 27 species (Chamberlin & Mathews 1970), Armenia with 19 species, Azerbaijan with 16 species (Goncharov & Borissova 1965) and Iraq with 7 species (Townsend and Guest 1974) are the most important diversification centers of the section. The first species of sect. *Incani*



Map1. Distribution map of *Astragalus* sect. *Incani* in Iran.

was introduced as *A. incanus* by Linnaeus in 1775 (Ranjbar *et al.* 2005). Bunge (1868-1869) introduced a new section namely *Proselius* (*illegitimate name*), for *Astragalus* group with bifurcate hairs from the subgenus *Cercidothrix*. Among the modified hairy *Astragalus* of this section are a natural group and include a monophyletic clade based on molecular evidence (ITS sequences of nrDNA). (Kazempour Osaloo *et al.* 2003).

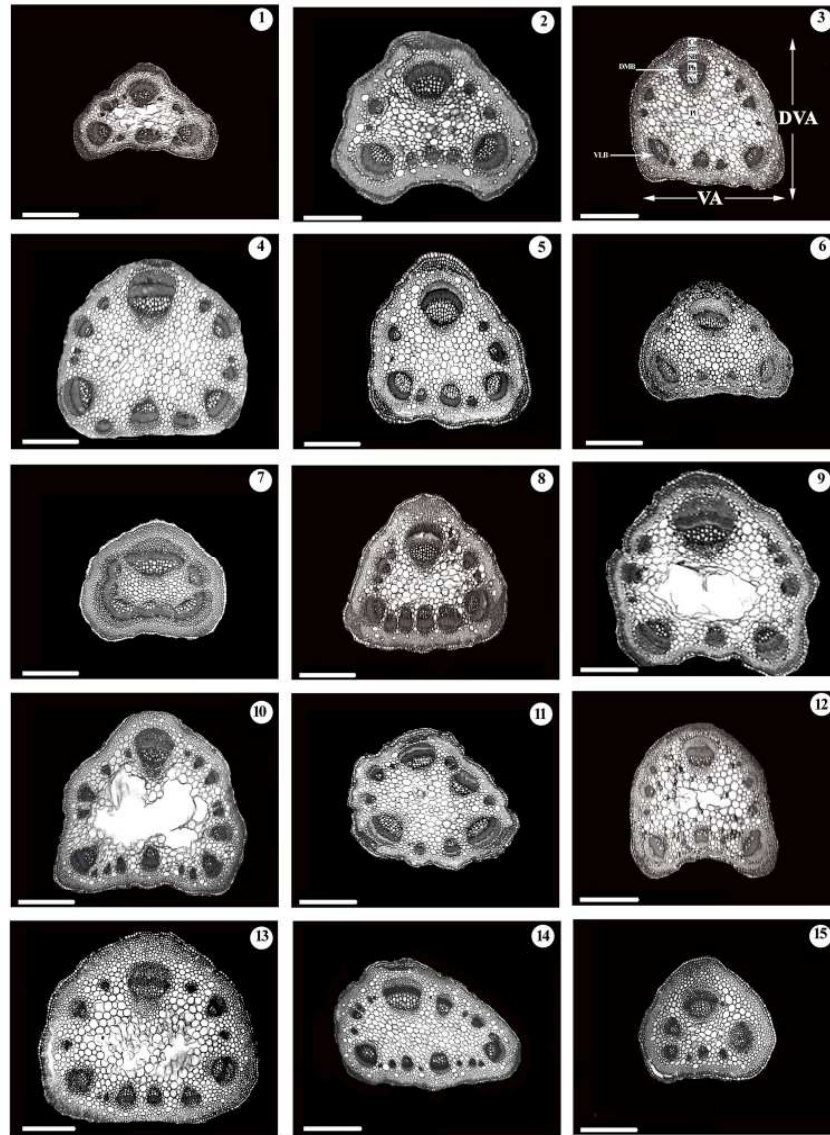
Material and methods

This study has been carried out on 24 species which to be included in Sect. *Incani* that distributed in Iran. Petioles from approximately 36 herbarium specimens of 24 species were surveyed. They were removed from herbarium specimens (see table1) at MSB, TUH, HSBU, TARI and IRAN (abbreviations according to Holmgren *et al.* 1990). We removed a part of the middle of mature petioles and smoothed by cooking in 5 minutes. Subsequently we fixed them in absolute FAA. After 24 hours petioles were removed and washed with fresh water, then inserted in Ethanol (70%). Cross sections were made by hand using commercial razor blades. The sections stained with Carmum and Methylen blue. Then we dehydrated them in ethanol gradient and finally toluene (99.5%). Later on mounted section on slides using Canada balsam and studied using an Olympus microscope CH2.

Results

There are three kinds of tissue in each cross section (see figs 1-15). The parenchymatous tissue at the periphery of petiole, which is the exterior, and is by single cell-layer of epidermis, called cortex. In the centre of the petiole there is a primarily parenchymatous tissue called pith. Vascular bundles are the third tissue. The length of ventral and dorsiventral axis, thickness of DMB (dorsal median bundle), VLBs (ventral lateral vascular bundle) and pith area, and number of bundles (first and second series) are measured in this research.

Data has been represented in table 2. Cross sections of petioles are shown in figs. 1-15. This structure is very variable in these species. Quantitative characters are as follows: The length of ventral axis (fig. 3) is from 0.29 μm (*A. siahcheshmehensis*) to 1.08 μm (*A. zanjanensis*). The length of dorsiventral axis varies from 0.28 μm (*A. siahcheshmehensis*) to 1.03 μm (*A. zanjanensis*). The thickness of DMB varies from 100 μm (*A. siahcheshmehensis*) to 250 μm (*A. fridae*). The thickness of VLB varies from 50 μm (*A. siahcheshmehensis*) to 200 μm (*A. ordubadensis*, *A. zanjanensis*). The thickness of pith area (cell layers) is between 3 (*A. incanus*) to 17 (*A. askius*). Number of bundle (first and second series) are between 4 (*A. subglaberrimis*, *A. siahcheshmehensis*) to 12 (*A. subglaberrimis*, *A. fridae*). Pith (Paranchymatous cells)



Figs: 1-15 Cross section of the petiole anatomy in selected species of *Astragalus* sect. *Incani*. Scale bars =0.1mm. 1- *A. abnormalis* Rech. f., 2- *A. cyclophyllos* Beck., 3- *A. demavendicus* Boiss. & Buhse., 4- *A. divandarrehensis* Podlech., 5- *A. dopolitanicus* Podlech., 6- *A. fuliginosus* Beck., 7- *A. glaucophyllus* Bunge., 8- *A. gudrunensis* Boiss. & Hausskn. 9- *A. homandicus* Maassoumi. & Podlech. 10- *A. ordubadensis* Grossh., 11- *A. paucifoliolatus* Podlech., 12 *A. punctatus* Bunge. 13- *A. rostratus* C. A. Mey., 14- *A. safavi* Podlech. & Maassoumi., 15- *A. supervisus* (Kuntze) Sheld.

Table 1. Herbarium vouchers of *Astragalus* sect. *Incani*.

Species	Locality	Date	Collector(s)	Herbarium no.
<i>A. abnormalis</i> Maassoumi & Podlech	Hamadan: Aq Bula Mountains, 100 km N Hamadan Alt.: 2000 m	1.7.1960	Rioux & Golvan	MSB-001901
<i>A. askius</i> Bunge	Tehran: Chalus road, toward Gadjereh, 3000 m	23.7.2005	Zarre, Mehrabian & Fritsch	HSBU-2005-100
<i>A. askius</i> Bunge	Tehran: Shemshak toward Deezin, Alt.: 3200 m	5.8.2005	Zarre & Mehrabian	HSBU-2005135
<i>A. askius</i> Bunge	Tehran: Jagrood, 5 km (N), toward high way, Alt.: 2000 m	25.5.2002	Mehrabian & Shafiee	HSBU-2002-130
<i>A. cyclophyllus</i> Beck.	Hamadan: Delijan to Taherlu, Jamshid-abad and Gonbad, Alt.: 1900 m	8.6.1988	Mozaffarian	TARI-64598
<i>A. cyclophyllus</i> Beck.	Hamadan: 30 km to Avaj	5.5.1996	Attar & Okhovat	TUH-19561
<i>A. demavandicus</i> Boiss. & Buhse	Elburz: Gatchsar toward Gajereh, Varang-round, Alt.: 2240-2500 m	1.6.1977	Termeh & Matin	IRAN-36733
<i>A. demavandicus</i> Boiss. & Buhse	Elburz: Gatchsar toward Gajereh, Varang-round, Alt.: 2240-2500 m	1.6.1977	Termeh & Matin	IRAN-36733
<i>A. divandarrehensis</i> Podlech	Hamadan: 45 km SW Hamadan, Alt.: 2150 m	9.6.1959	H. Pabot	MSB-001915
<i>A. dopolanicus</i> Podlech.	Hamadan: 45 Km SW Hamadan, 2150 m	9.6.1959	H. Pabot	MSB-001915
<i>A. fridae</i> Rech. f.	Semnan: ca 15 km from Semnan to Firouzkuh, Alt.: 1400 m	22.5.1987	Ghahreman & Mozaffarian	TUH-5823
<i>A. fridae</i> Rech. f.	Semnan: 20 km. quest de Semnan, pres de Momen-abad collines de Gypus, Alt.: 1200-1300 m	24.4.1975	Iranshahr	IRAN-40865
<i>A. fuliginus</i> Beck.	Guilan: Mendgele toward Zandjan, 18 km E Alt.: 1300 m	31.5.1971	-	IRAN-44009
<i>A. fuliginus</i> Beck.	Tehran: Damavand, road from Polour to Firouzkuh, Lasum, Alt.: 1900-2400 m	28.8.1991	Ghahreman & Mozaffarian	TUH-9952
<i>A. glaucophyllus</i> Bunge	Turkey: Amasya in valle Kryrass-dere pr. Amasia, 400 m	15.5.1890	Bornmüller	MSB-2802
<i>A. gudrunensis</i> Boiss. & Hausskn.	Kurdistan: ad radices montis Hamzeh Arab SE Bijar Alt.: 2000 m	-	-	MSB-0090721
<i>A. homandicus</i> Maassoumi & Podlech	Tehran: 1 km on the road to Demavand, main diviation	1.6.2002	Marofi & Maassoumi	MSB-001900
<i>A. homandicus</i> Maassoumi & Podlech	Tehran: Jajrod toward Rudehen	25.5.2004	Mehrabian & Yosefzadi	SBUH-2004-287
<i>A. incanus</i> L.	6 km SW Santa Cruz de Moya an der Straße nach Landete, 1.130 m	28.5.1988	Podlech	MSB-44141

Species	Locality	Date	Collector(s)	Herbarium no.
<i>A. longicuspis</i> Bunge	Azarbaijan Sharqi: N slopes of mt Kiamaki Dagh	6.6.2002	Maassoumi & Safavi	MSB-001918
<i>A. micrancistrus</i> Boiss & Hausskn.	Kurdistan: between Dizili, Alt.: 1500-2000 m	29.6.2001	Maassoumi & Safavi	MSB-1.16959
<i>A. ordubadensis</i> Grossh.	Azarbaidjan Sahrqi: 1 km from Harrangh to Duzel	16.4.1995	Ghahraman, Kasebi & Amini	MSB-001894
<i>A. paucifoliolatus</i> Podlech	Markazi: between Arak And Malayer, Mohageran Village, Alt.: 2000 m	5.6.2003	Mehraban	HSBU-326
<i>A. paucifoliolatus</i> Podlech	Hamadan: 15-29 km SE Hamasan, Yalfan Alt.: 1959-2200 m	10.6.1987	Termeh, Delghandi & Karavar	MSB-001889
<i>A. pseudorobustus</i> Podlech & Maassoumi	Kurdistan: Marivan, Tang-e-Dezli; 2km to Dezli	7.5.1996	Attar & Okhovat	TUH-19528
<i>A. punctuatus</i> Bunge	Azarbaijan Sharqi: Arpa, Darcocci, ca. 6 km E Tabriz, 1680 m	5.6.2002	Maassoumi, Safavi & Ghahremani	MSB-82501
<i>A. rostratus</i> C. A. Mey.	Guilan: Assalem Toward Khalkhal, 16 km Khalkhal Alt.: 2000 m	22.5.74	Termeh & Moussavi	IRAN-16482
<i>A. rostratus</i> C. A. Mey.	Azarbaijan: Talish, Dist. Lirik, Amburdore valley of river Orand-Tschaj	14.6.1979	Menschshkij & Toh. Popova	MSB-001883
<i>A. safavi</i> Podlech & Maassoumi	Zanjan: Ca 57 km on the road from Zanjan to Bigar Alt.: 1600 m	4.6.2002	Maassoumi & Safavi	MSB-001884
<i>A. subalpinus</i> Boiss & Buhse	Semnan: Shahrud, Kuh-e-Ghatry, S. slopes, Alt.: 2600 m	8.6.1973	Froroughi	MSB-001882
<i>A. subglaberrimus</i> Podlech & Massoumi	Tehran: Jajroud, 5km N of High way	7.6.2003	Mehraban & Azizi	HSBU-2003-198
<i>A. supervius</i> (Kuntze) Sheld.	Tehran: Darakeh, Kara Alt.: 2100 m	15.5.2003	Mehraban	HSBU-2003-105
<i>A. supervius</i> (Kuntze) Sheld.	Tehran: Sohanak Alt.: 1900 m	24.5.2004	Mehraban	HSBU-2004-235
<i>A. supervius</i> (Kuntze) Sheld.	Markazi: Arak, 15 km west of Arak, Maadan, Alt.: 1950 m	2.6.2003	Mehraban	HSBU-2003-248

consists of 4 layers (*A. siahcheshmehensis*, *A. subalpinus*) to 12 layers (*A. subglaberrimis*, *A. fridae*). In qualitative characters: The shape of cross section is variable from elliptic-ovate (*A. fridae*, *A. homandicus*), broadly ovate (*A. punctuatus*, *A. subalpinus*) to orbicular (*A. judrunensis*, *A. supervius*). The shape of DMB can be orbicular (*A. siahcheshmehensis*, *A. demavendicus*), suborbicular (*A. cyclophyllus*, *A. homandicus*), to elliptic (*A. fuliginosus*, *A. glaucophyllus*). In *A. fuliginosus* bundle sheath approximately is a connective ring.

Discussion

Anatomical characters have been the subject of only a few researches on the systematics of this genus.

Zarrinkamar (1996) studied gum conducting tissue in stem in Tragacanthic species. Hadad and Barnett (1989) and Engle (1991) studied many species of this genus with systematical approach (Table 3). A few studies have been carried out on the species of this section (Kazempoor Osaloo *et al.* 2003, Zarre *et al.* 2005; Ranjbar *et al.* 2005; Pirani *et al.* 2006). This is the first systematic study on this section which is based on phylogeny. Hadad & Barnett (1989) recognized two major patterns in petiole anatomy. The first group identified by small pith made up of thick walled parenchymatous cells along large amounts of sclerenchyma around the vascular bundle. The second group characterized by large pith composed of thin

Table 2. Selected characters from petiole anatomy of *Astragalus* Sect. *Incani*.

Species	character	Length of ventral axis (mm)	Length of dorsiventral axis (mm)	Diameter of DMB (μm)	Diameter of VLBs (μm)	Diameter of the pith (cell layers)	Number of bundles (first and second series)
<i>A. abnormalis</i>		0.48	0.38	100	60	9	6
<i>A. askius</i>		0.74	0.68	150	145	17	6
<i>A. cyclophyllus</i>		0.59	0.50	160	120	6	7
<i>A. demavendicus</i>		0.57	0.53	110	100	11	6
<i>A. divandarehnsis</i>		0.78	0.66	190	150	9	6
<i>A. doplanicus</i>		0.66	0.62	180	120	9	6
<i>A. fridae</i>		0.86	0.86	260	170	10	12
<i>A. fuliginosus</i>		0.49	0.37	100	100	11	6
<i>A. glaucophyllus</i>		0.40	0.45	154	120	7	6
<i>A. gudruensis</i>		0.65	0.40	150	110	6	6
<i>A. homandicus</i>		0.40	0.48	140	70	9	6
<i>A. incanus</i>		0.44	0.34	155	70	3	5
<i>A. longicuspis</i>		0.50	0.40	100	70	6	6
<i>A. micrancistrus</i>		1.06	1.02	210	200	16	7
<i>A. ordubadensis</i>		0.40	0.41	120	100	11	6
<i>A. paucifolius</i>		0.59	0.55	150	120	12	6
<i>A. pseudorobustus</i>		0.82	0.80	220	130	14	6
<i>A. punctuatus</i>		0.50	0.55	120	90	9	6
<i>A. rostratus</i>		0.84	0.71	190	110	7	8
<i>A. safavi</i>		0.72	0.50	150	80	9	8
<i>A. siahcheshmehensis</i>		0.29	0.28	100	50	4	4
<i>A. subalpinus</i>		0.48	0.38	120	80	4	4
<i>A. subglaberrimis</i>		0.85	0.86	170	120	15	12
<i>A. supervisus</i>		0.51	0.44	150	80	5	5
<i>A. zanzanensis</i>		1.08	1.03	220	200	14	8

Table 3. Character polarity of petiole anatomy in *Astragalus* (Engle 1991).

	Character evolution trend	Original character state	Derived character state
1	Increase in thickness of the outer sclerenchymatous sheath of all vascular bundles	Thinner than vascular tissue	Several times thicker than vascular tissue
2	Dominance of the median vascular bundle over the others	Little differences in size between the major bundles	Median bundle several times bigger than the biggest lateral bundles
3	Reduction of pith parenchyma	Cross section area of pith several times bigger than that of the median bundle	Cross section area of the median bundle several times bigger than that of the pith

Table 4. Character polarity in petiole anatomy in selected species of *Astragalus* sect. *Incani* in Iran based on Engle (1991).

Character evolution trend	Original character state	Derived character state
Species		
<i>A. abnormalis</i>	1,2	3
<i>A. askius</i>	1,3	2
<i>A. cyclophyllus</i>	-	1,2,3
<i>A. demavendicus</i>	2	1,3
<i>A. divandarehnsis</i>	2	1,3

<i>A. doplanicus</i>	3	1,2
<i>A. fridae</i>	-	1,2,3
<i>A. fuliginosus</i>	3	1,2
<i>A. glaucophyllus</i>	1,3	2
<i>A. gudrunensis</i>	3	1,2
<i>A. homandicus</i>	1,3	2
<i>A. incanus</i>	3	1,2
<i>A. longicuspis</i>	1,3	2
<i>A. micrancistrus</i>	1	2,3
<i>A. ordubadensis</i>	1	2,3
<i>A. paucifoliatus</i>	1,3	2
<i>A. pseudorobustus</i>	1,3	2
<i>A. punctuatus</i>	1,2,3	-
<i>A. rostratus</i>	3	1,2
<i>A. safavi</i>	1,3	2
<i>A. siahcheshmehensis</i>	1,3	2
<i>A. subalpinus</i>	1,3	2
<i>A. subglaberrimus</i>	3	1,2
<i>A. supervisus</i>	1	2,3
<i>A. zanzanensis</i>	1,2,3	-

walled parenchymatous cells along small amounts of sclerenchyma around the vascular bundle. Character polarity in 25 selected species was studied. Among the studied species evolutionary direction is variable (table 4). Dominance of median vascular bundle over others is an apomorphic character as in *A. zanzanensis*, *A. supervisus*, *A. askius*, *A. cyclophyllus* (fig. 2), *A. doplanicus* (fig. 5), *A. fridae*, *A. fuliginosus* (fig. 6), *A. glaucophyllus* (fig. 7), *A. gudrunensis* (fig. 8), *A. longicuspis*, *A. micrancistrus*, *A. paucifoliolatus* (fig. 11), *A. punctuatus* (fig. 12), *A. pseudorobustus*, *A. rostratus*, *A. safavi*, *A. siahcheshmehensis*, *A. subglaberrimus* which show this status and the other species show plesiomorphic status. The reduction of the outer sclerenchymatous which observed in *A. supervisus*, *A. ordubadensis*, *A. micrancistrus*, *A. cyclophyllus*, *A. micrancistrus* is an apomorphic character and other species show plesiomorphic status (increasing of pith parenchymatous). The increasing of the outer sclerenchymatous or sheath is an apomorphic character that exist in *A. zanzanensis*, *A. rostratus*, *A. subglaberrimus*, *A. demavendicus*, *A. fridae*, *A. fuliginosus*, *A. gudrunensis* and other species show plesiomorphic status (the reduction of the outer sclerenchymatous). Presence of connective bundles in *A. glaucophyllus* is an autapomorphic character. In *A. fuliginosus*, and *A. fridae* all characters show apomorphic status. In *A. punctuatus* all characters show plesiomorphic status. In *A. fridae*, *A. zanzanensis*, *A. micrancistrus*, *A. subglaberrimus*, *A. askius*, and *A. zanzanensis* there are similarities in anatomical evidences but they conflict in morphological characters

thus there are not suitable evidence for sharply grouping and determining of all of natural groups. *A. gudrunensis*, *A. demavendicus*, *A. doplanicus*, *A. punctuatus* are similar in anatomical character but they are differing in their morphological characters. The results reveal that petiole anatomy is a suitable evidence for taxonomical differentiation in species level and some taxonomical complexes and delimiting small natural groups but not useful for circumscribing sections. Thus all of the evidences are necessary for grouping in section and its phylogenetical situation in other sections of *Astragalus*.

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