

## Morphological Analysis of *Rubus* Seed

Sugae Wada<sup>1</sup> and Barbara M. Reed<sup>2</sup>

<sup>1</sup> Department of Horticulture, Oregon State University, 4017 Ag and Life Sciences Bldg, Corvallis, OR 97331-7304, USA

<sup>2</sup> USDA-ARS National Clonal Germplasm Repository, 33447, Peoria Rd, Corvallis, OR 97333-2521, USA

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### Abstract

The genus *Rubus* exhibits morphological diversity and a wide range of reproductive systems and habitats. We examined seed coat ultrastructural morphology of seed accessions of 10 subgenera preserved at the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository (NCGR), Corvallis, Oregon, using scanning electron microscopy (SEM). SEM images were taken of 56 seed samples collected worldwide. Macromorphological characteristics differed among groups at the subgenus level. *Chamaemorus*, *Cyclactis* (except *R. saxatilis* L.), *Idaeobatus*, *Lampobatus*, *Malachobatus*, and *Michranthobatus* have similar exomorphic patterns. *R. odoratus* L. and *R. parviflorus* Nutt. (subg. *Anoplobatus*) had a unique hilar end hole. *R. saxatilis* had seed coat sculpturing inconsistent with its assigned subg. (*Cyclactis*) and appeared more in common with subg. *Idaeobatus*. The subg. *Rubus* and *Idaeobatus* showed conspicuous patterns of reticulate and rugose surface relief of the outer cell walls. Species belonging to the subg. *Rubus* had steeper-edged truncate or acute lateral ridges with a wide and protruded raphal region, while *Idaeobatus* had smoothly curved rounded ridges and raphal region. The two species in subg. *Chamaemorus* showed areticate, finely textured surface with flat or no secondary cell wall protrusion and a ridged raphe. For *R. arcticus* L., subg. *Cyclactis*, accessions from three geographical regions had consistent microsculpture patterns. These morphological characteristics of *Rubus* seed revealed by SEM provide additional information to identify infrageneric levels.

### INTRODUCTION

The genus *Rubus* includes ~750 species (Robertson, 1974; Thompson, 1995) and occurs on all continents except Antarctica (Focke, 1914; Gustafsson, 1943; Hummer, 1996). The largest subgenus *Rubus*, the blackberries, is further subdivided into 12 sections. *Rubus* taxonomy is difficult and controversial. Stem armature and leaf morphology are key characters, however both are highly homoplastic and have limited phylogenetic value among the *Rubus* subgenera (Alice and Campbell, 1999). *Rubus* seed is enclosed in a hard stony endocarp or testa. The testa structure, maternal origin tissue, is a major constraint to radicle emergence in reduced seed dormancy phenotypes of *Arabidopsis* (Debeaujon et al., 2000). Descriptions of *Rubus* seed are scarce. Corner (1976) cited Topham's (1970) report on the seed coat morphology of two *Rubus* species, *R. fruticosus* L., agg. and *R. idaeus* L. stating "integument is 6 cells thick, the persistent seed coat of thin-walled cells, the middle layer is crushed, and the endosperm is 6 cells thick". Robertson (1974) reported "single seeded drupelets on a dry or spongy, often elongated receptacle, the drupelets falling individually or coalescent and either falling from the receptacle as a unit or with it; stones hard, variously textured; seeds filling the stone; embryo small, the radicle superior".

Seed coat morphology provides important taxonomic information in many plant families. Garnock-Jones (1991) prepared a generic key for Brassicaceae using seed morphology, testa anatomy, and embryos for several New Zealand genera. Clear differences in seed coat morphology were evident among the genera investigated. Recently Dowidar et al. (2003) studied the ultrastructure of seed coats and/or achenes

using SEM and certain selected macromorphological characters for 47 taxa of the Rosaceae. This study suggested that the Rosaceae needs taxonomic revision.

Ultrastructural pattern analysis of the seed coat observed under the SEM is reliable for evaluating phenotypic relationships and clarifying taxonomy (Bouman, 1975; Barthlott, 1981; Tobe et al., 1987; Vaughan and Whitehouse, 1971; Zou et al., 2001). Seed morphology provides various practical applications which perform an important role in many areas of seed biology (Jensen, 1998). A crucial application is the identification of seeds for gene bank management. The objective of our study was to characterize the seed coat characters revealed by SEM of *Rubus* seed at the NCGR. We examined the surface exomorphology of 56 *Rubus* seed accessions representing 51 taxa in 10 subgenera (USDA-ARS, 2006).

## MATERIALS AND METHODS

The seed accessions for this study were collected from 15 countries (Table 1). Samples of 100 seed per accession were obtained from seed storage (-20°C) at NCGR. SEM images were taken using an AmRay3300 FE Field Emission SEM in the Department of Botany and Plant Pathology, Oregon State University. Seeds were mounted on aluminum stubs with two-sided carbon conductive-adhesive tape and sputter coated for 2 minutes with a thin layer of the alloy, 60% gold and 40% palladium (Edwards S150B, U.K.). All supplies for the SEM were purchased from Ted Pella, Inc. (Redding, CA). Terminology of Barthlott (1981) based on SEM observations of epidermal and seed coat surfaces in 5000 species of seed plant was applied and that of Koul et al. (2000) as modified from Murley (1951) was also used. Further terms were added to describe specific seed coat morphology for this genus. Accession identifying numbers are in parentheses, plant identification (PI) numbers are listed in Table 1.

## RESULTS

### Morphological Analysis by Subgenus

The basic cell shape is mostly scale-like with anticlinal undulations and sinuate margins. The micropyle is located on the protuberance of the raphal region which is more or less wider and shallowly or highly raised depending on the species. Seed dimensions range from  $1.4 \times 1.2 \times 0.81$  mm to  $6.0 \times 3.5 \times 2.35$  mm.

**1. Subg. *Rubus* (Fig. 1-a).** *R. adenotricos* Schldt. (1250), *R. allegheniensis* Porter (552), *R. argutus* Link (1818), *R. caesius* L. (2167), *R. canadensis* L. (791), *R. canescens* DC. (941), *R. hirtus* Waldst. & Kit. (905), *R. hispidus* L. (2022), *R. kennedyanus* Fernald (525), *R. lacinatus* Willd. (2224), *R. robustus* C. Presl. (1789), *R. sanctus* Schreb. (1057), *R. sp.* (1909), *R. separianus* Genev. (754), *R. ulmifolius* Schott. (943), *R. ursinus* Cham. & Schldt. (2041): Most seed samples of subg. *Rubus* have conspicuously reticulated secondary periclinal wall sculpturing which appears as rough seed surfaces. Four groups share common traits of microsculpturing pattern. Subg. *Rubus* commonly has more conspicuous and steeper-edged truncate or acute ridges in the lateral view with wider and well projected raphal regions.

*Group 1:* *R. allegheniensis*, *R. argutus*, *R. caesius*, *R. kennedyanus*, *R. robustus*, *R. separianus*, *R. sp.* Outlines of the cells are mainly round or curved with conspicuous markings such as a circle, half moon, heart and omega shape. The rounded, curved outline cells are surrounded by the secondary periclinal wall sculpturing; those are distinctly reticulated with somewhat more rounded structures, which continue from concave to U-shaped depressions.

*Group 2:* *R. canadensis*, *R. canescens*, *R. lacinatus*. Outlines of the cells are smaller than other groups. The outlines are round, elongated in one direction or have a curved C-shape. The cells are surrounded with fairly thick and flat-topped wide, secondary periclinal walls. These walls are irregularly reticulated, consisting of the various shapes, including round, elongated in one direction with curve or without, triangular or rectangular.

*Group 3: R. adenotricos, R. hirtus, R. hispidus, R. sanctus, R. ursinus.* Outline of the cells are wavy and undulate rather than reticulate. Cell shapes include round, tri- or tetragonal and eye-shaped (ocellate). The cells are surrounded by the secondary periclinal wall and have narrower and somewhat more pointed (inverted V-shape) sculpturing in a wavy striped pattern. Shallower concave depressions are reflected in the cell wall sculpturing.

*Group 4: R. ulmifolius, R. robustus.* Outlines of the cells are mostly curved ranging from round to half moon-, triangular-, or square- shaped. The cells are surrounded by flat-topped wider secondary walls with relatively regular patterns of reticulation (common feature of reticulation shown in subg. *Idaeobatus*). Shallower, flattened C-shaped and concave depressions are continued to the secondary periclinal walls.

**2. Subg. *Cyclactis* (Fig. 1-b).** *R. arcticus* L. (1894: Alaska/1919: China/2238: Sakhalin), *R. pedatus* Sm. (1895), *R. saxatilis* L. (2173): The basic cell type is scale-like, finely textured somewhat round, half moon, isodimetric, or elongated in one direction in outline. *R. arcticus* and *R. pedatus* have similar patterns of seed coat sculpturing. These patterns have neither secondary wall structures of surface reticulation, depressions, nor protruded raphal regions and ridges but present an entirely flat and smooth surface. For *R. arcticus*, subgenus *Cyclactis*, three accessions were examined from different geographical regions (1894: Alaska: *R. a.* subsp. *stellatus*, 1919: China: *R. a.* subsp. *arcticus*, 2238: Sakhalin). These samples revealed a consistent microsculpturing pattern.

**3. Subg. *Idaeobatus* (Fig. 1-c).** *R. aurantiacus* Focke (1961), *R. coreanus* Miq. (1636), *R. crataegifolius* Bunge (2283), *R. hawaiiensis* A. Gray (2191), *R. hoffmeisterianus* Kunth and C.D. Bouche (1079), *R. idaeus* L. (2177): The cell outline is round, elongated in one direction, and/or curved C-shape, which is similar to that of subg. *Rubus*. The secondary periclinal walls are also distinctly round edged with C-shaped structures that appear rotated 90° to the right and have surface sculpturing between foveate and reticulate. When compared to subg. *Rubus*, seeds of *Idaeobatus* have smaller and more regular spaced depressions. Most subgenus *Idaeobatus* taxa investigated here (except *R. crataegifolius* and *R. hoffmeisterianus*) show smoothly curved round-ridges in the lateral view with flatter or less protruding raphal regions. *R. crataegifolius* and *R. hoffmeisterianus* have exceptionally narrow and acute ridges. Two accessions of *R. idaeus* (2177: Armenia, 2302: Georgia) and *R. strigosus* (1690: Canada, 1978: US, Oregon) showed consistent cell types and sculpturing pattern of reticulation of the secondary wall.

**4. Subg. *Lampobatus* (Fig. 1-d).** *R. bogotensis* Kunth (1283), *R. briarceus* Focke (1809), *R. glaucus* Benth. (2095), *R. megalococcus* Focke (1800), *R. nubigenus* Kunth (1249), *R. roseus* Poir. (1281): The cell outlines are rounded, elongated in one direction, or triangular to pentagonal. The cell boundaries range from irregularly straight to regularly curved. Secondary walls appear flat topped more than other subgenera and circular in shape for most species (*R. briarceus*, *R. glaucus*, *R. megalococcus*, *R. nubigenus*, and *R. roseus*), except *R. bogotensis* (more angled, thicker, wider periclinal walls with deeper and larger depressions). *R. bogotensis* has one of the most spectacular, unique and a species specific seed coat sculpturing for all of *Rubus* (Fig. 1-d). *R. nubigenus* and *R. roseus* exhibit similar sculpturing pattern in cell types, secondary periclinal wall structure, and total seed shape.

**5. Subg. *Chamaemorus* (Fig. 1-e).** *R. pseudochamaemorus* Tolm. (2243), *R. chamaemorus* L. (2241): Subg. *chamaemorus*, consisting of only two species (some authors report it as monotypic), has distinctive seed coat morphology that is finely textured, areticated and entirely flat without protruded secondary wall sculpturing. These seed coats have neither significant ridges nor raphal regions in the lateral view.

**6. Subg. *Michranthobatus* (Fig. 1-f).** *R. cissoids* A. Cunn. (772), *R. schmidelioides* A. Cunn. (741): In both taxa the secondary periclinal walls are sulcate or striated and areticated. Instead of reticulation, striated transverse wall sculpturing toward the embryonic axis is exhibited on the seed coat of *R. cissoides* (this characteristic striation is also observed as a unique feature from several taxa, *R. lambertianus*, *R. multibracteatus*, *R. tephrodes* of the subg. *Malachobatus*). *R. schmidelioides* has more scarcely and irregularly folded and/or verrucated surface sculpturing.

**7. Subg. *Malachobatus* (Fig. 1-g).** *R. hillii* F. Muell. (1199), *R. lambertianus* Ser. (2133), *R. multibracteatus* H. Lev. & Vaniot (1642), *R. tephrodes* Focke (1713), *R. setchuenensis* Bureau & Franch. (1695, 1696), *R. swinhoei* Hance (1671): Three different trends of seed coat sculpturing pattern are observed.

*Group 1:* *R. lambertianus*, *R. multibracteatus*, *R. tephrodes*. Secondary walls are areticulate and with transversely striate or sulcate periclinal sculpturing to the embryonic axis which is straight and/or somewhat curved. These are unique seed coat features compared to those of the other subgenera. Only one species in *Michranthobatus*, *R. cissoides* (772) collected from New Zealand, had these transverse striations.

*Group 2:* *R. hillii*, *R. swinhoei*. Total seed coat morphology was similar to the common pattern of sculpturing of the subg. *Idaeobatus*, but shallowly reticulate and continued by periclinal walls with milder and shallower depressions. *R. hillii* has similar seed coat sculpturing and total seed shape like *R. hayata-koizumii* in the subg. *Chamaebatus*.

*Group 3:* *R. setchuenensi*. Bureau & Franch: Both accessions (1695, 1696) have flat and non-sculptured surfaces. They have flatter reticulations than other subgenera and/or have areticulated surface sculpturing.

**8. Subg. *Anoplobatus* (Fig. 1-h).** *R. odoratus* L. (2215), *R. parviflorus* Nutt. (1738): Seed coat sculpturing of both taxa is somewhat more delicate but similar to the common features of the subg. *Idaeobatus*. However, both have a distinctively unique hilar end hole which is a visibly raised and rimmed structure. This structure is observed only in seeds of this subgenus.

**9. Subg. *Dalibardastrum* (Fig. 1-i).** *R. tsangorum* Han.–Mazz. (1674): The cell outlines are round, elongated in one direction or dichotomous, curved, triangular, or tetra to pentagonal. The secondary periclinal walls are foveate-reticulate, flat topped or widely rounded, with apparent rough surface sculpturing. The depressions are small, V-shaped or concave, and are continued by the secondary wall.

**10. Subg. *Chamaebatus*: (Fig. 1-j).** *R. hayata-koizumii* Naruh. (178): The cell outlines range from mostly round, elongated in one direction, to triangular. The secondary periclinal walls are foveate-reticulate with flat topped, widely rounded surface sculpturing. Shallow depressions are concave into and continued by the secondary wall sculpturing.

## DISCUSSION

Barthlott (1984) emphasized and our observations confirmed that seeds of *Rubus* exhibit a complex and high level of morphological and micromorphological diversity. This study provides valuable taxonomic information concerning genetic-phylogenetic differences. Satomi and Naruhashi (1971) utilized seed coat characteristics to re-classify *R. trifidus* from subg. *Anoplobatus* to subg. *Idaeobatus* because of their closely analogous seed coat morphology. Seeds of related species possess similar microsculpturing, suggesting that differences in microsculpturing can correspond to divergent taxonomical classification (Clark and Jernstedt, 1978).

Our study observed distinct exomorphic patterns in each of the subg. *Chamaemorus*, *Cyclactis* (except *R. saxatilis* L.), *Idaeobatus*, *Lampobatus*, *Malachobatus*, and *Michranthobatus*. Subg. *Rubus* and *Idaeobatus* exhibited a conspicuous pattern of reticulate and rugose surface relief of the outer cell walls. Species seeds within subg. *Rubus* had more projected and steeper-edged truncate or acute ridges on their lateral view with a wider raphal region in contrast with those of *Idaeobatus* which had rather smoothly curved rounded ridges. However, several distinct groups of shared sculpturing patterns existed within both subgenera. Subg. *Rubus* and *Idaeobatus* possibly have polyphyletic origins (Alice and Campbell, 1999). We found *R. saxatilis* to have seed coat sculpturing inconsistent with its assigned subgenus (*Cyclactis*) and more in common with subg. *Idaeobatus*. Therefore, we suggest that *R. saxatilis* should be moved into subg. *Idaeobatus* which is consistent with recent molecular criteria. Alice and Campbell (1999) documented that *R. saxatilis* should be transferred to the subg. *Idaeobatus*, proximal to *R. crataegifolius*. Although the taxon has fruit that dehisces with the receptacle it is most

likely a tetraploid derivative of *R. idaeus*. Both *R. odoratus* and *R. parviflorus*, in subg. *Anoplobatus*, can be distinguished by their hilar end hole, unique in this genus.

Seed exo-micromorphological characteristics of genus *Rubus* as revealed by SEM in this study provided consistent key polymorphic traits, and useful information compared to other morphological markers employed to identify specific subgenera and/or species. Our SEM investigation of seed coat characters of the genus *Rubus* shows the diversity among the species and demonstrated common traits within subgenera. Further study through broader sampling of species could improve taxonomic consistency.

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## Tables

Table 1. Origin and identifying numbers of *Rubus* accessions.

<i>Rubus</i> Taxa	Acc. number	P I number	<i>Rubus</i> Taxa	Acc. number	P I number
<b>Australia</b>			<b>New Zealand</b>		
<i>R. hillii</i>	1199	553226	<i>R. cissoides</i>	772	553163
<b>Armenia</b>			<i>R. hispidus</i>	2022	618367
<i>R. caesius</i>	2176	643953	<i>R. schmidelioides</i>	741	553883
<i>R. idaeus</i>	2177	638211	<i>R. separinus</i>	754	553887
<i>R. saxatilis</i>	2173	638210	<b>Georgia</b>		
<b>Bolivia</b>			<i>R. idaeus</i>	2302	643957
<i>R. briaceus</i>	1809	618475	<b>Japan</b>		
<i>R. megalococcus</i>	1800	618473	<i>R. crataegifolius</i>	2284	638306
<i>R. robustus</i>	1989	618466	<i>R. mesogaeus</i>	2321	643958
<b>Bulgaria</b>			<i>R. sachalinensis</i>	2282	553870
<i>R. sp.</i>	1909	618410	<b>Pakistan</b>		
<b>Canada</b>			<i>R. hoffemeisterianus</i>	1079	553241
<i>R. lacinatus</i>	2224	638255	<i>R. sanctus</i>	1057	553877
<i>R. strigosus</i>	1690	606474	<b>Russia</b>		
<b>China</b>			<i>R. arcticus</i>	2238	638266
<i>R. arcticus</i>	1919	608837	<i>R. chamaemorus</i>	2241	638269
<i>R. aurantiacus</i>	1961	606538	<i>R. pseudochamaemorus</i>	2243	638271
<i>R. coreanus</i>	1636	618520	<b>Taiwan</b>		
<i>R. lambertianus</i>	2133	643936	<i>R. hayata-koizumii</i>	178	553899
<i>R. multibracteatus</i>	1642	606459	<b>USA</b>		
<i>R. parvifolius</i>	1664	606467	<i>R. allegheniensis</i>	552	553093
<i>R. setchuenensis</i>	1695	604616	<i>R. arcticus</i>	1894	606526
<i>R. setchuenensis</i>	1696	604617	<i>R. argutus</i>	1818	606490
<i>R. tephrodes</i>	1713	604621	<i>R. canadensis</i>	791	553136
<i>R. swinhoei</i>	1671	606471	<i>R. hawaiiensis</i>	2191	638225
<i>R. tsangorum</i>	1674	618534	<i>R. kennedyanus</i>	525	265790
<b>Ecuador</b>			<i>R. occidentalis</i>	2211	638243
<i>R. adenotricos</i>	1250	548889	<i>R. odoratus</i>	2215	265790
<i>R. bogotensis</i>	1283	548895	<i>R. parviflorus</i>	1738	606477
<i>R. glaucus</i>	2095	618275	<i>R. pedatus</i>	1895	606527
<i>R. niveus</i>	2092	618305	<i>R. spectabilis</i>	2045	618357
<i>R. nubigenus</i>	1249	548908	<i>R. ursinus</i>	2041	618353
<i>R. roseus</i>	1281	548921	<b>Yugoslavia</b>		
			<i>R. canescens</i>	941	370253
			<i>R. hirtus</i>	905	370217
			<i>R. ulmifolius</i>	943	370255

## Figures

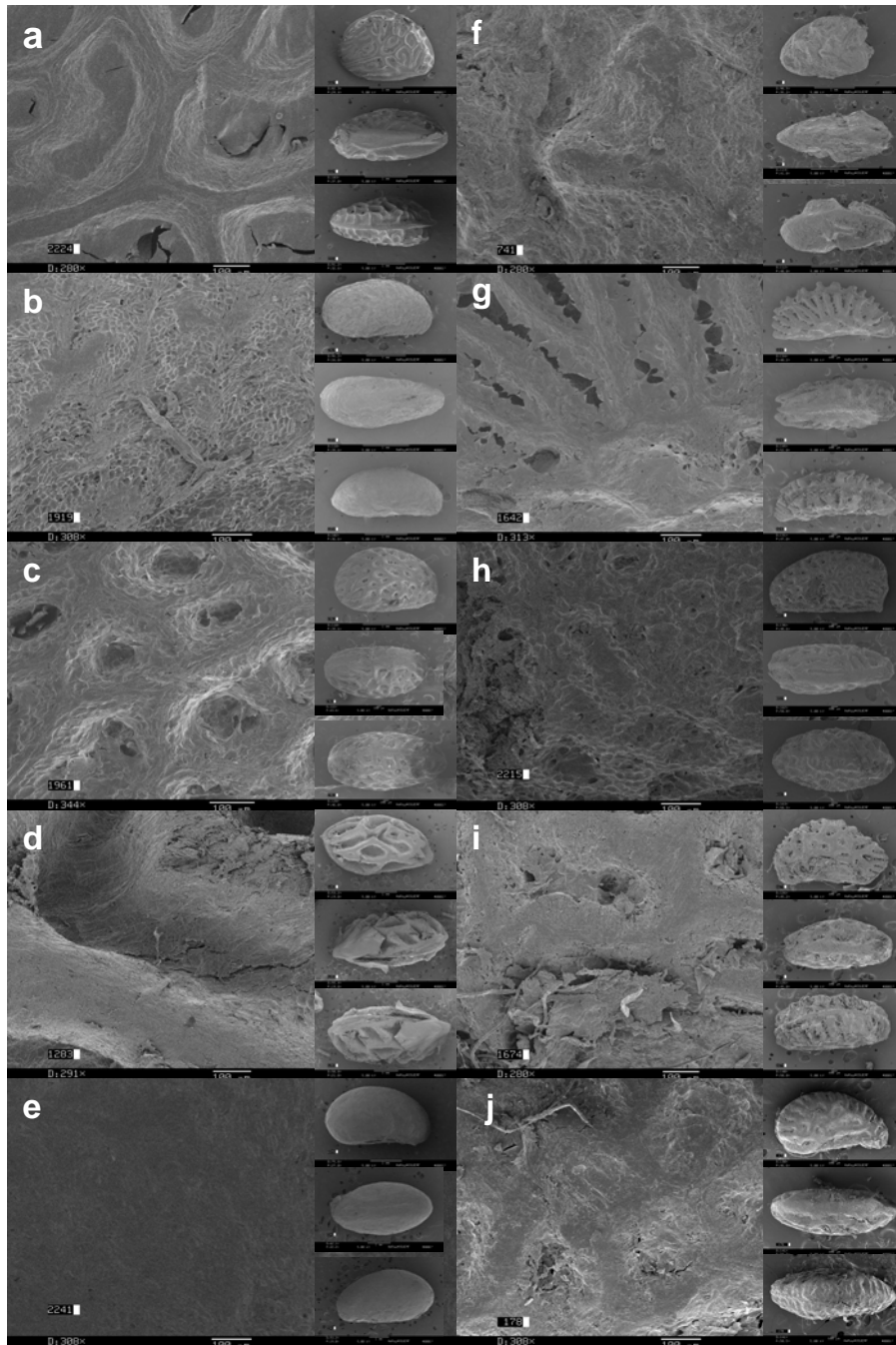


Fig. 1. SEM images: For each letter (a-j) there is a surface view in higher magnification (the scales are shown below each) with smaller images of (upper) seed overview, (middle) view of a micropylar on the raphal region and (lower) view of a seed edge (back side of raphal region). a. Subg. *Rubus* (*R. lacinatus*), b. Subg. *Cyclactis* (*R. arcticus*), c. Subg. *Idaeobatus* (*R. aurantiacus*), d. Subg. *Lampobatus* (*R. bogotensis*) e. Subg. *Chamaebatus* (*R. chamaemorus*), f. Subg. *Michranthobatus* (*R. schimiloides*), g. Subg. *Malachobatus* (*R. multibracteatus*), h. Subg. *Michranthobatus* (*R. odoratus*), i. Subg. *Dalibardastrum* (*R. tsangorum*), j. Subg. *Chamaebatus* (*R. hayata-koizumii*).