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Quantitative Anatomical Characteristics of the Leaf Blades of the Several Species of *Crataegus* L.

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Abstract

Introduction. The *Crataegus* L. (Hawthorn) is a common herb in numerous Pharmacopoeias. The State Pharmacopoeia of the Russian Federation provides hawthorn fruits and flowers for medical utilization. With that, the literature data confirms the medical utility of hawthorn leaves since the "leaves" and the "flowers with leaves" have pharmacopoeial status worldwide. Therefore, those are considered as prospective forms of *Crataegus* raw material for Russian pharmaceutical production. However, most species remain poorly pharmacognostically investigated regarding the quantitative microscopic characteristics (the sizes of stomatal apparatus (SA) and epidermal leaf blade (LB) trichomes), which could be substantial for establishing the authenticity of the raw material.
Aim. Examine epidermal anatomy of *Crataegus* spp. Leaf blades (LBs) and perform a comparative study of several quantitative diagnostic features of LBs of hawthorn plants from the sect. *Sanguineae* and the sect. *Crataegus*, growing in diverse regions of the Russian Federation.
Materials and methods. Samples of hawthorn leaves (*C. sanguinea*, *C. maximowiczii*, *C. dahurica*, *C. rhipidophylla*, *C. monogyna* and *C. pallasii*) were collected in natural habitats in Western Siberia (Kemerovo) and in arboretums of Botanical Gardens (Moscow, Stavropol). Measurements of anatomical structures were carried out using a light microscope accompanied by an ocular micrometre.
Results and discussion. The LB surface phenotypic diversity within hawthorn species and sections was studied. The LBs were described in terms of meterages (longitude and width) of SA, meterages and shape of sedentary multicellular leaf teeth glands. The peculiarities of pubescence and the sizes of simple unicellular non-glandular trichomes were also observed.
Conclusion. The results of quantitative anatomical examination provided the characteristic features determining these elements at the species and section levels. Thus, it may facilitate authentication and quality control of whole or ground *Crataegus* medicinal raw material.

Keywords: *Crataegus* species, leaf blade, leaf teeth glands, stomatal apparatus, simple unicellular trichomes

Conflict of interest. The authors declare that they have no obvious and potential conflicts of interest related to the publication of this article.

Contribution of the authors. Valentina Sagaradze and Elena Babaeva developed the design of the experiment and performed a comparative microscopic study of epidermal features of leaf blades, processed the data. All authors collected raw materials of *Crataegus* spp. and participated in the discussion of the article, contributed to the final manuscript.

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Количественные анатомические характеристики листовых пластинок некоторых видов *Crataegus* L.

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Резюме

Введение. Сырье растений рода *Crataegus* L. (Боярышник) включено во многие Фармакопеи мира. Государственная фармакопея Российской Федерации предусматривает использование плодов и цветков боярышника. Вместе с тем, литературные данные подтверждают возможность использования в медицинских целях листьев боярышника, поскольку «листья» и «цветки с листьями» имеют фармакопейный статус во всем мире. Они рассматриваются как перспективные виды сырья боярышника для российского фармацевтического производства. Однако большинство видов остаются плохо изученными в отношении количественных микроскопических характеристик (размеры устьичного аппарата (УА) и трихом эпидермиса листовых пластинок), которые могут иметь большое значение для установления подлинности сырья.

Цель. Изучить анатомическое строение эпидермиса листовых пластинок *Crataegus* spp. и провести сравнительное исследование количественных диагностических признаков листовых пластинок растений боярышника из секции *Sanguineae* и секции *Crataegus*, произрастающих в разных регионах Российской Федерации.

Материалы и методы. Образцы листьев боярышника (*C. sanguinea*, *C. maximowiczii*, *C. dahurica*, *C. rhipidophylla*, *C. monogyna* и *C. pallasii*) были заготовлены в естественных местообитаниях в Западной Сибири (Кемерово) и в дендрариях ботанических садов г. Москвы и г. Ставрополя. Измерения анатомических структур проводились с помощью светового микроскопа с окулярным микрометром.

Результаты и обсуждение. Проведено сравнительное исследование морфолого-анатомического строения листовых пластинок (ЛП) на уровне видов и секции боярышника. Приведены размеры (длина и ширина) для УА, сидячих многоклеточных железок и простых одноклеточных трихом. Описана форма железок и установлено различие на уровне секции. Отмечены особенности опушения.

Заключение. Результаты количественного анатомического исследования позволили выявить характерные отличительные признаки по размерам УА и форме желёзок в зависимости от вида боярышника и на уровне секций. Таким образом, это может облегчить установление подлинности и контроль качества целого или измельченного лекарственного сырья *Crataegus*.

Ключевые слова: виды *Crataegus*, листовая пластинка, желёзки на зубцах листа, устьичный аппарат, простые одноклеточные волоски

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Вклад авторов. В. А. Сагарадзе и Е. Ю. Бабаева разработали план эксперимента и провели сравнительное микроскопическое исследование анатомических признаков листовой пластинки, произвели обработку данных. Все авторы принимали участие в заготовке сырья *Crataegus* spp., а также в обсуждении статьи и подготовке окончательной редакции рукописи.

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ABBREVIATIONS

EPh – European Pharmacopoeia; LB – leaf blade; MBG – Tsytsin Main Botanical Garden; MRM – Medicinal raw material; RAS – Russian Academy of Sciences; RSPh XIV – The State Pharmacopoeia of the Russian Federation; SA – stomatal apparatus; USP – United States Pharmacopeia; WHO – World Health Organization.

INTRODUCTION

The herbal preparations of *Crataegus* L. (Hawthorn), family Rosaceae Juss., are well-known regarding the beneficial health properties [1] and widely prescribed as the prevention or concomitant therapy for chronic heart failure of the I-III functional class (New York Heart Association (NYHA) Functional Classification). These are the following indications: coronary heart disease, atrial fibrillation, paroxysmal tachycardia, hypertension, angioneurosis, insomnia and atherosclerosis [2-10].

The complex raw material "hawthorn leaves and flowers" (*Crataegi folium cum flore*) and hawthorn fruits (*Crataegi fructus*) are the most widespread medicinal raw material (MRM) registered in Pharmacopoeias in Europe and America [3, 11–13]. "Hawthorn leaves" (*Crataegi folium*) are also investigated and utilized in China [14–17]. Moreover, it occurs as an object of study in various researches [18, 19]. In several countries, including Russia, "Hawthorn flowers" is officially approved MRM of hawthorn [20].

The genus *Crataegus* L. estimates approximately 250 species, more than a half of which grow in North America, while the rest grow in the Old World [21]. The genus *Crataegus* is characterized by a complicated taxonomic structure [21, 22]. Several studies displayed that majority of species were united into subgenera and sections based on the morphological features of leaves and fruits. According to literature, five subgenera were distinguished: *Crataegus*, *Sanguineae* Ufimov subgen. nov., *Americanae* El-Gazzar, *Mespilus* (L.) Ufimov & T. A. Dickinson, *Brevispinae* (Beadle) Ufimov & T. A. Dickinson, which mostly coincide with geographical zones [23, 24]. Each subgenus included several sections [21, 22, 24, 25]. Despite great diversity, only a few species are registered in pharmacopoeial monographs. For example, ten hawthorn species and two hybrids are approved for harvesting medicinal plant raw materials in the current State Pharmacopoeia of the Russian Federation the XIVth edition (RSPh

XIV) [20]. At the same time, approximately 40 species of wild hawthorn were found in the eastern regions of forest, forest-steppe and steppe zones of the European and Asian parts of the Russian Federation [26].

The majority of hawthorn species included in the current RSPh XIV belong to the sect. *Crataegus* of the subgen. *Crataegus* [20]. *C. monogyna* Jacq. (also included in EPh and USP) [11, 22], *C. rhipidophylla* Gand., *C. pallasii* Griseb. and other European species and their hybrids are the ordinary representatives of the sect. *Crataegus* [21]. Their growth area covers European countries, the middle zone of the European part of Russia, the Caucasus, Krasnodar and Stavropol Territories and the Crimea [21, 25].

The species from sect. *Sanguineae* Zabel ex C. K. Schneider of the subgen. *Sanguineae* are widespread in the Asian part of the Russian Federation. However, only four species are listed in the RSPh XIV. *C. sanguinea* Pall remains the most extensive representative of the sect. *Sanguineae* [20]. It grows in Siberia, in the east of the European zone of Russia, in Zavolzhye, in the Middle and the South Urals and Altai Territory. *C. dahurica* Koechne ex Schneid. and *C. maximowiczii* C.K. Schneid. grow in Siberia, Amur and Primorye equally belong to the sect. *Sanguineae* [27–30].

Anatomical studies of medicinal products of *Crataegus* spp., especially leaves, are limited, furthermore quantitative microscopic characteristics are usually ungiven. Anatomical diagnostic features of Bashkortostan *C. sanguinea* leaves were studied by S. V. Trofimova [31]. The morphology and anatomy of *C. sanguinea* and *C. monogyna* petioles were described by N. A. Volkova [32]. T. A. Rezanova and S. A. Bakshutov had presented the results of morphological and anatomical investigation of leaves of 20 *Crataegus spp*. collected in Belgorod region during the beginning of fruiting, including three species from the sect. *Sanguineae* and three species from the sect. *Crataegus* [33]. V. Sharipova had investigated the leaf structure of *C. korolkowii* (sect. *Sanguineae*), collected in two different environmental locations [34]. The description of anatomic-morphological examination of leaves, flowers and fruits of the endemic plant *C. almaatensis* Pojark growing Almaty region of the Republic of Kazakhstan was given by E. N. Bekbolatova et al. [35].

Quantitative characterization of anatomical features is substantial for establishing the authenticity of the MRM. The sizes and density of microscopic diagnostic features commonly are unnormalized in microscopy atlases. Therefore, proceeded type of MRM could not be accurately characterized [36]. In some cases, it is necessary to normalize sizes or determine the density of anatomical features [37], considering the plasticity of diagnostic features in different environmental conditions [34, 38–42].

In this connection, the studies of anatomical features of medicinal products of various representatives of the genus *Crataegus* L. have become relevant. We set up a task to carry out the comparative study of some quantitative epidermal features of leaf blades (SA, glands and epidermal non-glandular trichomes) of hawthorn plants from the sect. *Sanguineae* and the sect. *Crataegus*, growing in diverse regions of the Russian Federation. Accompanying our previous study of flavonoid content in "*Crataegi folia cum flores*" further results can be used to refine the authenticity and quality criteria of the promising medicinal raw material [43].

MATERIAL AND METHODS

Plant material

The leaves of *C. sanguinea, C. maximowiczii, C. dahurica, C. monogyna, C. rhipidophylla* and *C. pallasii* were collected during the mass flowering period in May-early June 2016 in the arboretum of Tsytsin Main Botanical Garden of the Russian Academy of Sciences (MBG RAS), Moscow (Central District of the Non-Chernozem Zone), Kemerovo (south of Western Siberia) and the Stavropol Botanical Garden (Central part of the Ciscaucasia). Taxonomic identification of the plants was established by taxonomist R. A. Ufimov, PhD. Voucher specimens were deposited in the Herbarium of Vascular Plants of the Komarov Botanical Institute of the RAS, Saint-Petersburg (LE) (table 1). The plant material was air-dried and stored at room temperature.

Table 1. The list of hawthorn leaf samples included in the comparative analysis

№ voucher specimens	Species	Location
Section *Sanguineae Zabel ex C.K. Schneider*		
LE01020969	*C. sanguinea*	the arboretum of the MBG RAS, Moscow (55°50'21" N, 37°36'03" E)
LE01020943	*C. sanguinea*	Kemerovo, Kedrovsky coal mine (56°32' N, 86°3' E)
LE01020981	*C. dahurica*	the arboretum of the MBG RAS, Moscow (55°50'21" N, 37°36"03" E)
LE01020939	*C. dahurica*	the arboretum of Kuzbass Botanical Garden, Kemerovo (55° 21.2981' 0" N, 86° 5.2388' 0" E)
LE01020970	*C. maximowiczii*	the arboretum of the MBG RAS, Moscow (55°50'21" N, 37°36'03" E)
LE01020948	*C. maximowiczii*	Suchaya rechka village, Kemerovo (55°14'21" N, 86°06'56" E)
Section *Crataegus*		
LE01020972	*C. monogyna*	the arboretum of the MBG RAS Moscow (55°50'21" N, 37°36'03" E)
LE01020974	*C. rhipidophylla**	the arboretum of the MBG RAS Moscow (55°50'21" N, 37°36'03" E)
LE01020976	*C. rhipidophylla**	the arboretum of V.V. Skripchinskiy Stavropol Botanical Garden, Stavropol (45° 2.6701' 0" N, 41° 58.1439' 0" E)
LE01020982	*C. pallasii*	the arboretum of the MBG RAS Moscow (55°50'21" N, 37°36'03" E)

Note. *syn. – *C. curvisepala.*

Microscopy

Microscopic samples of leaf surface were studied. Stem leaves from the middle tier of main shoot were observed. It is known that the number of stomata per surface unit varies within different parts of a leaf. The sites approaching the average number of stomata are located in the middle third of LB: between ribs and a midrib [44]. Therefore, this site was taken for anatomical investigations and measurements. For *C. sanguinea*, *C. maximowiczii*, *C. dahurica* and *C. rhipidophylla* two specimens per species and for *C. monogyna* and *C. pallasii* one specimens per species were observed. At least 3 individual leaves were used. For each sample 3 measurements were made. Microscopic samples of LB were prepared according to the General Pharmacopoeial Monograph 1.5.3.0003.15 RSPh XIV "Technique of microscopic and microchemical studies of medicinal plant materials and herbal medicines" [45]. The samples were observed under the light Mikmed-1 microscope with AU-12 1.5x binocular (10x eyepiece, WF10x wide-angle eyepiece with a scale, 8x, 10x, 20x, 40x lenses) (Lomo, Russia) and the MSP-1 binocular (Lomo, Russia). Pictures were taken with Fugifilm DIGITAL CAMERA FinePix JX500 (Fugifilm, Japan) and edited in Picasa (ver. 3.9 141.259, Google, USA).

Anatomical diagnostic features were studied according to the requirements of General Pharmacopoeial Monograph 1.5.1.0003.15 "Folia" in RSPh XIV [45]. Measurements of anatomical structures were carried out using an ocular micrometre according to the method described in the General Pharmacopoeial Monograph 1.5.3.0003.15 "Technique of microscopic and microchemical studies of medicinal plant materials and herbal medicines" [45]. The linear size between two most distant points (the cell wall of the periostic epidermal cells), measured horizontally was considered as the length of the SA [46]. The linear size across the cell walls of the periosteum cells of the epidermis, measured horizontally was considered as the width of SA [47].

Statistical analysis

Statistical calculations were carried out using Excel (ver. 2016, Microsoft, USA) and RStudio for Windows (ver. Desktop 1.4.1103, RStudio PBC, USA) software package. The normality of all data was analyzed by Shapiro-Wilk's test. All normally distributed data were analyzed using ANOVA with post-hoc Tukey HSD Test for intergroup comparisons. In other cases, Nemenyi non-parametric all-pairs comparison test for Kruskal-type ranked data was applied. Comparisons between two sections were performed using Mann-Whitney U-test. Data on figures are presented as medians and 25^{th}-, 75^{th}-percentiles. Data in tables are presented as means±SD. Student t-test with Bonferroni correction for multiple comparisons was applied for two-group comparison if the data were normally distributed; $p \leq 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Leaves of observed species were hypostomatic. Stomata were elongated, rarely rounded, surrounded by 5–7 periostic cells (anomocytic type of SA). The striations of cuticle were folding around stomata (figure 1).

Figure 1. Surface section of Crataegus monogyna leaf. Scale bar – 100 µm

The stomatal length varied from 20.1 to 50.3 µm and width varied from 10.1 to 36.9 among investigated species. Significant differences regarding SA width among the sect. *Sanguineae* representatives were detected neither among distinct species nor within the species harvested in different environmental conditions. However, C. *dahurica* from Kemerovo had significantly greater SA longitude than other studied accessions and C. *dahurica* from Moscow as well. The *C. rhipidophylla* and *C. pallasii* (the sect. *Crataegus*) had the equivalent SAs meterages and pretended to have significantly wider and longer SAs than *C. monogyna* SA (table 2).

Comparing the data on both sections, we found that the sect. *Crataegus* representatives had significantly greater SA meterages (figure 2).

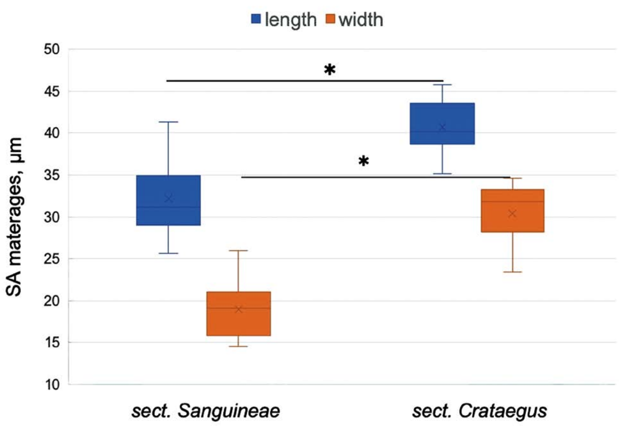

Figure 2. The meterages of stomatal apparatus in the sect. Sanguineae and the sect. Crataegus. The data are presented in the form of a median, mean and percentiles (25 %, 75 %). *U-test, p ≤ 0.05

Multicellular marginal glandular trichomes were typical for *Crataegus* species LBs. Similar trichomes were previously described for several genera in fami-

Table 2. Morphometric parameters of SA on hawthorn leaf surface, µm

Section	*Sanguineae*											
Species	***C. sanguinea* (Kemerovo)**		***C. sanguinea* (Moscow)**		***C. maximowiczii* (Kemerovo)**		***C. maximowiczii* (Moscow)**		***C. dahurica* (Kemerovo)**		***C. dahurica* (Moscow)**	
	length	width	length	width	length	width	length	width	length	width	length	width
min	26.8	13.4	23.5	13.4	20.1	10.1	20.1	10.8	23.5	10.1	23.5	10.1
max	36.9	30.2	40.2	30.2	40.2	26.8	40.2	33.5	50.3	30.2	40.2	26.8
average	31.4 ± 1.6^{a}	21.8 ± 3.8	29.1 ± 2.4^{a}	19.4 ± 3.4	28.6 ± 1.9^{a}	17.6 ± 2.2	31.5 ± 3.3^{a}	18.2 ± 2.2	40.4 ± 1.9^{b}	21.1 ± 0.9	32.4 ± 2.5^{a}	17.1 ± 1.3

Section	*Crataegus*							
Species	***C. monogyna* (Moscow)**		***C. rhipidophylla* (Moscow)**		***C. rhipidophylla* (Stavropol)**		***C. pallasii* (Moscow)**	
	length	width	length	width	length	width	length	width
min	26.8	16.8	33.5	23.5	33.5	26.8	33.5	13.4
max	50.3	33.5	50.3	36.9	50.3	36.9	50.3	40.2
average	37.4 ± 1.6^{a}	25.2 ± 1.9^{b}	40.8 ± 2.1^{a}	31.2 ± 1.5^{c}	43.5 ± 1.9^{a}	33.2 ± 1.1^{c}	41.3 ± 1.6^{a}	32.2 ± 1.4^{c}

Note. ^{a,b,c} ANOVA, Tukey HSD grouping, $p \leq 0.05$.

ly Rosaceae: *Prunus* L., *Malus* L., etc. [48]. Chin and co-authors had presented the classification of multicellular glands and had described their structural variants for several species from the genus *Prunus* [49]. According to that classification, we had observed leaf teeth glands located near vascular zones. The epidermis of the gland was formed by palisade cells. The hypodermal zone was intensely brown coloured (figure 3).

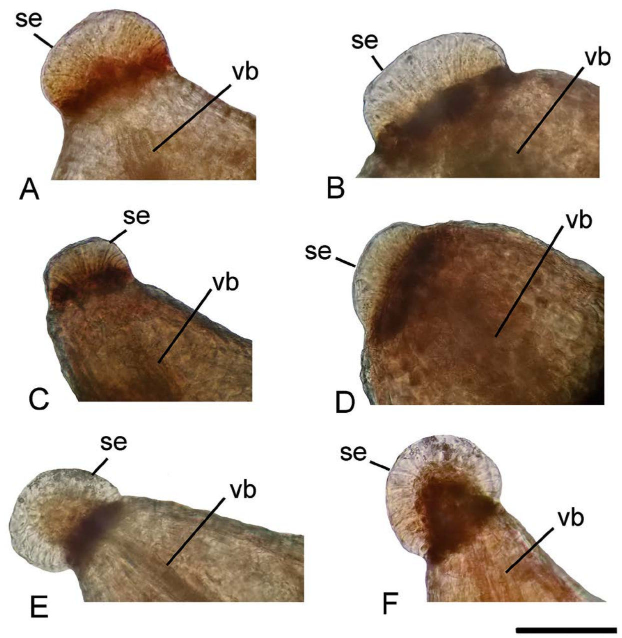

Figure 3. Leaf teeth gland of *Crataegus*.

A – *Crataegus sanguinea* (Moscow); B – *Crataegus sanguinea* (Kemerovo); C – *Crataegus dahurica* (Moscow); D – *Crataegus dahurica* (Kemerovo); E – *Crataegus rhipidophylla* (Moscow); F – *Crataegus rhipidophylla* (Stavropol). vb – vascular bundle; se – secretory epidermis. Scale bar – 100 µm

The secretion of glandular trichomes of flowering plants naturally includes various classes of secondary metabolites. Terpenoids are the most studied [50]. Phenolic compounds, particularly flavonoids, are considered the most common components in glands secretion of woody plants [51, 52]. The chemical composition of the leaf teeth glands exudation in hawthorns is scarce [53].

The sizes of leaf teeth glands in *C. sanguinea, C. dahurica*, and *C. maximowiczii* (sect. *Sanguineae*) from two regions: Moscow (central region of the Non-Chernozem Zone) and Kemerovo (south of Western Siberia) were studied comparatively. *C. dahurica* (Kemerovo) had the widest leaf teeth glands in comparison to *C. maximowiczii* from the same location and the species from Moscow. *C. sanguinea* (Kemerovo) could be significantly distinguished from Moscow specimens: *C. dahurica* and *C. maximowiczii* ($p \leq 0.05$), *C. sanguinea* ($p \leq 0.1$) (table 3).

The specimens from Moscow and *C. maximowiczii* from Kemerovo had equally wide leaf teeth glands. Regarding the longitude of leaf teeth glands: within the

Table 3. Morphometric parameters of hawthorn leaf teeth glands, µm

Section	*Sanguineae*											
Species	*C. sanguinea* (Kemerovo)		*C. sanguinea* (Moscow)		*C. maximowiczii* (Kemerovo)		*C. maximowiczii* (Moscow)		*C. dahurica* (Kemerovo)		*C. dahurica* (Moscow)	
	length	width	length	width	length	width	length	width	length	width	length	width
min	73.7	107.2	30.2	67.0	33.5	93.8	23.5	63.7	63.7	120.6	30.2	50.6
max	87.1	187.6	83.8	167.5	83.8	144.1	50.3	97.2	194.3	251.3	137.4	120.6
average	79.9 ± 3.8[a]	144.1 ± 11.8[b,c*]	49.2 ± 3.6[a]	104.5 ± 11.5[c*,d]	52.4 ± 8.6[a]	117.2 ± 10.4[c,d]	35.2 ± 1.4	82.4 ± 1.8[d]	126.1 ± 17.5	171.5 ± 16.2	50.9 ± 14.4[a]	83.0 ± 10.6[d]
Section	**Crataegus**											
Species	*C. monogyna* (Moscow)		*C. rhipidophylla* (Moscow)		*C. rhipidophylla* (Stavropol)		*C. pallasii* (Moscow)					
	length	width	length	width	length	width	length	width				
min	36.9	36.9	26.8	60.3	30.2	67.0	33.5	36.9				
max	80.4	107.2	53.6	93.8	70.4	130.65	73.7	97.1				
average	55.6 ± 3.3	67.7 ± 1.9	40.4 ± 3.3	79.4 ± 1.9	50.0 ± 2.2	72.5 ± 2.2	53.5 ± 3.3	81.9 ± 1.9				

Note. [a]Nemenyi All-Pairs Rank Comparison Test, $p \leq 0.05$; [b,c,d]ANOVA, Tukey HSD grouping, $p \leq 0.05$; c* – $p \leq 0.1$.

same harvesting location, significant differences among species were not found in both sections, except difference between *C. maximowiczii* (Moscow) and Siberian *C. dahurica*.

Within the sect. *Sanguineae,* the length/width ratio of the leaf teeth gland varied among species. *C. sanguinea* had 0.47–0.55 length/width ratio, while *C. maximowiczii* samples demonstrated comparable results: 0.43–0.45. Thus, the glands were twice as wide as long. The length/width ratio of *C. dahurica* leaf teeth glands varied: the sample from Kemerovo – 0.72 and the sample from Moscow – 0.67. So, the shape of these glands was close to cubic (in the plane – square).

Among species within the sect. *Crataegus* and individual species collected in different regions (e.g., *C. rhipidophylla* from Moscow and Stavropol) significant differences in leaf teeth glands meterage were unfound. Unlike the sect. *Sanguineae*, hawthorns from the sect. *Crataegus* mainly had a spherical (in the plane – rounded) leaf teeth gland shape with 0.78 ratio – *C. monogyna*. The length/width ratio of 0.65 was *C. pallasii* and 0.67 – *C. rhipidophylla* (Stavropol). The *C. rhipidophylla* from the arboretum of the MBG RAS (Moscow) is distinguished by more elongated glands – 0.51 length/width ratio. The sizes of leaf teeth glands of specimens collected in the arboretum of the MBG RAS (Moscow) were comparable in both sections (table 3). With that, sect. *Sanguineae* plants had significantly wider leaf teeth glands (figure 4). Our data is consistent with the results obtained in the Belgorod region [33].

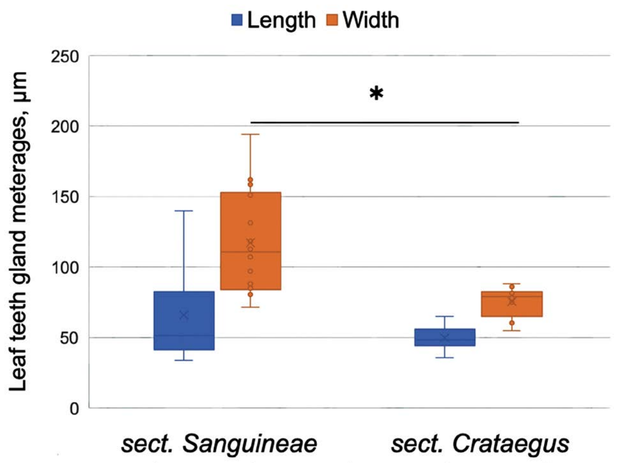

Figure 4. Leaf teeth meterages in the sect. Sanguineae and the sect. Crataegus. The data are presented in the form of a median, mean and percentiles (25 %, 75 %). *U-test, p ≤ 0.05

According to the Pharmacopoeias and WHO Monographs on medicinal plants, epidermal non-glandular trichomes on *Crataegus* leaf blade surface are considered as a diagnostic feature [2, 11–13, 16, 20]. Observed leaf blades were covered with simple unicellular trichomes of 117–660 µm long (table 4), excluding the *C. rhipido-*

Table 4. **Morphometric parameters of epidermal simple unicellular trichomes on hawthorn leaves, µm**

Section	***Sanguineae***											
Species	***C. sanguinea* (Kemerovo)**		***C. sanguinea* (Moscow)**		***C. maximowiczii* (Kemerovo)**		***C. maximowiczii* (Moscow)**		***C. dahurica* (Kemerovo)**		***C. dahurica* (Moscow)**	
Adaxial side	length	width	length	width	length	width	length	width	length	width	length	width
min	197.7	13.4	211.1	13.4	144.1	13.4	154.1	16.8	–	–	–	–
max	579.6	26.8	428.8	26.8	412.1	30.1	247.9	30.2	–	–	–	–
average	384.4 ± 21.5	20.6 ± 0.4[g]	296.8 ± 18.7[a]	23.5 ± 0.7[e]	227.5 ± 28.1[b]	20.9 ± 3.4[h]	199.8 ± 5.7[c]	24.2 ± 1.1[i]	–	–	–	–
Abaxial side	length	width	length	width	length	width	length	width	length	width	length	width
min	426.4	13.4	311.6	13.4	117.6	13.4	264.7	16.8	284.8	16.3	268.0	16.8
max	688.8	26.8	582.2	23.5	304.9	30.2	616.4	30.2	552.8	26.8	660.0	30.2
average	535.1 ± 6.0	19.5 ± 1.0[g]	456.0 ± 51.6[a]	18.7 ± 1.0[e]	320.9 ± 12.9[b]	20.4 ± 1.0[h]	385.9 ± 39.1[c]	23.0 ± 2.0[i]	445.8 ± 89.2	21.1 ± 4.2	512.7 ± 8.1	20.2 ± 0.9

Section	***Crataegus***							
Species	***C. monogyna* (Moscow)**		***C. rhipidophylla* (Moscow)**		***C. rhipidophylla* (Stavropol)**		***C. pallasii* (Moscow)**	
Adaxial side	length	width	length	width	length	width	length	width
min	264.7	16.8	–	–	227.8	10.1	244.6	16.8
max	603.0	36.9	–	–	495.8	23.5	462.3	26.8
average	407.3 ± 28.1[d]	27.1 ± 5.2[j]	–	–	313.3 ± 71.0[e]	20.4 ± 3.2[k]	327.5 ± 26.7[f]	22.1 ± 0.9[l]
Abaxial side	length	width	length	width	length	width	length	width
min	304.9	16.8	–	–	258.0	10.1	254.6	10.1
max	649.9	33.5	–	–	495.8	23.5	492.5	26.8
average	442.8 ± 54.1[d]	26.1 ± 4.6[j]	–	–	357.4 ± 63.7[e]	19.1 ± 2.3[k]	363.1 ± 18.8[f]	21.1 ± 0.7[l]

Note. [a,b,c,d,e,f,g,h,i,j,k,l] t-test with Bonferroni correction for multiple comparisons.

phylla (arboretum of the MBG RAS, Moscow), which was glabrous. Single trichomes were detected on the abaxial side of *C. dahurica* LB. The other species were pubescent on both sides. It was noted that the trichomes on the abaxial side of LB of *C. sanguinea* were longer in comparison to those on the adaxial side. The other specimens were pubescent with trichomes of equal longitude on both sides. Nearby the midribs simple unicellular trichomes commonly arranged in clusters (figure 5). The pubescence features (type, length and density of the trichomes) on the petiole were similar to the pubescence of the LB.

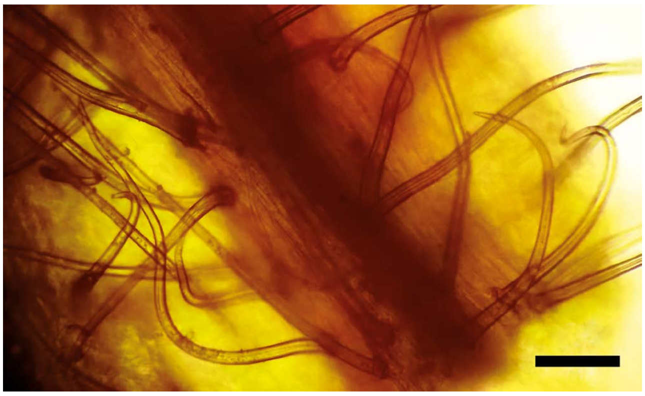

Figure 5. Surface section of Crataegus maximowiczii leaf. Groups of simple unicellular trichomes near the midrib. Scale bar – 100 μm

CONCLUSIONS

The anatomical study of SA and trichomes on leaf epidermis of *C. sanguinea, C. dahurica*, *C. maximowiczii, C. monogyna*, *C. rhipidophylla*, *C. pallasii* LBs was performed. Microscopy has revealed characteristic features: hypostomatic leaf type, anomocytic SA, multicellular glands, simple unicellular hairs. Studied species had comparable sizes of LBs' SA within both sections. With that, plants from the sect. *Crataegus* from both regions demonstrated significantly greater sizes of SA in comparison with the sect. *Sanguineae* plants.

Both sections did not demonstrate significant differences in leaf teeth glands longitude among species collected within the same location. Comparing the sections concerning leaf teeth glands length the difference was also unfound. Furthermore, significantly wider leaf teeth glands differentiated *C. dahurica* and *C. sanguinea* (Kemerovo) from the other species of the sect. *Sanguineae*, collected in Moscow. The sections *Sanguineae* and *Crataegus* were also distinguished by the leaf teeth gland shape: cuboid and spherical, respectively. With that, in the sect. *Sanguineae* plants had significantly wider leaf teeth glands.

The studied species also had various features of pubescence of LB and the sizes of simple unicellular non-glandular trichomes. The species of both sections were pubescent with trichomes of equal length on both sides, except Siberian *C. sanguinea,* which had longer trichomes on the adaxial side. Within both sections, non-glandular trichomes assembled in clusters in the region of the midrib.

Identified distinctive features could be applicable for authentication of the MRM of hawthorn ("leaves" or "flowers with leaves") harvested in different geographical zones. Possible variations of studied parameters on species and sections should be taken into account during the quality control process of hawthorn MRM since it will help screen out the herbal adulterants.

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