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Diagnostic micromorphological features of leaf surface of selected species of the genus *Artemisia* L. (Asteraceae)

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Key words: *Artemisia*, micromorphological features, leaves, light microscopy, scanning electron microscopy.

Ključne besede: *Artemisia*, mikromorfološke značilnosti, listi, svetlobna mikroskopija, vrstična elektronska mikroskopija.

Abstract

The leaves of different species of the genus *Artemisia* show considerable inner and external structural diversity. This paper presents a comparative study of the micromorphological features of leaf surface for seven species from different regions of Ukraine: *A. absinthium* L., *A. annua* L., *A. dracunculus* L., *A. marschalliana* Spreng., *A. vulgaris* L., *A. argyi* H.Lév. & Vaniot, and *A. verlotiorum* Lamotte. The vegetative organs of these plants have potentially significant value as medicinal raw materials. This study was carried out using both light and scanning electron microscopy. The main diagnostic characteristics for each species – in terms of form and structure of epidermal cells, stomata, and trichomes (glandular and non-glandular) – were described and illustrated. Using combinations of selected qualitative micromorphological characteristics of the investigated species of *Artemisia,* it is possible to detect the species identity of these raw materials.

Izvleček

Listi različnih vrst iz rodu *Artemisia* so glede na notranjo in zunanjo zgradbo precej raznoliki. V članku je predstavljena primerjalna raziskava mikromorfoloških značilnosti listnih površin pri sedmih vrstah, ki rastejo v različnih regijah v Ukrajini: *A. absinthium* L., *A. annua* L., *A. dracunculus* L., *A. marschalliana* Spreng., *A. vulgaris* L., *A. argyi* H.Lév. & Vaniot in *A. verlotiorum* Lamotte. Vegetativni organi teh rastlin so potencialno pomembni kot surovina za uporabo v medicini. Raziskavo smo izvedli s pomočjo tako svetlobne kot elektronske mikroskopije. Predstavili smo poglavitne diagnostične značilnosti vsake od vrst – z vidika oblike in strukture celic povrhnjice, listnih rež ter trihomov (žleznih in nežleznih). Z uporabo kombinacije izbranih kvalitativnih mikromorfoloških značilnosti preiskovanih vrst iz rodu *Artemisia* je v teh surovinah za uporabo v medicini mogoče določiti, katere vrste so prisotne.

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Introduction

The genus *Artemisia* L. (wormwood) is one of the largest genera in the family Asteraceae (Compositae). It has about 500 species and is an important object of study for taxonomists and florists in almost all countries. There are 30 species of *Artemisia* in Ukraine; 9 of them have been studied as medicinal plants (Mosyakin & Fedoronchuk, 1999; Minarchenko, 2005). The State Pharmacopoeia of Ukraine (SPhU) has an entry only on *Artemisia absinthium* L. (Naukovo-ekspertnyi farmakopeynyit sentr, 2016). The large content of biologically active substances, including essential oils, suggests a wide range of applications of species of *Artemisia* genus in medical and veterinary practice and in the food industry. *Artemisia* is a taxonomically complicated genus because some species have similar morphology and even very similar microscopic structures. It can be difficult to identify related species, especially as dry raw materials (Hayat et al., 2009b). The species of this genus are also characterized by phytochemical variability. Different chemotypes and cytotypes synthesize different compounds with different biological activity (Ivănescu et al., 2015).

Taxonomists use a variety of morphological characters to determine *Artemisia* species, including the life form, shape, and dissection of the leaf blade, the general shape of the inflorescence, the aggregation of the heads, the length of the stem of the head, the nature of pubescence, and so on. The parameters of these structures in wormwood, for example, are variable. Therefore, the issue of clear demarcation of all species in this group still remains controversial (Hayat et al., 2009a). In addition, the identification of whole plants and raw materials of species involves a number of variables. The raw materials usually contain only parts of plant organs, and one of the requirements for the quality of raw materials is the absence of coarser parts of plants (stems, roots, etc.). The raw material of wormwood contains mostly leaves with a small proportion of inflorescences and stems, and it is important to identify the main species-specific morphological or anatomical features of these organs, which would allow a clear determination of their species affiliation. There are no summary data of such characteristics for different species of wormwood in Ukraine.

This paper presents for the first time a comparative characterization of diagnostic features of the leaf epidermis of 7 species of genus *Artemisia* that are used as sources of valuable biologically active compounds: *A. absinthium* L., *A. annua* L., *A. dracunculus* L., *A. marschalliana* Spreng., *A. vulgaris* L., *A. argyi* H.Lév. & Vaniot, and *A. verlotiorum* Lamotte. The two last invasive species, *A. argyi* and *A. verlotiorum,* are of particular interest. They have appeared relatively recently in Ukraine and in recent decades have shown a tendency to expand into natural phytocenoses (Mosyakin et al., 2019 and the references therein). Moreover, the dry raw material of *A. argyi* is morphologically similar to *A. absinthium* and so can potentially occur as an impurity in the raw material of the latter species. Likewise, the raw material of *A. verlotiorum* is similar to *A. vulgaris*. The raw materials of these two species contain many important biologically active compounds that are widely studied and used in medicine in Asia and Europe. Moreover, the raw material of *A. verlotiorum* is more valuable than *A. vulgaris* (Carnat et al., 2001), and there are cases of hybridization between these two species (James et al., 2000).

Materials and methods

This study is based on materials collected during many years of field research by the authors in different regions of Ukraine, with the additional use of herbarium specimens that were deposited in the National Herbarium of Ukraine (KW) (Table 1).

Table 1: Specimens of the *Artemisia* species analyzed in the study.

Tabela 1: Primerki vrst rodu *Artemisia*, proučevani v raziskavi.

Species	Locality	Collecting Date	Collectors, Herbarium code
Artemisia absinthium	Odesa region, Ovidiopol district, the village of Sukhyi Lyman, the roadsides	10.6.2005	Bondarenko O.J., KW № 120226
	Sumy region, near the town of Hlukhiv, the dry slopes	21.8.2010	Koval L.V., KW № 154497
	Kyiv region, Obukhiv district, the village of Kozyn, the roadsides	2.7.2019	Tymchenko I.A., Dvirna T.S., KW № 155601
	Poltava region, Poltava district, near the village of Novi Sanzhary, the steppe meadows	6.8.2019	Dvirna T.S., KW № 155600
	Kyiv region, Makariv district, near the village of Mykolaivka, the abandoned fields	15.7.2020	Minarchenko V.M., KW № 155599

A. annua	Kharkiv region, near the village of Kehychivka, the forest strip along the railway	19.9.2015	Davydov D.A., KW № 123866
	Vinnytsya region, Chechelnyk district, western suburbs of the village of Bondurivka, the forest road	28.9.2016	Kolomiychuk V. P., KW № 123463
	Poltava region, Poltava district, near the village of Lelyukhivka, the vegetable gardens	26.7.2019	Dvirna T.S., KW № 155594
	Kyiv region, Obukhiv district, near village Kozyn, floodplain meadow of Kozynka river, the roadsides	7.8.2020	Tymchenko I.A., KW № 155595
	Poltava region, Lubny district, near the village of Berezotocha, the roadsides	10.8.2020	Glushchenko L.A., KW № 155610
A. argyi	The city of Kyiv, the railway station of Darnytsya	18.9.1989	Мосякін С.Л., KW № 155609
	Zhytomyr region, Ovruch district, the railway station of Ihnatpil	7.8.2010	Orlov O.O., KW № 092379
	The city of Kyiv, Pushcha-Vodytsya, near the lake of Synye, the forest margin	22.8.2018	Bagatska T.S., KW № 134301
	The city of Kyiv, the roadsides of ring road	14.8.2020	Minarchenko V.M., KW № 155602
A. dracunculus	Kyiv region, Makariv district, near the village of Mykolaivka, the forest margin	24.7.2019	Minarchenko V.M., Makhynia L.M., KW № 155604
	Kyiv region, Obukhiv district, near the village of Kozyn, the floodplain of Kozynka river, the abandoned vegetable gardens	20.7.2020	Tymchenko I.A., KW № 155603
	Zhytomyr region, the town of Berdychiv, the railway station, ruderal places	25.7.2021	Orlov O.O., Shynder O.I., KW № 153832
A. marschalliana	Chernihiv region, Borzna district, near the village of Hryshivka, the floodplain meadow of lake Trubyn	6.8.2004	Shyian N.M., KW № 00112743
	Volyn region, Ratne district. Near the town Ratne, the roadsides	21.8.2008	Minarchenko V.M., Tymchenko I.A., KW № 155596
	Zhytomyr region, Narodychi district, near the village of Velyki Klishchi, Drevlyanskyi nature reserve, the forest glades	18.8.2015	Kolomiychuk V. P., KW № 116426
	The city of Kyiv, Syretsky Park, the roadsides	11.9.2020	Minarchenko V.M., KW № 155598
	Kyiv region, Makariv district, near the village of Khmilna, the wastelands	26.9.2020	Minarchenko V.M., KW № 155597
A. verlotiorum	Crimea, the city of Simferopil, the mountain of Salhirka	14.7.2004	Shevera M.V., KW № 090866
	Crimea, near the town of Hurzuf, Ay-Danil, the weed in the vineyard	11.10.2013	Ryff L.E., KW № 148035
	Zakarpattya region, the city of Uzhhorod, Hrushevskoho street, ruderal areas	12.9.2019	Shevera M.V., KW № 145936
	The city of Kyiv, Syretsky Park, the roadsides	11.9.2020	Minarchenko V.M., Boyko G.V., KW № 155605
A. vulgaris	Chernihiv region, Bobrovytsya district, the village of Nova Basan, the roadsides of the forest margin	26.7.2002	Ilyinska A.P., Futorna O.A., Dyachenko I.I., Nitsenko L.M., KW № 007317
	Khmelnytskyi region, the town of Slavuta, Suvorova street	21.8.2002	Gubar L.M., KW № 060927
	Kyiv region, Makariv district, near the village of Mykolaivka, the abandoned fields	24.7.2019	Minarchenko V.M., Makhynia L.M., KW № 155606
	Poltava region, Poltava district, near the village of Novi Sanzhary, the roadsides	6.8.2019	Dvirna T.S., KW № 155608
	Kyiv region, Obukhiv district, the village of Kozyn, the roadsides	8.8.2020	Tymchenko I.A., KW № 155607

Light microscopy (LM)

Fragments of dried leaves (basal stem and from the middle of the inflorescence) of 10 samples (individuals) of each species were obtained for morphological and anatomical analysis. The raw materials were boiled in water or 5% sodium hydrochloride solution for 2–5 minutes. The specimens were then prepared for light microscopy (LM). Micropreparations were produced from fresh plants in some cases. The dense layer of trichomes on the leaf blade was removed with adhesive tape in order to analyze the epidermal surfaces. Microscopic examination was performed 10 times for each organ from the different parts of the sample. Observations and photomicrographs were performed using an Olympus CX23 light microscope and a Philip Harris stereomicroscope, using Levenhuk M1000 PLUS camera software.

Scanning electron microscopy (SEM)

Investigations of diagnostic features of model species were also performed using a scanning electron microscope (SEM) JSM-6060LA according to standard methods. The main terminology used in describing the diagnostic features of raw materials is based on classical works in this field (Hayat et al., 2009a; Hussain, 2019). The paper also uses the classic terms of characterization of plant organs micromorphology.

Results and discussion

Features of the epidermis of leaves play a decisive role in the identification of raw materials, as the share of leaves in raw materials of *Artemisia* species is 60–80%. The main microdiagnostic characteristics of wormwood leaves in raw materials are the shape of epidermal cells, as well as the presence/absence and structure of non-glandular and glandular (or glands) trichomes; slightly less important in identification are the structure, size, and location of the stomata.

Epidermal cells

According to our research, the cells of the leaves' abaxial epidermis, in the most studied species of *Artemisia* genus, are characterized by an irregular shape with wavy walls (Figure 1a–f) and mostly smooth cuticles. The abaxial epidermis of *A. marschalliana* leaves is characterized by a polygonal shape of cells with slightly wavy or smooth walls and an elongated-comb cuticle in some samples (Figure 1g). The tortuosity of the anticline walls of the adaxial epidermis is well expressed only in *A. annua* and *A. vulgaris*, somewhat weaker in *A. verlotiorum* (Figure 1i, l–n); in *A. absinthium*, *A. dracunculus*, *A. argyi,* and *A. marschalliana*, the shape of the cells is polygonal (Figure 1h, j, o). In *A. verlotiorum* and *A. vulgaris*, the shapes of the cells of upper and lower leaf epidermis are very similar, but in the latter species, the tortuosity of the anticline walls of epidermal cells is much higher on both sides. Probably, this characteristic has considerable variability, as in some works we find opposite data (Bini Maleci & Bagni Marchi, 1983).

Stomata

The leaf blade of *A. absinthium*, *A. annua*, *A. dracunculus* and *A. marschalliana* is amphistomatic with anomocytic-type stomata, although *A. marschalliana* has a predominance of tetracytic stomata (Figure 1g, o). Stomata occur much less frequently on the adaxial epidermis of *A. absinthium*, *A. annua* (Figure 3b, d), while in *A. dracunculus* (Figure 1c, j) and *A. marschalliana* (Figure 1g, o) the density of stomata is approximately the same on the both epidermises. We have found that leaves of *A. argyi*, *A. verlotiorum* and *A. vulgaris* have hypostomatic type with numerous stomata in the lower epidermis, but according to some data (Kondratieva & Kondratieva, 2010), stomata on the leaves *A. argyi* can be on both sides.

The shape of the stomata and the length of the stomatal slit are characterized by moderate variability. Thus, a common feature for the stomata of the leaves of the analyzed species is their rounded or elongated shape with 3–6 side cells; they are slightly sunken, raised, or located at the level of epidermal cells. In general, the stomata are larger in size on the adaxial epidermis in all analyzed species with amphistomatic leaf type. The range of ostiole lengths for some species is species-specific. It is the smallest in *A. absinthium* (17–20 µm), and the largest in *A. argyi* (13–23 µm) and *A. marschalliana* (18–28 µm). The size of the ostiole in *A. annua* was 10–17 µm, in *A. dracunculus* 12.7–18.5 µm, in *A. verlotiorum* and *A. vulgaris*, this figure ranged from 13–20 µm. In the last two species, the stomata clearly protrude above the epidermis and have a rounded shape (Figure 4d–e). The size of individual structures (epidermal cells, stomata, glandular and non-glandular trichomes) is different in samples of wormwood collected from different places, and often overlap in a certain range, so a greater diagnostic role in raw materials plays a combination of qualitative (shape, location, presence/absence) and quantitative characteristics

In *A. absinthium*, *A. annua*, *A. argyi*, *A. verlotiorum* and *A. vulgaris*, the stomata are divergent, often convex or elongated. The stomata of *A. marschalliana* are predominantly unidirectional along the leaf axis; they are located at the level of the epidermis or slightly sunken with protruding anticline walls of the closing cells (Figure 3a, 4f). On the abaxial epidermis of *A. annua*, the stomata are multidirectional, and on the adaxial epidermis they are

Figure 1: Diagnostic features of the leaf epidermal cells of different *Artemisia* species as seen under LM.

Slika 1: Diagnostične značilnosti epidermalnih celic listov različnih vrst iz rodu *Artemisia* kot so vidne s pomočjo svetlobnega mikroskopa.

Legend: abaxial epidermis (abaksialna povrhnjica):
a – *A. absinthium,* ×400; b – *A. annua,* ×400; c – *A. dracunculus,* ×100; d – *A. argyi,* ×400; e – *A. verlotiorum,* ×400; f – *A. vulgaris,* ×400; g – *A. marschalliana,* ×100; adaxial epidermis (adaksialna povrhnjica): h – *A. absinthium,* ×400; i – *A. annua,* ×400; j – *A. dracunculus,* ×100; k – *A. argyi,* ×400; l – *A. verlotiorum,* ×400; m–n – *A. vulgaris,* ×400; o – *A. marschalliana*, ×100.

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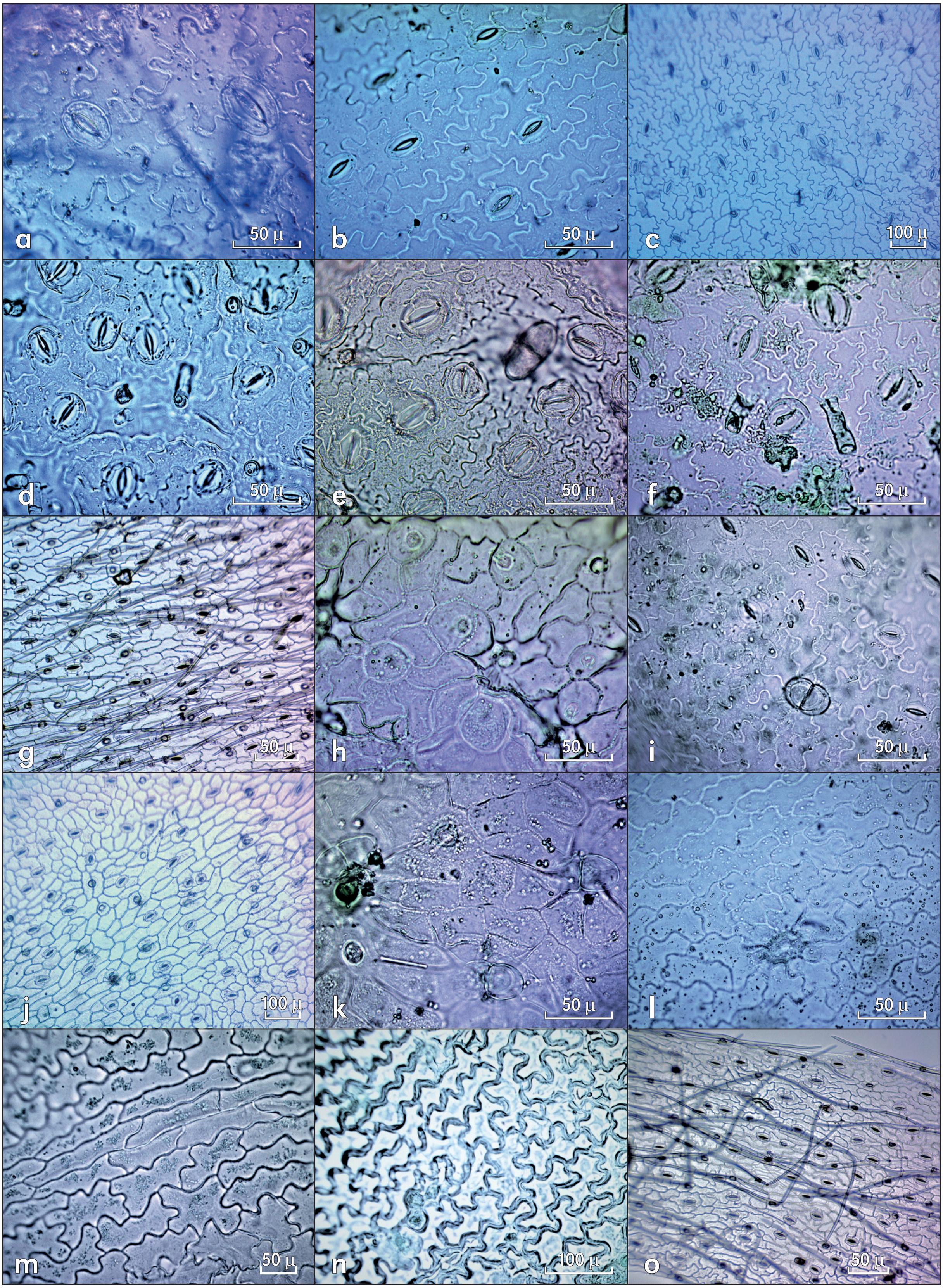
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mainly oriented along the leaf axis (Figure 3b, 4b). In *A. dracunculus*, the stomata are oriented mainly along the leaf axis on both sides and are located at the level of epidermal cells (Figure 3g, 4h). The density of stomata on the leaf surface exhibits a very significant variability within each species, which is confirmed by the correlation of this indicator with environmental conditions (Ochirova et al., 2015).

Glandular and non-glandular trichomes

One of the most important characteristics of the epidermis of wormwood leaves is the presence/absence and structure of glandular (glands) and non-glandular trichomes. In most members of the genus *Artemisia,* indicators of density of glandular trichomes on the leaf surface are characterized by significant variability in plants according to different environmental conditions and different stages of development. Therefore, it is likely that quantitative indicators of the density of glandular trichomes cannot play a decisive role in the identification of raw materials.

Multicellular peltate or biseriate trichomes, differentiated into basal, basic, and apical cells, are dominant among the glandular trichomes in the studied species (Figure 2a). They are the site of synthesis and storage of various types of secondary metabolites, the most noted of which are terpenoids and flavonoids (Nigam et al., 2019). The most valuable compound in wormwood species is artemisinin (Xiao et al., 2016), which is synthesized and concentrated in glandular trichomes – this indicator is important in determining the quality of raw materials of a particular species (Ferreira & Anick, 2009).

It is believed that biseriate glandular trichomes of *Artemisia* species consist of 10 pairs of secretory cells arranged in 2 rows (Duke et al. 1994; Appezzato-da-Glória et al., 2008), while Cui et al. (2020) suggest that *A. argyi* has 8-celled biseriate glands. *A. absinthium* glands are large, 25–50 μm long in the frontal plane. They are localized in the recesses of the adaxial and abaxial epidermis under a dense layer of T-shaped non-glandular trichomes (Figure 2a–b, 3d–f). In general, their density on the adaxial surface is significantly higher than on the abaxial surface, which is characteristic of most of the studied species.

On the epidermis of *A. argyi* leaves, we found several morphotypes of glandular trichomes. Biseriate trichomes dominate, especially on the adaxial leaf surface (Figure 3h, i). Moreover, their density is much higher than in *A. absinthium*. The length of these glands in the frontal plane is 28–57 μm. They are basically more or less sunken into the epidermis (Figure 3i). There are also 4-celled glandular trichomes with vertical cell placement in *A. argyi*; in our study, they were found rarely on the adaxial epidermis between biseriate trichomes. There are also small round glands with a diameter of 10–16 μm with secretory content. They are embedded in the epidermis (Figure 2c). It was noted that the density of glandular and non-glandular trichomes in this species is lower in mature leaves compared to young leaves (Cui et al., 2020). An important feature of the epidermis of *A. dracunculus* leaves is also the presence of peltate glands, but they are somewhat different from those in other species of the genus. Peltate glands on the leaves of *A. dracunculus* are large, lyre-shaped, and the longest in the frontal plane among the analyzed species (37–63 μm) (Figure 4g, h). Occasionally, on the epidermis of *A. dracunculus* leaves, there are also classic peltate (Figure 2e) and unicellular glands embedded in the epidermis, and their diameter varies in the range of 10–16 μm.

The surface of *A. annua* leaves on both sides and on the stem is abundantly covered with peltate glandular trichomes. There are unicellular glands among them (Figure 2d, 3c). The size of peltate trichomes of *A. annua* in the frontal plane varies between 29 and 47 μm. They are usually sunken at the base into the abaxial and adaxial epidermis of the leaf. Duke et al. (1994) reported that there are two biotypes of *A. annua*: with and without secretory trichomes; plants without glandular trichomes lack artemisinin.

Peltate glands are present mainly on the abaxial epidermis under a dense cover of non-glandular T-shaped trichomes in the studied samples of *A. verlotiorum* (Figure 4i, j). On the adaxial epidermis there are occasionally peltate and unicellular glands, as well as single flat multicellular glands, which rise slightly above the epidermis (Figure 3j). The density of glandular trichomes on the abaxial epidermis of *A. verlotiorum* leaves was 12–18 pieces/mm^2, while in other studies this figure reached 30 pieces/mm^2 (Bini Maleci & Bagni Marchi, 1983). We can explain this difference by the limiting environmental conditions in our area, given the southern origin of this species. Peltate glands are sunken at the base into the epidermis, large, up to 63 μm long in the frontal plane, generally divided in half at the apex (Figure 4j). The glands of *A. vulgaris* are sunken at the base into the epidermis, up to 35 μm long in the frontal plane, generally divided in half at the apex (Figure 2j, 4k). Glandular trichomes of the capitate type are also rarely present here (Hayat et al., 2009b) without constriction in the terminal part (Figure 4l). Note that in *A. vulgaris*, the peltate glands are almost half the size of those in *A. verlotiorum*, and their density is also about half lower (Figure 4m). Among the studied samples of *A. marschalliana* plants, small peltate glands were found mainly on the leaves of pubescent forms (Figure 2f). Note that in our study, the size of the peltate glands (length in the frontal plane) differed significantly on the leaves from different fragments of the shoot in all species. They also differ from those in some publications (Karbalaei et al., 2021). In addition, the size of the glandular trichomes often overlap in related species. This may confirm the weak diagnostic value of quantitative values of the size of these trichomes.

Another diagnostic feature of all analyzed species of the genus *Artemisia* is also the presence/absence of non-glandular trichomes and their structure. These trichomes are classified according to their morphology and the studied species are dominated by T-shaped (or dolabriform) non-glandular trichomes. They are present on the aboveground parts of all studied species but their localization,

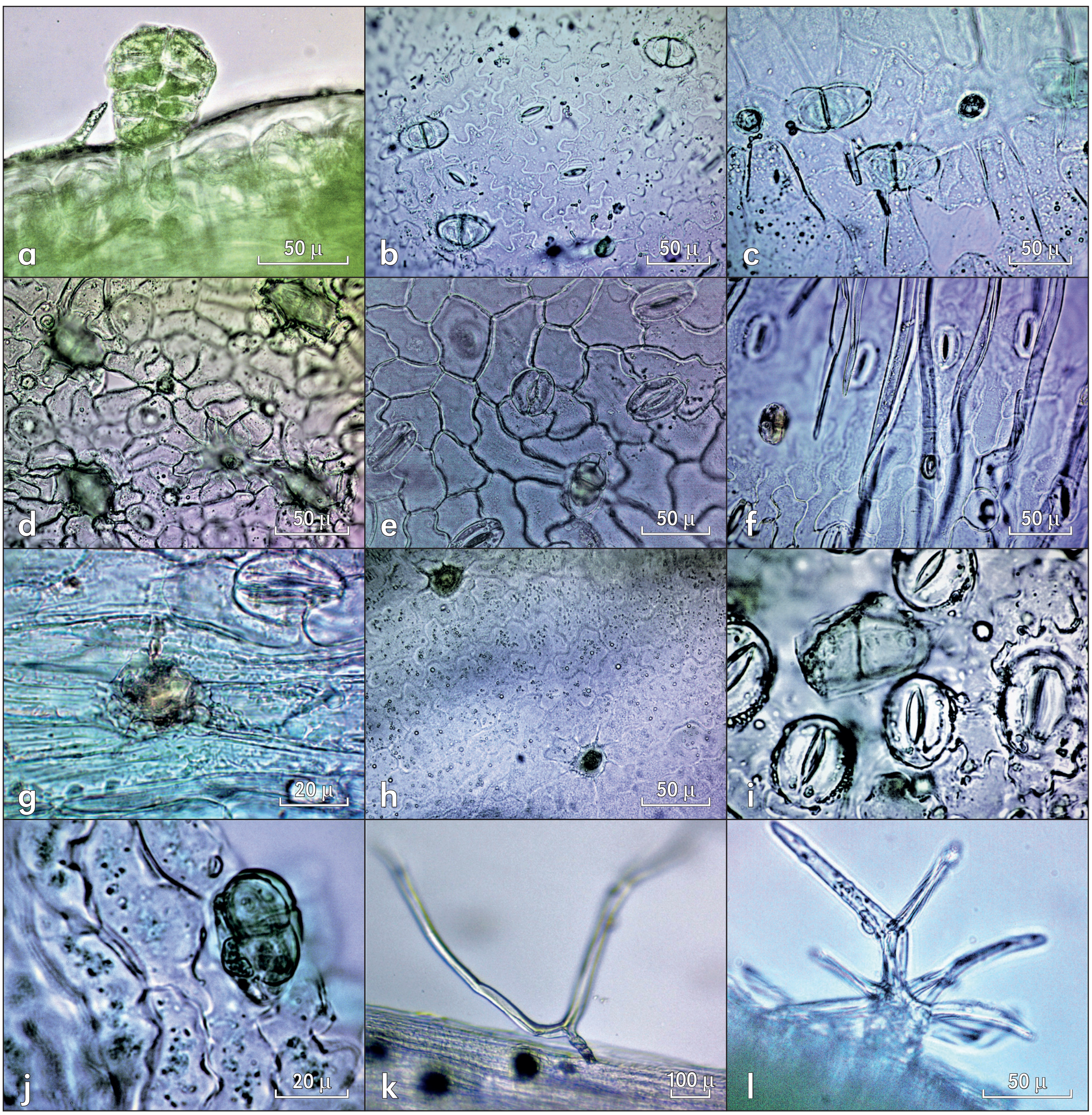

Figure 2: Variations in glandular and non-glandular trichomes on the leaf surfaces of some *Artemisia* species as seen under LM.

Slika 2: Razlike v žleznih in nežleznih trihomih na listnih površinah nekaterih vrst iz rodu *Artemisia* kot so vidne s pomočjo svetlobnega mikroskopa.

a–b – *A. absinthium*, ×400; c – *A. argyi*, ×400; d – *A. annua*, ×400; e – *A. dracunculus,* ×400; f – *A. marschalliana,* ×400; g – *A. marschalliana,* ×1000; h – *A. verlotiorum*, ×400; i – *A. verlotiorum*, ×1000; j – *A. vulgaris*, ×1000; k – *A. vulgaris*, ×100; l – *A. dracunculus,* ×400.

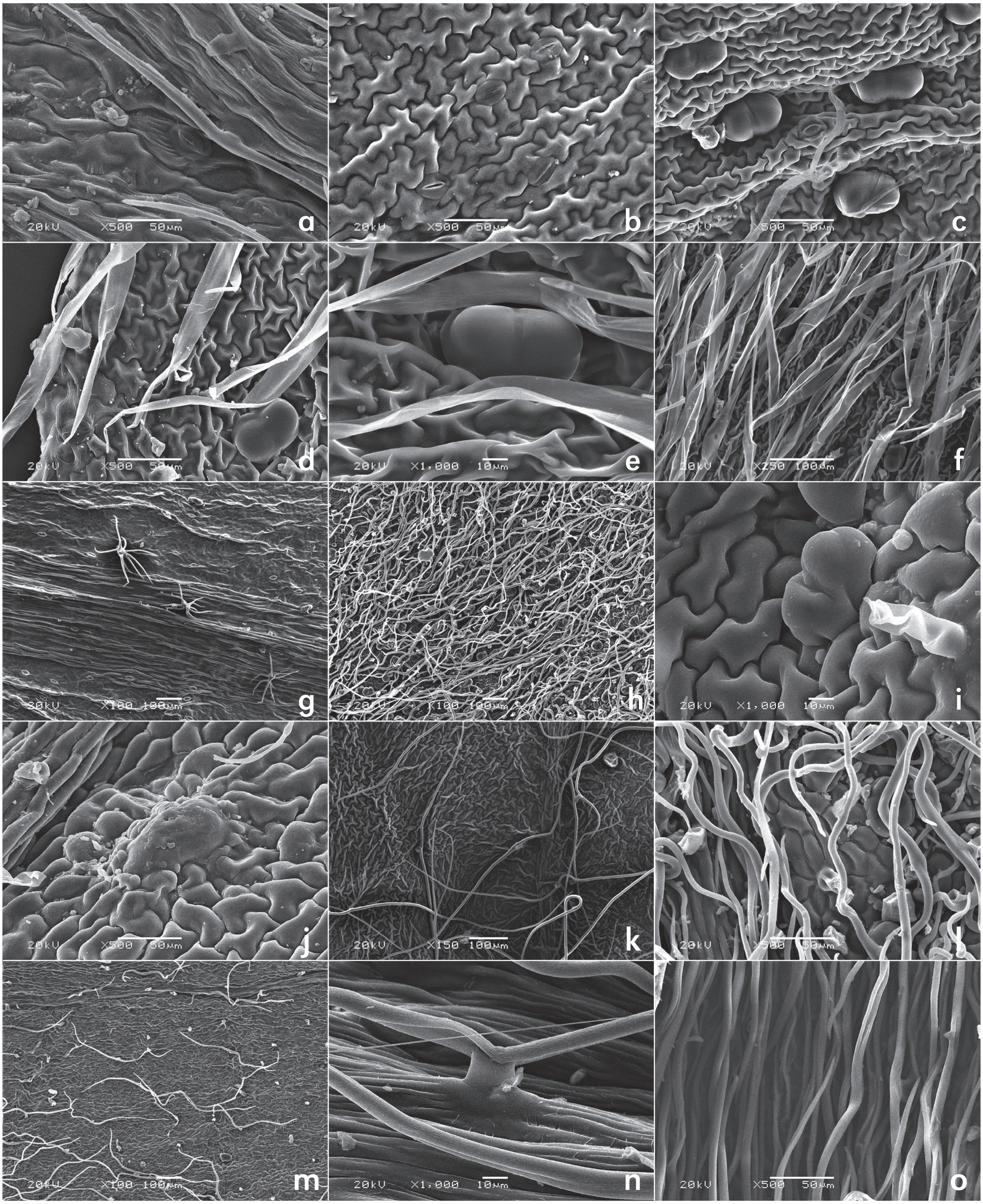

Figure 3: Variations in stomata, glandular and non-glandular trichomes of adaxial leaf epidermis of different *Artemisia* species by means of SEM.

Slika 3: Razlike v listnih režah, žleznih in nežleznih trihomih adaksialne povrhnjice listov različnih vrst iz rodu *Artemisia* kot so vidne s pomočjo elektronskega mikroskopa.

a – *A. marschalliana,* ×500 ; b–c – *A. annua,* ×500; d – *A. absinthium,* ×500; e – *A. absinthium,* ×1000; f – *A. absinthium,* ×250; g – *A. dracunculus,* ×100; h – *A. argyi,* ×100; i – *A. argyi,* ×1000; j – *A. verlotiorum,* ×500; k – *A. vulgaris,* ×150; l – *A. argyi,* ×500; m – *A. verlotiorum,* ×100; n – *A. marschalliana,* ×1000; o – *A. marschalliana,* ×500.

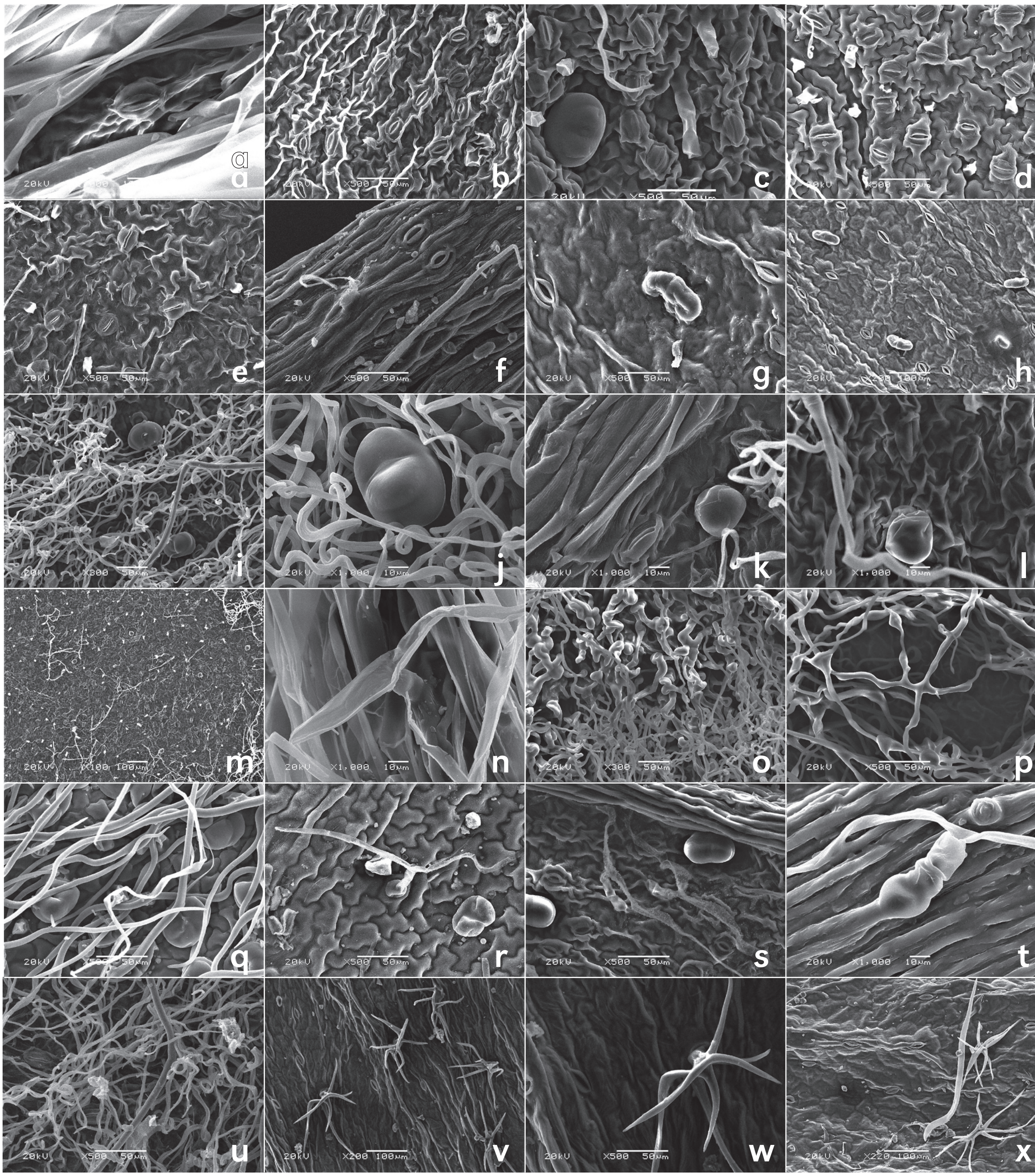

Figure 4: Variations in stomata, glandular and non-glandular trichomes of abaxial leaf epidermis of different *Artemisia* species as seen under SEM.
Slika 4: Razlike v listnih režah, žleznih in nežleznih trihomih abaksialne listne povrhnjice različnih vrst iz rodu *Artemisia* kot so vidne s pomočjo elektronskega mikroskopa.
a – *A. absinthium,* ×1000; b – *A. annua,* ×500; c – *A. argyi,* ×500; d – *A. verlotiorum,* ×500; e – *A. vulgaris,* ×500; f – *A. marschalliana,* ×500; g – *A. dracunculus,* ×500; h – *A. dracunculus,* ×200; i – *A. verlotiorum,* ×300; j – *A. verlotiorum,* ×1000; k – *A. vulgaris,* ×1000; l – *A. vulgaris,* ×1000; m – *A. vulgaris,* ×100; n – *A. absinthium,* ×1000; o – *A. argyi,* ×300; p–q – *A. argyi,* ×500; r–s – *A. annua,* ×500; t – *A. verlotiorum,* ×1000; u – *A. vulgaris,* ×500; v – *A. dracunculus,* ×200; w – *A. dracunculus,* ×500; x – *A. dracunculus,* ×220.

structure, density and orientation have species-specific features. A common feature of these non-glandular trichomes is their placement on the same level with the epidermal cells, or with base slightly convex, in contrast to the glandular trichomes, which are usually sunken into the epidermis. T-shaped non-glandular trichomes are elongated generally parallel to the surface of the epidermis terminal cell in the form of a compass needle, with a stem base formed by 1–4 cells. The basal cell in these trichomes is usually enlarged. The site of fastening has the form of rounded rollers.

A characteristic feature of T-shaped non-glandular trichomes of *A. absinthium* is the presence of a wide (up to 18 µm in the middle) and long (2–5 mm) tape-shaped terminal cell with a saucer-shaped depression at the site of attachment to the stem (Figure 4n). The stem part of the trichome is usually short (1–2 cells) with an enlarged basal cell, which is attached at the level of the epidermis. In some trichomes, the number of stem cells can reach four. T-shaped trichomes are mostly placed unidirectionally (Figure 3d, f), densely covering the leaf blade on both sides with a higher density on the abaxial epidermis.

A. argyi is similar to *A. absinthium* in pubescence. All aboveground organs of plants of this species are also glaucous from dense pubescence with T-shaped trichomes, but their structure is somewhat different. We found some morphological differences in the T-shaped trichomes of *A. argyi*. In particular, the felt covering on the abaxial side of the leaf is formed mainly by T-trichomes with long filamentous nodular apical cells, which sometimes grow together and form anastomoses (Figure 4p). They are placed so densely that it is difficult to notice the glands under them. The terminal T-trichome cell on the adaxial epidermis is long, thick, smooth or slightly tortuous (Figure 4q). T-trichomes with a ribbon-like apical cell appear here singly. The stem part of both morphotypes is composed of 2–3 cells with a thickened basal cell. On the adaxial epidermis, T-shaped trichomes are less tortuous and less densely spaced, with peltate glands clearly visible between them.

A. annua presents a different type of T-shaped trichomes than those described above. These are 5-celled trichomes with a ribbon-like apical cell, but much shorter and narrower than in *A. absinthium*; the stem part is 2–4 celled, and the basal cell is large and barrel-shaped (Figure 4r), as in *A. verlotiorum* (Figure 4t). Such trichomes are rarely present on both sides of the leaf blade of *A. annua* (Figure 4s). T-shaped trichomes are present on *A. verlotiorum* on the abaxial epidermis with a long terminal filamentous cell and a saucer-shaped depression in the center (Figure 4i–j). They have a number of similar features to those described above: their base is 2–3 celled, as in *A. absinthium*, but the basal cell is large, sometimes barrel-shaped, especially along the veins (Figure 4t); on the adaxial surface of the leaf blade, these trichomes are shorter and almost flat without a clear orientation (Figure 3m), as in *A. vulgaris* (Figure 3k). Radial placement of adjacent epidermal cells is often present around the base of the trichome on the adaxial surface. The abaxial epidermis of *A. vulgaris* is dominated by filamentous T-shaped trichomes, which form a less dense cover than in *A. verlotiorum*, and occasionally there are T-shaped trichomes with a thick, long, slightly tortuous or flat terminal cell (Figure 4u). Their stem base is formed by 2–4 cells. T-trichomes of *A. vulgaris* on the adaxial epidermis are similar to those in *A. verlotiorum*, although the terminal cell is longer in *A. vulgaris.*

Of particular note is the pubescence of the epidermis in leaves of *A. marschalliana*. This species is characterized by huge variations of this feature: from dense pubescence of all aboveground organs of plants to the complete absence of T-shaped trichomes. Moreover, plants that are pubescent, sparsely pubescent, or even glabrous can grow together where there is predominance of one kind in the coenopopulation. The only thing that was established in this study was the predominance of pubescent plants of *A. marschalliana* in the populations in Southeast Ukraine, whereas in Polissya the plants are weakly pubescent or bare. T-shaped trichomes with a unicellular broad base and a long flat terminal cell (Figure 3n) are usually oriented along the axis of the leaf on both sides.

Non-glandular trichomes of *A. dracunculus* are different from those in other studied species. Specific macromorphic multibranched trichomes (Figure 2l, 3g, 4v–x), which are also a unique diagnostic feature of *A. persica* (Hayat et al., 2009b), were found scattered on the leaf in the samples that we studied. These are large branched trichomes with a broad short base and various numbers of branches. They are scattered in *A. dracunculus* on both sides of the leaf blade. Typical for most species of the genus *Artemisia,* non-glandular T-shaped trichomes with almost equally long terminal cells were very rare (Figure 4x) in the samples we studied.

Conclusions

We found that the leaf surfaces of *A. absinthium, A. annua, A. dracunculus, A. marschalliana, A. vulgaris, A. argyi* and *A. verlotiorum* have some common and species specific features in the structure of epidermis, stomata, glandular and non-glandular trichomes. Comprehensive analysis of these characteristics of *Artemisia* species allows us to determine their species affiliation clearly. Targeted falsification or incorrect identification of a particular species of *Artemisia* during the microscopic analysis of raw materials is unlikely. Among the analyzed structures of leaf surfaces of *Artemisia* species, the species specificity is more pronounced in the morphology and localization of the glandular and non-glandular trichomes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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