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Fruit morphology in *Galium* section *Platygalium* (Rubiaceae) and its potential taxonomic significance

Морфология плодов подмаренников (*Galium*, Rubiaceae) секции *Platygalium* и её значение для систематики

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Ключевые слова: Rubiaceae, *Galium*, *Platygalium*, fruit, morphology, mericarp surface, key.

Summary. Fruit (mericarp) morphology of nine species from section *Platygalium* of the genus *Galium* from Russia were examined with light microscope and scanning electron microscope (SEM). Macro- and micromorphological characters, including fruit shape, size, and types of mericarp surface and indumentum are presented. Three types of mericarp surface were observed, glabrous, hairy with either hooked or straight hairs and tuberculate. The results agree with those of Pobedimova (1958), who classified the section *Platygalium* into three series. The mericarp characters also appear to be useful for species delimitation in this group.

Аннотация. Изучена морфология плодов (мерикарпиев) девяти видов секции *Platygalium* рода *Galium* с территории России под световым и сканирующим электронным микроскопом. Рассмотрены макро- и микроморфологические признаки, включая форму и размеры, а также типы поверхности и опушения мерикарпиев. Установлены три типа поверхности мерикарпиев: гладкая, опушенная крючковатыми или прямыми волосками и бугорчатая. Результаты согласуются с классификацией Е. Г. Победимовой (Pobedimova, 1958), разделявшей секцию на три ряда. Признаки мерикарпиев оказываются полезными также и для разграничения видов в этой группе.

Introduction

Rubiaceae (coffee family) is the fourth-largest angiosperm family, comprising approximately 660 genera and 11,500 species classified into 42 tribes (Robbrecht, Manen, 2006). *Galium* L. is one of the largest genera of the tribe *Rubieae* Baill. It comprises more than 600 species worldwide, mostly occuring in temperate areas, but also in alpine and arctic regions to subtropical and tropical zones at higher elevations (Chen and Ehrendorfer, 2011).

Galium is a typical member of *Rubieae*, sharing both morphological and molecular synapomorphies of this monophyletic group (Backlund et al., 2007; Bremer, Eriksson, 2009; Rydin et al., 2009a). However, infratribal taxonomy and classification of *Rubieae* is problematic, especially for the larger genera *Asperula* and *Galium*. A number of taxa within *Asperula* appear morphologically similar to *Galium*, differing only in corolla tube length, and these have been transferred from *Asperula* to *Galium* (Ehrendorfer, 1958; Ehrendorfer et al., 2005; Natali et al., 1995). *Galium* itself is problematic taxonomically, because taxa from different sections exhibit similar habit, many species are widely

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distributed and polymorphic, and species groups are often poorly differentiated both morphologically and geographically (Pobedimova, 1958).

Galium section *Platygalium* is a large and morphologically diverse group. No comprehensive, worldwide treatment exists for this section (Ehrendorfer et al., 2005). However, from a review of various floras, the section comprises at least 70 species (Dempster, 1981, 1982; Dempster, Stebbins, 1968; Ehrendorfer et al., 2005; Ehrendorfer et al., 1976; Ehrendorfer, Schönbeck-Temesy, 1982; Pobedimova, 2000; Yamazaki, 1993). The section is distributed worldwide with centers of diversity in eastern Asia, eastern North America, the Mediterranean, and the Caucasus (Ehrendorfer et al., 2005). Taxa are hermaphroditic and bear dry fruits that are glabrous or covered with hooked to straight hairs (Ehrendorfer et al., 1976; Ehrendorfer et al., 2005; Ehrendorfer, Schönbeck-Temesy, 1982; Pobedimova, 1958, 2000).

In «Flora Orientalis» Boissier (1881) reported 91 species of the genus *Galium* divided into three sections: section (I) *Eugalium*, section (II) *Aparine*, and section (III) *Cruciata*. Section (I) *Eugalium* is classified into four unranked groups: 1. *Platygalia*, 2. *Leiogalia*, 3. *Chromogalia*, and 4. *Ceratocarpa*. The group (1) *Platygalia*, includes the following species: *G. boreale* L., *G. rubioides* L., and *G. valantioides* Bieb. (Tab. 1). Pobedimova (1958) in the «Flora USSR» regarded *Platygalia* at the rank of section and reported 11 species within it, divided into 3 series, *Rubioidea*, *Borealia* and *Valantioidea* (Tab. 1). Series *Rubioidea* includes *G. rubioides*, *G. articulatum* Lam., *G. physocarpum* Ledeb., *G. volgense* Pobed., and *G. ussuriense* Pobed.; series *Borealia* includes *G. boreale, G. amblyophyllum* Schrenk, *G. turkestanicum* Pobed., *G. amurense* Pobed., *G. septentrionale* Roem. et Schult., and *G. mugodsharicum* Pobed.; series *Valantioidea* includes only *G. valantioides*.

According to the World Checklist of Rubiaceae (Govaerts et al., 2011), most of the species reported by Pobedimova (1958) in the «Flora USSR» have been reduced to synonyms of either *G. boreale* or *G. rubioides*. Thus, *G. articulatum*, *G. physocarpum*, *G. volgense*, and *G. mugodsharicum* are regarded there as synonyms of *G. rubioides*, while *G. ussuriense*, *G. amurense*, and *G. septentrionale* as synonyms of *G. boreale.*

Galium section *Platygalium* has not been treated systematically and monographically to date. The systematic treatment of this group is difficult for the following reasons: species are often similar in habit, highly polymorphic, and weakly differentiated geographically. *Galium boreale* and *G. rubioides* belong to a polyploid complex with a widespread circumpolar distribution in the northern Hemisphere. The complex comprises rare diploids (2n = 2x = 22), tetraploids (2n = 4x = 44), hexaploids (2n = 6x = 66), and higher polyploids

Table 1

List of the studied species of *Galium* and their taxonomic positions according to different treatments

№	Taxon	Boissier (1881)	Pobedimova (1958)	Ehrendorfer et al. (1976)	Govaerts et al. (2011)
1	*G. articulatum*	-	Section: *Platygalia* Series: *Rubioidea*	-	Synonym of *G. rubioides*
2	*G. rubioides*	Section *Eugalium Platygalia*	Section: *Platygalia* Series: Rubioidea	Section: *Platygalium*	Accepted
3	*G. physocarpum* (= *G. volgense*)	-	Section: *Platygalia* Series: Rubioidea	-	Synonym of *G. rubioides*
4	*G. ussuriense*	-	Section: *Platygalia* Series: Rubioidea	-	Synonym of *G.boreale*
5	*G. boreale*	Section *Eugalium Platygalia*	Section: *Platygalia* Series *Borealia*	Section: *Platygalium*	Accepted
6	*G. amurense*	-	Section: *Platygalia* Series *Borealia*	-	Synonym of *G.boreale*
7	*G. mugodsharicum*	-	Section: *Platygalia* Series *Borealia*	-	Synonym of *G. rubioides*
8	*G. septentrionale*	-	Section: *Platygalia* Series *Borealia*	-	Synonym of *G.boreale*
9	*G. valantioides*	Section. *Eugalium* Subsections: *Platygalia*	Section: *Platygalia* Series *Valantioidea*	-	Accepted

(12x). 4x and 6x cytotypes occur throughout Europe (Kliphuis, 1986), 12x cytotype has been reported for *G. rubioides*. Many morphological intermediates occur throughout Eurasia. The status of all these taxa remains uncertain without detailed cytotaxonomic analyses (Ehrendorfer et al., 1976).

The scanning electron microscope (SEM) has been used by various authors during the past years for investigating details of the surface structure of pollen and seed. The SEM microscopy of seeds has become a routine technique for furnishing information on seed coat morphology (Heywood, 1969). Abdel Khalik et al. (2008) recommended SEM in their studies of the structure of fruits and seeds ornamentation in the genus *Galium* as being useful for taxonomic studies. In particular, Balde (2013) proved and distinguished the specific independence of *G. amurense* from closely related species *G. boreale* on the basis of morphological characteristics revealed with SEM.

The present study deals with micro- and macro-morphological characters of fruit (mericarps) sculpture and indumentum in taxa of the section *Platygalium* from Russia in order to establish their usefulness for further taxonomic work. Fruits of *Galium* are of schizocarpic nature, composed of two mericarps, each mericarp having only one seed. They are usually regarded as important for *Galium* taxonomy at infrageneric and species level.

Materials and methods

The present study is based on the materials kept at the Herbarium (MHA) of the Main Botanical Garden of Russian Academy of Sciences. For the purposes of the study, all the specimens were determined according to the narrow treatment by Pobedimova (1958) using the key and descriptions given in the «Flora USSR». Only mature fruits were taken for investigation. Dried fruits were soaked in boiling water for 2–4 minutes, to compensate for shrinkage and examined by light microscope, with 10–15 fruits for each taxon chosen to cover the range of variation. Measurements were standardized with ocular micrometer. The fruit surface microsculpture and indumentum characters were examined with a LEO 1430 VP Scanning Electron microscope.

List of *Galium* specimens used for light and scanning electron microscope studies:

1. *G. articulatum*: Volgograd Prov., 19 IX 1974, E. Gogina, A. Matzenko .

2. *G. rubioides*: Volgograd Prov., 03 VIII 1993 I. Schanzer, G. Klinkova.

3. *G. physocarpum*: Volgograd Prov., 30 VIII 1985, N. Belyanina.

4. *G. ussuriense*: Polar Urals, 19 VIII 1964, A. Skvortsov, V. Filin.

5. *G. boreale*: Estonia, 15–16 VIII 1986, A. Skvortsov, V. Bochkin, N. Shevyreva.

6. *G. amurense*: Khabarovsk surroundings, 10 VII 1980. V. Kuvaev.

7. *G. mugodsharicum*: Smolensk Prov., 18 IX 1971, V. Makarov, G. Proskuryakova.

8. *G. septentrionale*: Polar Urals, Tyumen Prov., 04 VIII 1991, T. Konovalova, N. Shevyreva.

9. *G. valantioides*: N Caucasus, Pyatigorsk neighbourhood, 03 VII 1976, E. Gogina.

Results

Mericarp shape and size

Mericarp shape among the investigated taxa is more or less the same, however it may be either subglobose or subglobose inflated (swollen), with the pericarp loosely surrounding seeds (Tab. 2). It is subglobose in *G. ussuriense, G. boreale, G. amurense, G. valantioides, G. septentrionale*, and *G. mugodsharicum,* while being to different degree inflated (swollen) in *G. physocarpum*, *G. articulatum,* and *G. rubioides* (Tab. 2). Mericarp shape shows no highly significant differences among the studied taxa.

Mericarp dimensions do not vary greatly among the examined taxa. They range from 1.0–1.5 × 1.0–2.0 mm in *G. mugodsharicum* to 3.0–5.0 × 2.5–3.5 mm in *G. amurense* (Tab. 2). However, we can say that among the studied species those having fruits with inflated pericarp, do have larger fruits as well.

Mericarp indumentum

Among the studied species, mericarp surface is glabrous in those with inflated pericarp, i.e. *G. articulatum* (Plate I: 1A, B)*, G. rubioides* (Plate I: 2A, B), *G. physocarpum* (Plate I: 3A, B), and *G. ussuriense* (Plate I: 4A, B). In other species it is covered with hairs or tubercules of various length, shape and density. Thus, in *G. boreale* mericarps are densely covered with uncinate hairs 0.3–0.4 mm long (Plate II: 5A, B). In *G. amurense* mericarps are densely setose with long straight hairs of 0.50–0.65 mm long (Plate II: 6A, B). They are scabrous with short hooked setae 0.10–0.15 mm in *G. mugodsharicum* (Plate II: 7A, B); sparsely covered with hooked hairs 0.20–0.25 mm in *G. septentrionale* (Plate II: 8A, B) and glabrous to tuberculate in *G. valantioides* (Plate III: 9A, B).

Table 2

Mericarp descriptions of studied *Galium* species

№	Taxon	Mericarp Shape	Mericarp size in (mm)	Mericarp Surface
1	*G. articulatum*	Subglobose	1.0–1.5 x 2.0–2.5	Glabrous
2	*G. rubioides*	Subglobose	1.5–2.0 x 2.5–3.0	Glabrous
3	*G. physocarpum* (= *G. volgense*)	Subglobose	1.5–2.0 x 2.0–3.0	Glabrous, inflated (swollen), pericarp loosely surrounding the seeds
4	*G. ussuriense*	Subglobose	3.0–5.0 x 2.5–3.5	Glabrous
5	*G. boreale*	Subglobose	1.5–2.0 x 2.0–2.5	Densely uncinate
6	*G. amurense*	Subglobose	1.0–1.5 x 1.5–2.0	Densely setose with long straight hairs
7	*G. mugodsharicum*	Subglobose	1.0–1.5 x 1.0–2.0	Scabrous with short hooked setae
8	*G. septentrionale*	Subglobose	1.0–1.5 x 1.0–2.0	Sparsely hooked hair
9	*G. valantioides*	Subglobose	1.0–2.0 x 1.5–2.5	Glabrous to tuberculate

Discussion

Our results seem to prove the correspondence between the mericarp characters and the traditional classification within the section *Platygalium* (Pobedimova, 1958). Based on the main external fruit morphology, two groups have traditionally been distinguished, with glabrous and hispid fruits (Boissier, 1881). Our results based on morphological characters of mericarps agree with classification of the section *Platygalium* into three series (Pobedimova, 1958), since three types of mericarp surface were revealed, glabrous (Plate I), hairy with either hooked or straight hairs (Plate II), and tuberculate (Plate III). The glabrous type of mericarps is characteristic of *G. rubioides*, *G. articulatum*, *G. physocarpum* and *G. ussuriense* which belong to the series *Rubioidea*, while *G. boreale*, *G. amurense*, *G. septentrionale* and *G. mugodsharicum* with hairy mericarps belong to the series *Borealia* (Pobedimova, 1958). The series *Valantioidea* includes *G. valantioides* which has tuberculate mericarp surface.

Moreover, we found out that detailed characteristics of mericarp hairiness were useful to delimitation of four closely similar species (*G. boreale*, *G. amurense*, *G. septentrionale* and *G. mugodsharicum*) from the series *Borealia*. Thus, *G. boreale* has mericarps densely covered with hooked hairs 0.3 – 0.4 mm long, *G. amurense* has mericarps covered with long, dense, straight, hairs 0.50–0.65 mm long, while in *G. septentrionale* mericarps are sparsely covered with short hooked hairs 0.20–0.25 mm long. Finally, *G. mugodsharicum* has mericarps scabrous with short hooked setae 0.10–0.15 mm long. Mericarp characters were also found useful to distinguish between *G. physocarpum* and some other closely related species of the series *Rubioidea*. Here, however, *G. articulatum, G. ussuriense* and *G. rubioides* seem to be indistinguishable from each other neither by mericarp shape and dimensions, nor by the details of its surface. We suggest treating them all under *G. rubioides*.

Within the framework of this study, it has become clear that the present species concept for the section *Platygalium* is still very vague and needs further careful and critical morphological, palynological, karyological and molecular examination. Furthermore, the existence of hybridization between both closely related species and species from different subsections leading to appearance of plants with intermediate character combinations cannot be excluded. Due to these recognized problems, both the key below, and the data on mericarp dimensions in tables 1 and 2 are preliminary, and only enable to recognize typical members of the taxa studied.

Key to the studied species based on mericarp characters:

1a. Mericarps hairy 2
1b. Mericarps glabrous or tuberculate 5
2a. Mericarps covered with long, dense, straight hairs ..... *G. amurense*
2b. Mericarps covered with hooked hairs 3
3a. Mericarps covered with dense hooked hairs ..... *G. boreale*
3b. Mericarps scabrous or sparsely hooked hairs 4
4a. Mericarps scabrous with short hooked setae .....*G. mugodsharicum*
4b. Mericarps covered with sparsely hooked hairs ..... *G. septentrionale*
5a. Mericarps tuberculate ..... *G. valantioides*
5b. Mericarps glabrous 6
6a. Pericarp loosely surrounding the seed .... *G. physocarpum*
6b. Pericarp more or less adhere to the seed, rugose ....*G. rubioides, G. articulatum, G. ussuriense* 7

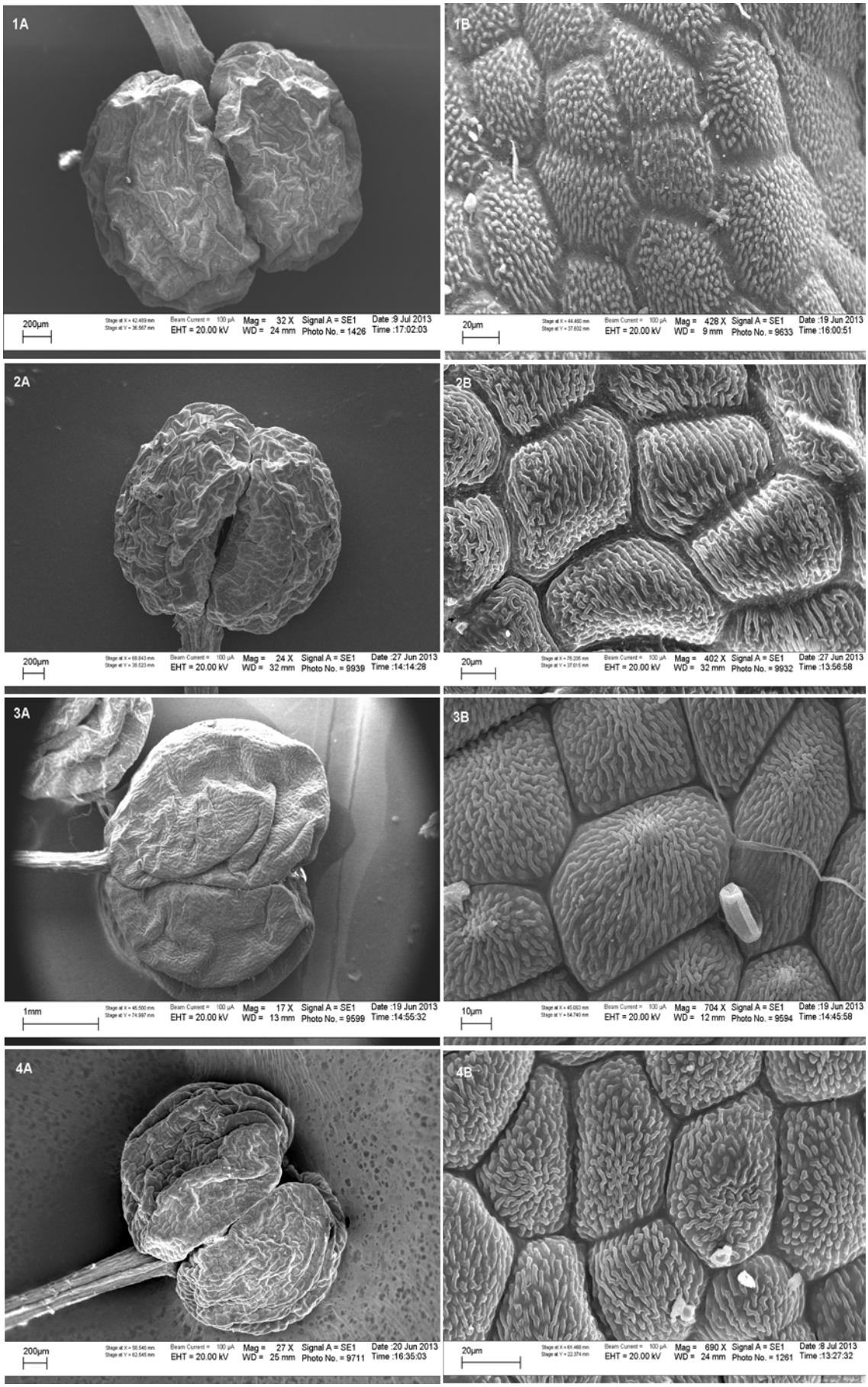

Plate I. SEM photographs of mericarps. A – entire mericarps, B – part of mericarp surface, enlarged.
1A, B – *Galium articulatum*; 2A, B – *G. rubioides*; 3A, B – *G. physocarpum*; 4A, B – *G. ussuriense.*

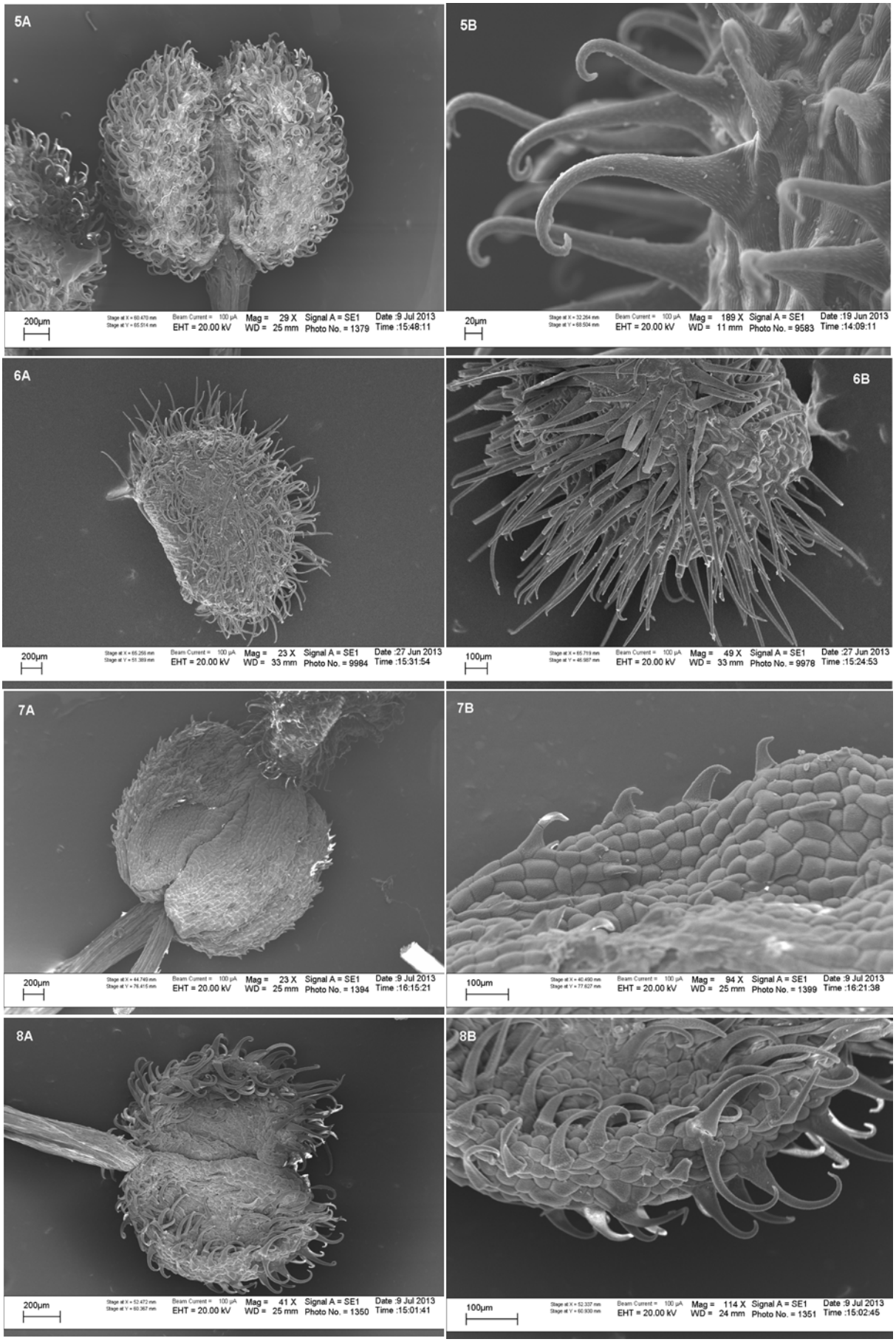

Plate II. SEM photographs of mericarps. A – entire mericarps, B – part of mericarp surface, enlarged.
5A, B – *Galium boreale*; 6A, B – *G. amurense*; 7A, B – *G. mugodsharicum*; 8A, B – *G. septentrionale.*

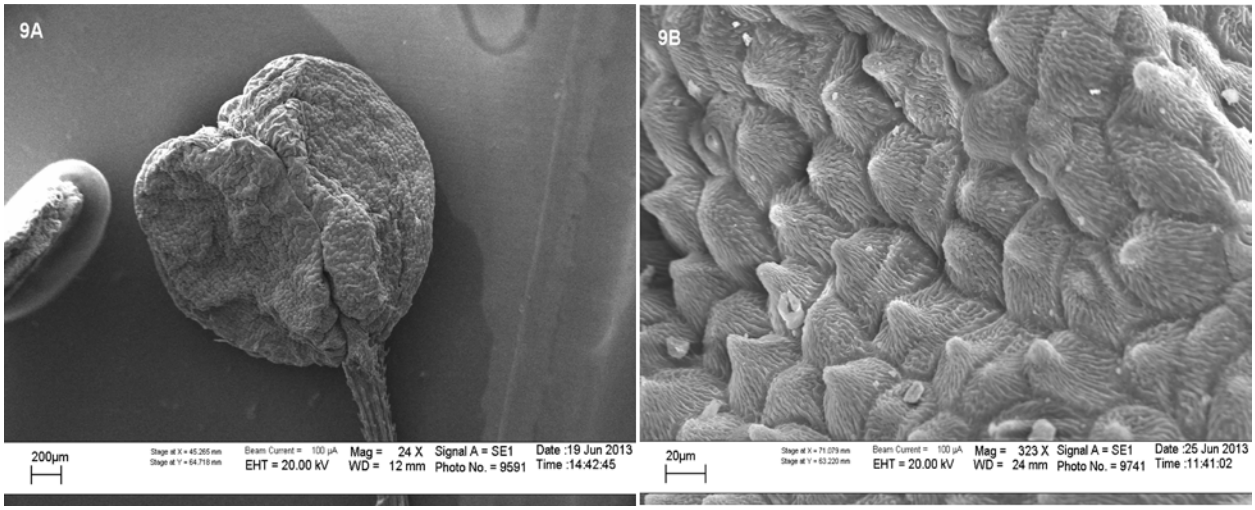

Plate III. SEM photographs of mericarps. A – entire mericarps, B – part of mericarp surface, enlarged.
9A, B – *Galium valantioides.*

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