ANATOMY-TAXONOMY STUDIES OF THE SPECIES OF SECTION SERRATULOIDEAE (COUSINIA, COMPOSITAE) IN IRAN

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Cousinia section Serratuloideae has 14 species in Iran including two new species "C. sheidaiana and C. elburzensis" to be described and one transfered species "C. adenostegia". All species of this section except C. pterocaulos are endemic to Iran. Comparative anatomy of the leaf (except C. olivieri and C. alfredii) and stem (some of the species) of the species of the section Serratuloideae is presented. Stem structure, with the exception of some minor differences, was the same for all the species. Most variation belonged to leaves and the structure of their midrib. It is therefore, easy to distinguish species, on the basis of these structural characteristics. The coloured photographs for each species and an indentification key based on the anatomical characteristics is presented.

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Key words. Taxonomy, anatomy, Asteraceae, Cousinia, Serratuloideae, endemic, Iran.

مطالعه آناتومی – تاگزونومی گونههای بخش سراتولوئیده (جنس کوزینیا، خانواده کاسنی) در ایران

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بخش سراتولوئیده در ایران دارای ۱۶ گونه است، از این تعداد در این تحقیق ۲ گونه جدید .C. elburzensis و C. sheidaiana به آن اضافه و گونه C. pterocaulos نیز به آن منتقل میشود. همه گونه های آن به جز گونه C. pterocaulos اندمیک ایران هستند. تشریح مقایسهای ساختار برگ (به جز گونه های C. alfredii) اندمیک ایران هستند. تشریح گونهها) گونههای بخش سراتولوئیده از جنس کوزینیا مطالعه شده صفات تشریحی اندامهای مختلف برای هر گونه شرح داده شده است. ساختار تشریحی ساقه همه گونهها به جز برخی اختلافات جزئی تقریبا در همه یکسان است. بیشترین اختلاف مربوط به برگ و ساختار تشریحی رگبرگ میانی آن است.

بر اساس همین صفات میتوان به راحتی گونههای مختلف این بخش را از یکدیگر جدا کرد. عکسهای رنگی برای هر گونه و کلید شناسائی بر اساس صفات آناتومیک اراثه شده است.

INTRODUCTION

The genus *Cousinia* Cass, has nearly about 690 species(Attar et al. 1999-2004) throughout the world and nearly all of them occur in the central and southwestern Asia. Of this number, 240 species are listed from Iran in the Flora-Iranica (Rechinger 1972, 79) under 48 sections. Since 1995 various sections of the genus *Cousinia* including *Serratuloideae* have been taxonomically studied. This section had formerly 11 species which all, except one, are endemic to Iran. On the basis of new studies one species transferred to this section and also two new species are recognized for the section. The anatomical studies in this section is accomplished for the first time. In this paper

the anatomic attributes of the species have been described and compared.

MATERIALS AND METHODS

To undertake anatomic study of the species, materials were kept in FAA for a few months. They were then boiled a few hours in a waterglycerin solution before cross-sectioning, Methyl Green and Bismarck Brown colors were used for staining (Mahdigholi, 2003). Then they were studied and photographed by Olympus Light Microscope. The sample plants under study were all taken from type localities. Cross sections were made at the middle of stems ad leaves in mature plants. Voucher specimens are shown in table 1 and anatomical characters in table 2.

	Studied	Location						
C. crispa Jaub. & Spach	Stem, leaf	Tehran: Kandovan, 2700m, Attar & Mahdigholi, 27825- TUH						
C. $pierocaulos$ (C \land Mey) Rech. f.	Leaf	Gilan: Talesh, Aghvelar village, 1600m, Attar & Mahdighon, TUH						
C_pinarocephala Boiss.	Stem, leaf	f Mazandaran: Rudbarak, Alam- kun,2350m, Attai & Mahdigholi, 28699-TUH						
C albumonic su nov.	Leaf	Mazandaran, Mahdigholi & Attar, 32192-TUH						
C. rechingerae Bornm.	Stem, leaf	Golestan: Moraveh-Tappeh, 300m, Attar & Mahdigholi, 28689-TUH						
C. Journal august Briss	Stem, leaf	Tehran: Kandovan, 2500m, Attar & Mahdigholi, 27824-10H						
C. serratuloides Boiss.	Leaf	Tehran: Lasem village, 2100m, Attar & Mahdigholi, 32205- TUH						
C_irritans Rech. f.	Stem, leaf	Semnan: road of Shahrud to Azad shahr, 1600m, Attar & Mahdigholi, 21893-TUH						
C. sheidaiana sp. nov.	Leaf	Markazi: Ashtian to Tafresh, 20km. to Tafresh, Ghahreman & Attar, 21817-TUH						
C discolar Bunge	Leaf	Khorasan: Neyshabour, Dizbad, 2200m, Attar & Mahdigholi, 25396-TUH						
C. concolor Bunge	Stem, leaf	Khorasan: Neyshabour, Pivehgen village.2100m, Attar & Mahdigholi, 27647-TUH						
C. adenostegia Rech. f.	Stem, leaf	Khorasan: Neyshabour, Barfriz village, 2300m, Heidary 32203-TUH						

Table 1. Voucher specimens of Cousinic species.

Table 2- Anatomical comparison of the species of cousinia section Serratuloideae.

	Crispa	Reching.	Pin	Pte	Elb	Нур	Ser.	lrr	Shei	Dis	Con	Ade
Midrib shape												
	Trian-trilobed	Trian -trilobed	Trian-trilobed	Triantrilobed	Trianone-lobe	Subr	Irregular elliptic	Irregular rounded	Subroun	Elliptic	Subroun.	
Limb diameter µm	160	200	200	280	300	240	200	400	240	300	280	250
Series of pallisad parenchyma	2	1	2	2	3-2	3-2	2	3	2	2	2	2
Series of spongeneous parychyma	4-3	5-4	9-8	7-6	4-3	8-7	3-2	2	3-2	3-2	2-1	6-5
Arachnoid hairs of upper surface	Lax	Lax	Lax	Lax	Lax	Lax	Lax	Lax	Lax	Dense	Not	Not
Arachnoid hairs of lower surface	Dense	Dense	Dense	Dense	Dense	Dense	Dense	Dense	Dense	Dense	Not	Not
Number of vascular bundles	3	3	3	3	2-1	6	5	3	3	10	3	5
Form of cell wall of pallisad parenchyma	Entir- seret	Entire	Entire	Entire	Serrate	Entire	Entire	Entire	Entire	Entire	Serrate	Sinus- Serrat
Cuticle of lower surdace of leaf µm	1	12.5	8	4	6	15-12	4	12.5	16-14	10	16	12 5
Cuticle of upper surdace of leaf µm	1	12 5	8	4	6	15-12	12.5	12 5	16-14	10	16	12.5

Abbreviation: Trian =Triangular; Subr.=Subrounded; Pin =pinarocephala; Pte -pterocaulos; Elb =elburzensis; Hyp =hypoleuca; Ser.=serratuloides; In =irritans; Shei,-sheidatana; Dis =discolor; Con =concolor; Ade.=adenostegia

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ANATOMICAL DESCRIPTIONS OF THE SPECIES

Group A (The species with herbaceous texture of leaves):

1. Cousinia crispa Jaub. & Spach (Fig. 1) Stem: T.S. of the stem nearly circular, with few layers of tangential collenchyma (about 40 under epidermis. thickness) um in thin and Parenchymatous area of cortex consisting of 2-3 layers. Vascular bundles elliptical-shaped. Scirenchymatous fibres above the external and internal phloem 70 µm and 45 µm respectively. Lignose parenchyma is presented between vascular bundles.

Lamina: T. S. lamina nearly 160 μ m in thickness. Epidermis with simple multicellular hairs and clavate stipe. Cuticle 10 μ m in thickness. Stomata superficial. Mesophyll consisting of 2 layers of palisade cells in upper surface and length of each cell about 30 μ m. Cell wall entire or serrate. Spongiose parenchyma 30-60 μ m, 3-4 layers, cells rectangular or circular.

Midrib: T.S. midrib triangular, 3-lobed, 0.8×1 mm, with three bundles. Collenchyma of lower and upper epidermis 300 μ m and 120 μ m respectively.

2. Cousinia rechingerae Bornm. (Fig. 2) Stem: T.S. of the stem about circular, with tangential collenchyma (100-150 μ m in diam.) under epidermis. Parenchymatous area very thin, consisting of 2-3 layers. Vascular bundles numerous, elliptic. Sclrenchymatous fibres above the external and internal phloem 50-80 μ m and 25-50 μ m respectively, between vascular bundles with ligonse parenchyma.

Lamina: Hairs of epidermis simple, multicellular with clavate or cubic stipe. Cuticle 12.5 μ m in diam. Epidermis cells rather large, 25-40 μ m wide. Somata superficial. Lamina 200 μ m in thickness. Palisade cells one layer, 65 μ m long, cell wall

irregularly sinuate; spongiose cells 4-5 layers, cubic to circular, $65 \ \mu m$ in thickness.

Midrib: T.S midrib triangular, 3-lobed, 1x0.9mm, distance of vascular bundles from lower and upper epidermis 400 μ m and 200 μ m. Collenchyma in lower epidermis 60-100 μ m in thickness.

3. Cousinia pinarocephala Boiss. (Fig. 3) Stem: T. S. of stem nearly rounded, irregularly epidermis simple. sinuate. Hairs of secretory-glandular. rarelv multicellular. Collenchyma of under epidermis tangential and 40-60 µm with large schizogenous canals. Parenchymatous area very thin, consisting of 2-3 layers. Vascular bundles numerous. elliptic, sclerenchymatous fibres above the phloem 50-70 µm. Lignose parenchyma is presented between vascular bundles.

Lamina: about 200 μ m in thickness. Cuticle 4 μ m in thickness on the lower and upper epidermis. Hairs simple, multicellular, rarely secretory-glandular. Stomata superficial. Palisade cells two layers, 80 μ m in diam., spongiose parenchyma consisting of 8-9 layers, 100 μ m in diam.

Midrib: T.S. of midrib nearly triangular, 3lobed, 1.5x1.5mm. Distance of vascular bundles from lower and upper epidermis 400 and 500 μ m respectively. Collenchyma of lower and upper surfaces tangential and 50-60 μ m in thickness.

4. Cousinia pterocaulos (C. A. Mey.) Rech. f. (Fig. 4)

Lamina: 280 μ m in thickness. Simple hairs multicellular, with cubic large stipe, glandular-secretory hairs with a 5-6 cellular stipe and a swelling terminal cell. Cuticle 8 μ m in diam. on the lower and upper surfaces. Stomata superficial. Mesophyll consisting of 2 layers of palisade cells (70-80 μ m in diam.) and 6-7 layers of cubic, spherical cells (spongiose). Cell wall entire or rarely serrate.

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Midrib: T.S. midrib nearly triangular, 3-lobed 1.5x1.25mm. Collenchyma of lower and upper epidermis tangential and 38-75 μ m. Distance of vascular bundles from lower and upper epidermis 500 and 625 μ m. Numbers of vascular bundles three, each surrounded by sclerenchymatus fibres. Central part of midrib with a cavity. Cell wall of parenchymatous area irregularly sinuate.

5. Cousinia elburzensis sp. nov. (Fig. 5)

Lamina: $300 \ \mu\text{m}$ in diam. Hairs simple, multicellular with a large stipe. Cuticle $6\mu\text{m}$ in diam. on the lower and upper epidermis. Stomata superficial regularly scattered in the lower and upper surpfaces. Palisade cells consisting of 2-3 layers, 50-100 μm in thickness, spongiose cells irregular, compact, cubic or spherical. Cell wall completely serrate.

Midrib: T.S. midrib triangular, one-lobed, 0.8×1.25 mm. Upper and lower epidermis with simple multicellular hairs. Number of vascular bundles 1-2, sometimes with 1-2 cavities around them. Distance of vascular bundles from lower and upper epidermis 625 and 375 μ m respectively.

Group B (species with leathery texture)

6. C. hypoleuca Boiss. (Fig. 6 and 7)

Stem: T.S. stem nearly rounded, irregularly sinuate. Hairs simple and multicellular, rarely secretory-glandular, with 30-50 μ m tangential collenchyma under epidermis. Cuticle 4 μ m in thickness. Small and large resiniferous schizogenous cavities are presented in the collenchyma. Parenchymatous area very thin consisting of 2-3 layers. Vascular bundles numerous, elliptic, sclerenchymatous fibres of around the external and internal phloem 50-70 μ m and 30-50 μ m respectively. Area of between vascular bundles accompanied by lignose parenchyma.

Lamina: 240µm in thickness. Cuticle of lower and upper epidermis 12-15µm. Stomatal cells superficial. Palisade parenchyma 2-3 layers, 48-100μm in diam., spongiose cells compact.

Midrib 2.T midrih semiorbicular 1.25x1.7mm lower surface convex and crisped, upper surface entire. Hairs of lower surface simple, multicellular, with basal cubic cell and 2-4 cellular terminal part. Cuticle of upper and lower epidermis 6µ in diam. Collenchyma under lower epidermis 80-100um in diam., tangential, collenchyma with small and large schizogenous resiniferous cavities. Distance of vascular bundles from upper and lower surfaces 175 and 75µm respectively. Numbers of vascular bundles nearly 6.

7. C. serratuloides Boiss. (Fig. 8)

Lamina: 180-200 μ m in thickness. Hairs of lower epidermis dense, simple, multicellular, with cubic stipe, hairs of lower surface sparse, glandular-secretory. Cuticle of upper epidermis 12.5 μ m and lower epidermis 4 μ m. Stomata superficial. Palisade cells two layers, 50-62 μ m, with entire cell wall, spongiose cells 2-3 layers, 15-25 μ m, entire or rarely serrate.

Midrib: T.S. midrih irregular elliptic. 0.7x1mm. Cuticle of lower and upper epidermis 10µm in diam. Collenchyma of the lower epidermis tangential, 60-120µm, with small and large schizogenous resiniferous cavities. Distance of the vascular bundles from the lower and upper surfaces 250 and 500µm. Distance of between vascular bundles accompanied by sclerenchymatous fibres. Numbers of vascular bundles five.

8. C. irritans Rech. f. (Fig. 9)

Stem: T.S. stem about irregular rounded. Cuticle 5μ m in diam. Collenchyma under epidermis tangential. 100 μ m in diam., with small and large schizogenous resiniferous cavities. Parenchymatous area very thin and consisting of 2-3 layers. Vascular bundles numerous, elliptic. Sclerenchymatous fibres of external and internal phloem 80-150 and 30-

70μm. Area between the vascular bundles with ligonse parenchyma.

Limb: $350-400\mu$ m in thickness. Cuticle of upper and lower epidermis 12.5μ m in thickness. Stomata superficial. Palisade cells of upper surface three layers and $38-63\mu$ m in thickness., of lower surface two layers and $18-33\mu$ m in thickness.

Midrib: T.S midrib irregular rounded, or angular, 0.8x1.2mm. Cuticle of upper and lower epidermis with $7\mu m$ in thickness. Collenchyma under upper and lower epidermis $60-70\mu m$, with schizogenous resiniferous cavities. Vascular bundles three and distance of them from lower and upper epidermis 250-750 μm respectively.

9. C. sheidaiana sp. nov. (Fig. 10)

Limb: $240\mu m$ in thickness. Cuticle layer 14-16 μm in thickness. Stomata superficial. Palisade cells two layers with 20-30 μm in diam. and sinuate cell wall.

Midrib: T.S. midrib almost rounded, 1.3x1.5mm. Collenchyma of lower epidermis tangential and $80-100\mu$ m in diam., sometimes with schizogenous resiniferous cavities. Numbers of vascular bundles 3 and distance of them from lower and upper epidermis 300 and 700µm respectively.

10. C. discolor Bunge (Fig. 11)

Limb: 250-300µm in thickness. Cuticle of lower and upper epidermis 10µm. Hairs of lower epidermis dense, simple, multicellular and with clavate stipe. Stomatal cells superficial. Upper epidermis with sparse simple multicellular hairs. Mesophyll of upper surface consisting of 2 layers of palisade cells with 50-100µm in thickness and entire wall. Spongious cells of lower surface 2-3 layers, compact and without space of intercellular.

Midrib: T.S. midrib elliptic, 0.7x1.3mm. Collenchyma under lower epidermis tangential, 30-40µm in thickness, with schizogenous resiniferous cavities. Numbers of vascular bundles 10 and distance of them from lower and upper epidermis 100 and 370µm respectively.

11. C. concolor Bunge (Fig.12 and 13)

Stem: T.S. stem nearly rounded and irregularly sinuate. Hairs of epidermis 3-4 cellular. secretory-glandular and with a 5um in terminal swelling cell. Cuticle Collenchyma under epidermis thickness. tangential and about 80um in thickness and with schizogenous resiniferous cavities. Parenchymatous area very thin and consist of 2-3 lavers. Vascular bundles numerous. elliptic, sclerenchymatous fibres of external and internal phloem 80 and 40-50µm in thickness. Areas between of vascular bundles accompanied with lignose parenchyma and sometimes with sclerenchymatous fibres.

Lamina: 280µm in thickness. Cuticle of lower and upper epidermis 16µm. Stomatal cell superficial. Mesophyll of upper surface consisting of two layers of palisade cells with 60-68µm in thickness. Cell wall serrate

Midrib: T.S. midrib semiorbicular, 1x1.25mm. Lower surface convex, crisped and upper surface convex and entire. Hairs of lower epidermis secretory-glandular, 3-4cellular with a terminal swelling cell. Numbers of vascular bundles three and distance of them from lower and upper epidermis 250 and 625µm.

12. C. adenostegia Rech. f., (Fig.14)

Stem: T.S. stem about rounded. Cuticle layer thickness. Collenchyma under 5um in epidermis tangential, 100µm in thickness, with resiniferous schizogenous cavities. Parenchyma area very thin, consisting of 1-2 layers. Vascular bundles numerous, elliptic. External and internal sclerenchymatous fibres and 70-100um respectively. 100-140um Intervascular areas accompanied by ligonse parenchymtous and sclerenchymatous fibres.

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Lamina: $220-250\mu$ m in thickness. Cuticle of lower and upper epidermis nearly 12.5μ m. Stomatal cells superficial. Mesophyll of upper surface consisting of two layers of palisade cells with length of $80-100\mu$ m, cell wall sinuate-serrate.

Midrib:T.S. midrib semiorbicular, 1.4x0.9mm. Lower surface slightly sinuate and upper surface entire. Hairs of epidermis secretory-glandular, with multicellular stipe and a terminal swelling cell. Cuticle of lower and upper epidermis 70-125 μ m. Collenchyma under epidermis with small and large schizogenous resiniferous cavities. Numbers of vascular bundles five and distance of them from lower and upper epidermis 150 μ m and 375 μ m.

DISCUSSION

Since all of the species in this section occur in regions. they show anatomic dry characteristics of the xerophyte plants, for example: thickness of cuticle, compact spongiose parenchyma and density of hairs. Due to characteristics and diversity of their leaf structures, the study of these plants has been focused on this organ. Based on their anatomic characteristics, these plants can be divided into two types; the leathery leaf type at both sides of its blade, presence of palisade parenchyma (one or two layers in lower surface), and herbaceous leaf type which occurs only on upper surface. Another anatomic attributes among these plants belonging to this genus is the shape of the midrib. The shape of midrib, number and arrangements of vascular bundles is variable in different species (Attar et al., 2000).

T.S. of midrib is elliptic in *C. discolor*, and nearly orbicular in *C. serratuloides*, *C. hypoleuca*, *C. concolor*, *C. irritans*, *C. sheidaiana*, *C. adenostegia* (group of leathery leaves) and is trilobed in *C. pinarocephala*, *C. pterocaulos*, *C. crispa*, *C. rechingerae* and one-lobed in *C. elburzensis* (group of herbaceous leaves).

Cuticle is thick in C. concolor, C. irritans, C. sheidainana, C. adenostegia, C. discolor, C. serratuloides and C. hypoleuca and is thin in C. crispa, C. rechingerae and C. elburzensis.

Palisade mesophyll in group of herbaceous leaves is one layer in *C. rechingerae*, two layers in *C. crispa*, *C. pinarocephala*, *C. pterocaulos* and two or three layers in *C. elburzensis*.

Arachnoid hairs is present on lower and upper surfaces of leaves in *C. discolor* and only in lower surface of *C. hypoleuca*, *C. serratuloides*, *C. irritans* and *C. sheidaniana*. Leaves of *C. concolor* and *C. adenostegia* are completely glabrous.

Number of vascular bundles are variable in different species: in group of herbaceous leaves are three or four except *C. elburzensis* which is one or two, in group of leathery leaves are more than ten in *C. discolor* and four to nine in others.

Identification key to the species based on the anatomical characteristics

1. T.S. of midrib nearly triangular, deeply lobed 2

- T.S. of midrib nearly semiorbicular to elliptic, without deep lobes or scarcely sinuate 6

2. Palisade cells in upper surface 1-layered, cells of upper epidermis large *C. rechingerae* - Palisade cells in upper surface 3-layered:

cells of upper epidermis smaller 3

- 3. Midrib with cavities 4
- Midrib not as above 5

4. Vascular bundles of midrib 1-3; cell wall of the palisade mesophyll completely serrate

C. elburzensis

- Vascular bundles of midrib 3-5; cell wall of the palisade mesophyll entire-serrate

C. petrocaulos

5. Lower epidermis completely crisped; number of layers of spongiose mesophyll 4-5

- Lower epidermis not crisped; layers of

spongenous mesophyll 8-9 C. pinarocephala 6. Midrib shape nearly elliptic, numbers of vascular bundles 10-12 C discolor - Midrib shape not elliptic; numbers of vascular bundles less than 10 7 7. Hairs of lower surface of leaf dense, long, multicellular, with basal clavate cell 8 Hairs of lower surface of leaf almost _ glabrous, sometimes with short glandular hairs (terminal cell nearly spherical) 11 8. Epidermal cell almost rectangular 9 - Epidermal cell transversely oblong 10 9. Palisade cells of upper surface 1-3 layers, in lower part 2-3 and shorter than the uppers C. sheidaiana - Palisade cells of upper surface 2-4 layers, in lower part 2 and shorter than the uppers C.irritans 10.T.S. of midrib almost semiorbicular. sclerenchyma only present above the vascular bundles and also in thin layers around them C.hypoleuca - T.S. of midrib elliptic, sclerenchyma in thick layer above and around the vascular bundles *C. serratuloides* 11.Vascular bundles of midrib 3; palisade 2-3 layers and spongenous mesophyll mesophyll 2-3 layers C. concolor

Vascular bundles of midrib 5; palisade mesophyll 3 layers and spongenous mesophyll 3-4 layers C. adenostegia

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Fig. 1. C. crispa; A, a part of stem; B,C, a part of lamina; D, midrib; E, a part of midrib and trichomes. Scale bars = $100 \mu m$.



Fig. 2. C. rechingerae; A, midrib; B, a part of stem; C, D, lamina. Scale bars =100µm.



Fig. 3. C. pinarocephala; A, C, a part of stem; B, midrib; D, lamina. Scale bars =100µm.



Fig. 4. *C. pterocaulos*; A, C, lamina; B, a part of midrib and multicellular trichomes; D, trichomes of leaf; E, midrib. Scale bars =100µm.

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Fig. 5. C. elburzensis; A, multicellular trichomes; B, D, lamina; C, midrib. Scale bars =100µm.



Fig. 6. C. hypoleuca; A, midrib; B, a part of midrib; C, D, lamina. Scale bars =100µm.



Fig. 7. C. hypoleuca; A, stem; B, D, E, a part of stem; C, multicellular trichomes. Scale bars =100µm.



Fig. 8. C. serratuloides; A, B, lamina; C, midrib. Scale bars =100µm.



Fig. 10. C. sheidaiana; A, midrib; B, a part of midrib; C, lamina. Scale bars =100µm.



Fig. 9. C. *irritans*; A, stomatal cells; B, a part of lamina; C, midrib; D, a part of stem. Scale bars $=100 \mu m$.





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Fig. 12. *C. cocolor*; A, stomatal cells; B, a part of lamina; C, stomata and stomatal chamber; D. lamina; E, midrib. Scale bars =100 μ m, except in C where scale bar = 10 μ m.

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Fig. 13. C. concolor; A, C, D, a part of stem; B, stem. Scale bars =100µm.



Fig. 14. *C. adenostegia*; A, midrib; B, D, a part of stem; C, a part of midrib; E, F, a part of lamina. Scale bars =100µm.