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MICROMORPHOLOGICAL AND QUANTITATIVE CHARACTERISATION OF LEAVES OF WILD GROWING *PYRUS COMMUNIS* L. (ROSACEAE)

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Abstract

The leaf surface micromorphology of wild *Pyrus communis* L. was investigated using light and scanning electron microscopy. Secretory structures (colleters) were identified on the apical and lateral teeth of leaf blades and stipules. Cuticular folds were present on both the surfaces, more developed near stomata and veins. Leaves are hypostomatic, with stomata approximating the stephanocytic type. Leaves of *P. communis* exhibited stomatal dimorphism even at the intrabud stage, featuring larger primary stomata surrounded by smaller secondary stomata. Leaf size and shape varied considerably across the specimens from southern Russia, the Caucasus, and Crimea. Factor analysis revealed two main groups of correlated variables: one related to leaf size (petiole length, blade length and width) and another to leaf morphology (apex elongation, margin, and blade shape). The study revealed novel micromorphological characteristics with diagnostic potential for discriminating closely related *Pyrus* species.

Key words: Micromorphology; Scanning electron microscopy CryoSEM; Colleters; Cuticular folds; Stomatal dimorphism

Introduction

The study of micromorphological structures of plant surfaces in search of additional taxonomic and diagnostic characters are particularly relevant for taxa whose ranks have been repeatedly revised in recent years due to molecular phylogenetic evidence. The family *Rosaceae* is one such example (Potter *et al*., 2007; Takhtajan, 2009; Phipps, 2014, 2016; Xiang *et al*., 2017; Wang *et al*., 2024; Jin *et al*., 2024). Various micromorphological characteristics of leaf surfaces, such as specific wax deposits and folded microrelief, heterogeneity of stomatal complexes, and types of trichomes (including colleters), are considered valuable diagnostic features for distinguishing taxa of different ranks within *Angiospermae* (Carr and Carr, 1990; Fontenelle *et al*., 1994; Zamani *et al*., 2017; Ullah *et al*., 2021; Bahadur *et al*., 2023; Choi *et al*., 2023; Song *et al*., 2024; Idrees *et al*., 2025).

Wild *Pyrus communis* L. is a key progenitor of the cultivated European pear (Challice & Westwood, 1973; Quinet & Wesel, 2019) and is widely used in horticulture as a rootstock. According to the POWO database, there are numerous synonymous for P. *Communis* L. including: *Malus communis* (L.) Poir.; *Pyrus asiae-mediae* Popov; *Pyrus balansae* Decne.; *Pyrus bourgaeana* Decne.; *Pyrus communis* subsp. *bourgaeana* (Decne.) Nyman; *Pyrus communis* var. *mariana* Willk.; *Pyrus domestica* Medik.; *Pyrus elata* Rubtzov; *Pyrus medvedevii* Rubtzov.

Hybridisation is widespread among *Pyrus* L. species, with many *P. communis* samples exhibit hybrid characteristics resulting from crosses with local European pear species such as *P. nivalis* Jacq. (Aldasoro *et al*., 1996; Paganová, 2003). The genetic diversity of wild *P. communis* has been extensively studied by Volk *et al*., (2006). Edible European pears (*P. communis* subsp. *communis*) originated from wild relatives found in the mountainous regions of the Caucasus and Eastern Europe. The pedigree of numerous cultivated *P. communis* subsp. *communis* varieties includes hybrids between wild subspecies of *P. communis* and *P. nivalis* (snow pear).

Pyrus communis subsp. *pyraster* and *P. communis* subsp. *caucasica* exhibit remarkable phenotypic similarity (Aldasoro *et al*., 1996). Historically, these subspecies have been primarily distinguished based on their geographical distribution. *P. communis* subsp. *pyraster* is native to western regions and south of the Black Sea (including the Balkans, Turkey, and other European countries), whereas wild forms originating from the Caucasus Mountain region (southwestern Russia, Crimea, Georgia, Armenia, and Azerbaijan) are classified as *P. communis* subsp. *caucasica*. Genetic analyses have revealed significant differentiation between these wild subspecies, which are recognized as ancestors of cultivated European pears (Volk *et al*., 2006; Korotkova *et al*., 2018). This genetic distinctiveness supports the classification of *P. communis* as a distinct species within the genus *Pyrus*.

Currently, *P. communis* is classified within the genus *Pyrus*, subtribe *Malinae* (formerly *Pyrinae*, *Maloideae*) of the family *Rosaceae* (Campbell *et al*., 2007; Potter *et al*., 2007; Takhtajan, 2009; Phipps, 2014; Xiang *et al*., 2017; Korotkova *et al*., 2018; Wang *et al*., 2024; Jin *et al*., 2024). As part of the European *Pyrus* group, *P. communis* naturally occurs across Europe (including the Caucasus), North Africa, Asia Minor, Central Asia, Iran, and Afghanistan (Fiala *et al*., 2024). In Europe, these trees can thrive at elevations of 1800–

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2000 meters above sea level (Zhukovskiy, 1971). The Caucasus region, along with Asia Minor (including the Caucasus, Iran, and Turkmenistan), serves as the center of diversity for wild *P. communis* (Vavilov, 1931). According to him N.I. Vavilov, the diverse habitats found in the Caucasus Mountains contribute to the rich genetic variability of these plants.

Wild *Pyrus communis* (2n=34) is a long-lived deciduous tree (with individuals living 150–300 years), reaching heights of 20–30 meters. Characterized by a spreading, broad, irregular crown that can be broadly pyramidal or rounded, and dark bark, it features erect branches with numerous short prickles. The leaves are large, slightly glossy, simple, equipped with deciduous or persistent linear-lanceolate stipules and long petioles. In natural habitats, *P. communis* predominantly grows on plains, in deciduous and occasionally coniferous forests, and forest steppes, often forming pure stands.

Despite the informative nature of genetic studies, detailed micromorphological analysis of vegetative organs remains underexplored yet essential area for diagnostic and phylogenetic research. This study aimed to investigate the micromorphological and quantitative characteristics of leaves in wild *Pyrus communis* and to assess their potential for providing additional diagnostic traits to differentiate species within the genus *Pyrus* L.

Materials and Methods

Morphometric data: Leaf blades and stipules from wild *Pyrus communis* L. were studied. Fresh samples (partially and fully unfolded young leaves, defined as 50–80% of mature leaf size) were collected in triplicate from the mid-crown of trees in the N.V. Tsitsin Main Botanical Garden arboretum (April–June). Additionally, herbarium specimens from the Lomonosov Moscow State University were examined (Table 1). Ten typical leaves per specimen were selected for morphometric analysis to ensure robust statistical evaluation.

Microscopy: Fresh and herbarium samples were examined using a Keyence VHX-1000 E digital microscope. For scanning electron microscopy (SEM), 1 cm² segments from leaf margins (including teeth) and interveinal areas were mounted on copper plates using thermal paste (Ryabchenko & Babosha, 2012). Fresh samples were examined via cryo-SEM (Deben Cool stage) at -25 to -30°C. Herbarium material (MW 0 677 990) was sputter-coated with silver in a cathodic sputtering chamber (Bal-Tec SCD 005, Balzers, Liechtenstein). The samples were examined using a LEO-1430 VP SEM at 20 kV. Stomatal dimensions and density were measured from micrographs using ImageJ 1.48v.

Morphometric analysis: Digital images of herbarium specimens were used to measure leaf blade length (L), half-width (W), and petiole length (P). The ratio L/2W was calculated. Qualitative traits (leaf blade, apex, base, margin) were scored on a scale of 1–3 (Table 2).

Images of herbarium specimens were used to assess the shape of the leaf blade apex, base and margin, and the shape of the leaf blade as a whole. The results were presented as scores from 1 to 3. The leaf shape variable 'leaf' took values for ovate (1), oval or broadly ovate (2) and obovate (3) leaves depending on the degree of displacement of the greatest leaf width from base to apex. The apex shape variable assessed the degree of elongation of the apex as rounded (1), acuminate (2), cuspidate or elongated-cuspidate (3). The base shape variable assessed the degree of elongation of the base as heart-shaped or rounded (1), cuspidate (2) and acuminate (3). The degree of pronounced denticles (variable 'line') distinguished leaves with entire (1), crenate (2) or serate (3) margins.

Statistical analysis

Statistical calculations were performed using jamovi 2.3.28 (ANOVA, correlation, factor analysis, GLM mediation analysis), PAST 4.03 (principal component analysis), and MS Excel (descriptives and simple tests). Results are reported as mean ± SE. Statistical significance was set at $p<0.05$.

Table 1. Herbarium specimens of *Pyrus communis* L. used in the work.

No.	Region	Collection sites	Voucher
1.	Volgograd region	Kalachevsky steep left bank of the Goluboy river near the mouth of the Sukhaya Golubaya gully	MW 1 052 628
2.	Georgia	Adigen district, ascent to Godersky pass. Deciduous forest	MW 0 677 992
3.	Georgia	Gombor Pass, left. Bank of the York River. Beech and hornbeam forest	MW 1 010 623
4.	Kalmykia	Neighbourhood of Obilnoye village, Kamennaya gully	MW 1 064 964
5.	Karachay-Cherkessia	t. Cherkessk, Green Island Park on soot	MW 0 677 991
6.	Krasnodar Krai	Anapa district, Black Sea coast. Cape Malyi Utrish area, mouth of Lobanova Gap. Forest edge of a hornbeam forest	MW 0 677 990
7.	Krasnodar Krai	Taman Peninsula, Oak Market tract, north-eastern slope, oak woodland.	MW 0 677 989
8.	Krasnodar Krai	Gelendzhik district, 1 km south-east of Afonka farm. Beech and hornbeam forest	MW 1 006 992
9.	Crimea	Karadag. Along the northern slope of the pass between Planerskaya and Karadag biostation. Forest of downy oak	MW 0 612513
10.	Crimea	Eastern slope of Chatyrdag. Young rare hornbeam-beech forest	MW 0 612514
11.	Crimea	Eastern Crimea, Sudak neighbourhood. Sudak valley. On the bank of the Suuk-su river	MW 0 612535
12.	Crimea	Between Chatyrdag and Tyrke, along the watershed ridge between Salgir and Demerdzhi	MW 0 612 536
13.	Crimea	Kuibyshev district, ascent from Sokolinoye to Ai-Petri. Forest edge of hornbeam-beech forest	MW 0 612 538
14.	Crimea	Eastern Crimea. Karaden. Forest between Syuryu-kai and Legener	MW 0612 509
15.	Crimea	Chatyrdag State Reserve. Western slope, along the road to the Middle Plateau	MW 0612 517
16.	Crimea	Eastern Crimea, vicinity of the village. Gromovka. Western slope of Kuchla. Rake thickets	MW 0612 528
17.	Crimea	Eastern Crimea, vicinity of the village. Gromovka. Among thickets of hornbeam on mountain slopes	MW 0612 529
18.	Rostov region	The neighbourhoods of Taganrog and Novocherkaska	MW 0 384 747
19.	Rostov region	Between N.-Kurmoyarskaya and Nachavskaya st. Thorn bushes on a hillock in the Don floodplain	MW 0 384 748
20.	Rostov region	The vicinity of the village. Zavetnoe. Forest plantation	MW 0 384 749

Table 2. Morphological characteristics of leaf blades of herbarium specimens of *Pyrus communis* L.

Voucher	Leaf shape (Leaf)	Apex shape (Apex)	Base shape (Base)	Leaf edge (Line)	Leaf length (L), cm	1/2 Leaf width (W), cm	Petiole length (P), cm	Length to width ratio (L/2W)
MW 1 052 628	Oval	Acuminate	Rounded	Crenate	3.7 ± 0.2	1.6 ± 0.11	3.4 ± 0.5	1.17 ± 0.04
MW 0 677 992	Oval	Acuminate	Acuminate	Crenate	6.5 ± 0.3	2 ± 0.07	3.7 ± 0.4	1.63 ± 0.05
MW 1 010 623	Ovate	Elongated-cuspidate	Rounded	Crenate	3.7 ± 0.2	1.26 ± 0.06	2.2 ± 0.1	1.48 ± 0.03
MW 1 064 964	Oval	Cuspidate	Cuspidate	Serrate	5.8 ± 0.9	1.6 ± 0.05	5.5 ± 0.4	1.85 ± 0.34
MW 0 677 991	Obovate	Acuminate	Acuminate	Crenate	5.9 ± 0.1	1.88 ± 0.06	3.3 ± 0.5	1.56 ± 0.03
MW 0 677 990	Ovate	Cuspidate	Acuminate	Serrate	5.5 ± 0.3	1.67 ± 0.08	2.4 ± 0.5	1.63 ± 0.03
MW 0 677 989	Ovate	Cuspidate	Rounded	Crenate	6.2 ± 0.5	1.99 ± 0.19	2.8 ± 1.1	1.6 ± 0.12
MW 1 006 992	Ovate	Cuspidate	Rounded	Serrate	3.3 ± 0.2	1.28 ± 0.05	1.9 ± 0.2	1.31 ± 0.05
MW 0 612 513	Oval	Acuminate	Acuminate	Crenate	4.5 ± 0.1	1.47 ± 0.05	2.7 ± 0.2	1.54 ± 0.04
MW 0 612 514	Oval	Acuminate	Rounded	Crenate	2.7 ± 0.1	1 ± 0.07	1.3 ± 0.3	1.4 ± 0.09
MW 0 612 535	Ovate	Cuspidate	Rounded	Crenate	4.5 ± 0.1	1.68 ± 0.04	3 ± 0.2	1.34 ± 0.03
MW 0 612 536	Oval	Acuminate	Cuspidate	Crenate	3.5 ± 0.1	1.07 ± 0.05	1.5 ± 0.2	1.67 ± 0.05
MW 0 612 538	Obovate	Rounded	Acuminate	Crenate	5.6 ± 0.1	1.61 ± 0.05	1.7 ± 0.1	1.76 ± 0.1
MW 0612 509	Obovate	Rounded	Acuminate	Entire	4.3 ± 0.1	1.5 ± 0.04	3.2 ± 0.2	1.44 ± 0.04
MW 0612 517	Oval	Acuminate	Rounded	Crenate	4.6 ± 0.3	1.75 ± 0.03	3.3 ± 0.4	1.31 ± 0.09
MW 0612 528	Oval	Acuminate	Rounded	Crenate	4.6 ± 0.2	1.5 ± 0.07	2.8 ± 0.2	1.59 ± 0.11
MW 0612 529	Oval	Acuminate	Cuspidate	Crenate	4.8 ± 0.1	1.6 ± 0.07	3.3 ± 0.2	1.55 ± 0.1
MW 0 384 747	Oval	Acuminate	Heart-shaped	Crenate	4.5 ± 0.2	1.68 ± 0.04	2.8 ± 0.5	1.36 ± 0.06
MW 0 384 748	Oval	Acuminate	Heart-shaped	Crenate	3.2 ± 0.1	1.5 ± 0.05	3.8 ± 0.3	1.07 ± 0.04
MW 0 384 749	Oval	Cuspidate	Cuspidate	Crenate	4.5 ± 0.8	1.19 ± 0.04	1.8 ± 0.1	1.95 ± 0.4

Results

Morphological characterisation of the shoot and leaf of *Pyrus communis* L. Two types of shoots were recognized: elongated (auxiblasts) and shortened (brachyblasts). The auxiblasts shoots had well-spaced and long internodes. Brachyblasts shoots had closely spaced nodes (Fig. 1A).

Leaves are simple, petiolate with persistent stipules (Fig. 1A-C). Mature blades were dark green and glossy adaxially, pale abaxially, sometimes pubescent on veins. Blade shape varied from ovate to obovate; apices were rounded, acuminate, or cuspidate; bases ranged from rounded to cuspidate or elliptic-cuspidate (Table 2). Apical and lateral teeth often bore colleters (Fig. 4C; 5A, C-H). Margins were toothed, less commonly crenate or serrate, rarely entire (in one specimen only). Lateral denticles were acuminate. Long, thin petioles sometimes showed arcuate curvature, while linear-lanceolate stipules with sharply pointed, entire to slightly toothed margins persisted in mature leaves (Fig. 1A, C; 4D-F).

Variation in leaf size and shape: Quantitative leaf parameters varied widely among specimens (Table 2). Mean leaf length of samples varied from 2.7 to 6.5 cm, leaf half-width from 1.3 to 2.0 cm, petiole length from 1.3 to 5.5 cm, and leaf length to width ratio was from 1.1 to 1.95.

Principal component analysis of variables characterizing leaf size and shape revealed three components explaining 79.7% of the total variance. Figure 2 illustrates the distribution of herbarium specimens in the coordinate space defined by the first and second principal components. Specimens were distributed in three distinct groups primarily differentiated by leaf shape, apex characteristics, and margin features. Group 1, located in the upper left quadrant, exhibited ovate leaf shape with cuspidate apex. Group 3, positioned in the lower right quadrant, comprised three specimens characterized by obovate leaves. The distribution patterns of the more abundant groups 1 and 2 showed elongation along the vectors of quantitative variables, indicating a wide and approximately equal range of variation in leaf blade length and width, as well as petiole length within these groups.

Correlation analysis (Table 3) revealed a strong positive relationship between leaf length and width (r = 0.804, $p<0.01$). Both parameters demonstrated moderate correlations with petiole length. However, only leaf width exhibited a direct influence on petiole length, while the effect of leaf length was indirect, primarily mediated through the correlation between leaf length and width (Table 6). Table 3 also showed that the shift of maximum leaf width (position of maximum width) toward the apex in obovate leaves was correlated with the development of a rounded apex and, to a lesser extent, with a more elongated leaf base and a margin featuring less pronounced denticles.

The data in Table 3 indicate a certain dependence of leaf length and shape on base morphology. Analysis of mean values across specimen groups with rounded, cuspidate, and acuminate bases revealed an increase in leaf length and elongation with progressive base elongation from rounded to acuminate forms (Table 7).

Factor analysis results (Tables 4 and 5) identified three principal factors corresponding to these correlations. Factor 1 encompassed correlated variables describing apex elongation, margin characteristics, and overall leaf shape. Factor 2 was formed by variables related to blade size and petiole length. Factor 3 was associated with the blade length-to-width ratio, showing moderate correlations with blade length, base characteristics, margin features, and overall blade shape. Referring back to Figure 2, Factor 1 of qualitative leaf characteristics distinguished groups 1, 2, and 3, while variables of Factor 2 (leaf dimensions) exhibited approximately equal variation, particularly within the more comprehensively represented groups 1 and 2.

Micromorphological structures of the leaf surface of *Pyrus communis* L.

Trichomes and colleters: Leaf structures (leaf blades and stipules) at the intra-bud development stage were covered with numerous long simple (covering) trichomes (Fig. 3A-E). These trichomes formed quite dense pubescence along the margins of the leaf blades (Fig. 3C-E). As the leaves developed, the number of trichomes gradually decreased; they dried out and fell off, leaving traces. On mature leaves, trichomes were observed mainly along the margins in the tooth area and on the petiole (Fig. 5A-D; Fig. 7A).

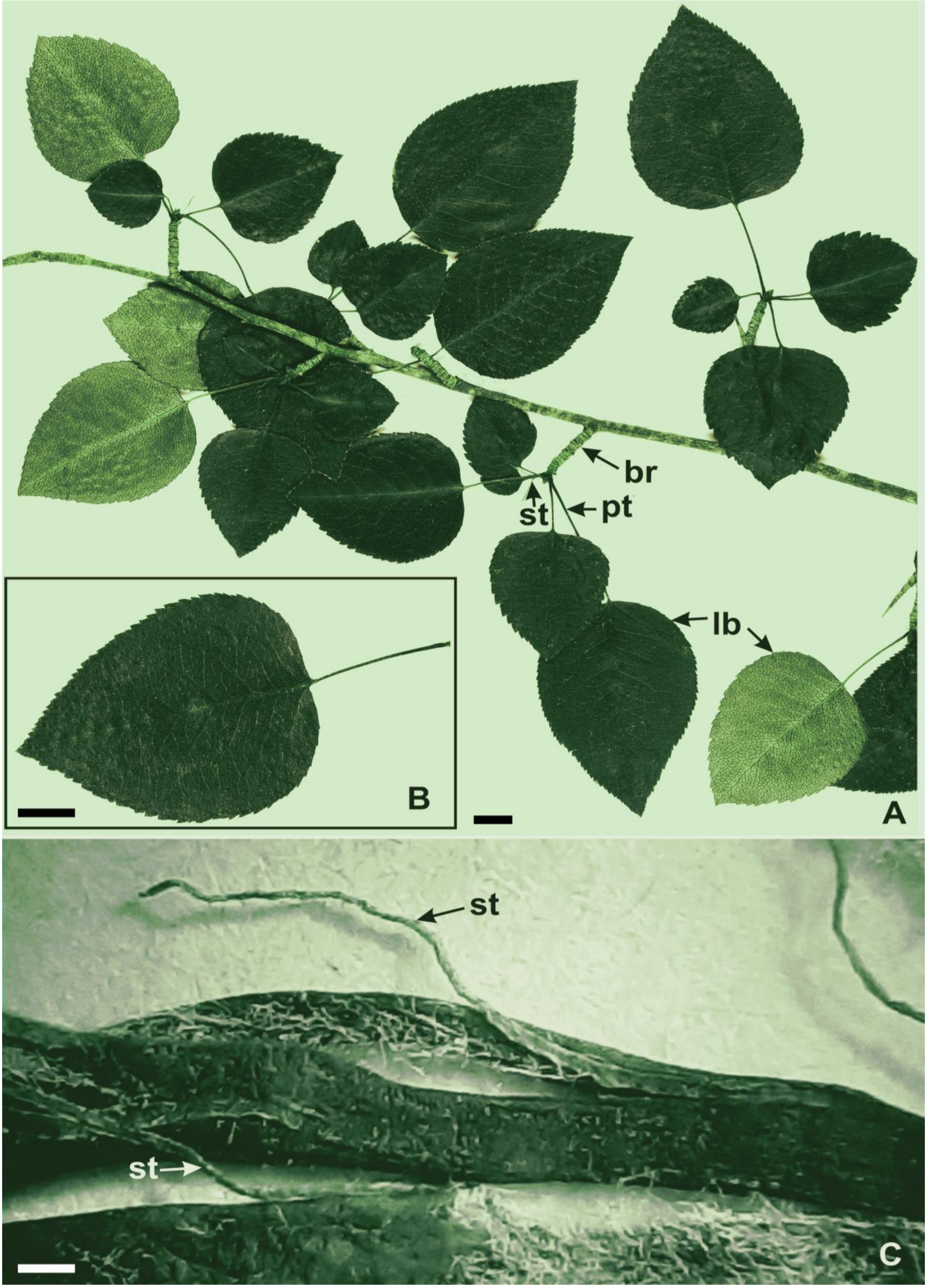

Fig. 1. Morphology of *Pyrus communis* L. shoot (herbarium specimen MW 0 677 990, Keyence light digital microscope): **A** - shoot fragment; **B** - enlarged leaf with serrated margin and petiole; **C** - stipules. Designations: br - brachyblast, lb - leaf blade, st - stipule, pt – petiole. Scale bars: **A, B** – 1 cm; **C** – 1 mm.

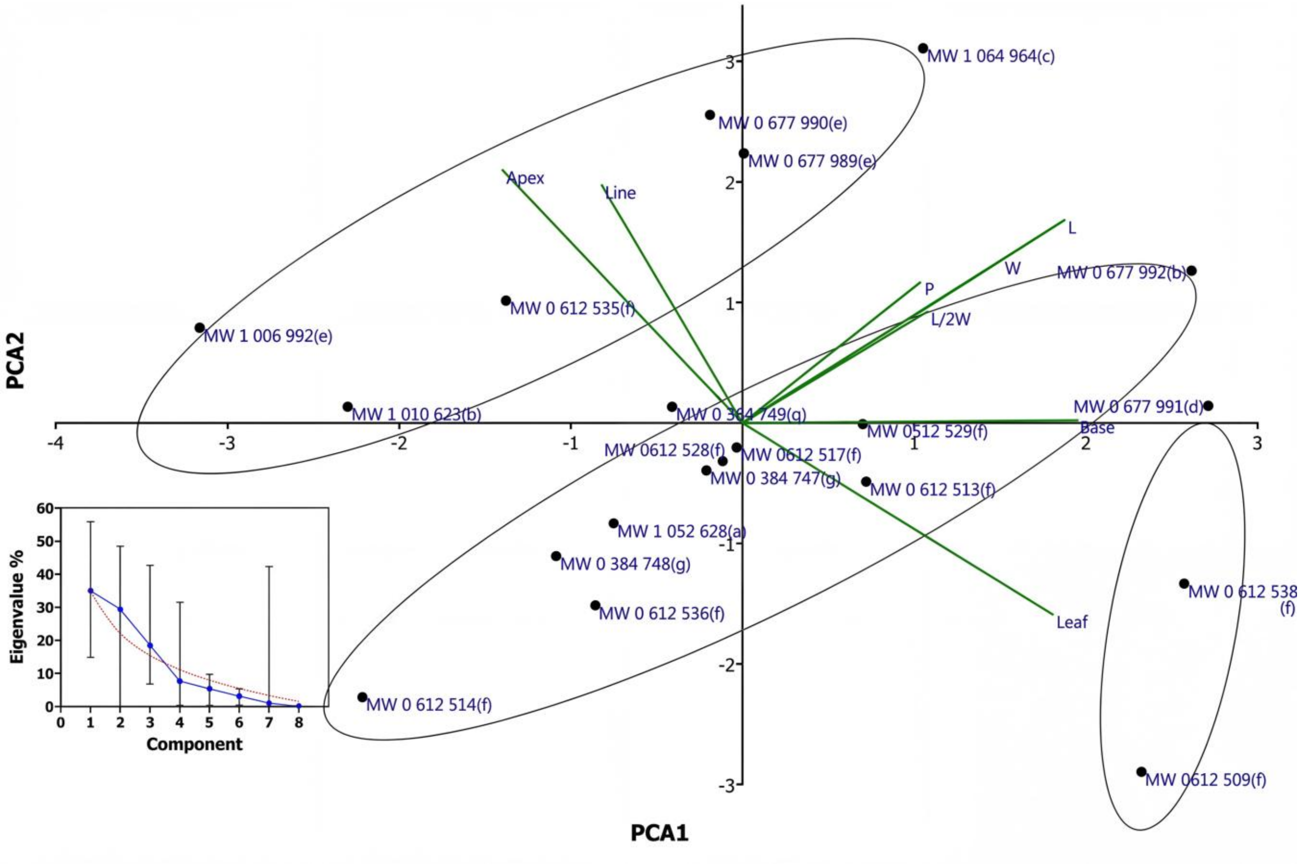

Fig. 2. Principal component analysis of leaf morphology variables. The dots indicate the position of the individual samples in the coordinates of the first two principal components (PCA1 and PCA2). Insets: 'Biplot' projection of the original variables onto a scatterplot (top right) and an eigenvalue plot (top left), the red dashed line indicates the expected eigenvalues in the random model (broken stick). Ellipses highlight groups of similar samples.

Table 3. Correlation matrix of leaf parameters.

	Leaf	Apex	Base	Line	L	W	P	L/2W
Leaf	1							
Apex	-0.836*	1						
Base	0.514	-0.369	1					
Line	-0.514	0.645*	-0.079	1				
L	0.131	0.055	0.529	0.119	1			
W	0.057	-0.042	0.227	-0.015	0.804*	1		
P	0.140	0.046	0.076	0.124	0.433	0.589*	1	
L/2W	0.163	0.150	0.533	0.174	0.566*	-0.027	-0.068	1

Note. Critical values of the Pearson correlation coefficient for n=20: 0.44 ($p<0.05$); 0.56 ($p<0.01$).

* - for these pairs of variables, Spearman and Kendall rank correlation coefficients are also reliable

Table 4. Exploratory factor analysis (factor loadings).

Variables	Factor 1	Factor 2	Factor 3	Uniqueness
L		0.817	0.465	0.00199
W		1.022		-0.02453
P		0.564		0.66968
L/2W			0.911	0.14990
Leaf	-0.815		0.308	0.28209
Apex	0.865			0.23354
Base	-0.314		0.488	0.69825
Line	0.726		0.389	0.26134

Note. The ‘Principal Axis Factorisation’ extraction method was used in conjunction with the ‘oblimin’ rotation

Among the numerous trichomes covering the margins, finger-like projections with green or light brownish contents - colleteres - were observed on leaf teeth (Fig. 3E). In newly developed leaves, colleters were present on apical and lateral denticle tips, exhibiting light green or almost transparent secretions (Fig. 5C, E, D). Stipules also bore colleters: elongated apical structures with light brown content and finger-shaped formations along the perimeter and base (Fig. 4D-F; 5F; 6D-F). SEM analysis revealed that young leaf colleters were multicellular formations actively secreting substances (Fig. 6A-C). In mature fresh and herbarium leaves, colleters occurred on apex and lateral teeth, sometimes in sinuses (Fig. 5H), appearing as reddish-brown callous formations (Fig. 5G, H). Over time, some colleters withered, leaving scars on denticle tops (Fig. 7A-D).

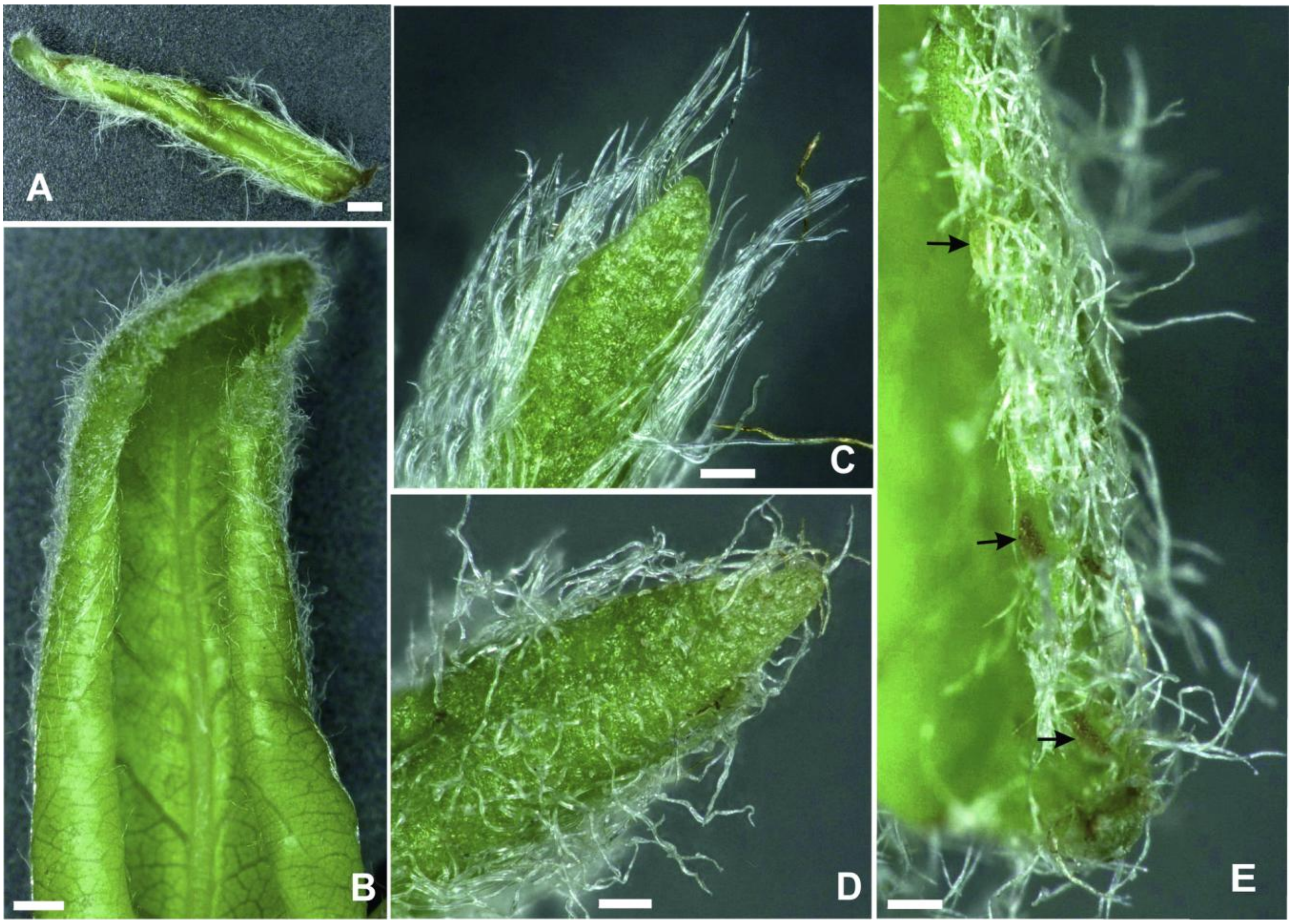

Fig. 3. Bud stage of leaf blade development of *Pyrus communis* L. (Keyence light digital microscope): **A, B** - general view of undeveloped leaf blade covered with trichomes and its apex in enlarged view; **C, D** - apex of leaf blade at the stage of unfolding with trichomes; **E** - edges of leaf blade covered with trichomes with young colleters. The arrow indicates colleters. Scale bars: **A** – 600 µm; **B** – 500 µm; **C, D, E –** 150 µm.

Table 5. Confirmatory factor analysis.

Factor	Variables	Weight	SE	p
1	Leaf	0.535	0.1397	<0 .001
	Apex	-0.592	0.1185	<0 .001
	Line	-0.363	0.0900	<0 .001
	Base	0.241	0.1843	0.192
2	L	0.828	0.1368	< 0.001
	W	0.272	0.0420	<0 .001
	P	0.552	0.1935	0.004
3	L/2W	0.205	0.0348	<0 .001
	L	0.618	0.0939	<0 .001
	Base	0.411	0.1727	0.017
	Line	0.158	0.0762	0.039
	Leaf	0.182	0.1131	0.107

Stomata and cuticular ornamentation: The leaf blade of *P. communis* exhibited hypostomatic characteristics with abundant stomata distributed on the abaxial surface. The adaxial epidermis consisted primarily of pavement cells featuring nearly straight walls, with simple trichomes observed predominantly in young leaves (Fig. 8A). The abaxial epidermis comprised several cell types: pavement cells (constituting the majority of the tissue), stomata, and trichomes. The pavement cells displayed coarsely undulating anticlinal walls, becoming increasingly sinuous with the development of the leaf blade (Fig. 8B, C). The stomata of *P. communis* predominantly exhibited a stephanocytic structure (Fig. 8B). A distinctive feature of mature stomata was the presence of a more or less pronounced rosette formed by 5 or more cells surrounding the guard cells. The guard cells were slightly elevated relative to adjacent epidermal cells.

Stomatal dimorphism was already evident at the bud stage: larger single primary stomata were surrounded by smaller secondary (normal) stomata (Fig. 8 B). The size of secondary stomata in fresh young leaves was slightly smaller than in herbarium leaves. They were 23.6±0.4 and 25.7±0.4 µm long and 16.2±0.5 and 18.0±0.3 µm wide, respectively. In fresh young leaves and mature leaves of herbarium specimens, the proportion of primary stomata was comparable: 16.9±2.3 and 17.4±0.6%. The sizes of primary stomata exceeded those of secondary stomata: length 34.3±1.5 and 29.3±0.5 µm, width 16.5±0.8 and 20.9±0.6 µm in fresh young and mature herbarium leaves, respectively. However, the density of both types of stomata in young leaves was about 2 times lower than in the herbarium material: 78.7±3.9 and 153.0±10.2 stomata per 1 mm^2 of abaxial leaf surface.

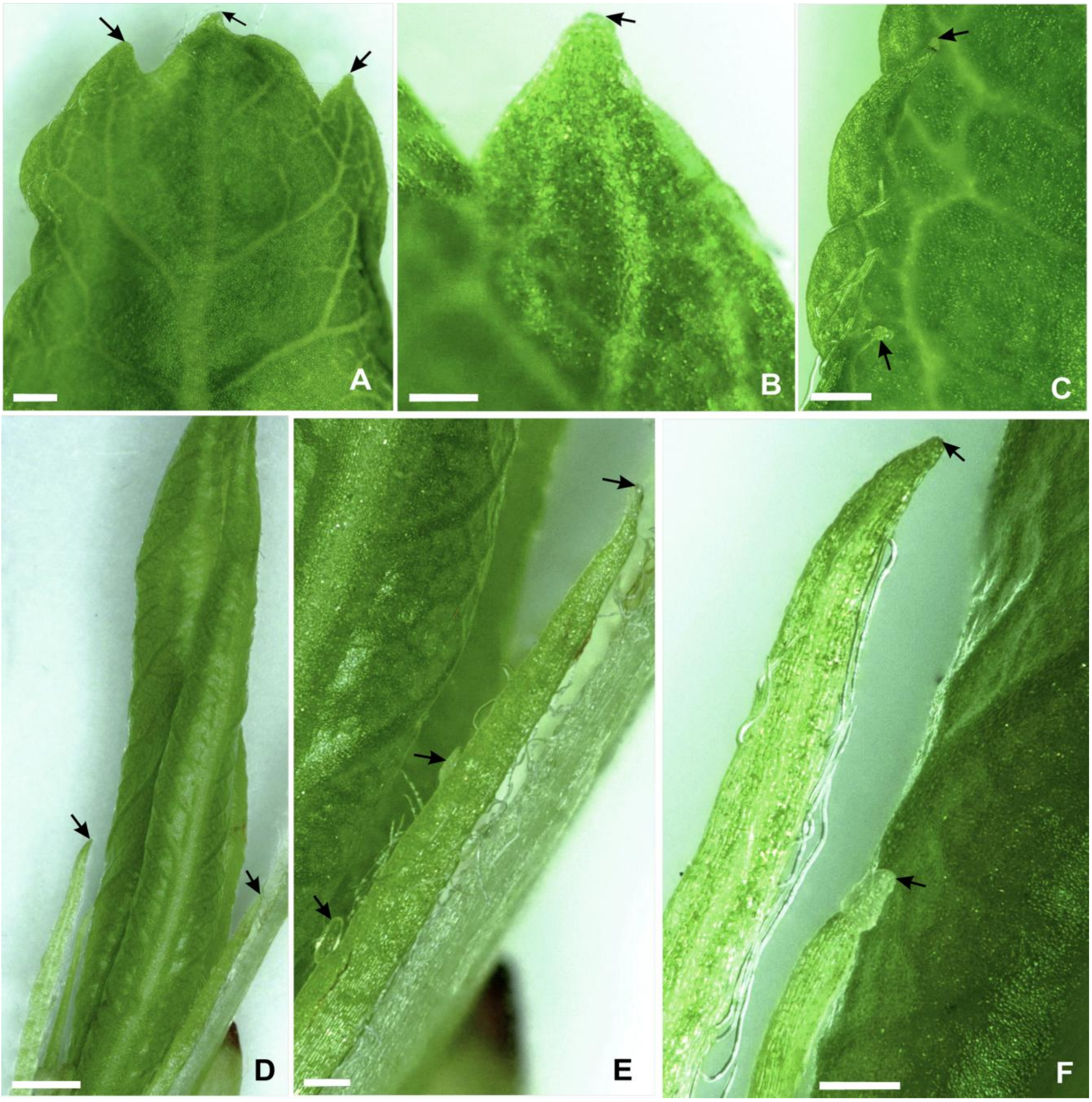

Fig. 4. Young leaf structures of *Pyrus communis* L. at the unfolding stage (Keyence light digital microscope): **A, B** - apex of leaf blade with teeth; **C** - segment of leaf blade margin with young colleters on teeth; **D** - stipules linear-lanceolate; **E** - stipule with apical and lateral finger-like colleters; **F** - stipule with terminal young colleter with light green content. The arrow indicates colleters. Scale bars: **A** – 500 µm; **B** – 100 µm; **C –** 200 µm; **D –** 1 mm; **E, F –** 200 µm.

Table 6. Indirect and total effects of correlated leaf length and width on petiole length (GLM mediation analysis).

Type	Effect	Estimate	SE	95% C.I. (a)		β	z	p
				Lower	Upper			
Indirect	W ⇒ L ⇒ P	-0.319	0.865	-2.014	1.375	-0.0901	-0.369	0.712
Component	W ⇒ L	3.056	0.506	2.065	4.048	0.8038	6.043	<0 .001
	L ⇒ P	-0.105	0.282	-0.658	0.449	-0.1121	-0.370	0.711
Direct	W ⇒ P	2.407	1.074	0.302	4.511	0.6787	2.241	0.025
Total	W ⇒ P	2.087	0.658	0.798	3.376	0.5886	3.174	0.002

Type	Effect	Estimate	SE	95% C.I. (a)		β	z	p
				Lower	Upper			
Indirect	L ⇒ W ⇒ P	0.509	0.2421	0.0343	0.983	0.546	2.101	0.036
Component	L ⇒ W	0.211	0.0350	0.1428	0.280	0.804	6.043	<0 .001
	W ⇒ P	2.407	1.0737	0.3021	4.511	0.679	2.241	0.025
Direct	L ⇒ P	-0.105	0.2824	-0.6580	0.449	-0.112	-0.370	0.711
Total	L ⇒ P	0.404	0.1928	0.0264	0.782	0.433	2.097	0.036

Note. Confidence intervals computed with Delta method. Betas are completely standardized effect sizes

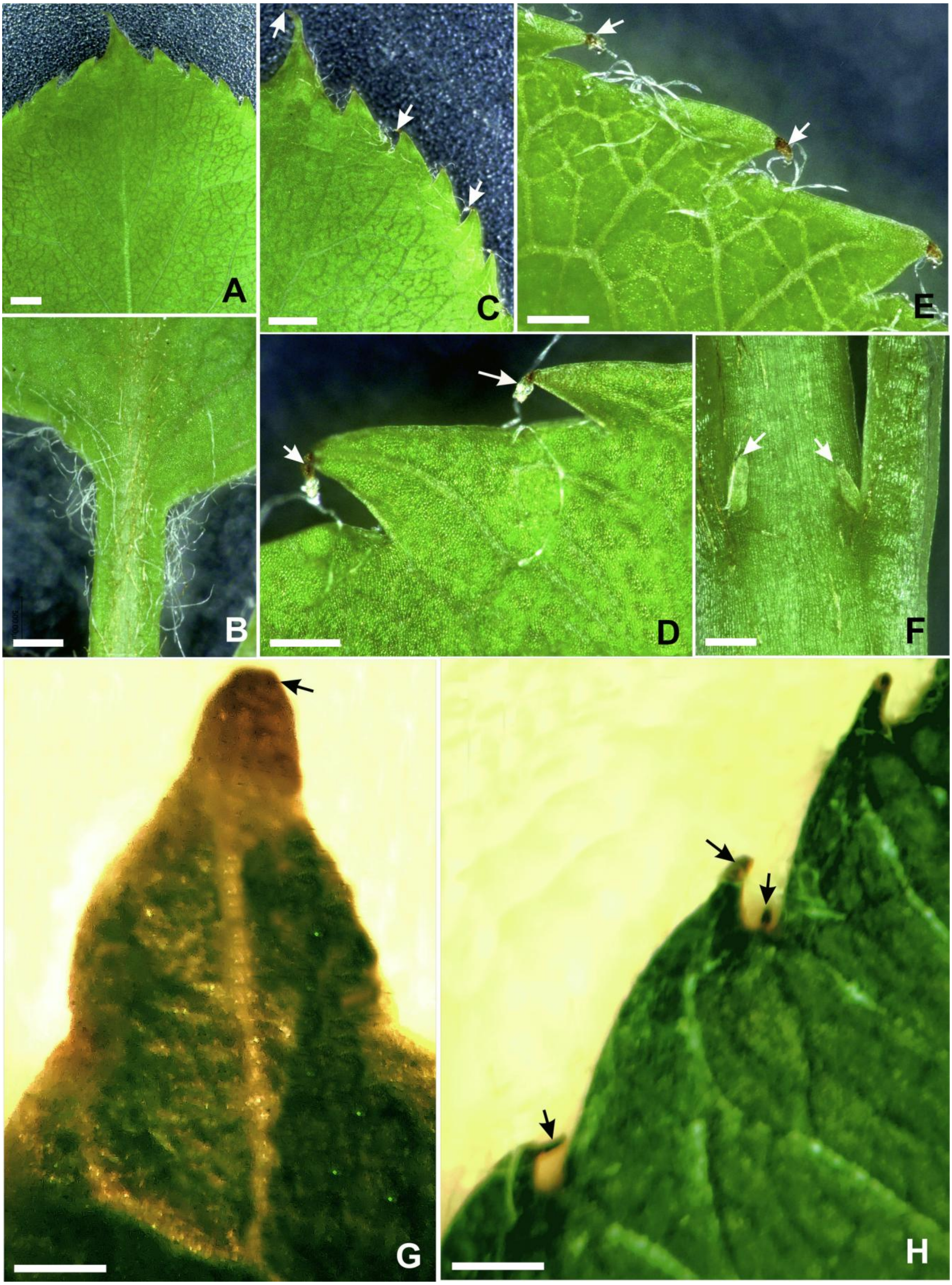

Fig. 5. Leaf of *Pyrus communis* (Keyence light digital microscope): **A, B** - apex and base of leaf blade with trichomes at margins; **C** - segment of apex and margin of leaf blade with colleters; **D, E** - segments of leaf blade margins with secreted colleters at tips of teeth; **F** - fragment of stipules base with secreted colleters; **G** - apex of leaf blade with finger-shaped colleter; **H** - enlarged fragment of leaf blade margin with hook-shaped colleters. The arrow indicates colleters. Scale bars: **A** – 1 mm; **B** – 500 µm; **C – 1** mm; **D, E, F –** 300 µm; **G –** 100 µm; **H –** 300 µm.

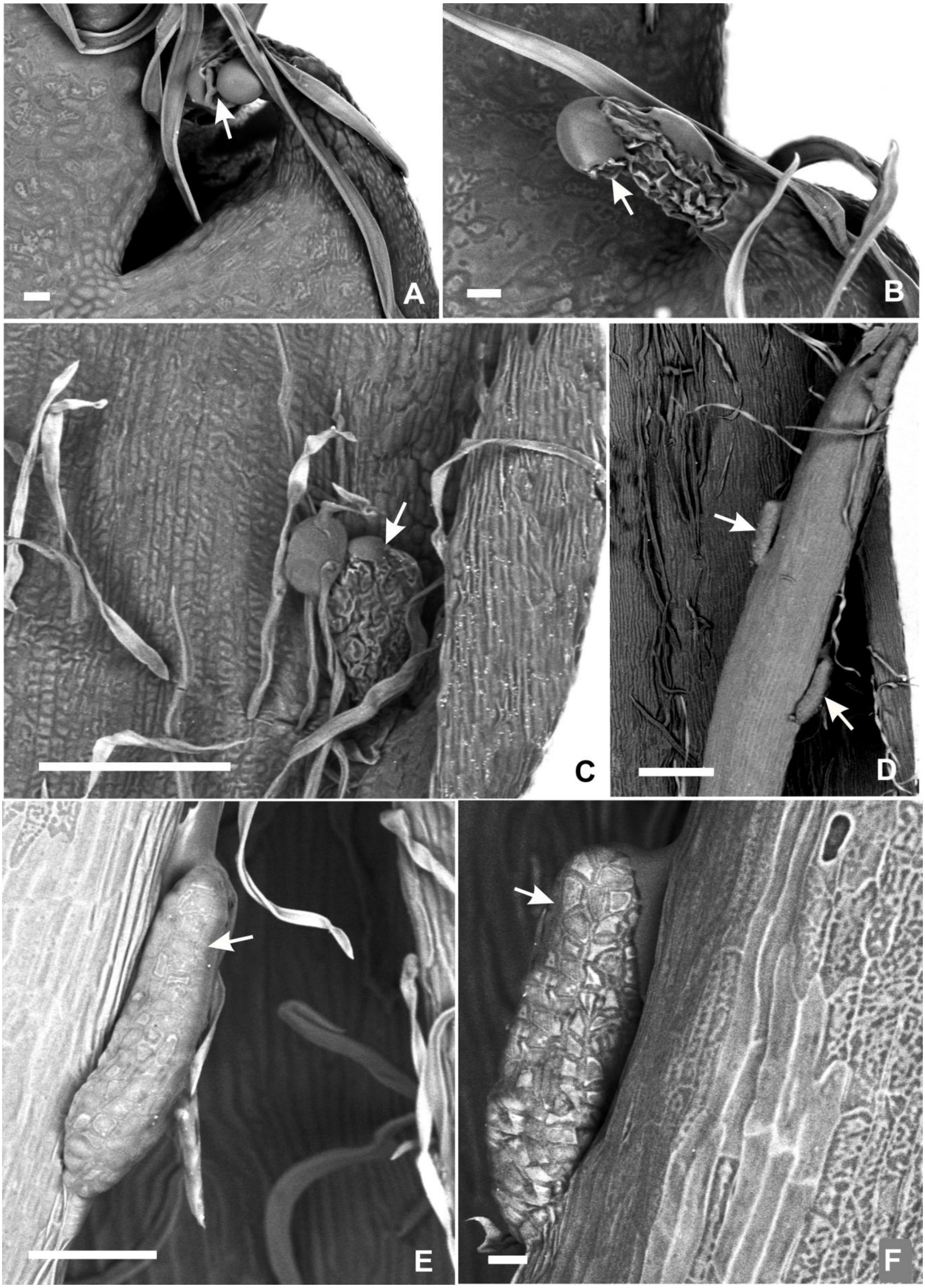

Fig. 6. Secreted colleters of a young leaf of *Pyrus communis* L. (cryoSEM): **A, B** - tip of lateral denticle; **C** - colleter at the base of stipule; **D** - stipule with colleters along the margin; **E, F** - finger-like multicellular colleters of stipule in magnified view. The arrow indicates colleters. Scale bars: **A, B** - 20 µm; **C** - 200 µm; **D** - 300 µm; **E** - 100 µm; **F** - 20 µm.

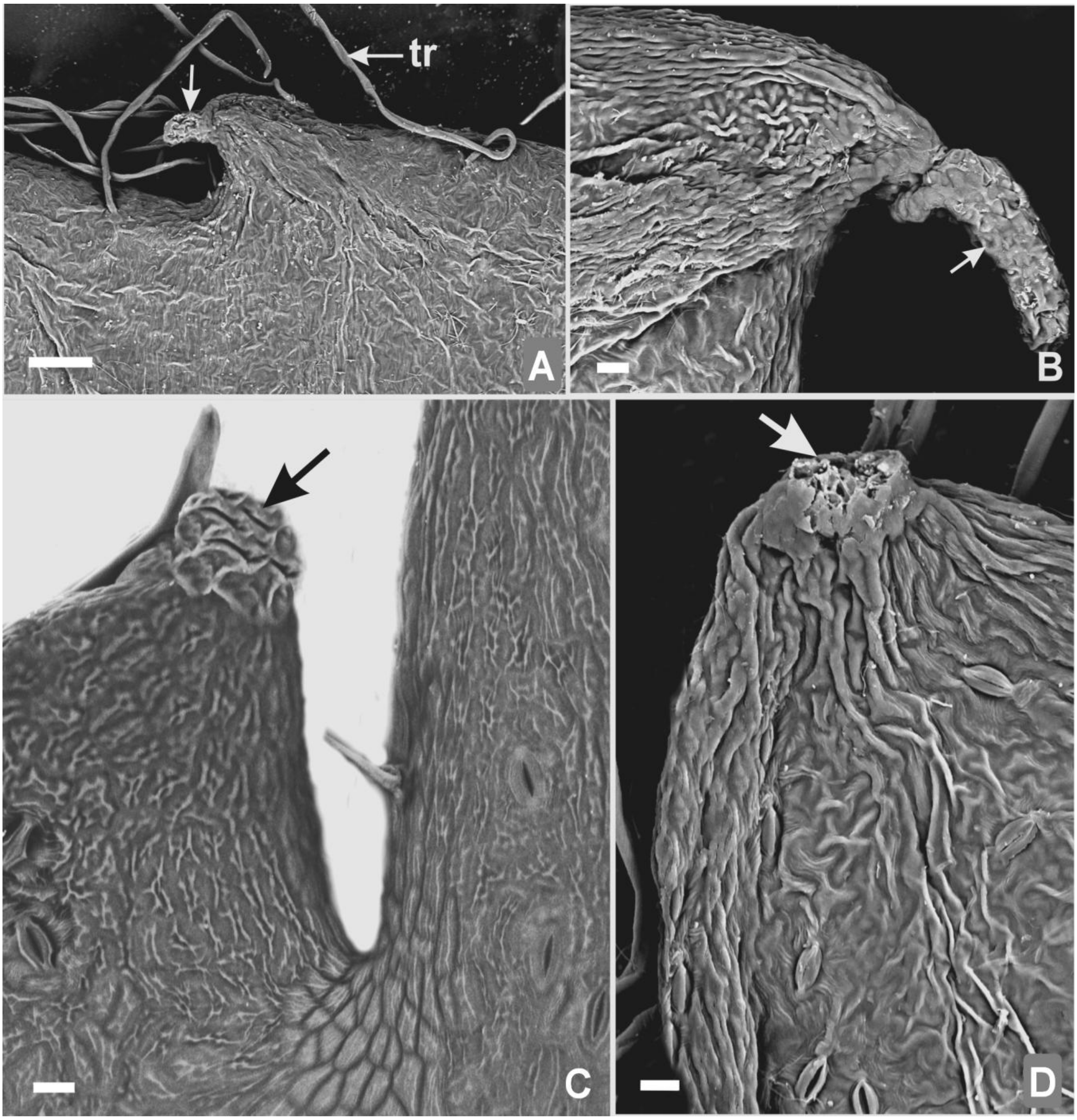

Fig. 7. Micromorphology of the leaf surface of *Pyrus communis* L., herbarium specimen in cryoSEM: **A** - margin of leaf blade, colleter and trichome; **B** - enlarged hook-shaped colleter on lateral denticle; **C** - dried colleter, **D** - base of fallen colleter with spreading secretion on leaf denticle surface. Designations: tr - trichoma. The arrow indicates colleters. Scale bars: **A** - 100 µm; **B** - 20 µm; **C** - 20 µm; **D** - 20 µm.

Table 7. Influence of leaf base shape on leaf size and length to width ratio.

Base shape	n	L	W	L/2W	P
Rounded	10	4.12±0.32a	1.52±0.09a	1.36±0.05a	2.7±0.24
Cuspidate	4	4.68±0.46b	1.37±0.14b	1.75±0.09b	3.04±0.92
Acuminate	6	5.36±0.34b	1.69±0.09ab	1.59±0.04b	2.84±0.29

Values are means ± SE. Means in a column without a common superscript letter differ ($p<0.05$), as analyzed by one-way ANOVA (L/2W) or covariance analyses (L, W) and the Tukey test

On the surface of the adaxial and abaxial epidermis we observed more or less developed cuticular ornamentation (Fig. 8A, D-F). On the adaxial side there were areas having well-developed continuous cuticular folds, often passing from one cell to another (Fig. 8A). On the abaxial side, more pronounced folding was observed on veins and in the area of stomata (Fig. 8D-F). The folding on the veins was similar to that on the adaxial surface. Radial cuticular folds, diverging from the guard cells in different directions, were observed on most of the stomata of the leaves of the herbarium specimens (Fig. 8 D-F). In most cases, 2 or 3 such radial folds were observed in one stoma. The most pronounced radial folds were present in the area of the primary stomata. In addition, cuticular stomatal rings and rings of ridges were sometimes observed as part of the stomatal complexes (Fig. 8E, F).

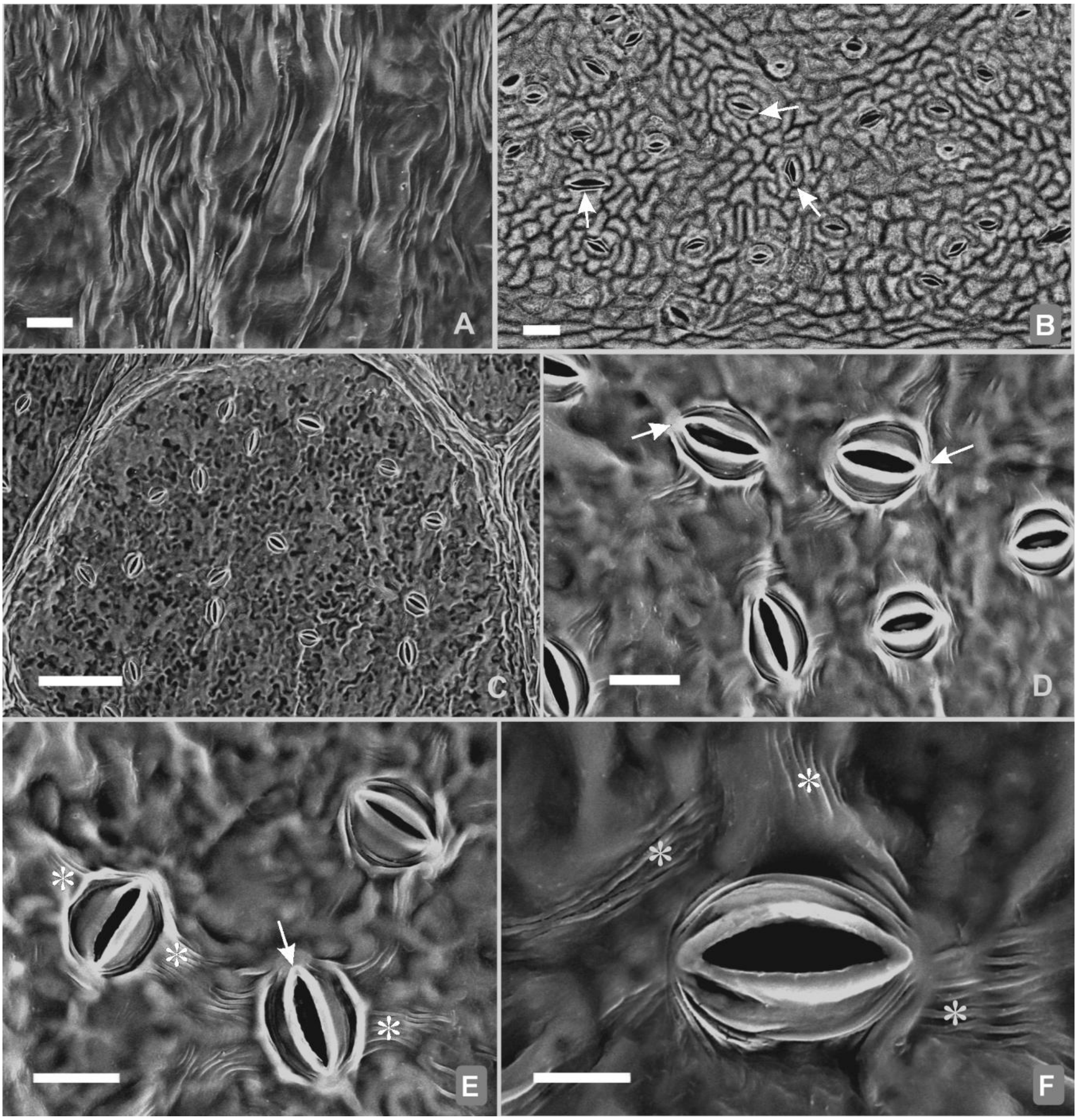

Fig. 8. Adaxial and abaxial sides of *Pyrus communis* L. leaf (A, C-F - herbarium specimen, B - fresh young leaf), (cryoSEM): **A** - cuticular folding on adaxial surface; **B** - abaxial surface at bud stage of leaf development; **C** - abaxial epidermis with tortuous anticlinal cell walls; D - primary and secondary stomata; **E, F** - stomatal radial and concentric cuticular folds. The arrow indicates primary stomata; asterisks marked radial cuticular folds. Scale bars: **A** - 10 µm; **B** - 30 µm; **C** - 100 µm; **D** - 20 µm; **E** - 20 µm; **F** - 10 µm.

Discussion

Species of the genus *Pyrus* are known for their morphological plasticity, gradual transitions and intermediate states of plant characters (Korotkova *et al.*, 2018). Polymorphism among individuals of most *Pyrus* species is due to the lack of geographic isolation and reproductive barriers, which favour the emergence of multiple hybrid forms (Zheng *et al.*, 2014). Morphological variability with multiple intermediate forms and frequent hybridisation within *Pyrus* pose challenges in defining species boundaries (Wu *et al.*, 2018; Mu *et al.*, 2022).

The Asian species had significantly larger leaves than the European species (Fiala *et al.*, 2024). *P. communis*, among other species of the genus, was characterised by relatively small leaf sizes, especially compared to cultivated forms (Fiala *et al.*, 2024). In the wild specimens we examined, leaf sizes were similar to or slightly smaller than those previously reported for *P. communis* and some other wild species of the Caucasus, but sizes varied widely in different herbarium specimens. The variability was of the same order as for samples of different pear species (Magomedmirzaev, 1990). We also noted a marked variability in the morphological characteristics of the shape of the apex, margin, base and the shape of the leaf blade as a whole. Variables related to leaf morphology were correlated with each other. For example, specimens with an ovate leaf shape were also characterised by an elongated acuminate apex. However, in the groups that stood out among the herbarium specimens we examined for leaf morphological traits, leaf sizes varied within approximately the same limits.

Previous studies have reported varying correlations among leaf parameters in pear species. In a sample comprising cultivated and wild pear species, a strong correlation was observed between leaf blade length and width (r = 0.84). The correlations between petiole length and leaf dimensions were relatively weak but statistically significant (0.31 and 0.38) (Zarei *et al.*, 2019). A different pattern was noted in another study of wild pears, where a correlation existed between petiole length and leaf width, but no relationship was found between leaf length and width (Magomedmirzaev, 1990). Our investigation of wild *P. communis* specimens also revealed a similar pattern: we observed a high correlation between leaf length and width, a relatively low correlation between leaf width and petiole length, and confirmed that the low correlation between petiole length and blade length was entirely mediated through the relationship between leaf length and width.

In *P. communis* studied by us, we noted the presence of secretory formations - colleters, which have been previously noted in other members of the Malinae (Kumachova *et al.*, 2021, 2023, 2024; Vinogradova *et al.*, 2024). A similar structure has been described in *P. communis* as glandular leaf teeth (Metcalfe & Chalk, 1965). In our study, these secretory structures were observed in large numbers on leaf blades and stipules at the bud stage of their development. The colleters were located at the tips of apical and lateral denticles. According to literature data, they synthesise a bitter mucilaginous hydrophobic secretion that serves as a protective coating for leaf bud rudiments as well as for developing young leaves against feeding (Evert, 2015; Canaveze & Machado, 2015).

Anticlinal walls of abaxial epidermal cells had strongly sinuous outlines. The stomata of *P. communis* were closer to the stephanocytic type; having pronounced cuticular stomatal radial and concentric ornamentation. It was manifested as 2-4 folds radially diverging and girdling the closing cells. Similar cuticular folds were previously described by us in other species of the subtribe Pyrinae (currently Malinae) (Kumachova *et al.*, 2021). The leaf blades of *P. communis* were hypostomatic, as in most species of the subtribe Malinae (Rosaceae).

Stomatal dimorphism is quite common. It has also been observed in several species of the Malinae subtribe related to *P. communis* (Carr & Carr, 1990; Boldt & Rank, 2010). Primary stomata were larger, had more pronounced radial cuticular folds and were usually surrounded by normal (secondary) stomata. In the specimens we studied, the proportion of primary stomata was approximately 17%, manifesting high variability of this parameter, is consistent with data obtained for other species of the subtribe (Kumachova *et al.*, 2021, 2023; Vinogradova *et al.*, 2024). Stomatal dimorphism was already evident in the early stages of leaf development. The length of primary stomata in our study corresponded approximately to literature data for total stomatal sampling in a number of pear species, including cultivated forms of this species (Fiala *et al.*, 2024), while the size of secondary stomata in mature leaves of wild *P. communis* was slightly smaller.

The combination of distinctive traits - including colleter morphology on leaf and stipule teeth, hypostomatic leaves with stephanocytic stomata, pronounced stomatal dimorphism with characteristic primary stomata proportions, and specific cuticular fold patterns (particularly radial folds around stomata) - establishes a reliable micromorphological profile for *P. communis*. These characteristics may potentially enable differentiation from closely related wild species and cultivated forms (varieties) of *Pyrus*, offering a possible solution to challenges posed by morphological convergence and hybridisation.

Conclusions

The leaf surface of wild ~~plants~~ species of *Pyrus communis* L. is characterised by pronounced cuticular folding on both sides of the leaf blade. The leaf blades are hypostomatic. The stomata are stephanocytic and are surrounded by radial cuticular folds. Stomatal dimorphism is also well expressed. The anticlinal walls of the pavement cells in the abaxial epidermis exhibited pronounced sinuous outlines. The secreting structures were represented by colleters at the apex and sometimes at the lateral leaf teeth, which are formed in young leaf structures at the bud stage. The micromorphological structure of the surface of *P. communis* leaves, as well as the size of the stomata, were found to be similar to other species of the genus *Pyrus*. The shape and size of leaves from herbarium specimens from the south of Russia, the Caucasus and Crimea varied. Factor analysis identified a factor of leaf size parameters (petiole length, leaf blade length and width) and a factor of leaf morphological features (apex elongation, edge shape and leaf blade shape), which corresponded to 2 groups of correlated variables.

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