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MORPHOLOGICAL AND MICROMORPHOLOGICAL INVESTIGATIONS REGARDING THE LEAVES OF SEVERAL *ROSA* L. SPECIES

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Abstract: Multicellular secretory glands are present on *Rosa* L. species, at least on the stipellae edge; some of them have a larger number and different particularities.
The researches have been made on leaves of the following species: *Rosa agrestis*, *R.* x *damascena*, *R. multibracteata*, *R. pimpinellifolia* and *R. rubiginosa*.
Micromorphological studies emphasized a large number of cells which forming both terminal secretory part and foot, even though their dimensions are small.
These researches highlight the micromorphological aspect of these glands, tector hairs and epicuticular wax. All of them were examined through scanning electron microscopy method.

Key words: epicuticular wax, micromorphology, *Rosa*, secretory glands, tector hairs.

Introduction

The classical literature concerning to morpho-anatomy of *Rosa* L. genus mentioned both names, either "secretory gland" (especially in plant morphology papers) [BELDIE & PRODAN, 1956; CIOCÂRLAN, 2009], or "glandular trichomes" (especially in plant anatomy papers without express reference to *Rosa* genus) [FAHN, 1987]. The multitude of writings about *Rosa* L. species places secondly after cereals [BELDER & MISSONE, 1994]. Furthermore, there still are things to clarify.

The present scientific literature present many electron-microscopy researches for many genera and, some of them, are about rings of few *Rosa* species or varieties. Likewise, epicuticular wax has been studied at a limited number of species [WERLENMARK & al. 1999; WISSEMANN, 2000]

Often, secretory emergencies from *Rosa* are treated as "glandular trichomes" [CAISSARD & al. 2006; HASHIDOKO & al. 2001; SULBORSKA & WERYSZKO-CHMIELEWSKA, 2014].

It can be observed that these emergencies are pediculous formations, which has a foot with variable length, branched or not, multiseriate (generally 12-13 series of cells), multicellular (each series being composed of 8-12 cells for short pediculous glands from the teeth of leaflets and for some stipellae, 30-40 cells in glands with branched foot from *R.* x *damascena*, *R. multibracteata*, *R. pimpinellifolia*). These pediculous formations shows, most often, terminal but sometimes from the side, on their pediculous ramifications, a secretory part more or less globulous or hairy-shaped; this character varies with species. The external

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layer of secretory part consists of at least 40 cells. For these reasons we agree with the term "secretory gland" instead of the term "glandular trichomes" [GOSTIN & ADUMITRESEI, 2010]. Secretory emergences found in *Rosa L* are structures more massive than the ones described as glandular trochomes from *Acer campestre* [ASCENSAO & PAIS, 1985], *Hibiscus permambucensis* [ROCHA & MACHADO, 2009], *Salvia officinalis* [VENCLATACHALAMK & al 1984].

Material and methods

The **investigated material** is from collection of The Botanical Garden "Anastasie Fătu" from Iaşi. We have studied the following species: *Rosa agrestis* Savi, *R.* x *damascena* Herrm., *R. multibracteata* Hemsl. & E. H. Wilson, *R. pimpinellifolia* L. (syn. *R. spinosissima* L.), *R. rubiginosa* L. Among them, *R. agrestis*, *R. pimpinellifolia*, *R. rubiginosa* are spontaneous species from Romanian Flora and *R.* x *damascena* and *R. multibracteata* are non-indigenous species. The investigated species belong to a polyploid series. Some of them are tetraploid species (for example *R.* x *damascena*, considered by many authors with hybrid origin), *R. multibracteata* and *R. pimpinellifolia*, or pentaploid species (for example *R. agrestis* and *R. rubiginosa*).

Microphotographs that refer to morphologic aspects of a leaf have been made at Karl Zeiss Jena eyeglass with an Olympus SP 500UZ camera.

Scanning electron microscopy (SEM) investigations – fragments of leaves, rachis and stipellae were fixed in FAA in ethanol 50% for 48 hours, stored in 70% ethanol (Johansen 1940). After dehydration in a graded ethanol series, the material was dried at critical point with CO_2, sputter-coated with a thin layer of gold (30 nm) and, finally, examined in a scanning electron microscopy (Tescan Vega II SBH) at an acceleration voltage of 27.88 kV.

Results and discussion

The leaf is odd-pinnate composed by 5-9 leaflets, several at R. *multibracteata*, *R. pimpinellifolia*, and *R. rubiginosa*.

The leaflets shape is different from oblong-elliptic to cuneate-obovate at *R. agrestis*, oblong-ovate at *R.* x *damascena*, wide-ovate at *R. multibracteata*, elliptical at *R. pimpinellifolia*, and ovate at *R. rubiginosa*.

The leaflets edge is either double-glandulous serrate (at *R. agrestis*, *R. pimpinellifolia* and *R. rubiginosa*) or simple serrate (at *R.* x *damascena* and *R. multibracteata*). Most often, on the teeth of leaflets are present glands or hydathodes.

The leaflets have different size: from 1.4/0.7 cm (at *R. pimpinellifolia*) and 2.2/3 cm (at *R. multibracteata*), which are the smallest leaflets, up to 6/3 cm (at *R.* x *damascena*), which are the largest leaflets.

The leaflets surface is glabrous on both sides (i.e. *R.* x *damascena* and *R. multibracteata*), short-hairy on the upper surface (i.e. *R. agrestis*, *R. pimpinellifolia* and *R. rubiginosa*), hairy and glandulous on the lower surface (at *R. agrestis*, *R. pimpinellifolia* – which have spherical glands with a foot slightly longer than in other species, or *R. rubiginosa* – which have glands located only on the nervure of all orders). On the median nervure, both hairs and glands are more numerous than in other segments of leaf, for all investigated species.

Usually, leaf rachis presents thorns typical for species on abaxial surface, very common at *R.* x *damascena* (it has thorns with a disposition in 2-3 parallel rows and some of them ending with a gland), and at *R. pimpinellifolia* (with acicular thorns and some glands have either a relatively short foot or an extremely long and lignified foot, seeming to be acicular-like with a gland on extremity). Generally, hairs and glands are more abundant on rachis than on leaflets surface, except the adaxial channel, where they are missing or they are rarely and shorter. Also, hairs and glands have an increased frequency on petiolules in the case of species where they are present.

The stipellae are fused with leaf petiole and they have a specific shape for each species. Thus, at *R. agrestis* stipellae are slightly rounded in the middle, with auricles oriented apart, with a rich-glandulous and hairy edge. The glands are situated side by side, which secretory spherical part and foot with different lengths (but not too long). The glands are reddish with a translucent secretion that smells like green apples (Pl. I, Fig. 1). In the same time, the stipellae of *R.* x *damascena*, are long and not too wide (2.1/0.6 – 0.7 cm), with parallel edges and auricles with a parallel disposition to leaf rachis; edges presents glands and tector hairs. At *R. multibracteata*, stipellae are very short (less than 1 cm length) and relatively narrow, with parallel edges, which have relatively rare glands, but more frequent on auricles. They have short foot, are approximately spherical and red colored at maturity. During the young stage, gland secretion is translucent (Pl. I, Fig. 2) and at *R. pimpinellifolia* stipellae are narrow, with widened auricles and with an external orientation, with glands both on their edges and lower side. Stipellae have glands on terminal side. They are almost spherical, slightly flattened on the top, and the foot has variable length (Pl. IV, Fig. 21). At *R. rubiginosa*, stipellae have parallel edges and rounded auricles, with outward disposition with medium size (1.5 - 0.5 cm), they have high-glandulous edges with spherical gland and foot with different length (but relatively short compared to that of other species and varieties). The secretion of these glands smells like green apples and it is translucent (Pl. V, Fig. 25, 26).

We have to mention that, glands begin to regress at some point, and in most cases it overlap with dropping flowers and, rarely, the glands remain active much later - *R. agrestis*, *R. pimpinellifolia*, *R. rubiginosa*. The regression only partially and somewhat later than flowering of glands from leaf can be explained by the fact that a significant part of these gland secretions are sesquiterpenes, which are not characteristic secretions of flower, leaf by going to its metabolism beyond the flower (flavones and volatile oils are characteristic for both leaves and petals) [ADUMITRESEI & al. 2009; CAISSARD & al. 2006; HASHIDOKO & al. 2001; ZAMFIRACHE & al. 2006].

Micromorphology of leaf appearance highlights the peculiarities of the investigated species. Thus, the species more closely related genetically are *R. agrestis* and *R. rubiginosa* and they have some similarities.

R. agrestis presents clavate secretory glands (stipitate) on leaflets underside, along some different orders of nervure, leaflets teeth, stipellae edges, petiole and rachis (Pl. I, Fig. 3-6).

On the lower surface of leaflets, on petiole and rachis glands alternate with tector hairs: on leaflets are more glands, while on rachis there are more hairs; only glands are present on stipellae edges.

At the young leaf, glands are very close to each other; as much as leaf grows and epidermal cells between nervures are dividing, these distances became larger.

Glands have pluriseriate and pluricellular feet; their terminal part are under-globulous and it has a small central concavity at the apical extremity. The glands are of two

types: some of them are big, with a foot length between 40-50 μm, and the diameter of secretory part is between 110-130 μm; the others have the diameter of secretory part between 80-100 μm. Glands situated on rachis have slightly longer foot, reaching up to 65 μm. On the stipellae edges a gland with a longer foot alternating with a small one with a shorter foot.

Glands situated on leaflets teeth have a very short foot (15-20 μm).

The number of cells from the external layer are more than 40.

The tector hairs are numerous on the upper surface of leaflets and rachis; they are missing on stipellae (Pl. I, Fig. 3-6) and they are very rare on the leaflets underside. The hairs from those two epidermis from leaflets are relatively long (200-250 μm) and flexuous. The hairs from rachis are of two types: some are long (250-500 μm) and rare, and the others are short (30-90 μm) and more frequently.

Epidermal cells from around the base of tector hairs form a slight protuberance around them.

At *R. rubiginosa*, clavate secretory glands are present in the same leaf segments with some peculiarities. Both on rachis and underside leaflets, and on stipellae edges, the glands alternate with tector hairs; on rachis, hairs are more frequent than glands, and on leaflets and stipellae proportions of these parts are balanced, tilting slightly for glands (Pl. V, Fig. 25, 26, 28, 30).

The glands of this species are somewhat smaller compared with *R. agrestis* having foot length of 40-50 μm, and diameter of secretory part is around 100 μm. At *R. agrestis*, glands from leaflets teeth have an extremely short foot (about 20 μm), and some glands from rachis have a foot much longer than the majority of them (about 180 μm), as the secretory part (170 μm).

Form of the secretory part is almost globulous (in the case of glands situated on leaflets and stipellae, but even some glands situated on rachis) with a slight central protuberance on apical pole and under-globulous protuberance on glands with a long foot on the rachis.

The number of cells from external layer of secretory part varies between 20 and 50, and this number is correlated with gland size.

On leaflets teeth hydathodes are present (Pl. V, Fig. 29).

Tector hairs are present on both sides of leaflets, on stipellae edges and rachis. Also, some of them are long (up to 200 μm) and flexuous, while others are shorter (40-100 μm) and straight (Pl. V, Fig. 27, 30).

Rosa pimpinellifolia also shows a large number of glands on all leaf segments, and it has a specific series of particular aspects.

Some glands have a relatively short foot, and others had extremely long and lignified foot (seeming to be a needle-like emergence with a gland at its extremity) (Pl. IV, Fig. 19, 20, 22, 23).

On the stipellae edges under-globulous glands are present, with a foot of different size, gland diameter varies between 120 and 150 μm.

Also, at this species the branching of foot glands can be noticed; the ramifications ends with the same type of glands. The foot which shows lateral branches is extremely long (up to 1600 μm), and it have stretched strong base (270-300 μm), but also its upper part is considerably widened (about 150 μm) in which the foot thickness is adjusted, this part is similar with gland foot for the most investigated species. Such foot shows 2-3 lateral branches (on both sides) with typical glands (Pl. IV, Fig. 21).

In addition, on stipellae edges can be observed globulous glands; they have a long foot (420-680 μm) and widened to near the top (140-100 μm), and also common globulous glands with short foot (90-100 μm) or average (250 μm) and with the same thickness throughout its length, gland diameter varying around 110-120 μm. The upper part of the gland is slightly concave.

R. x *damascena* and *R. multibracteata* species presents only few glands located on stipellae edges, and on rachis at *R.* x *damascena* species.

Thus, at *R.* x *damascena* on stipellae edge, both secretory glands and tector hairs are present, these last in a smaller number.

The glands are of two types: some are globulous (with short foot by 80 mm, and gland by 80-90 μm) and others are piriform (with foot long 300 μm, and 150 x gland axes 90-110 μm). The bottom part of the pyriform gland foot is stretched. The top of globulous gland is, in this case, as a perfect sphere (Pl. II, Fig. 9-12).

Tector hairs have a medium length (250-270 μm).

At *R. multibracteata*, in the adaxial channel of the petiole (the area where it accrete with stipellae) are present short and thick hairs, which are rarer on rachis.

The rachis presents glands (rare and only on lower surface) and tector hairs, especially on insertion points of leaflets; tector hairs are present in adaxial channel.

This specie presents on the stipellae edges both pyriform and globulous glands, both categories are active in young leaf, the pyriform glands ending his their activity first (Pl. III, Fig. 15-18).

The pyriform glands have a foot slightly widened at the bottom, with a medium length (450-600 μm) and glands with axes 250-260 x 100 μm.

Globulous glands have a shorter foot (150 μm) and the secretory part about 100 μm being strongly curved at the top.

Epicuticular wax is of granular type at *R. agrestis* (Pl. I, Fig. 6), *R.* x *damascena* (Pl. II, Fig. 12) and *R. multibracteata* (Pl. III, Fig. 18) species, and of triangular type at *R. pimpinellifolia* (Pl. IV, Fig. 24).

Stomata length varies between 15 μm at *R. agrestis* (among the lowest compared to other investigated species), 20 μm at *R.* x *damascena* and *R. multibracteata*, 25 μm at *R. pimpinellifolia*; in the case of *R. rubiginosa* stomata have variable lengths, between 15 and 20 μm.

Conclusions

Secretory glands are spread on leaves in all cases, the differences are concerning to number, shape and scattered thereof (only on the stipellae edges and/or leaflets teeth, rachis, median nervure, etc.). Presence, shape, size and disposition of glands (characteristic for some other species of Rosaceae family) are characters used for diagnosis and they are very interesting by their own great variability within the *Rosa* genus.

On the stipellae edge glands are present in all species. At some species (*R. agrestis*, *R. pimpinellifolia* and *R. rubiginosa*), both hairs and glands are present in all leaf segments, in different proportions. However, in most species, leaflets are hairless, tectors and secretory formations are located only on the stipellae edges, rachis, and sometimes on median nervure and leaflets teeth.

The shape of secretory glands is generally elliptical or under-globulous, with variations between them, sometimes encountered both forms at the same taxa (*R.* x *damascena* and *R pimpinellifolia*).

Secretory glands are present in many cases even on rachis at *R. agrestis*, *R.* x *damascena*, *R. pimpinellifolia* and *R. rubiginosa*, their frequency considerably increases at their insertion points of leaflets and on their petiolules.

On rachis, glands can alternate with hairs as at the following species: *R. agrestis*, *R.* x *damascena*, *R. multibracteata* (where glands are rare) and *R. rubiginosa*.

The leaflets teeth are ending with glands, as in the following species: *R. agrestis*, *R. pimpinellifolia*, *R. rubiginosa* (where glands alternating with hydathodes). The presence of hydathodes in large numbers on leaflets teeth was observed at *R. rubiginosa*.

Secretory glands at *Rosa* present a pluriseriate and multicellular foot, and its clavate terminal part is either globulous or under-globulous in some cases; either elliptical-oblongated or piriform in some other cases. Among the investigated taxa, some of them show only under-globulous to globulous glands (*R. agrestis*, *R. rubiginosa* and *R. pimpinellifolia*).

R. x *damascena* and *R. multibracteata* species present both type of glands: more or less globulous and elliptical to piriform.

The branching of foot in elliptical or pyriform gland is present at *R. multibracteata* and *R. pimpinellifolia*.

The length of the foot glands vary within some wide limits, from 20 μm in under-globulous glands from leaflets teeth of *R. agrestis*, to 1600 μm from stipellae edges glands from *R. pimpinellifolia*. Broadly, globulous and under-globulous glands (unless the case shown above) have shorter foot which ranging from 20 to 200 μm, and elliptical glands present a foot longer ranging from 300 to 600 μm.

The diameter of under-globulous to globulous secretory part varies between 80 μm (at *R. agrestis*) and 180 μm to those on the rachis (at *R. rubiginosa*).

The dimensions of secretory part of elliptical or piriform glands ranges from 90 to 110 μm for minor axis and from 150 and 260 μm for major axis.

In terms of number of cells which forming external layer of secretory glands part, it varies between 40 and 50, and it is correlated with glands size.

The number of cells (in cross-section) that forms a foot gland is approximatively 12-13, the foot external layer which consists of some series by at least 8 to 12 cells.

The tector hairs have different lengths ranging: from 30 to 90 μm for short hairs (*R. agrestis* and *R. rubiginosa*); from 250 to 270 μm for long hairs (*R.* x *damascena*); 200-500 μm for hairs present on rachis (*R. agrestis*). At the same species was observed both short hairs and long hairs.

Generally, epidermal cells forming a slight protuberance around tector hairs.

The stomata are either irregular dispersed among epidermal cells (*R. agrestis*, *R. pimpinellifolia* and *R. rubiginosa*), or with a grouped disposition on hybrids (*R.* x *damascena*).

In terms of cell stomata length, we distinguished species with smaller stomata (15-20 μm): *R. agrestis* (not exceeding 15 μm); *R.* x *damascena*, *R. multibracteata* and species with larger stomata (greater than 25 μm) as *R. pimpinellifolia*.

The epicuticular wax could be of granular type (*R. agrestis*, *R.* x *damascena*, *R. multibracteata* and *R. rubiginosa*), and by triangular type (*R. pimpinellifolia*).

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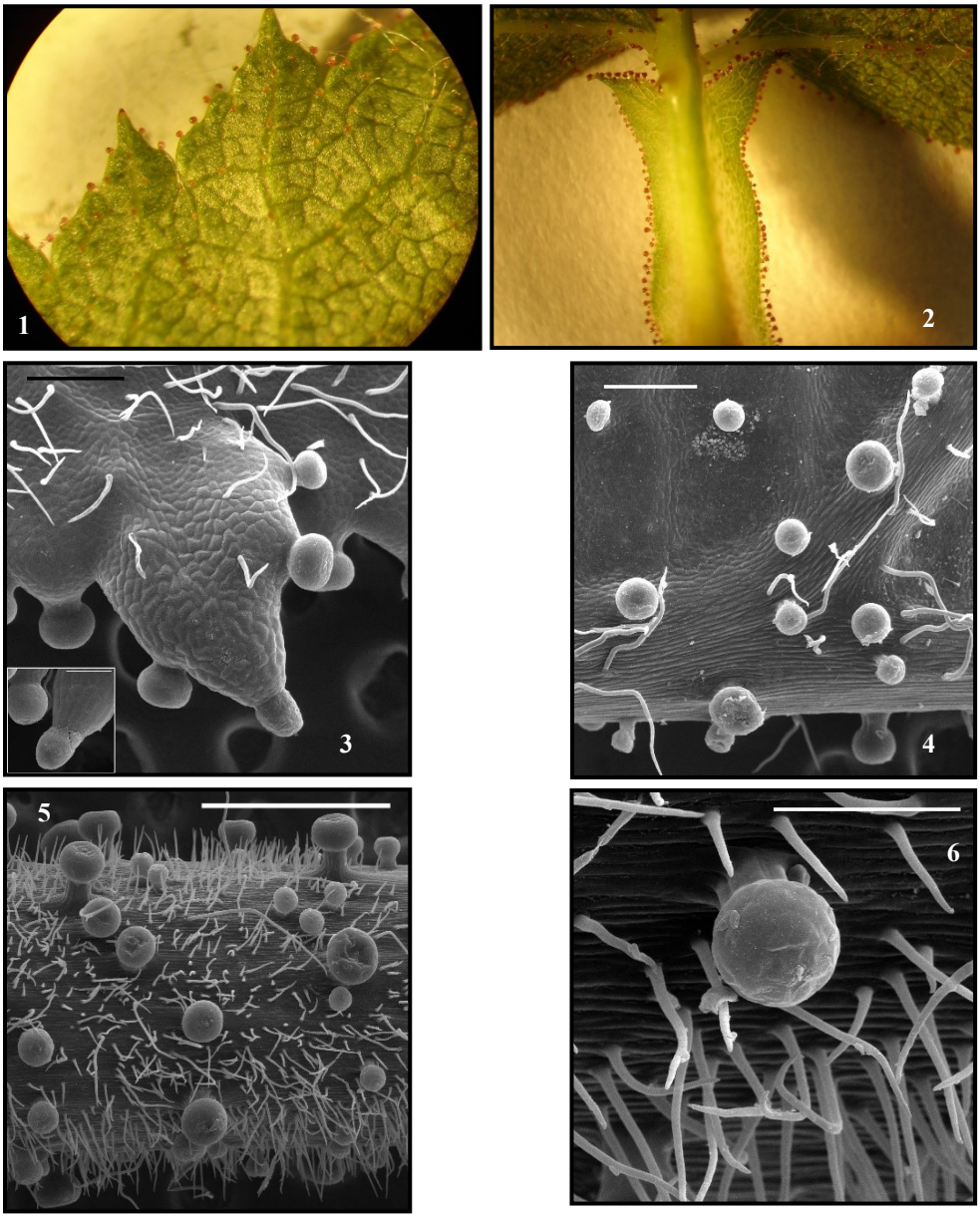
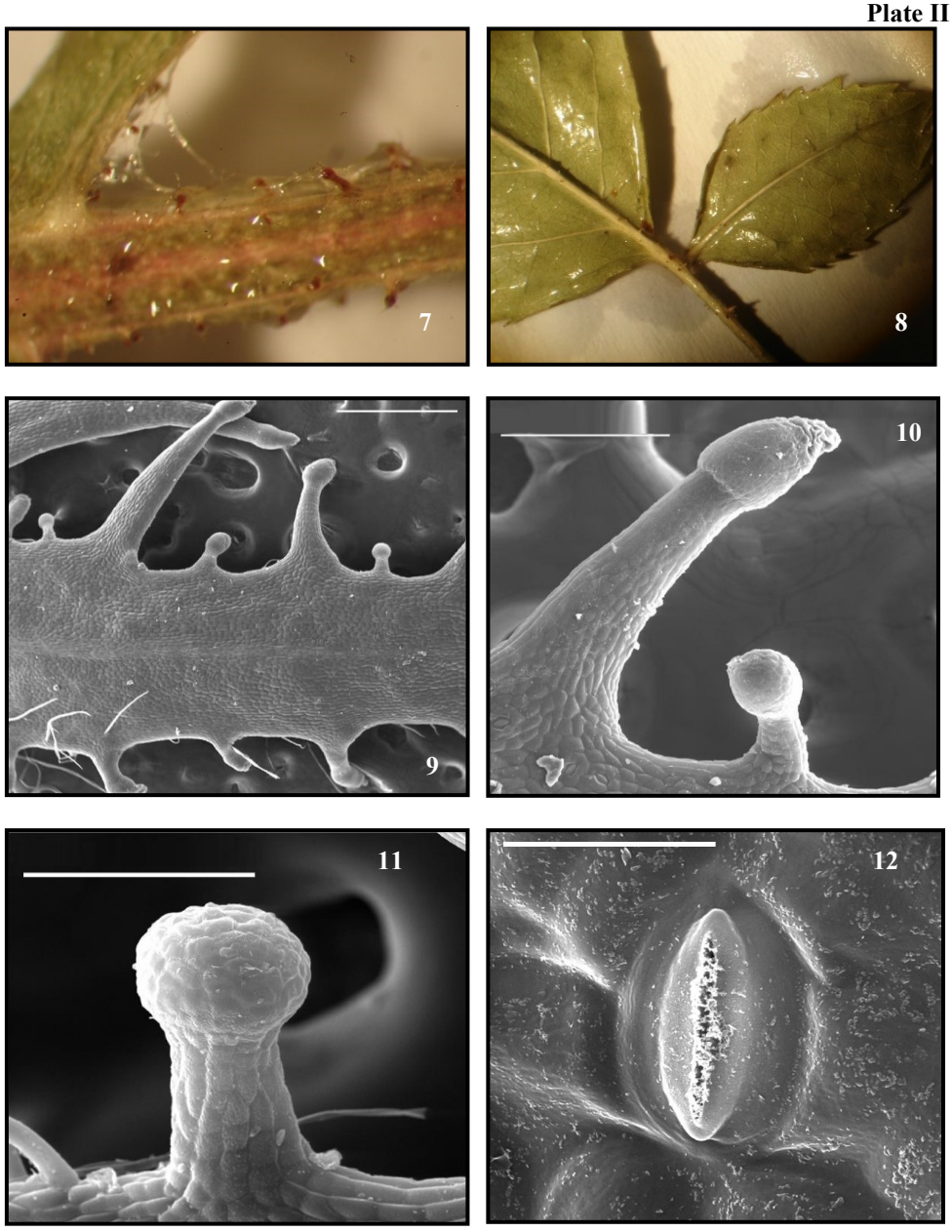
Plate I



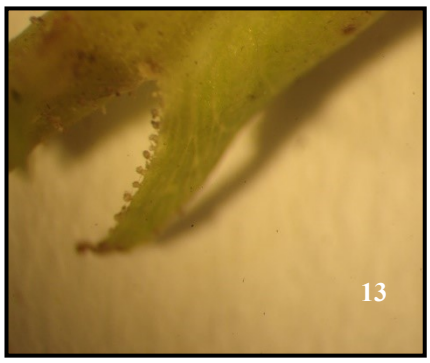
***Rosa agrestis*: 1)** leaflet, lower epidermis (scale bar 6.3 x 2.5); **2)** leaflet (6.3 x 1.6); **3)** margin of leaflet (200 µm, details = 100 µm) (ADUMITRESEI & al. 2009); **4)** leaflet (200 µm) (ADUMITRESEI & al. 2009); **5)** rachis (500 µm); **6** rachis (100 µm).

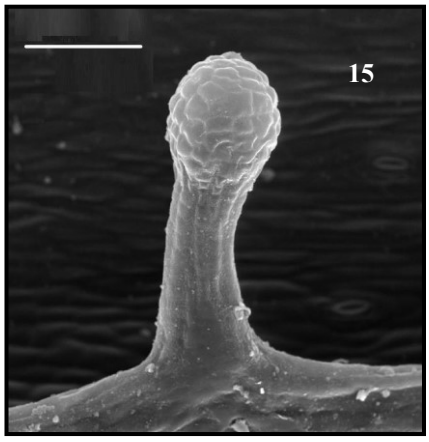
Plate II

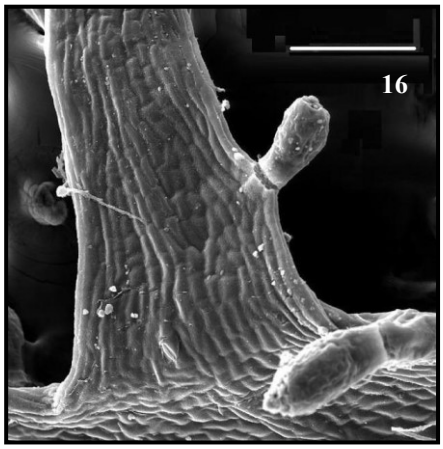


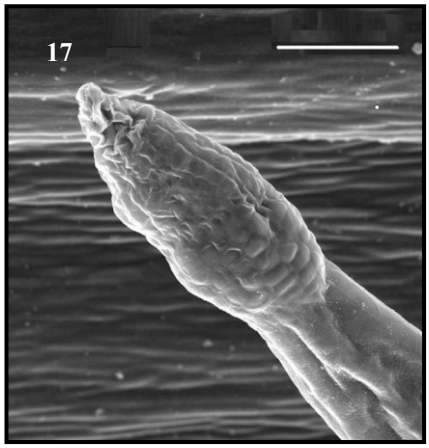
***Rosa* x *damascena*: 7)** stipellae and rachis (6.3 x 4); **8)** leaflets (6.3 x 1.6); **9)** stipellae (500 µm); **10)**, **11)** stipellae glands (100 µm); **12)** epicuticular wax and stomata (20 µm).

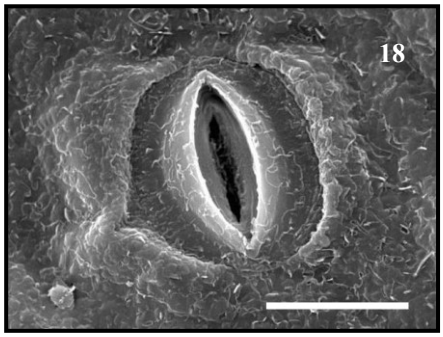
Plate III





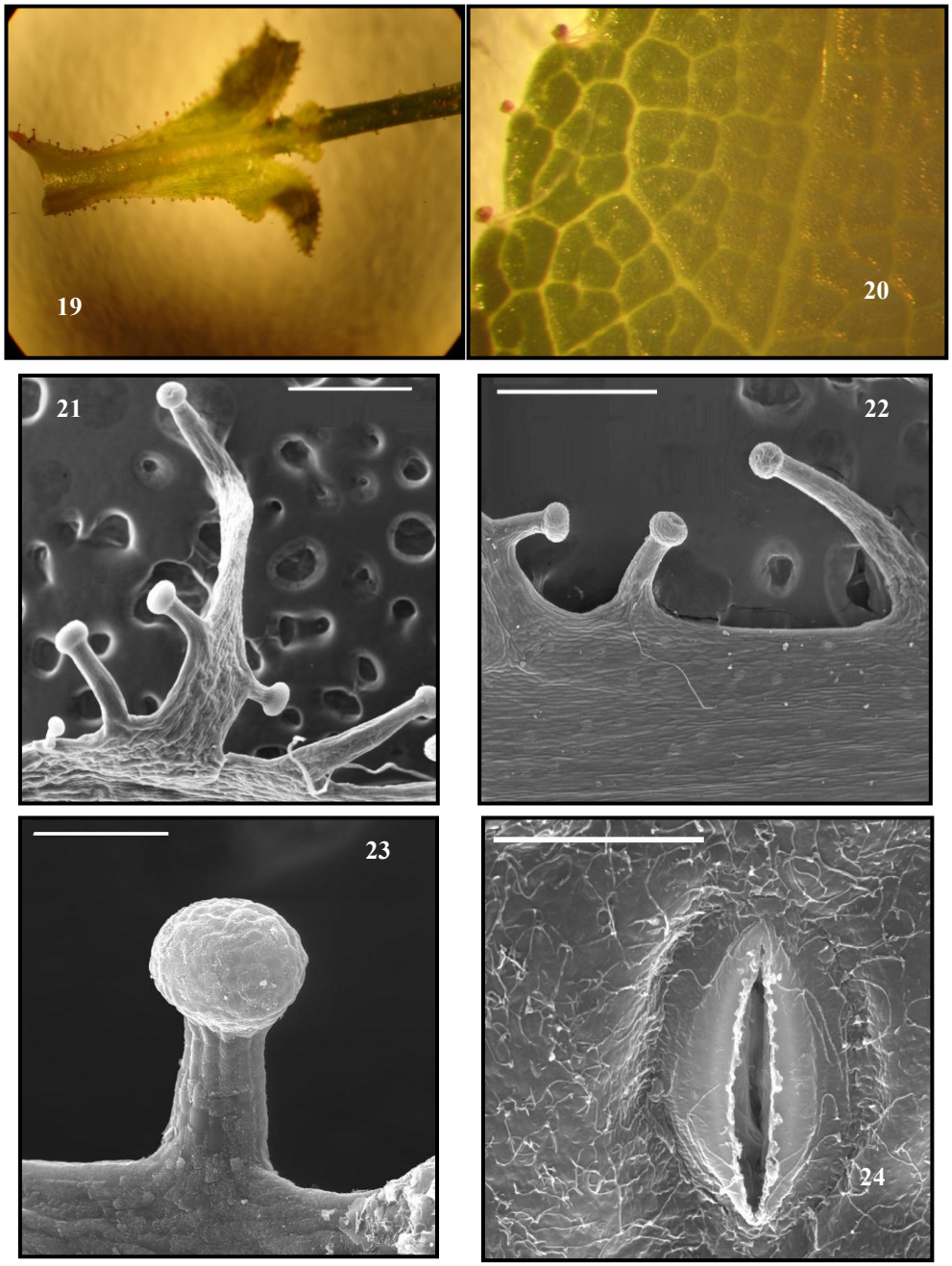






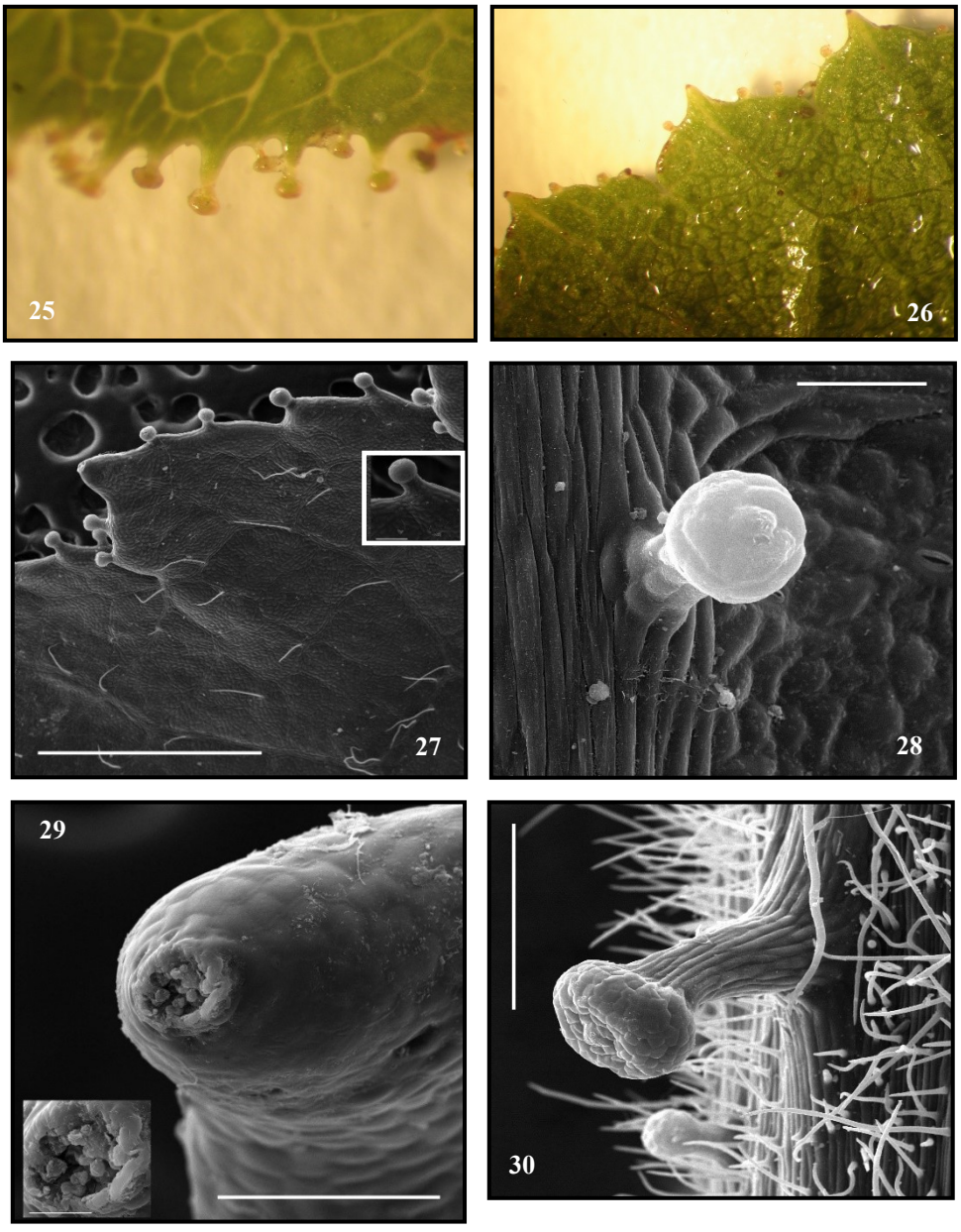
***Rosa multibracteata*: 13)** stipellae (6.3 x 4); **14)** leaflet (6.3 x 1.6); **15)** stipellae (200 μm); **16), 17)** stipellae glands (100 μm); **18)** epicuticular wax and stomata (20 μm)

Plate IV



***Rosa pimpinellifolia*: 19)** stipellae (6.3 x 1.6); **20)** leaflet (6.3 x 4); **21)** stipellae glands (50 µm); **22)** stipellae glands (500 µm); **23)** stipellae glands (100 µm); **24)** epicuticular wax and stomata (20 µm).

Plate V



***Rosa rubiginosa*: 25)** leaflet (500 µm); **26)** mature leaflet (50 µm); **27)** mature leaflet (1 mm and 100 µm) (ADUMITRESEI & al. 2009); **28)** leaflet gland (500 µm); **29)** leaflet hydathode (100 µm and 20 µm), **30)** rachis (500 µm).