

MICROMORPHOLOGY OF TRICHOMES IN THE FLOWERS OF THE HORSE CHESTNUT *Aesculus hippocastanum* L.

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Abstract

Aesculus hippocastanum L. is an ornamental tree appreciated for its beautiful flowers and leaves. The flowers of this species contain secondary metabolites exhibiting pharmacological activity. They also produce essential oils and coloured “nectar guides”, which enable insects to reach nectar and pollen. The aim of the study was to investigate the types and characteristics of chestnut flower trichomes, which may contain biologically active substances. The analyses were performed using light, fluorescence, and scanning electron microscopy. Three types of trichomes were found on the sepals and the surface of the ovary, whereas the corolla petals exhibited two types of hairs and papillae. The hairs differ in terms of their length and number of cells. The perianth and pistil had no capitate hairs, whereas the ovary exhibited the presence of collectors.

Histochemical assays revealed that all the types of trichomes and papillae contained lipids or essential oils; hence, they can be classified as glandular structures. The “nectar guides” were characterised by higher density of secretory hairs than that on the rest of the petal surface, which implies that these petal fragments may emit stronger fragrance.

Key words: *Aesculus hippocastanum*, sepals, petals, ovary, hairs, characteristics, histochemistry

INTRODUCTION

The horse chestnut *Aesculus hippocastanum* L. (Sapindaceae) is a representative of medicinal and honey-bearing plants. It is also highly valued for its decorative features [1–3].

The chestnut flowers, fruits, seeds, bark, and leaves are a source of the glycoside esculin, which seals capillary walls, reduces capillary wall fragility, and improves their flexibility [4–6]. The chestnut raw material was found to contain flavonoids, tannins,

polyphenolic acids, sugars, and a toxic triterpene saponin – escin [1,5,7]. Chestnut inflorescences, which are the raw material, are harvested at the full bloom stage. Next, individual flowers are separated and dried, and thus weakly-scented raw material is obtained [1].

The chestnut pollen and nectar provide *Apis mellifera* L., *Bombus* sp., and *Osmia rufa* L. with nourishment [8]. The pollen of the species contains saponin, which is harmful to bees. Insects are likely to be intoxicated when chestnut trees are the primary source of pollen in a given area [3,9].

Diagonal zygomorphy, characteristic for many representatives of Sapindaceae, is observed in *A. hippocastanum* flowers [10]. Individual flowers are horizontally arranged in the inflorescence [11]. They are composed of free dimorphic petals (4–5). The two upper petals are narrower than the lateral two [12]. The adaxial surface of the petals bears yellow or carmine spots – nectar indicators (nectar guides), which are larger on the upper petals. The nectar guides in *A. hippocastanum* smell more intensely than the other corolla parts, which is important in pollination ecology [10,11].

Forest et al. [12] report the presence of densely distributed, different coloured (red, yellow, white) glandular trichomes in the representatives of the genus *Aesculus*. The trichomes are composed of a multicellular stalk and a multicellular head. In contrast, Hardin [13,14] describes unicellular and multicellular hairs on *A. hippocastanum* leaves. Multicellular mechanical trichomes and glandular trichomes were detected by Weryszko-Chmielewska and Haratym on the surface of leaf veins in this species [15,16]. The adaxial surface of chestnut bud scales bears collectors secreting a mixture of terpenes and mucilage [17,18].

The aim of the study was to determine the types of hairs present on the surface of the sepals, petals, and ovary in *A. hippocastanum* flowers. Since the flower structures in this species contain secondary metabolites with pharmacological activity, knowledge of the morphology and properties of secretory structures may help to find the location of bioactive substances. The presence of lipids in the hairs was detected with two histochemical tests. The distribution of the hairs on the surface of the nectar guides and on the remaining petal surface was compared.

MATERIALS AND METHODS

In 2011–2012, the micromorphology of trichomes present on the surface of the elements of the perianth and the ovary was investigated in *Aesculus hippocastanum* L. Trees from which the flowers were collected were growing in the Centre of Lublin. The surface of the epidermis was observed using light (LM), fluorescence (FM), and scanning electron (SEM) microscopy.

Stereoscopic microscope. Preliminary observations of perianth and pistil ovary hairs were performed with the use of a stereoscopic microscope coupled with a NIKON COOLPIX 4500 photographic camera.

Light microscope. Cross-sections were manually made from fresh and ethanol-fixed (70%) elements of the perianth (sepals, corolla petals, and ovary). In histochemical tests, the sections were treated with the Sudan red reagent staining lipids red and Nile blue staining acidic lipids blue and neutral lipids pink [19–21]. Epidermal cells were observed under a Nikon Eclipse E400 light microscope.

Fluorescence microscope. Hand-made sections of fragments of fresh corolla petals were embedded in a drop of 0.01 % auramine O fluorochrome [22]. The preparations were observed under a Nikon Eclipse 90i microscope equipped with a FITC filter (excitation light 465–495 nm) and a barrier filter (wavelength 515–555 nm).

Scanning electron microscope. Fragments of the investigated flower elements were sampled in the initial phase of flowering. The plant material (sepals, corolla petals, pistil ovary) was fixed in 4% glutaraldehyde for 6 hours and in 0.1 M phosphate buffer pH 7.0 at 4°C for 24 hours. The fixed plant samples were dehydrated in a series of increasing acetone concentrations. After critical-point drying in liquid CO₂, the samples were coated with gold in an EMITECH K550X sputter-coater. The observation and documentation of the epidermis surface were performed under a TESCAN VEGA II LMU scanning electron microscope (SEM). The length of nine types of hairs growing on

the epidermis of sepals, corolla petals, and the ovary ($n = 15$) was measured.

RESULTS

Numerous hairs are visible on the elements of the *Aesculus hippocastanum* flowers (sepals, corolla petals, pistils, and stamens) both in the early and late anthesis phases (Fig. 1A). The hairs differ in their morphological characteristics and represent a few types. No capitate trichomes were found on the sepals and petals; in turn, only short, conical, or elongated hairs were visible on these elements. Our preliminary histochemical analysis suggests that they may be classified as secretory hairs (Fig. 1G). Multicellular globular hairs – colleters were observed on the ovary (Figs 1C–E, 5A, C–F). Three types of hairs were distinguished on the sepals and ovary, and 2 types of hairs and papillae were visible on the petals (Table 1).

Sepals. The hairs growing on the sepals differ in their length, size, number of cells, and distribution (Fig. 2A–D). We have classified them into three different types (Table 1).

Type A is represented by viable highly elongated 3–5-celled hairs. They are only present on the adaxial surface of the sepals and exhibit considerable variation in length (Table 1). Shorter hairs are usually erect, while longer ones are crooked or spirally twisted (Fig. 2B, C).

Type B comprises subulate or cylindrical trichomes composed of 1–3 viable cells with a varied length (Fig. 1G, 2A, D, Table 1). The hairs densely cover the abaxial surface of the petals (Fig. 2A). Granular or elongated outgrowths are visible on the surface of the hair cell walls (Fig. 2D).

Type C is represented by the shortest, conical, unicellular trichomes with a bulbous portion in the basal part (Fig. 2B, C, Table 1). A secretion was observed on the surface of many of these hairs growing on the adaxial and abaxial side of sepal epidermis.

The histochemical analysis performed using Sudan red revealed the presence of lipids, which was visualised by red staining in all the types of sepal trichomes. In addition, the treatment of sepal tissues with the Nile blue reagent stained all the trichomes blue, which confirms the presence of lipid compounds.

Petals. Fig. 3 and Table 1 show the types of trichomes present in the corolla petal epidermis. Within the coloured nectar guide, we observed higher density of hairs in the lower part of the adaxial surface of the petals (Fig. 3A). The trichomes were aligned in compact rows along the vascular bundles (Fig. 3A, C). The longest hairs grew at the petal margins (Fig. 1A, B, E, 2A, B).

Type D comprises viable subulate or cylindrical 1–3-celled trichomes. The outer walls of the hair cells are covered by granular or elongated outgrowths (Fig. 3D, E). A secretion was visible on some trichomes on both sides of the petal.

Type E is represented by viable, short, unicellular, cylindrical or oval hairs (Fig. 3E, G, H; Table 1). Remains of secretion were typically observed on these hairs, and their cell walls exhibited outgrowths.

Type F comprises conical or oval papillae located on the adaxial and abaxial epidermis of the petals (Figs 1H, I, 3 E–G). The highest papillae were found in the central part of the petals. Cuticle striation was observed on the surface of the papillae (Fig. 3F, G).

The histochemical assays applied (Sudan red, Nile blue) allowed detection of lipid substances in both types of petal trichomes and papillae (Fig. 1I). Similarly, fluorescence microscope images show fluorescence of auramine O-treated secretion and de-

monstrate secretory activity of the above-mentioned structures (Fig. 1H).

Pistil. Both the ovary and the style bear trichomes (Figs 1C, D, 4A–F). Three types of hairs were identified on the ovary surface (Table 1).

Type G are colleters classified as glandular trichomes and composed of a multicellular head on a long multicellular stalk (Figs 1D, E, 4C–F). They are the longest hairs growing of the ovary surface (Table 1).

Type H is represented by viable subulate 1–3-celled hairs with a varied length (Table 1, Fig 4 C–F). Cell wall outgrowths are visible on the surface of these trichomes (Fig. 4E).

Type I comprises viable short trichomes with a cylindrical shape and varied length (Fig. 4D, Table 1). Their apical part was bulged, probably due to secretion accumulating under the cuticle.

Likewise in the case of sepal and petal trichomes, the histochemical analysis revealed presence of lipids in all the types of ovary trichomes.

Table 1
Characteristics of trichomes
in the *Aesculus hippocastanum* flowers

Part of flowers	Type of trichomes	Length (μm)	Features of trichomes
Sepal	A Elongate, thin	130–990	Long, straight or crooked, twisted, serpentine-like, 3–5 cellular
	B Subulate or cylindrical	40–140	With long, gradual taper, 1–3 cellular
	C Conical	18–40	Shot, unicellular, with a bulbous portion in the base
Petal	D Subulate or cylindrical	260–780	Long, sharp ended or rounded, 1–3 cellular
	E Cylindrical or oval	66–80	Short, unicellular
	F Papillae	21–40	Rounded or conical
Ovary	G Colleters	360–830	With a multicellular head on a long multicellular stalk
	H Subulate	85–620	Long, sharp ended, 1–3 cellular
	I Cylindrical	10–26	Short, round ended, unicellular

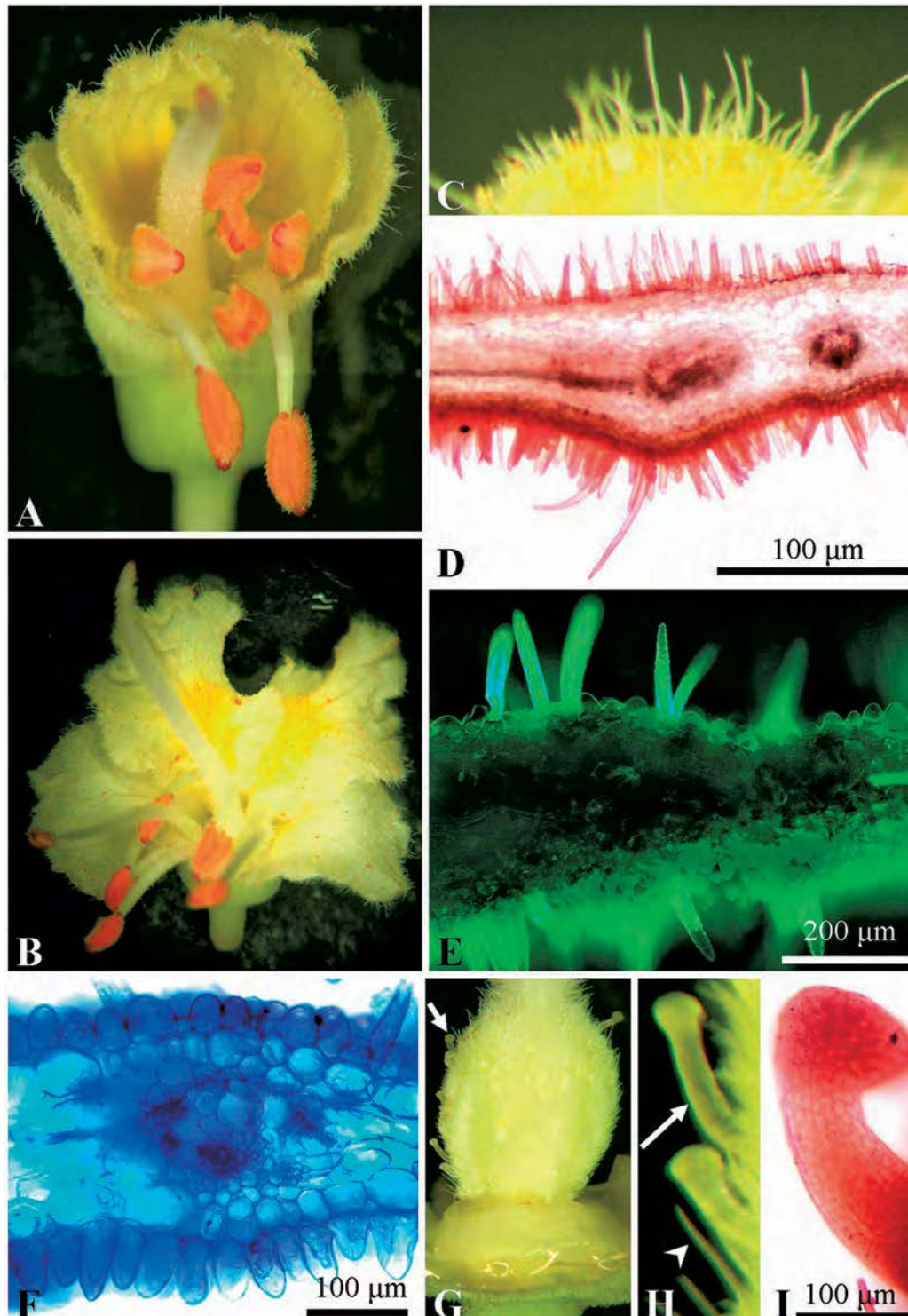


Fig. 1. Flowers and flower parts of *Aesculus hippocastanum* (LM and FM):

A, B – flowers at different development stages

C – trichomes in the apical part of a petal (LM)

D – cross section of a sepal with visible trichomes, after treatment with Sudan Red

E – part of a cross section of a petal, visible fluorescence of the epidermal papillae and hairs after treatment with auramine O

F – part of a cross section of a petal with papillae on the adaxial and abaxial surfaces, after treatment with Nile blue

G – ovary with visible hairs on its surface (arrow)

H – different types of hairs on the ovary surface: colletters (arrow) and subulate hairs (arrowhead)

I – colletter after treatment with Sudan Red.

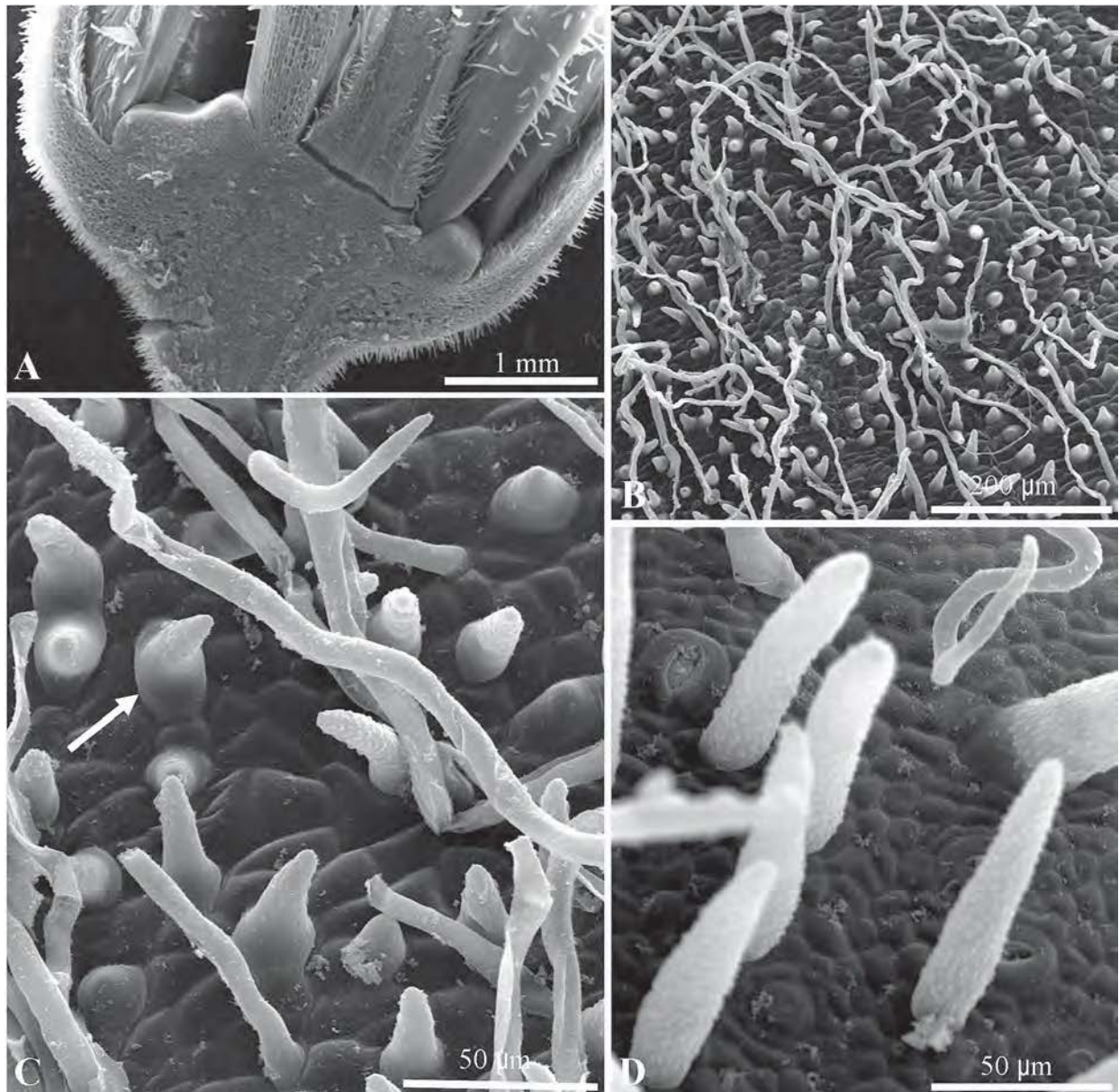


Fig. 2. Part of a longitudinal section of a flower and the sepal epidermis with hairs in *Aesculus hippocastanum* (SEM):

- A – hairs on different parts of a flower viewed in longitudinal section
- B – different types of hairs on the adaxial sepal surface
- C – long curly hairs and conical hairs with the bulbous portion in the basal part (arrow)
- D – hairs on the abaxial sepal surface.

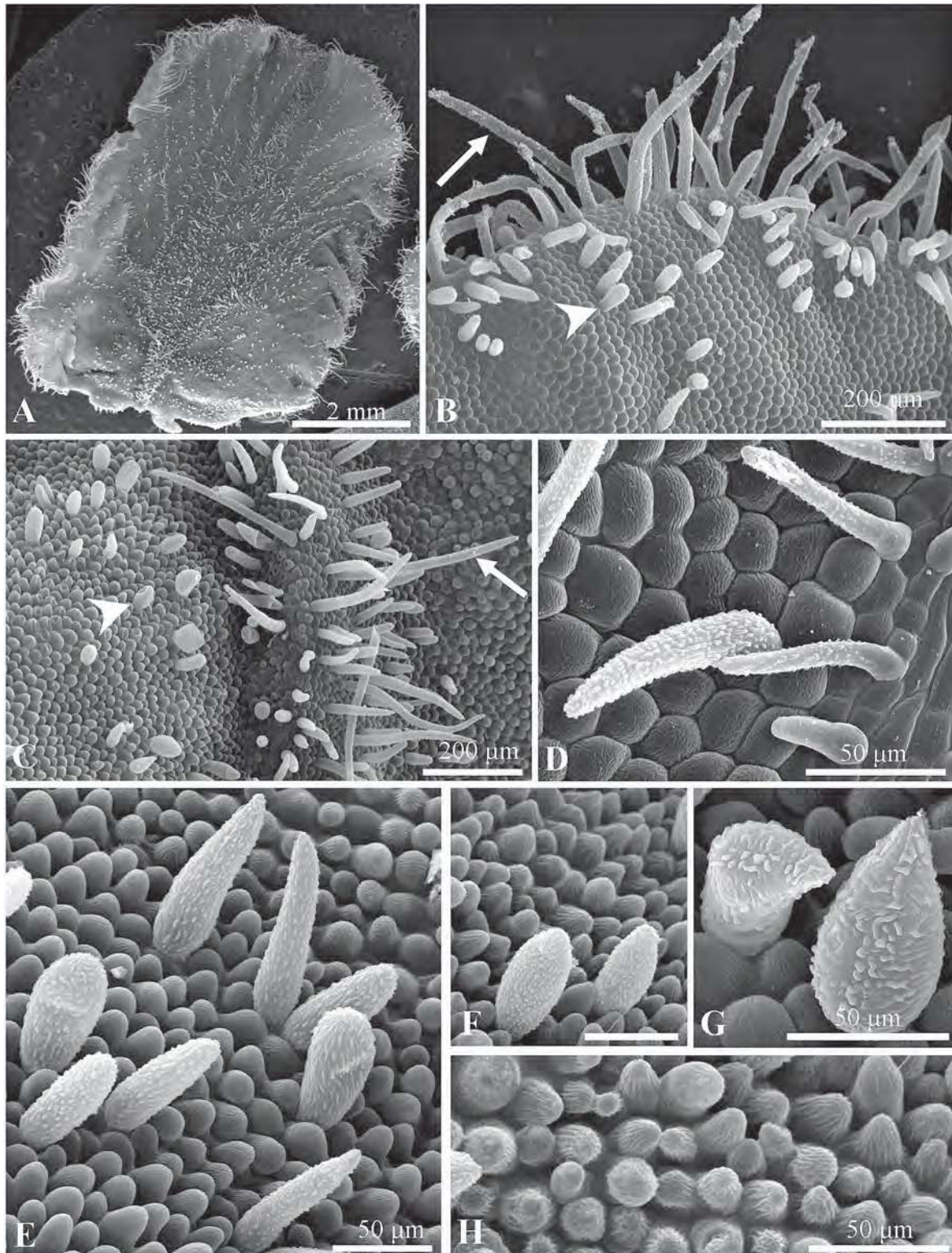


Fig. 3. Petals of *Aesculus hippocastanum* (SEM):

- A – petal with hairs on the adaxial surface, visible is a larger concentration of trichomes along the veins and in the central part, at the place of a coloured spot
- B, C – subulate (arrow) and capitate (arrowhead) hairs as well as papillae on the adaxial petal surface
- D–G – hairs and papillae on the adaxial surface
- H – papillae in the adaxial petal epidermis.

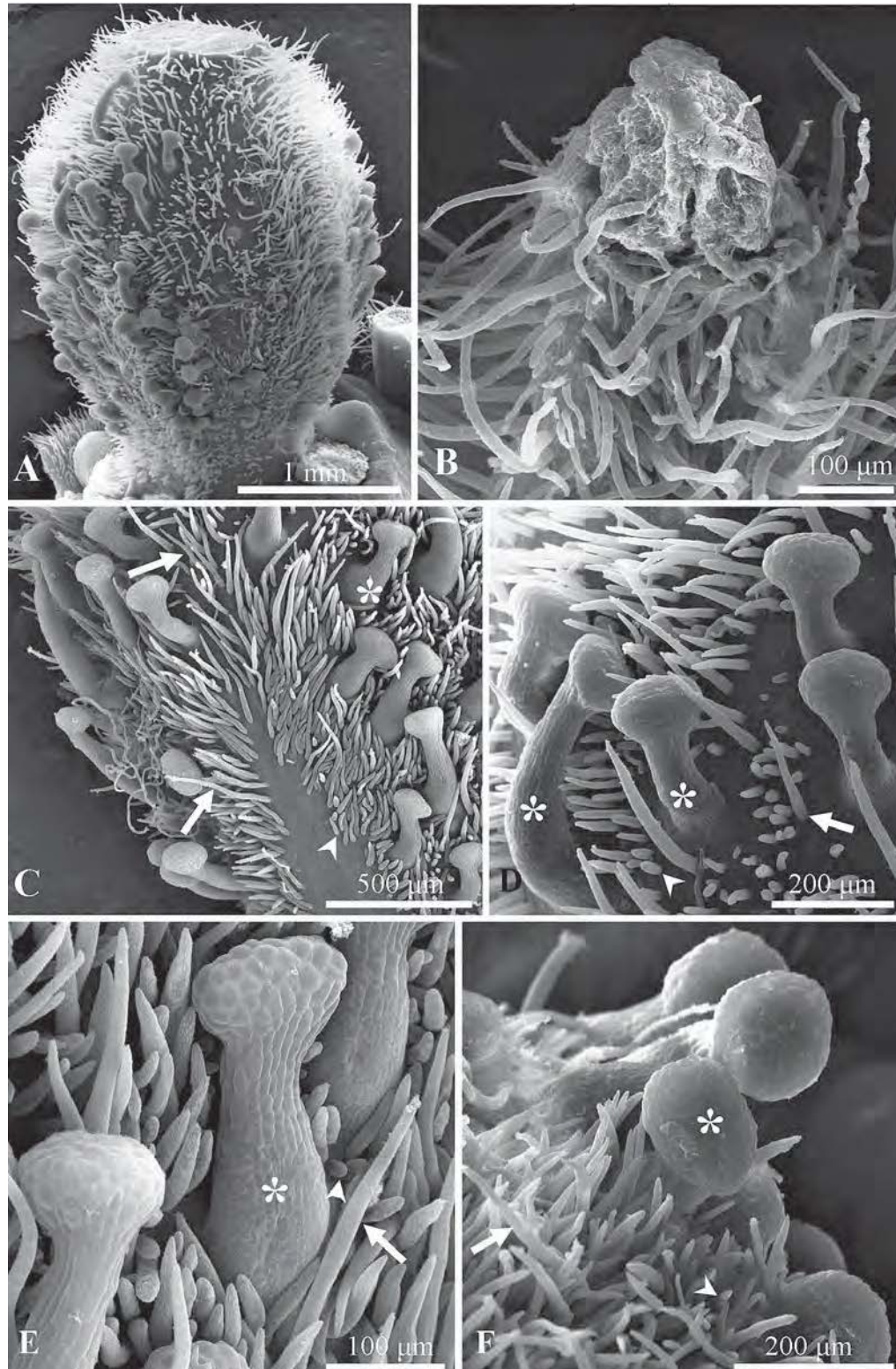


Fig. 4. Parts of a pistil of *Aesculus hippocastanum*:
 A – different types of hairs on the ovary surface
 B – elongated hairs on the style
 C–F – multicellular glandular collectors (arrows), subulate hairs (arrows) and short secretory hairs (arrowhead).

DISCUSSION

No capitate trichomes, usually observed in plants from other families e.g. Lamiaceae [23–27], Boraginaceae [28], and Verbenaceae [29], were found in *Aesculus hippocastanum* flowers. In turn, unicellular and multicellular subulate or cylindrical hairs were found on the *A. hippocastanum* sepals and corolla petals. Their cells exhibited lipid content in the histochemical assays and fluorescence of cell components under the fluorescence microscope. On the fresh flower petals, we did not find clearly coloured, densely distributed glandular trichomes reported in the genus *Aesculus* by Forest et al. [12].

The described subulate and cylindrical hairs growing in *A. hippocastanum* flowers are similar to trichomes observed previously on the leaves of this species [15,16]. However, they were located only on the abaxial surface of the leaf blades.

Our observations of the distribution of trichomes in the area of the “nectar guides” on the upper horse chestnut petals indicate that the density of trichomes within these coloured spots is higher than that on the other parts of the petals. We have shown that the trichomes are viable and exhibit secretory activity (secretion on the hair surface and positive results of the histochemical tests), which confirms previous findings of other authors who report that these fragments of *A. hippocastanum* petal give off a more intensive odour [10,11].

On the *A. hippocastanum* ovary, we found numerous massive colleters producing a long multicellular stalk and a multicellular head. These secretory structures were intensely stained red and blue with Sudan red and Nile blue, respectively. Particularly intense staining was visible in the centrally located colleter head and the basal part of the stalk, which implies a high concentration of lipids in these areas. Colleters present on bud scales in this species were described by Charriere-Landreix [17] and Hejnowicz [18]. However, the bud scale colleters were characterised by a different structure with a very short stalk and a substantially enlarged head.

The epidermis of both surfaces of the chestnut petals exhibited densely arranged papillae containing lipid compounds. The Sudan red reagent applied as a histochemical assay indicates the presence of essential oils as well. The positive reaction (red staining) to Sudan red confirms the presence of essential oils in these structures. In many other plant species, papillae present in petal epidermis are sites of fragrance emission [24,30–33].

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Authors' contributions

Concept of the study: EW-C; microscopical analysis: EW-C, MC; writing of the manuscript: EW-C, MC, AS; photographs: AS, MM.

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Mikromorfologia włosków w kwiatach kasztanowca *Aesculus hippocastanum* L.

Streszczenie

Aesculus hippocastanum L. jest cenionym drzewem ozdobnym ze względu na piękne kwiaty i liście. Kwiaty tego gatunku zawierają wtórne metabolity wykazujące aktywność farmakologiczną. Wytwarzają również olejki eteryczne oraz barwne „nectar guides”, które ułatwiają owadom dotarcie do nektaru i pyłku. Celem pracy było zbadanie typów i właściwości włosków występujących w kwiatach kasztanowca, w których mogą być zlokalizowane substancje biologicznie aktywne. Badania przeprowadzono przy użyciu mikroskopii świetlnej, fluoroescencyjnej i skaningowej elektronowej. Stwierdzono, że na działkach kielicha oraz na powierzchni zalążni występują po trzy typy włosków, natomiast na płatkach korony dwa typy włosków oraz papille. Włoski te różnią się długością i liczbą tworzących je komórek. Na okwiecie i słupku nie znaleziono włosków główkowatych, natomiast na zalążni stwierdzono obecność koleterów.

Z zastosowania testów histochemicznych wynika, że wszystkie rodzaje włosków i papille zawierają lipidy lub olejki eteryczne, należą zatem do struktur wydzielniczych. Na powierzchni „nectar guides” obserwowaliśmy większe zagęszczenie włosków wydzielniczych niż na pozostałej powierzchni płatków, co wskazuje, że te fragmenty płatków mogą emitować silniejszy zapach.

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