

## LEAF AND STEM ANATOMY IN IRANIAN *BOLBOSCHOENUS* SPECIES (CYPERACEAE), AS RELATED TO THEIR TAXONOMY AND ECOLOGY

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Anatomical features of Iranian species, *Bolboschoenus affinis*, *B. glaucus*, *B. maritimus*, *B. planiculmis* and *B. schmidii*, were compared in an attempt to identify diagnostic characters that support morphological differentiation and to discover possible relationships between the anatomical and ecological differentiation of species. The leaf and stem anatomy visible on cross sections was studied to complement formerly used diagnostic characters in fruit anatomy. The presence and arrangement of bulliform cells in the leaf epidermis and subepidermal layers appeared to vary between some species, as did the presence of air cavities between the vascular bundles. We found that stem anatomy characters were not that much decisive in the differentiation of species. Although most of the anatomical characters supported the taxonomical differentiation based on morphology and fruit anatomy, they would not be decisive when used alone. In contrast, anatomical adaptations to habitat conditions seem to be clear in certain species and correspond to their ecology, i.e., the adaptation to drought and salinity or flooding conditions.

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**Key words.** *Bolboschoenus*, habitat conditions, leaf, stem, anatomy, taxonomy, Iran.

مطالعه تشریحی برگ و ساقه در گونه‌های ایرانی جنس *Bolboschoenus*، در ارتباط با تاکسونومی و اکولوژی

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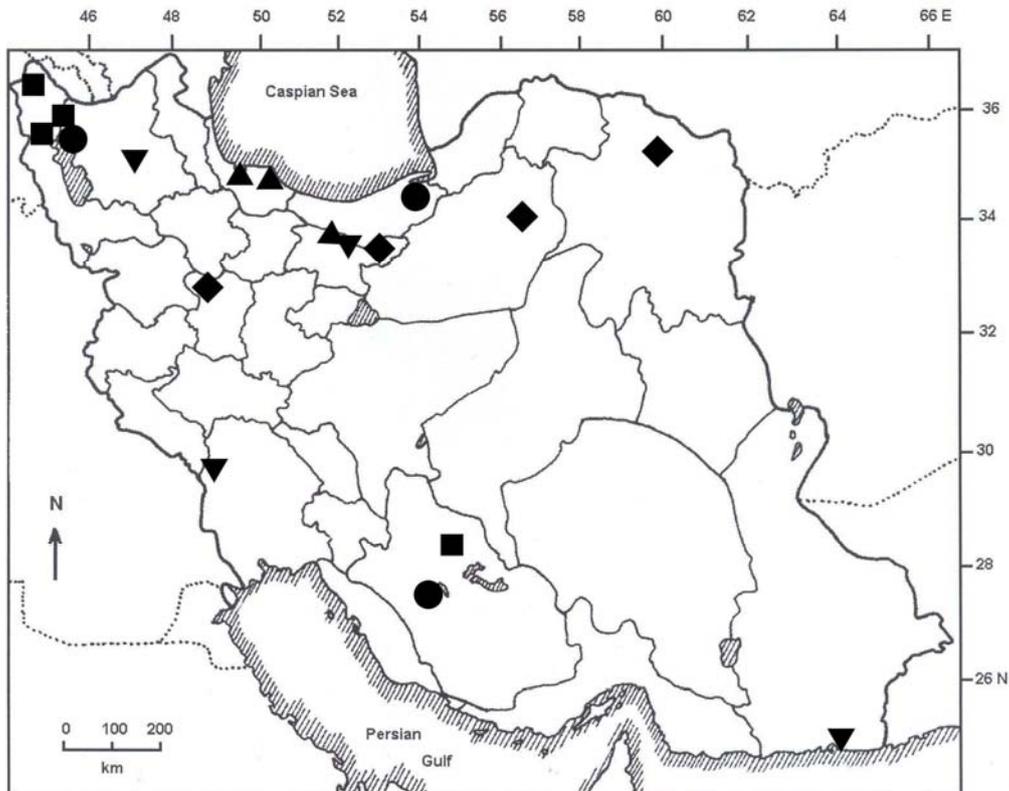
ساختار تشریحی برگ و ساقه ۵ گونه از جنس *Bolboschoenus* مورد بررسی قرار گرفتند که هدف از این تحقیق، شناسایی خصوصیات تشخیصی با توجه به تمایز مورفولوژیکی و یافتن روابط موجود بین تفاوت‌های ساختاری و اکولوژیکی این گونه‌ها بود. ساختار تشریحی در برش عرضی برگ و ساقه برای بدست آوردن صفات تشخیصی جهت تکمیل مطالعات قبلی انجام گرفته در برش عرضی میوه این گونه‌ها مطالعه شدند. حضور و ترتیب سلولهای بولیفرم در لایه‌های اپیدرمی و زیراپیدرمی و همینطور حضور حفرات هوا بین دستجات آوندی در بین گونه‌ها متغیر بود. همچنین مشخص گردید که ساختار تشریحی ساقه دارای صفات تشخیصی مناسب در تشخیص گونه‌ها نیست. اگرچه اغلب صفات تشریحی تایید کننده تفاوت‌های مورفولوژیکی و تشریحی میوه می‌باشند لیکن آنها به تنهایی نمی‌توانند موثر باشند. در مقابل، سازگاریهای آناتومیکی نسبت به شرایط زیست محیطی در مورد هر گونه در ارتباط با اکولوژی آنها (از جمله سازش با شرایط خشکی، شوری و ..) کاملاً مشخص است.

### INTRODUCTION

Formerly included in the *Scirpus* genus, *Bolboschoenus* (Ascherson) Palla (*Cyperaceae*), has been accepted for more than a decade as a distinct taxon based on Goetghebeur & Simpson (1991). The *Bolboschoenus* genus includes approximately 15 species throughout the world (Browning & Gordon-Gray 2000, Tatanov 2007).

However, distinguishing among the individual species within *Bolboschoenus* is difficult in some cases due to the high variation in quantitative morphological characters and possible absence of decisive diagnostic characters in the flowering plants (Browning et al. 1995, 1996; Smith 2002).

In most Floras (Maire 1957, Raymond 1965, DeFilipps 1980, Tan 1985, Kukkonen 1998, 2001),



Map. 1. Distribution map of the localities of the studied specimens: ■ *Bolboschoenus maritimus*, ▲ *B. planiculmis*, ▼ *B. glaucus*, ● *B. affinis*, ◆ *B. schmidii*.

infrageneric classifications were based on various morphological characters, such as the inflorescence structure, spikelet size, glume colour, number of stigmas and perianth bristles, achene shape and colour. Oteng-Yeboah (1974) had first mentioned the importance of fruit shape and anatomy in differentiation at the infrageneric level. Browning & Gordon-Gray (1993) used fruit characters for distinguishing the species within this genus in the southern regions of Africa. According to Browning et al. (1995), achene surface features and anatomy provide a very useful suite of characters to supplement the rather few morphological characters readily available to taxonomists studying this genus. Recently, fruit characters have been used in many studies as distinguishing characters of *Bolboschoenus* species (Browning & Gordon-Gray 2000, Browning et al. 1997a, b, Hroudová et al. 1998, Tatanov 2004, 2007, Amini Rad et al. 2010). In addition, it appeared that the pericarp structure influences fruit buoyancy, resulting in the differential ability of species to distribute via water (Hroudová et al. 1997). However, with regard to recent knowledge on species differentiation, the leaf

and stem anatomy in *Bolboschoenus* has not yet been studied.

The oldest and the most comprehensive anatomical study of the *Cyperaceae* family is that of Metcalfe (1971), comprising the anatomy of the leaves and stems of 280 species belonging to 90 genera. Among them, the anatomical structure of *Scirpus maritimus* L. has been presented. The importance of anatomical studies for taxonomy was emphasised by Standley (1990) who used anatomical data concerning the structure of achene epidermal cells and the anatomy of leaves for the taxonomical and phylogenetical classification within the genus *Carex*.

Regarding the distribution of *Bolboschoenus* in Iran, the oldest report was published by Boissier (1882) who recorded *Scirpus maritimus* L. [= *Bolboschoenus maritimus* (L.) Palla] as being widespread in Baluchestan. Parsa (1950) reported *S. maritimus* in two forms, f. *compactus* Krock and f. *monostachys* nom. nud., and Mobayen (1975) also reported *Scirpus maritimus* from Iran. In Flora Iranica (Kukkonen 1998), *B. affinis* (Roth) Drobow was confirmed from Iran in addition to *B. maritimus*. Amini Rad (2003) first

Table 1. List of studied specimens, herbarium number and localities.

Species	Locality
<i>Bolboschoenus affinis</i>	Mazandaran: Behshahr, 10 km N Ghaleh Payan, Zehzad (IRAN 52991). E. Azarbaijan: Tabriz, Bandar-e Sharafkhaneh, 1300 m, Amini Rad & Torabi (IRAN 54855). Fars: 25 km to Shiraz, Lake Maharlou, Amini Rad et al. (IRAN 35550)
<i>B. maritimus</i>	E. Azarbaijan: 16 km Marand to Jolfa, alt. 1325 m, Amini Rad (IRAN 53893). W. Azarbaijan: Makou to Buralan, 1380 m, Amini Rad & Pahlevani (IRAN 48931); 28 km Khoy to Qotur, alt. 1470 m, Amini Rad & Pahlevani (IRAN 48868). Fars: Qader Abad, 8 km S. of the bridge, 2070 m, J. Sádlo (IRAN 32501)
<i>B. glaucus</i>	Khuzestan: Shoush, Amini Rad & Eskandari (IRAN 43970). E. Azerbaijan: 5 km Gharaeh-Chaman to Mianeh, Amini Rad & Eskandari (IRAN 37288). Baluchestan: 18 km Chabahar to Rask, Amini Rad & Torabi (IRAN 43966). Tehran: Roudehen, 2–3 km Mehrabad to Zaridar, alt. 1650–1700 m, Amini Rad (IRAN 47727).
<i>B. planiculmis</i>	Gilan: Langerud, Chamkhaleh, Hassanbekandeh village, Amini Rad (IRAN 37227); Rasht, Rice Research Station, Amini Rad & Tehrani (IRAN 38640). Tehran: Pardisan Park, Amini Rad (IRAN 47725)
<i>B. schmidii</i>	Hamedan: Shahnaz dam, Yalphan (mt.), alt. 1890–2200 m, Termeh & Mussavi (IRAN 30712). Khorasan: Neyshabour, Bar, 1615–1650 m, Amini Rad et al. (IRAN 47728). Semnan: Biarjomand, Touran protected area, Kuh-e Majerad, Kalat-Asbe, 950–970 m, Amini Rad et al. (IRAN 47729). Tehran: 5 km Firuzkuh to Semnan, alt. 1990 m, Amini Rad et al. (IRAN 47730).

reported *B. glaucus* (LAM.) S.G. Smith for the flora of Iran, and Amini Rad & Hroudová (2007) reported *B. schmidii* (Raymond) Holub and *B. planiculmis* (F. Schmidt) T.V. Egorova as new records from Iran. Recently, Amini Rad et al. (2010) have revised the genus of *Bolboschoenus* in Iran, recognising the following taxa using fruit features (fruit shape and pericarp anatomy) as the main diagnostic characters: *B. affinis*, *B. maritimus*, *B. glaucus*, *B. planiculmis* and *B. schmidii*. However, other anatomical studies carried out on the *Cyperaceae* family in Iran are very rare. Zarrinkamar et al. (2002) have investigated the leaf anatomy of the *Carex* genus from the Arasbaran protected area of East Azarbaijan, Iran. Amini Rad & Sonboli (2008) investigated the stem and leaf anatomy of 10 species belonging to the *Cyperus* subgenus *Cyperus*. Overall, comparative anatomical studies of Iranian *Bolboschoenus* are lacking.

Although characters in fruits represent the features that are generally used for taxonomic classification within this genus, we propose that the anatomical structure of vegetative organs might also provide valuable accompanying features. We attempted to complete the knowledge of Iranian *Bolboschoenus* by a comparison of the stem and leaf anatomy as possible additional features to distinguish species. The aims of the present research of anatomical studies within *Bolboschoenus* are: 1) to ascertain whether differences in anatomy may be found among *Bolboschoenus* taxa in Iran, supporting morphological differences, 2) to determine whether possible anatomical peculiarities might be related to the habitat

conditions of individual species.

## MATERIAL AND METHODS

For the anatomical study of *Bolboschoenus* species, plants from 3 or 4 populations of each species were analysed. The localities were selected to cover as much geographical distribution and different habitats as possible (Fig. 1). A list of the localities of the studied specimens has been provided in the Table 1.

Sections from the middle of the stem and from the middle of the second leaf or leaf before the flag leaf were used. The sections were placed in water for approximately 24 hours. The material was then fixed in glycerine-alcohol (ratio 1:1) for 72 hours and then transferred to 70% alcohol.

The transversal sections of the stems and leaves were cut by hand. The sections were cleared with sodium hypochlorite (10–15 minutes), washed with distilled water and stained with carmine (10–15 minutes) and methyl green (30 seconds). After washing with distilled water, the sections were transferred to microscope slides and photographed using an Olympus BX-51 microscope equipped with a camera. The sizes of air cavities were measured on the photos; the cavity diameter and leaf thickness at the same position were measured for all of the cavities of a given leaf visible on the photo.

## RESULTS

### *Bolboschoenus* (L.) Palla

Selected anatomical characters in leaf and stem cross sections are given in Table 2.

Table 2. Selected anatomical characters in leaf and stem cross sections distinguishing *Bolboschoenus* species.

character	<i>B. affinis</i>	<i>B. glaucus</i>	<i>B. maritimus</i>	<i>B. planiculmis</i>	<i>B. schmidii</i>
sclerenchyma strands in midrib (abaxial)	3-5	5-9	3	1-3	3-5
sclerenchyma strands in margins	2 and 1	2 and 1	1 and 1	1 and 1	1 and 1
number of small vascular bundles in midrib (behind the main vascular bundle)	1	1-2	0-1	1	0
size of adaxial epidermal cells in midrib (presence of bulliform cells)	bulliform cells in epidermis	small cells not different from other epidermal cells, or cells only slightly inflated	size variable; the same size as other epidermal cells, or inflate (bulliform cells) in epidermis	bulliform cells in epidermis	slightly greater or of the same size than other epidermal cells
arrangement of bulliform cells	2 layers	no layers, grouping of inflate cells between epidermis and main vascular bundle	1 to 2 layers (bulliform cells in hypodermis)	2 layers	1 to 2 layers of inflate cells (bulliform cells in hypodermis)
presence of air cavities in leaf lamina	in less than one half of all gaps	in more than ¾ gaps	2/3-3/4 gaps	2/3-4/5 gaps	¾ to all gaps
size of air cavities in leaf lamina (cavity diameter : leaf thickness ratio)	(1/7-)1/5-1/4(-1/3)	(1/3-)2/5-1/2(-3/5)	(1/8-)1/4-1/3(-1/2)	(1/4-)1/3-2/5(-3/5)	(1/4-)1/3(-1/2)
stem shape in cross section	obtusely to acutely triangular	acutely triangular	acutely obtusely triangular	acutely to obtusely triangular	acutely triangular
margins (stem sides)	concave to flat or convex	concave to flat or convex	concave or flat	concave or slightly convex	concave
presence of peripheral cavities	0 (no cavities)	0-42	0-15	0-11	8-30

leaf

stem

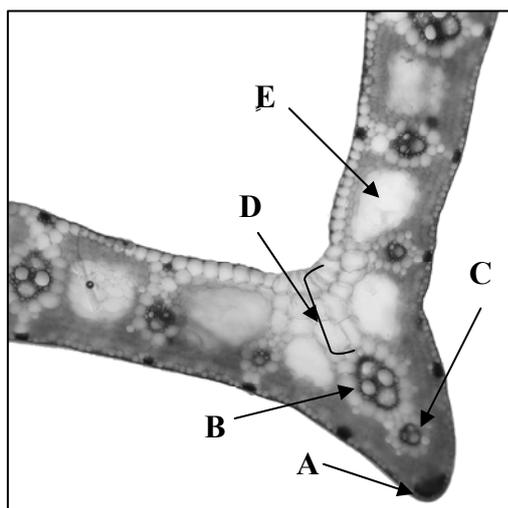


Fig. 2. Leaf anatomical parts in *Bolboschoenus* genus: A, Sclerenchyma strand; B, Large vascular bundle; C, Small vascular bundle; D, Bulliform cells; E, Air cavity

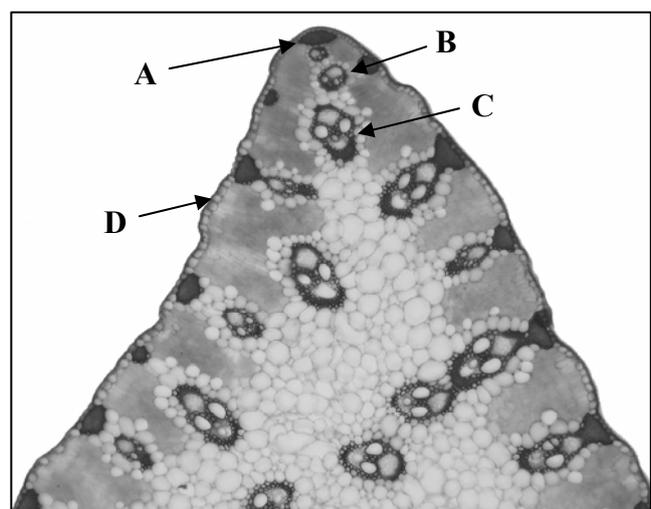


Fig. 3. Stem anatomical parts in *Bolboschoenus* genus: A, Sclerenchyma strand; B, Small vascular bundle; C, Large vascular bundle; D, Epidermis (translucent cells)

*Leaf.* The leaves are V-shaped or flanged V-shaped in cross section, keeled or acutely keeled. The epidermis is composed of one layer of translucent cells or accompanied by one to more layers of translucent cells; the epidermal cells overlying the sclerenchyma strands are smaller than those overlying the chlorenchyma. Bulliform cells in the epidermis and other inflated cells below the epidermis are usually developed on the adaxial side of the midrib. Air cavities containing lobed or (less frequently) stellate cells or arising by their breakdown are frequently present between the vascular bundles. The stomata are paracytic, with 1–3 between sclerenchyma strands, and are not seen in the median nerve area; subsidiary cells (annex) are dumbbell shaped. The bundle sheath is 2-layered; the inner sheath is fibrous and outer sheath is parenchymatous. The leaf characters were almost identical in all of the studied specimens of each species (Fig. 2).

*Stem.* The stems are triangular or acutely triangular in outline. The epidermis is formed of one layer of translucent cells. The stomata are paracytic, with 1–3 between sclerenchyma strands; the subsidiary cells (annex) are dumbbell shaped. The central ground tissue is spongy, with small irregular intercellular cavities, but the structure is not net-like. The sclerenchyma is composed of subepidermal strands of various sizes and shapes opposite the vascular bundles. The vascular bundles are numerous, peripheral and are sometimes penetrating deeply towards the centre of the stems. The bundle sheath is 2-layered: the inner bundle sheath is fibrous and the outer bundle sheath is parenchymatous. (Fig. 3)

***Bolboschoenus affinis* (Roth) Drobow**

*Leaf.* The leaves are 3.4–4.9 mm in width and keeled. The margins rounded and almost equal, but in some cases are unequal (one margin rounded and the other flat). The epidermis on the adaxial and abaxial sides is formed of one layer of translucent cells; the adaxial cells in the midrib are inflated, forming bulliform cells, and accompanied by one to two layers of translucent inflated cells. A group of other inflated cells similar to bulliform cells is also present in the adaxial midrib, continuing to parenchyma sheaths around the vascular bundles. Sclerenchyma strands are located below the epidermis, accompanying the vascular bundles. The median vascular bundle is accompanied by 3–5 abaxial strands; other sclerenchyma strands correspond to the locations of vascular bundles in the leaf lamina. The mesophyll is composed of chlorenchyma tissue between the vascular bundles and immediately subjacent to the epidermis. Air cavities are developed in the centre of the chlorenchyma between the vascular bundles and also close to the median nerve, except near the leaf margins and midrib area. The cavities between

the vascular bundles are only partially developed in many cases (visible in less than one half of the gaps between vascular bundles), frequently representing a transition to aerenchyma tissue. The sizes of the cavities are small,  $(1/7-1/5-1/4-1/3)$  of the leaf thickness. The median nerve is formed of one or two vascular bundles (one small and the other large), and other vascular bundles are situated in parallel lines in the leaf lamina (9–10 vascular bundles in one half, and 10–11 vascular bundles in other half) (Figs. 4 A, B and 5 A, B).

*Stem.* The stems are 1.8–2.8 mm in diameter, triangular in outline, and flat or convex sides. Vascular bundles are distributed in a peripheral layer under the stem surface (34–40, small and large) and also scattered in the central tissue (6–10, most large), with 3–4 vascular bundles usually grouped in corners. Sclerenchyma tissue is formed of 32–48 subepidermal strands of various size and shape, located opposite the vascular bundles; the strands are mostly triangular, crescent or cap shaped or sometimes flat in corners and sometimes connected to the vascular bundles (Figs. 6 A, B and 7 A, B).

***Bolboschoenus glaucus* (Lam.) S.G. Smith**

*Leaf.* The leaves are 5–9 mm in width and are acutely keeled. Leaf margins are equal and arrow-shaped. The epidermis on the adaxial and abaxial sides is represented by one layer of translucent cells and the adaxial epidermal cells in the midrib are little inflated (though not similar to bulliform cells). The inflated cells form a group in the adaxial midrib below epidermis, and are connected with sheaths of vascular bundles. Sclerenchymatous tissue accompanies the median vascular bundle in 5–9 abaxial strands; other abaxial and adaxial strands are located opposite the vascular bundles in the leaf lamina, with the strands on the adaxial side being larger than those on the abaxial side. The mesophyll is composed of chlorenchyma tissue occurring between the vascular bundles and immediately subjacent to the epidermis. Air cavities occur between the vascular bundles in the leaf lamina, with two cavities in midrib area, except near the leaf margins; they contain aerenchyma (stellate cells with numerous intercellular spaces) or broken tissue. The air cavities are highly visible, occurring in more than  $3/4$  of the gaps between vascular bundles; cavities are large, mostly  $2/5-1/2$  (range of  $1/3$  to  $3/5$ ) of the leaf thickness. The vascular bundles are usually 3–5 in the median nerve (median large and 3 or 4 lateral small) and other in the lines in the leaf lamina – 12 vascular bundles in one half and 11–14 vascular bundles in the other half. (Figs. 4 C, D and 5 C, D).

*Stem.* The stems are 2.5–4.7 mm in diameter, acutely triangular in outline, and the sides are concave or flat.

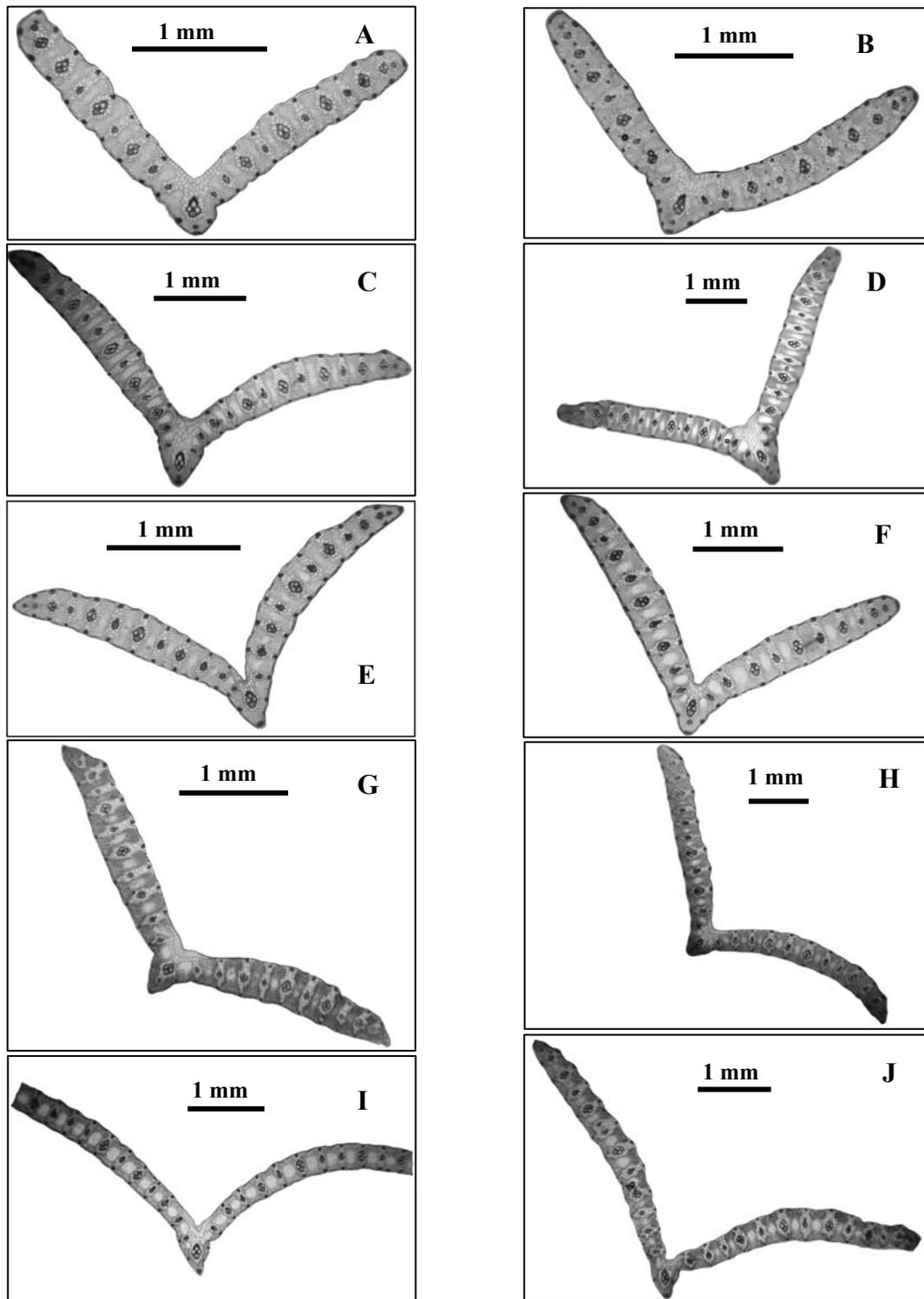


Fig. 4. Transverse section of leaf: *Bolboschoenus affinis* (A: 35550-IRAN, B: 54855-IRAN); *B. glaucus* (C: 37288-IRAN, D: 43966-IRAN); *B. maritimus* (E: 32501-IRAN, F: 48931-IRAN); *B. planiculmis* (G: 37227-IRAN, H: 47725-IRAN); *B. schmidii* (I: 47728-IRAN, J: 47729-IRAN).

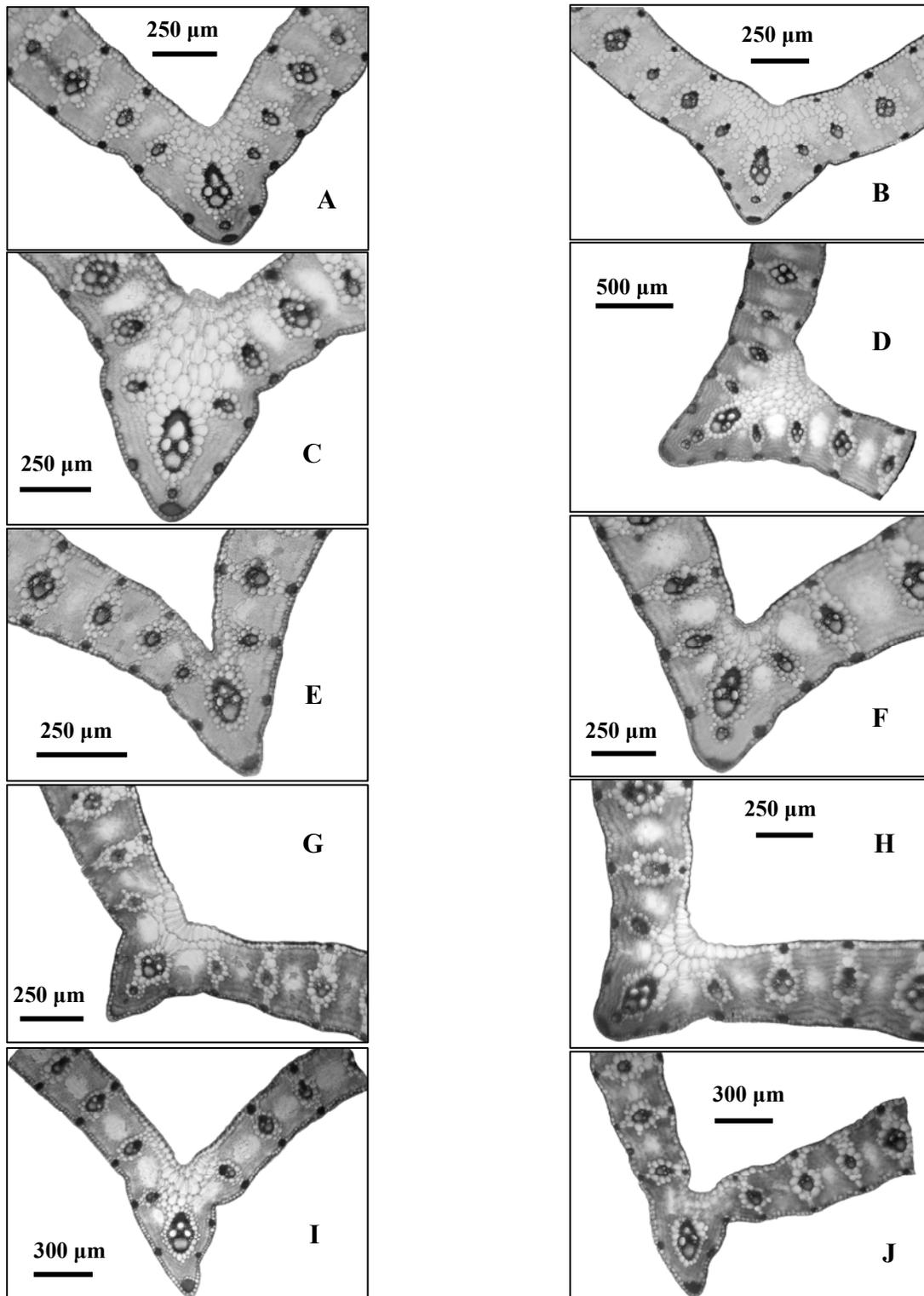


Fig. 5. Transverse section of leaf: *Bolboschoenus affinis* (A: 35550-IRAN, B: 54855-IRAN); *B. glaucus* (C: 37288-IRAN, D: 43966-IRAN); *B. maritimus* (E: 32501-IRAN, F: 48931-IRAN); *B. planiculmis* (G: 37227-IRAN, H: 47725-IRAN); *B. schmidii* (I: 47728-IRAN, J: 47729-IRAN).

The central ground tissue is formed of inflated cells with small irregular intercellular cavities but not of net-like structure. Air cavities are developed in some cases in the peripheral part of the stem in rows between the vascular bundles (Fig. 4 C, D); some small may be scattered in the central part of the stem. The vascular bundles are located in a row in the peripheral layer below the stem surface (33–47 small and large bundles), in corners (2–3), and 10–15 (relatively large) are scattered in the central part of the stem. The sclerenchyma is formed of 40–59 subepidermal strands of various sizes, opposite and connected to the vascular bundles. The strands are mostly triangular or obtusely triangular in cross section (Figs. 6 C, D and 7 C, D).

**Bolboschoenus maritimus** (L.) Palla

*Leaf.* The leaves are 2.5–4.7 mm in width and keeled. The margins may be subequal, with one margin rounded and the other margin acute. Epidermis on the adaxial and abaxial sides represented by one layer of translucent cells; the adaxial cells in the midrib are of the same size as the other cells or slightly inflated (similar to bulliform cells), accompanied by one to two layers of translucent inflated (bulliform) cells. The median vascular bundle is accompanied by 3 abaxial sclerenchyma strands; other abaxial and adaxial strands of different size are located opposite to the vascular bundles, with one strand in the leaf margins. Mesophyll is composed of chlorenchymatous cells, filling spaces between the vascular bundles and immediately subjacent to the epidermis. Air cavities are developed between the vascular bundles except near the leaf margins, and are composed of stellate cells with intercellular spaces. The cavities occur in most gaps between the vascular bundles (in  $2/3$ – $3/4$  of gaps), and two cavities are located in midrib area. The size of the cavities is small, taking  $(1/8)$ – $1/4$ – $1/3$  ( $-1/2$ ) of the leaf thickness. One or two vascular bundles (one large and the other small) are located in the median nerve; 7–10 vascular bundles in one half and 8–11 vascular bundles in the other half of the lamina (Figs. 4 E, F and 5 E, F).  
*Stem.* The stems are 1.5–2.5 mm in diameter, acutely or obtusely triangular in outline, and the sides are concave or flat. Air cavities are usually small and scattered in the central part, but larger cavities may be present in the centre of the stem; the cavities are composed of stellate cells with intercellular spaces and may also be present irregularly in a row in the peripheral parts. Vascular bundles of different sizes are located in numbers of 23–35 in the peripheral parts of the stem, with 4–9 relatively large bundles in the central part. Vascular bundles (1–2) are located in corners. The sclerenchyma forms 23–38 subepidermal strands accompanying the vascular bundles but is

usually not connected with them; the strands are of various sizes, rounded to triangular in cross section, and crescent or cap shaped in corners (Figs. 6 E, F and 7 E, F).

**Bolboschoenus planiculmis** (F. Schmidt) T. V. Egorova

*Leaf.* The leaves are 4–6 mm in width and keeled. The margins are subequal, acute or obtuse. The epidermis on the adaxial and abaxial sides is represented by one layer of translucent cells, on adaxial midrib forming well-developed inflated (bulliform) cells, which are accompanied by other one or two layers of bulliform cells. The median vascular bundle is accompanied by 1–3 sclerenchyma abaxial strands; other abaxial and adaxial strands of different sizes are located opposite the vascular bundles throughout leaf lamina, with one final strand in each margin. The mesophyll is composed of chlorenchymatous cells, occurring around vascular bundles and immediately subjacent to the epidermis. Air cavities are composed of stellate cells with intercellular spaces and occur regularly in the row in the gaps between the vascular bundles (in  $2/3$ – $4/5$  gaps), with two cavities situated in the midrib area. The cavities are mostly highly visible, occupying  $(1/4)$ – $1/3$ – $2/5$  ( $-3/5$ ) of the leaf thickness. Vascular bundles (2, one large and one small) are located in the median nerve, with 11 vascular bundles in each half of the lamina (Figs. 4 G, H and 5 G, H).

*Stem.* The stems are 1.9–3 mm in diameter and triangular or acutely triangular in outline. The sides are concave, flat or convex. Air cavities are small and scattered in the central part of the stem, only rarely and slightly visible between the vascular bundles in the peripheral parts. The vascular bundles are relatively large when located in the central part (9–12 of them), 29–40 bundles of different sizes are located in the peripheral parts, and 1–2 vascular bundles are located in corners. The sclerenchyma forms 29–45 hypodermal strands opposite the vascular bundles; they are of various sizes, rounded to triangular, only rarely connected to vascular bundles and crescent or cap shaped in corners (Figs. 6 G, H and 7 G, H).

**Bolboschoenus schmidii** (Raymond) Holub

*Leaf.* The leaves are 4.6–6.9 mm in width, flanged, acutely keeled, and the margins are subequal and acute. The epidermis on the adaxial and abaxial sides is represented by one layer of translucent cells; the adaxial cells in the midrib are of the same size as the other epidermal cells or little inflated (not similar to bulliform cells) but accompanied by 1–2 layers of more inflated (bulliform) cells below the epidermis. The median vascular bundle is accompanied by 3–5

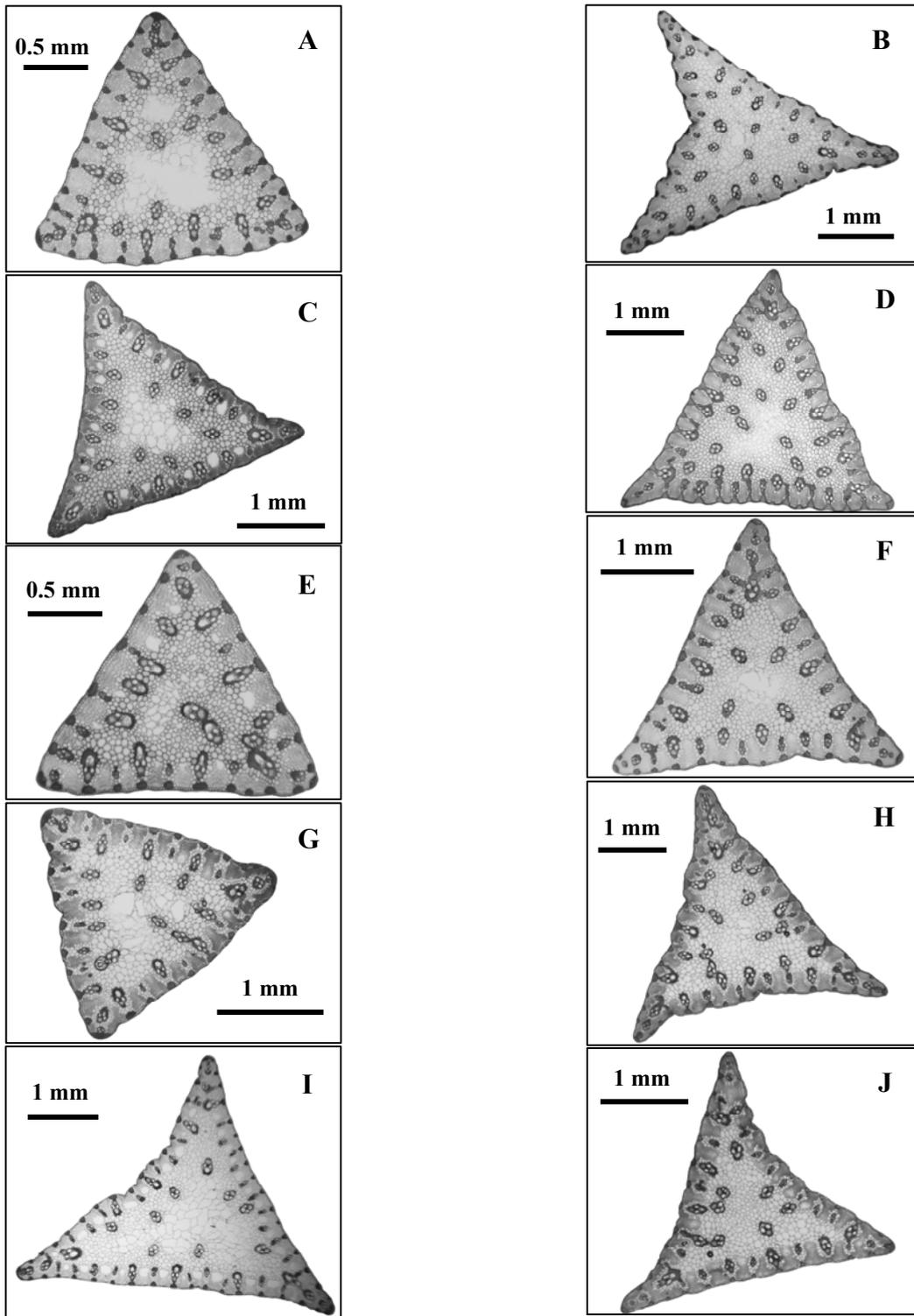


Fig. 6. Transverse section of stem: *Bolboschoenus affinis* (A: 35550-IRAN, B: 52991-IRAN); *B. glaucus* (C: 37288-IRAN, D: 43970-IRAN); *B. maritimus* (E: 32501-IRAN, F: 48931-IRAN); *B. planiculmis* (G: 37227-IRAN, H: 47725-IRAN); *B. schmidii* (I: 47728-IRAN, J: 47729-IRAN).

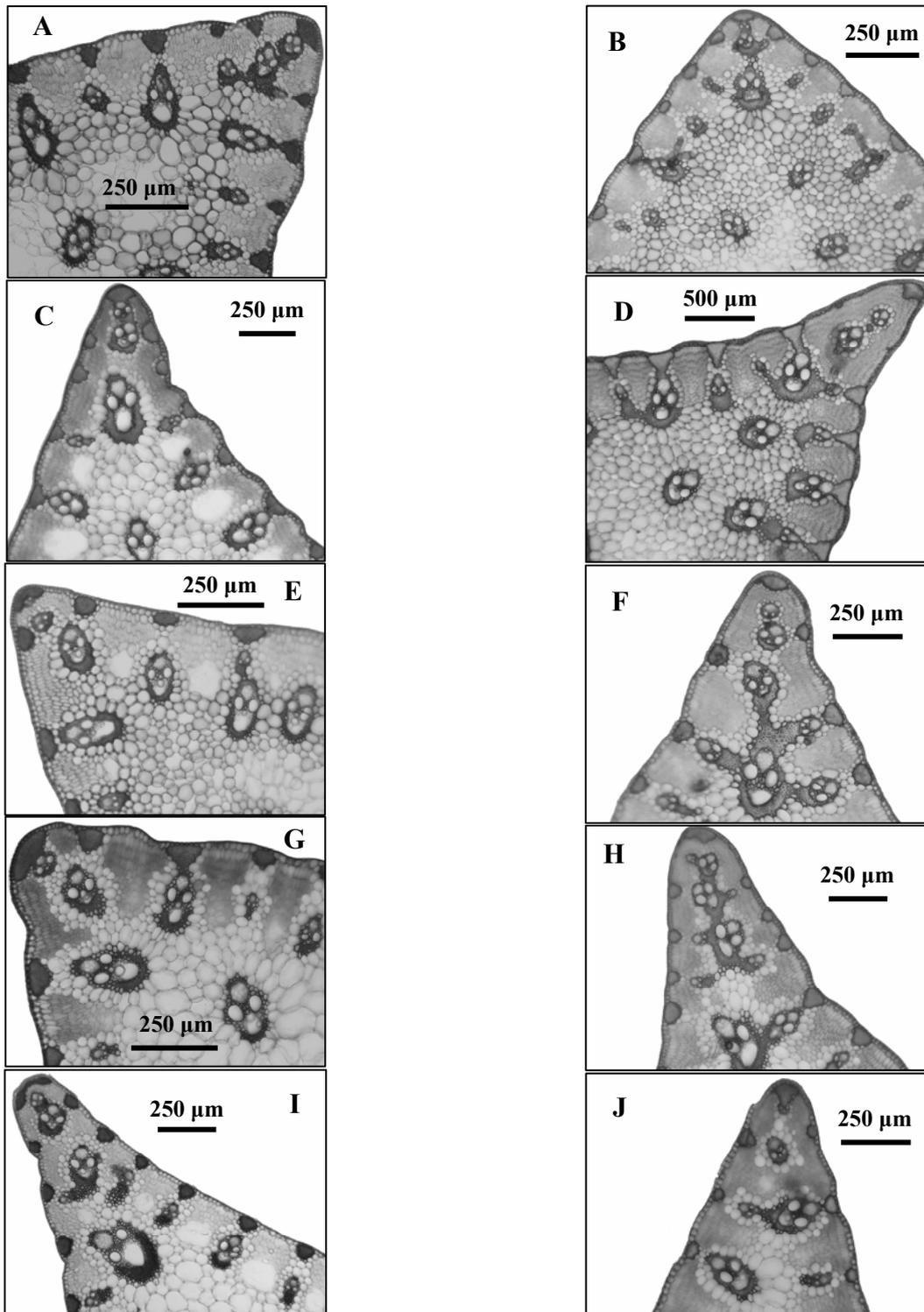


Fig. 7. Transverse section of stem: *Bolboschoenus affinis* (A: 35550-IRAN, B: 54855-IRAN); *B. glaucus* (C: 37288-IRAN, D: 43970-IRAN); *B. maritimus* (E: 32501-IRAN, F: 48931-IRAN); *B. planiculmis* (G: 37227-IRAN, H: 47725-IRAN); *B. schmidii* (I: 47728-IRAN, J: 47729-IRAN).

sclerenchyma abaxial strands; other abaxial and adaxial strands are of similar size, located opposite to the vascular bundles, with one strand in each margin. The mesophyll is composed of chlorenchymatous cells located between the vascular bundles, immediately subjacent to the epidermis. Air cavities are mostly highly visible between the vascular bundles and composed of stellate cells with inter-cellular spaces; they occur in 3/4 of all of the gaps between bundles, with two cavities of similar size in the midrib area. The cavities usually occupy (1/4–) 1/3 (–1/2) of the leaf thickness. One vascular bundle is located in the median nerve, 11–15 vascular bundles in one half and 11–14 vascular bundles in the other half of the lamina (Figs. 4 I, J and 5 I, J).

*Stem.* The stems are 2–3.5 mm in diameter and acutely triangular in outline, with concave sides. Air cavities occur regularly in a row between the peripheral vascular bundles, in some cases also in the central part of the stem. They are mostly large and composed of stellate cells with numerous intercellular spaces. The vascular bundles located in the peripheral areas are small and large, 31–44 in number; 7–10 large vascular bundles are located in the central area, with 2 vascular bundles in corners. Sclerenchyma is present in 30–52 hypodermal strands opposite to the vascular bundles, in some cases connected to them. Subepidermal strands are of various sizes, almost triangular, crescentiform in corners (Figs. 6 I, J and 7 I, J).

## DISCUSSION

In Iranian *Bolboschoenus*, the following characters common to the anatomy of *Cyperaceae* (Standley 1990) were found: the leaves are dorsiventral and V-shaped in cross-sectional outline, with a single row of vascular bundles, of which the median and some of the lateral ones are enlarged; a two-layered bundle sheath, with the inner being sclerenchymatous and the outer being parenchymatous; there is a median adaxial group of inflated bulliform cells; air cavities extend the length of the leaf between the vascular bundles; adaxial epidermal cells are larger than the abaxial cells; the stomata are seen in abaxial and adaxial side, with the subsidiary and guard cells level with the epidermal surface; and epidermal cells lack papillae.

In accordance with *Bolboschoenus* being  $C_3$ -photosynthetic plants (Soros & Bruhl 2000), no Kranz anatomy was found; the vascular bundles were surrounded by a mestome (fibrous) sheath and parenchymatous bundle sheath, as shown by Soros & Dengler (1998) for  $C_3$  *Cyperaceae*.

When compared with the previous anatomical data on *Bolboschoenus*, some differences were found. Metcalfe (1971) reported only anatomical characters of

*B. maritimus* (*Scirpus maritimus* L.), which probably only concerned *B. maritimus* s. str. (the most frequent species in Great Britain, Hroudová unpublished). The following differences were found when comparing our results and those of Metcalfe l.c. for *B. maritimus*, enabling us to imagine a variation in the range of individual characters: the stems are triangular with flat sides or acutely triangular with concave sides (our results) versus triangular with flat or convex sides (Metcalfe l.c.); there is a rare occurrence of air cavities in the stem (our results) versus the presence of 45 air cavities (Metcalfe l.c.); the sclerenchyma forms round to triangular strands in the stem (our results) versus flat-shaped (Metcalfe l.c.); the leaves are V-shaped (our results) versus widely V-shaped (Metcalfe l.c.) and there are three vascular bundles in the midrib (our results) versus two vascular bundles (Metcalfe l.c.). However, in specimen number 32501, the stem profile was similar to the specimens studied by Metcalfe l.c.: obtusely triangular in cross section, with large air cavities visible between the vascular bundles in the peripheral parts of the stem. It is also necessary to take into account that the width of the V-shape of the leaf might be influenced by leaf folding under different environmental conditions.

When comparing the anatomical characters of the studied species (Table 2), differences were found in the size of the adaxial epidermal cells in the midrib, the presence of bulliform cells in the epidermis, the presence and number of inflated cell layers beneath the epidermis, the number and shape of sclerenchyma strands, the number of vascular bundles in the midrib, and number and size of air cavities in the leaf lamina.

The presence and development of bulliform cells appear to be valuable diagnostic characters among the studied species and corresponds to their ecology. Bulliform cells are primarily found in grasses (*Poaceae*) and function in leaf folding (for decreased water loss) under dry conditions or, according to another hypothesis, function to unroll the leaves during bud opening (Pazourek & Votrubová 1997). Similarly, these cells are also found in some *Cyperaceae* as an adaptation to an amphibious lifestyle in temporary lakes (Leite et al. 2009); bulliform cells are also found as characters distinguishing some *Cyperus* species (Hefler & Longhi-Wagner 2010). According to Metcalfe (1971: Page 22), bulliform cells are important in the epidermis and hypodermis and generally constitute part of the epidermis, but, in a few cases, cells of the hypodermis layer may be more inflated than those of the epidermis itself. However, Martins et al. (2012) studied the development of subepidermal layers in *Cyperoideae* during leaf ontogeny, and found that several cell layers below epidermis in the studied

species did not represent hypodermis, but multiple epidermis, originated from periclinal cell division of the protodermis. Correspondingly to their findings, we have found multiple translucent cell layers in *Bolboschoenus* on the adaxial side in the midrib of leaves.

The following features were found to characterise the *Bolboschoenus* species: (1) *B. glaucus*: the absence of inflated (bulliform) cells in the epidermis, greater cells below epidermis in irregular layers or forming a group of unordered inflated cells in the midrib between the adaxial epidermis and the main vascular bundle; (2) *B. affinis*, *B. schmidii* and *B. maritimus*: epidermal cells only slightly inflated, with one or two subepidermal layers of cells greater than epidermal cells (bulliform cells); (3) *B. planiculmis*: well developed inflated (bulliform) cells in epidermis and also in one to two subepidermal cell layers. Similar shape and parallel position of the cells of surface epidermal layer with those of subepidermal layers indicate a multiple epidermis in *Bolboschoenus planiculmis*, and probably also in *B. maritimus*, *B. affinis* and *B. schmidii*. In *B. glaucus* the nonaligned arrangement of the cells makes the interpretation of multiple epidermis difficult, and together with small epidermal cells distinguishes *B. glaucus* from other *Bolboschoenus* species.

Differences between the species may also be found in the proportions of the air cavities in the leaf lamina: the rare occurrence and small size of air cavities were observed in *B. affinis*; however, in *B. glaucus* and *B. schmidii*, air cavities were present in nearly all of the gaps between the vascular bundles and were in the largest in *B. glaucus*. Although some differences also appeared in the other variables measured, their ranges mostly overlapped; possible variation dependent on environmental conditions should be taken into account for all of the quantitative characters (including air cavities). No crucial differences in the anatomical structure of the stem were found among the Iranian *Bolboschoenus* species, reliably enabling their differentiation (Fig. 4, Table 2). Thus, the studied anatomical characters may be considered to support differentiation based on morphological characters and fruit anatomy and not as strictly distinguishing characters alone.

Although *Bolboschoenus* species are primarily wetland plants, they frequently grow in habitats with fluctuating water levels, temporarily dry or temporarily flooded, which requires adaptations to both drought and flooding (see Leite et al. 2009). In Iran, two main habitat factors may influence *Bolboschoenus* and could induce anatomical adaptations: drought and salinity. The anatomical adaptations to both of these factors may be identical in some cases, leading to the reduction of

transpiration (Fahn & Cutler 1992). Of the Iranian *Bolboschoenus*, *B. affinis* and *B. maritimus* frequently inhabit saline habitats; the others are freshwater plants that inhabit various wetland habitats: *B. schmidii* occurs along streams and water sources, in channels and near villages; *B. glaucus* is the most frequent species occurring from streams and lake shores to semi-desert swamps and secondary habitats near villages; and *B. planiculmis* is a rare plant, introduced in secondary habitats (Amini Rad et al. 2010).

The presence of well-developed bulliform cells in epidermis is a qualitative character, common especially to *Bolboschoenus planiculmis*, as well as in *B. affinis*, *B. maritimus* and *B. schmidii*. This character indicates the ability of the leaf to fold or the involution of the leaf lamina when a decrease in transpiration is necessary. The presence of bulliform cells might be considered as a phylogenetically developed adaptation to the drought, which could be also connected with an increase in the salt concentration in the soil solution in saline habitats. No other special anatomical adaptations to salinity were observed in *B. maritimus*, a species that is probably adapted to saline habitats mainly through physiology, i.e., by osmotic adjustment (Hroudová et al. in prep.). In *B. planiculmis*, bulliform cells might develop as adaptive response to water shortage, enabling this species to survive during summer drought in temporarily flooded field depressions in the steppe zone throughout its Eurasian area of distribution (Egorova & Tatanov 2003, Hroudová et al. 2007). *B. glaucus* represents a wetland plant with a wide ecological amplitude that is well adapted to flooding, and is distinguished by well-developed air cavities in the leaf lamina and partially also in the stem periphery, and by the absence of typical bulliform cells in the epidermis. The presence of air cavities may be related to environmental conditions, as intercellular spaces in the leaf and stem enable gas transport and exchange in plants growing under flooded conditions (Cronk & Fennessy 2001).

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