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Anatomical findings of *Onobrychis* sect. *Heliobrychis* (Fabaceae) in Iran and their taxonomic implications

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Abstract: Peduncle anatomy was investigated in 20 species of *Onobrychis* Miller sect. *Heliobrychis* Bunge from Iran by light microscopy. Ten quantitative and qualitative characters were studied in the peduncle cross sections. Results showed that qualitative characters represent considerable variations within the section, some of which such as outline shape of the peduncle cross section, density of papillae on hair surface, and position of cavities in peduncles are taxonomically important characters to differ annual species from perennials in the section. Four groups were distinguished within the perennial species according to peduncle anatomical characters. In addition, a key is provided for the taxa based on these characters.

Key words: Anatomical characters, cross section, Leguminoseae, *Onobrychis, Heliobrychis*

Introduction

The genus *Onobrychis* Miller (Hedysareae, Fabaceae), with about 130 species in 2 subgenera and 9 sections, includes annual or perennial herbs or shrubs. The genus is mainly distributed in northern temperate regions; however, centres of its genetic diversity are in the eastern Mediterranean area and south-west Asia (Rechinger, 1984; Lock & Simpson, 1991). *Onobrychis* sect. *Heliobrychis* Bunge belongs to the subgenus *Sisyrosema* Bunge and includes annual or perennial plants, caulescent or acaulescent, with sub-orbicular ecristate and setose fruits. The section comprises 3 subsections (Širjaev, 1926). It is predominantly distributed in the Zagros Mountains and north-west Iran. The section with about 24 species is the biggest section in Iran. Its majority species are endemic and important as forage legumes (Rechinger, 1984; Aktoklu, 2001; Ranjbar et al., 2004, 2010b; Amirabadizadeh et al., 2007).

Due to the high polymorphism in morphological characters, the section is taxonomically the most problematic group and the boundaries of its species are not completely clear. Several studies have been made to evaluate interspecific relationships within the genus *Onobrychis* in Iran using morphological, palynological, and karyological characters (Karamian et al., 2009, 2010; Ranjbar et al., 2009, 2010b). However, there is no report on anatomy of the genus. Anatomical characters are not always as useful as morphological characters for plant identifications; however, they are well-established criteria and can offer significant assistance in plant taxonomy (Güvenç & Duman, 2010; Ranjbar et al., 2010a; Güvenç et al., 2011). In the present study, peduncle anatomy is used

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to assess interspecific relationships within *Onobrychis* sect. *Heliobrychis* and its taxonomic significance is discussed.

Materials and methods

Samples of *Onobrychis* sect. *Heliobrychis* were prepared from fresh materials collected in the field or from herbarium specimens. Voucher specimens (Table 1) were deposited in the herbaria BASU and TARI. Since some acaulescent species present in the section, cross sections were made only from peduncles. Mature peduncles from dried specimens were chosen and softened in a mixture of distilled water/glycerine/ethanol 70% (1:1:1) for 2 weeks. Cross sections were made from the middle part of peduncles using commercial razor blades. The sections were stained with methyl blue and carmine and mounted on the slides using Canada balsam. Then, they were examined using an Olympus BX-

Table 1. Voucher specimens of *Onobrychis* sect. *Heliobrychis* used in this study.

Taxa	Altitude (m)	Locality and date of collection	Collector name and number
O. andalanica Bornm.	1520	Kurdistan: Dehgolan to Kamyaran, 18.5.2004	*Ranjbar* 6138, BASU
O. atropatana Boiss.	1700	Azerbaijan Sharqi: Khoy to Siahcheshmeh, 28.6.2003	*Ranjbar* 5346, BASU
O. aucheri Boiss. subsp. *teheranica* (Bornm.) Rech. f.	1700	Semnan: Ahovan, 6.6.1996	*Taherian* 2828, TARI
O. aurea Ranjbar, Amirabadizadeh & Ghahremani	1720	Azerbaijan Sharqi: Tabriz to Moshkanbar, 13.6.2005	*Mozaffarian* 87151, TARI
O. buhseana Bunge ex Boiss.	1900	Azerbaijan Sharqi: Tabriz to Sperkhan, 23.6.1994	*Mozaffarian* 72734, TARI
O. gaubae Bornm.	1800	Tehran: Karaj, 29.5.2002	*Mozaffarian* 82403, TARI
O. gypsicola Rech. f.	700	Khuzistan: Ramhormoz to Izeh, 19.4.1982	*Assadi* & *Abuhamzeh* 38791, TARI
O. haussknechtii Boiss.	1200	Kermanshah: Tazehabad to Azgaleh, 23.5.2005	*Ranjbar* 7584, BASU
O. heliocarpa Boiss.	1300	Zanjan: Halab, 26.5.2006	*Ranjbar* 9962, BASU
O. heterophylla C.A.Mey.	1600	Azerbaijan Sharqi: Hurand, 3.6.2004	*Ranjbar* 6024, BASU
O. kermanensis (Širj. & Rech. f.) Rech. f.	1380	Hormozgan: Hajiabad to Sirjan, 8.4.1998	*Amirabadi* & *Abbasi* 7053, TARI
O. lunata Boiss.	1500	Kermanshah: Sarpolezahab, 24.5.2005	*Ranjbar* 7563, BASU
O. melanotricha Boiss. var. *melanotricha*	2100	Isfahan: Chadegan, 2.6.1981	*Nowruzi* & *Etemadi* 647, TARI
O. oxyptera Boiss.	1800	Fars: Marvdasht, Arsanjan, 25.5.1997	*Zandi* 11775, TARI
O. plantago Bornm.	3200	Yazd: Shirkuh, 21.6.1975	*Foroughi* & *Assadi* 17948, TARI
O. psoraleifolia Boiss. var. *Psoraleifolia*	2200	Isfahan: Kashan, Ghamsar, 28.5.1982	*Mozaffarian* 42116, TARI
O. scrobiculata Boiss.	1900	Hamedan: Tajabad village, 5.6.2005	*Ranjbar* & *Moradi Behjou* 7239, BASU
O. sojakii Rech. f.	1950	Fars: Doshman-Ziary, 1.6.1983	*Mozaffarian* 45899, TARI
O. subacaulis Boiss.	550	Azerbaijan Sharqi: Siahrud to Jolfa, 5.6.2004	*Ranjbar* 6058, BASU
O. szovitsii Boiss.	1500	Azerbaijan Sharqi: Tasudj, 7.5.1987	*Yousefi* 973, TARI

41 photomicroscope at 40-400× magnifications and photographed by an Olympus digital camera.

Anatomical characters, which were selected and quantified here, included outline shape of the peduncle cross section, the shape of epidermal cells, hair surface, the number of collenchyma layers, the number of parenchyma layers in cortex, the shape of endodermis cells, the number of vascular bundles, the number of pericyclic fibre layers, the shape of parenchymatous cells in pith, and the position of cavities (Fahn, 1990; Hasan & Heneidak, 2006; Mehrabian et al., 2007).

Results

Outline shape of the peduncle cross section in studied taxa is circular, elliptic, pentagonal, or hexagonal with a sinuate or smooth border. The epidermis consists of a single layer of sub-circular to sub-rectangular cells covered sparsely with hairs or rarely glabrous (e.g., *Onobrychis szovitsii*). The hair surfaces are loosely to densely papillose, or are rarely smooth (e.g., *Onobrychis gaubae*). There is a continuous ring composed of 4-7 layers of tangential collenchymatous cells below the epidermis that are discontinued by cortex parenchyma. The cortex is composed of 4-6 layers of sub-circular to sub-rectangular parenchymatous cells. A single continuous endodermis exists at the end of the inner cortex. The central vascular cylinder consists of 5-13 discrete collateral primary bundles individually distinct arranged in an elliptic ring around the pith. These bundles are separated by narrow medullar rays. Pericyclic fibres occur in 5-13 layers in pile above the vascular bundles. The pith is more or less circular and composed of polygonal or large isodiametric cells with conspicuous intercellular spaces. There are some cavities around pericyclic fibres and also within the cortex parenchyma (Figure 1, Table 2).

The results of this study revealed that quantitative characters show a few variations by a continuous range and are stable within the section. However, qualitative characters, such as outline shape of the peduncle cross section, density of papillae on the hair surface and the number of cavities, are significant. These characters have good taxonomic values and can be useful to evaluate interspecific relationships within the section. Results are summarised as follows:

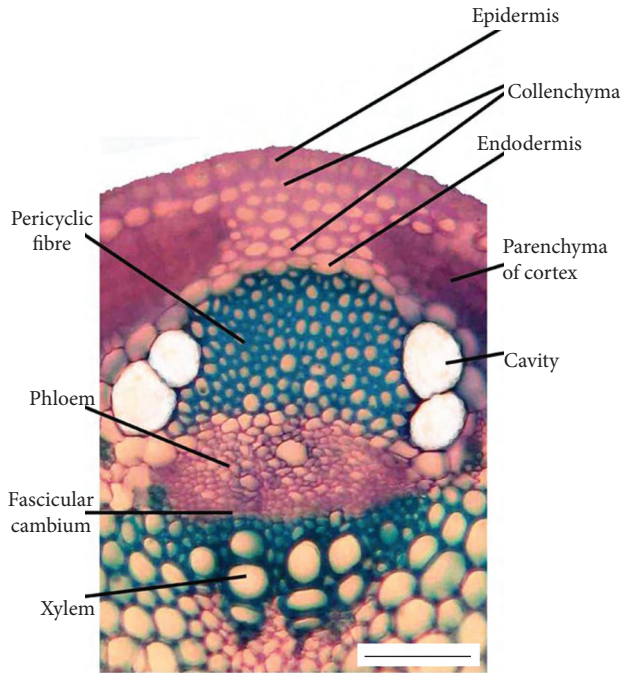

Figure 1. Peduncle cross section of *Onobrychis atropatana* showing quantified characters. Scale bar: 100 µm.

Perennial species develop metaxylem elements in the xylem tissue. Some cavities were observed within cortex parenchyma and also around pericyclic fibres. They can be divided into 4 groups:

A. Group 1 consists of *Onobrychis szovitsii, O. aurea, O. atropatana, O. andalanica, O. scrobiculata, O. buhseana, O. heterophylla, O. gaubae,* and *O. psoraleifolia,* represents a circular shape in peduncle outlines covered with hairs, and are loosely papillose on their surfaces or rarely smooth (Figures 2-4).

B. Group 2 consists of *Onobrychis lunata*, *O. melanotricha, O. kermanensis,* and *O. sojakii,* shows elliptic shape in peduncle outlines covered with hairs, and are densely papillose on their surfaces (Figures 4 & 5).

C. Group 3 consists of *Onobrychis oxyptera* and *O. plantago,* shows pentagonal shape in peduncle outlines covered with hairs, and are densely papillose on their surfaces (Figure 5).

D. Group 4 consists of *Onobrychis haussknechtii* and *O. gypsicola,* shows pentagonal or hexagonal shape in peduncle outlines covered with hairs, and are loosely papillose on their surfaces (Figure 5).

Table 2. Differential characters related to the peduncle cross section in *Onobrychis* sect. *Heliobrychis*.

Species	Outline shape of the peduncle cross section	Hair surface	Number of collenchyma layer	Number of cortex parenchyma layer	Number of vascular bundle	Number of pericyclic fibre layer	Number of cavity position
Perennial species							
Group A							
O. szovitsii	circular	-	4-5	4-5	12	9-11	2
O. aurea	± circular	loosely papillose	4-5	4-5	9	10-13	2
O. atropatana	± circular	loosely papillose	5-6	5-6	12	10-11	2
O. andalanica	± circular	loosely papillose	5-7	5-6	9-10	10-12	2
O. scrobiculata	circular	loosely papillose	4-5	5-6	8	9-11	2
O. buhseana	circular	loosely papillose	5-7	5-6	7-8	9-11	2
O. heterophylla	± circular	loosely papillose	4-6	4-5	8	8-9	2
O. gaubae	circular	smooth	4-5	4-5	10	7-9	2
O. psoraleifolia	± circular	loosely papillose	5-6	4-6	9-10	8-9	2
Group B							
O. lunata	± elliptic	papillose	4-5	4-5	8-9	9-10	2
O. melanotricha	± elliptic	densely papillose	4-5	4-5	10-11	6-7	2
O. kermanensis	± elliptic	densely papillose	4-5	4	13	10-13	2
O. sojakii	± elliptic	densely papillose	4-5	4-5	9	10-13	2
Group C							
O. oxyptera	pentagonal	densely papillose	6-7	5-6	11-12	9-11	2
O. plantago	pentagonal	densely papillose	4-5	4-5	7-8	5-6	2
Group D							
O. haussknechtii	hexagonal	loosely papillose	4-6	4-5	8	9-10	2
O. gypsicola	pentagonal	loosely papillose	4-6	4-5	10-13	7-9	2
Annual species							
O. aucheri	pentagonal	densely papillose	4-5	4-5	5	6-8	1
O. subacaulis	hexagonal	densely papillose	5-6	4-5	8	5-6	1
O. heliocarpa	hexagonal	densely papillose	4-5	4-5	8	8-9	1

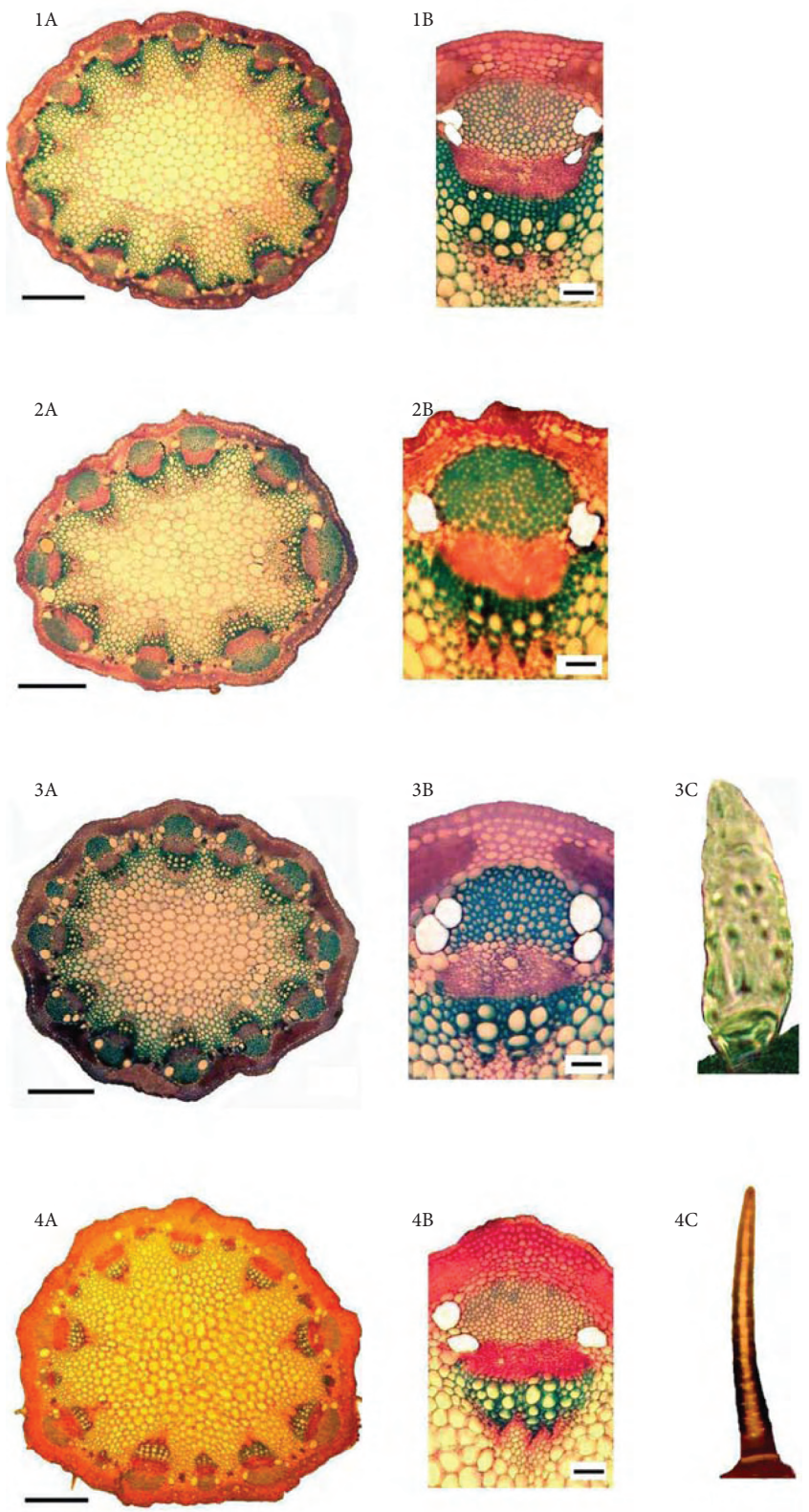

Figure 2. Photomicrographs of the peduncle cross sections. A- entire section. B- enlargement showing internal structure. C- hair. 1- *Onobrychis szovitsii*, 2- *O. aurea*, 3- *O. atropatana*, 4- *O. andalanica*. Scale bars: A = 0.5 mm, B = 50 µm.

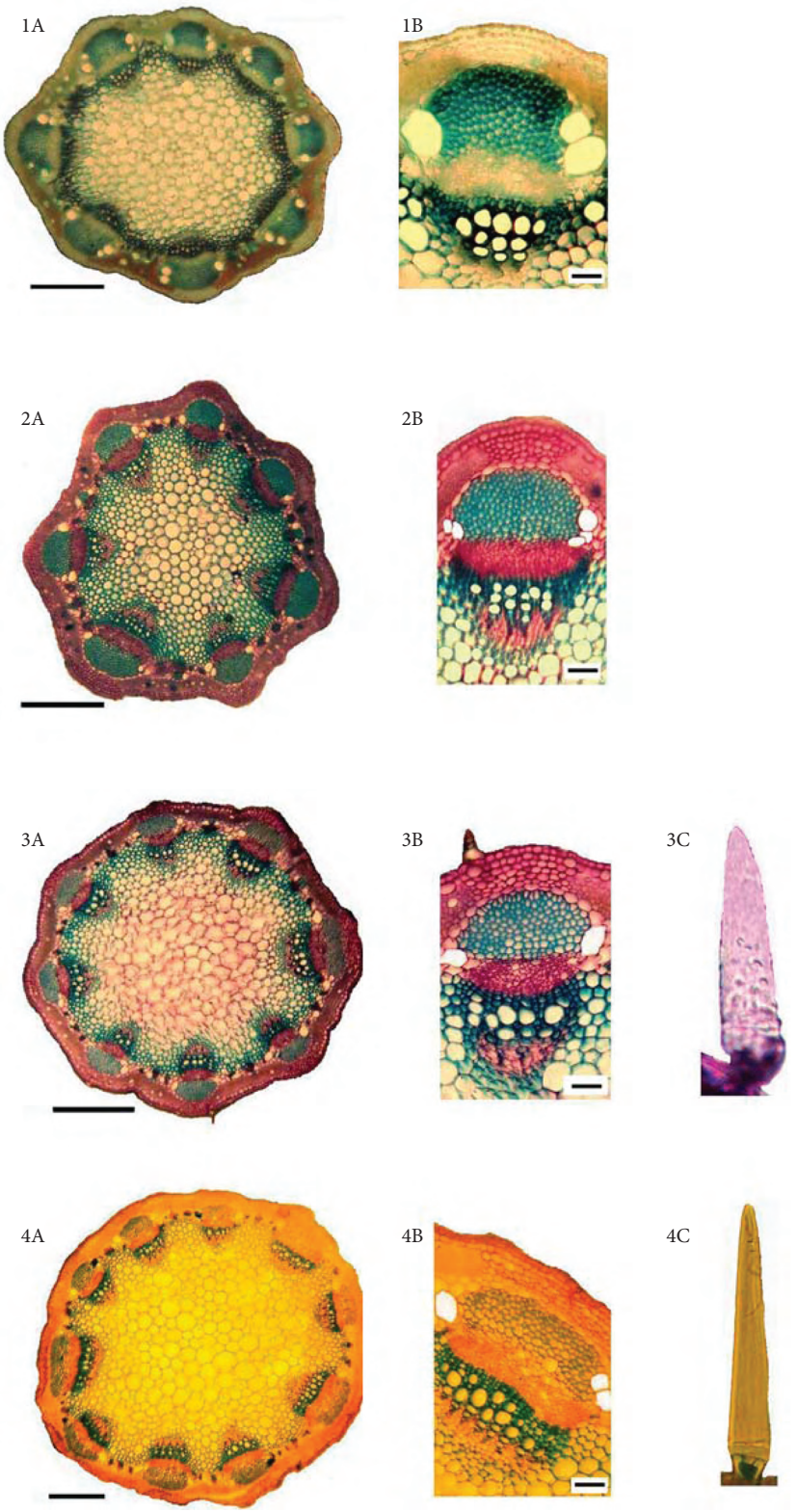

Figure 3. Photomicrographs of the peduncle cross sections. A- entire section. B- enlargement showing internal structure. C- hair. 1- *Onobrychis scrobiculata*, 2- *O. buhseana*, 3- *O. heterophylla*, 4- *O. gaubae*. Scale bars: A = 0.5 mm, B = 50 µm.

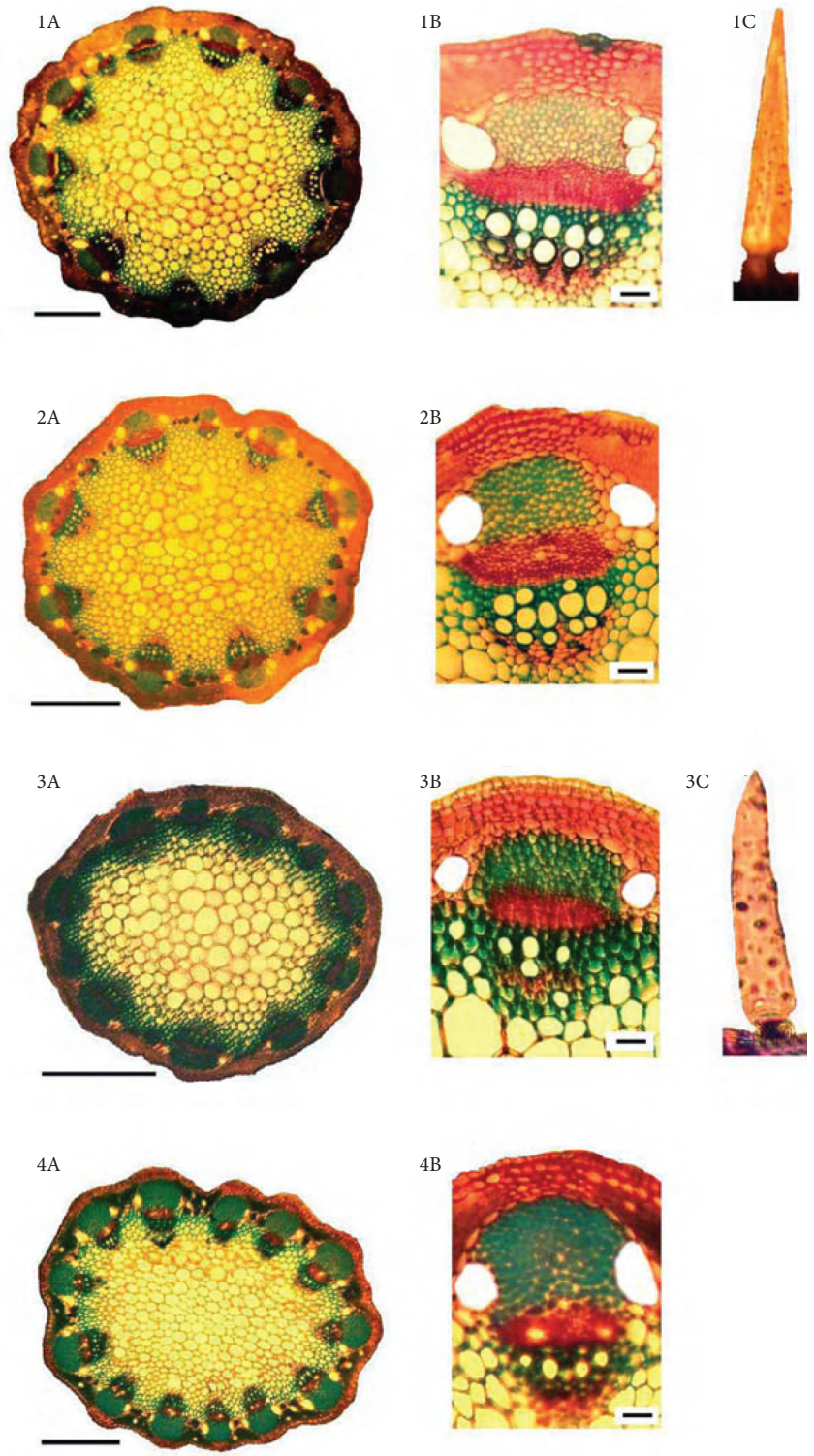

Figure 4. Photomicrographs of the peduncle cross sections. A- entire section. B- enlargement showing internal structure. C- hair. 1- *Onobrychis psoraleifolia*, 2- *O. lunata*, 3- *O. melanotricha*, 4- *O. kermanensis*. Scale bars: A = 0.5 mm, B = 50 µm.

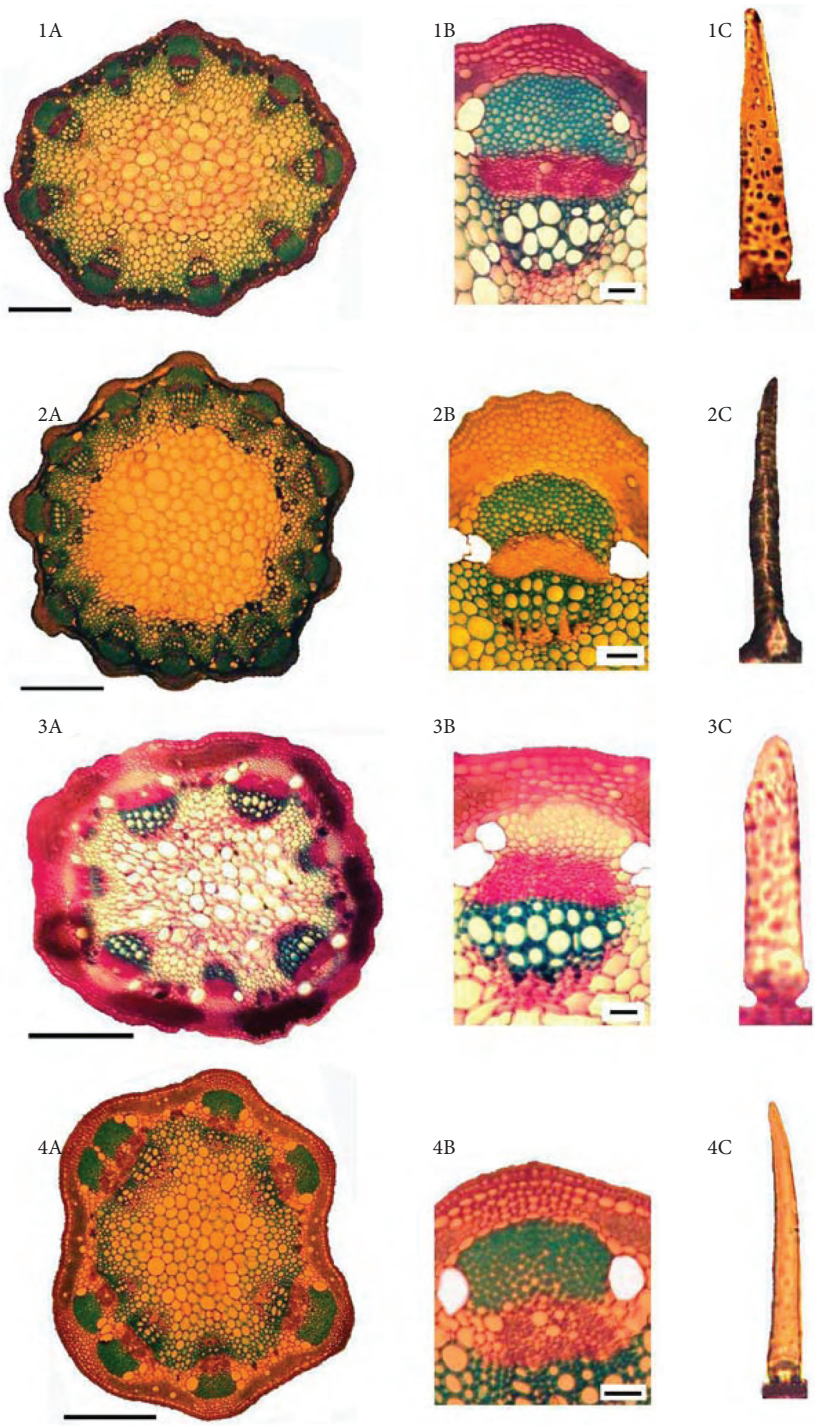

Figure 5. Photomicrographs of the peduncle cross sections. A- entire section. B- enlargement showing internal structure. C- hair. 1- *Onobrychis sojakii*, 2- *O. oxyptera*, 3- *O. plantago*, 4- *O. haussknechtii*. Scale bars: A = 0.5 mm, B = 50 μm.

Annual species do not develop metaxylem elements in the xylem tissue. The cavities were observed only around pericyclic fibres. They include *Onobrychis aucheri*, *O. subacaulis,* and *O. heliocarpa,* show pentagonal or hexagonal shapes in peduncle outlines covered with hairs, and are densely papillose on their surfaces (Figure 6).

Key to the studied species of *Onobrychis* sect. *Heliobrychis* based on peduncle anatomy:

1a. Metaxylem elements, well-developed; cavity position, within cortex parenchyma and around pericyclic fibres ……..…… 2

1b. Metaxylem elements, not well-developed; cavity position, only around pericyclic fibres ..…..… 18

2a. Outline shape of the peduncle cross section, circular or elliptic ……..…..…..……. 3

2b. Outline shape of the peduncle cross section, pentagonal or hexagonal …..…..……. 15

3a. Epidermis layer, hairy ……..…… 4

3b. Epidermis layer, glabrous ……..……. *O. szovitsii*

4a. Outline shape of the peduncle cross section, circular; hair surface, loosely papillose or rarely smooth …...……..……...……... 5

4b. Outline shape of the peduncle cross section, elliptic; hair surface, densely papillose ….…….. 12

5a. Hair surface, loosely papillose ……...…… 6

5b. Hair surface, smooth …… *O. gaubae*

6a. Pericyclic fibre, thick and composed of more than 10 layers ……..……..... 7

6b. Pericyclic fibre, thin and composed of less than 10 layers ……..…… 11

7a. Number of vascular bundle, more than 9 …….. 8

7b. Number of vascular bundle, 9 or less than 9 9

8a. Number of vascular bundle, 12 ….. *O. atropatana*

8b. Number of vascular bundle, 9-10 ... *O. andalanica*

9a. Number of vascular bundle, 9 …… *O. aurea*

9b. Number of vascular bundle, 7-8 ……...…… 10

10a. Collenchyma composed of 5-7 layers .....*O. buhseana*

10b. Collenchyma composed of 4-5 layers ……..…… *O. scrobiculata*

11a. Number of vascular bundle, 9-10 ……..……. *O. psoraleifolia*

11b. Number of vascular bundle, 8 …… *O. heterophylla*

12a. Number of vascular bundle, 10-13…….. 13

12b. Number of vascular bundle, 8-9…….. 14

13a. Number of vascular bundle, 13 ……..…….. *O. kermanensis*

13b. Number of vascular bundle, 10-11 ……..……*O. melanotricha*

14a. Pericyclic fibre, thick and composed of 10-13 layers ……..…..…..……. *O. sojakii*

14b. Pericyclic fibre, thin and composed of 9-10 layers ……..……. *O. lunata*

15a. Hair surface, densely papillose .....……..….. 16

15b. Hair surface, loosely papillose ……...……. 17

16a. Collenchyma composed of 6-7 layers ……..……. *O. oxyptera*

16b. Collenchyma composed of 4-5 layers ……..…… *O. plantago*

17a. Outline shape of the peduncle cross section, hexagonal; number of vascular bundle, 8 ……..……..... *O. haussknechtii*

17b. Outline shape of the peduncle cross section, pentagonal; number of vascular bundle, 10-13 …...……..……. *O. gypsicola*

18a. Outline shape of the peduncle cross section, hexagonal ……..……...…….. 19

18b. Outline shape of the peduncle cross section, pentagonal ….……..……. *O. aucheri*

19a. Pericyclic fibre, thick and composed of 8-9 layers ……..……..…… *O. heliocarpa*

19b. Pericyclic fibre, thin and composed of 5-6 layers ……..……...……. *O. subacaulis*

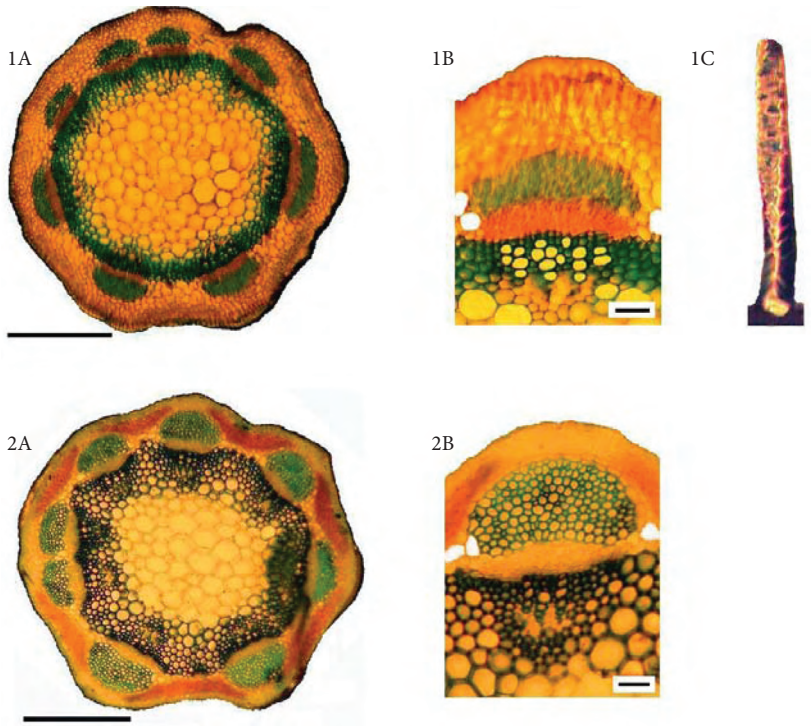

Figure 6. Photomicrographs of the peduncle cross sections. A- entire section. B- enlargement showing internal structure. C- hair. 1- *Onobrychis subacaulis*, 2- *O. heliocarpa*. Scale bars: A = 0.5 mm, B = 50 µm.

Discussion

The perennial and annual species of *Onobychis* sect. *Heliobrychis* were separated well by anatomical characters related to peduncle. It can be concluded that perennial and annual species are 2 main groups in the section as justified by Širjaev (1926). *Onobychis* sect. *Heliobrychis* has been divided into 3 subsections, namely *Szovitsianae*, *Boissierianae,* and *Persicae*, by Širjaev (1926). The 2 former subsections are perennials, but the latter includes annual plants. The subsection *Szovitsianae* includes the isolated species of *O. szovitssi*, which is distinguished easily by its glabrous habit. The subsection *Boissierianae* includes the remaining perennial species of the section. According to our results, *Szovitsianae* cannot be considered as a distinctive subsection and may be included in the subsection *Boissierianae*. This is supported well by results from pollen morphology of the *Onobychis* sect. *Heliobrychis* (Karamian et al., 2009).

Perennial species of the section were divided into 4 groups based on anatomical characters. This grouping is in agreement with the results from morphology (unpublished data). Group A includes the species with predominantly well developed stems. In contrast, groups B and C include the species that are predominantly acaulescent. Group D includes The species show disjunctive distribution in south-west Iran at an altitude range of 300-1200 m, unlike The remaining members.

The other interesting anatomical character in *Onobychis* sect. *Onobrychis* is the presence of papillae on hair surface. This character has been reported as an ancestral state against smooth surface occurring in some species of legumes, such as thorny *Astragali* (Zarre, 2003). The members of the section are predominantly papillose on the hair surfaces, but are rarely smooth. In addition, based on the density of papillae, 2 character states can be distinguished. Thus, 3 character states can be considered, e.g., smooth, loosely papillose, and densely papillose for the hair surfaces. However, the significance of the characters related to hairs can be assessed exactly for taxonomy and phylogenetic reconstruction of the section, when they have been investigated precisely by scanning electron microscopy.

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