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FOLIAR ANATOMICAL STUDIES OF AJUGA CHAMAECISTUS (LAMIACEAE) FROM IRAN

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Ajuga chamaecistus Ging. ex Benth. (Lamiaceae) is a small subshrub, widely distributed in Iran. It has four subspecies including *chamaecistus*, *scoparia*, *tomentella* and *euphrasioides*, all endemics of Iran. In this study, three subspecies were collected from 16 provinces, totally 28 regions and their leaf anatomical structure was studied. The studied anatomical traits were leaf type, trichome type (glandular/non-glandular), stomata position, number of the upper and lower palisade parenchyma, number of vascular bundle, presence or absence of the fibre- Sclerenchyma, presence or absence of the vascular sheath, tissue of vascular sheath, upper and lower collenchyma as qualitative traits and thickness of the upper and lower palisade parenchyma, thickness of the upper and lower cuticle, mesophyll/ vascular bundle, diameter of lamina, adaxial and abaxial stomatal density, adaxial and abaxial stomatal length, adaxial and abaxial stomatal width as quantitative traits. This study provided valuable information on the leaf anatomical structure of *A. chamaecistus*. The results showed that the anatomical structure of the studied subspecies are very similar. Although PCA analysis separated some subspecies, the anatomical traits of the leaf are not enough to separate different subspecies, to do this, other studies, such as anatomical traits of other plants structures such as petiole and stem as well as other biosystematics studies may be helpful.

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Key words: *Ajuga chamaecistus*; subspecies; Lamiaceae; anatomy; leaf; Iran

مطالعه آناتومیکی برگ گونه *Ajuga chamaecistus* Ging. ex Benth. از تیره نعنائیان

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گونه Ajuga chamaecistus یکی از گونه‌های جنس .Ajuga L و متعلق به تیره Lamiaceae می‌باشد که گسترش وسیعی در ایران دارد. این گونه دارای چهار زیرگونه chamaecistus، scoparia، tomentella و euphrasioides است که همگی انحصاری ایران می‌باشند. در این تحقیق، از سه زیرگونه موجود در 16 استان کشور و مجموعاً 28 نمونه جمع‌آوری گردید و برای اولین بار ساختار تشریحی برگ آنها مورد مطالعه قرار

گرفت. صفات تشریحی مورد مطالعه عبارت بودند از: تیپ برگ، نوع کرک (غده‌ای یا غیر غده‌ای)، موقعیت روزنه، تعداد لایه‌های پارانشیم فوقانی و تحتانی، وجود کانال ترشحی، تعداد دستجات آوندی رگبرگ، وجود یا عدم وجود فیبر- اسکلرانشیم، وجود یا عدم وجود غلاف آوندی، نوع بافت غلاف آوندی، وجود کلانشیم فوقانی و تحتانی به عنوان صفات کیفی و ضخامت پارانشیم فوقانی و تحتانی، ضخامت کوتیکول فوقانی و تحتانی، نسبت مزوفیل به آوند، قطر پهنک، تراکم روزنه فوقانی و تحتانی، طول روزنه فوقانی و تحتانی و عرض روزنه فوقانی و تحتانی به عنوان صفات کمی. این تحقیق اطلاعات ارزشمندی در مورد ساختار تشریحی برگ گونه Ajuga chamaecistus فراهم نمود. نتایج نشان داد که ساختار تشریحی زیرگونه‌های مطالعه شده بسیار به یکدیگر شباهت دارند و اگرچه با واکاوی‌های چند منظوره(PCA) تعدادی از زیرگونه‌ها جداسازی شدند، اما صفات تشریحی برگ به تنهایی برای جداسازی زیرگونه‌های مختلف کافی نیست و حتماً همراه با آن صفات تشریحی اندام‌های دیگری مانند دمبرگ و ساقه و همچنین بررسی‌های بیوسیستماتیکی نیز باید مورد توجه قرار گیرند.

INTRODUCTION

Ajuga chamaecistus Ging. ex Benth. is one of the species of *Ajuga* L. (Lamiaceae). This genus has six species in Iran, some of which include several infra-specific taxa. *Ajuga chamaecistus* is the most wide-spread species in Iran. The plant is a small shrub, with violet flowers. It is also distributed in Afghanistan, Central Asia, east Turkey, the Caucasus and Iraq and usually grows in mountainous or rocky slopes. There are four endemic infraspecific taxa in Iran as follows:

-subsp. ***chamaecistus***: It is an endemic subspecies of Iran and is distributed in the northwest, west and center of Iran (Azarbaijan, Kurdestan, Hamedan, Lorestan, Markazi, Gilan, Kermanshah, Isfahan, Chaharmahal & Bakhtiari, Fars, Alborz and Tehran provinces).

-subsp. ***scoparia***: It is also an endemic subspecies of Iran and its habitat is in west, center and south of Iran (Isfahan, Chaharmahal & Bakhtiari, Markazi, Yazd, Fars, Kerman, Semnan and Tehran provinces).

-subsp. ***tomentella***: It is also an endemic subspecies of Iran and its habitat is in the west, center and south of Iran (Isfahan, Chaharmahal & Bakhtiari, Markazi, Hamedan, Gazvin, Alborz and Tehran provinces).

-subsp.***euphrasioides***: This taxon is endemic of Iran and is distributed in center of Iran (Isfahan and Chaharmahal & Bakhtiari provinces), (Jamzad, 2012). It should be mentioned that in this study no plants of this subspecies could be collected, so it is not included in our study.

The high morphological variation in the species has led to describing infra-specific taxa. There are overlapping characters in different taxa which makes it difficult to define the boundary of each taxon. We studied the anatomical characters to see if there is diagnostic traits that can help to define the taxa. Anatomical studies may help in this regard. Today, all aspects of plant anatomy are considered by plant taxonomists and many findings have been obtained in this area (Akhani and Forther, 1994; Ai, 1989; Cutter, 1971). Some scientists such as Metcalf & Chalk (1985), Heywood (1985), Carlquist (1961) and Rudall (1994) beleived that anatomical studies are very important and should not be ignored. Carlquist (1975) emphasizes anatomical-systematical relationships. Vast anatomical studies are performed in order to better understanding the relationships between the different taxa of Lamiaceae family. The application of anatomical traits of plants in the mint family has often led to the solution of the taxonomical problems (Bokhari and Hedge, 1971).

Although a few species of the genus *Ajuga* are investigated anatomically (Akçin *& al*., 2006; Ghitä *& al*. 2012; Çali, 2014, Sönmez and Köse, 2017), there is no anatomical study of *Ajuga chamaecistus* in the literatures. In the present research we give detailed description of anatomical characteristics of the leaves of the species and its subspecies.

MATERIALS AND MEDHODS

A total of 28 specimens of three subspecies of *Ajuga chamaecistus* were collected from different provinces, where the species was distributed, during the flowering period. A list of the specimens, systematic positions, locality and geographical coordinates of the corresponding subspecies is given in table 1. The fresh specimens were kept in 70% alcohol. Transverse sections were made by hand using commercial razor blades. The cuttings were cleared with sodium hypochlorite, stained with carmine-vest and methyl green and mounted in gelatin. To study the density, length and width of the stomata, a piece of the leaf epidermis was separated. The prepared samples were studied and measured by light microscope model CH30. Pictures were taken by a Nikon digital camera model COLPIX P90. Qualitative and quantitative data were analyzed by principal component analysis (PCA) using version 16 of minitab software and position of specimens on the coordinate axes and ordination of

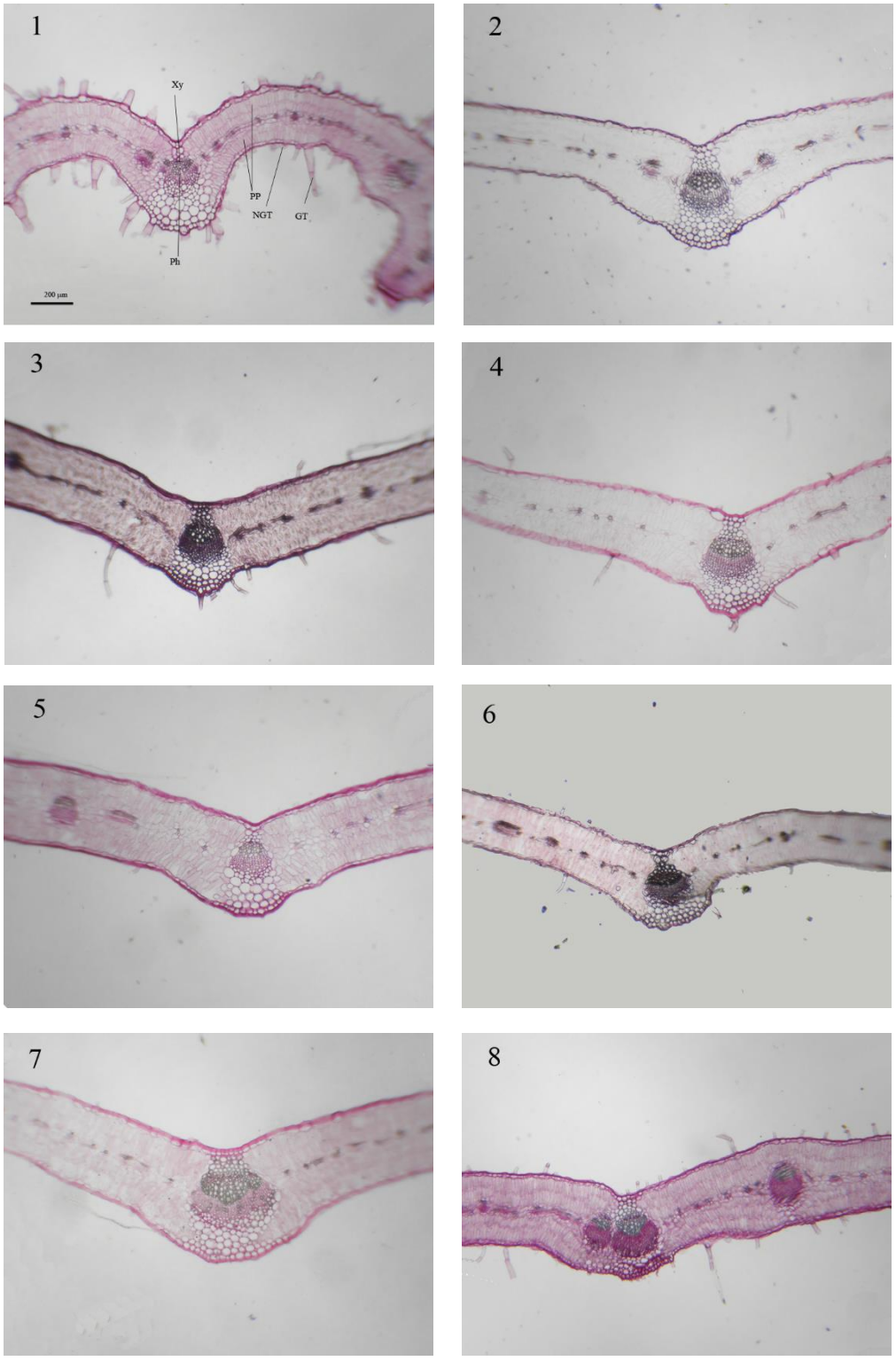
them was performed. For analysis of the qualitative traits, zero and one method were used. The trait of the palisade parenchyma layers was considered as a qualitative trait, because the number of the layers varied between one and three. Therefore, for this trait, if the number of layers was a mixture of 1 or 2, the code zero, and a mixture 2 or 3, the code 1 were considered. Trait of the density of the glandular trichomes were considered as a qualitative trait, either as low or high numbers, and were analyzed by zero and one method.

Table 1. The studied specimens of *Ajuga chamaecistus* and its subspecies, collection data and geographical coordinates of the corresponding subspecies. Subsp. *tomentella* x *scoparia* is a sample with intermediate characters.

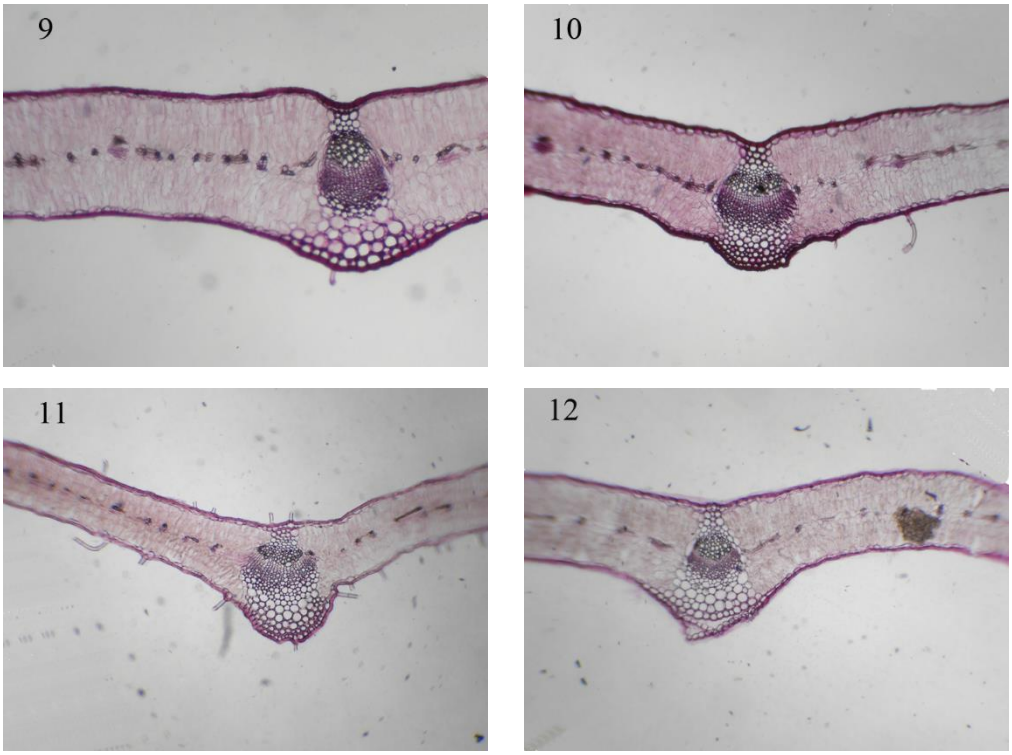
Subspecies	Variety	Collection data	Latitude and Longitude
chamaecistus		Tehran:Damavand to Firoozkooh, beginning of Arou Road, 2200, Kazemi Saeed and Mohebbi, 107177 (TARI).	N: 35 38 79.0 E: 052 24 08.4
chamaecistus		Alborz:Joustan, between Taleghan and Evank, 1963, Kazemi Saeed and Mohebbi, 107154 (TARI).	N: 36 10 93.4 E: 050 52 53.4
chamaecistus		Hamedan: Siah kamar, 2321, Kazemi Saeed and Sadeghi, 107159 (TARI).	N: 34 45 20.3 E: 048 48 31.3
chamaecistus		Kermanshah: Sanghor to Bistoon, after Karghsar, north of Moineh village, 1700, Kazemi Saeed and Mohebbi, 107162 (TARI).	N: 34 42 83.8 E: 047 21 54.4
chamaecistus		Fars: 30 km of west of Shiraz, Hosseinabad station, 1980, Kazemi Saeed, hatami and Bazrafkan, 107166 (TARI).	N: 29 36 59.2 E: 052 13 45.0
chamaecistus		Isfahan: Frieden, Darreh Bid, 2674, Kazemi Saeed and Feizi, 107150 (TARI).	N: 33 06 40.1 E: 050 24 10.5
chamaecistus		Gilan: Jirandeh to kelishom, 3 km to Kelishom, 1770, Kazemi Saeed and Moradi, 107168 (TARI).	N: 36 43 15.7 E: 049 55 38.0
chamaecistus		Markazi: Hesar, Rasband Mount., 1933, Kazemi Saeed and Haghshenas, 107157 (TARI).	N: 33 59 27.6 E: 049 20 53.8
chamaecistus		Chaharmahal&Bakhtiari: Ardal, Kooranabad, 2050, Kazemi Saeed and Mohebbi, 107164 (TARI).	N: 32 12 42.0 E: 050 51 95.9
chamaecistus		Lorestan: Northwest of Khorramabad, 10 km to Alashtar, 1634, Kazemi Saeed and Mohebbi, 107161 (TARI).	N: 33 27 84.6 E: 048 22 46.8
chamaecistus		Kordestan: 35 km from Sanandaj to Kamyaran, mountains around Naran village, 2150, Kazemi Saeed and Mohebbi, 107176 (TARI).	N: 35 08 56.2 E: 047 07 31.0
chamaecistus		west Azarbaijan: Mahabad, behind the dam, 1537, Kazemi Saeed and Mohebbi, 107175 (TARI).	N: 36 73 08.1 E: 045 59 97.5
scoparia		Yazd: Herat to Chennaraz, Ghorogh-e-Shadi, 2115, Kazemi Saeed and Hosseni, 107173 (TARI).	N: 29 48 21.3 E: 054 08 49.2
scoparia		Fars: Sarvestan, Post Chenar, 1843, Kazemi Saeed, hatami and Bazrafkan, 107167 (TARI).	N: 29 12 12.3 E: 053 20 07.3
scoparia		Isfahan: Hardang, 2090, Kazemi Saeed and Feizi, 107153 (TARI).	N: 32 15 47.4 E: 051 11 01.4
scoparia		Tehran: Old Qom Road, at the beginning of the Nalbandan neck, 1288, Kazemi Saeed and Ashrafi, 107152 (TARI).	N: 35 14 05.5 E: 050 59 20.8
scoparia		Semnan, Enzo, 2018, Kazemi Saeed and Taherian, 107160 (TARI).	N: 35 45 38.8 E: 053 24 46.8
scoparia		Kerman: Bazenjan, Rabor, Ghedar-e_ Archani, 2661, Kazemi Saeed and pourmirzaei, 107169 (TARI).	N: 29 17 26.5 E: 056 49 88.9
Scoparia		Markazi, margin of Latehdar to Shaegh road, 2258, Kazemi Saeed and Mirdavoodi, 107156 (TARI).	N: 34 00 28.7 E: 050 13 08.7
scoparia		Chaharmahal&Bakhtiari:Borujen, Martaeh-e- Chah Neghahdar, 2486, Kazemi Saeed and Mohebbi, 107163 (TARI).	N: 32 05 40.5 E: 051 20 52.8
tomentella		Tehran: near of Jajrood, opposite the bus terminal, 1626, Kazemi Saeed and Mohebbi, 107172 (TARI).	N: 35 43 79.6 E: 051 40 89.1
tomentella		Alborz: Not reached to Chalous, Sirachal, 1919, Kazemi Saeed and Mohebbi, 107171 (TARI).	N: 36 01 48.6 E: 051 09 82.3
tomentella		Ghazvin: Sagharan to Arteshabad, After warm water, 1710, Kazemi Saeed and Mohebbi, 107155 (TARI).	N: 35 42 71.3 E: 049 19 96.9
tomentella		Hamedan, Hamedan Road to Sanandaj, Vinsar village, 1920, Kazemi Saeed and Mohebbi, 107178 (TARI).	N: 35 00 26.2 E: 048 05 16.9
tomentella		Isfahan: Ghaheiz Protected Station, 2450, Kazemi Saeed and Feizi, 107151 (TARI).	N: 33 00 13.1 E: 050 36 58.7
tomentella		Markazi: Shazand, 2248, Kazemi Saeed and Haghshenas, 107158 (TARI).	N: 33 46 25.2 E: 049 39 18.2
tomentella	*tomentella*	Chaharmahal&Bakhtiari: Ardal, Beheshtabad, 1750, Kazemi Saeed and Mohebbi, 107165 (TARI).	N: 32 02 44.8 E: 050 63 23.2
tomentella x *scoparia*		Tehran: Lashkarak road, opposite of Telo rehabilitation center, 1723, Kazemi Saeed and Ashrafi, 107170 (TARI).	N: 35 45 87.5 E: 051 37 67.4

RESULTS

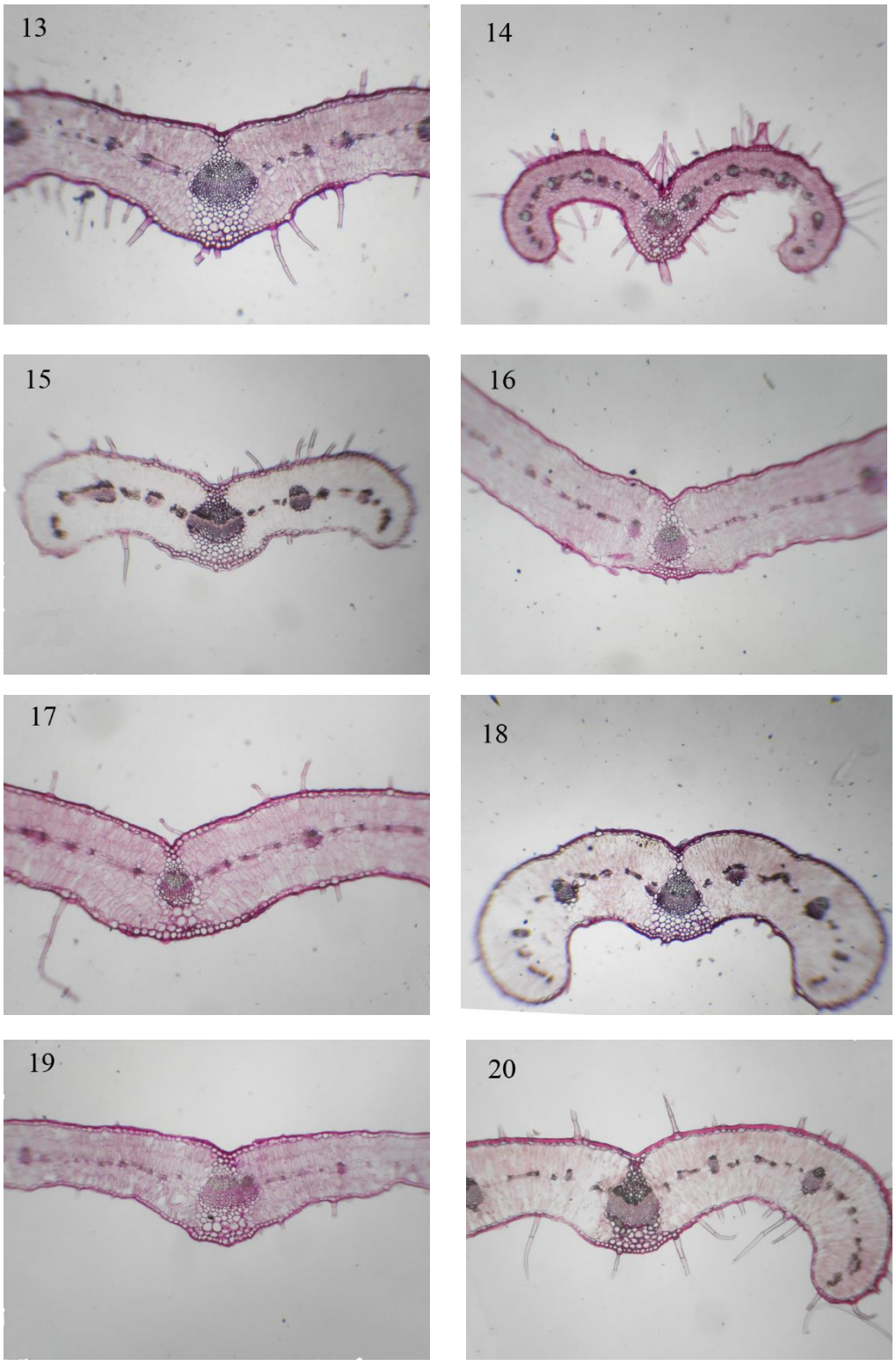
The results of the leaf anatomical studies of the 28 samples are presented in the tables 2 and 3 and their images in the figs. 1-40. The leaf type in all samples was isobilateral. However, in some samples it tends to become spongy. The highest diameter of lamina was related to subsp. *scoparia* of Bezenjan specimen with 469.2 µm and the lowest to subsp. *tomentella* of Jajrood specimen with 214.2 µm. Leaves of all samples had stomata at both upper and lower surfaces. In the adaxial surface, subsp. *chamaecistus* of Mahabad sample with 190.78 and the same subspecies of kelishom with 68.4 number per unit area (mm^2) had the highest and lowest stomatal density, respectively. In the abaxial surface, subsp. *chamaecistus* of Mahabad and subsp. *scoparia* of Post Chenar sample with 215.93 had the highest number of stomata per unit area (mm^2), while the subsp. *scoparia* of the Enzo sample with 60.53 had the lowest. Regarding to stomatal length of the upper surface, the subsp. *tomentella* f the Ghahiz sample with 37.5µm and subsp. *chamaecistus* of the Mahabad sample with 26.25 µm had the highest and lowest stomatal length, respectively. At the lower surface, subsp. *chamaecistus* of the kelishom specimen with 38.33µm had the highest and subsp. *tomentella* x *scoparia* of the Telo sample with 22.5 µm had the lowest stomatal length. The maximum amount of the stomatal width in the adaxial surface was observed in subsp. *tomentella* of the Beheshtabad sample with 25.83 µm and minimum in subsp. *tomentella* of the Sirachal with 19.17µm. In the abaxial surface, the highest stomata width belonged to the subsp. *chamaecistus* of the kelishom sample with 25.83 and the lowest belonged to subsp. *tomentella* of the Sirachal with 17.5 µm. The stomata type was mostly anemocytic, although the anisocytic type was observed. The stomata were mostly flat and in some cases a little prominent. The number of the upper palisade parenchyma layers was in the most cases 2-3 layers. Except in subsp. *tomentella* of the Sirachal sample, the number of the layers was mostly two. The subsp. *tomentella* of the Sirachal sample often had 3 layers. Other samples had only two or both 1and 2 layers. In the lower surface, the number of the palisade parenchyma layers were mostly both 2 and 3, or only two layers. A few samples had both 1 & 2 layers. The subsp. *tomentella* of the Sagharan sample had 1 & 2 & 3 layers. The subsp. *tomentella* of the Sirachal sample had three and the subsp. *scoparia* of the Post Chenar had only one layer. The upper palisade parenchyma thickness was the highest in subsp. *tomentella* of the Sirachal sample with 168.13 µm and the lowest in subsp. *tomentella* of the Behestabad sample with 83.2 µm. The subsp. *scoparia* of the Latehdar sample with 171.6 and subsp. *tomentella* of the Jajrood with 64.13 µm showed the highest and the lowest thickness in the lower palisade parenchyma, respectively. There was one vascular bundle in the midrib and veins in all specimens. All specimens had collenchyma at both of the lower and upper surfaces, although the number of layers varied among different subspecies. Only 7 of the 28 samples lacked fibre- Sclerenchyma tissue. Also, Sclerenchyma and fibre cells were observed above the phloem of subsp. *scoparia* of Martaeh-e-Chah Neghahdar sample. The subsp. *tomentella* of Sirachal, Ghaheiz and Behestabad Samples had fiber cap. In subsp. *tomentella* x *scoparia* of Telo sample, fiber cap was being formed. Vascular bundles in all specimens completely or partially were surrounded by the vascular sheath that its tissue was parenchyma. Leaves had glandular and non- glandular trichomes on both surfaces. Non- glandular trichomes were present in all samples, while glandular trichomes were observed in most specimens, often on the lower and in some cases on both surfaces. Except subsp. *chamaecistus* of Kordestan sample which had glandular trichome only on the upper surface. Also, subsp. *chamaecistus* of Moineh, Kooranabad and Alashtar samples lacked any glandular trichome. The highest cuticle thickness of the upper surface was observed in subsp. *chamaecistus* of Kooranabad sample with 17.5 and the lowest in subsp. *chamaecistus* of Joustan, Rasband and subsp. *scoparia* of Enzo samples with 7.5 µm. In the lower surface, subsp. *chamaecistus* of Kooranabad and subsp. *tomentella* of Jajrood samples with 18.33 and subsp. *chamaecistus* of Joustan with 5.83 µm had the highest and lowest cuticle thickness, respectively. The highest ratio of the mesophyll to vascular bundle in the midrib was observed in subsp. *chamaecistus* of Arou sample with 4.07 and the lowest in subsp. *chamaecistus* of Darreh Bid with 2.1.



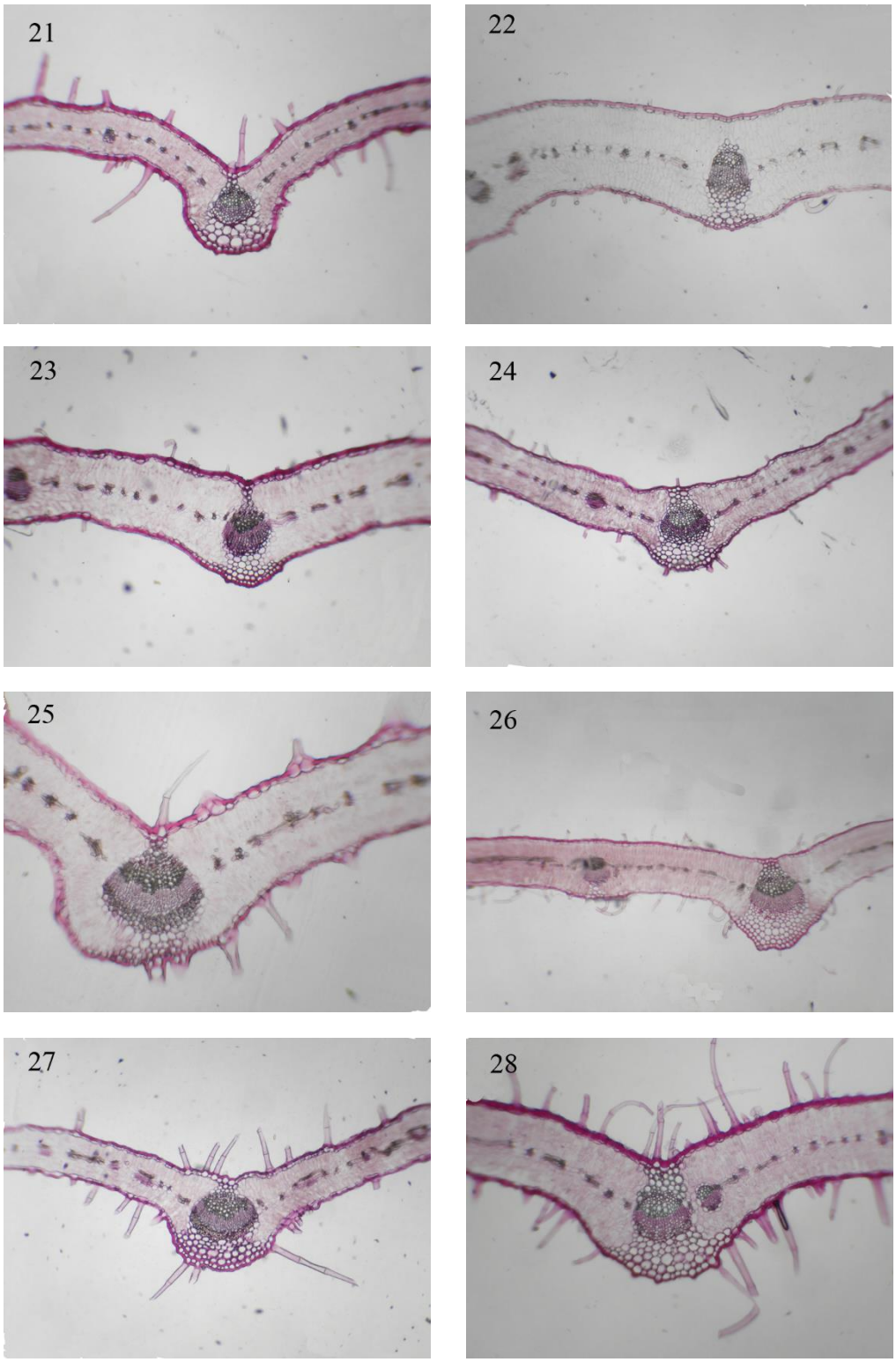
Figs. 1-8. Transverse section of leaves of studied populations of *Ajuga chamaecistus* subsp. *chamaecistus*: 1, Arou; 2, Joustan; 3, Siahkamar; 4, Moineh; 5, Hosseinabad; 6, Darreh Bid; 7, Kelishom; 8, Rasband populations.



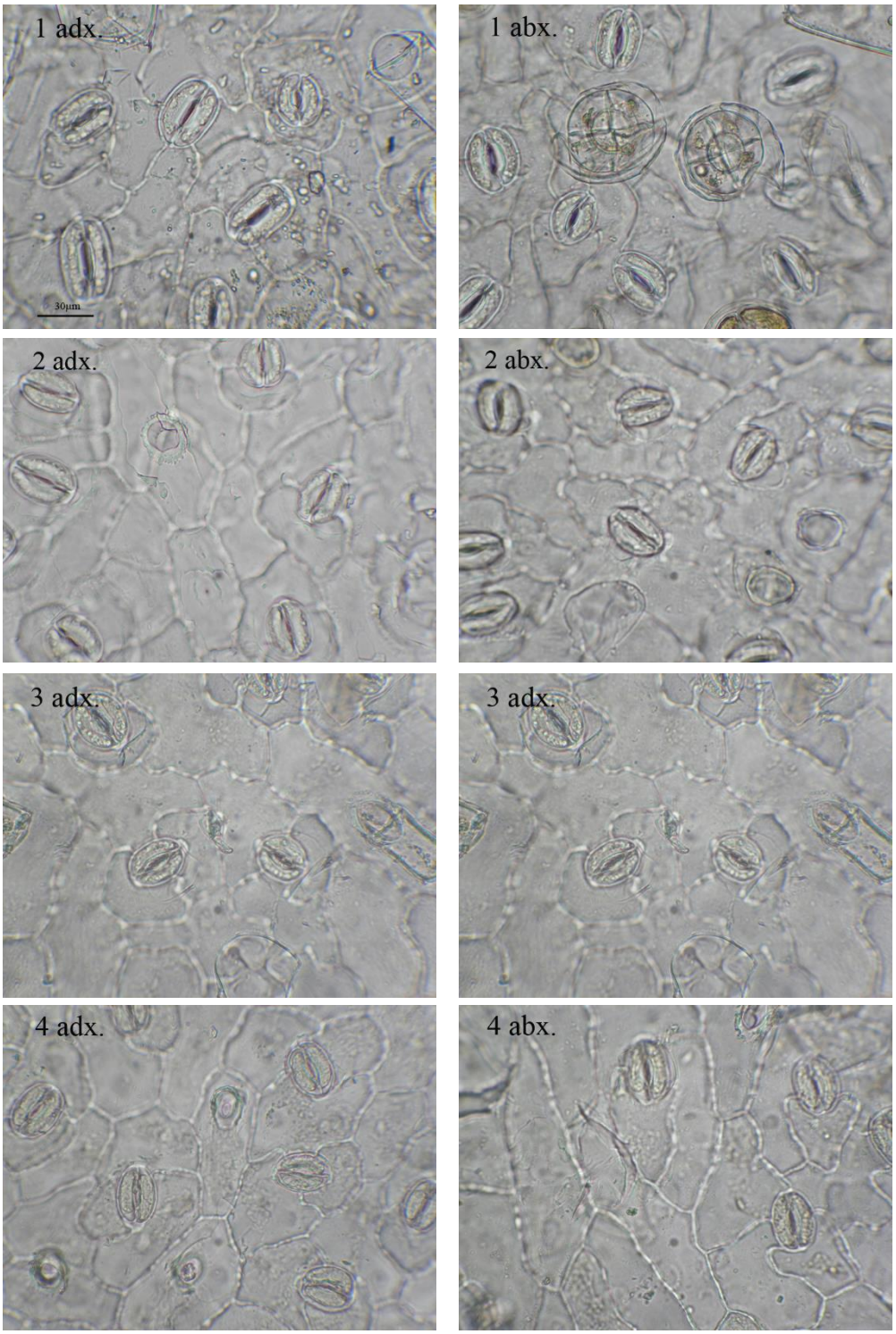
Figs. 9-12. Transverse section of leaves of studied populations of *Ajuga chamaecistus* subsp. *chamaecistus*: 9, Kooranabad; 10, Alashtar; 11, Kordestan; 12, Mahabad populations. Abbreeviations: Xy=Xylem; Ph=Phloem; PP=Palisade Parenchyma; Co=Collenchyma; Scl= Sclerenchyma.



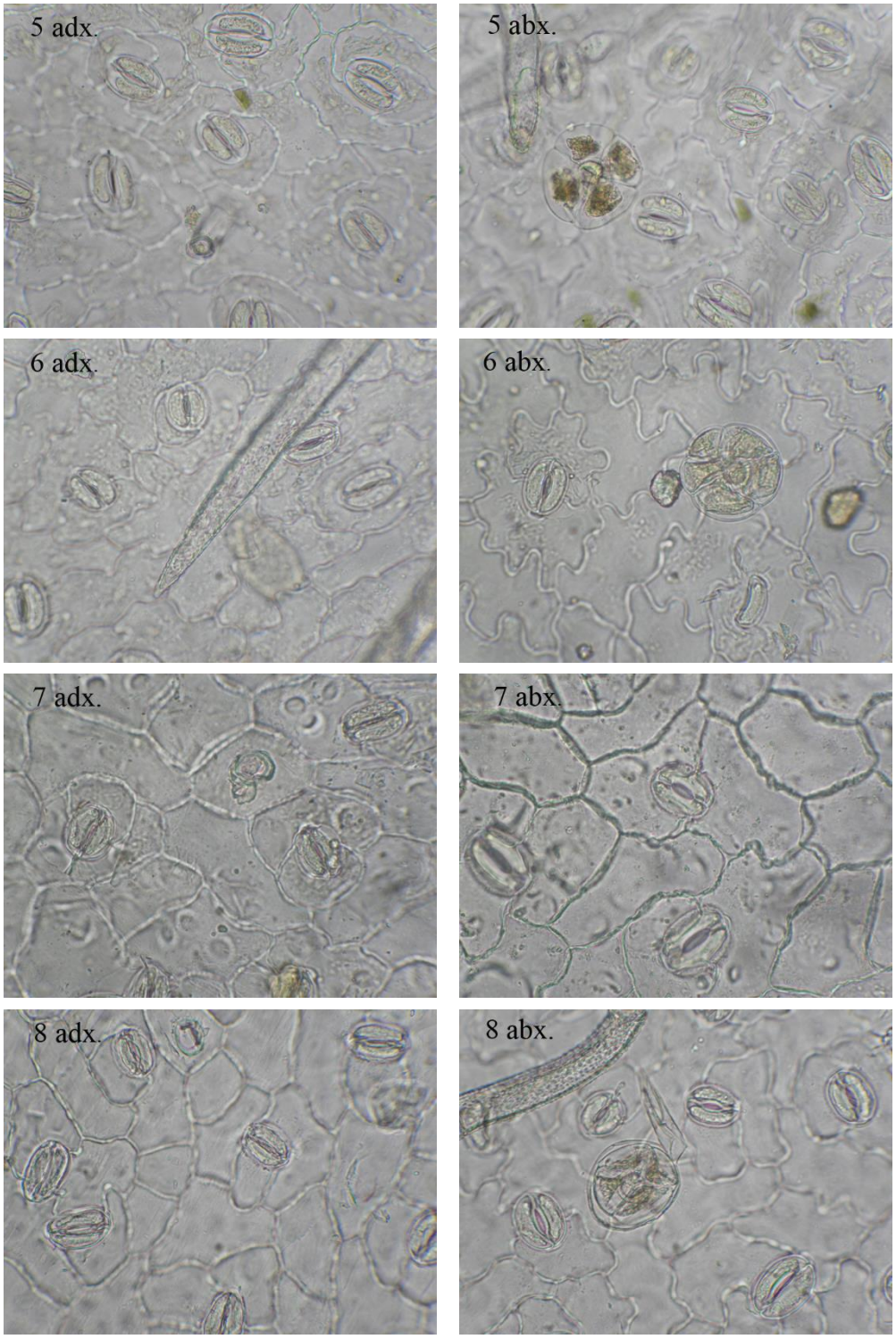
Figs. 13-20. Transverse section of leaves of studied populations of *Ajuga chamaecistus* subsp. *scoparia*: 13, Ghorogh-e-Shadi; 14, Post Chenar; 15, Hardang; 16, Nalbandan; 17, Enzo; 18, Bazenjan; 19, Latehdar; 20, Martaeh-e- Chah Neghahdar populations.



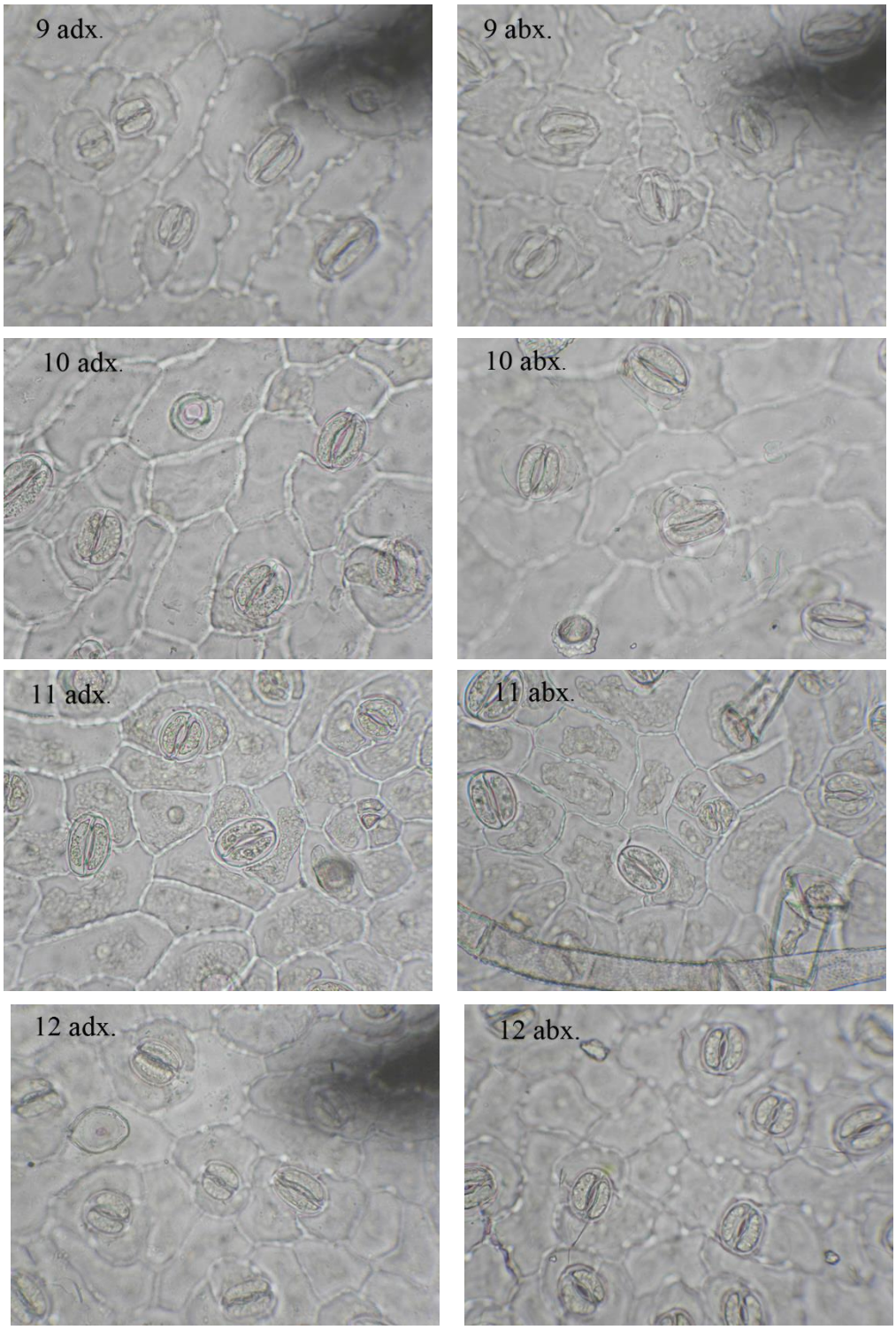
Figs. 21-28. Transverse section of leaves of studied populations of *Ajuga chamaecistus* subsp. *tomentella*: 21, Jajrood; 22, Sirachal; 23, Sagharan; 24, Vinsar; 25, Ghaheiz; 26, Shazand; 27, Beheshtabad; 28, Telo (*tomentella* x *scoparia*) populations.



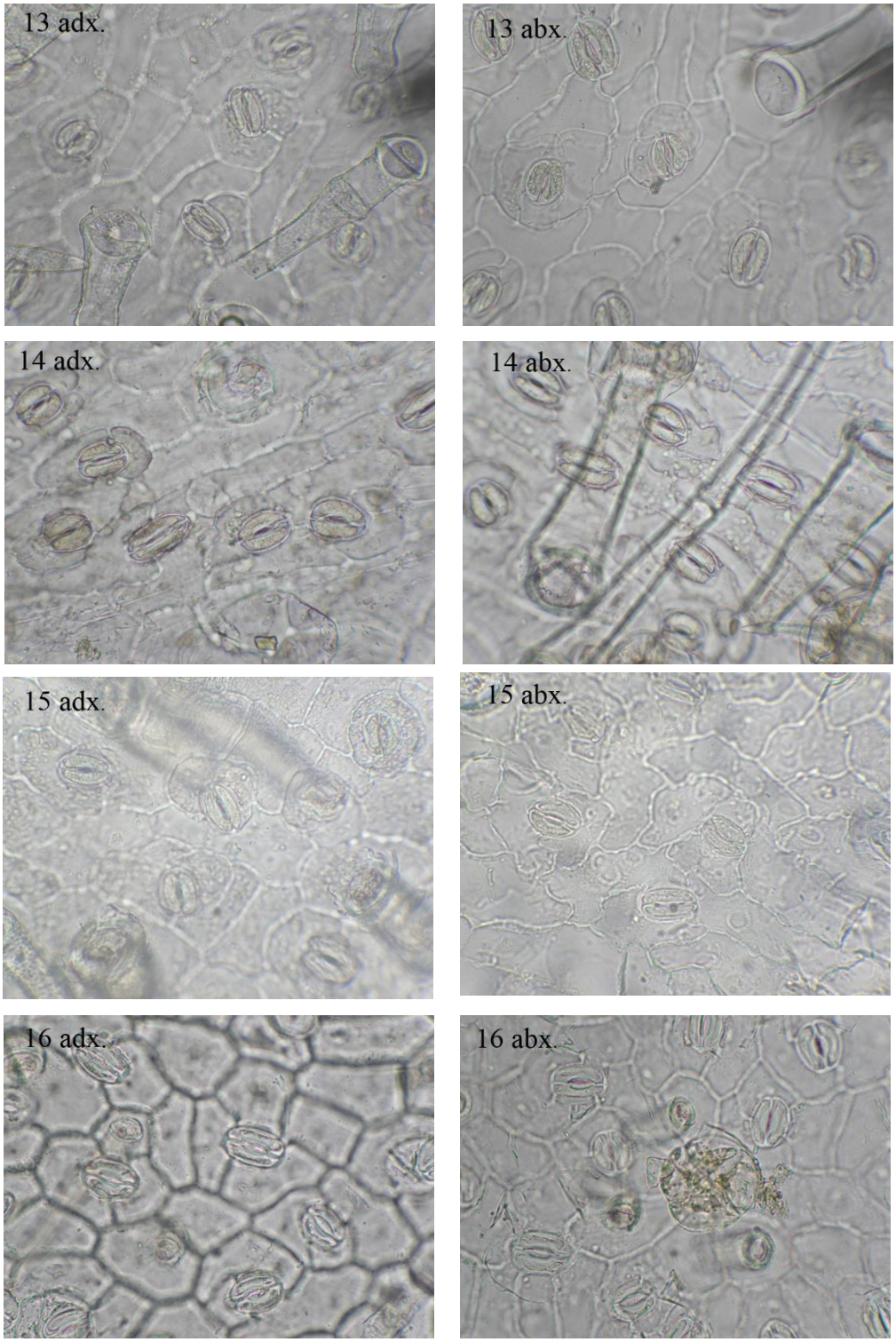
Figs. 1-4. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *chamaecistus*: 1, Arou; 2, Joustan; 3, Siahkamar; 4, Moineh populations.



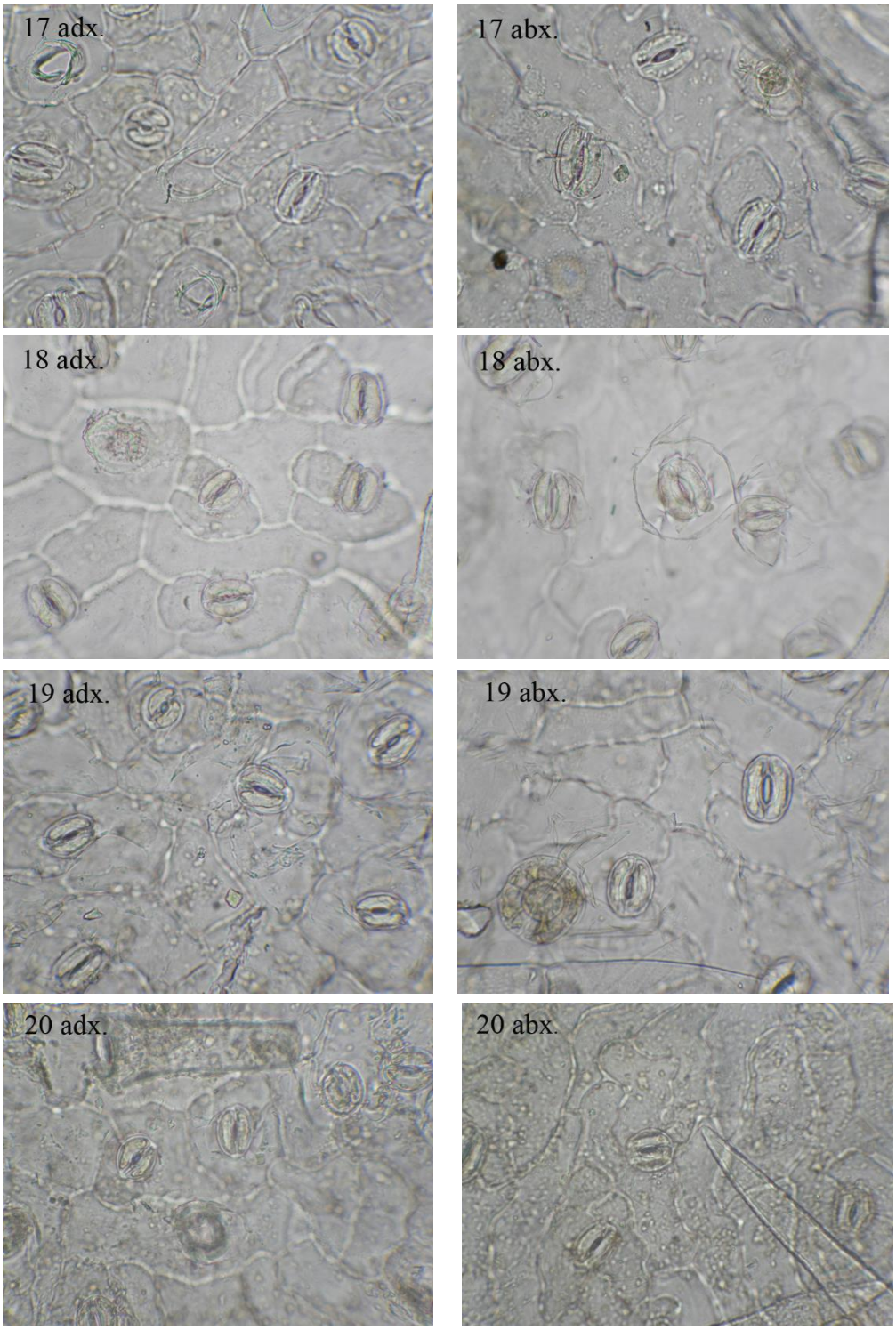
Figs. 5-8. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *chamaecistus*: 5, Hosseinabad; 6, Darreh Bid; 7, Kelishom; 8, Rasband populations.



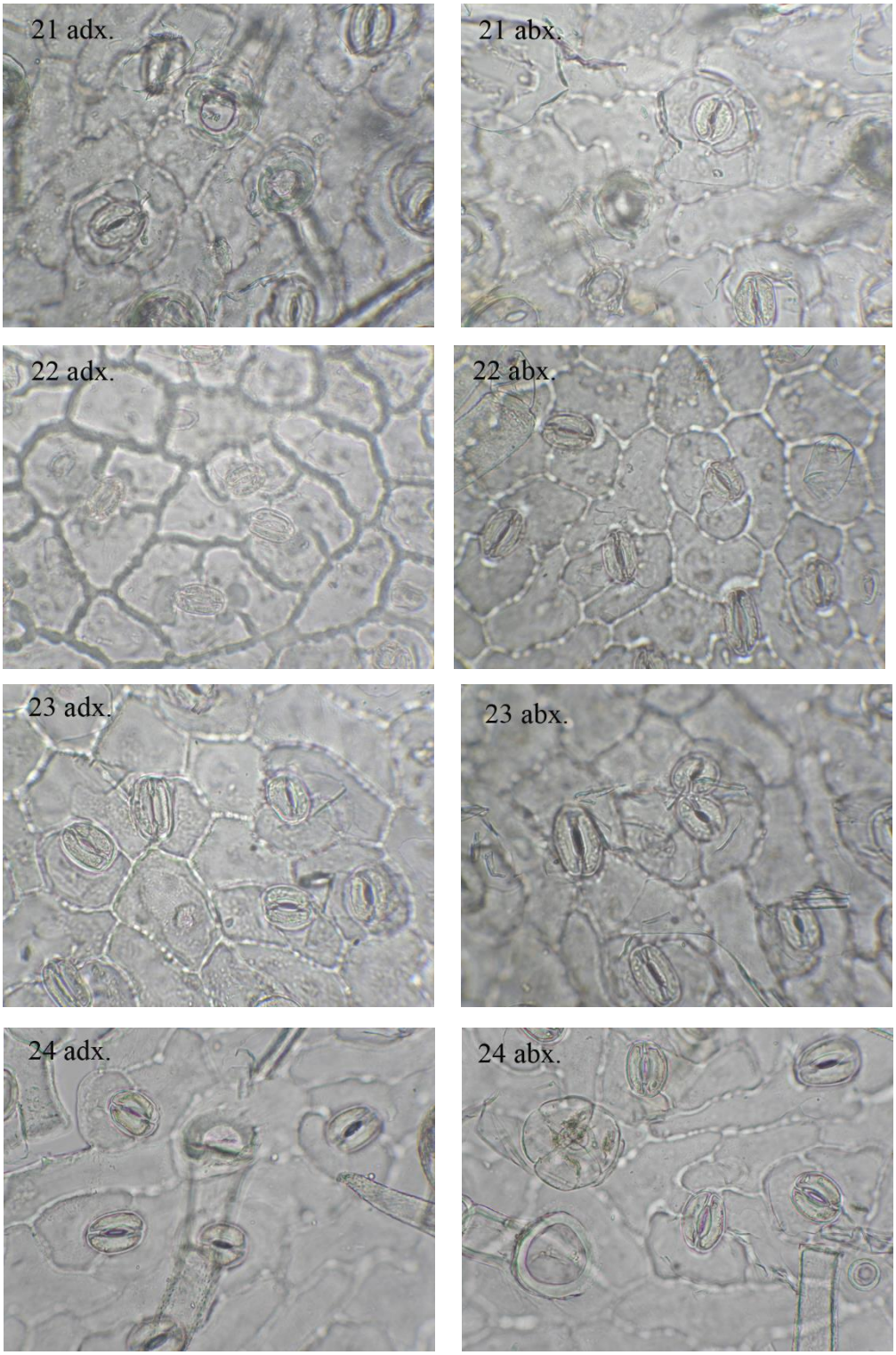
Figs. 9-12. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *chamaecistus*: 9, Kooranabad; 10, Alashtar; 11, Kordestan; 12, Mahabad populations.



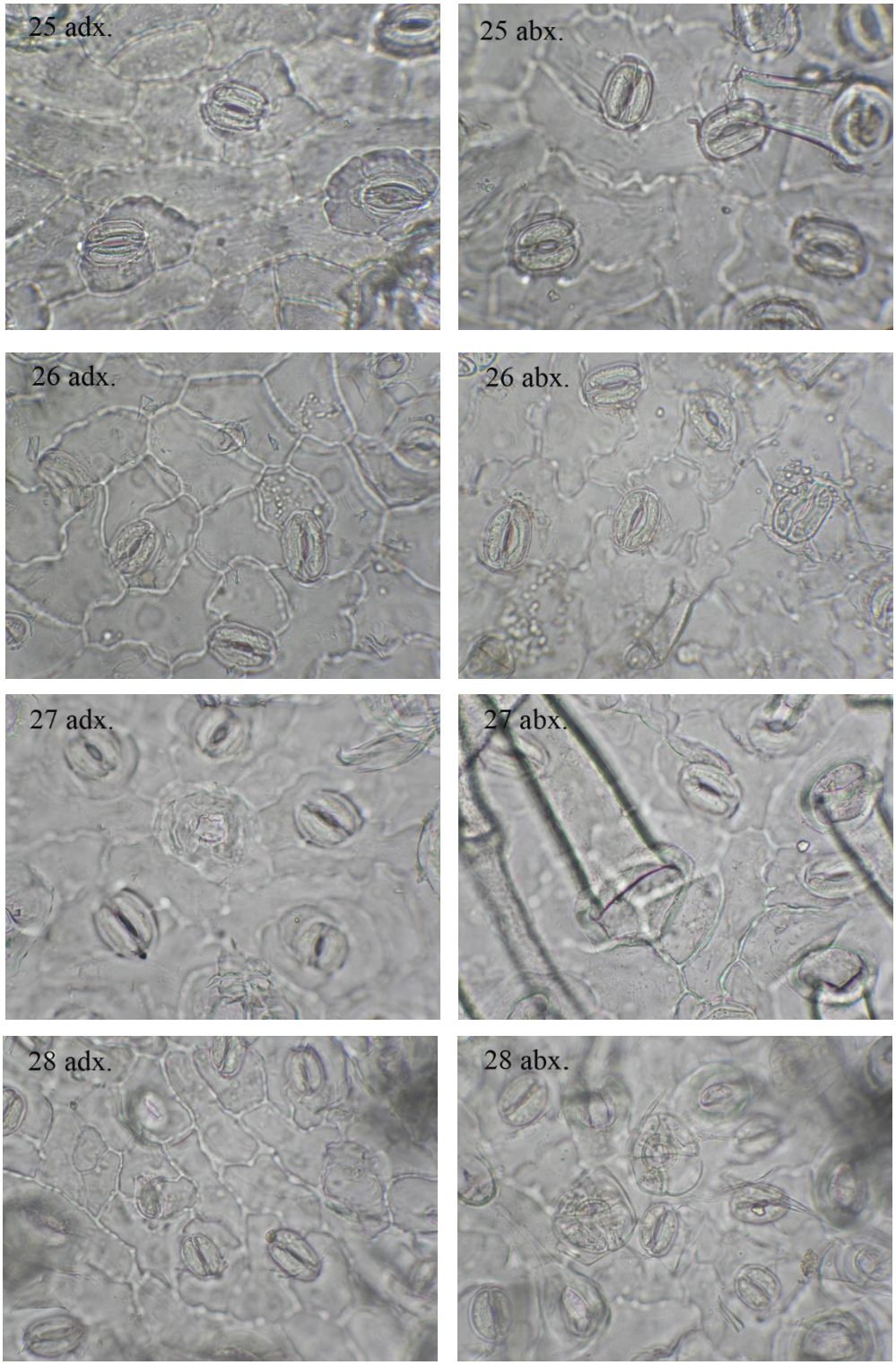
Figs. 13-16. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *scoparia*: 13, Ghorogh-e-Shadi; 14, Post Chenar; 15, Hardang; 16, Nalbandan populations.



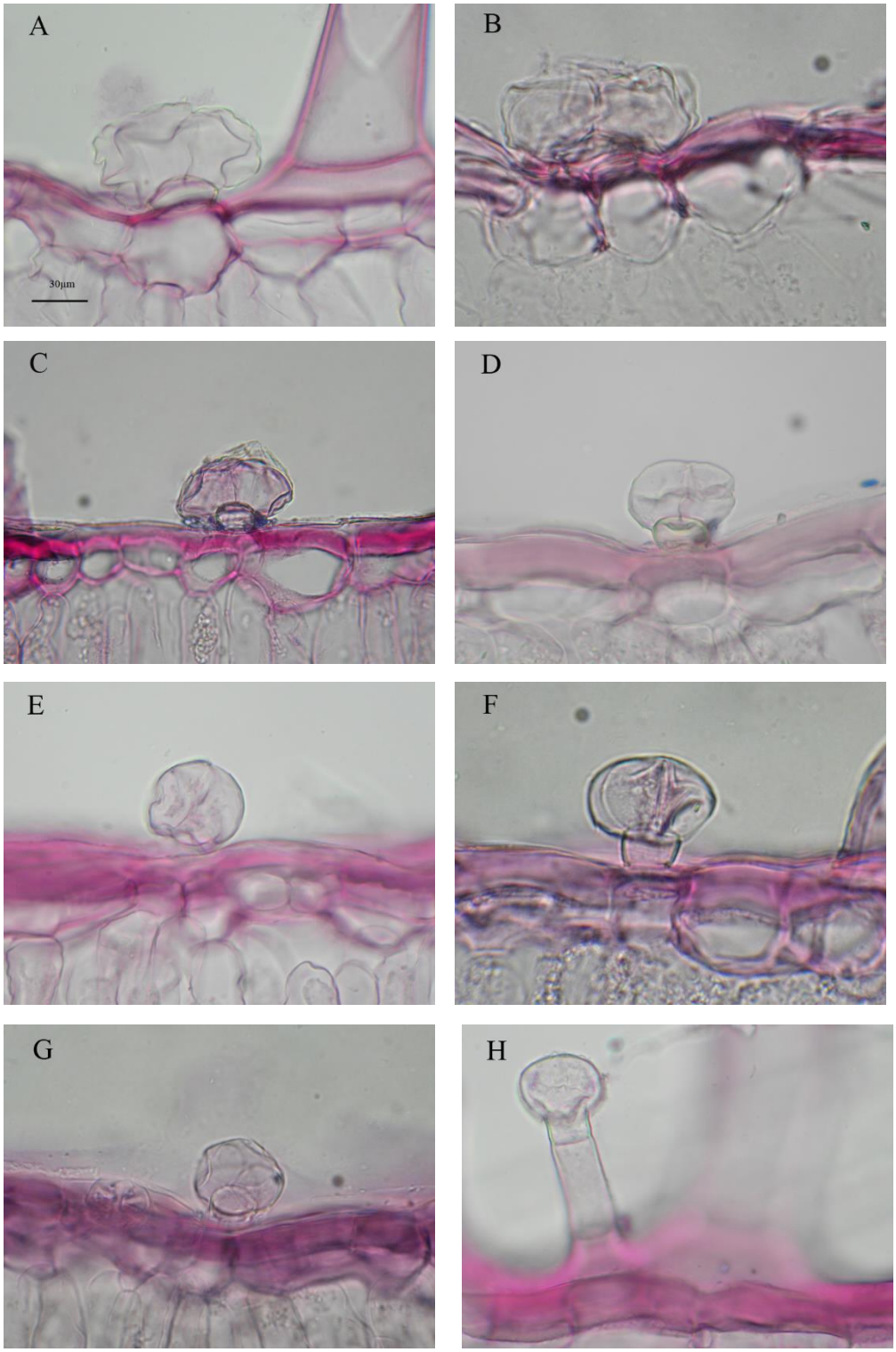
Figs. 17-20. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *scoparia*: 17, Enzo; 18, Bazenjan; 19, Latehdar; 20, Martaeh-e- Chah Neghahdar populations.



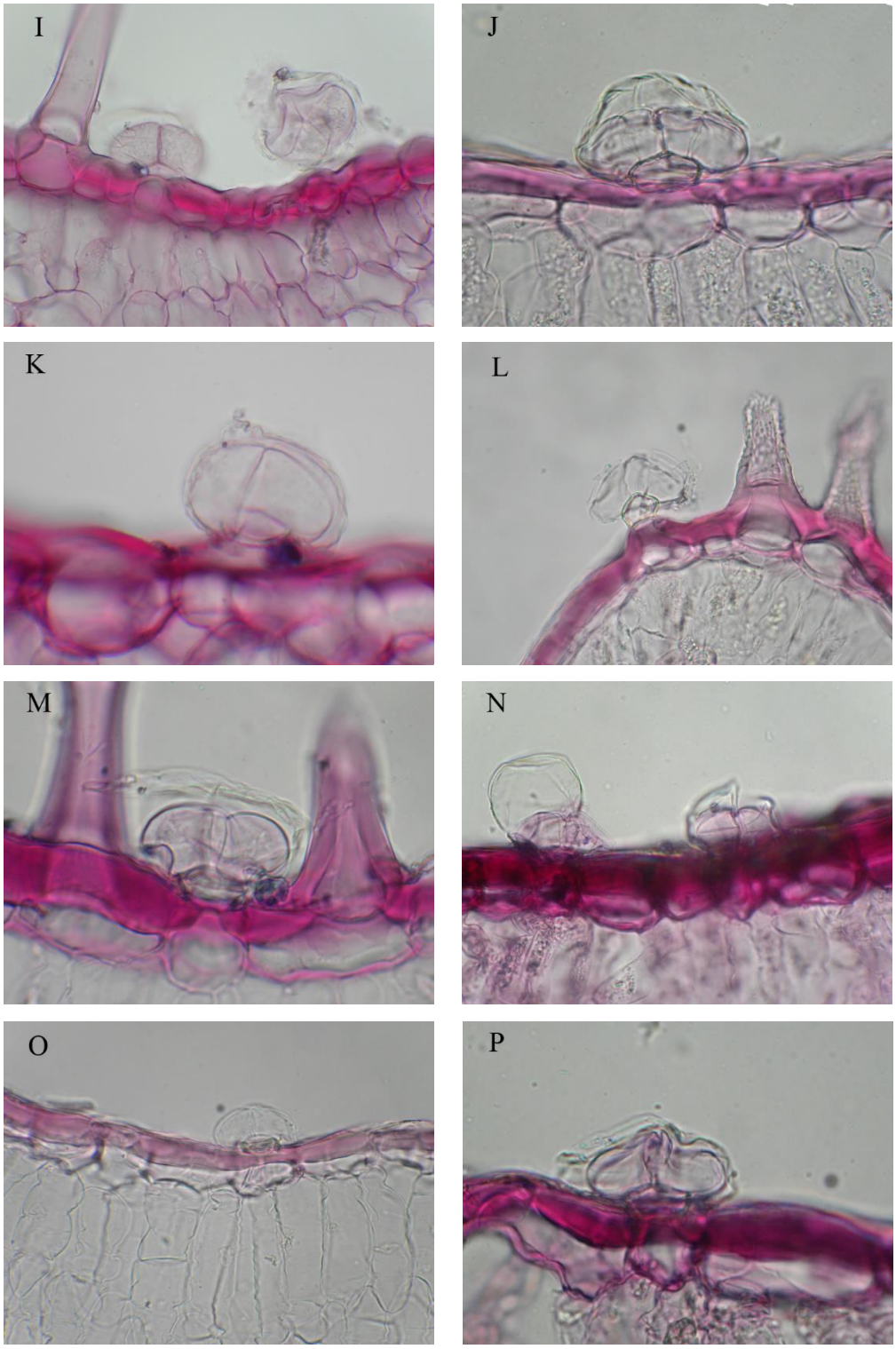
Figs. 21-24. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *tomentella*: 21, Jajrood; 22, Sirachal; 23, Sagharan; 24, Vinsar populations.



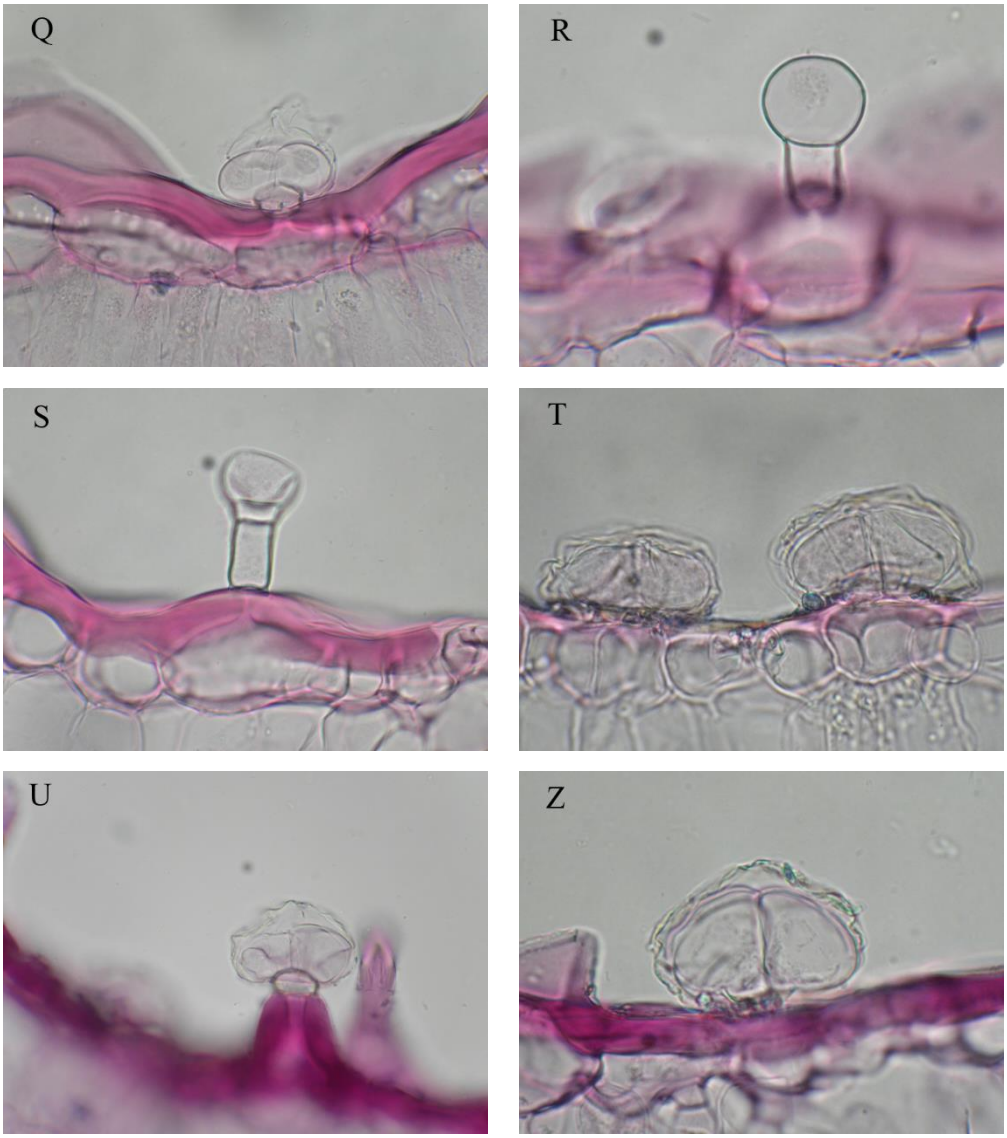
Figs. 25-28. Stomata of the upper and lower surfaces of leaves of studied populations of *Ajuga chamaecistus* subsp. *tomentella*: 26, Shazand; 27, Beheshtabad, 28, Telo (*tomentella* x *scoparia*) populations.



Figs. 29. Glandular trichome of leaves of studied populations of subsp. *chamaecistus*: A, Arou; B, Joustan; C, Siahkamar; D, Kelishom; E, Hosseinabad; F, Kordestan; G, Mahabad; subsp. *scoparia*: H, Post Chenar1 populations.



Figs. 29. Continued. Glandular trichome of leaves of studied populations of subsp. *scoparia*: I. Post Chenar2; J, Bazenjan; K, Enzo; L, Martaeh-e- Chah Neghahdar populations; subsp. *tomentella*: M, Jajrood; N, Sagharan; O, Sirachal; P, Vinsar populations.



Figs. 29. Continued. Glandular trichome of leaves of studied populations of subsp. *tomentella*: Q, Ghaheiz1; R, Ghaheiz2; S, Ghaheiz3; T, Shazand; Z, Beheshtabad; subsp. *tomentella* x *scoparia*: U, Telo populations.

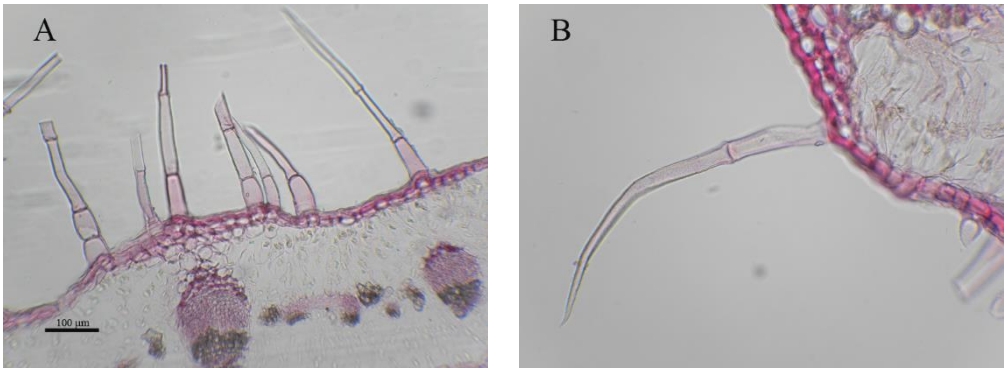


Fig. 30. Non-glandular trichome of leaves in different populations. subsp. *scoparia*: A, Hardang; B, Martaeh-e- Chah Neghahdar populations.

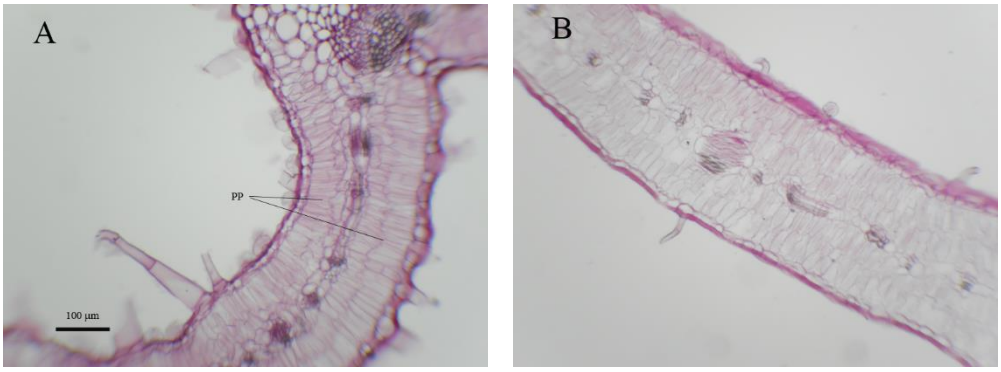


Fig. 31. Isobilateral leaf type of populations of subsp. *chamaecistus*: A, Arou; B, Hosseinabad populations.

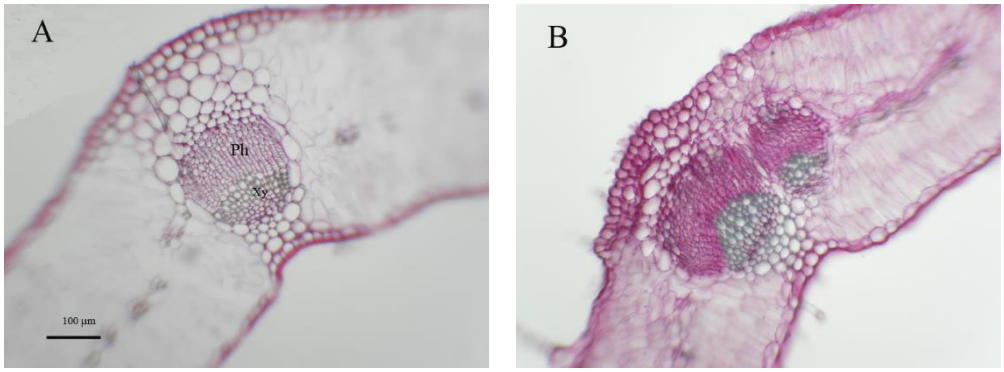


Fig. 32.Vascular sheath of leaves in different populations of subsp. *chamaecistus*: A, Hosseinabad; B, Rasband populations.

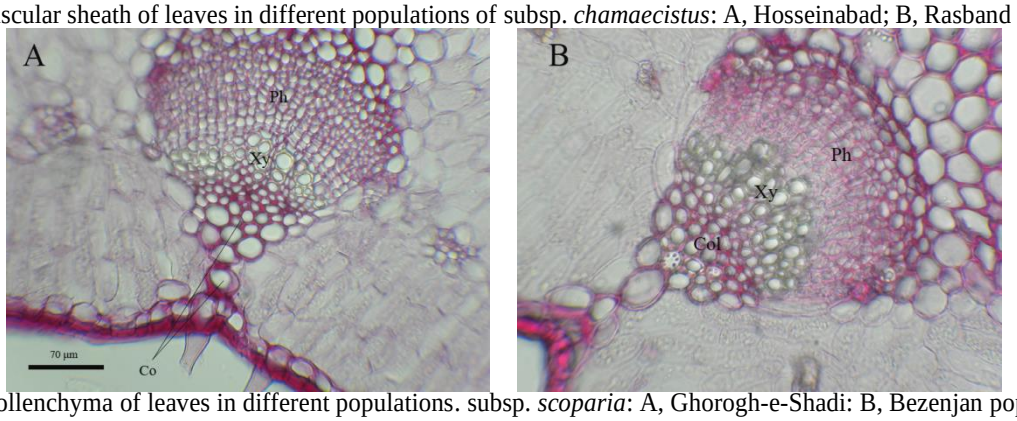


Fig. 33. Collenchyma of leaves in different populations. subsp. *scoparia*: A, Ghorogh-e-Shadi: B, Bezenjan populations.

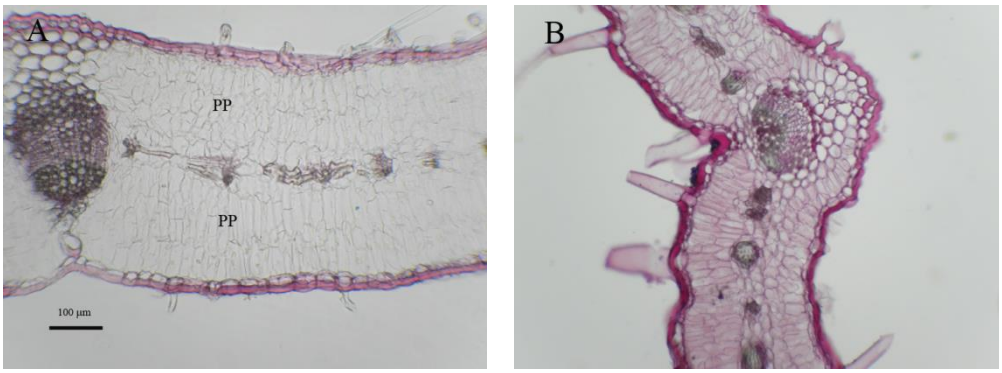


Fig. 34. Three and one layers palisade parenchyma of leaves in different populations of subsp. *tomentella*: A, Sirachal; B, subsp. *scoparia*: Post Chenar (lower surface) populations.

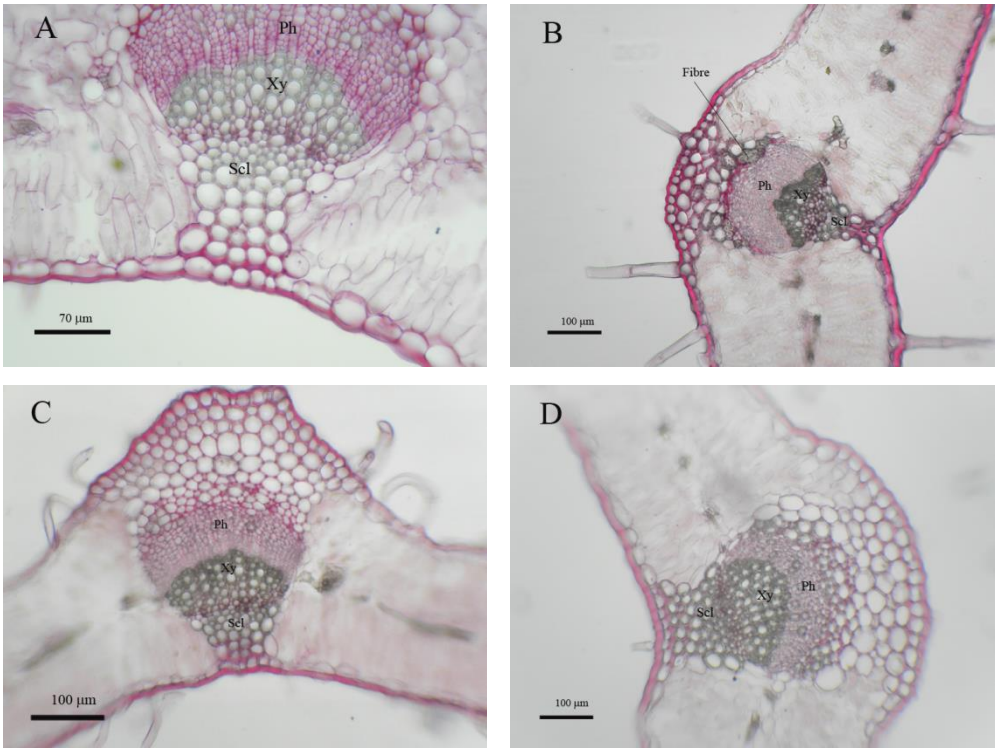


Fig. 35. Sclerenchyma of leaves in different populations of subsp. *scoparia*: A, Latehdar; B, Martaeh-e- Chah Neghahdar; subsp. *tomentella*: C, Shazand; subsp. *chamaecistus*: D, Kelishom populations.

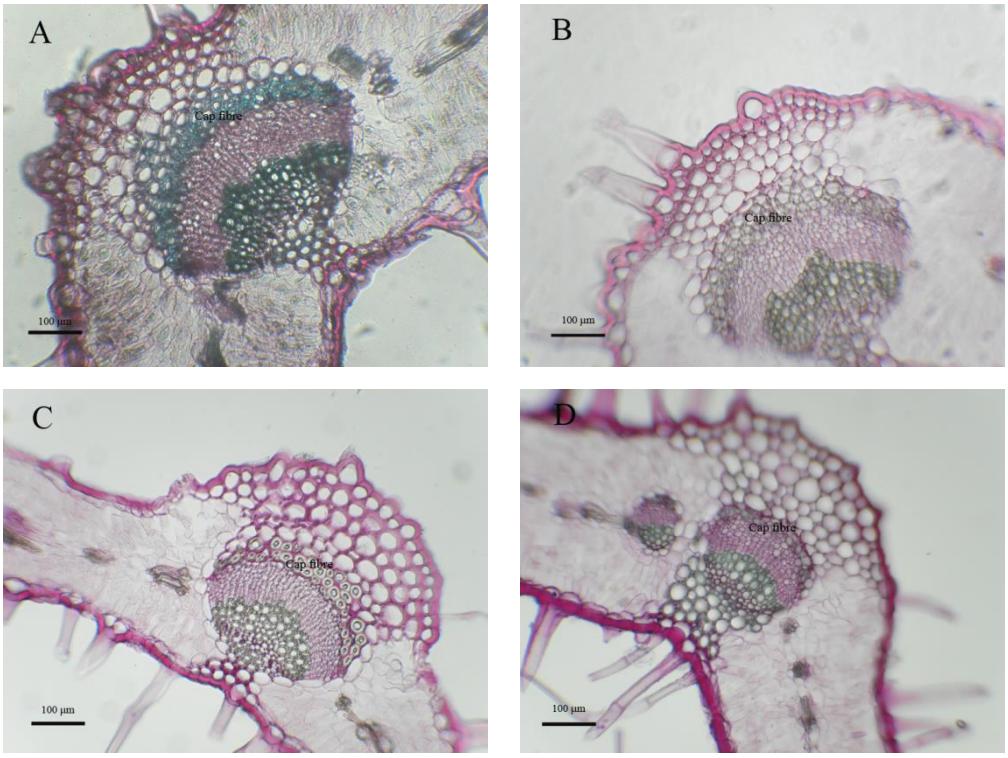


Fig. 36. Cap fibre above the phloem of leaves in different populations of subsp. *tomentella*; A. Sirachal; B. Ghaheiz; C. Beheshtabad; subsp. *tomentella* x *scoparia*: D, Formation of cap fibre above the phloem, Telo populations.

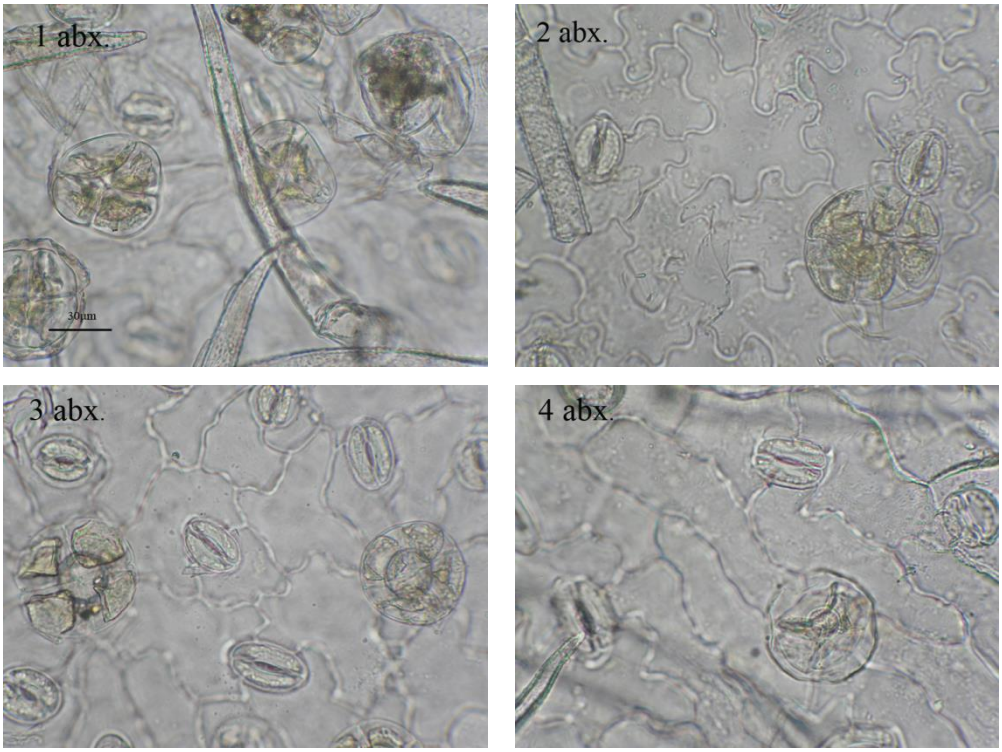


Fig. 37. Glandular trichome of leaves in different populations. subsp. *chamaecistus*: 1, Arou; 2, Darreh Bid; 3, Rasband; subsp. *scoparia*: 4, Enzo;subsp. *tomentella*: 5, Beheshtabad populations.

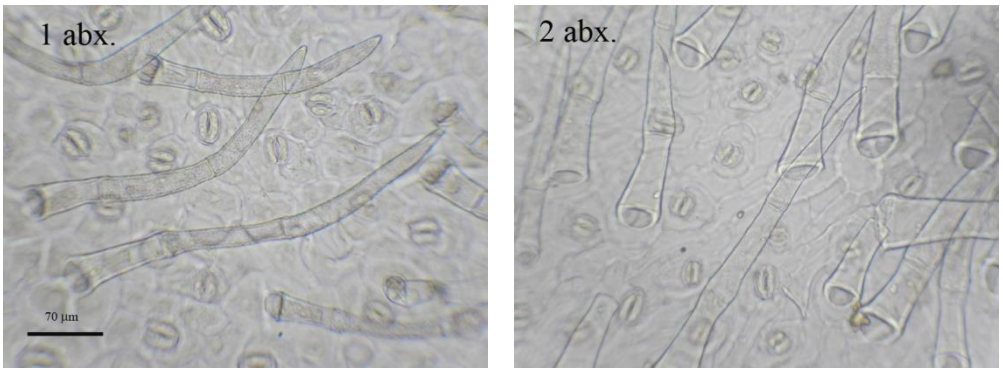


Fig. 38. Non-glandular trichome phloem of leaves in different populations, 1, Kordestan,(subsp. *chamaecistus*); 2, Ghorogh-e-Shadi (subsp. *scoparia*) populations.

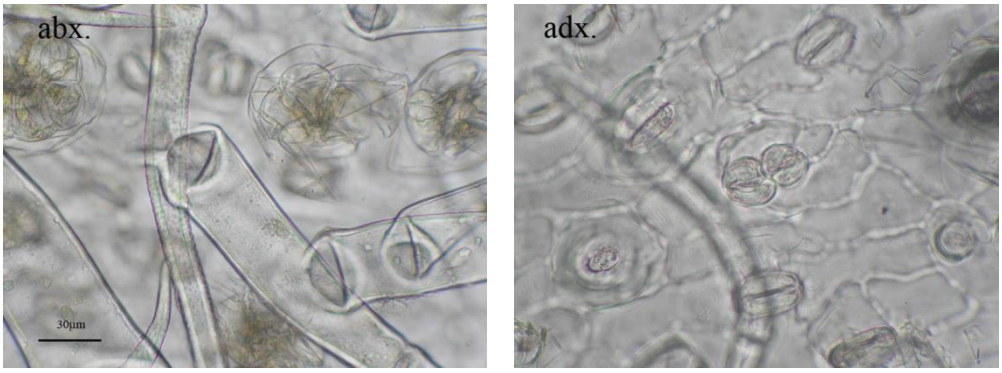


Fig. 39-40. Non-glandular and glandular trichomes of leaves of subsp. *tomentella* x *scoparia*: in Post Chenar Population and Two stomata next to each other of leaves in Telo population.

Qualitative and quantitative data were analyzed by principal component analysis (PCA) using version 16 of minitab software and position of specimens on the coordinate axes and ordination of them was performed. Based on the results obtained from qualitative data, the eigenvalues for the first and second axes (components) were 1.93 and 1.59, respectively. The first component (axis) with 0.24 percent of the data variance, denoted that in the separation of the subspecies, presence of the glandular trichomes on the lower epidermis, presence of the glandular trichomes on the upper epidermis, density of the glandular trichomes on the lower epidermis and the presence or absence of the vascular sheath had positive and the number of the upper palisade parenchyma layers had negative correlation. Also, on the second component (axis) with 0.20 percent of the data variance, presence of the glandular trichomes on the upper epidermis, presence or absence of fibre-Sclerenchyma and the number of the lower palisade parenchyma layers had positive correlation (table. 4). In the biplot diagram as well as the dendrogram, subsp. *chamaecistus* of Sirachal and subsp. *Scoparia* of Post Chenar specimens each separately and Kooranabad, Alashtar and Moineh samples as a group, were distinguished from the other samples (figs. 41 & 42). As shown in the biplot diagram, Enzo, Nalbandan, Hardang, Latehdar, Bezenjan and Ghorogh-e-shadi samples belong to subsp. *scoparia* are rather close to each other (fig. 41). Based on the results obtained from quantitative data, the eigenvalues for the first and second axis (components) were 3.47 and 2.86, respectively. The first component (axis) with 0.27 percent of the data variance, specified that in the separation of the subspecies, the density of the stomata on both of the upper and lower surfaces, had positive and the length and width of the stomata negative correlation. Also, on the second component (axis) with 0.22 percent of the data variance, thickness of the upper and lower palisade parenchyma and the diameter of the lamina, had negative correlation (table. 5). In the biplot diagram as well as the dendrogram, subsp. *tomentella* of Jajrood sample individually, subsp. *scoparia* of Enzo and subsp. *chamaecistus* of Arou samples together and subsp. *tomentella* of Beheshtabad and Ghahiz samples, subsp. *chamaecistus* of Joustan, Siahkamar, Alashtar and Kelishom specimens were grouped (figs. 43 & 44).

Table 2. Qualitative anatomical features in the specimens.

	Subspecies	variety	Sample	Leaf type	Trichome type	Stomata position	Number of the upper palisade parenchyma	Number of the upper palisade parenchyma	Number of vascular bundle	Fibre- Sclerenchyma	Vascular sheath	Tissue of vascular sheath	Upper collenchyma	Lower collenchyma
1	*chamaecistus*		Arou	Isobilateral		Flat	1-2 Mostly 2	1-2 Mostly 2	1	-	Vascular sheath/Semi-	Parenchyma	*	*
2	*chamaecistus*		Joustan	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	1-2 Mostly 2	2-3 Mostly 2	1	-	Vascular sheath/Semi-	Parenchyma	*	*
3	*chamaecistus*		Siahkamar	Isobilateral	Glandular/non-glandular(both Surfaces)	Flat/ Prominent	1-2 Mostly 2	2	1	*	Semi-	Parenchyma	*	*
4	*chamaecistus*		Maineh	Isobilateral	non- glandular	Flat	2	2-3 Mostly 2	1	*	Semi-	Parenchyma	*	*
5	*chamaecistus*		Hosseinabad	Isobilateral	Glandular/non-glandular(Lower Surface)	flat/ prominent	1-2 Mostly 2	2	1	-	Vascular sheath	Parenchyma	*	*
6	*chamaecistus*		Darreh Bid	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat/ Prominent	1-2 Mostly 2	1-2	1	*	Vascular sheath/Semi-	Parenchyma	*	*
7	*chamaecistus*		Kelishom	isobilateral	Glandular/non-glandular(Lower Surface)	Flat/ Prominent	1-2 Mostly 2	2-3 Mostly 2	1	*	Vascular sheath/Semi-	Parenchyma	*	*
8	*chamaecistus*		Rasband	isobilateral	Glandular/non-glandular(Lower Surface)	Flat/ Prominent	2-3 Mostly 2	2-3 Mostly 2	1	*	Semi-	Parenchyma	*	*
9	*chamaecistus*		Kooranabad	isobilateral	non- glandular	Flat/ Prominent	2-3	2	1	-	Semi-	Parenchyma	*	*
10	*chamaecistus*		Alashtar	Isobilateral	non- glandular	Flat	2-3	2-3	1	*	Semi-	Parenchyma	*	*
11	*chamaecistus*		Kordestan	Isobilateral	Glandular/non-glandular(upper Surface)	Flat	2	2	1	-	Semi-	Parenchyma	*	*
12	*chamaecistus*		Mahabad	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	2	2	1	-	Vascular sheath	Parenchyma	*	*
13	*scoparia*		Ghorogh-e-Shadi	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	2-3	2-3	1	*	Semi-	Parenchyma	*	*
14	*scoparia*		Post Chenar	Isobilateral	Glandular/non-glandular(both Surfaces)	Flat	1-2 Mostly 2	1	1	*	Vascular sheath/Semi-	Parenchyma	*	*

Table 2. Continued.

	Subspecies	variety	Sample	Leaf type	Trichome type	Stomata position	Number of the upper palisade parenchyma	Number of the upper palisade parenchyma	Number of vascular bundle	Fibre- Sclerenchyma	Vascular sheath	Tissue of vascular sheath	Upper collenchyma	Lower collenchyma
16	*scoparia*		Nalbandan	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	2-3 Mostly 2	2	1	*	Vascular sheath	Parenchyma	*	*
17	*scoparia*		Enzo	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	2-3	2	1	-	Vascular sheath/Semi-	Parenchyma	*	*
18	*scoparia*		Bazenjan	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	2-3	2-3	1	*	Semi-	Parenchyma	*	*
19	*scoparia*		Latehdar	Isobilateral	Glandular/non-glandular(lower surface)	Flat	2-3 Mostly 2	2-3 Mostly 2	1	*	Vascular sheath/Semi-	Parenchyma	*	*
20	*scoparia*		Martaeh-e-ChahNeghahdar	Isobilateral	Glandular/non-glandular(lower surface)	Flat	2	2-3 Mostly 2	1	*	Semi-	Parenchyma	*	*
21	*tomentella*		Jajrood	Isobilateral	Glandular/non-glandular(lower surface)	Flat	1-2 Mostly 2	1-2	1	*	Vascular sheath/Semi-	Parenchyma	*	*
22	*tomentella*		Sirachal	isobilateral	Glandular/non-glandular(both surfaces)	Flat/ Prominent	2-3 Mostly 3	3	1	*	Semi-	Parenchyma	*	*
23	*tomentella*		Sagharan	Isobilateral	Glandular/non-glandular(both Surfaces)	flat/ prominent	2	1-2-3	1	*	Vascular sheath/Semi-	Parenchyma	*	*
24	*tomentella*		Vinsar	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat/ Prominent	2	2	1	*	Semi-	Parenchyma	*	*
25	*tomentella*		Ghaheiz	isobilateral	Glandular/non-glandular(Lower Surface)	Flat	2	2	1	*	Semi-	Parenchyma	*	*
26	*tomentella*		Shazand	Isobilateral	Glandular/non-glandular(lower surface)	Flat/ Prominent	2-3 Mostly 2	2-3 Mostly 2	1	*	Semi-	Parenchyma	*	*
27	*tomentella*	*tomentella*	Beheshtabad	Isobilateral	Glandular/non-glandular(Lower Surface)	Flat	1-2	2	1	*	Vascular sheath	Parenchyma	*	*
28	*tomentella* x *scoparia*		Telo	Isobilateral	Glandular/non-glandular(lower surface)	Flat	2-3	2	1		Semi-	Parenchyma	*	*

Table 3. Quantitative anatomical features in the specimens.

	Subspecies	variety	Sample	Thickness of theupper palisade parenchyma (µm)	Tthickness of thelower palisade parenchyma (µm)	Thickness of theuppercuticle(µm)	Thickness of thelower cuticle(µm)	Mesophyll/ Vascular bundle	Diameter of lamina (µm)	Adaxial stomatal density(mm^{-2})	abaxial stomatal density(mm^{-2})	Adaxial stomatal length(µm)	Adaxial stomatallength(µm)	Adaxial stomatal width (µm)	Adaxial stomata width (µm)
1	*chamaecistus*		Arou	105.73	102.27	8.33	9.17	4.07	312.8	144.65	176.10	36.88	36.67	25	25
2	*chamaecistus*		Joustan	97.07	109.2	7.5	5.83	2.67	309.4	105.87	156.18	34.17	33.33	24.17	21.67
3	*chamaecistus*		Siahkamar	100.53	110.93	12.5	12.5	2.42	343.4	92.24	100.63	35	31.67	25	23.33
4	*chamaecistus*		Maineh	128.27	123.07	11.67	11.67	2.31	319.6	147.80	133.65	30.83	31.25	19.58	23.13
5	*chamaecistus*		Hosseinabad	95.33	121.33	10.83	11.67	2.79	351.9	178.20	187.63	31.67	34.17	20.83	23.33
6	*chamaecistus*		Darreh Bid	86.67	109.2	8.33	11.67	2.1	278.8	99.84	70.75	31.25	32.5	21.88	21.88
7	*chamaecistus*		Kelishom	116.13	138.67	15	15	2.38	353.6	68.40	82.81	35	38.33	21.25	25.83
8	*chamaecistus*		Rasband	147.33	145.6	7.5	7.5	2.61	391	153.04	175.05	30.83	32.5	22.5	20.83
9	*chamaecistus*		Kooranabad	143.87	130	17.5	18.33	2.17	401.2	146.23	165.62	32.5	30.83	19.58	20
10	*chamaecistus*		Alashtar	133.47	143	16.67	13.33	3.06	408	128.93	141.51	32.5	35	22.5	25
11	*chamaecistus*		Kordestan	88.4	104	13.33	15	3.03	293.25	165.09	180.03	31.67	32.5	22.5	23.75
12	*chamaecistus*		Mahabad	112.67	98.8	12.5	11.67	3.12	346.8	190.78	215.93	26.25	32.5	21.25	22.5
13	*scoparia*		Ghorogh-e-Shadi	110.93	124.8	13.33	10.83	2.6	306	157.23	167.45	27.5	25.63	20.83	21.25
14	*scoparia*		Post Chenar	90.13	79.3	14.17	12.5	3.59	312.8	166.67	215.93	30.83	29.17	20.83	20
15	*scoparia*		Hardang	124.8	117.87	12.5	10	2.49	367.2	148.85	122.64	28.33	26.67	20	20
16	*scoparia*		Nalbandan	150.8	140.4	15	12.5	2.98	384.2	181.34	202.31	26.67	29.17	20.83	20.42
17	*scoparia*		Enzo	133.47	152.53	7.5	7.5	4.01	357	124.74	60.53	30.83	34.17	22.08	23.75
18	*scoparia*		Bazenjan	157.3	163.8	16.88	11.67	3.17	469.2	113.84	114.26	30	32.92	20.5	20.42
19	*scoparia*		Latehdar	159.47	171.6	12.5	11.25	3.14	408	121.59	84.91	28.33	34.38	21.67	23.13

Table 3. Continued.

	Subspecies	variety	Sample	Thickness of theupper palisade parenchyma (µm)	Tthickness of thelower palisade parenchyma (µm)	Thickness of theuppercuticle(µm)	Thickness of thelower cuticle(µm)	Mesophyll/ Vascular bundle	Diameter of lamina (µm)	Adaxial stomatal density(mm^{-2})	abaxial stomatal density(mm^{-2})	Adaxial stomatal length(µm)	Adaxial stomatallength(µm)	Adaxial stomatal width (µm)	Adaxial stomata width (µm)
20	*Scoparia*		Martaeh-e-ChahNeghahdar	100.53	126.53	13.33	10	2.2	340	99.58	99.06	33.33	30.83	20	20
21	*tomentella*		Jajrood	93.6	64.13	15.83	18.33	2.91	214.2	98.53	98.53	29.17	28.33	21.67	20.83
22	*tomentella*		Sirachal	168.13	154.27	13.33	11.67	3.55	425	147.80	193.92	30	27.5	19.17	17.5
23	*tomentella*		Sagharan	117.87	114.4	15	13.33	3.47	360.4	124.74	161.16	32.5	30	22.92	21.88
24	*tomentella*		Vinsar	98.8	91.87	13.33	12.5	3.42	244.8	121.07	161.43	29.17	28.33	22.92	23.33
25	*tomentella*		Ghaheiz	124.8	130	14.38	14	2.71	387.6	73.38	93.29	37.5	30	25	25
26	*tomentella*		Shazand	143.87	124.8	10	12.5	2.58	397.8	129.72	107.97	32.5	34.17	21.25	23.75
27	*tomentella*	*tomentella*	Beheshtabad	83.2	79.73	13.33	10.83	2.7	258.4	140.46	140.72	34.17	33.75	25.83	25.63
28	*tomentella* x *scoparia*		Telo	105.73	100.53	15	12.5	2.82	278.8	159.33	170.85	30	22.5	20.42	18.33

Table 4. Eigenvalues, variance proportion of Leaf qualitative anatomical featuresvariables of *Ajuga chamaecistus* taxa in PCA analysis.

Leaf anatomical features	PCA_1	PCA_2
The number of the upper palisade parenchyma layers	**-0.54**	-0.01
Presence of the glandular trichomes on the lower epidermis	**0.40**	0.14
Presence or absence of the vascular sheath	**0.43**	-0.23
Density of the glandular trichomes on the lower epidermis	**0.36**	0.24
Presence of the glandular trichomes on the upper epidermis	**0.40**	**0.44**
Presence or absence of the Fibre- Sclerenchyma	-0.23	**0.52**
The number of the lower palisade parenchyma layers	-0.18	**0.50**
Stomata position	-0.02	0.36
Eigenvalues	1.93	1.59
Variance Proportion	0.24	0.20

Table 5. Eigenvalues, variance proportion of Leaf quantitative anatomical featuresvariables of *Ajuga chamaecistus* taxa in PCA analysis.

Leaf anatomical features	PCA_1	PCA_2
tal density	**0.34**	0.18
The lower stomatal density	**0.29**	0.26
The upper stomatal length	**-0.40**	-0.12
The upper stomatal width	**-0.42**	0.06
The lower stomatal length	**-0.33**	-0.28
The lower stomatal width	**-0.44**	-0.07
The upper palisade parenchyma thickness	0.27	**-0.47**
The lower palisade parenchyma thickness	0.14	**-0.55**
The diameter of the lamina	0.18	**-0.51**
The ratio of the mesophyll to vascular bundle	0.06	0.00
The upper cuticle thickness	0.18	0.04
The lower cuticle thickness	0.05	0.12
Eigenvalues	3.47	2.86
Variance Proportion	0.27	0.22

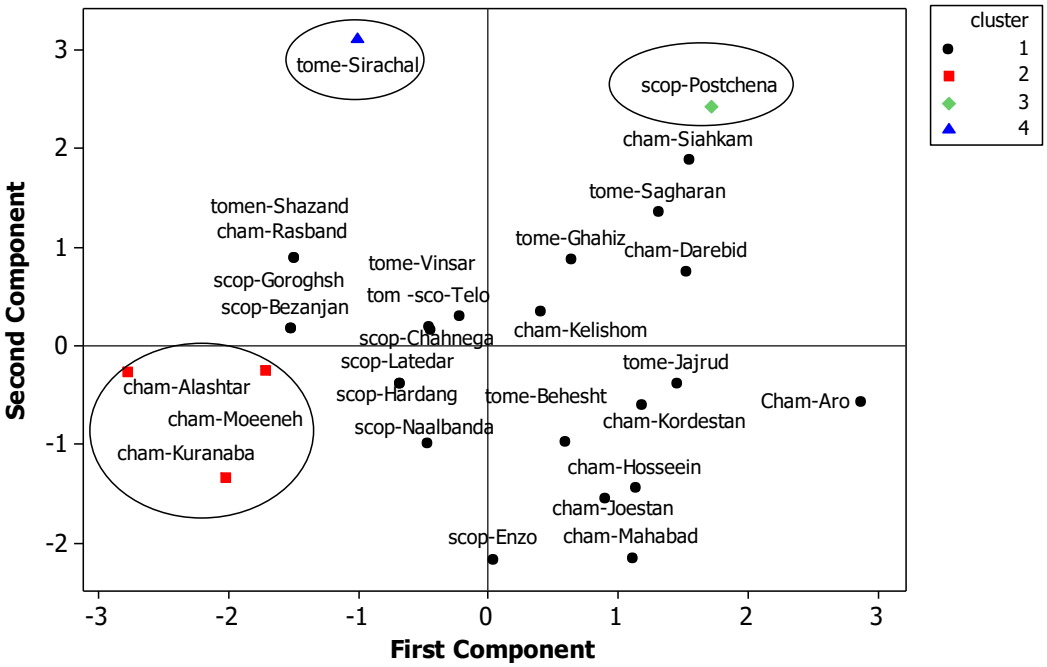


Fig. 41. The biplot diagram of the qualitative anatomical features indicating the position of the studied subspecies relative to each other.

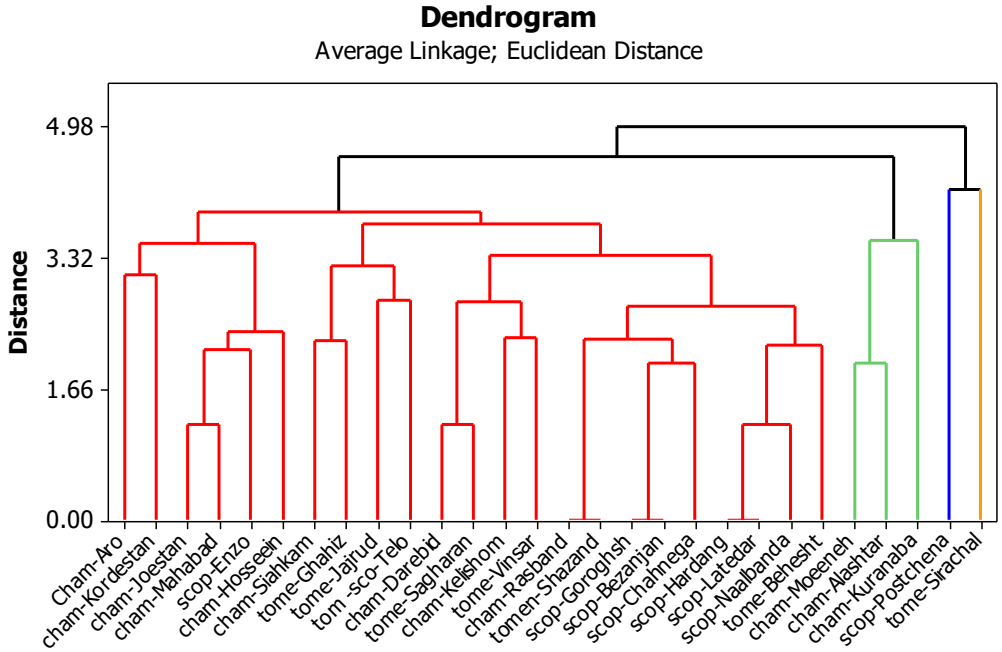


Fig. 42. The cluster analysis diagram of the studied subspecies based on the qualitative anatomical features. Abreviations: cham: subsp. *chamaecistus*; scop: subsp. *scoparia*; Tom: subsp. *tomentosa* and their relevant populations.

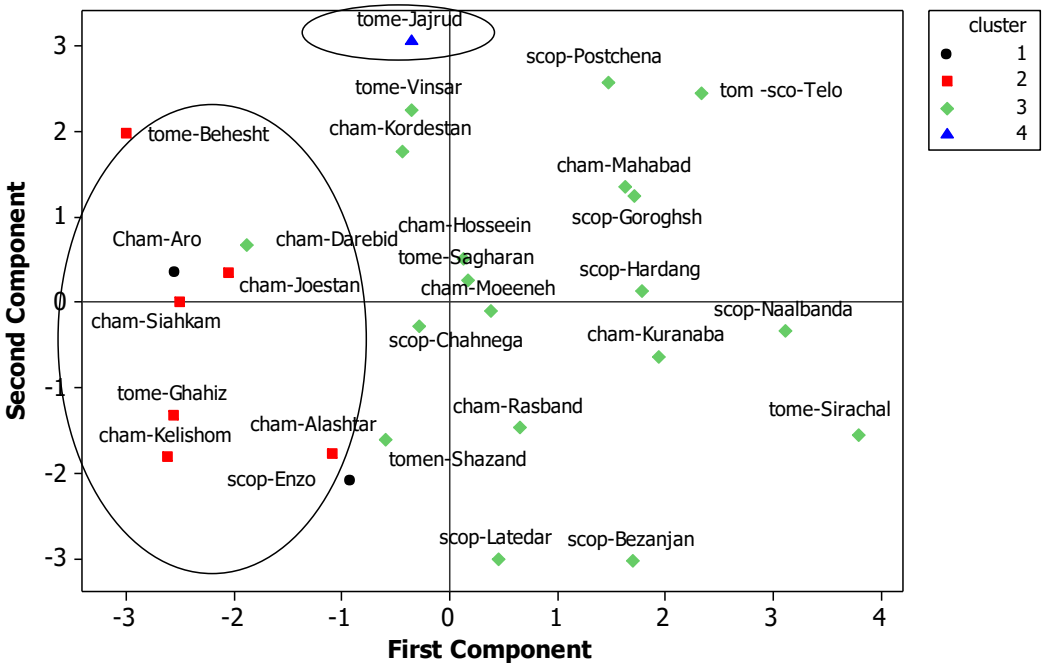


Fig. 43. The biplot diagram of the quantitative anatomical features indicating the position of the studied subspecies relative to each other.

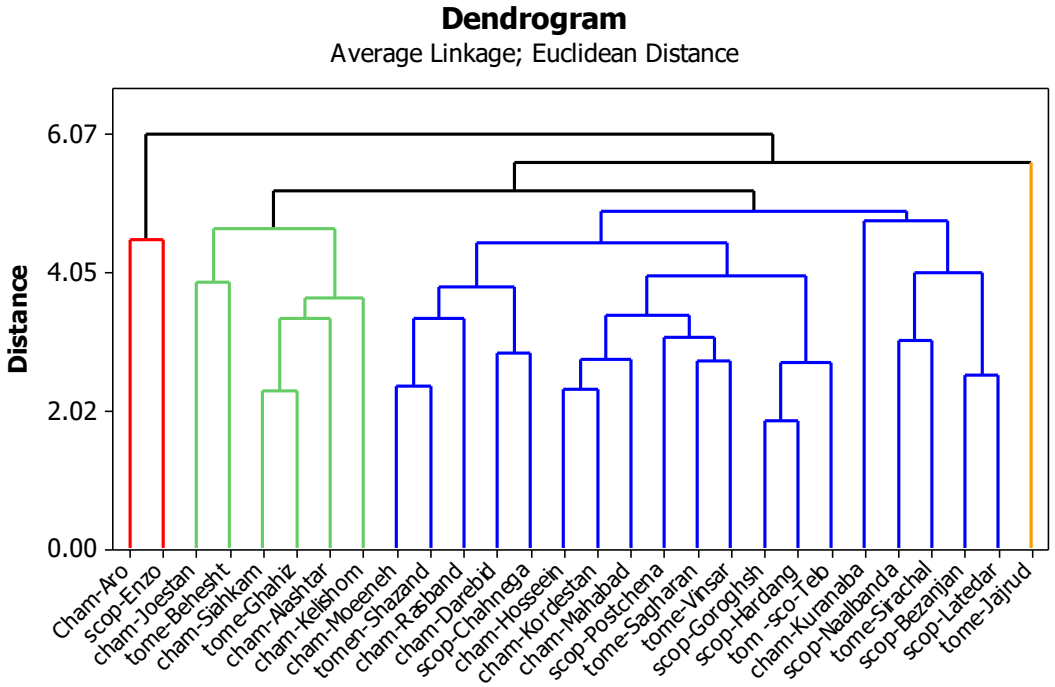


Fig. 44. The cluster analysis diagram of the studied subspecies based on the quantitative anatomical features.

DISCUSSION

The leaf type in mint family are usually dorsiventral (Metcalfe and Chalk, 1950). Our results showed that the leaf type in all samples was isobilateral. However, in some samples, lower palisade parenchyma tended to become spongy. This type of the palisade parenchyma has also been reported in various species of the genera such as *Teucrium*, *Salvia* and *Lallemantia* (Dinç and Doğu, 2012; Polat, 2015; Lakusˇic *& al*, 2006; Rahimi *& al*, 2018). Çali (2014) also observed the same leaf type in *Ajuga orientalis* L. Our results are in agreement with his findings. Sönmez and Köse (2017) reported dorsiventral leaf type for *Ajuga postii* and *A. relicta*, but they mentioned that the palisade and spongy parenchyma cells of the mesophyll of *A.postii* are not differentiated. Also, Akçin and his colleagues (2006) founded that *Ajuga reptans* L. and *A. chamaepitys* L. had dorsiventral leaf type. The same result was observed in *Ajuga genevensis* L. and *A. reptans* L. (Ghitä *& al*. 2012).The extensive palisade parenchyma is one of the characteristics of the Xerophytes. Environmental conditions, such as the intensity of the sun's radiation cause the formation of the palisade parenchyma (Fahn, 1982). Therefore, it seems due to windy and dry climate and high intensity of UV rays in the highlands, the presence of the isobilateral mesophyll is a kind of adaptation for tolerance to water stress and radiation (Van der Werme, 1994).

The stomata were presented on the both of the upper and lower surfaces of all samples. The same result was observed in *Ajuga reptans* L., *A. chamaepitys* L. (Akçin *et. al*., 2006), *A. orientalis* L. (Çali, 2014), *A. relicta* (Sönmez and Köse, 2017), *A. genevensis* and *A. reptans* (Ghitä *& al*. 2012). But *A. postii* had stomata only on the lower surface of the leaf. Stomata of *Lamiaceae* are specific with diacytic types (Metcalfe and Chalk, 1950). The stomata type in our samples was mostly anemocytic, although the anisocytic type was also observed. Stomata type were reported in *A. chamaepitys* diacytic rarely anemocytic and in *A. reptans* diacytic and rarely anisocytic (Akçin *& al*., 2006). Also, Çalı (2014) observed diacytic type in *A. orientalis*. The stomata were mostly flat, but in some cases a little prominent. In *A. genevensis* in both epiderms, especially in the inferior one, stomata were prominent (Ghitä *& al*. 2012).

Generally, the number of palisade parenchyma layers were 2 in most cases at both surfaces. The subsp. *tomentella* of Sirachal specimen with three layers, was an exception. Ghitä *& al*. (2012) reported three layers for upper palisade parenchyma in *A. genevensis.* The upper palisade parenchyma thickness was the highest in subsp. *tomentella* of Sirachal with 168.13 µm. As the palisade parenchyma of this sample was three layers, this result was expectable. Some species of the mint family are clearly covered by a fiber cap above the phloem (Metcalfe and chalk, 1979). Seyedi and Salmaki (2016) pointed to the presence of a fibre cap in *Phlomis tuberosa* from *Phlomides* section. In the midrib and veins of the leaf of the numerous angiosperms the vascular bundles are surrounded, in whole or in part, by a distinct bundle sheath comprising one or more layers of compact parenchyma cells (Metcalfe and chalk, 1979). In our research, vascular bundles in all the specimens, completely or partially, were surrounded by the vascular sheath that its tissue was parenchyma. In the most specimens, the thickness of the upper cuticle was higher than the lower cuticle. According to Panawala (2017) cuticle layer thickness in the upper epidermis is more than the lower epidermis. The highest ratio of mesophyll to vascular bundle in the midrib was observed in subsp. *chamaecistus* of Arou sample with 4.07 and the lowest in subsp. Darreh Bid with 2.1. The whole study showed that the anatomical structure of the studied subspecies was very similar. Although PCA analysis separated some subspecies, but only anatomical traits of the leaf are not enough to separate different subspecies and must be studied along with the other anatomical traits such as petiole and stem and compared with morphological traits.

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