

ANATOMICAL STUDIES ON FOURTEEN SPECIES OF THE GENUS *COTONEASTER* L. (ROSACEAE) IN IRAN

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In this study, anatomical features of blade and midrib region in 14 species of genus *Cotoneaster* belonging to two sections *Cotoneaster* and *Chaenopetalum* are presented. These species include *Cotoneaster nummularioides*, *C. kotschyi*, *C. turcomanicus*, *C. multiflorus*, *C. nummularius*, *C. hissaricus*, *C. luristanicus*, *C. suavis*, *C. morulus*, *C. ovatus*, *C. discolor*, *C. assadii*, *C. melanocarpus* and *C. persicus*. Among 30 studied characters, five characters (quantitative features such as presence of fiber, presence of cuticle and external xylem) are constant in different species. Twenty four characters 24 are variable in several species and several individuals of the same species. Only one character (i.e. number of palisade parenchyma layer) was variable and useful in separation of species.

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مطالعات تشریحی جنس شیرخشت در ایران

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در این مطالعه خصوصیات تشریحی مربوط به پهنک و رگبرگ میانی ۱۴ گونه از جنس شیرخشت *Cotoneaster* که مربوط به دو بخشه *Cotoneaster* و *Chaenopetalum* می‌باشند مورد مطالعه قرار می‌گیرد. این گونه‌ها شامل *C. kotschyi*، *C. nummularioides*، *C. ovatus*، *C. morulus*، *C. suavis*، *C. luristanicus*، *C. hissaricus*، *C. nummularius*، *C. multiflorus*، *C. turcomanicus*، *C. persicus* و *C. melanocarpus* می‌باشند. از بین ۳۰ صفت آناتومیکی، تعداد ۵ صفت (صفات کیفی از قبیل وجود فیبر، وجود کوتیکول و گزیم خارجی) در گونه‌های مختلف ثابت، ۲۴ صفت در گونه‌های مختلف و جمعیت‌های مختلف یک گونه متنوع بوده، و تنها یک صفت (تعداد لایه‌های پارانشیم نردبانی) در گونه‌های مختلف دارای ارزش کاربردی در جداسازی گونه‌ها می‌باشند.

Introduction

The genus *Cotoneaster* Medicus is a shrub member of subtribe *Pyrinae* (formerly *Maloideae*), Family *Rosaceae* (Campbell et al. 2007). *Cotoneaster* consists of approximately 260 species in temperate regions of the northern hemisphere (Mabberley 1997) that 19 of them occur in most regions of Iran, but its main distribution range includes Alborz Mts. and elevations in NW Iran (Azerbaijan province). Among these species, three ones (*C. assadii*, *C. esfandiarrii* and *C. persicus*) are endemic to Iran. Habitats of the genus are in open forests (*C. nummularioides*), step forests (*C.*

hissaricus) and scrublands (*C. ovatus*). Regarding anatomy, there are a few studies (Metcalf & Chalk 1957) that focuses on the anatomical features of several genera of the family *Rosaceae*. In the case of the genus, some common characters have been reported which main of them are as follow: isolated strands of fibers with intervening unlignified parenchyma, presence of fiber in secondary phloem and papillose indumentum of lower epidermis. Therefore, because of lacking of anatomical data about the genus, this study aims to present detailed anatomical information on some

species of the genus and to assess these data's taxonomical implication.

Materials and methods

For this study, about 200 specimens were collected from several localities of Iran. Voucher specimens (Table 1) are deposited in TUH (acronyms according to Holmgren et al. 1990) Among 19 species of the genus, 14 species examined in this anatomical survey. Dried leaves of mature fruit were fixed in alcohol-glycerin (1:1) for two months. Then cross sections were made in the middle region with a razor, dehydrated with sodium hypochlorite and stained with methyl green and Bismark brown colors. After preparation of slides, images were observed with Leitz light microscope model Wetzlar, photographed by Nikon camera model Coolpix S10 and then required characters were measured with Measurepro software version HASP 2.17. Totally 30 characters were measured in this study (Table 2).

Results

Main anatomical characters are summarized in Table 2. Leaves consist of midrib and blade regions that their features are as follow:

Midrib

Shape of midrib ranges from orbicular (Figs. 1a, 10a, 15a), semi-orbicular (Figs. 6a, 8a, 11a, 17a, 21a) to oblong (Figs. 3a, 12a, 14a, 16a, 19a, 20a, 22a, 23a). Angle between two sides of midrib (A) ranges from 85° in *C. kotschy* to 150° in *C. morulus*. In the most specimens (e.g. Figs. 3c, 7c, 8c, 10c, 17c) unicellular hairs were recognized easily. Upper cuticle thickness (MUCT) is remarkably more than lower cuticle (MLCT, Table 2) so that its thickness ranges from 12 µm in *C. kotschy* to 19 µm in *C. morulus* while lower cuticle thickness ranges from 5 µm in *C. suavis* to 10 µm in *C. turcomanicus*. Thickness of upper epidermis (MUET in Table 2) that consists of quadrangular large cells in comparison with smaller and usually orbicular cells of lower epidermis, ranges from 10 µm in *C. assadii* to 25 µm in *C. turcomanicus* while lower epidermis thickness (MLET in Table 2) ranges from 6 µm in *C. multiflorus* to 13 µm in *C. melanocarpus*. Regarding collechyma layer, upper one consists of two (Fig. 24b), three (Fig. 22b, 23b) to four (e.g. Fig. 9b) layers of angular cells and its thickness (UCOT) ranges from 45 µm in *C. multiflorus* to 112 µm in *C. discolor* while lower one is composed of two (e.g. Figs. 23a, 24a) to three (e.g. Figs. 2b) layers of regularly arranged tangential (plate) cells and its thickness (LCOT) ranges from 46 µm in *C. luristanicus* to 90 µm in *C. assadii*. One other common feature of midrib is the presence of

parenchyma layer. This layer composed of several layers with irregular arrangement so that upper parenchyma is an indistinct part composed of one (e.g. Fig. 10b) to two (e.g. Fig. 12b) layers while lower part is a distinct one consisting of two (e.g. Fig. 18b) to four layers (e.g. Fig. 24a). The later inner tissue is protective tissue of phloem. This tissue is fiber, composed of mostly a united aggregate of cells (e.g. Fig. 3b, 6b) or rarely of separated aggregates (e.g. Fig. 9b), its thickness (MPPTT) ranges from 22.5 µm in *C. discolor* to 46 µm in *C. morulus*. In spite of presence of this tissue around phloem, xylem lacks of protective tissue (e.g. Figs. 2b, 3b, and 4b). Shape of vascular bundles is a variable character so that some shapes such as semi-elliptic (e.g. Figs. 13b, 15b, 22b), elliptic (e.g. Figs. 3b, 6b, 8b), semi-orbicular (e.g. Figs. 17b), and irregular (e.g. Figs. 1b, 9b, 12b) can be recognized. Length of xylem (MXL) and phloem (MPL) ranges from 165 µm and 190 µm in *C. multiflorus* (Fig. 11b) to 280 µm and 360 in *C. assadii* (Fig. 12b), respectively. One other important is the presence of external xylem. In some specimens, this tissue is distinctive (e.g. Figs. 11b, 15b, 22b) while in some others is indistinctive (e.g. Figs. 1b, 3b, 6b).

Blade

The common structure in the blade includes upper and lower epidermis with a thick cuticle layer, palisade and spongy layer. Palisade layer consists of 2-4 layers. On the basis of number of layers of palisade layer, studied species can be divided into three groups as: a) with two layers that includes *C. assadii* (Fig. 12c) and *C. melanocarpus* (Figs. 23c, 24c), b) with 2-3 layers that includes *C. discolor* (Figs. 13c, 14c), *C. luristanicus* (Fig. 18c), *C. morulus* (Fig. 19c), *C. suavis* (Fig. 20), *C. turcomanicus* (Figs. 21c, 22c), *C. nummularioides* (Figs. 1c, 2c), *C. ovatus* (Figs. 7c, 8c, 9c) and *C. persicus* (Figs. 10c), c) with 3 layers that includes *C. hissaricus* (Figs. 15c, 16c) and *C. kotschy* (Fig. 17c).

Discussion

On the basis of Flora Iranica (Riedl 1969), the genus *Cotoneaster* has two sections in Iran "*Chaenopetalum* and *Cotoneaster*". The former section includes *C. nummularioides*, *C. multiflorus*, *C. kotschy*, *C. nummularius*, *C. turcomanicus*, *C. suavis*, *C. ovatus*, *C. discolor*, *C. assadii*, *C. hissaricus*, *C. persicus*, *C. luristanicus* and *C. morulus* and the later section has *C. melanocarpus*, *C. integerrimus* and *C. esfandiarii*. These sections have some morphological differences. The importance of anatomical characters and their fitness for the most actual subgeneric taxonomic grouping are discussed in the following.

Table 1. Voucher specimens of *Cotoneaster* species used in anatomical studies.

Taxa	Origin of the taxa
<i>C. assadii</i>	Tehran: Karaj to Chalous, ca 20 km after Gachsar to Chalous ; 11.9.2007; Attar, Zamani & Raei Niaki; 37633-TUH
<i>C. hissaricus</i>	Nothern Khorassan: Ca 20 km to Ashkhaneh from Golestan National Park, deviation road of Darkesh village to Haver village; 8.9.2007; Attar, Zamani & Raei Niaki; 37668-TUH
<i>C. hissaricus</i>	Azerbaijan: Kaleybar, After Aquyeh village to Bouqalamun station; 21.9.2007; Attar, Zamani & Raei Niaki; 37711-TUH
<i>C. kotschyi</i>	Northern Khorassan: Ca 20 km to Ashkhaneh from Golestan National Park , deviation road of Darkesh village to Haver village, 8.9.2007; Attar, Zamani & Raei Niaki; 37658-TUH
<i>C. luristanicus</i>	Lorestan: Aleshtar, Dareh-Tang; 18.9.2007; Zamani, Raei Niaki & Malaki; 37564-TUH
<i>C. melanocarpus</i>	Azerbaijan: Marand, Zunuz, Kuh-kamar; 24.6.1994; Ghahreman & Mozaffarian; 17421-TUH
<i>C. melanocarpus</i>	Tehran: 70 km of Ghaem-Shahr from Firuzkuh; 7.9.2007; Attar, Zamani & Raei Niaki; 37647-TUH
<i>C. morulus</i>	Northern Khorassan: Ca 20 km to Ashkhaneh from Golestan National Park , deviation road of Darkesh village to Haver village; 8.9.2007; Attar, Zamani & Raei Niaki; 37671-TUH
<i>C. multiflorus</i>	Mazandaran: Gheam-shahr, ca. 20 km from Pole'e-Sefid to Alasht; 28.8.1991; Ghahreman & Mozaffarian; 9982-TUH
<i>C. nummularius</i>	Nothern Khorassan: Ca. 20 km to Ashkhaneh from Golestan National Park, deviation road of Darkesh village to Haver village; 8.9.2007; Attar, Zamani & Raei Niaki; 37665-TUH
<i>C. nummularius</i>	Tehran: Karaj to Chalus, ca. 20 km after Gachsar to Chalous; 11.9.2007; Attar, Zamani & Raei Niaki; 37625-TUH
<i>C. nummularius</i>	Azerbaijan: Ca 2 km after Kaleibar to Eskanloo; 21.9.2007; Attar, Zamani & Raei Niaki; 37707-TUH
<i>C. ovatus</i>	Tehran: Ca 78 km to Gachsar from Chalous; 7.9.2007; Attar, Zamani & Raei Niaki; 37643-TUH
<i>C. ovatus</i>	Nothern Khorassan: Ca 20 km to Ashkhaneh from Golestan National Park, deviation road of Darkesh village to Haver village; 8.9.2007; Attar, Zamani & Raei Niaki; 37669-TUH
<i>C. ovatus</i>	Nothern Khorassan: Ca 20 km to Ashkhaneh from Golestan Nasional Park, deviation road of Darkesh village to Haver village, 8.9.2007., Attar ,Zamani & Raei Niaki;37673-TUH
<i>C. persicus</i>	Kerman:110 km SW Kerman , Gughar village., Mirtadzh;23467-TUH
<i>C. suavis</i>	Northern Khorassan: Ca 20 km to Ashkhaneh from Golestan National Park, deviation road of Darkesh village to Haver village; 8.9.2007; Attar ,Zamani & Raei Niaki; 37670-TUH
<i>C. turcomanicus</i>	Tehran: Ca 75 km to Ghaem-Shahr from Firuzkuh; 7.9.2007; Attar & Zamani; 37644-TUH
<i>C. turcomanicu</i>	Tehran: Firuzkuh, Veresk village; 7.9.2007; Attar,Zamani & Raei Niaki; 37649-TUH

Section *Cotoneaster*

The section that includes only *C. melanocarpus*, *C. integerrimus* and *C. esfandiarii* in Iran, in anthesis has erect and mostly pink petals (Riedl 1969). Among these species, only *C. melanocarpus* (Table 1) was examined in this study. On the basis of anatomy, this species is recognized by three (rarely two or four) styles, pink or red (rarely white) and erect petals, black fruit covered with waxy membrane (Khatamsaz 1992). Regarding distribution, this species is distributed in northwest of Iran (Azerbaijan province). According to anatomical studies, *C. melanocarpus* indicates some common characters as observed in other species of the genus (Table 2). It seems that the most remarkable character in this field is the number of palisade parenchyma layer (column PN in Table 2) so that in *C. melanocarpus* two layers is observed.

Section *Chaenopetalum* Koehne

This section that contains most species of the genus in Iran, has spread and mostly white petals (Riedl 1969).

Regarding morphology, the most important distinguishing characters belong to shape and size of leaf, size of petiole, color of fruit, erectness of sepals in fruit, density and length of inflorescence (Khatamsaz 1992) so that some close species are distinguished with the following characters: *Cotoneaster nummularioides* and *C. kotschyi* are separated in length of leaf (up to 9 mm and 7 mm, respectively) (Khatamsaz 1992), *C. discolor* has oblong-elliptic leaves with cuneate base, while *C. persicus* has narrow oblong-elliptic leaves with widely cuneate basis (Riedl, 1969), *C. nummularius* consists of light red and 6-8 mm long fruit, appressed sepals in fruit while *C. ovatus* has dark red and 8-10 mm long fruit, more or less erect sepals (Khatamsaz 1992). Moreover according to distribution, some species such as *C. nummularioides*, *C. kotschyi*, *C. ovatus* and *C. nummularius* are distributed in most localities of west, northwest, north, northeast, east and center of Iran but some others such as *C. assadii* and *C. suavis* are distributed only in central Alborz and northeast of Iran (Khatamsaz 1988; Khatamsaz 1992). As noted above, the best separating character belongs to the number of palisade parenchyma layer. On the

basis of this character (column PN in Table 2), species of the section are divided to three groups as: a) with two layers that includes *C. assadii* (Fig. 12c) and *C. multiflorus* (Fig. 11c), b) with two to three layers that includes *C. discolor* (Figs. 13c, 14c), *C. luristanicus* (Fig. 18c), *C. morulus* (Fig. 19c), *C. nummularius* (Figs. 3c, 4c, 5c, 6c), *C. nummularioides* (Figs. 1c, 2c), *C. ovatus* (Figs. 7c, 8c, 9c), *C. persicus* (Fig. 10c), *C. suavis* (Fig. 20c), *C. turcomanicus* (Figs. 21c, 22c) and c) with three layers that includes *C. hissaricus* (Figs. 15c, 16c) and *C. kotschy* (Fig. 17c).

Conclusion

The genus *Cotoneaster* is a morphologically complex and difficult to name many specimens with corresponding references. As discussed above, anatomical characters present only a few evidences not only for separating several species, but also in sectional level.

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Table 2. Anatomical characters of the studied species of the genus *Cotoneaster*.

Species	ML/W	MUCT	MLCT	MUET	MLET	MUCOT	MLCOT	MUPT	MLPT	MXL	MXW	MPL	MPW	MPPT	A	BUCT	BLCT	BUET	BLET	PN	PT	ST	FPT	SPT	TPT	PNM	
<i>C. Sect. Chaenopetalum</i>																											
<i>C. assadii</i>	680-700/600	16	7.5	10.2	9.6	110	90	20	21	280	140	360	97	45	115	11	13.5	16	7.5	2	100	81	44	39	-	3	
<i>C. discolor</i>	440-600/420-520	16.5	5.2	10	8	112	82.5	14	26.5	170	82.5	260	64	22.5	137.5	18.5	11.5	24	9.5	2-3	100	95	49.5	42	26	3-4	
<i>C. hissaricus</i>	460-700/400-600	16.5	7	15.5	6.7	95	73	18.5	23.5	225	112.5	285	73	35	115	11	9.5	23.5	9.75	3	130	110.5	56	44.5	34.5	3	
<i>C. kotoahyi</i>	480/350	12	9.5	13	8	50	72	13	23	200	94	240	49	23	85	10	10	26	10	3	130	110	53	41	32	3	
<i>C. luristanicus</i>	630/430	18.5	7.5	10	12	80	46	25	44	230	130	355	66	25	125	10.5	9.5	23	10	2-3	100	90	46	38	27	3	
<i>C. morulus</i>	500-600/450-500	19	6	15.5	9.3	88.5	48.5	22.5	25.5	225	125	285	88	46	150	4.5	8.5	25.5	8.5	2-3	150	95	49.5	51	40	3	
<i>C. multiflorus</i>	400/330	13.2	5.5	14	6	45	62	14	17	165	77	190	56	35	135	12	8.5	20	7.7	2	100	78	51	46	-	2-3	
<i>C. nummularius</i>	370-750/350-600	18.7	7	15.5	9.25	82	82.5	19	23	225	95	275	75.5	27	145	18.5	10.5	19.5	9.25	2-3	110	82.5	42	37.5	26.5	3-4	
<i>C. nummularioides</i>	410-450/350-400	13.7	6.5	16	6.75	68	64.5	14.5	14	175	85	200	46.5	37.5	110	14	7.8	25.5	8.2	2-3	108.5	78.5	41.5	41	25	3	
<i>C. ovatus</i>	560-650/380-500	18.5	6.5	12.5	8.2	70	58	13.5	17	220	105	280	77	27.5	135	18.8	9.5	22	10.4	2-3	140	104.5	65	42.5	39	2-3	
<i>C. persicus</i>	440/360	15	7.5	17	9.8	74	60	13.2	19	180	82	210	51	33	135	10.5	10.5	24	6.2	2-3	120	120	47	41	35	3-4	
<i>C. suavis</i>	550-700/550	13.5	5	29	6.8	94.5	55	26	39	220	112.5	315	56	43.5	142.5	12	8.5	22.5	8.2	2-3	115	91	52	43	22.5	2-3	
<i>C. turcomanicus</i>	440-450/360-420	15.5	10	25	7.6	76	62	19	29.5	175	60.5	225	45	28.5	112.5	11	10	25	10.1	2-3	122	83	62.5	47	26.5	2-3	
<i>C. Sect. Cotoneaster</i>																											
<i>C. melanocarpus</i>	500/310-450	14.5	8	11	13	56.5	49	20	31	220	83	275	58	36	100	13.5	12	20	9	2	92.5	100.5	46.5	40	-	2	

Abbreviations: ML(W), length (width) of midrib; MU(L)CT, midrib upper (lower) cuticle's thickness; MU(L)ET, midrib upper (lower) epidermis thickness; MU(L)COT, midrib upper (lower) collenchyma thickness; MU(L)PT, midrib upper (lower) parenchyma thickness; MXL(W), midrib xylem's length (width); MPL(W), midrib phloem's length (width); MPPTL(W), midrib phloem protective tissue's length (width); A, angle between two sides of midrib; BU(L)ET, blade upper (lower) epidermis thickness; PN, number of palisade parenchyma layer; PT, palisade parenchyma layer's thickness; FPT, first palisade parenchyma layer's thickness; SPT, second palisade parenchyma layer's thickness; TPT, third palisade parenchyma layer's thickness; PNM, number of palisade parenchyma near midrib region; -, lacking of the mentioned character.

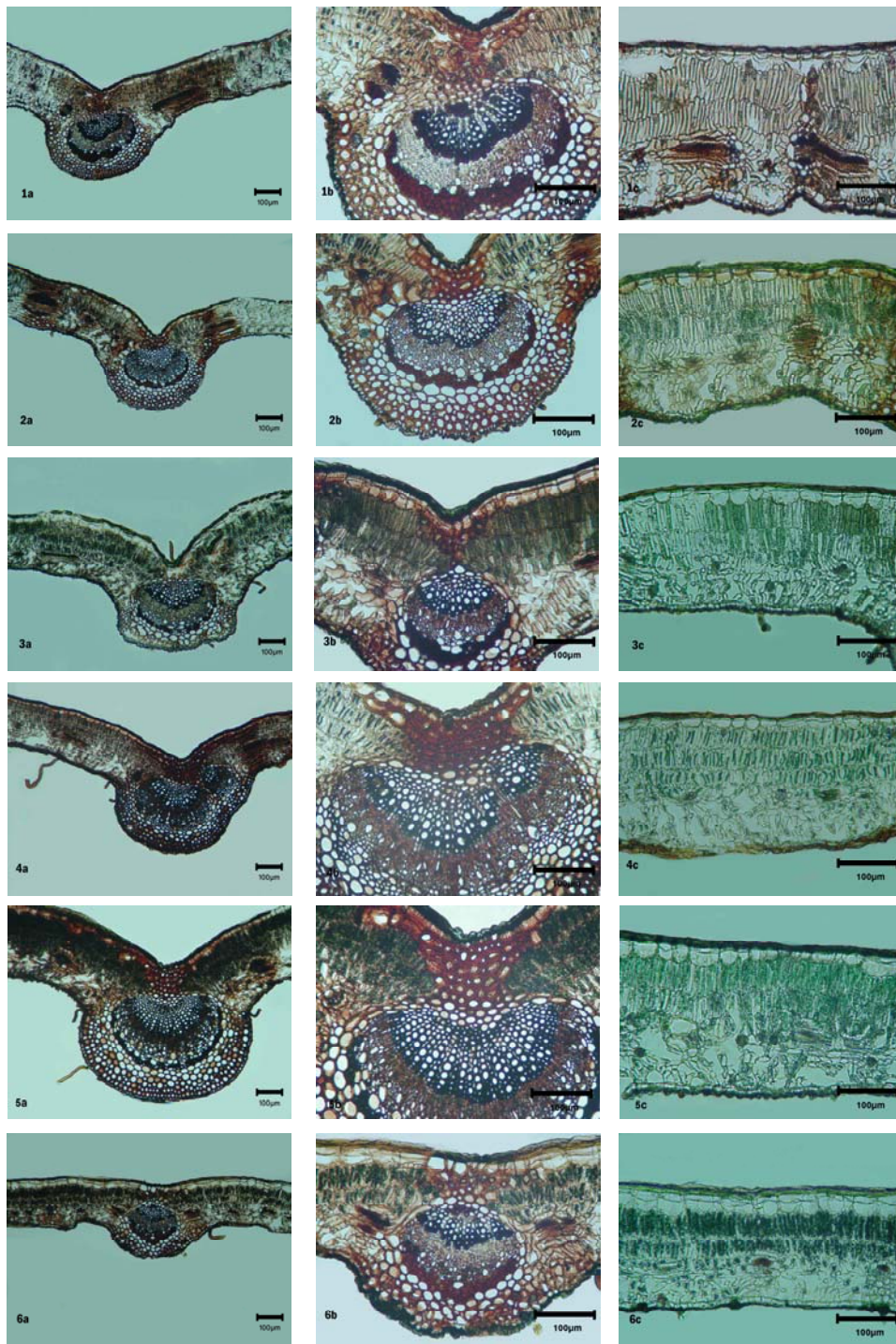


Fig. 1: *Cotoneaster nummularioides* (37618), Fig. 2: *C. nummularioides* (37715), Fig. 3: *C. nummularius* (18858), Fig. 4: *C. nummularius* (37625), Fig. 5: *C. nummularius* (37665), Fig. 6: *C. nummularius* (37707).

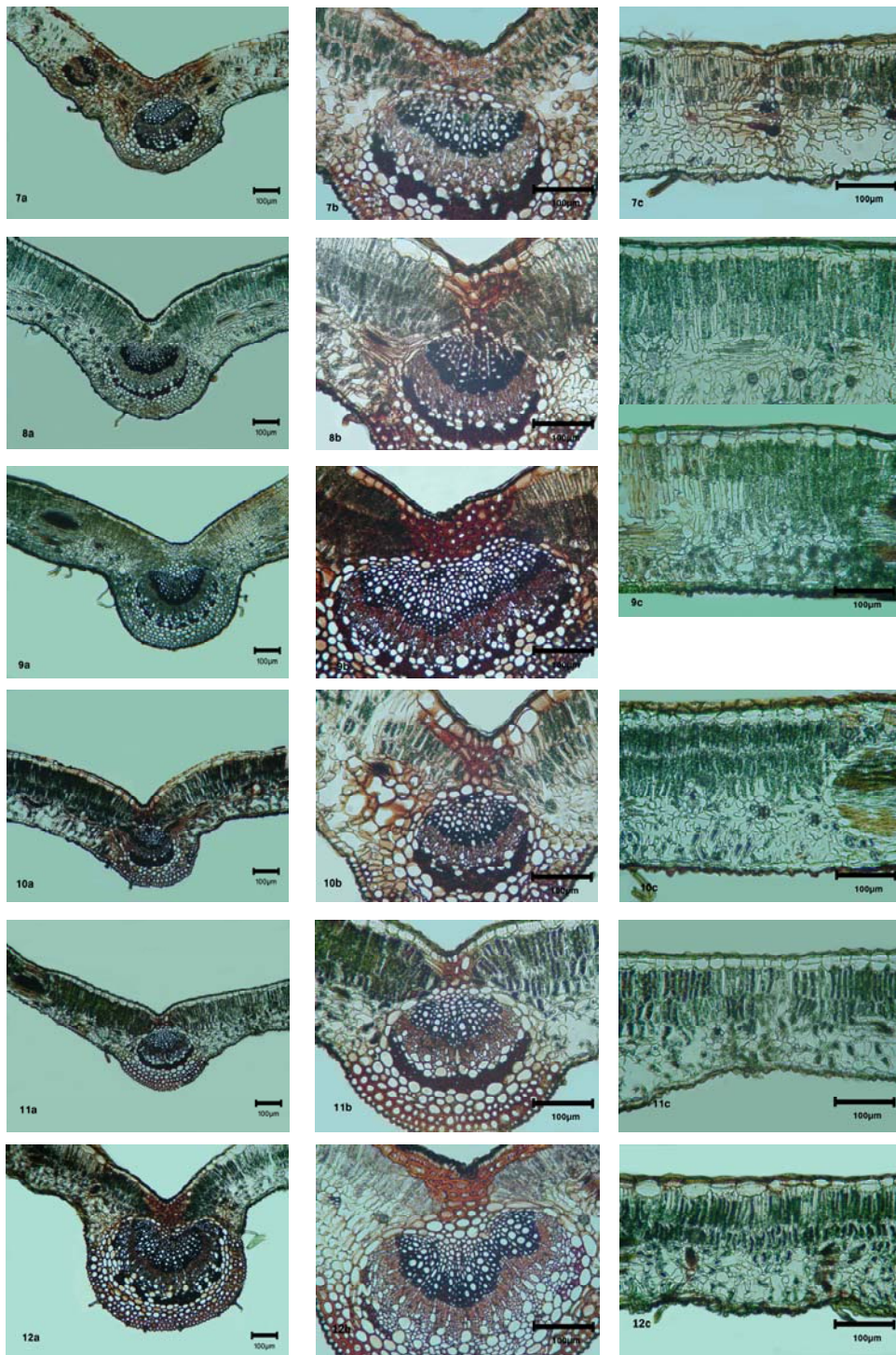


Fig. 7: *Cotoneaster ovatus* (37643), Fig. 8: *C. ovatus* (37669), Fig. 9: *C. ovatus* (37673), Fig. 10: *C. persicus*, Fig. 11: *C. multiflorus*, Fig 12: *C. assadii*.

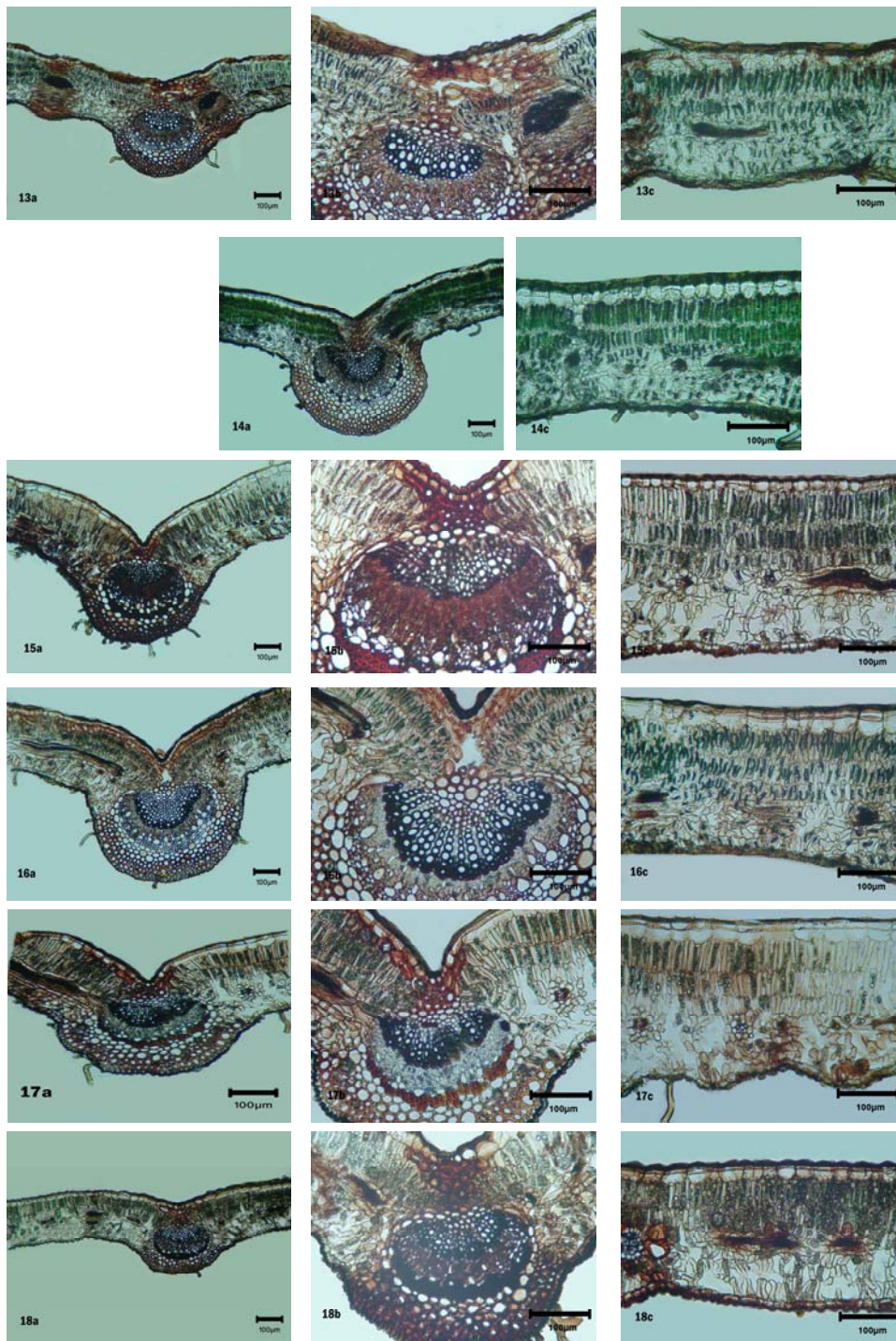


Fig. 13: *Cotoneaster discolor* (1989), Fig. 14: *C. discolor* (37646), Fig. 15: *C. hissaricus* (37711), Fig. 16: *C. hissaricus* (37668), Fig. 17: *C. kotschyi*, Fig. 18: *C. luristanicus*.

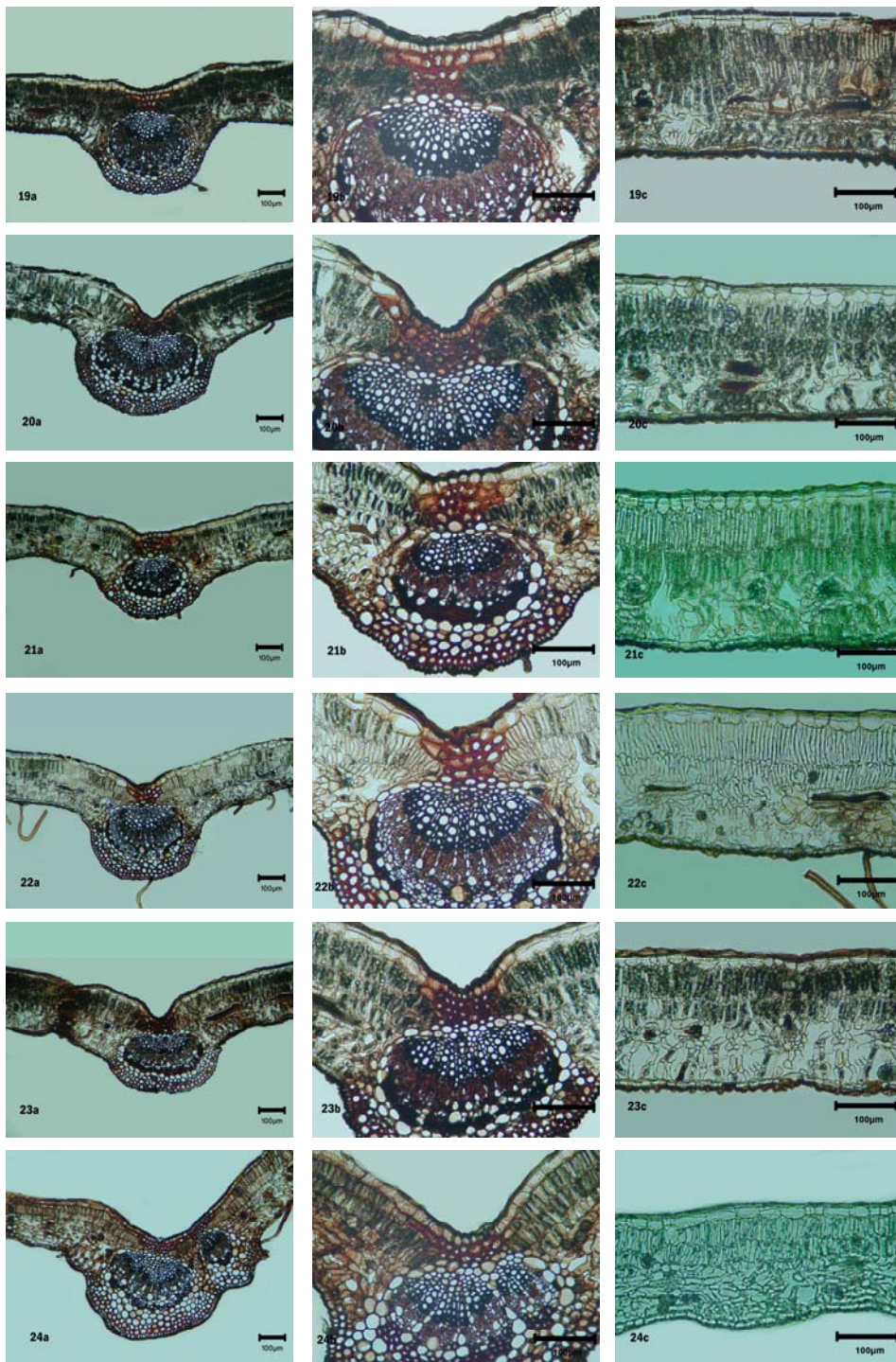


Fig. 19: *Cotoneaster morulus* (37715), Fig. 20: *C. suavis* (37670), Fig. 21: *C. turcomanicus* (37644), Fig. 22: *C. turcomanicus* (37649), Fig. 23: *C. melanocarpus* (37647), Fig. 24: *C. melanocarpus* (17421).