Leaf anatomy of the members of *Cornaceae* family in conditions of the Forest-Steppe of Ukraine

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Summary

There are researches of many years of biological characteristics (fertility, vegetative propagation and other) in introduced species of *Cornaceae* family in the Ukraine, while there are few data on leaf micromorphology of these plants. The aim of our work was to investigate the anatomy and morphology of leaves of *Cornaceae* species. Cross sections of leaves were prepared and staining using standard histological methods, light microscopy and scanning electron microscopy were made. It was shown that all investigated species have the similar leaf anatomy: single–layer upper and lower epidermis covered with a cuticle and trichomes, the stomata are on the lower leaf side, the mesophyll consists of the palisade and spongy parenchyma. In the same time, there are differences in the anatomical quantitative features: size and number of stomata, number of trichomes, size of cells, leaf blade thickness, and the palisade ratio. We can claim about successfully adaptation of the investigated species based on their anatomy without any destructive features and every year flowering and fruiting (according to the previous data). This work may serve as a basis for further investigations of introduced species of Cornus and monitoring of their condition under introduction.

Introduction

The species of the polymorphous family *Cornaceae* Bercht. et J. Presl. are weakly distributed and investigated in Ukraine. The most of dogwood species are valued as an ornamental and forest reclamation plants, some of them are used as fruits (Hutchinson, 1942). Plants of this family are widely used as the medicinal raw through their anti–inflammatory, tonic and astringent properties (Plotnykova, 1971). The cornel wood has high strength and is resistant to the biological destroyers (Klymenko, 1990). *Cornus mas* L., *Swida sanguinea* (L.) Opis. and *Swida australis* (C. A. Mey) Poyark. ex Grossh are indigenous species of Ukraine. Today in Ukraine more than 30 species of cornel from the different geographical origin are introduced in the botanical gardens and arboretums in different regions of Ukraine and recommended for green building.

Many taxonomists differently assess *Cornaceae* volume and share related connections (Ferguson, 1966; Murrell, 1993).

A. L. Takhtadzhyan (2009) considered *Cornaceae* as a natural family with 55–60 species. A. I. Poyarkova (Poyarkova, 1950; 1951) divided a heterogeneous Linnaean genus *Cornus* L. s. l. into six independent genera.

Considering the high value of dogwood species and the possibility of their
full utilization, the collection of these species has been started in M.M. Gryshko National Botanical Garden since 50s of the 20th century. In the first ten years the objects of introduction were mainly species of the genus *Swida*. In 70s researches have focused in the department of fruit plants acclimatization where were compile collections of *Cornus mas*, *Cornus officinalis*, *Swida ssp.*, and in 1993–2016 years were also introduced *Cornus sessilis*, several breeds of *Cornus officinalis*, *Cynoxylon japonica* (including some of very decorative breeds of it), *Cynoxylon capitata* and *Cynoxylon nuttalli*. Nowadays, more than 30 species of the family *Cornaceae* from the different floristic regions of East Asia and North America were introduced in M.M. Gryshko National Botanical Garden (Klymenko, 2013; 2015). Research of many years of biological characteristics, in particular fertility, the biochemical composition of fruits and leaves, vegetative propagation and winter hardiness in introduced species of genera *Cornus*, *Cynoxylon* and *Swida* in the Forest–Stepp region of Ukraine allowed the opportunity to assess adaptive capacities and strategy in the new conditions of existence (Klymenko, 1990; 2006; 2013). While there are few data on leaf micromorphology in *Cornus* species introduced in the Botanical garden (Klymenko & Klymenko, 2015). Therefore, the aim of our work was to investigate the anatomy and morphology of leaves of *Cornaceae* species from the different geographical regions as well as to value the significance of anatomical characteristics in the adaptive strategy of plants in the conditions of introduction.

*C. mas* is very close morphologically to both East Asian species – *C. officinalis* and *C. chinensis*. *C. sessilis* species from North California evolutionary placed far from the first three species. These species have fragmented habitat (Ferguson, 1966).

In Europe, three species grow in the west of the continent: *C. mas*, in the south–east, in the central regions of China – *C. chinensis*, in Japan – *C. officinalis*. Only one species of this genus – *C. sessilis* is located in North America (California). A significant portion of flora of the Chinese–Japanese subregion has a wide environmental range, and so they can grow, as well as others – could adapt successfully in regions significantly different in climatic conditions (Plotnikov, 1983). Therefore, the difference of the certain climatic parameters of introduced species natural origin areas *C. florida* and *C. cousa*, and conditions of the Right Bank Forest–Stepp region of Ukraine is not an obstacle to their introduction in the study area.

According to the introduction zoning of the territory of Ukraine (Kohno, 1994) Right–Bank of Ukraine belongs to the North–East of introduction area and Right–Bank introduction subarea, where introduction is possible and broad growth of all species of the northern regions of the Caucasus, Central China, Northeast China, Korea, northern Japan, the northern and central parts of the Atlantic and Pacific regions of North America. East Asia according to Alexeev (1935) is the main center of the formation of the temperate flora of the northern hemisphere. East Asia is the primary source of the speciation fruit crops.

**Material and methods**

**Plant material** *Cynoxylon florida*, which is native to southern Canada and the Atlantic region and the southern states of the central part of North America, is very decorative during flowering because of the large bright white or pink bract surrounding the inflorescence. In autumn leaves and fruits turn red. *C. florida* is cultivated in Central Europe for 200 years and is quite winter hardy, so it can be successfully cultivated in Ukraine. In Kyiv, this cultivar is flowering abundantly and fructifying for almost past 20 years (Klymenko, 2013). Greater attention should be given to Korean–Japanese cultivar of *Cornus officinalis*, which is successfully introduced in USA and widely used as an ornamental plant. This plant is not only decorative, but it has medical properties, as evidenced by its name. The second concerned Asian species is *Cynoxylon japonica*, which is growing during past 30 years in National Botanical Garden and started fructifying on 7–8 year of...
introduction. *C. officinalis* is quite winter hardy and can withstand temperatures down to −35 °C as European species *Cornus mas*, which is grown almost all over Ukraine: in the Crimea, in the south–east of the Right–bank Forest–Steppe, Forest–Steppe, in the West, Transnistria, the individual location recorded in the Carpathians. *C. mas* is undemanding to soil and enough drought–resistant. *Swida sanguinea* (L.) Opiz. is very wide and within Europe and Western Asia. Northern border of this plant crosses the middle part of the United Kingdom and southern Scandinavia. *Cornus sessilis* in Ukraine has not been studied and grows only in the National Botanical Garden from 2003 (table 1). This plant is growing well, but not yet bears fruit, because in 2011 it was suffered from frost.

*Conditions of introductions*

National Botanical Garden of NASU is located on the south-eastern outskirts of Kyiv on the low Pechersky slopes of Kyiv Upland (coordinates 50°22′ S, 30°33′ E). Kyiv is on the verge of two physical-geographic zones: to the north of the forest zone of Ukrainian Polissia, to the south – the Forest-Steppe zone with a predominance of elements of the steppe vegetation. Groundwater in the territory of the botanical garden is at a great depth, therefore the phenological phenomena and the introductory process are most influenced by the climate. The climate of Kyiv is temperate continental, the average annual temperature is +7.6 °C, the average temperature in January −5.5 °C, in July +20.4 °C. The absolute minimum is −32.2 °C, the maximum is +39.4 °C. Snow cover is formed almost every winter and lasts about 90 days. The summer months in Kyiv are characterized by moderate warmth and a sufficient amount of moisture. In the autumn, cloudy weather is observed, often with prolonged rains. The average amount of precipitation in Kyiv is 625 mm, in some years it ranges from 410 to 795 mm. Most precipitation falls in May-July. During the active vegetation period, up to 350–400 mm of precipitation falls.

**Table 1.** Place of origin of the investigated species and terms of their introduction

<table>
<thead>
<tr>
<th>Species</th>
<th>Place of origin</th>
<th>Year of introduction to the National botanical garden</th>
<th>Taking place for the introduction of plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cornus officinalis</em></td>
<td>Japan, Northeast China and Korea</td>
<td>1993</td>
<td>State Oregon, USA</td>
</tr>
<tr>
<td><em>Cynoxylon japonica</em></td>
<td>Japan, Hans Island, Shikoku, Kyushu and Tsushima, the southern and middle part of the peninsula of Korea</td>
<td>1993</td>
<td>State Oregon</td>
</tr>
<tr>
<td><em>Cornus sessilis</em></td>
<td>North America, California</td>
<td>2003</td>
<td>State Oregon, USA</td>
</tr>
<tr>
<td><em>Cynoxylon florida</em></td>
<td>states of North and South Carolina, Georgia, Florida, USA</td>
<td>2001</td>
<td>State North Carolina</td>
</tr>
<tr>
<td><em>Cornus mas</em></td>
<td>Central and South–Estern Europe and in Asia</td>
<td>In Ukraine this species grows in natural conditions</td>
<td></td>
</tr>
<tr>
<td><em>Swida sanguinea</em></td>
<td>Europe and Western Asia</td>
<td>In Ukraine this species grows in natural conditions</td>
<td></td>
</tr>
</tbody>
</table>

*Anatomy and ultrastructure of leaf surface*

Mature, healthy leaves without any visible changes were collected for investigations in...
August 2014 and June 2015. To examine the internal leaf structure, pieces 0.5x1 cm from the center of a lamina were fixed in 2.5 % glutaraldehyde (0.1 M cacodylate buffer, pH 7.3) for 12 h at ambient temperature, and then in 1 % OsO$_4$ in the same buffer for 12 h at 4°C. Samples then were dehydrated using a graded acetone series and embedded in epon–araldite resins. For light microscopy (LM), semi–thin sections (0.5–1 μm) were obtained using an ultramicrotome RMC MT–XL (USA) (Reynolds, 1963). Sections were stained with 0.12 % toluidine blue and examined with an NF light microscope (Carl Zeiss, Germany). Photos were made with Leitz light microscope (Leitz Dialux 20 EB, Leitz, Wetzlar, Germany) attached to a PC–based image processing system. Leaf thickness, height and width of epidermal and palisade parenchyma cells, fractional area of the intercellular air space were measured from digital photo using program Image J for Windows. 30 epidermal and palisade parenchyma cells from 3 leaves of each species were measured.

The tissue for scanning electron microscopy (SEM) was fixed in 2.5 % glutaraldehyde (0.1 M cacodylate buffer, pH 7.3) for 12 h at temperature 4°C, post fixation was made in 1% paraformaldehyde (0.1 M cacodylate buffer, pH 7.3). Samples then were dehydrated using a graded ethanol series and hexamethyldisilazane at the last stage (Sigma, USA) (Kuo, 2007). Dried samples sputter were coated with gold and observed with a JSM-35 scanning electron microscope (Japan). The height and width of stomata and amount on 1 mm$^2$ were measured on obtained photos.

**Analysis of data**

Determination of reliability of the difference of the data was carried out by Student's test (T–test) if independent samples are normally distributed and the Mann test (U–test) if independent samples with non–normal distribution. The level of significance was accepted at p < 0.05 (Lakin, 1990). All data are presented as M±m, where M – arithmetic average, m – arithmetic average deviation.

**Results and discussions**

**Anatomy of Asian species**

Leaves of *Cornus officinalis* Sieb. et Zuss. are rounded, rounded–ovate, dark green, 7.0–10.0 cm long, 5.0–8.0 cm wide. Epidermal cells of a leaf upper (adaxial) side is appreciably convex are covered with a cuticle forming nonparallel stripes. Stomata and trichomes on the upper side are absent (fig. 1A). Epidermal cells of the leaf lower side are convex, a cuticle forms parallel stripes around stomata, partially touched adjacent cells (fig. 1C). A leaf lower (abaxial) side is lustrous because it has rusty spots of siliceous trichomes.

Leaves of *Cynoxylon japonica* (Siebold & Zucc.) Nakai are elliptical–oval, 6.0–10.0 cm in length and 3.0–5.0 cm in width, peaked, with circular base. Epidermal cells of the leaf upper (adaxial) side are convex, covered with cuticle that forms parallel stripes around trichomes and on other surface of epidermal cells.

![SEM photographs of the surface of C. officinalis and C. japonica leaves.](image)
Trichomes are flat (not raised over surface), unicellular, have two arms and short stem (base), symmetrical, covers with papillose cuticle (fig. 1B). Epidermal cells on the lower side of leaves are convex. Cuticle forms parallel stripes around trichomes and waved stripes on the surface of epidermal cell and around stomata. Trichomes by its structure are similar the upper side of the leaf trichomes (fig. 1D). The number of trichomes is higher compared with *C. officinalis*, so leaf is downier. Length and width of *C. japonica* stomata is less than in *C. officinalis* but the number of stomata is greater (table 2).

*C. officinalis* and *C. japonica* have a similar anatomical structure: dorsiventral type of leaf anatomy, where mesophyll differentiated on palisade and spongy parenchyma (fig. 2A, 2B). The upper and lower epidermis consists of oval elongate cells and covers with a cuticle. Cells of the upper epidermis of *C. japonica* have significantly large length in comparison with *C. officinalis* cells (table 2). The cells of the
lower epidermis of the examined species are not statistically significant different. The palisade parenchyma of investigated species consists of 2–3 layers of cylindrical cells, length and width of which has no significant differences (table 2). C. officinalis characterized by greater leaf thickness compared with C. japonica and larger palisade coefficient (ratio of the thickness of palisade parenchyma to mesophyll thickness), lower partial volumes of intercellular spaces (greater cell density).

Anatomy of American species
Cornus sessilis Torr. leaves are elliptically–ovate, 5.0–7.0 cm long, on top – light green, slightly pubescent from below. Epidermal cells on the upper side are convex, covered with cuticle that formed bare number of parallel strips around trichomes, other space of epidermal cells is smooth. Trichomes are flat (not raised over surface), unicellular, have two arms and short stem (base), asymmetrical, covers with the warty cuticle. Stomata on the upper side of the leaf blade are absent (fig. 3A). Epidermal cells on the lower side of leaf are convex. Cuticle forms parallel strips around trichomes, partially takes adjacent cells, on other surface of the epidermal cells cuticle remains smooth. Trichomes are flat (not raised over surface), unicellular, have two arms and short stem (base), asymmetrical, covers with warty cuticle (fig. 3D).

Leaves of Cynoxylon florida (L.) Raf. species are elliptical, 5.0–8.0 cm long, 3.0–5.0 wide, smooth–edged, without stipule, with 3–5 arcuate parallel veins, dark green at upper side and albescent at lower side. Epidermal cells on the upper side of C. florida leaves are not convex, a cuticle forms parallel stripes around trichomes and waved stripes on other surface of the epidermal cell. Trichomes are flat (not raised over surface), unicellular, with two arms and short stem (base), symmetrical, covers with the warty cuticle. Stomata on the upper side of the leaf blade are absent (fig. 3B). Epidermal cells on the lower side of leaf are convex, a cuticle forms parallel strips around stomata. On the surface of epidermal cells cuticle forms the papillae. The part of trichomes are flat (not raised over surface) as well as other are raised over surface. All trichomes have two arms and short stem (base), symmetrical. Filamentous trichomes are found. All trichomes are unicellular, covers with the warty cuticle (fig. 3D). Length and width of C. florida stomata is greater than in C. sessilis species. In view of the outgrowths of cuticle, which covers the stomata, count their number is not possible (table 2).

![Fig. 3. SEM photographs of the surface of C. sessilis and C. florida leaves. A. The upper (adaxial) surface of C. sessilis leaf with convex cells and trichomes, around which cuticle formed bare number of parallel strips. B. On the lower (abaxial) surface of C. sessilis leaf cuticle forms parallel strips around flat symmetrical trichome and stomata and remains smooth on other surface of the epidermal cells. C. Epidermal cells on the upper (adaxial) side of C. florida leaf are around flat, cuticle forms stripes around flat symmetrical trichome and on another surface. D. Cuticle forms the papillae on the surface of epidermal cells on the lower (abaxial) surface of C. florida leaf. Flat symmetrical and filamentous trichomes are found. Bar: A, B, C – 50 μm, D – 100 μm.](image)

C. sessilis and C. florida also have a similar anatomical structure: dorsiventral type of leaf anatomy, where mesophyll is differentiated on palisade and spongy parenchyma. The upper and lower epidermis consists of oval elongate cells and it is covered with a cuticle (fig. 2C, 2D). Cells of the upper epidermis of C. florida have significantly large length compared with cells of C. sessilis. Cells of the lower epidermis of the examined species are not statistically significant different (table 2). Palisade parenchyma consists of 1 layer of cells, length
and width of which is significantly large than in *C. florida* (table 2). *C. sessilis* has a greater thickness of leaf blade compared with *C. florida* and larger partial volumes of intercellular spaces (less density of cell). Palisade coefficient (ratio of the thickness of palisade parenchyma to mesophyll thickness) has no significant differences in the compared species (table 2).

**Anatomy of European species**

The leaf blade of *Cornus mas* L. is oval or elliptic shape with sharp top, 4–9 cm length and 2–5 cm width. Epidermal cells on the upper side of *C. mas* leaves are slightly convex, a cuticle forms parallel stripes around trichomes and waved stripes on the surface of the epidermal cell. Trichomes are flat (not raised over surface), unicellular, have two arms and short stem (base), asymmetrical, covers with warty cuticle. Stomata on the upper side of the leaf blade are absent (fig. 4A). Epidermal cells on the lower side of leaf are convex. Cuticle forms parallel strips around stomata, partially takes adjacent cells (fig. 4C). Trichomes are flat (not raised over surface), unicellular, have two arms and short stem (base), symmetrical, covers with warty cuticle.

*Swida sanguinea* (L.) Opiz. leaves are broadly-elliptic or ovate, 4–10 cm long, 2–6 cm wide, pointed. Epidermal cells on the upper side of *S. sanguinea* leaves are not convex, a cuticle forms parallel stripes around trichomes and waved stripes on the surface of epidermal cell. Trichomes are flat (not raised over surface), unicellular, have two arms and short stem (base), symmetrical, covers with warty cuticle. Stomata on the upper side of the leaf blade are absent (fig. 4B). Epidermal cells on the lower side of leaf are slightly convex. Cuticle is smooth on all surface of leaf. Trichomes are filamentous, unicellular, locating along the leaf veins (fig. 4D). Length and width of *S. sanguinea* stomata is significantly smaller than in *C. mas* and the number of stomata is significantly greater (table 2).

*C. mas* and *S. sanguinea* also have a similar anatomical structure: dorsoventral type of leaf anatomy, where mesophyll is differentiated on palisade and spongy parenchyma. The upper and lower epidermis consists of oval elongate cells and it is covered with a cuticle (Fig. 2E, 2F).

**Fig. 4.** SEM photographs of the surface of *C. mas* and *S. sanguinea* leaves. **A.** The upper (adaxial) surface of *C. mas* leaf with slightly convex cells, cuticle that forms parallel stripes around trichomes and waved stripes on the surface of the epidermal cell. Trichome is flat asymmetrical, covers with warty cuticle. **B.** On the lower (abaxial) surface of *C. mas* leaf cuticle forms parallel strips around stomata. **C.** Cells on the upper (adaxial) side of *S. sanguinea* leaf are not convex, cuticle forms parallel stripes around trichomes and waved stripes on the surface of epidermal cell. Trichome is flat symmetrical, covers with warty cuticle. **D.** On the lower side of *S. sanguinea* leaf cuticle is smooth. Trichomes are filamentous, locating along the leaf veins. Bar: A, C – 50 µm, B – 20 µm, D – 100 µm.

Cells of the upper epidermis of *C. mas* have significantly large length compared with cells of *S. sanguinea*. Cells of the lower epidermis of the examined species are not statistically different (table 2). Palisade parenchyma consists of 1 layer of cells, length of which are not statistically significant difference between compared species. The width of the palisade parenchyma cells was significantly greater in *S. sanguinea* (table 2).

**Discussion**

In general, we shown that all investigated species have the leaf similar anatomy, independently of their different native geographical areas. This similarity of the leaf anatomy of investigated species may be explained with their close systematic relationship. The upper and lower epidermis
consists of a single layer of oval cells; stomata are on the lower side, leaves are covered with a cuticle and trichomes. Leaves have the dorsventral structure: palisade parenchyma consists of 1–3 layers of cells; spongy parenchyma consists of oval and irregularly shaped cells and large intercellular spaces. In the same time, there are differences in the anatomical quantitative sings: a size and a number of stomata, a number of trichomes, a size of epidermal and mesophyll cells, leaf blade thickness, and the palisade ratio (table 2). The differences in quantitative signs consist with the species geographic origin and adaptation to introduction conditions. Leaves of studied species vary in a size and density of stomata on the leaf surface: a size of guard cells is the smallest in *S. sanguinea* and the larger in *C. florida* but maximum of the stomata density per 1 mm² was observed in *S. sanguinea* and minimum in *C. mas* (table 2). The density of stomata in *C. florida* was not measured because of the large number of papillae that covering the stomata (fig. 3D). It would note that a size of stomata may vary depending on the leaf blade age and its position on a plant (Matveeva, 1980). Investigated species may be divided on the groups depending on a size of the upper epidermis cells: species with large cells – *C. japonica, C. sessilis, C. mas*, species with small cells – *S. sanguinea, C. florida*. *C. officinalis* occupies the intermediate position (table 2). Sizes of the lower epidermis cells have no statistically significant differences in all species (table 2). Convex epidermal cells are typical for the leaf lower side (Hardin & Murrell, 1997).

The leaf surface of all species is covered with the sheathing unicellular, T–shaped trichomes. In *C. florida*, trichomes are covered with crystals of calcium carbonate (fig. 3B, 3D) (Hardin & Murrell, 1997). There are no crystals of calcium carbonate and calcium oxalate on trichomes in other species, in which trichomes have micropapillae. Stem of trichomes is very short and lies in the plane of the epidermis cells, so it is difficult identified in the investigation by SEM. It is known that the sheathing trichomes protect plants from overheating, excessive transpiration and eating by animals. The youngest hairs, which originated before the formation of stomata, have a thin cuticle. Later, a cuticle became thicken, the protoplast of trichome died and the cell cavity filled with air (Lotova, 2011). It is known that a number of trichomes changes during growth and aging of leaves (Hardin & Murrell, 1997). Their maximum amount observed in *C. florida*, the lowest number of trichomes – in *C. officinalis*. Leaves of *C. florida* have papillae – low and wide outgrowths of epidermal cells, which cover the stomata and create the velvety surface of the blade protecting the plants from overheating (fig. 3D). The obtained data may be explained by the conditions in the native habitat of this species, namely state Florida, USA, where there is a large number of sunny hours (2400–3200 hours) as well as the highest average of rainfall. This fact also explains a smaller in the 1.8 times size of *C. florida* epidermal cells as compared to the studied species of genera *Cornus* and *Cynoxylon* from less arid habitats. Thus, in all investigated species the upper side of the leaf blade has less quantity of trichomes than the lower (data is not shown in the table). Thus, the differences between the micromorphology of the upper and lower side of the leaf blade are both qualitative and quantitative. Features of the leaf surface may reflect plant adaptation to the environment and/or protection from insects or herbivores.

Differences in the leaf blade thickness in the investigated species may also be considered as the features of plant adaptation to the climatic conditions of their habitats. Asian species *C. officinalis* and *C. japonica* have the thickest lamina due to a bigger amount of mesophyll layers number and larger cells of the upper epidermis in comparison with those in American and European species. The minimal thickness of a leaf blade was found in *S. sanguine* (table 2). This species is wide spread in Europe and may be found in the northern latitudes. *S. sanguinea* is frost–resistant and characterized by a smaller linear size of stomata and their high density, smaller epidermal and palisade parenchyma cells compared with other species of interest. Some of these features are inherent to the wide range of frost resistance plants: some of herbaceous frost–hardiness
species, for example, *Solanum* and *Secale* species, had smaller and thicker leaves. Increased leaf thickness of these plants was caused by increased mesophyll cell size and palisade coefficient (Palta & Li, 1979; Huner et al., 1981). In soft wheat (*Triticum aestivum* L.), high level of frost resistance was significantly correlated with a higher density of stomata and guard cells lesser length in 21% of varieties (Lamari et al., 2014). However, hardy cultivars of evergreen tree *Ilex opaca* Ait. had a significantly lower number of stomata per unit leaf area than cultivars that were not hardy (Knecht & Orton, 1970). Winter hardiness is a very important adaptive reaction of woody plants to the climatic conditions of the temperate zone. The Forest–Steppe of Ukraine is characterized by winters with an unstable snow cover, frequent thaws and abrupt fluctuations in air temperatures. The results of the assessment of the winter hardiness of investigated species in the previous studies have shown that the most winter–hardened ones in the Forest–Steppe are aboriginal plants *C. mas* and *S. sanguinea*, they showed no damage. 1–2 and 1–3 years old shoots of the introduced *C. officinalis* and *C. sessilis* in 2010–2011 were frozen. In subsequent years’ plants completely regenerated. 1–3 summer shoots of *C. japonica* in the winter of 2011–2012 were frozen, in other *Cynoxylon* plants only annual shoots were damaged (Klymenko & Klymenko, 2015).

*C. florida* also has thin leaf blades have small epidermal cells and intercellular spaces but unlike *S. sanguinea* this species grows in the Florida with the arid habitat and is characterized by the largest linear size of palisade parenchyma cells among the investigated species. Mesophyll cells of other studied species have the same height but differ slightly on the width (table 2).

The palisade ratio (the ratio of the thickness of the palisade parenchyma to a thickness of mesophyll) is the most in *C. officinalis* and the least in *S. sanguinea*. In other species there are not significant differences on this sign (table 2). Leaf anatomical features in all investigated species are typical for mesophytes: clear differentiation of mesophyll on the palisade and spongy parenchyma, a few number of palisade parenchyma layers, a low (30–40%) or medium (40–50%) palisade ratio, loose spongy parenchyma, the presence of stomata only on the lower side of a leaf blade (Poplavskaja, 1948). Moreover, leaves of all species have a quite thick cuticle and they are covered with trichomes that are typical for xerophytic plants. In *C. sessilis* and *C. florida*, which grow in the drier habitats (California and Florida) compare with other investigated species with a lot of sunny days and relatively high air temperature, xeromorphic features are expressed somewhat stronger: more trichomes and papillae, a small size of epidermal cells, and the multilayered palisade parenchyma. Described anatomical features are sustained for this species. Thus, the investigated plants belong to the ecological group of xeromesophytes—mesophytes which have xeromorphic features. As the studied species grow in the well–lighted habitats, the described xeromorphic signs of leaf are characteristic for the long–day plants (Vasiliev, 1988), although the palisade ratio ranges from 30 to 50% in leaves of plants growing under low light (Buinova et al., 2002).

**Conclusions**

Leaf is the most plastic organ of plant and its structure reflects the features of ecological adaptation to certain environmental conditions. Anatomical features of the leaf are very important for monitoring the condition of plants in a culture. It is known that the morphological and anatomical characteristics of plants may be altered in the processes of adaptation to the new conditions of growth (Huner et al., 1981). It was shown that the studied plants in the nature grows in the different ecotopes (different geographic zones), but are cultivated under the same environmental conditions in the National Botanical Garden. Our investigations showed that *Cornaceae* species introduced from North America, East Asia, as well as native species, have some anatomical features of xeromesophytes. In the Forest–Steppe zones of Ukraine dry habitats are in exceptional conditions.
conditions: on the southern exposed slopes, dry sandy slopes and other warmed areas. In the National Botanical Garden introduced plants get sufficient moisture by irrigation, and thus compared with the places of their naturally occurring in plants these enhanced features of mesomorphic (Poplavskaja, 1948). Based on our investigation of leaf anatomy we can made conclusion that the introduced species C. officinalis, C. sessilis, C. japonica along with native species C. mas and S. sanguinea withstand a wide range of temperatures and are tolerant to the low winter temperatures (up to −30–32 °C) in the Forest–Steppe of Ukraine, in which they are sufficient adapted, which is expressed in the annual fruit bearing and the formation of a large number of mature seeds (Klymenko & Klymenko, 2015). So we can claimed about successfully adaptation of C. mas, C. officinalis, C. sessilis, C. florida, C. cousa and S. sanguinea plants to the conditions of the National Botanical Garden based on their anatomy without destructive features, every year flowering and fruiting. The stability of the condition of plants, based on adaptability is a key indicator of the stability and capacity of plants to develop without significant destructions against the background of numerous environmental factors (Zhuchenko, 1988). It is known that stability of the system determine by the liability of parts. In biology this is phenomenon of phenotypic plasticity. Phenotypic plasticity is ability of genotype changes its expression and creates different phenotypes in response on different the environmental influences, so plant can adapt to the environmental fluctuations (Dubyna & Kordyum, 2015).

This work may serve as a basis for the further investigations of introduced species of Cornus and monitoring of their changes in the conditions of introduction.
Table 2.
Anatomical features of leaves of *C. officinalis*, *C. japonica*, *C. sessilis*, *C. florida*, *C. mas*, *S. sanguinea*

<table>
<thead>
<tr>
<th>Leaves</th>
<th>Leaf thickness, µm, ( M \pm m )</th>
<th>Lengths of upper epidermis cells, µm, ( M \pm m )</th>
<th>Lengths of lower epidermis cells, µm, ( M \pm m )</th>
<th>Partial volumes of intercellular spaces, %, ( M \pm m )</th>
<th>Palisade coefficient, %, ( M \pm m )</th>
<th>Lengths of palisade parenchyma cells, µm, ( M \pm m )</th>
<th>Width of palisade parenchyma cells, µm, ( M \pm m )</th>
<th>Lengths of stomata, µm, ( M \pm m )</th>
<th>Width of stomata, µm, ( M \pm m )</th>
<th>Number of stomata per 1 mm(^2), ( M \pm m )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cornus officinalis</em></td>
<td>262.63±2.49(^a)</td>
<td>17.65±1.16(^a)</td>
<td>13.66±1.22(^a)</td>
<td>12±0.65(^a)</td>
<td>47.30±1.68(^a)</td>
<td>25.01±1.06(^a)</td>
<td>8.50±0.37(^a)</td>
<td>23.9±0.63(^a)</td>
<td>13.35±0.8(^a)</td>
<td>125</td>
</tr>
<tr>
<td><em>Cynoxylon japonica</em></td>
<td>227.45±6.38(^b)</td>
<td>20.94±0.86(^b)</td>
<td>11.85±0.62(^b)</td>
<td>20.98±1.18(^b)</td>
<td>37.36±1.15(^b)</td>
<td>29.93±2.31(^b)</td>
<td>11.55±0.60(^b)</td>
<td>20.88±0.6(^b)</td>
<td>11.2±0.32(^b)</td>
<td>161.67±7.26</td>
</tr>
<tr>
<td><em>Cornus sessilis</em></td>
<td>173±2.51(^c)</td>
<td>21.75±0.98(^c)</td>
<td>10.92±0.76(^c)</td>
<td>43.50±1.19(^c)</td>
<td>40.22±1.54(^c)</td>
<td>25.87±2.47(^c)</td>
<td>14.71±0.57(^c)</td>
<td>17.54±0.5(^c)</td>
<td>11.29±0.3(^c)</td>
<td>137.5</td>
</tr>
<tr>
<td><em>Cynoxylon florida</em></td>
<td>159.33±2.11(^d)</td>
<td>14.50±0.82(^d)</td>
<td>12.57±0.77(^d)</td>
<td>15.73±0.95(^d)</td>
<td>37.11±0.98(^d)</td>
<td>72.98±3.17(^d)</td>
<td>25.12±1.15(^d)</td>
<td>24.73±0.6(^d)</td>
<td>18.44±0.6(^d)</td>
<td>———</td>
</tr>
<tr>
<td><em>Cornus mas</em></td>
<td>196.64±6.99(^e)</td>
<td>22.63±0.60(^e)</td>
<td>12.70±0.35(^e)</td>
<td>15.91±0.83(^e)</td>
<td>41.73±0.86(^e)</td>
<td>31.50±1.69(^e)</td>
<td>9.69±0.41(^e)</td>
<td>21.3±0.42(^e)</td>
<td>13.06±0.5(^e)</td>
<td>112.5±19.1</td>
</tr>
<tr>
<td><em>Swida sanguinea</em></td>
<td>137.3±3.63(^f)</td>
<td>12.64±0.6(^f)</td>
<td>10.98±0.35(^f)</td>
<td>33.02±1.35(^f)</td>
<td>34.96±1.16(^f)</td>
<td>31.95±1.67(^f)</td>
<td>14.65±0.75(^f)</td>
<td>15.42±1.5(^f)</td>
<td>12.11±1.4(^f)</td>
<td>262.5±25</td>
</tr>
</tbody>
</table>

Means with the same letter in the superscript within the same column are not significantly different at \( p < 0.05 \). Means with the same mark (*) in the superscript within the same row are not significantly different at \( p < 0.05 \). (Student \( t \)-test \( n = 50 \), Mann–Whitney \( u \)-test, \( n = 10 \)).
References

Alekseev, V.P.: China Plant resources. Nauka. (Leningrad), 236 pp., 1935.
Klymenko S.V.: Cornelian Cherry Varieties in Ukraine. Fytosotsyotsentr, (Kyiv), 32 pp., 2006.
