THE TAXONOMIC SIGNIFICANCE OF LEAF ANATOMY IN THE GENUS ONOSMA L. (BORAGINACEAE) IN IRAN

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The leaf anatomy of representative species of *Onosma* L. from Iran have shown that the type of hairs are characteristic features in distingushing species and group of species within the genus. In internal structure of lamina two distinct groups are recognized within *Onosma*; sections *Protonosma* M. Pop. and *Podonosma* (Boiss.) Gruck with dorsiventral mesophyll and sect. *Onosma* mainly isobilateral. Several anatomical characters are associated with xeromorphy. Leaf anatomy supports the division of the genus *Onosma* L. into 3 sections.

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اهمیت تاکسونومیکی ساختار تشریحی برگ در گیاهان جنس Onosma L. از ایران

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ساختار تشریحی گونه‌های مورد مطالعه از جنس *Onosma* L. در ایران نشان‌می‌دهد که نوع کرک‌ها یکی از صفات مشخص در شناسایی گونه‌ها و تفکیک بخشها در جنس *Onosma* می‌باشد ساختمان درونی پهنک برگ نیز دو گروه را بخوبی مشخص می‌سازد، در بخشهای Sect. *Podonosma* و Sect. *Protonosma* دارای مزوفیل یک طرفه (پارانشیم نرده‌ای فقط زیر اپی‌درم فوقانی) است ولی در بخش Sect. *Onosma* مزوفیل برگ به صورت دوطرفه با لایه‌های پارانشیم نرده‌ای متعدد در زیر هر دو اپی‌درم فوقانی و تحتانی است. در بخش Sect. Onsosma صفات متعدد تشریحی نشان دهنده خصوصیات گزروفیتی (خشکی پسند) است در حالیکه صفات تشریحی برگ گونه‌های دو بخش دیگر بیشتر مزوفیتی است. ساختار درونی برگ گونه‌های مطالعه شده تقسیم بندی مورفولوژیکی جنس *Onosma* را به سه بخش Sect. *Podonosma*، Sect. *Protonosma* و Sect. *Onosma* تأیید می‌نماید.

INTRODUCTION

The genus *Onosma* L. comprises about 150 species, distributed mainly in the Mediterranean and Irano-Touranian regions (Tutin & al. 1972; Zohary 1978). The genus has high diversity in Iran. It contains more than 50 species with wide geographical distribution and several endemic species in this area.

Riedle (1967) subdivided the genus into 3 sections: Sect. *Protonosma* M. Pop.; Sect. *Podonosma* (Boiss.) Gurcke, each section with one species only, as *O. rostellatum* Lehm. and *O. orientale* L. respectively, and Sect. *Onosma* with the rest of species. In previous taxonomic studies of *Onosma*, Boissier (1879) recognized Sect. *Podonosma* as a genus *Podonosma* Boiss. with only one species. He divided the genus *Onosma* into 3 sections based on the type of hairs or setae. Recently taxonomic accounts of the family have been evaluated by Khatamsaz (1992 & 1996) employing mainly morphological characters and several species have been recognized as new records for Iran (Ghahreman & Attar 1996).

Anatomical data of the genus *Onosma* are few and scattered in literature dealing more generally with the family *Boraginaceae* (Metcalfe & Chalk 1950, 1985). As noted by Metcalfe & Chalk (1950) various type of hairs (setae) occur mostly in *Boraginaceae* and it shows great variation within *Onosma* species. In recent years taxonomic importance of leaf anatomy of *Heliotropium* and *Cordia* species of *Boraginaceae* have been studied by Doaigey & al (1981), Roa & Kumar (1995), Azizian, Yusofi & Kasaian (1996). Since there is no comprehensive anatomical study of the genus *Onosma*, in this paper 19 species in 3 sections of *Onosma* from Iran have been investigated in order to see if anatomical character of leaves could be used in their identification and delimitation of the genus. Full descriptions can be found in Kasaian 1994.

MATERIALS AND METHODS

Specimens used in this study were collected in the field or from herbarium materials, (voucher specimens are deposited in the Central Herbarium of Iran (TARI)), A total of 30 specimens representative of 14 species of *Onosma* were examined listed in table 1. The leaves were fixed in FAA for about 72 hours and then preserved in 70 % ethanol. Herbarium specimens were boild gently before fixation. Sections were made

Table 1. Material used in anatomical studies of the genus *Onosma*.

Taxa	Origin & Collectors
O. rostellatum Lehm.	Fars: Bamou Protected Region, Cheshmeh Fil, 1900-2650m, Wendelbo & Froughi 17638; Esfahan. N. side of Kuh-e Dena, Gardaneh Bijan, 2800 m, Assadi & Abouhamzeh 46154; Esfahan: Semirom, above Deh-e Bid, 2200 m, Nouroozi 2981.
O. orientale L.	Fars: 34 Km. from Nurabad to Dogonbadan, 700 m, Assadi & Abouhamzeh 38465; Khuzestan: 38 Km Baghmalek to Izeh, 600 m, Foroughi 3591.
O. dichroanthum Boiss.	Kordestan: 12 Km. E. of Sanandaj, 2180 m, Fattahi 1312; Tehran: Firouzkuh pass, Gel-Ahak, Takht Chenar, 1750 m, Dini & Amin 1335; Tehran, Firouz-Kuh, Pol-e Veresk, 1600 m, Gheissari 1348.
O. longilobum Bge.	Khorassan: 50 Km. N. of Ghuchan, Chevenli, 2000-2100 m, Assadi & Maassoumi 21441; Semnan: 15 Km N. of Shahrud, Nekaraman, 2300 m, Assadi & Maassoumi 21039.
O. kotschyi Boiss.	Tehran: Abali, 1850 m, Dini & Arazm 1338; Tehran, Gezel Dareh, 2450-2500 m, Dini & Arazm 1330.
O. bulbotrichum DC.	Fars: Kazeron, Shahpur cave, Tange Chogan, 920 m, Foroughi 3588; Tehran: 123 Km W. of Tehran, 1200 m, Seraj 24455; Kermanshah: 32 Km S. of Islamabad, Gardaneh Gahlajeh, 1600 m, Hamzehee 1470.
O. macrophyllum Bornm.	Fars: 25 Km S. of Fasa, Salou village, Kuh-e Raz, 1600-2200 m, Mozaffarian 46792; Esfahan: Chadegan, Kuh-e Bidak, 2550 m, Nowroozi et al. 1088.
O. elwendicum Wettst.	Tehran: Vardvard valley, 1850 m, Wendelbo et al. 11768; Kordestan: 107 Km from Zanjan on the road to Bijar, 1750 m, Assadi & Amini 13575-A.
O. demawendicum Riedl	Tehran: 14 Km S. of Damavand, Akhorbadin, 1650-1800 m, Mozaffarian 53831.

Table 1. Continued.

O. kilouyense Boiss. & Hausskn.	Kermanshah: Gahvareh, Bezahoo mt., 1620 m, Hossaini 2666; Kermanshah: 150 Km N. of Kermanshah Mailek & Shoushtari 2683.
O. bilabiatum Boiss. & Buhse	Azerbaijan: S. slope of Kuh-e Sefid, Tang-e Farah-Kash, 1800-1900 m, Wendelbo & Assadi 16636.
O. albo-roseum Fisch. & Mey.	Tehran: Arak to Mahalat, Late-dar, 2100-2500 m Mozaffarian & Maassoumi 47935; Kermanshah: 150 Km NW. of Kermanshah, 1300 m, Shoushtari 2578; Kordestan, Saravand, 98 Km W. of Sanandaj, 1380 m, Fattahi 691.
O. hebebulbum DC.	Tehran, Gazvin to Hamadan, pass just after Avaj, 2100 m, Assadi & Mozaffarian 36665.
O. armenum DC.	Azerbaijan: Mahabad, Miandoab road, Borvy 2203.

from the middle part of lamina (midrib), cleared in parazone, stained with safranin and fast green. Subsequently mounted in glycerol solution. Epidermal peels, were prepared by using Jeffery,s solution (10% nitric acid and 10% chromic acid, 1: 1), or they were boiled in 1-5% KOH solution for a few minutes to separate the epidermis from the mesophyll. The prepared epidemis was washed and stained with safranin. A Camera Lucida was used for the drawing. Photographs were made using Olympus-BH2 microscope attached to a camera. Trichomes were obseved in transverse sections of leaves complemented with epidermal peel as well as stereoscopic observation of herbarium specimens.

RESULTS

Leaf surface (Fig 1, A-E)

Cuticule: thick adaxially than abaxially. Epidermal cells polygonal, more or less elongated, cell wall striaght, usually larger on adaxial surface. Stomata superficial, mainly anomocytic, more abundant abaxially.

Hairs: several types present on both surfaces.

Nonglandular hairs

1- Hairs (setae) unicellular with or without prominent base and long narrow apex, with thick warty and rough walls sometimes thin and soft wall. In some species few minute hairs attached to the base of setae (Fig. 2,

B, C). Various types of setae are characteristic and present within the genus except in *O. bilabiatum*, *O. albo-roseum*, *O. latifolium*, *O. hebebulbom* and *O. armenum.*

2- Short hairs one celled with sharp apex, mainly on adaxial surface. This form is confined to some species in Sect. *Onosma*, such as *O. dichroanthum, O. bilabiatum, O. hebebulbum* (Fig. 2, A).

3- Stellate hairs, sessile or subsessile, with one large erect central branch surrounded by short narrow branches. This form variable in size with short or large branches and thichened walls (Fig. 2, C, D_1, D_2). This form is confined to some species of the sect. *Onosma* but absent from sect. *Protonosma* and sect. *Podonosma.*

Glandular Hairs

1- Long 2-celled stalked glandular hairs, where the stalk shows a larger foot and the cell above shorter ending with a unicellular gland, this type is restricted to Sect. *Podonosma* with only one species *O. orientale* (Figs. 2, A, D & 3).

2- Short 1-celled stalk and 1-celled head. This form also restricted to *O. orientale* (Sect. *Podonosma*) and rarely in *O. dichroanthum* and *O. macrophyllum.* (Fig. 2, A-4).

Leaf T. S. (Lamina) (Fig. 3, A-F)

Outline dorsiventral to isobilateral, midrib prominent below, sometimes ± curved in the margin (Fig. 3B & Fig. 5B).

Hairs abundant on adaxial surface, but more frequent along the majar veins on abaxial surface. Hair forms as described for leaf surface. (Fig. 4, A-E). Cuticlue thick on adaxial surface. Epidermal cells usually in one layer often larger adaxially.

Mesophyll: Palisade tissue in one layer adaxially, often equlling about 1/2 thickness of mesophyll, in *O. rostellatum* and *O. orientale*, (sects. *Protonosma* and *Podonosma* respectively) which show more mesomorphic characters in these groups (Fig. 5C). But in sect. *Onosma* palisade cells comprising 2-3 layers adaxially with sinous walls and one layer abaxially, sometimes spongy parenchyma in between, which indicate xeromorphic characters e. g. in *O. armenum* and *O. elwendicum*. Spongy parenchyma usually lobed, abaxially in dorsiventrai leaf. (Fig. 3, B, C, E, F) & (Fig. 5B).

Vascular bundles: collateral, in midrib, crescentic form, subepidermal collenchyma

present, often well developed on both sides of midrib, except in *O. orientale* (sect. *Podonosma*).

Bundle sheath present as one layer of large parenchymatous cells surrounding the small vascular bundles, but absent from the largest bundles. (Fig. 5, A, C.). Crystal (calcium carbonate) present in two forms; a- deposited in cell wall of hairs, or b- located in the base of large hairs, which mentioned by Metcalfe & Chalk (1950) as hair with calcified wall and characteristic in *Boraginaceae* (Fig. 4E).

DISCUSSION

Since there has been no comprehensive study of the genus *Onosma*, in this investigation apart from gross morphology, particular attention has been paid to data from leaf anatomy. Anatomical characters of leaf support the morphological subdivision of the genus as Reidle (1967) considered in Flora Iranica. Epidermal cells are found to be similar in all species examined. Stomata are mainly anomocytic. The type of hairs are characteristic feature in this genus. Large hairs (setae) of various forms are diagnostic character within the sections; for example in *O. orientale* sect. *Podonosma*, setae have prominent base surrounded with few short hairs, in *O. rostellatum* sect. *Protonosma*, setae are with rough and warty wall, while in *O. chrysochaetum* and *O. longilobum* of sect. *Onosma* setae have no prominent base with thin and unicellular hairs around it. In some species of sect. *Onosma* such as *O. sericeum* and *O. macrophyllum* setae appears in position with few scattered short hairs. Large hairs (setae) absent from *O. bilabiatum*, *O. albo-roseum*, *O. latifolium*, *O. hebebulbum* and *O. armenum*, (sect. *Onosma*), which they have stellate hairs instead. Stellate hairs subsessile surrounded by short and unicellular hairs occur in *O. bilabiatum*, *O. armenum*. But sessile stellate hairs present in *O. albo-rosaum*, *O. latifolium* and *O. hebebulbum* (see table 2). Glandular hair with a long stalk is characteristic in *O. orientale* (sect. *Podonosma*) and absent from other species examined. The short-stalked glandular hairs occur in *O. orientale* as well. These types of hairs were used to separate sect. *Podonosma* from the genus *Onosma* by Boissier in 1879. Apart from hair characters, internal structure of leaves exhibit 2 distinct groups within the genus; in sects. *Protonosma* and *Podonosma*, leaf dorsiventral, palisade tissue in one layer adaxially. But in sect. *Onosma* leaf isobilateral, palisade tissue in 1-3 layers

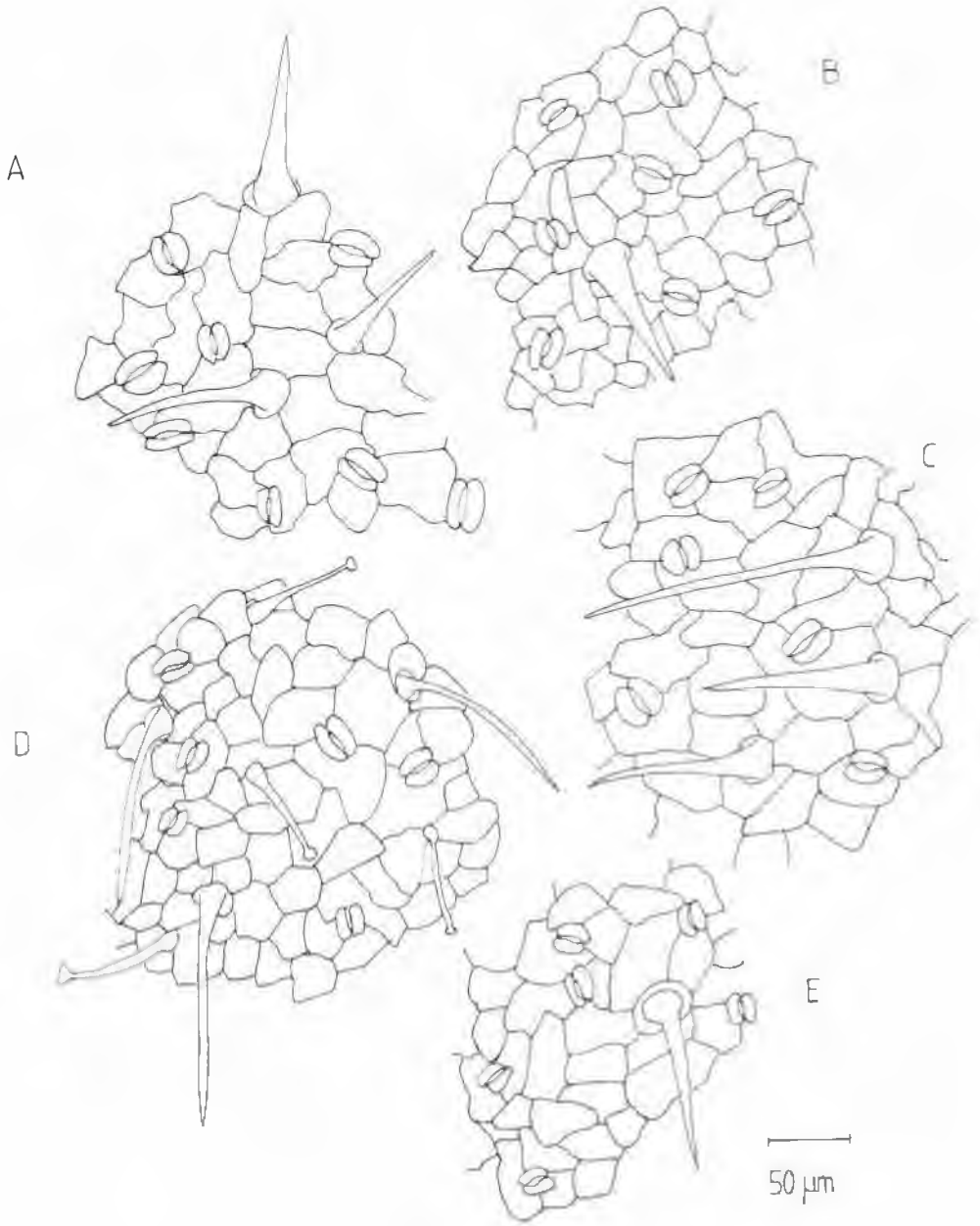

Fig. 1. Leaf surface, epidermal cells, stomata and hairs. A. *Onosma lanceolatum*, B. *O. kilouyense*, C. *O. dichroanthum*, D. *O. orientale*. E. *O. hebebulbum* .

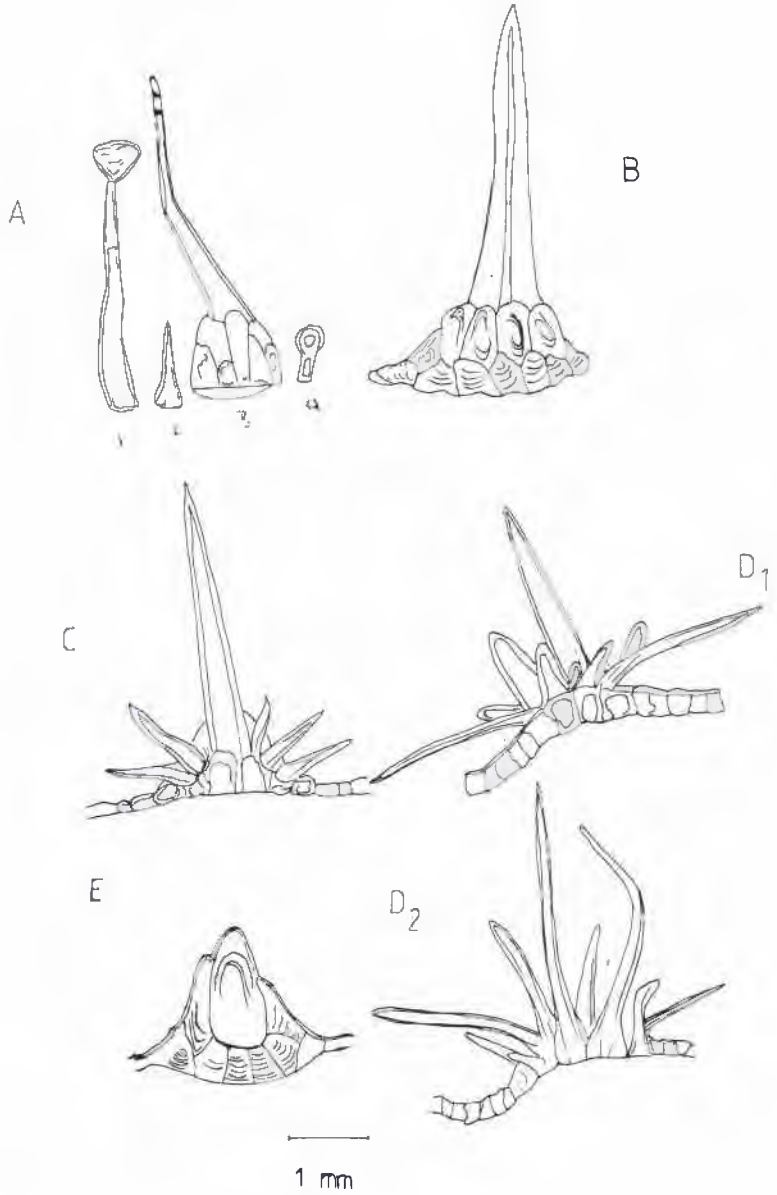

Fig. 2. Type of hairs. A. glandular long 2-celled stalk (1) and 1-celled stalk (4), short 1-celled hair and large hair (2, 3). - *Onosma orientale*; B. large hair with prominent base; C. Large hair surrounded with minute hairs; D1, D2. Stellate hairs; E. short hair with calcified wall.

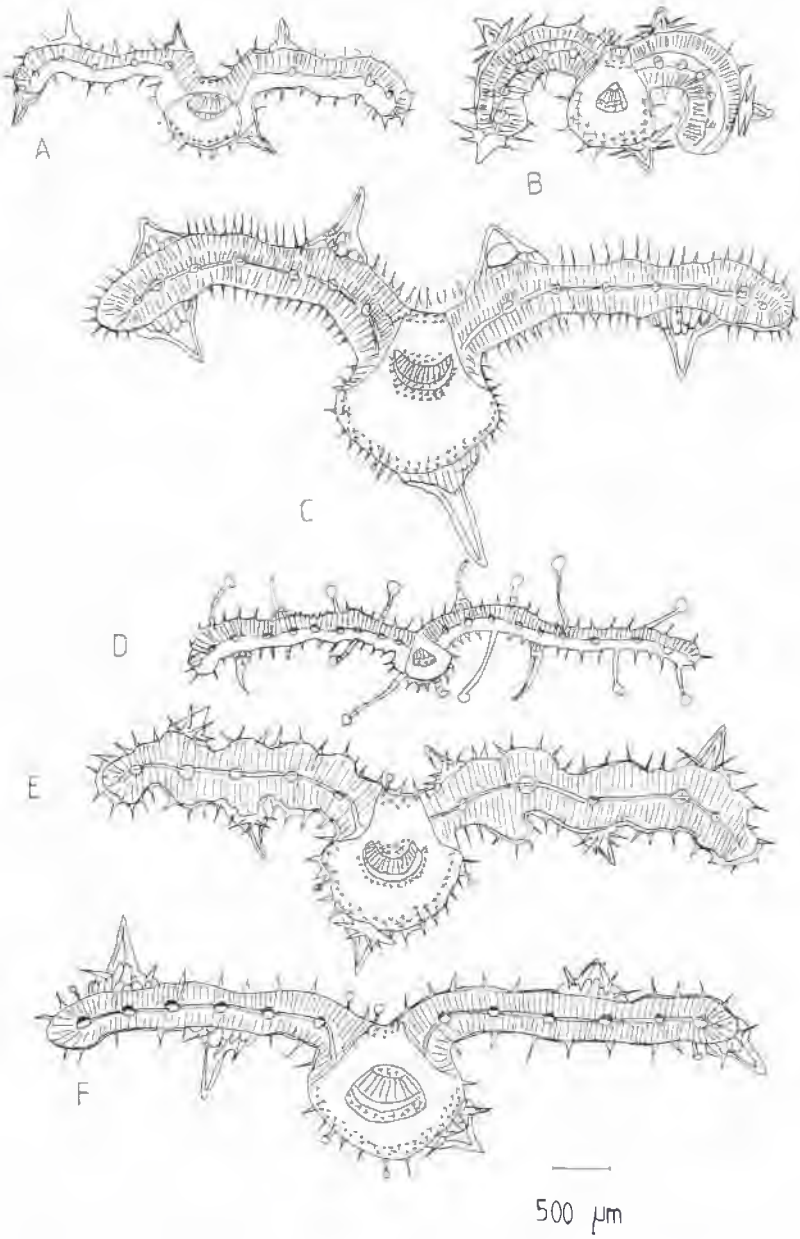

Fig. 3. Leaf lamina transverse section. A. *Onosma rostellatum*; B. *O. armenum*; C. *O. longilobum*; D. *O. orientale*; E. *O. kilouyense*; F. *O. elwendicum*. (palisade ▤ and spongy parenchyma □).

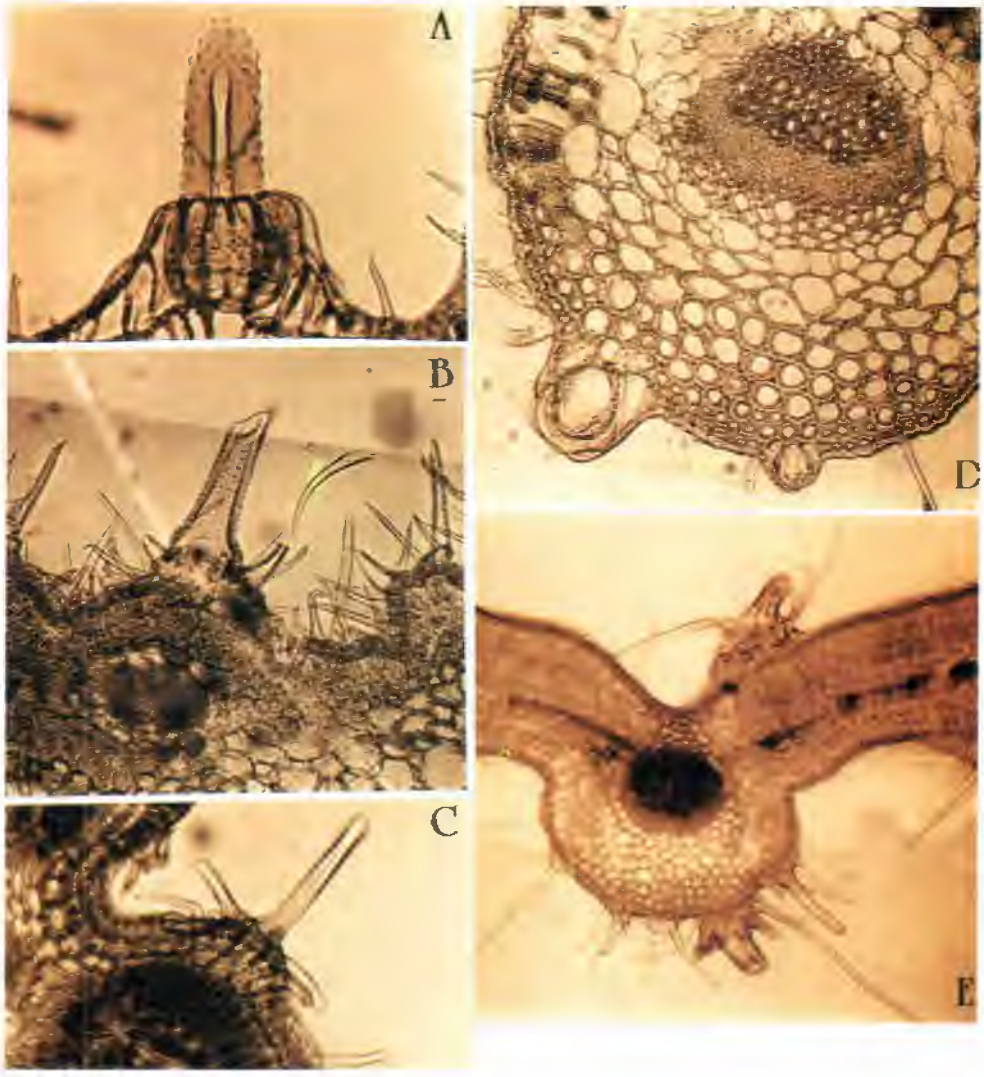

Fig. 4. Leaf lamina transverse section showing type of hairs in *Onosma* species A. short hairs with prominent basal cells, thick warty wall (*O. longilobum*) (X32); B, C. large hair with few minute hairs around the base (*O. armenum* & *O. elwendicum*) (X32); D. midrib, subepidermal collenchyma, vascular bundle crescentic (*O. macrophyllum*) (X32); E. midrib, different type of hairs on abaxial and adaxid surfaces, and subepidermal collenchyma extention on both sides of vascular bundle (*O. albo-roseum*) (X8).

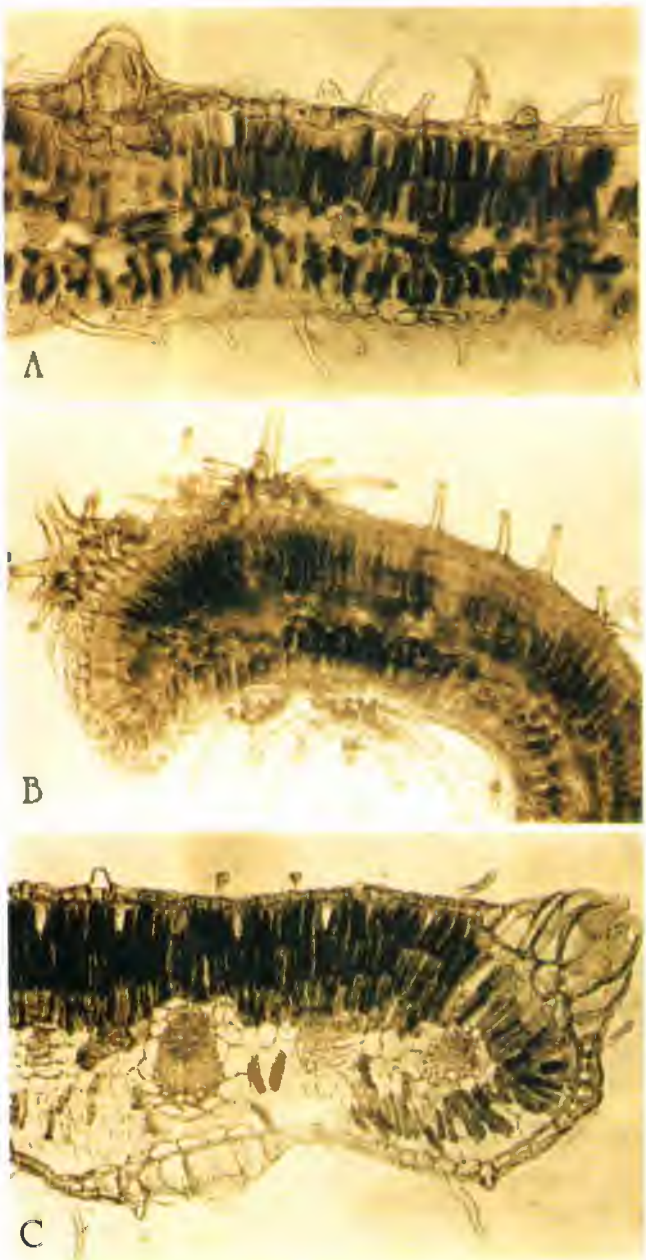

Fig. 5. Leaf lamina transverse section, light microscopy. A. *Onosma elwendicum*, isobilateral mesophyll and calcified, simple, unicellular hairs.B. *O. armenum*, isobilateral mesophyll and stellate hairs. C. *O. rostellatum*, dorsiventral mesophyll, with 3 layers of palisade adaxially and bundle sheath around small vascular bundle (X15).

Tab 2: Comparative anatomical leaf characters of *Onosma* species. pal: palisade, ad: adaxial, ab: abaxial, g. h.: glandular hairs, coll.: collenchyma

Species	Leaf shape	Mesophyll	Nonglandular hairs (Setae)	No. of ad. pal.	No. of ab. pal.	Glandular hairs	Coll. on midrib
O. rostellatum	lanceolate	dorsiventral	simple	1-2	–	short one celled	+
O. orientale	broadly lanceolate	"	"	1	–	long 2-celled	–
O. dichroanthum	linear-oblanceolate	isobilateral	"	2-3	1-2	short one celled	+
O. longilobum	spathulate-oblanceolate	"	"	2	1	"	+
O. kotschyi	linear-oblanceolate	"	"	2	1	"	+
O. bulbotrichum	"	"	"	2	1	"	+
O. macrophyllum	lanceolate-oblong	"	simple with minute haris	2	1-2	"	+
O. elwendicum	"	"	"	2-3	1	"	+
O. demawendicum	spathulate-oblanceolate	"	"	2-3	1-2	"	+
O. kilouyense	"	"	stellate	1-2	1	"	+
O. bilabiatum	"	"	"	2	1	"	+
O. albo-roseum	"	"	"	2-3	1-2	"	+
O. hebebulbum	"	"	"	2-3	2	"	+
O. armenum	linear-lanceolate	"	"	2-3	2	"	+

adaxially and one layer abaxially, which indicate of xeromorphic features in this group. Additional anatomical features, such as cuticule thickness, well developed patch of collenchyma on both side of midrib are associated with xeromorphy except in sect. *Podonosma* which shows more mesomorphic characters. In conclusion leaf anatomy in *Onosma*, is taxonomically of value and give additional support and distinctness of the species within 3 sections.

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