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Research Article

Pollen morphology and anatomy of *Rubus* L. (Rosaceae) in Thailand

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Abstract

Pollen morphology and anatomy were investigated in 19 species of the genus *Rubus* L. from Thailand by observation and analysis under a light microscope and a scanning electron microscope. The pollen grains are monads, with radial symmetry, circular shaped in polar view and isopolar. The sizes of the pollen grains are small and medium. Considering the species studied, the majority have medium sized pollen grains. There were different shapes detected viz., oblate, suboblate, prolate spheroidal, oblate spheroidal and prolate. The pollen grains have various apertures: tricolporate, tricolporate-tetracolporate, tricolpate and tricolpate-tetracolpate. There were five forms of exine sculpturing as follows: rugulate-perforate, striate-perforate, microverrucate-perforate, microverrucate and perforate. In the genus *Rubus* it is not possible to use the morphology of the pollen for the identification to species level. Eleven species had the morphology of the pollen studied for the first time. The anatomy of the leaf, petiole and stem were observed by transverse section. The anatomy can be used for the identification to species level. The results indicate that the anatomical characteristics used for the identification key are shape of leaf margin, shape of adaxial surface of midrib, pericyclic fibers, idioblast cell, shape of petiole, starch grain, number of vascular bundles, shape of stem and types of trichomes. In this study, the anatomy of 19 species was reported for the first time. The numerical analysis based on the pollen morphology and anatomy data did not support classification of the genus *Rubus* L.

Keywords: anatomy; palynology; Rosaceae; *Rubus*; Thailand

Introduction

The family Rosaceae has over 3,000 species from 90 genera and it distributed globally (Potter *et al.*, 2007). Classification within Rosaceae also varies based on the data. Schulze-Menz (1964) divided the Rosaceae using the characteristics of the fruit type into four subfamilies (Maloideae, Prunoideae, Rosoideae and Spiraeoideae). Hutchinson (1964) divided the Rosaceae into 20 tribes using the morphological characteristics of flower, fruit and seed. Morgan *et al.* (1994) classified the family Rosaceae into three subfamilies using

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molecular data as follows: Amygdaloideae, Maloideae and Rosoideae. Takhtajan (1997) classified the family Rosaceae into 11 subfamilies, 21 tribes and 10 genera. Kubitzki (2004) divided the family Rosaceae into 17 tribes and 85 genera. The family Rosaceae has been morphology studied by several authors: Thuan (1968); Vidal (1968); Kalman (1993); Lu *et al*. (2003). Thuan (1970) studied the family Rosaceae in Thailand and divided the Rosaceae into four subfamilies, 21 genera and 61 species. The new classification system based on molecular data by Potter *et al.* (2007) can separate the family Rosaceae into three subfamilies (Dryadoideae, Rosoideae and Spiraeoideae). *Rubus* is a genus of subfamily Rosoideae consisting of about 700 species. It is a large genus with a worldwide distribution, except for Antarctica, and central distribution in South America mainly with an altitudinal range between 1,000 and 3,000 m (Kalkman, 1993). The genus *Rubus* was first identified in Species Plantarum in the class Icosandria, order Polygynia consisting of 10 species (Linnaeus, 1753). Focke (1910, 1911, 1914) classified the genus *Rubus* using the morphological characteristics into 12 subgenera viz., *Anoplobatus, Chamaebatus, Chamaemorus, Comaropsis, Cylactis, Dalibarda, Dalibardastum, Idaeobatus, Lampobatus, Malachobatus, Orobatus* and *Rubus,* with 429 species. Alice and Campbell (1999) classified the genus *Rubus* based on molecular data into 12 subgenera, which is consistent with Focke (1910, 1911, 1914). The genus *Rubus* was studied by Kurz (1877) in Flora of British Burma and consisted of eight species; Thuan (1968) in the Flora of Cambodia, Laos and Vietnam described two subgenera (*Idaeobatus* and *Malachobatus*) with 49 species; Kalkman (1993) in Flora of Malesiana described five subgenera (*Chamaebatus, Idaeobatus, Malachobatus Micranthobatus* and *Rubus*) with 49 species; and Lu *et al*. (2003) in Flora of China described eight subgenera with 208 species. *Rubus*, which has 23 species throughout its two subgenera (*Idaeobatus* and *Malachobatus*), was reported by Thuan in the Flora of Thailand in 1970. The types of leaves, leaf shape (outline, leaf apex, leaf base and leaf margin), shape of stipule, bract and calyx, size of flowers and types of hair are used for the identification key of genus *Rubus* in Thailand (Thuan, 1970). There is great variation in the morphology of *Rubus* species, ranging from the structure of the leaf (outline of leaves, leaf base, leaf margin) and the shape of the bract and stipule; therefore, pollen morphology and anatomy are applied to study the plant taxonomy.

The study of pollen morphology is important for plant taxonomy in the Rosaceae. The first report studying the pollen morphology with a light microscope (LM) was by Erdtman (1966). He found that the grains had radial symmetry, were monad, isopolar and the sizes varied from small to large, while the shapes varied as follows: oblate-prolate, subptolate and oblate spheroidal, pollen aperture are tricolporate in genus *Adenostoms*, *Agrimonia*, *Aruncus*, *Rhodotypod* and *Rubus*, exine sculpture as follows: substriste (*Adenostoms*) and striate (*Aruncus, Rhodotypos* and *Rubus*). In the genus *Rubus*, pollen grains characteristics are suitable to be used for identification of species, for example, size, shape, aperture, syncolpate and exine sculpture. Tomlik-Wyremblewska (1995) has studied the morphology of the pollen from the genus *Rubus* in nine species (*R. apricus* Wimmer*, R. armeniacus* Focke*, R. divaricatus* P.J. Müller*, R. fabrimontanus* Spribille*, R. gracilis* J. Presl & C. Presl, *R. hirtus* Waldstein & Kitaibel*, R. laciniatus* Willdenow*, R. nessensis* W. Hall and *R. pedemontanus* Pinkwart) under light microscope and scanning electron microscope. The result showed that the pollen grains are monad, with radial symmetry, isopolar, small in size, tricolporate and striate. The morphology of the pollen of genus *Rubus* was considered in various reports as follows: Wang and Chen (2001) six species, Tomlik-Wyremblewska *et al*. (2004) 15 species, Wrońska-Pilarek *et al*. (2012) six species, Xiong *et al.* (2019) 155 species, Lechowicz *et al.* (2020) 58 species and Lechowicz *et al.* (2021) six species. The pollen morphology of eleven species of the genus *Rubus* has never been reported.

The basis for plant taxonomy study is the morphology. For identification, the key characteristics for the species of the genus *Rubus* are the flowers, but flowers are only found in some seasons. Many anatomical characteristics for the genus *Rubus* can be used for the identification of the species, namely types of trichomes, idioblast, starch grain, types of crystal and pericyclic fibers (Metcalfe and Chalk, 1950; Fell and Rowson, 1956, 1957, 1960, 1961; Wada and Reed, 2008; Tomlik-Wyremblewska *et al.,* 2010; Tomaszewski *et al.,* 2014;

Kasalkheh *et al.,* 2019). Therefore, this research's focus is to study genus *Rubus* pollen morphology and anatomy to provide classification and identification of some species in Thailand.

Materials and Methods

Plant materials

Plants from 19 species of *Rubus* were collected from the field in Thailand during 2014-2019 (Table 1). Plant specimens were pressed and fixed in 70% ethanol and deposited as reference specimens in the Mahasarakham University Herbarium. Specimens were studied palynologically and anatomical characters were obtained from spirit specimens.

Table 1. List of *Rubus* (Rosaceae) species studied in Thailand

No	Subgenus	Scientific name	Locality	Voucher specimen
1	Idaeobatus	*Rubus ellipticus* var. *obcordatus* Focke	Chiang Rai Province	K. Hanchana 26
2		*R. leucanthus* Hance	Chiang Rai Province	P. Srisanga 969
3		*R. niveus* Thunb	Chiang Rai Province	K. Hanchana 161
4		*R. sumatranus* Miq	Chiang Rai Province	K. Hanchana 160
5	Malachobatus	*R. alceifolius* Poir	Nakhon Nayok Province	K. Hanchana 68
6		*R. blepharoneurus* Cardot	Phetchabun Province	K. Hanchana 71
7		*R. chevalieri* var. *angkae* Thunb	Mae Hong Son Province	K. Hanchana 128
8		*R. clinocephalus* Focke	Phayao Province	K. Hanchana 60
9		*R. elongatus* Smith	Chiang Rai Province	K. Hanchana 184
10		*R. hasskarlii* Miq	Nakhon Nayok Province	K. Hanchana 176
11		*R. hastifolius* H.Lév. & Vaniot	Chiang Mai Province	K. Hanchana 137
12		*R. lasiotrichos* Focke	Chaiyaphum Province	K. Hanchana 173
13		*R. malvaceus* Focke	Chiang Rai Province	K. Hanchana 27
14		*R. moluccanus* L.	Chiang Mai Province	K. Hanchana 18
15		*R. pyrifolius* Smith	Chiang Rai Province	K. Hanchana 206
16		*R. rufus* Focke	Loei Province	K. Hanchana 178
17		*R. rugosus* Smith	Loei Province	K. Hanchana 23
18		*R. tiliaceus* Smith	Chiang Mai Province	K. Hanchana 124
19		*R. xanthoneurus* Focke	Chiang Rai Province	W. Pongamornkul 2513

Palynological studies

The pollen of 19 species of *Rubus* from Thailand were stored in 70% ethanol. An alcohol series (70, 80, 95 and 100%) was used to prepare the samples. The observations were carried out both with a light microscope (LM) and scanning electron microscope (SEM). For all species, voucher specimens were placed in the herbarium at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand. For LM analysis, three-five drops of silicone oil were placed onto the pollen grains in a vial, after that the samples were mounted on slides and sealed with paraffin. An Axio LabA1 LM was used for the examination of the pollen grains and it had a digital camera to take photographs and to determine the types of the pollen by taking measurements of 20 pollen grains per sample per specimen for the polar axis (P), equatorial axis (E), exine thickness and size of the aperture. During the analysis with the SEM, samples (stored in absolute alcohol) were transferred, mounted and dried on aluminum stubs using cellophane tape (double-sided), after which they were covered with gold. The pollen grains were examined with an SEM (JSM 6460 LV) microscope and digitally

photographed so the details could be identified for the aperture structure and exine sculpturing. Pollen grain classification follows Erdtman (1966) and terminology classes follow Punt *et al*. (2007).

Anatomical studies

The samples of 19 species of *Rubus* from Thailand were stored in 70% ethanol followed by transverse sectioning of the leaf, petiole and stem. The observations were carried out with a LM, but the type of trichome observations were carried out both with a LM and SEM. For LM analysis, the samples were placed in formalin acetic acid alcohol (FAA) and fixed before using tertiary butyl alcohol series for dehydration and then being embedded in paraffin following the methods of Thammathaworn (1996), sectioned on a rotary microtome at 5-10 μm thickness and stained in safranin and fast green for at least five slides for each sample, and then an Axio LabA1 LM was used for examination and to take digital photographs. For the SEM analysis, samples in absolute alcohol was transferred, mounted and dried on aluminum stubs with cellophane tape (double-sided) before being covered by gold. The trichomes were examined with an SEM (JSM 6460 LV) microscope and digitally photographed to determine the details of the trichome types. Abaxial surface thickness, adaxial surface thickness, leaf thickness, midrib length, midrib width, number of collenchyma layers in stem, number of collenchyma layers in petiole, number of large vascular bundles in petiole, number of palisade layers in leaf blade, number of small vascular bundles in petiole, number of spongy layers in leaf blade, number of vascular bundles in midrib and spongy mesophyll thickness, the ratio between adaxial surface thickness per abaxial surface thickness in leaf blade from each species were measured and the mean was calculated based on the range of the standard deviation. The type of trichome classification follows Kasalkheh *et al*. (2019) and Tomaszewski *et al*. (2014). Example specimens were deposited into the herbarium at the Department of Biology, Faculty of Science, Mahasarakham University, Thailand.

Statistical analysis

The morphological features of pollen grains and the anatomy of each species were assessed for morphometric analysis. In total eight quantitative characters for the pollen grains were analyzed (Colpus length: CL, Colpus width: CW, Distance between the apices of two ectocolpi: d, Equatorial axis; E, Exine thickness: ET, Polar axis: P, CL/CW ratio and E/CW ratio) (Table 2) and anatomy analyzed for six quantitative characters (Table 3). Factor and cluster analyses were used for the morphometric analysis. Principle component analysis (PCA) was used to perform the factor analysis. Euclidean distance was used for the cluster analysis (CA) and the unweighted pair-group methods using arithmetic average (UPGMA) was undertaken to form a phenogram. Statistical Package for the Social Science (SPSS version 14) was used to conduct the statistical analysis.

Table 2. Pollen morphological characters and character states used in morphometric analysis of *Rubus* (Rosaceae) in Thailand

No.	Characters	Character states
1	Equatorial axis (E)	Scale
2	Polar axis (P)	Scale
3	Exine thickness (ET)	Scale
4	Colpus length (CL)	Scale
5	Colpus width (CW)	Scale
6	CL/CW ratio	Scale
7	E/CW ratio	Scale
8	Distance between the apices of two ectocolpi (d)	Scale

Table 3. Anatomy characters and character states used in morphometric analysis of *Rubus* (Rosaceae) in Thailand

No.	Characters	Character states
1	Leaf thickness (LT)	Scale
2	Adaxial surface thickness (UET)	Scale
3	Abaxial surface thickness (LET)	Scale
4	Spongy mesophyll thickness (ST)	Scale
5	Midrib length (ML)	Scale
6	Midrib width (MW)	Scale

Results

Pollen morphology

LM and SEM were used to observe and analyze the morphological features of the pollen grains of *Rubus* from Thailand. Tables 4-5 contain the results. The general characteristics of the pollen grains are monads, radial symmetry, circular shaped in polar view and isopolar.

Pollen size

The sizes of the pollen grains ranged from small to medium (P = 17.73-40.97 μm, E = 18.82-43.48 μm). The pollen size can be separated into two types as follows: small size, including *Rubus ellipticus* var. *obcordatus* Focke, *R. elongatus* Smith, *R. hastifolius* H.Lév. & Vaniot, *R. leucanthus* Hance, *R. moluccanus* L.*, R. niveus* Thunb and *R. sumatranus* Miq and medium size, including *R. alceifolius* Poir, *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. clinocephalus* Focke *R. hasskarlii* Miq, *R. lasiotrichos* Focke, *R. malvaceus* Focke, *R. pyrifolius* Smith, *R. rufus* Focke, *R. rugosus* Smith, *R. tiliaceus* Smith and *R. xanthoneurus* Focke. *R. alceifolius* Poir had the largest pollen grains (P = 40.97 μm, E = 43.48 μm) and *R. ellipticus* var. *obcordatus* Focke had the smallest pollen grains (P = 17.73 μm, E = 22.14 μm) (Table 4). The medium size was found in the majority of the species studied.

Pollen shape

Considering the P/E ratio, when looking at the equatorial view, the pollen grain shapes can be classified into five types as follows:

Type I: Consists of the species with the oblate shape (100 x P/E ratio = 50.10-75.00), which includes *R. blepharoneurus* Cardot.

Type II: Consists of the species with the suboblate shape (100 x P/E ratio = 75.10-88.00), which includes *R. ellipticus* var. *obcordatus* Focke, *R. niveus* Thunb and *R. rugosus* Smith.

Type III: Consists of the species with the prolate spheroidal shape (100 x P/E ratio = 88.10-100.00), which includes *R. alceifolius* Poir, *R. clinocephalus* Focke, *R. hasskarlii* Miq, *R. lasiotrichos* Focke, *R. leucanthus* Hance, *R. malvaceus* Focke, *R. rufus* Focke, *R. sumatranus* Miq and *R. xanthoneurus* Focke.

Type IV: Consists of the species with the oblate spheroidal shape (100 x P/E ratio = 100.10-114.00), which includes *R. chevalieri* var. *angkae* Thunb, *R. elongatus* Smith, *R. hastifolius* H.Lév. & Vaniot, *R. moluccanus* L. and *R. tiliaceus* Smith.

Type V: Consists of the species with the prolate shape (100 x P/E ratio = 133.10-200.00), which includes *R. pyrifolius* Smith (Figures 1-4)

Table 4. Pollen morphological characteristic and measurements of *Rubus* (Rosaceae) in Thailand

No.	Sub genus	Scientific name	P (µm) mean ± SD (min-max)	E (µm) mean ± SD (min-max)	P/E ratio mean ± SD (min-max)	ET (µm) mean ± SD (min-max)	d (µm) mean ± SD (min-max)	CL (µm) mean ± SD (min-max)	CW (µm) mean ± SD (min-max)
1	Idaeobatus	*Rubus ellipticus* var. *obcordatus* Focke	17.73±1.42 (15.20-20.66)	22.14±1.91 (18.99-25.14)	0.80±0.04 (0.72-0.89)	1.10±0.22 (0.73-1.72)	5.94±1.38 (3.75-7.74)	11.20±0.74 (10.3-12.12)	2.60±0.25 (2.46-3.01)
2		*R. leucanthus* Hance	19.44±1.28 (17.34-21.82)	20.70±1.43 (18.12-23.48)	0.94±0.04 (0.86-0.99)	1.09±0.29 (0.62-1.62)	6.28±0.46 (5.66-6.66)	18.54±0.40 (18.02-19.01)	1.61±0.13 (1.45-1.77)
3		*R. niveus* Thunb	18.09±1.23 (16.76-19.04)	21.46±1.45 (19.32-24.06)	0.84±0.05 (0.76-0.93)	1.15±0.15 (0.76-1.48)	7.72±1.15 (6.26-9.38)	15.78±0.24 (15.49-16.11)	2.00±0.04 (1.9-2.1)
4		*R. sumatranus* Miq	18.22±1.28 (16.39-21.76)	18.82±1.36 (16.92-22.42)	0.96±0.04 (0.88-1.04)	0.72±0.15 (0.66-0.99)	6.41±0.48 (5.77-6.88)	13.38±0.12 (13.27-13.55)	1.96±0.39 (1.44-2.33)
5	Malachobatus	*R. alceifolius* Poir	40.97±4.29 (34.30-48.44)	43.48±4.46 (34.54-50.21)	0.94±0.05 (0.82-1.08)	2.55±0.27 (2.09-3.11)	-	27.25±3.01 (24.03-30.36)	1.85±0.50 (1.11-2.47)
6		*R. blepharoneurus* Cardot	26.63±1.61 (23.46-29.96)	36.96±1.37 (32.83-39.59)	0.72±0.03 (0.64-0.85)	2.09±0.35 (1.48-2.77)	15.44±0.64 (14.83-16.31)	12.60±2.13 (10.25-15.32)	1.50±0.20 (1.25-1.75)
7		*R. chevalieri* var. *angkae* Thunb	33.06±2.35 (28.81-36.95)	30.25±2.13 (27.68-34.79)	1.09±0.06 (0.99-1.24)	1.93±0.28 (1.16-2.36)	8.20±1.78 (5.67-10.57)	18.50±1.04 (17.12-19.82)	1.81±0.07 (1.71-1.91)
8		*R. clinocephalus* Focke	27.87±1.58 (24.74-30.80)	29.95±1.92 (24.44-32.22)	0.93±0.05 (0.82-1.03)	1.67±0.24 (1.17-2.13)	12.72±0.62 (11.91-13.58)	21.33±0.27 (21.00-21.66)	0.99±0.02 (0.97-1.03)
9		*R. elongatus* Smith	24.66±1.05 (22.69-26.58)	22.21±1.50 (19.41-24.74)	1.11±0.06 (0.98-1.24)	1.42±0.21 (1.03-1.85)	10.92±0.57 (9.96-11.68)	17.81±0.90 (16.50-18.62)	1.66±0.44 (1.3-2.33)
10		*R. hasskarlii* Miq	31.55±2.34 (26.13-36.39)	33.50±3.73 (26.83-41.12)	0.94±0.06 (0.84-1.10)	2.34±0.42 (1.71-3.43)	14.20±3.04 (9.64-18.07)	23.40±0.80 (22.66-24.59)	2.94±0.12 (2.84-3.12)
11		*R. hastifolius* H.Lév. & Vaniot	21.75±1.36 (18.90-23.91)	20.67±1.42 (16.91-22.6)	1.05±0.05 (0.96-1.19)	1.44±0.15 (1.12-1.77)	10.15±0.51 (9.38-11.17)	19.80±0.66 (18.97-20.55)	1.50±0.29 (1.2-1.90)
12		*R. lasiotrichos* Focke	33.98±2.54 (29.54-38.3)	37.11±2.47 (33.18-42.93)	0.91±0.04 (0.81-0.98)	2.40±0.29 (1.98-2.99)	-	23.72±0.32 (23.33-23.99)	1.50±0.29 (1.2-1.9)
13		*R. malvaceus* Focke	33.20±2.94 (28.46-40.28)	33.33±2.87 (28.90-33.33)	0.99±0.05 (0.87-1.11)	2.45±0.41 (1.92-3.35)	-	25.88±1.72 (23.76-28.07)	1.69±0.03 (1.66-1.73)
14		*R. moluccanus* L.	23.29±1.38 (23.29-21.04)	21.04±1.65 (21.04-17.99)	1.11±0.10 (0.98-1.32)	1.51±0.23 (1.11-1.97)	10.42±1.55 (8.15-12.54)	20.09±1.72 (18.69-22043	1.75±0.26 (1.38-2.04)
15		*R. pyrifolius* Smith	31.04±1.42 (28.25-33.74)	21.61±1.71 (19.52-23.93)	1.43±0.04 (1.30-1.63)	1.15±0.21 (0.82-1.70)	7.70±1.25 (5.87-9.39)	28.89±0.38 (28.41-29.32)	1.33±0.27 (1.00-1.66)
16		*R. rufus* Focke	28.99±1.63 (23.9-30.76)	30.71±1.78 (26.5-33.18)	0.94±0.05 (0.87-1.05)	1.91±0.26 (1.47-2.49)	10.09±0.82 (9.00-11.66)	20.34±0.26 (20.00-20.66)	3.50±0.13 (3.33-3.66)
17		*R. rugosus* Smith	26.97±1.77 (23.12-29.91)	31.17±2.53 (24.58-34.78)	0.86±0.06 (0.76-1.02)	1.67±0.17 (1.26-2.04)	9.98±1.09 (8.41-11.92)	23.48±0.73 (22.8-24.79)	2.94±0.56 (2.41-4.05)
18		*R. tiliaceus* Smith	31.44±1.57 (28.39-35.06)	28.56±1.62 (24.50-31.85)	0.11±0.06 (0.97-1.28)	1.65±0.22 (1.31-2.06)	11.65±2.24 (9.53-15.38)	24.07±1.2 (22.74-25.59)	2.02±0.16 (1.81-2.22)
19		*R. xanthoneurus* Focke	22.15±1.05 (19.43-25.11)	24.81±1.19 (22.14-28.32)	0.89±0.05 (0.82-0.97)	1.47±0.21 (0.97-1.86)	8.62±1.37 (6.78-9.91)	24.28±0.87 (23.24-25.33)	1.99±0.28 (1.66-2.33)

Key to abbreviations: CL = Colpus length, CW = Colpus width, d = Distance between the apices of two ectocolpi, E = Equatorial axis, ET = Exine thickness, P = Polar axis, - = Absent

Table 5. Pollen morphological characteristic of *Rubus* (Rosaceae) in Thailand

No.	Subgenus	Scientific name	Size	Equatorial shape	Aperture	Syncolpate	Exine sculpture
1	Idaeobatus	*Rubus ellipticus* var. *obcordatus* Focke	Small	Suboblate	Tp	-	Rugulate-perforate
2		*R. leucanthus* Hance	Small	Oblate spheroidal	Tp	-	Striate-perforate
3		*R. niveus* Thunb	Small	Suboblate	Tp	-	Striate-perforate
4		*R. sumatranus* Miq	Small	Oblate spheroidal	Tp	-	Striate-perforate
5	Malachobatus	*R. alceifolius* Poir	Medium	Oblate spheroidal	TTe	✓	Microverrucate-perforrate
6		*R. blepharoneurus* Cardot	Medium	Oblate	T	-	Microverrucate-perforrate
7		*R. chevalieri* var. *angkae* Thunb	Medium	Prolate spheroidal	TpTep	-	Microverrucate-perforrate
8		*R. clinocephalus* Focke	Medium	Oblate spheroidal	Tp	-	Microverrucate-perforrate
9		*R. elongatus* Smith	Small	Prolate spheroidal	Tp	-	Rugulate-perforate
10		*R. hasskarlii* Miq	Medium	Oblate spheroidal	TpTep	-	Microverrucate-perforrate
11		*R. hastifolius* H.Lév. & Vaniot	Small	Prolate spheroidal	Tp	-	Perforate
12		*R. lasiotrichos* Focke	Medium	Oblate spheroidal	TpTep	✓	Microverrucate-perforrate
13		*R. malvaceus* Focke	Medium	Oblate spheroidal	T	✓	Microverrucate
14		*R. moluccanus* L.	Small	Prolate spheroidal	Tp	-	Rugulate-perforate
15		*R. pyrifolius* Smith	Medium	Prolate	T	-	Striate-perforate
16		*R. rufus* Focke	Medium	Oblate spheroidal	Tp	-	Microverrucate-perforrate
17		*R. rugosus* Smith	Medium	Suboblate	Tp	-	Microverrucate
18		*R. tiliaceus* Smith	Medium	Prolate spheroidal	Tp	-	Microverrucate-perforrate
19		*R. xanthoneurus* Focke	Medium	Oblate spheroidal	Tp	-	Striate-perforrate

Key to abbreviations: T = Tricolpate, Tp = Tricolporate, TpTep = Tricolporate and Tetracolporate, TTe = Tricolpate and Tetracolpate, ✓ = Present, - = Absent

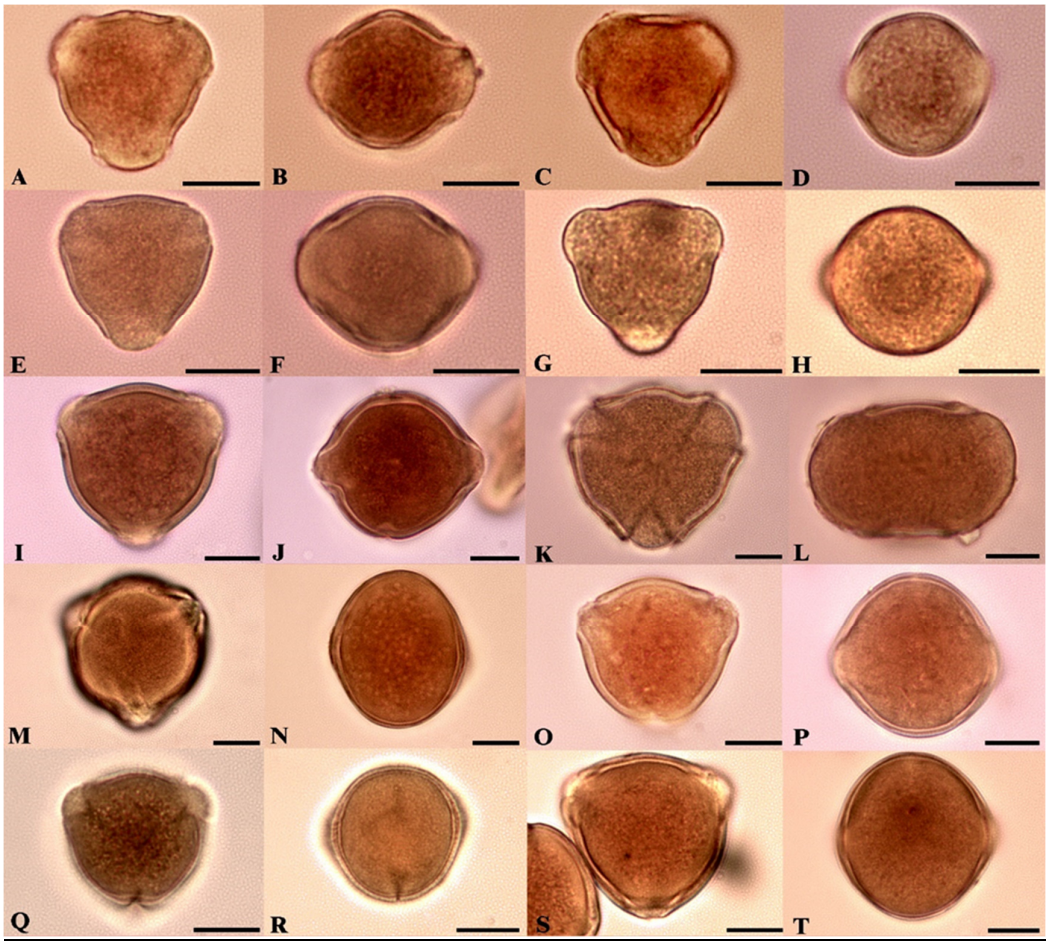

Figure 1. LM of pollen grains from *Rubus* species studied (A-B) *R. ellipticus* var. *obcordatus* Focke; (C-D) *R. leucanthus* Hance; (E-F) *R. niveus* Thunb; (G-H) *R. sumatranus* Miq; (I-J) *R. alceifolius* Poir; (K-L) *R. blepharoneurus* Cardot; (M-N) *R. chevalieri* var. *angkae* Thunb; (O-P) *R. clinocephalus* Focke; (Q-R) *R. elongatus* Smith; (S-T) *R. hasskarlii* Miq (Scale = 10 µm)

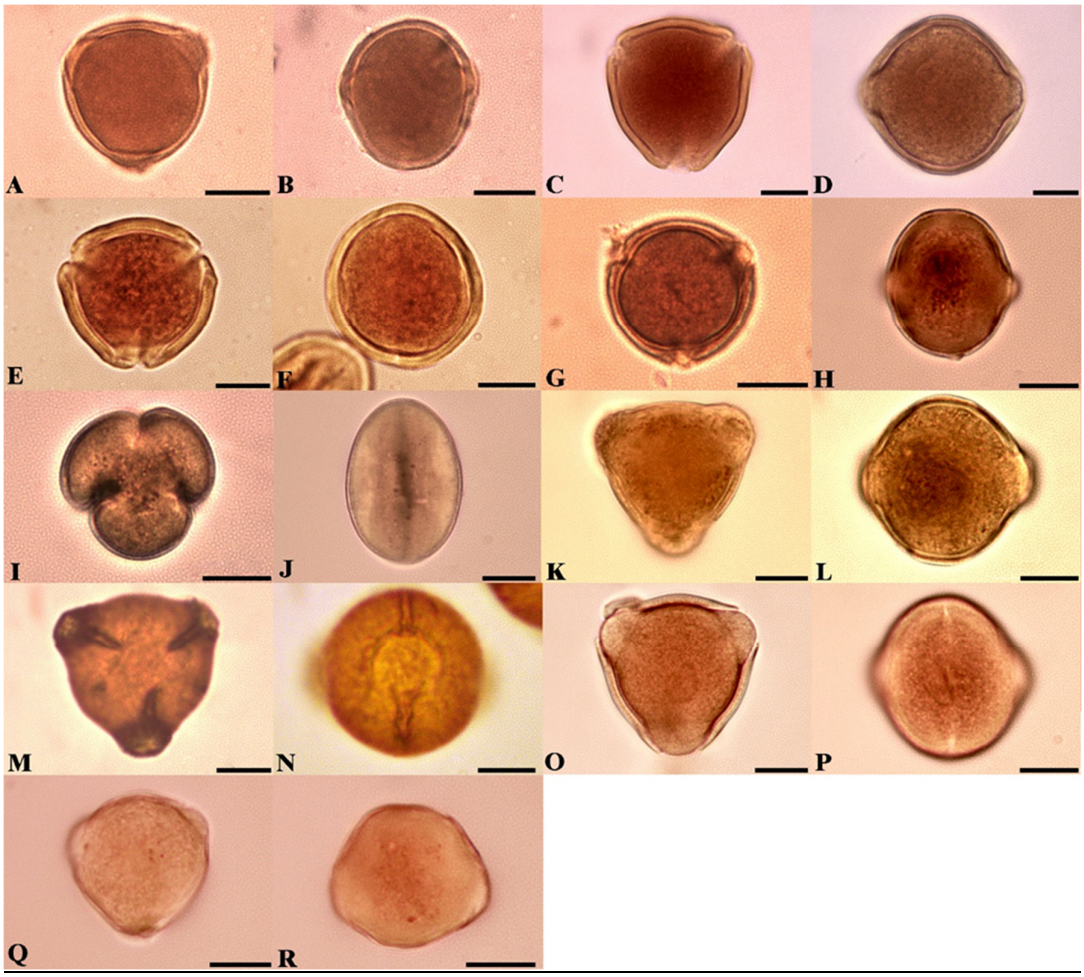

Figure 2. LM of pollen grains from *Rubus* species studied (A-B) *R. hastifolius* H.Lév. & Vaniot; (C-D) *R. lasiotrichos* Focke; (E-F) *R. malvaceus* Focke; (G-H) *R. moluccanus* L., (I-J); *R. pyrifolius* Smith; (K-I) *R. rufus* Focke; (M-N) *R. rugosus* Smith; (O-P) *R. tiliaceus* Smith; (Q-R) *R. xanthoneurus* Focke (Scale = 10 µm)

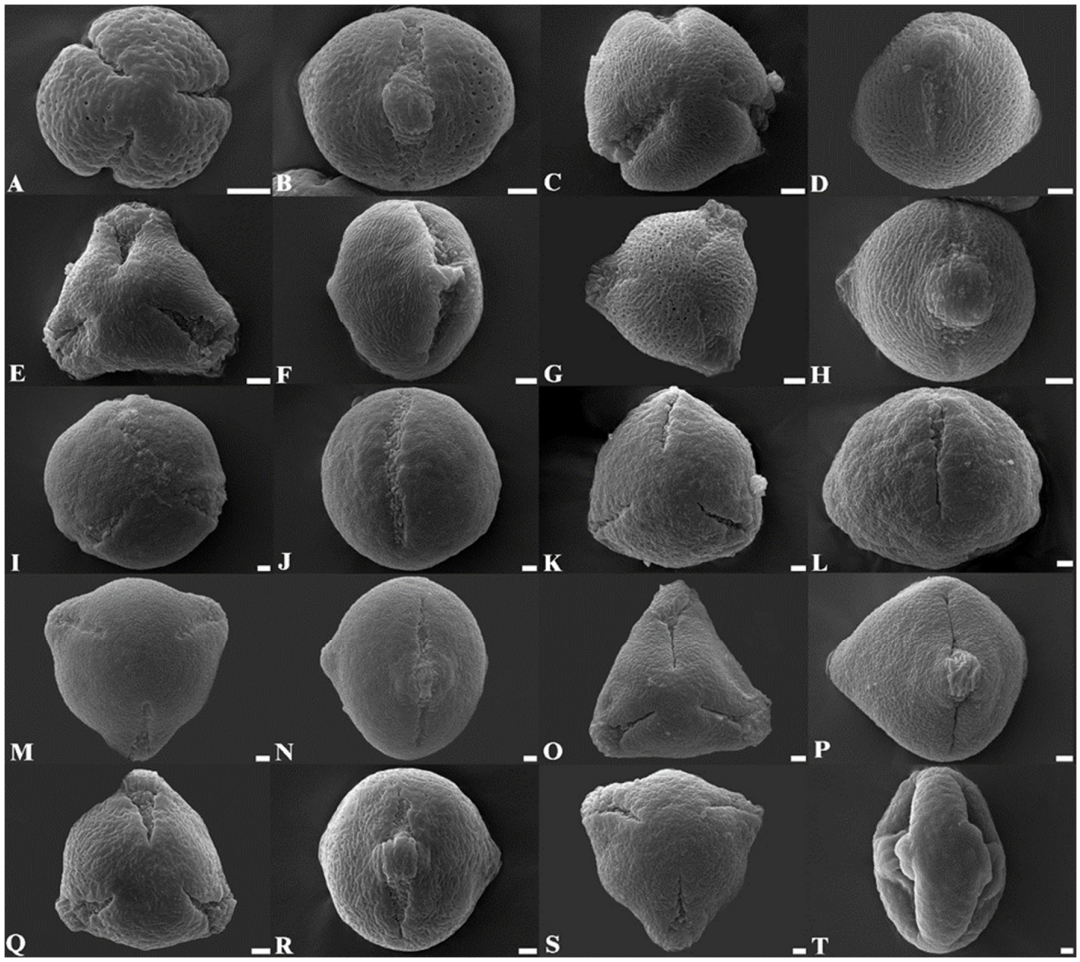

Figure 3. SEM of pollen grains from *Rubus* species studied. Variation of polar shape and equatorial shape including (A-B) *R. ellipticus* var. *obcordatus* Focke; (C-D) *R. leucanthus* Hance; (E-F) *R. niveus* Thunb; (G-H) *R. sumatranus* Miq; (I-J) *R. alceifolius* Poir; (K-L) *R. blepharoneurus* Cardot; (M-N) *R. chevalieri* var. *angkae* Thunb; (O-P) *R. clinocephalus* Focke; (Q-R) *R. elongatus* Smith; (S-T) *R. hasskarlii* Miq (Scale = 2 μm)

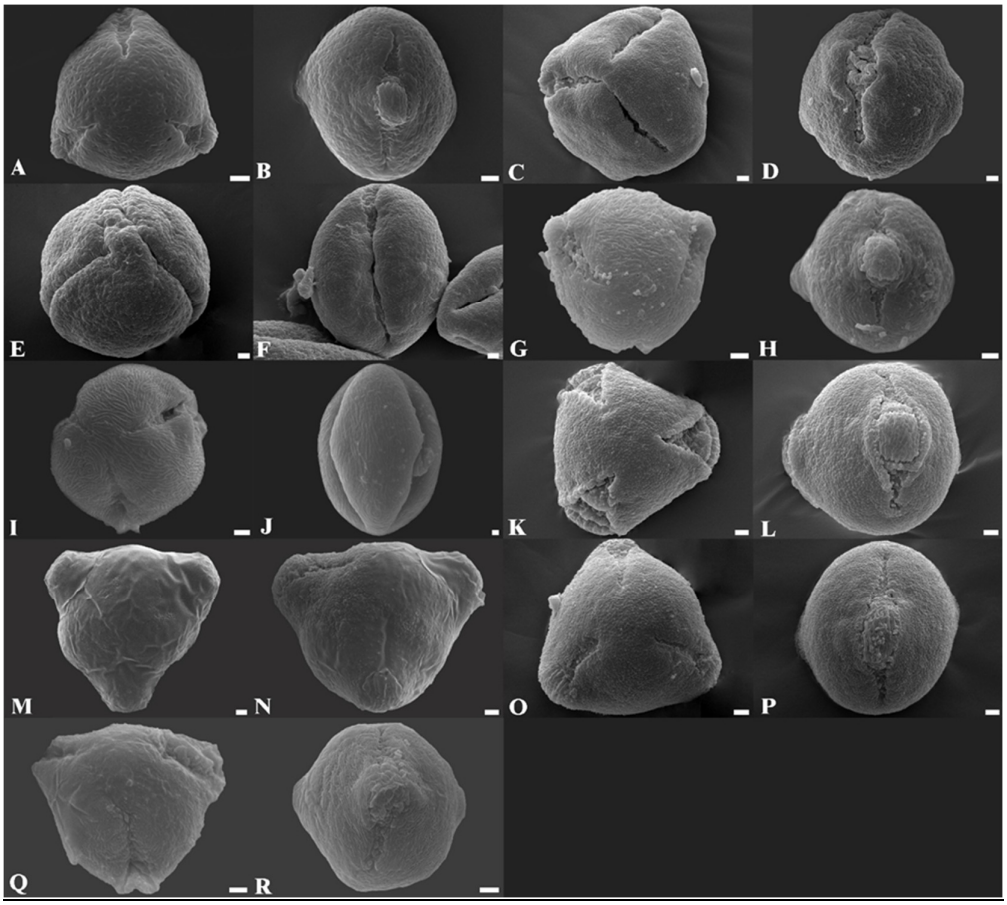

Figure 4. SEM of pollen grains from *Rubus* species studied. Variation of polar shape and equatorial shape including (A-B) *R. hastifolius* H.Lév. & Vaniot; (C-D) *R. lasiotrichos* Focke; (E-F) *R. malvaceus* Focke; (G-H) *R. moluccanus* L.; (I-J) *R. pyrifolius* Smith; (K-I) *R. rufus* Focke; (M-N) *R. rugosus* Smith; (O-P) *R. tiliaceus* Smith; (Q-R) *R. xanthoneurus* Focke (Scale = 2 µm)

Pollen aperture

The colpus length varies from 11.20 µm (*R. ellipticus* var. *obcordatus* Focke) to 28.89 µm (*R. pyrifolius* Smith), and the colpus width varies from 0.99 µm (*R. clinocephalus* Focke) to 3.50 µm (*R. rufus* Focke). The porus length varies from 2.78 µm (*R. niveus* Thunb) to 15.86 µm (*R. clinocephalus* Focke) and the porus width varies from 3.73 µm (*R. niveus* Thunb) to 13.01 µm (*R. rugosus* Smith). The pollen aperture can be separated into four types as follows:

Type I: Consists of the species with the tricolpate type, and includes *R. blepharoneurus* Cardot, *R. malvaceus* Focke and *R. pyrifolius* Smith.

Type II: Consists of the species with the tricolporate type, and includes *R. ellipticus* var. *obcordatus* Focke, *R. clinocephalus* Focke, *R. elongatus* Smith, *R. hastifolius* H.Lév. & Vaniot, *R. leucanthus* Hance, *R. moluccanus* L, *R. niveus* Thunb, *R. rufus* Focke, *R. rugosus* Smith, *R. sumatranus* Miq, *R. tiliaceus* Smith and *R. xanthoneurus* Focke.

Type III: Consists of the species with the tricolpate and tetracolpate types, and includes *R. alceifolius* Poir.

Type IV: Consists of the species with the tricolporate and tetracolporate types, and includes *R. chevalieri* var. *angkae* Thunb, *R. hasskarlii* Miq and *R. lasiotrichos* Focke (Figures 1-4).

Exine sculpturing of pollen

For all samples, thickness of the exine varies from 0.72 µm (*R. sumatranus* Miq) to 2.55 µm (*R. alceifolius* Poir). There were five types of exine sculpturing found as follows:

Type I: Consists of the regulate-perforate type species, and the sexine elements were longer than 1 µm when arranged in an irregular pattern and in the tectum the holes were less than 1 µm in diameter, which includes *R. ellipticus* var. *obcordatus* Focke, *R. elongatus* Smith and *R. moluccanus* L.

Type II: Consists of the striate-perforate type species, and the sexine elements were elongated and the parallel elements separated by grooves, and in the tectum the holes were less than 1 µm in diameter, which includes *R. leucanthus* Hance, *R. niveus* Thunb, *R. pyrifolius* Smith, *R. sumatranus* Miq and *R. xanthoneurus* Focke.

Type III: Consists of the species with the microverrucate-perforate type, which is a sexine element that is wart-like and smaller than 1 µm wide while being wider than it is tall without constrictions at the base and the tectum has holes smaller than 1 µm in diameter, which includes *R. alceifolius* Poir, *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. clinocephalus* Focke, *R. hasskarlii* Miq, *R. lasiotrichos* Focke, *R. rufus* Focke and *R. tiliaceus* Smith.

Type IV: Consists of the species with the microverrucate type, which is a sexine element that is wart-like and smaller than 1 µm wide while being wider than it is tall and without constrictions at the base, which includes *R. malvaceus* Focke and *R. rugosus* Smith.

Type V: Consists of the species with the perforate type, in which the tectum has holes with a diameter of less than 1 µm, which includes *R. hastifolius* H.Lév. & Vaniot. (Figures 5-7).

Principal component analysis (PCA) of pollen morphology

Table 6 shows the results for the 19 *Rubus* species from the eigen analysis and factor loading scores from eight characters. As shown in Table 7, 81.11% of the pollen morphological characters were explained by three principal components. The first principal component explained 44.84% of the variation and associated with the Colpus length (CL), Equatorial axis (E), Exine thickness (ET) and Polar axis (P). The second principal component explain 22.64% of the variation and associated with the Colpus length per Colpus width ratio (CL/CW ratio), Colpus width (CW) and equatorial axis per Colpus width ratio (E/CW ratio). The third principal component explain 13.63% of the variation and associated with the distance between the apices of the two ectocolpi (d) (Tables 6-7).

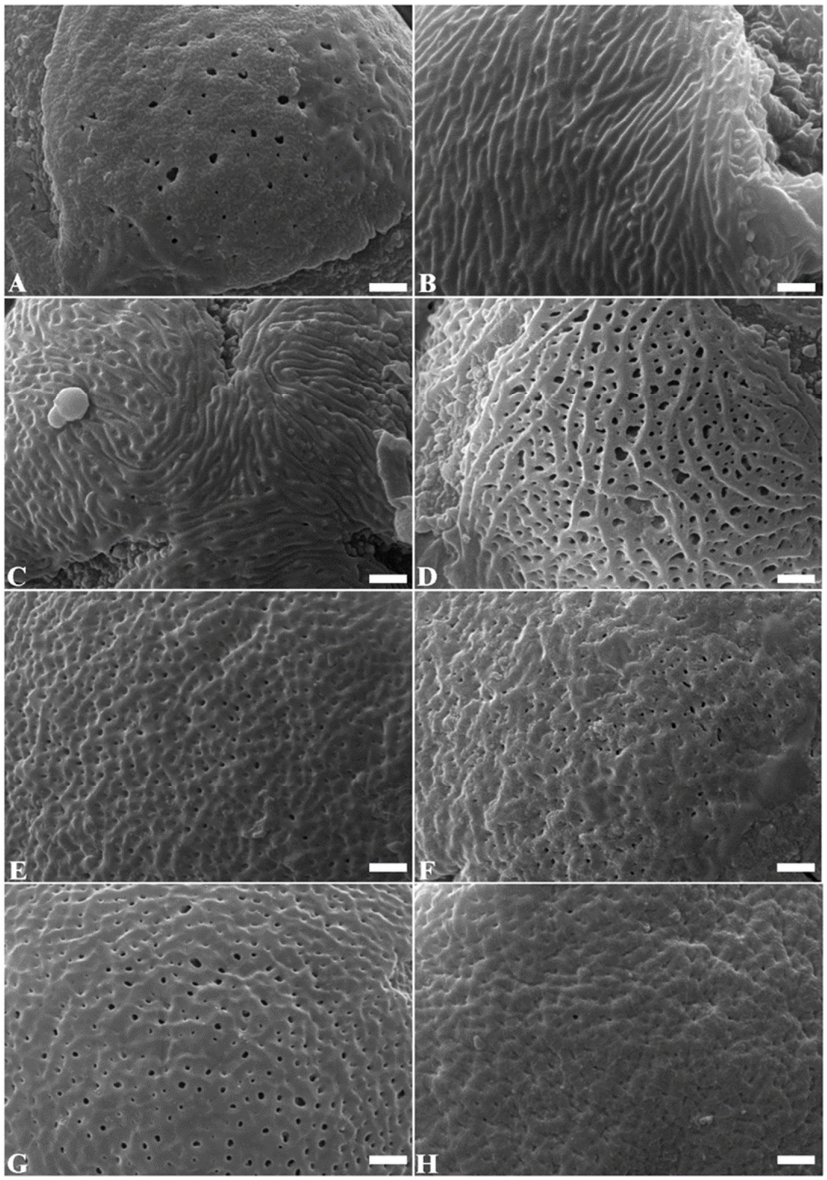

Figure 5. SEM of variation of exine sculpturing from *Rubus* species studied (A) *R. ellipticus* var. *obcordatus* Focke; (B) *R. leucanthus* Hance; (C) *R. niveus* Thunb; (D) *R. sumatranus* Miq; (E) *R. alceifolius* Poir; (F) *R. blepharoneurus* Cardot; (G) *R. chevalieri* var. *angkae* Thunb; (H) *R. clinocephalus* Focke (Scale = 1 µm)

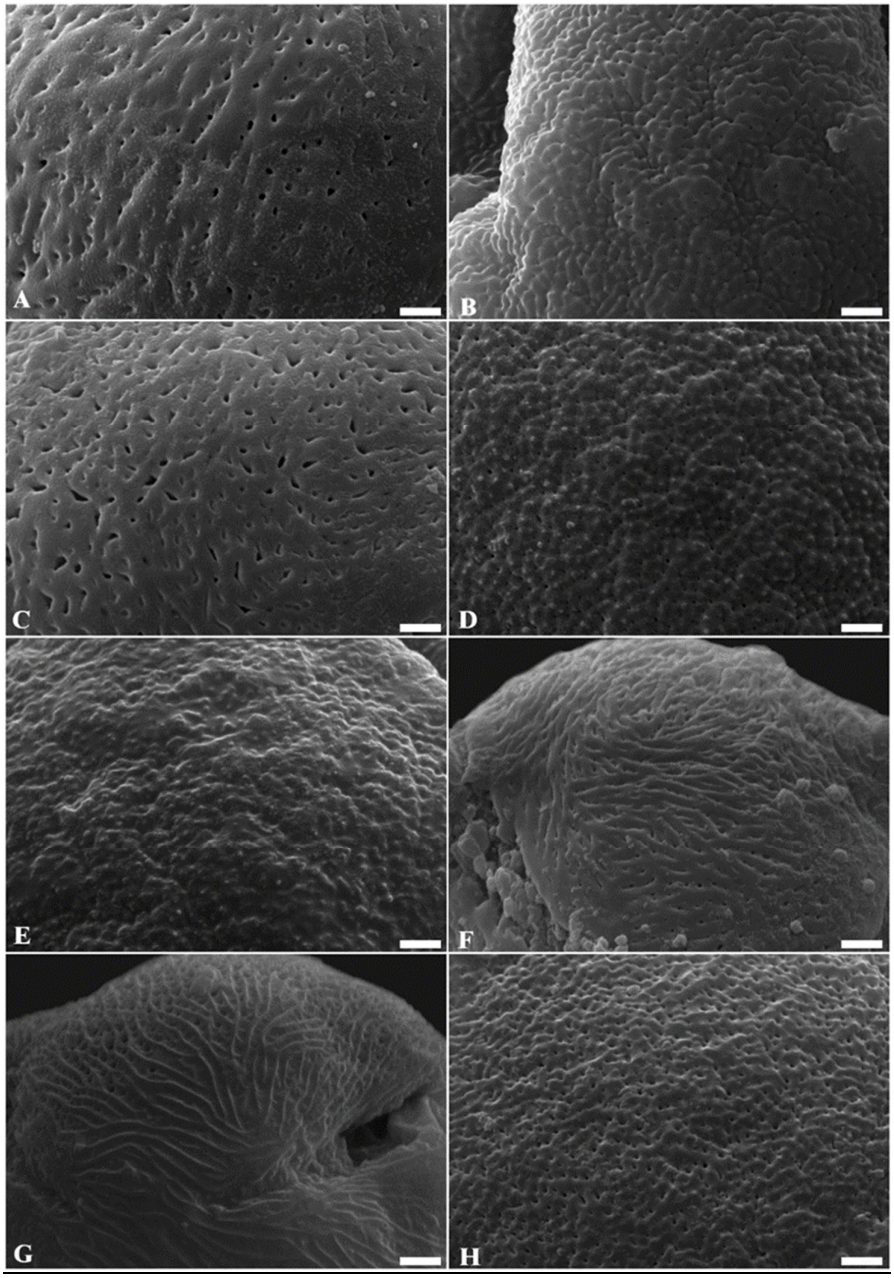

Figure 6. SEM of variation of exine sculpturing from *Rubus* species studied (A) *R. elongatus* Smith; (B) *R. hasskarlii* Miq; (C) *R. hastifolius* H.Lév. & Vaniot; (D) *R. lasiotrichos* Focke; (E) *R. malvaceus* Focke; (F) *R. moluccanus* L.; (G) *R. pyrifolius* Smith; (H) *R. rufus* Focke (Scale = 1 µm)

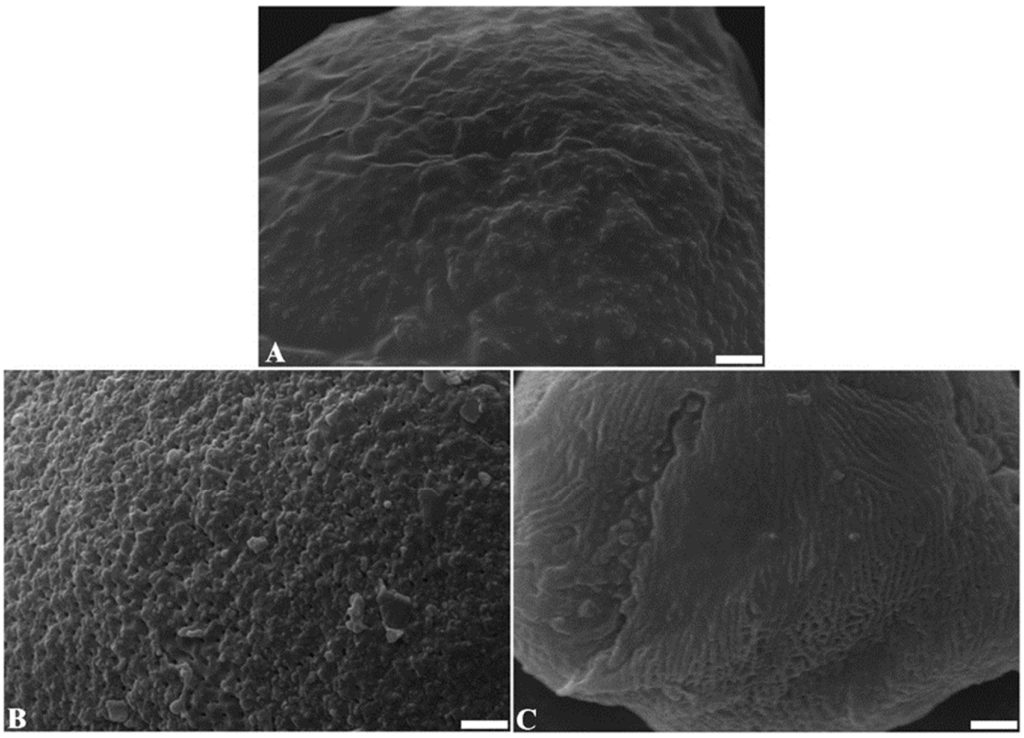

Figure 7. SEM of variation of exine sculpturing from *Rubus* species studied (A) *R. rugosus* Smith; (B) *R. tiliaceus* Smith; (C) *R. xanthoneurus* Focke. (Scale = 1 μm)

Table 6. Factor loadings demonstrating relationships of variables and indicating most intrinsic characters for separating pollen morphology of studied *Rubus* (Rosaceae) in Thailand

Variable	Principal components		
	1	2	3
ET	0.94	0.10	
P	0.93	0.12	-0.15
E	0.93		
CL	0.61	0.25	-0.33
E/CW	0.15	0.93	0.19
CL/CW	0.16	0.92	
CW		0.58	0.26
d			0.93

Key to abbreviations: CL = Colpus length, CW = Colpus width, d = Distance between the apices of two ectocolpi, E = Equatorial axis, ET = Exine thickness, P = Polar axis

Table 7. Eigen analysis of correlation matrix of pollen morphology of *Rubus* (Rosaceae) inThailand

Component	Eigenvalues		
	Total	Percent of variance	Cumulative percent
1	3.50	44.84	44.84
2	1.81	22.64	67.48
3	1.09	13.63	81.11

The principal component analysis scatter plot is created by factor score 1 (principal component 1) at X-axis and factor score 2 (principal component 2) at Y-axis. The *Rubus* species in Thailand are placed into three groups as shown in Figure 8. The first group comprises fourteen species: *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. ellipticus* var. *obcordatus* Focke, *R. elongatus* Smith, *R. hasskarlii* Miq, *R. hastifolius* H.Lév. & Vaniot, *R. leucanthus* Hance, *R. niveus* Thunb, *R. pyrifolius* Smith, *R. rufus* Focke, *R. rugosus* Smith, *R. sumatranus* Miq, *R. tiliaceus* Smith and *R. xanthoneurus* Focke. The second group comprises two species: *R. clinocephalus* Focke and *R. moluccanus* L. The third group comprises three species: *R. alceifolius* Poir, *R. lasiotrichos* Focke and *R. malvaceus* Focke.

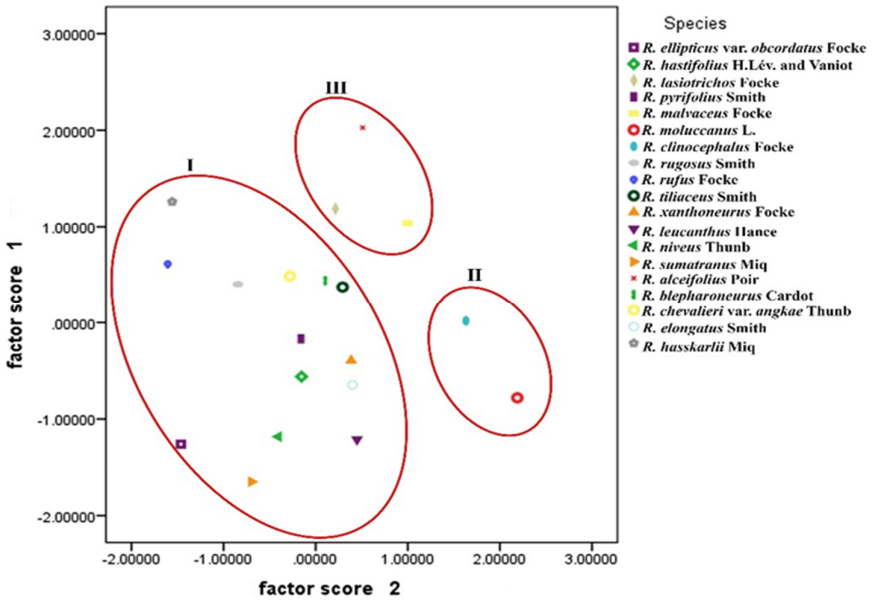

Figure 8. PCA scatterplot built from characters of pollen morphology of species of *Rubus* from Thailand

Cluster analysis

Figure 9 shows the dendrogram from the cluster analysis for the unweighted pair-group method calculated from the arithmetic average (UPGMA) method. Within the dendrogram there were three major clusters as determined by the characteristics of the pollen morphology. The first group consists of fourteen species: *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. ellipticus* var. *obcordatus* Focke, *R. elongatus* Smith, *R. hasskarlii* Miq, *R. hastifolius* H.Lév. & Vaniot, *R. leucanthus* Hance, *R. niveus* Thunb, *R. pyrifolius* Smith, *R. rufus* Focke, *R. rugosus* Smith, *R. sumatranus* Miq, *R. tiliaceus* Smith and *R. xanthoneurus* Focke. The second group consists of two species: *R. clinocephalus* Focke and *R. moluccanus* L. The third group consists of three species: *R. alceifolius* Poir, *R. lasiotrichos* Focke and *R. malvaceus* Focke.

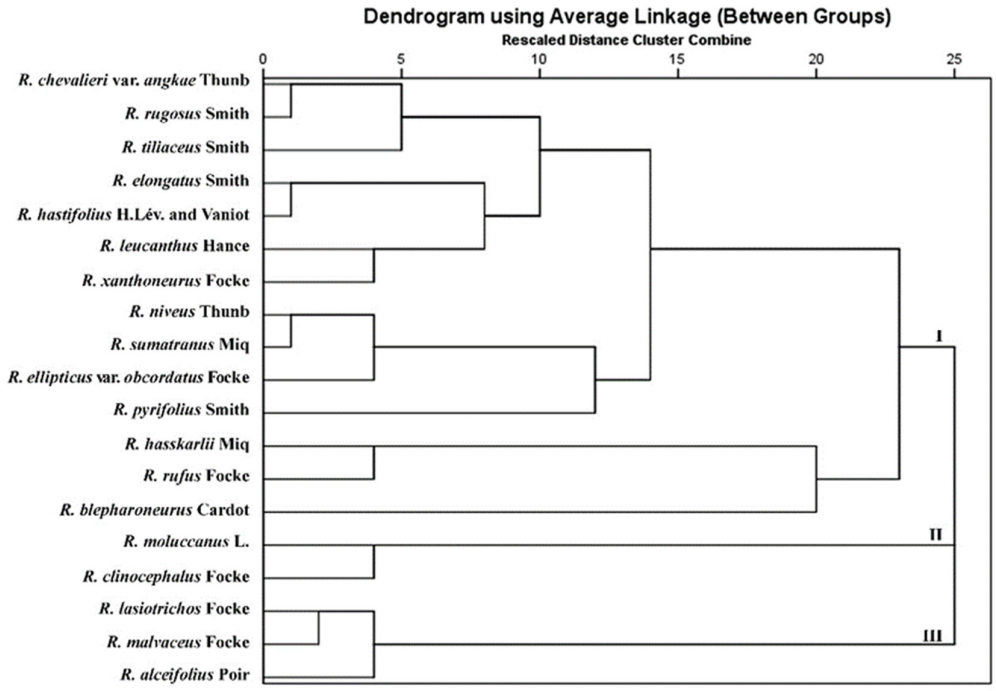

Figure 9. UPGMA dendrogram of cluster analysis of pollen morphological characters of *Rubus* in Thailand

Anatomical studies

The transverse sections of the leaf, petiole and stem anatomy showed some variation and some similarities within the genus *Rubus* in Thailand (Tables 8-12). A summary of the results of the anatomical characteristics studied in this investigation is presented in Tables 8-12.

Table 8. Anatomy characteristics and measurements of *Rubus* (Rosaceae) in Thailand

No.	Sub genus	Scientific name	LT (µm) mean ± SD (min-max)	UET (µm) mean ± SD (min-max)	LET (µm) mean ± SD (min-max)	ST (µm) mean ± SD (min-max)	ML (µm) mean ± SD (min-max)	MW (µm) mean ± SD (min-max)
1	Idaeobatus	*Rubus ellipticus* var. *obcordatus* Focke	101.75±3.34 (95.28-108.91)	14.54±1.98 (11.82-18.18)	6.30±1.00 (4.58-8.16)	28.51±4.17 (22.31-36.25)	711.86±4.64 (700.95-724.09)	909.01±4.52 (901.72-921.18)
2		*R. leucanthus* Hance	102.39±11.09 (87.56-119.13)	21.63±2.27 (18.33-24.90)	15.49±2.21 (12.24-18.85)	19.76±3.38 (15.25-26.48)	562.42±10.49 (547.49-581.57)	614.22±9.65 (595.92-628.44)
3		*R. niveus* Thunb	79.20±4.10 (71.94-90.21)	11.16±0.75 (9.54-12.61)	7.83±0.58 (6.51-8.96)	14.10±2.06 (10.19-18.30)	509.02±1.56 (505.90-514.04)	603.37±12.09 (563.13-620.08)
4		*R. sumatranus* Miq	105.23±3.66 (96.20-114.04)	21.87±1.66 (18.84-26.43)	11.33±0.40 (10.73-12.67)	26.09±2.11 (22.86-33.12)	395.22±6.35 (374.78-405.34)	444.87±4.80 (431.25-457.31)
5	Malachobatus	*R. alceifolius* Poir	140.01±6.38 (124.72-154.32)	28.29±1.29 (25.95-31.68)	11.25±0.75 (8.96-12.58)	32.08±1.43 (28.58-35.02)	1260.18±13.47 (1227.79-1280.67)	1266.23±12.13 (1239.59-1300.90)
6		*R. blepharoneurus* Cardot	98.86±3.03 (91.1-104)	27.13±1.62 (22.93-31.58)	10.83±0.55 (9.24-12.2)	15.95±0.85 (13.75-17.84)	1190.40±4.76 (1179.24-1203.31)	1224.54±6.11 (1207.69-1235.83)
7		*R. chevalieri* var. *angkae* Thunb	92.41±0.89 (90.15-94.19)	18.54±1.94 (15.22-24.48)	9.62±0.64 (8.36-11.21)	12.66±1.16 (9.66-16.02)	899.93±5.53 (884.18-911.86)	1009.30±13.68 (976.08-1049.68)
8		*R. clinocephalus* Focke	103.96±4.32 (96.06-114.34)	11.52±1.07 (8.64-13.72)	9.43±0.67 (7.80-10.79	25.13±0.71 (24.19-27.42)	1093.25±10.42 (1065.61-1118.43)	995.16±3.50 (984.48-1001.05)
9		*R. elongatus* Smith	83.76±3.06 (74.89-90.66)	18.01±1.69 (13.74-21.34)	9.50±1.08 (6.84-12.21)	19.19±2.13 (12.53-23.84)	1198.85±9.67 (1167.22-1211.79)	1319.29±8.45 (1304.64-1345.24)
10		*R. hasskarlii* Miq	136.28±7.11 (121.14-158.52)	25.36±1.54 (23.03-30.38)	11.34±0.81 (9.64-13.48)	29.49±3.18 (22.48-38.15)	1446.82±9.49 (1428.21-1465.33)	1263.04±9.74 (1240.06-1282.23)
11		*R. hastifolius* H.Lév. & Vaniot	86.37±2.82 (78.91-93.92)	18.70±1.29 (15.32-21.92)	9.43±0.32 (8.65-10.20)	20.04±2.01 (17.3-26.95)	829.32±17.19 (789.44-869.14)	989.68±26.69 (945.73-1058.82)
12		*R. lasiotrichos* Focke	100.82±1.12 (98.28-103.85)	28.91±1.91 (24.94-34.68)	11.97±0.46 (10.73-13.21)	18.32±2.04 (14.85-24.94)	736.96±11.44 (703.91-758.99)	596.18±2.32 (602.92-590.80)
13		*R. malvaceus* Focke	77.37±2.57 (83.84-71.26)	19.04±1.59 (15.28-23.40)	9.10±0.89 (6.92-10.66)	10.61±1.08 (8.65-13.74)	996.87±3.26 (988.75-1004.76)	999.12±8.57 (984.47-1025.03)
14		*R. moluccanus* L.	124.13±0.87 (122.21-126.33)	35.51±2.13 (32.11-40.76)	11.16±0.77 (8.88-12.78)	28.52±1.25 (24.94-31.64)	848.31±3.03 (841.51-856.78)	931.00±2.86 (924.21-936.59)
15		*R. pyrifolius* Smith	142.74±4.38 (135.15-156.54)	38.65±1.32 (35.13-42.04)	10.85±0.41 (9.95-11.46)	29.50±1.77 (26.76-33.62)	924.98±5.65 (908.92-939.53)	786.47±5.93 (774.3-804.93)
16		*R. rufus* Focke	119.55±5.15 (107.71-129.98)	27.52±3.97 (17.74-36.22)	9.34±0.77 (7.59-11.71)	24.09±2.52 (19.82-32.08)	916.10±23.39 (866.62-980.77)	989.43±21.50 (944.46-1050.64)
17		*R. rugosus* Smith	142.34±3.69 (132.34-151.19)	16.87±1.40 (13.74-20.64)	11.29±0.82 (9.72-13.8)	26.08±1.28 (22.98-28.50)	859.02±12.46 (830.80-891.14)	1105.16±8.97 (1088.53-1122.74)
18		*R. tiliaceus* Smith	72.62±2.40 (65.16-77.76)	12.81±0.96 (10.53-15.28)	9.32±0.75 (8.21-11.67)	11.55±1.38 (8.18-14.9)	974.74±19.36 (936.00-1017.03)	872.19±21.01 (823.24-911.87)
19		*R. xanthoneurus* Focke	82.77±2.24 (78.37-87.55)	11.80±0.95 (9.67-13.74)	6.93±0.73 (5.56-8.73)	25.95±3.55 (21.39-38.15)	872.30±4.97 (862.96-885.09)	875.28±4.47 (866.18-884.54)

Key to abbreviations: LET = Abaxial surface thickness, LT = Leaf thickness, ML = Midrib length, MW = Midrib width, ST = Spongy mesophyll thickness, UET = Adaxial surface thickness

Table 9. Comparison of cross sections of leaf blades of *Rubus* (Rosaceae) in Thailand

No.	Sub genus	Scientific name	U/L (times)	Pa	Mesophyll		Type of trichomes		Idioblast cell		Type of crystals	BE
					NP	NS	Adaxial	Abaxial	PL	SL		
1	Idaeobatus	*Rubus ellipticus* var. *obcordatus* Focke	2	-	2-3	1-2	ST	ST, ST_C, MUT	✓	-	D, P	✓
2		*R. leucanthus* Hance	0	-	2-3	3-4	ST, GT_U	ST, GT_U	-	✓	D	-
3		*R. niveus* Thunb	2	-	2	2	ST	ST, ST_C	✓	✓	D, P	✓
4		*R. sumatranus* Miq	2	-	2-3	2-3	ST, GT_U	ST, MMT, GT_U, GT_M	✓	✓	D	✓
5	Malachobatus	*R. alceifolius* Poir	3	-	3	2	ST, MUT, GT_U	ST, MUT, GT_U	✓	-	D	✓
6		*R. blepharoneurus* Cardot	3	-	2-3	1-2	ST, MUT, GT_U	ST, ST_C, MUT, GT_U	✓	-	D	✓
7		*R. chevalieri* var. *angkae* Thunb	2	-	2	1-2	ST, GT_U	ST, ST_C, MUT	✓	-	D	✓
8		*R. clinocephalus* Focke	2	-	2	2	ST	ST, ST_C	✓	-	D, P	✓
9		*R. elongatus* Smith	2	-	2	2-3	ST. MUT	ST, MUT, GT_U	✓	-	P	✓
10		*R. hasskarlii* Miq	2	-	2	2	ST, MUT, GT_U	ST, ST_C, MUT	✓	-	D	✓
11		*R. hastifolius* H.Lév. & Vaniot	2	-	2	2-3	ST, MUT, GT_U	ST, MUT	✓	-	D, P	✓
12		*R. lasiotrichos* Focke	3	-	2	2	ST, MUT, GT_U	ST, ST_C, MUT	✓	-	D	✓
13		*R. malvaceus* Focke	2	-	2	2	ST, MUT, GT_U	ST, MUT, GT_U	✓	✓	D	✓
14		*R. moluccanus* L.	3	✓	2	3	ST, MUT	ST, ST_C, MUT	✓	-	P	✓
15		*R. pyrifolius* Smith	4	-	1-2	2	ST, MUT, GT_U	ST, ST_C, MUT	✓	✓	D	✓
16		*R. rufus* Focke	3	-	2	1-2	ST, MUT, GT_U	ST, ST_C, MUT	✓	-	D	✓
17		*R. rugosus* Smith	2	-	2-3	1-2	ST, GT_U	ST, ST_C	✓	-	D, P	✓
18		*R. tiliaceus* Smith	2	-	2	1-2	ST, GT_U	ST, ST_C, MUT	✓	-	D	✓
19		*R. xanthoneurus* Focke	2	-	2	2	ST, MUT, GT_U	ST, MUT, GT_U	✓	-	D	✓

Key to abbreviations: BE = Bundle sheath extension, D = Druse crystal, GT_M = Glandular trichome with multicellular multiseriate stalk, GT_U = Glandular trichome with unicellular uniseriate stalk , MUT = Multicellular uniseriate trichome, NP = Number of palisade layers, NS = Number of spongy layers , P = Prismatic crystal, Pa = Papillae, PL = Palisade layers, SL = Spongy layers, ST = Simple trichome, ST_C = Curled hairs, U/L = The ratio between adaxial surface thickness per abaxial surface thickness, ✓ = Present, - = Absent

Table 10. Comparison of cross sections of midribs and leaf margins of *Rubus* (Rosaceae) in Thailand

No.	Sub genus	Scientific name	Midrib							Shape of leaf margin
			Shape of adaxial	NB	PF	Type of trichomes		Type of crystals	Starch grain	
						Adaxial	Abaxial			
1	Idaeobatus	*R. ellipticus* var. *obcordatus* Focke	Convex	2	✓	ST	ST	D, P	-	CD
2		*R. leucanthus* Hance	Concave	1	-	ST	ST	D	-	CD
3		*R. niveus* Thunb	Convex	1	-	ST	ST	D, P	-	CD
4		*R. sumatranus* Miq	Concave	1	-	ST, GT_U	ST, GT_U, GT_M	D	-	Straight
5	Malachobatus	*R. alceifolius* Poir	Convex	2	-	ST	ST, MUT	D	-	CD
6		*R. blepharoneurus* Cardot	Convex	2	✓	ST, MUT, GT_U	ST	D, P	-	CD
7		*R. chevalieri* var. *angkae* Thunb	Convex	3	✓	ST, MUT, GT_U	ST	D, P	-	CD
8		*R. clinocephalus* Focke	Convex	3	✓	ST	ST	D	-	CD
9		*R. elongatus* Smith	Convex	1	✓	ST	ST	P	-	Straight
10		*R. hasskarlii* Miq	Convex	3	-	ST	ST, MUT	D, P	-	CD
11		*R. hastifolius* H.Lév. & Vaniot	Convex	1	✓	ST	ST, MUT	P	-	CD
12		*R. lasiotrichos* Focke	Convex	1	-	ST, MUT	ST, MUT	D	-	CD
13		*R. malvaceus* Focke	Convex	1	-	ST	ST	D	-	CD
14		*R. moluccanus* L.	Convex	1	✓	ST	ST	P	-	CD
15		*R. pyrifolius* Smith	Convex	2	✓	ST	ST	D	-	CD
16		*R. rufus* Focke	Convex	2	✓	ST	ST	D, P	✓	CD
17		*R. rugosus* Smith	Convex	1	✓	ST	ST	D, P	-	CD
18		*R. tiliaceus* Smith	Convex	2	✓	ST	ST, MUT	D	-	CD
19		*R. xanthoneurus* Focke	Convex	1	✓	ST, MUT	ST	D, P	-	CD

Key to abbreviations: CD = Curving downward, D = Druse crystal, GT_M = Glandular trichome with multicellular multiseriate stalk, GT_U = Glandular trichome with unicellular uniseriate stalk, MUT = Multicellular uniseriate trichome, NB = Number of vascular bundle, P = Prismatic crystal, PF = Pericyclic fibers, ST =Simple trichome, ✓ = Present, - = Absent

Table 11. Comparison of cross sections of petiole of *Rubus* (Rosaceae) in Thailand

No.	Sub genus	Scientific name	Shape		PF	Type of trichomes	NC	Type of crystals	Starch grain	Vascular bundle	
			Outline	Adaxial						NL	NSS
1	Idaeobatus	*R. ellipticus* var. *obcordatus* Focke	O	Convex	✓	ST, MMT	3-4	D, P	-	1	8
2		*R. leucanthus* Hance	Co	Concave	✓	ST	2	D, P	-	1	4
3		*R. niveus* Thunb	Co	Concave	✓	ST	2-3	D, P	✓	1	2
4		*R. sumatranus* Miq	Co	Concave	✓	ST, MMT, GT_M	2-3	D	-	1	2
5	Malachobatus	*R. alceifolius* Poir	Co	Concave	✓	ST, MUT	4-5	D, P	✓	1	6
6		*R. blepharoneurus* Cardot	Co	Concave	✓	ST	3	D, P	-	1	4
7		*R. chevalieri* var. *angkae* Thunb	C	Convex	✓	ST, MMT	3-4	D, P	✓	1	4
8		*R. clinocephalus* Focke	C	CC	✓	ST	4-5	D	-	1	6
9		*R. elongatus* Smith	C	Convex	✓	ST	3	P	-	1	3
10		*R. hasskarlii* Miq	O	Convex	✓	ST, MUT	4	D, P	-	1	6
11		*R. hastifolius* H.Lév. & Vaniot	C	Convex	✓	ST, MUT	4	D, P	✓	1	4
12		*R. lasiotrichos* Focke	Co	Concave	-	ST, MUT	4	D, P	-	2	4
13		*R. malvaceus* Focke	O	Concave	✓	ST, MUT	4	D, P	-	1	4
14		*R. moluccanus* L.	Co	Convex	-	ST, MUT	5	D	-	1	4
15		*R. pyrifolius* Smith	C	Straight	✓	ST, MUT	3-4	D	-	1	6
16		*R. rufus* Focke	O	Convex	✓	ST, MUT, GT_U	4	D	-	1	6
17		*R. rugosus* Smith	C	Straight	✓	ST	3	D	-	2	6
18		*R. tiliaceus* Smith	Co	Concave	✓	ST, MUT	4	D, P	✓	1	7
19		*R. xanthoneurus* Focke	Co	Concave	✓	ST	4	D, P	✓	1	2

Key to abbreviations: C = Circular shape, CC = Alternate convex and concave, Co = Cordate shape, D = Druse, GT_M = Glandular trichome with multicellular multiseriate stalk, GT_U = Glandular trichome with unicellular uniseriate stalk , NL = Number of large vascular bundle, NSS = Number of small vascular bundle, MMT = Multicellular multiseriate trichome, MUT = Multicellular uniseriate trichome, NC = Number of collenchyma layers, O = Ovate shape, P = Prismatic, PF = Pericyclic fibers, R = Rectangular shape, ST = Simple trichome, ✓ = Present, - = Absent

Leaf blade

The adaxial surface and abaxial surface consisted of one layer. A thin cuticle is present on both the adaxial and the abaxial surfaces. The shapes of the parenchyma cells are polygonal. The adaxial surface (11.16-38.65 μm) is larger than the abaxial surface (6.30-15.49 μm) (Table 8). On the adaxial surface there are no stomata, while on the abaxial surface there are typical stomata and raised stomata on all the plant species. On the abaxial surface, papillae are present in *R. moluccanus* L. (Figure 10). Five types of trichomes can be recognized as follows:

Type I: Simple trichome (ST). The characteristic is unicellular, thicker at the base of the trichome, lignified walls, tapering and sharp, which was found in all species on both surfaces studied (Figures 11A and 12).

Type II: Multicellular uniseriate trichome (MUT). The characteristic is multicellular, one row of cells and rounded tip, which was found in almost all species, except *R. clinocephalus* Focke, *R. leucanthus* Hance, *R. niveus* Thunb and *R. sumatranus* Miq (Figures 11B and 12).

Type III: Multicellular multiseriate trichome (MMT). The characteristic is multicellular, many rows of cells, tapering and sharp, which was found only on the abaxial surface of *R. sumatranus* Miq. (Figures 11D and 12).

Type IV: Curled hairs (ST_C). The characteristic is unicellular, very curled and similar to instant noodles, which was found on the abaxial surface in almost all species, except some species, which includes *R. alceifolius* Poir, *R. elongatus* Smith, *R. hastifolius* H.Lév. & Vaniot, *R. leucanthus* Hance, *R. malvaceus* Focke, *R. sumatranus* Miq and *R. xanthoneurus* Focke (Figures 11C and 12).

Type V: Glandular trichome with unicellular uniseriate stalk (GT_U). The characteristic has a uniseriate stalk and sphearoidal tip, which was found on both surfaces in almost all species, except some species including *R. clinocephalus* Focke, *R. ellipticus* var. *obcordatus* Focke, *R. moluccanus* L. and *R. niveus* Thunb (Figures 11E and 12).

Type VI: Glandular trichome with multicellular multiseriate stalk (GT_M). The characteristic has a multiseriate stalk and sphearoidal tip, which was found only on the abaxial surface of *R. sumatranus* Miq. (Figures 11F and 12).

The mesophyll is dorsiventral and consists of palisade cells and spongy cells. The palisade cells have one to three layers that are continuous in transverse sections and anticlinal close to the adaxial surface. There is a range of one to four layers of the spongy cells that are loosely arranged, periclinal, irregularly shaped with polygonal cells by being close to the abaxial surface. Palisade and spongy cells contain idioblast cells containing calcium oxalate as crystals in clusters, including druse and prismatic crystals. The vascular bundles have two types (large and small). The large vascular bundles are surrounded by about seven to eight parenchyma cells. The small vascular bundles are surrounded by about four to five parenchyma cells. The bundle sheath extension is present in almost all species, except *R. leucanthus* Hance. (Table 9, Figure 10 and Figure 11).

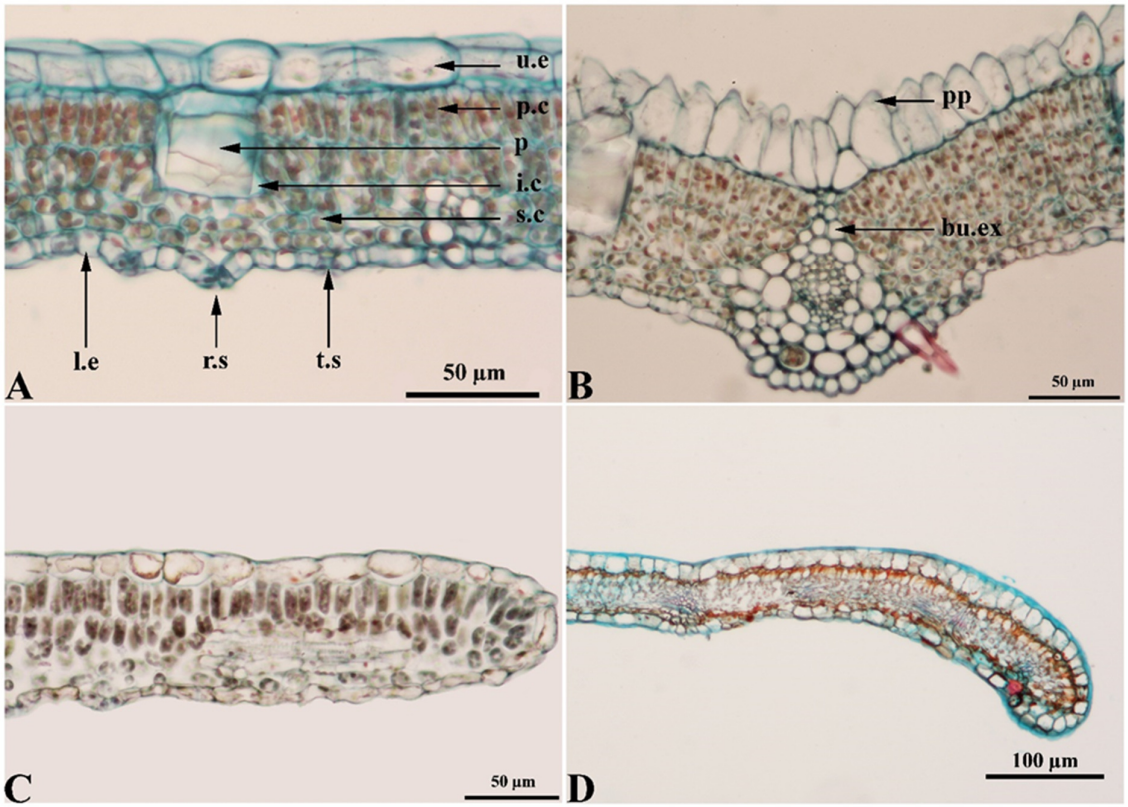

Figure 10. Transverse section of leaf blade and leaf margin of *Rubus* species (A) Leaf blade in *R. elongatus* Smith; (B) Leaf blade in *R. moluccanus* L.; (C) Straight leaf margin in *R. sumatranus* Miq; (D) Curved down leaf margin in *R. leucanthus* Hance

bu. ex, bundle sheath extension; i.c, idioblast cell; l.e, abaxial surface; p, prismatic crystal; p.c, palisade cell; pp, papillae; r.s, raised stomata; s.c, spongy cell; t.s, typical stomata; u.e, adaxial surface

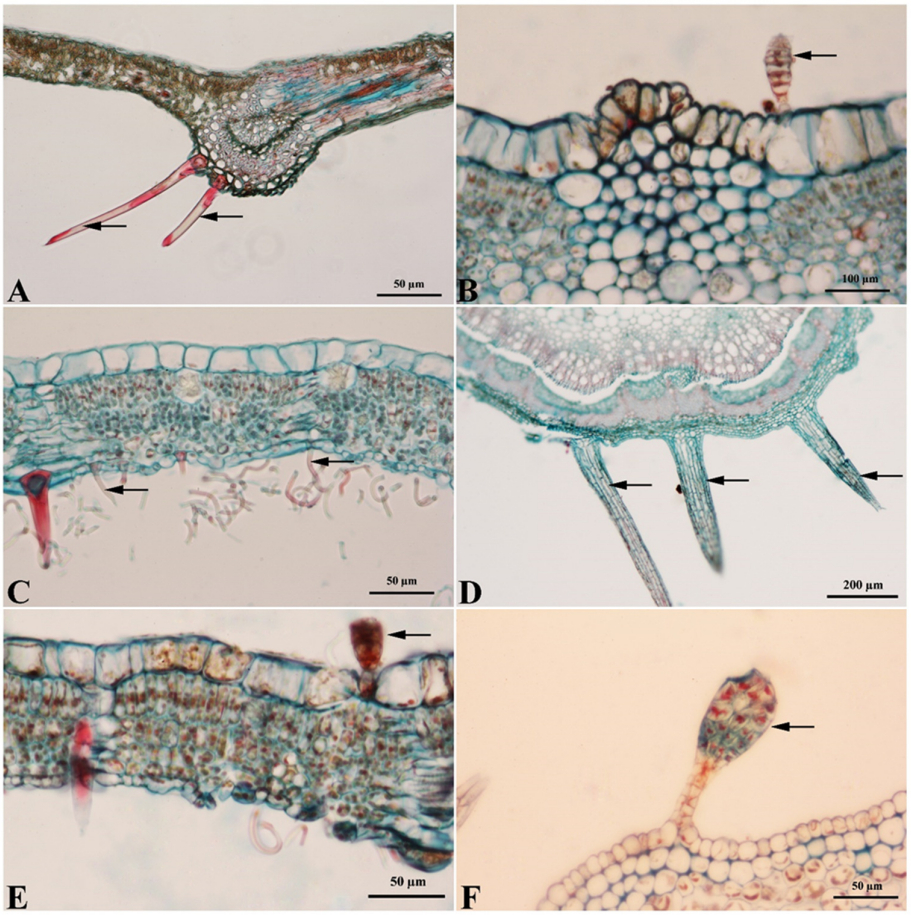

Figure 11. LM of variation of trichomes from *Rubus* species studied (A) Simple trichome in *R. xanthoneurus* Focke; (B) Multicellular uniseriate trichome in *R. lasiotrichos* Focke; (C) Curled hairs in *R. blepharoneurus* Cardot; (D) Multicellular multiseriate trichome in *R. ellipticus* var. *obcordatus* Focke; (E) Glandular trichome with unicellular uniseriate stalk in *R. lasiotrichos* Focke; (F) Glandular trichome with multicellular multiseriate stalk in *R. sumatranus* Miq (arrow indicates trichomes)

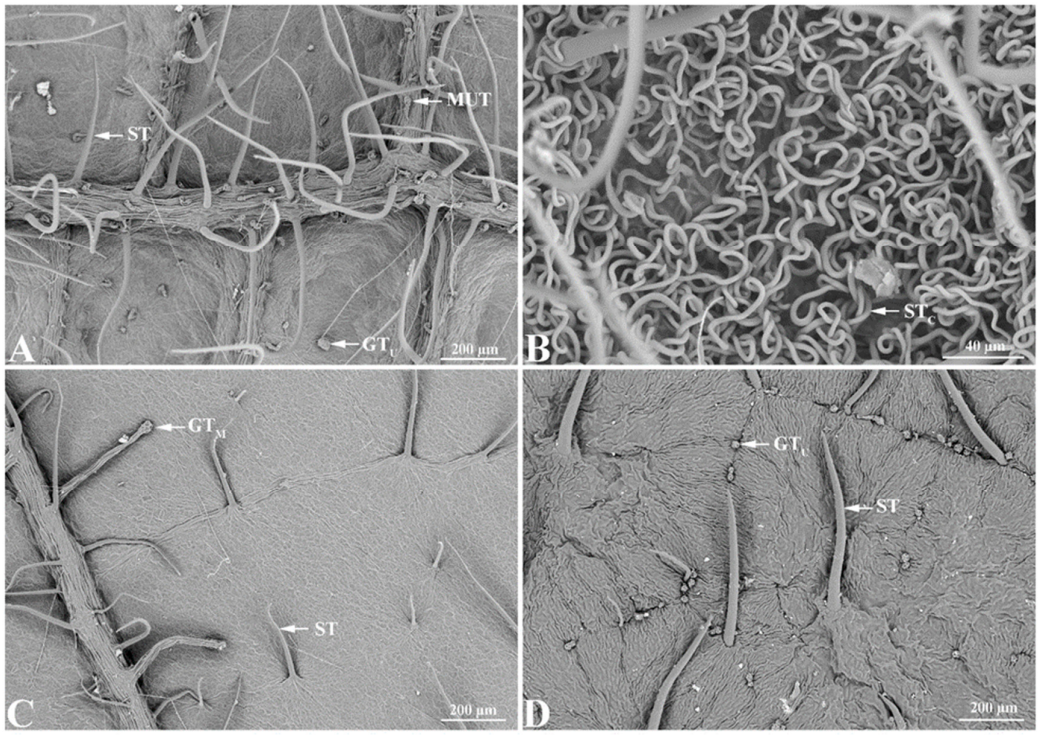

Figure 12. SEM micrographs of trichomes of *Rubus* species studied (A) Outline on abaxial surface of *R. alceifolius* Poir; (B) Outline on abaxial surface of *R. tiliaceus* Smith; (C) Outline on abaxial surface of *R. sumatranus* Miq; (D) Outline on adaxial surface of *R. blepharoneurus* Cardot

GT_M, Glandular trichome with multicellular multiseriate stalk; GT_U, Glandular trichome with unicellular uniseriate stalk; MUT, Multicellular uniseriate trichome; ST, Simple trichome; ST_C, Curled hairs (arrows indicate trichomes)

Midrib

The midrib from the adaxial surface has an outline that is convex in the transverse section, which was found in almost all species, while the abaxial surface is U-shaped, which was found in all species. The adaxial and abaxial surfaces consist of one layer. A thin cuticle is present on both surfaces. The shapes of parenchyma cells are polygonal. On the abaxial surface there are stomata but they are absent from the adaxial one, while typical stomata and raised stomata are present on the abaxial surface of all the species. Trichomes are present on both surfaces. Simple trichomes are present on all the species, but glandular trichomes with multicellular multiseriate stalk (GT_M) are found only on the abaxial surface in *R. sumatranus* Miq. The cortex contains collenchyma cells and parenchyma cells. Collenchyma cells beneath both surfaces are about two to eight layers thick. Parenchyma predominates in ground tissue, irregularly shaped and polygonal cells, some cells contain starch grains and cluster crystals of calcium oxalate including druse and prismatic. The vascular bundles have two types (large and small). The large vascular bundle is a collateral bundle and crescent shaped. The small vascular bundles are collateral bundles that consist of 1-2 separate bundles and are ovate shaped. The vascular bundles are surrounded by parenchyma cells. The xylem consists of vessels and small cell parenchyma, alternating with medullary rays. In all species, below the phloem of the collateral bundle there are pericyclic fibers in nearly all the species (Table 10 and Figure 13).

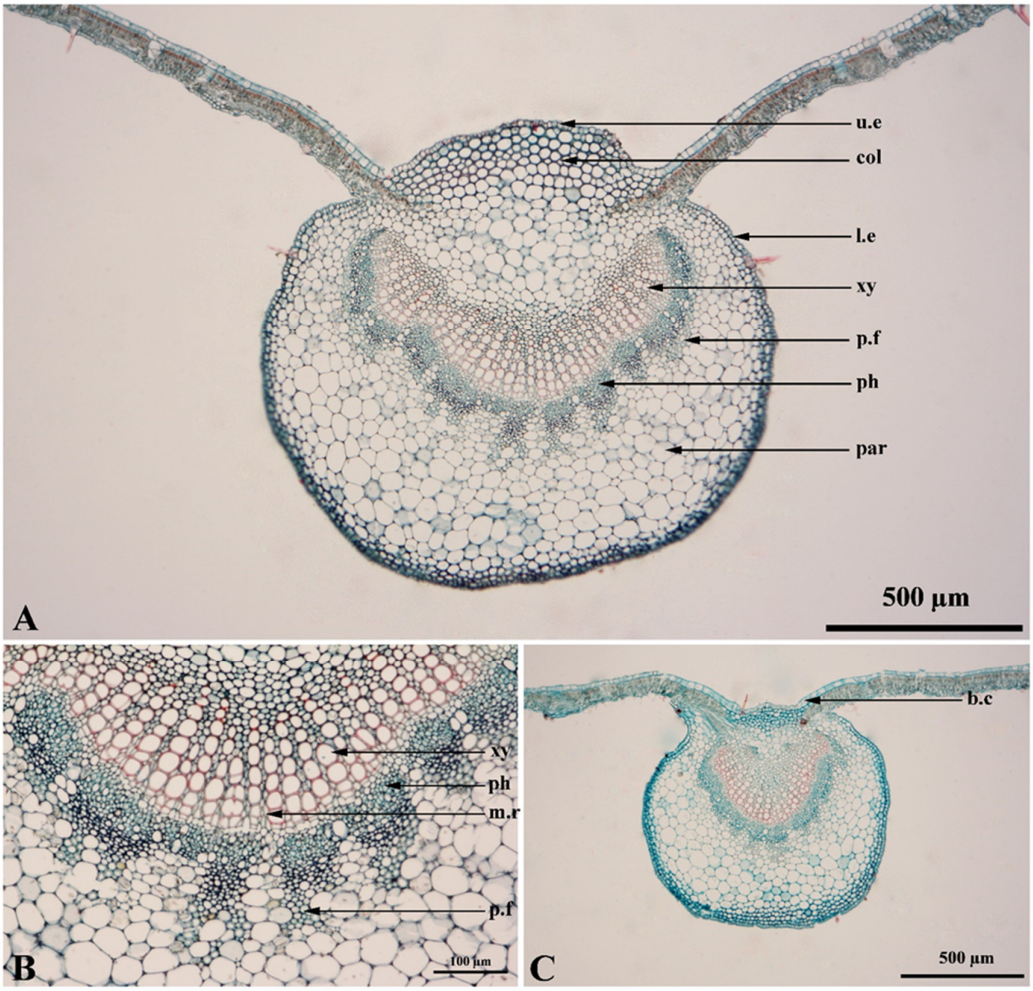

Figure 13. Transverse section of midrib of *Rubus* species (A) Outline of *R. elongatus* Smith; (B) Vascular bundle of *R. elongatus* Smith; (C) Outline of *R. chevalieri* var. *angkae* Thunb showing presence of bulliform cells

b.c, bulliform cell; col, collenchyma; l.e, abaxial surface; m.r, medullary rays; par, parenchyma; p.f, pericyclic fibers; ph, phloem; u.e, adaxial surface; xy, xylem

Leaf margin

The outline of the leaf margin in transverse section curves down, which was present in almost all species, except *R. elongatus* Smith and *R. sumatranus* Miq that have straight shapes (Table 10 and Figure 10).

Petiole

The outline of the petiole in transverse section can be divided into three types as follows: circular shaped, cordate shaped and ovate shaped, while the adaxial surface can be divided into three types as follows: convex shaped, concave shaped and straight shaped, while the abaxial surface is U-shaped in the whole species. The adaxial and abaxial surfaces consist of one layer. A thin cuticle is present on surfaces. The shapes of the parenchyma cells are polygonal. The stomata are typical stomata and raised stomata occur on the surface of all species. Trichomes are present on surfaces including simple trichome, multicellular uniseriate trichome, multicellular multiseriate trichome, glandular trichome with unicellular uniseriate stalk and glandular trichome with multicellular multiseriate stalk. Simple trichomes are present in all species. The cortex contains collenchyma cells and parenchyma cells. Under the epidermis is a zone of two to five layers of collenchyma.

Parenchyma predominates in ground tissue, irregularly shaped and polygonal cells, some cells contain starch grains and cluster crystals of calcium oxalate, including druse and prismatic. The vascular bundles have two types (large and small). The large vascular bundle is larger two times more than the small vascular bundles. The large vascular bundle is a collateral bundle and crescent shaped. The small vascular bundles are collateral bundles that consist of two to eight separate bundles and are ovate shaped. The vascular bundles are surrounded by parenchyma cells. The xylem consists of vessels and small cell parenchyma, alternating with medullary rays. Under the phloem there are pericyclic fibers in the collateral bundle, in almost all species, except *R. lasiotrichos* Focke and *R. moluccanus* L. Most of the parenchyma cells of the ground tissue contain starch grains and cluster crystals of calcium oxalate, including druse and prismatic (Table 11 and Figure 14).

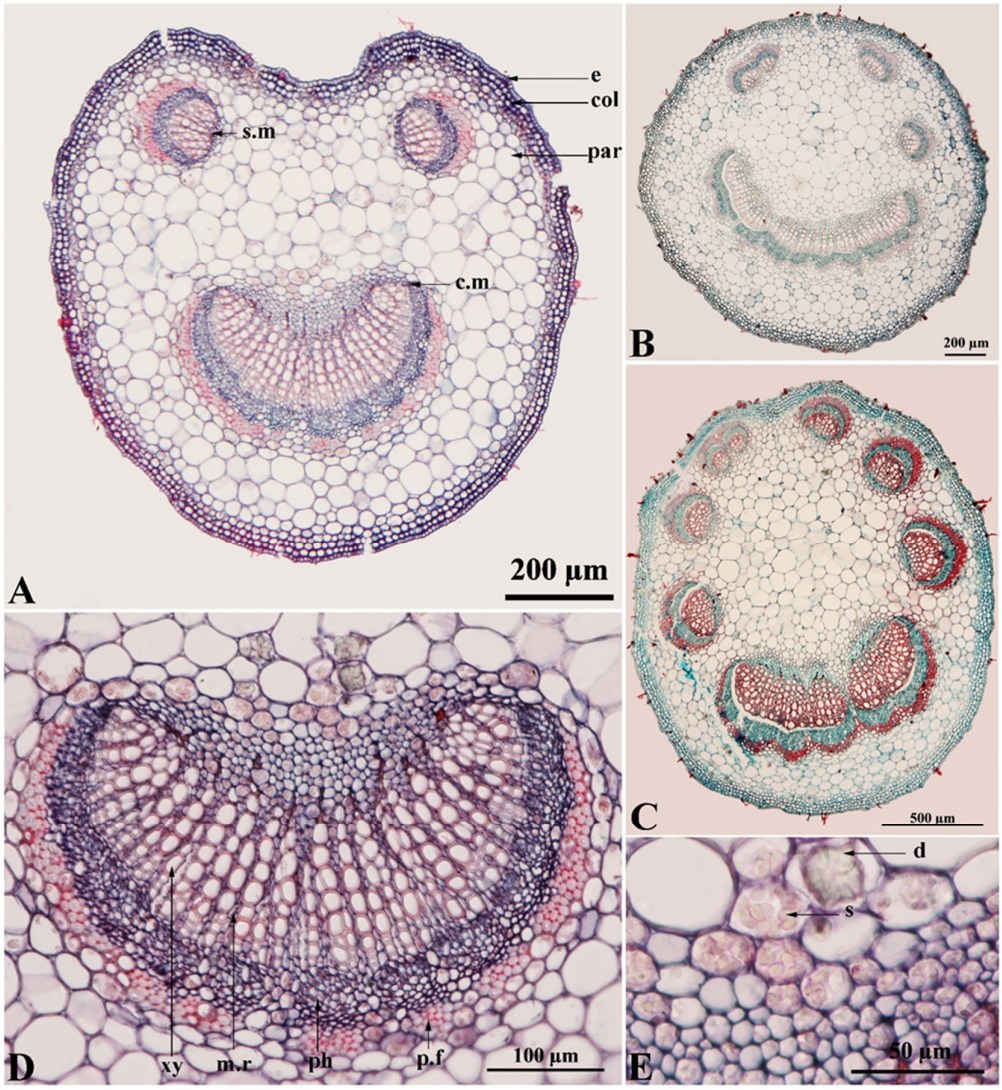

Figure 14. Outline of petiole in transverse section from *Rubus* species studied (A) Cordate shape in *R. niveus* Thunb; (B) Circular shape in *R. elongatus* Smith; (C) Ovate shape in *R. hasskarlii* Mip; (D) Central meristele of *R. niveus* Thunb; (E) Starch grain and Druse in *R. niveus* Thunb

c.m, central meristele; col, collenchyma; d, druse; e, epidermis; m.r, medullary rays; par, parenchyma; p.f, pericyclic fibers; ph, phloem; s, starch grain; s.m, small meristeles

Stem

The outline of the stem in transverse section can be divided into three types as follows: circular shapes and ovate shapes are present in almost all species, except *R. ellipticus* var. *obcordatus* Focke and *R. moluccanus* L. that are rectangular shaped. The adaxial and abaxial surfaces consist of one layer. A thin cuticle is present on surfaces. The shapes of parenchyma cells are polygonal. The stomata are typical stomata and raised stomata occur on the surface of all the species. Trichomes are present on surfaces including simple trichome, multicellular uniseriate trichome, multicellular multiseriate trichome, glandular trichome with unicellular uniseriate stalk and glandular trichome with multicellular multiseriate stalk. Simple trichomes are present in almost all species, except *R. leucanthus* Hance. The cortex contains collenchyma cells and parenchyma cells. Under the epidermis is a zone of two to five layers of collenchyma. Parenchyma predominates in ground tissue, irregularly shaped and polygonal cells, with some cells containing starch grains and cluster crystals of calcium oxalate including, druse and prismatic. The stele is dictyostele (eustele types) surrounded by parenchyma cells. The xylem consists of vessels and small cell parenchyma, alternating with medullary rays. Pericyclic fibers are present below the phloem, which was found in almost all species, except *R. rugosus* Smith (Table 12 and Figure 15).

Table 12. Comparison of cross sections of stems of *Rubus* (Rosaceae) in Thailand

No.	Sub genus	Scientific name	Shape	PF	Type of trichomes	NC	Type of crystals	Starch grain
1	Idaeobatus	*R. ellipticus* var. *obcordatus* Focke	R	✓	ST, MMT	2-3	D, P	-
2		*R. leucanthus* Hance	O	✓	-	3	D	-
3		*R. niveus* Thunb	C	✓	ST	3	D, P	✓
4		*R. sumatranus* Miq	O	✓	ST, MMT, GT_M	3	D	-
5	Malachobatus	*R. alceifolius* Poir	O	✓	ST, MUT	4-5	D	✓
6		*R. blepharoneurus* Cardot	O	✓	ST	4	D, P	-
7		*R. chevalieri* var. *angkae* Thunb	C	✓	ST, MMT	3-4	D	✓
8		*R. clinocephalus* Focke	C	✓	ST	4	D	✓
9		*R. elongatus* Smith	O	✓	ST	3	P	-
10		*R. hasskarlii* Miq	O	✓	ST	4	D, P	-
11		*R. hastifolius* H.Lév. & Vaniot	C	✓	ST	5	D, P	✓
12		*R. lasiotrichos* Focke	O	✓	ST, MUT	4	D, P	✓
13		*R. malvaceus* Focke	C	✓	ST, MUT	4	D, P	-
14		*R. moluccanus* L.	R	✓	ST, MUT	4	D, P	-
15		*R. pyrifolius* Smith	O	✓	ST, MUT	4	D	✓
16		*R. rufus* Focke	O	✓	ST, MUT, GT_U	4	D	✓
17		*R. rugosus* Smith	C	-	ST	4	D, P	-
18		*R. tiliaceus* Smith	O	✓	ST, MUT	4	D, P	✓
19		*R. xanthoneurus* Focke	C	✓	ST	4	D, P	-

Key to abbreviations: C = Circular shape, Co = Cordate shape, D = Druse, GT_M = Glandular trichome with multicellular multiseriate stalk, GT_U = Glandular trichome with unicellular uniseriate stalk , MMT = Multicellular multiseriate trichome, MUT = Multicellular uniseriate trichome, NC = Number of collenchyma layers, NVB = Number of vascular bundle, O = Ovate shape, P = Prismatic, PF = Pericyclic fibers, R = Rectangular shape, ST = Simple trichome, ST_C = Curled hairs, T = Tannin, ✓ = Present, - = Absent

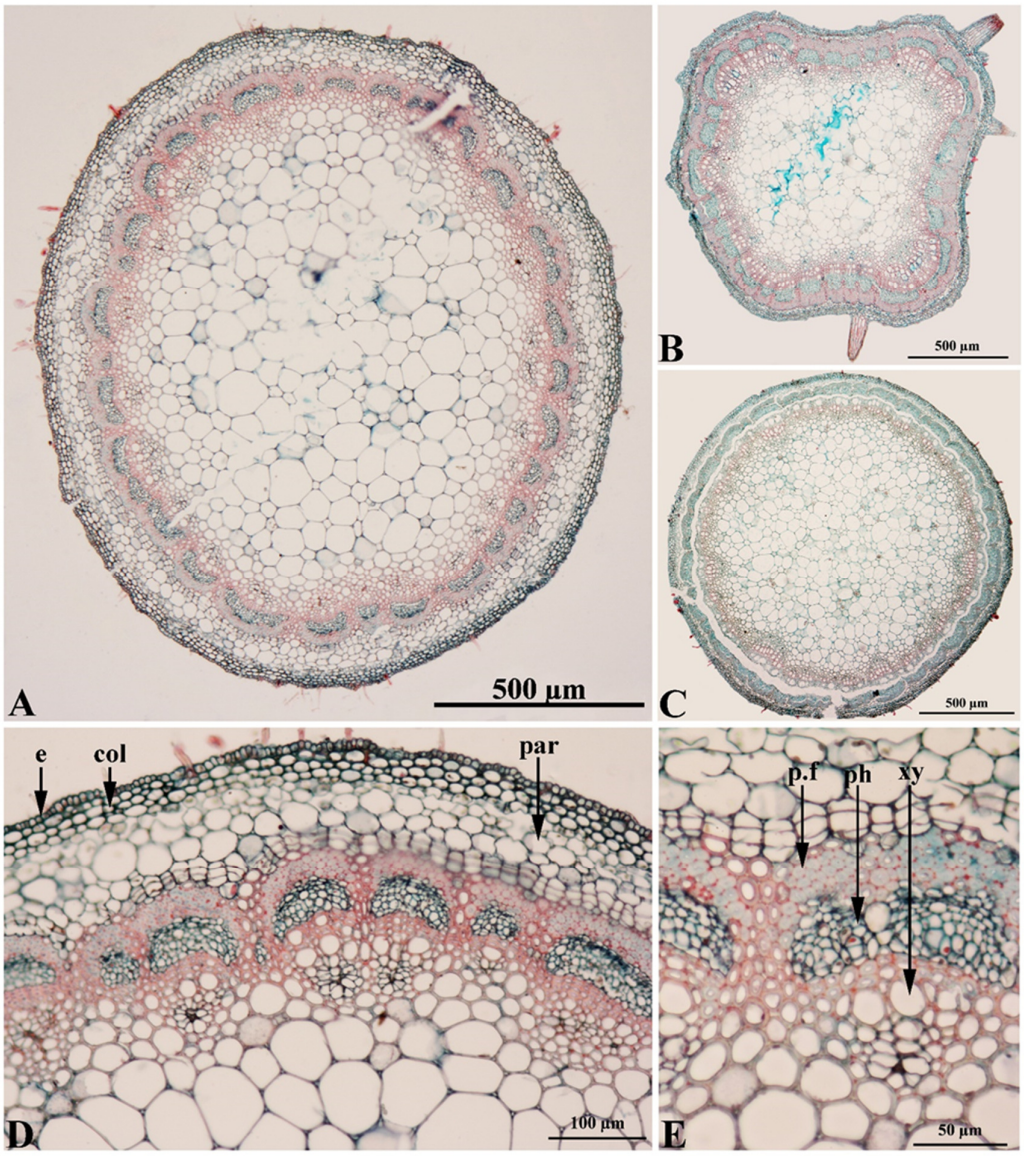

Figure 15. Outline of stem in transverse section from *Rubus* species studied (A) Ovate shape in *R. elongatus* Smith; (B) Rectangular shape in *R. ellipticus* var. *obcordatus* Focke; (C) Circular shape in *R. malvaceus* Focke; (D-E) Outline of *R. elongatus* Smith

col, collenchyma; e, epidermis; par, parenchyma; p.f, pericyclic fibers; ph, phloem; xy, xylem

Principal component analysis (PCA) of anatomical studied

Table 3 contains the results of 19 *Rubus* species from six characters obtained from scores of factors loading and eigen analysis. In total, 74.25% of the total variance for the anatomical character come from two principal components. Of these, the primary component gave 44.1% of the variation linked to the leaf thickness (LT), adaxial surface thickness (UET), abaxial surface thickness (LET) and spongy mesophyll thickness (ST). The second principal component explained 30.15% of the total variation and associated with midrib length (ML) and midrib width (MW) (Tables 13, 14).

The principal component analysis scatter plot comes from scores 1 and 2 for components 1 and 2 for the x and y axes, respectively. The *Rubus* species in Thailand are placed into two groups, as shown in Figure 16.

Table 13. Factor loadings indicating relationships between variables including the characters for differentiation of anatomically studied *Rubus* L. (Rosaceae) in Thailand

Variable	Principal components	
	1	2
LT	0.97	0.23
UET	0.82	
ST	0.71	0.20
LET	0.66	-0.25
MW		0.96
ML		0.94

Key to abbreviations: LET = Abaxial surface thickness, LT = Leaf thickness, ML = Midrib length, MW = Midrib width, ST = Spongy mesophyll thickness, UET = Adaxial surface thickness

Table 14. Eigen analysis of correlation matrix of anatomically studied *Rubus* L. in Thailand

Component	Eigenvalues		
	Total	Percent of variance	Cumulative percent
1	2.64	44.10	44.10
2	1.80	30.15	74.25

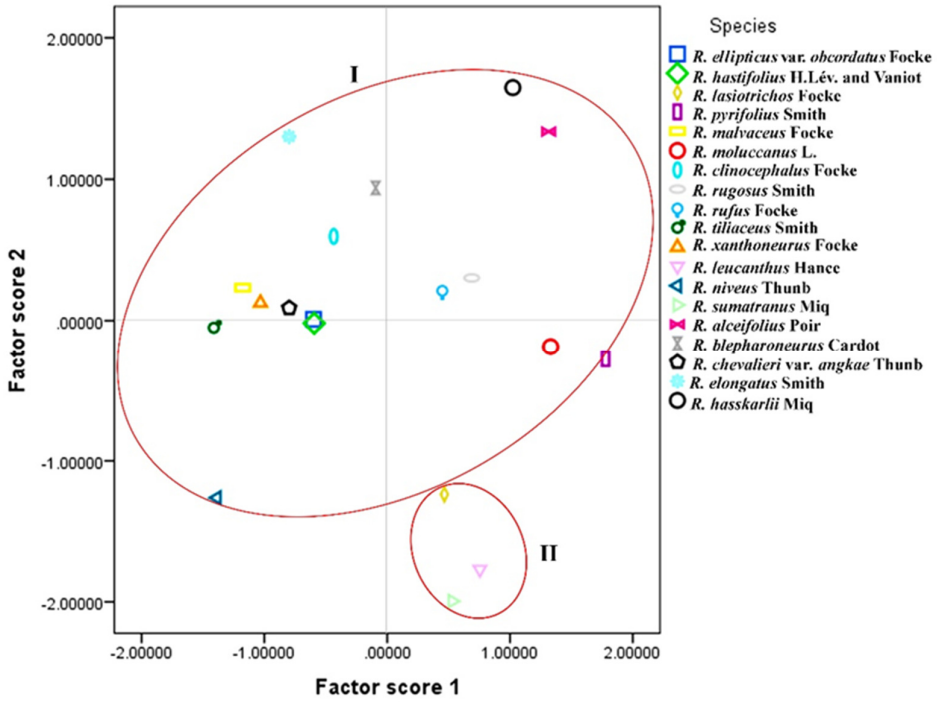

Figure 16. PCA scatterplot of *Rubus* species in Thailand based on anatomical characters

The first group comprises 16 species: *R. alceifolius* Poir, *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. clinocephalus* Focke, *R. ellipticus* var. *obcordatus* Focke, *R. elongatus* Smith, *R. hasskarlii* Miq, *R. hastifolius* H.Lév. & Vaniot, *R. malvaceus* Focke, *R. moluccanus* L., *R. niveus* Thunb, *R. pyrifolius* Smith, *R. rufus* Focke, *R. rugosus* Smith, *R. tiliaceus* Smith and *R. xanthoneurus* Focke. The second group comprises three species: *R. lasiotrichos* Focke, *R. leucanthus* Hance and *R. sumatranus* Miq.

Cluster analysis

Figure 17 contains a dendrogram of the cluster analysis from the unweighted pair-group method using arithmetic average (UPGMA), with two major clusters based on the anatomical character. Group I comprises 16 species namely *R. alceifolius* Poir, *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. clinocephalus* Focke, *R. ellipticus* var. *obcordatus* Focke, *R. elongatus* Smith, *R. hasskarlii* Miq, *R. hastifolius* H.Lév. & Vaniot, *R. malvaceus* Focke, *R. moluccanus* L., *R. niveus* Thunb, *R. pyrifolius* Smith, *R. rufus* Focke, *R. rugosus* Smith, *R. tiliaceus* Smith and *R. xanthoneurus* Focke. Group II comprises three species namely *R. lasiotrichos* Focke, *R. leucanthus* Hance and *R. sumatranus* Miq.

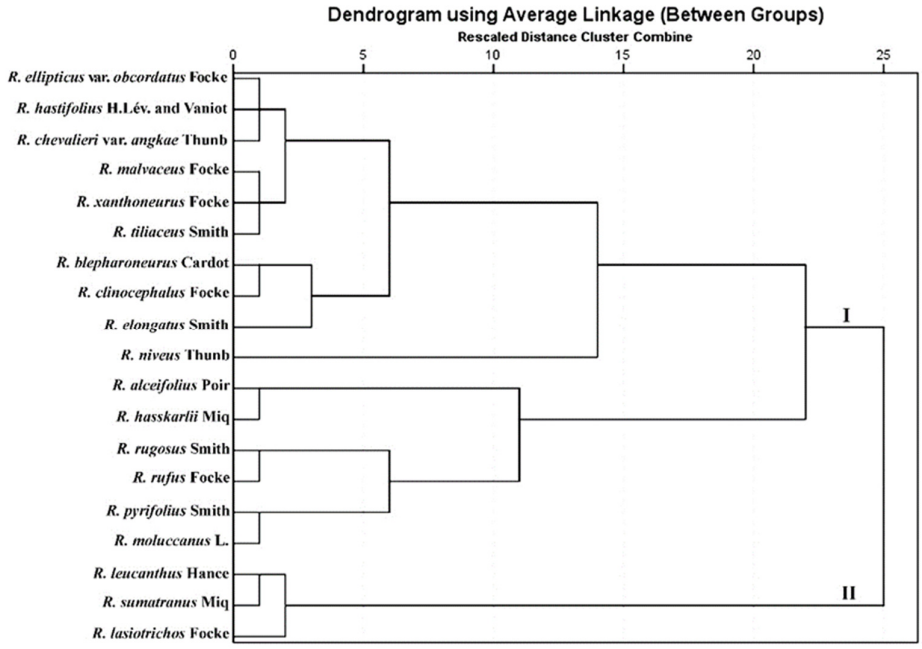

Figure 17. UPGMA dendrogram of cluster analysis of anatomical characters of *Rubus* in Thailand

Discussion

The generalized pollen morphology of all examined species in the present study is monad, with radial symmetry and isopolar. The size of pollen grains varies from small to medium. The present study recorded 12 species (63.15%) with medium pollen size and seven species (36.85%) with small pollen size. The largest pollen grain is *R. alceifolius* Poir (P = 40.97 μm, E = 43.48 μm) from flowers with a diameter more than 2 cm and smallest pollen grain is *R. ellipticus* var. *obcordatus* Focke (P = 17.73 μm, E = 22.14 μm) from flowers with a diameter less than 1 cm. The data regarding the pollen morphology agrees with older studies in genus *Rubus* by Tomlik-Wyremblewska (1995) who reported small sized pollen in nine species of genus *Rubus*, while Wang and Chen (2001) reported small to medium sized pollen in Rosaceae family and found that *R. sumatranus* Miq are small sized and consistent with this study. Tomlik-Wyremblewska *et al.* (2004) reported small to medium sized pollen in subgenus *Chamaebatus* L. and subgenus *Idaeobatus* L., while *R. ellipticus* var. *obcordatus* Fock and *R. niveus* Thunb are small sized and consistent with this study (Table 14). Wrońska-Pilarek *et al.* (2012) reported pollen with small to medium sizes in six species of genus *Rubus*. Ghosh and Saha (2017) reported pollen with small to medium sizes in six species of genus *Rubus*. Xiong *et al.* (2019) reported the pollen sizes in

155 species of genus *Rubus*, and *R. alceifolius* Poir*, R. ellipticus* var. *obcordatus* Fock, *R. leucanthus* Hance, *R. rufus* Focke and *R. xanthoneurus* Focke are small to medium sized, which is consistent with this study. Lechowicz *et al*. (2020) reported pollen with small sizes in 58 species of genus *Rubus* In our present study, the pollen sizes of three species (*R. hastifolius, R. niveus* Thunb and *R. sumatranus* Miq) are small sized while Xiong *et al.* (2019) reported a medium size. There was a range of shapes in the pollen grains that varied from prolate spheroidal (47.36%) to oblate spheroidal (26.31%), suboblate (15.78%), oblate (5.26%) and prolate (5.26%). Most species have the prolate spheroidal shape for their pollen, which is inconsistent with the report by Xiong *et al.* (2019) who found the most common pollen grain shapes as spheroidal and prolate in genus *Rubus*. The findings are inconsistent with some species from the study of Tomlik-Wyremblewska *et al.* (2004) who reported that prolate and subprolate shaped pollen in *R. ellipticus* var. *obcordatus* Focke and *R. niveus* Thunb, but in the present study the suboblate shape was found. Wang and Chen (2001) reported the subprolate-prolate pollen shape in *R. sumatranus* Miq, but the present study obtained an oblate spheroidal shape. Xiong *et al.* (2019) reported pollen shapes from eight species of the genus *Rubus* namely *R. alceifolius* Poir, *R. ellipticus* var. *obcordatus* Focke, *R. hastifolius* H.Lév. & Vaniot, *R. leucanthus* Hance, *R. niveus* Thunb, *R. rufus* Focke, *R. sumatranus* Miq and *R. xanthoneurus* Focke, which are prolate, prolate-spheroidal and subprolate for their pollen and inconsistent with this present study.

The four main types of pollen aperture are as follows: tricolporate (63.15%), tricolporate-tetracolporate (15.78%), tricolpate (15.78%) and tricolpate-tetracolpate (5.26%). Most species have the tricolporate type for their *Rubus* pollen. The data about the pollen aperture agrees with that from studies that have been conducted in the past on genus *Rubus* by Erdtman (1966) who reported the tricolporate pollen aperture from some species of Rosaceae family. Tomlik-Wyremblewska (1995); Wang and Chen (2001); Tomlik-Wyremblewska *et al.* (2004); Wrońska-Pilarek *et al.* (2012); Xiong *et al.* (2019); Lechowicz *et al*. (2020) confirmed that tricolpate, tricolpate-tetracolrate, tricolporate and tricolporate-tetracolporate pollen represent the common characters of pollen of the genus *Rubus*. Only one species (*R. ellipticus* var. *obcordatus* Focke) presents pollen apertures that are tricolrate and tetracolpate, while Xiong *et al.* (2019) reported tricolporate apertures. The observed exine sculpturing in this study were microverrucate-perforate (42.10%), striate-perforate (26.31%), rugulate-perforate (15.78%), microverrucate (10.52%) and perforate (5.26%). Most species have the microverrucate-perforate type for their pollen. These exine sculpture data agree with past work on genus *Rubus* by Tomlik-Wyremblewska *et al.* (2004). Xiong *et al*. (2019) found exine sculpture as striate-perforate in *R. niveus* Thunb. Wang and Chen (2001) and Xiong *et al.* (2019) reported exine sculpture that was striate-perforate in *R. sumatranus* Miq. Xiong *et al*. (2019) pointed out that in *R. ellipticus* var. *obcordatus* Focke and *R. leucanthus* Hance the exine sculpture are regulate-perforate and striate-perforate, which is consistent with our present study (Table 15). The findings are inconsistent with five species of the genus *Rubus*: *Rubus alceifolius* Poir, *R. ellipticus* var. *obcordatus* Focke, *R. hastifolius* H.Lév. & Vaniot, *R. rufus* Focke and *R. xanthoneurus* Focke that previously had their pollen morphology studied. In our present study, the exine sculpture of *R. ellipticus* var. *obcordatus* Focke was regulate-perforate, while Tomlik-Wyremblewska *et al.* (2004) reported striate-perforate. Xiong *et al*. (2019) reported the exine sculpture of *R. alceifolius* Poir*, R. hastifolius* H.Lév. & Vaniot, *R. rufus* Focke and *R. xanthoneurus* Focke that was inconsistent with our present study. Eleven species of the genus *Rubus* had their pollen morphology studied for the first time, namely *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. elongatus* Smith, *R. hasskarlii* Miq, *R. lasiotrichos* Focke, *R. malvaceus* Focke, *R. moluccanus* L., *R. pyrifolius* Smith, *R. clinocephalus* Focke, *R. rugosus* Smith and *R. tiliaceus* Smith (Table 15).

In the second part of this study, there was an investigation in Thailand of the detailed anatomical features of the leaf, petiole and stem of the genus *Rubus* The leaf blade anatomical features include the presence of one thin cuticle layer with shapes that are polygonal on both surfaces. Most species have the adaxial surface as larger than the abaxial surface, especially, the ratio between the adaxial surface thickness and the abaxial

surface thickness is four times in *R. alpestris* Blume and *R. pyrifolius* Smith, while in *R. leucanthus* Hance the adaxial surface is a similar size to the abaxial surface. Typical stomata and raised stomata are present on the adaxial surface of all species. The findings are consistent with the studies of Fell and Rowson (1956, 1957, 1960, 1961). Kasalkheh *et al.* (2019) reported that stomata are present on the adaxial surface of the leaf, except for *R. hirtus* Waldstein and Kitaibel where they are present on both surfaces. Trichomes were studied and six types were detected viz., simple trichome (100%), multicellular multiseriate trichome (78.94%), multicellular uniseriate trichome (73.68%), curled hairs (57.89%), glandular trichome with unicellular uniseriate stalk (5.26%) and glandular trichome with multicellular multiseriate stalk (5.26%). Simple trichomes are present on both surfaces of all species. The findings are consistent with the studies of Fell and Rowson (1956, 1957, 1960, 1961); Tomaszewski *et al.* (2014); Kasalkheh *et al.* (2019). Papillae are present on the abaxial surface of *R. moluccanus* L. The mesophyll consists of palisade cells and spongy cells composed of 1-4 layers. The idioblast cells are inserted in mesophyll layers of all species. Most species have the idioblast cells inserted in the palisade layers, except in *R. malvaceus* Focke, *R. niveus* Thunb, *R. pyrifolius* Smith and *R. sumatranus* Miq. in which the idioblast cells are inserted in both the mesophyll layers and *R. leucanthus* Hance in which the idioblast cells are inserted in the spongy layers. The idioblast cells contain cluster crystals of calcium oxalate, including druse and prismatic. The findings are in accordance with the studies of Fell and Rowson (1956, 1957, 1960, 1961). The vascular bundles are two types (large and small). Most species have two types of vascular bundles, except in *R. leucanthus* Hance that have one type of vascular bundle. Bundle sheath extensions are present in almost all species, except in *R. leucanthus* Hance.

On the adaxial surface, the transverse section of the midrib had an outline that is convex shaped and present in almost all species, except in *R. leucanthus* Hance. and *R. sumatranus* Miq, which are concave shaped, while the abaxial surface is U-shaped, and present in all species. The midrib's anatomical features include the presence of one thin cuticle layer with polygonal shapes on both surfaces. The adaxial surface has typical stomata and raised stomata for all species. Trichomes were studied and there are four types, namely simple trichome (100%), multicellular uniseriate trichome (42.01%), glandular trichome with unicellular uniseriate stalk (15.78%) and glandular trichome with multicellular multiseriate stalk (5.26%). Simple trichomes are present on both surfaces of all species. Multicellular uniseriate trichomes are present on the adaxial surface in *R. blepharoneurus* Cardot, *R. chevalieri* var. *angkae* Thunb, *R. lasiotrichos* Focke and *R. xanthoneurus* Focke but in *R. alceifolius* Poir, *R. hasskarlii* Miq, *R. hastifolius* H.Lév. & Vaniot, *R. lasiotrichos* Focke and *R. tiliaceus* Smith they are present on the abaxial surface. Glandular trichomes with unicellular uniseriate stalk are found on both surfaces of *R. sumatranus* Miq, and in some species, *R. blepharoneurus* Cardot and *R. chevalieri* var. *angkae* Thunb, they are present on the adaxial surface. Glandular trichomes with multicellular multiseriate stalk are found on the abaxial surface of *R. sumatranus* Miq. The ground tissue contains collenchyma cells and parenchyma cells surrounding the vascular bundles. Some parenchyma cells contain starch grains and cluster crystals of calcium oxalate. The starch grains found in *R. cochinchinensis* Tratt and *R. rufus* Focke are consistent with Fell and Rowson (1956) who found starch grains in midrib of *R. idaeus* L. and *R. plicatus* Weihe and Nees. The vascular bundles are collateral bundles with two types (large and small). Below the phloem are the pericyclic fibers in the collateral bundle of almost all species, except in *R. alceifolius* Poir, *R. hasskarlii* Miq, *R. lasiotrichos* Focke, *R. leucanthus* Hance, *R. malvaceus* Focke, *R. niveus* Thunb and *R. sumatranus* Miq where they are not found in the pericyclic fibers in the midrib.

The outline of the leaf margin in the transverse section curves down and are present in almost all species, except *R. elongatus* Smith and *R. sumatranus* Miq where they are straight shaped. The outline shapes of the petiole in transverse section are as follows: cordate (47.36%, found in nine species: *R. alceifolius* Poir, *R. blepharoneurus* Cardot, *R. lasiotrichos* Focke, *R. leucanthus* Hance, *R. moluccanus* L., *R. niveus* Thunb, *R. sumatranus* Miq, *R. tiliaceus* Smith and *R. xanthoneurus* Focke), circular (31.57%, found in six species: *R. chevalieri* var. *angkae* Thunb, *R. clinocephalus* Focke, *R. elongatus* Smith, *R. hastifolius* H.Lév. & Vaniot, *R.*

pyrifolius Smith and *R. rugosus* Smith) and ovate (21.05%, found in four species *R. ellipticus* var. *obcordatus* Focke, *R. hasskarlii* Miq, *R. malvaceus* Focke and *R. rufus* Focke). There are concave and convex shapes for the transverse section of the petiole outline for the adaxial surface, except in *R. pyrifolius* Smith and *R. rugosus* Smith that are straight shaped and in *R. clinocephalus* Focke that alternate convex-concave, while the abaxial surface is U-shaped in all species. The petiole's anatomical features include the presence of one thin cuticle layer with polygonal shapes on the surfaces. The stomata are typical stomata and raised stomata are present on the surface of all species. Trichomes were studied and there are five types, namely simple trichome (100%), multicellular uniseriate trichome (47.36%), multicellular multiseriate trichome (15.78%), glandular trichome with unicellular uniseriate stalk (5.26%) and glandular trichome with multicellular multiseriate stalk (5.26%). Simple trichomes are present on the surfaces of all species. The ground tissue contains collenchyma cells and parenchyma cells surrounding the vascular bundles. Some parenchyma cells contain starch grains and cluster crystals of calcium oxalate. The starch grains are found in *R. alceifolius* Poir, *R. chevalieri* var. *angkae* Thunb, *R. hastifolius* H.Lév. & Vaniot, *R. niveus* Thunb, *R. tiliaceus* Smith and *R. xanthoneurus* Focke. The vascular bundles are collateral bundles with two types (large and small). Below the phloem pericyclic fibers are present in the collateral bundle in almost all species, except *R. lasiotrichos* Focke and *R. moluccanus* L. in which the pericyclic fibers are not found in the petiole. The findings are consistent with the studies of Fell and Rowson (1956, 1957, 1960, 1961).

The outline shapes of the stem in transverse section are as follows: ovate (52.63%), circular (36.84%) and rectangular (10.52%). The stem anatomical features include the presence of one thin cuticle layer with polygonal shapes on surface. The stomata are typical stomata and raised stomata are present on the surface of all species. Trichomes were studied and five types are present viz., simple trichome (94.73%), multicellular uniseriate trichome (36.84%), multicellular multiseriate trichome (15.78%), glandular trichome with unicellular uniseriate stalk (5.26%) and glandular trichome with multicellular multiseriate stalk (5.26%). Simple trichomes are present on surfaces of almost all species, except in *R. leucanthus* Hance. The ground tissue contains collenchyma cells and parenchyma cells surrounding vascular bundles. Some parenchyma cells contain starch grains and cluster crystals of calcium oxalate. The starch grains are found in *R. alceifolius* Poir, *R. chevalieri* var. *angkae* Thunb, *R. clinocephalus* Focke, *R. hastifolius* H.Lév. & Vaniot, *R. lasiotrichos* Focke, *R. niveus* Thunb, *R. pyrifolius* Smith, *R. rufus* Focke and *R. tiliaceus* Smith. The stele is dictyostele (eustele types). Pericyclic fibers are present below the phloem in almost all species, except *R. rugosus* Smith (Table 16). The findings are consistent with the studies of Fell and Rowson (1956, 1957, 1960, 1961). For the first time, the anatomies of 19 species were studied.

The PCA scatterplot is classified into three groups based on pollen morphological characters, which are all the same as in the UPGMA dendrogram. The species of the genus *Rubus* are placed in group 1, which is separated from the other groups. The prominent pollen morphological characters of group 1 are exine thickness, colpus length, equatorial axis and polar axis. Group 2 are equatorial axis per colpus width ratio, colpus width per colpus width ratio and colpus width. Group 3 are distance between the apices of two ectocolpi.

The PCA scatterplot is classified into two groups based on anatomy characters, which are all the same as in the UPGMA dendrogram. The species of the genus *Rubus* are placed in group 1, which is separated from the other groups. The prominent anatomical characters of group 1 are leaf thickness, adaxial surface thickness, spongy mesophyll thickness and abaxial surface thickness. Group 2 are midrib length and midrib width.

Table 15. Comparison of some characteristics of pollen morphology of the genus *Rubus*

No	Sub genus	Scientific name	Characteristic	Wang and Chen (2001)	Tomlik-Wyremblewska *et al.* (2004)	Xiong *et al.* (2019)	In this study
1	Idaeobatus	*R. ellipticus* var. *obcordatus* Focke	Size	-	Small	Small	Small
			Equatorial shape	-	Prolate	Subprolate	Suboblate
			Aperture	-	Tricolporate	Tricolporate	Tricolporate
			Exine sculpture	-	Striate-perforate	Rugulate-perforate	Rugulate-perforate
2		*R. leucanthus* Hance	Size	-	-	Small	Small
			Equatorial shape	-	-	Prolate	Oblate-spheroidal
			Aperture	-	-	Tricolporate	Tricolporate
			Exine sculpture	-	-	Striate-perforate	Striate-perforate
3		*R. niveus* Thunb	Size	-	Small	Medium	Small
			Equatorial shape	-	Subprolate	Prolate	Suboblate
			Aperture	-	Tricolporate	Tricolporate	Tricolporate
			Exine sculpture	-	Striate-perforate	Striate-perforate	Striate-perforate
4		*R. sumatranus* Miq	Size	Small	-	Medium	Small
			Equatorial shape	Prolate-prolate	-	Prolate	Oblate spheroidal
			Aperture	Tricolporate	-	Tricolporate	Tricolporate
			Exine sculpture	Striate-perforate	-	Striate-perforate	Striate-perforate
5	Malachobatus	*R. alceifolius* Poir	Size	-	-	Medium	Medium
			Equatorial shape	-	-	Subprolate	Oblate-spheroidal
			Aperture	-	-	Tricolporate	Tricolrate and tetracolpate
			Exine sculpture	-	-	Scabrate-perforate	Microverrucate-perforrate
6		*R. hastifolius* H.Lév. & Vaniot	Size	-	-	Medium	Small
			Equatorial shape	-	-	Prolate	Prolate spheroidal
			Aperture	-	-	Tricolporate	Tricolporate
			Exine sculpture	-	-	Striate-perforate	Perforate
7		*R. rufus* Focke	Size	-	-	Medium	Medium
			Equatorial shape	-	-	Prolate spheroidal	Oblate-spheroidal
			Aperture	-	-	Tricolporate	Tricolporate
			Exine sculpture	-	-	Scabrate-perforate	Microverrucate-perforrate
8		*R. xanthoneurus* Focke	Size	-	-	Medium	Medium
			Equatorial shape	-	-	Prolate	Oblate-spheroidal
			Aperture	-	-	Tricolporate	Tricolporate
			Exine sculpture	-	-	Rugulate-perforate	Striate-perforate

Key to abbreviations: - = Absent

Table 16. Comparison of some characteristics of anatomy of the genus *Rubus*

Organ	Characteristics	Metcalfe and Chalk (1950)	Fell and Rowson (1956-1960)	Tomaszewski *et al.* (2014)	Kasalkheh *et al.* (2019)	Chwil M and Kostryco (2020)	In this study
Leaf	Papillae	-	-	-	-	-	✓
	Hypodermis	✓	-	-	-	-	-
	Type of trichome						
	- Branchy stalk	-	-	-	✓	-	-
	- GT_M	-	✓	-	✓	-	✓
	- GT_U	-	✓	✓	✓	✓	✓
	- MUT	-	✓	✓	✓	-	✓
	- MMT	-	-	-	-	-	✓
	- ST	-	✓	✓	✓	✓	✓
	- ST_C	-	✓	✓	-	✓	✓
	- Stellate	-	✓	✓	✓	-	-
	- Trifid branchy	-	-	-	✓	-	-
	Idioblast	-	✓	-	-	✓	✓
	DP	✓	✓	-	-	-	✓
	Starch grain	-	✓	-	-	-	✓
	BE	-	-	-	-	-	✓
	Pericyclic fibers	-	✓	-	-	-	✓
Petiole	Type of trichome						
	- GT_M	-	-	-	-	-	✓
	- GT_U	-	-	-	-	-	✓
	- MMT	-	-	-	-	-	✓
	- MUT	-	-	-	-	-	✓
	- ST	-	-	-	✓	-	✓
	- Stellate	-	-	-	✓	-	-
	DP	✓	-	-	-	-	✓
	Starch grain	-	-	-	-	-	✓
	Pericyclic fibers	-	-	-	-	-	✓
Stem	Type of trichome						
	- GT_M	-	-	-	-	-	✓
	- GT_U	-	-	-	-	-	✓
	- MMT	-	-	-	-	-	✓
	- MUT	-	-	-	-	-	✓
	- ST	-	-	-	-	-	✓
	DP	✓	-	-	-	-	✓
	Starch grain	-	-	-	-	-	✓
	Pericyclic fibers	-	-	-	-	-	✓

Key to abbreviations: BE = Bundle sheath extension, DP = Druse and prismatic crystals, GT_M = Glandular trichome with multicellular multiseriate stalk, GT_U = Glandular trichome with unicellular uniseriate stalk, MMT = Multicellular multiseriate trichome, MUT = Multicellular uniseriate trichome, ST = Simple trichome, ST_C = Curled hairs, ✓ = Present, - = Absent

Conclusions

The present study is based on the pollen morphology and anatomy of the genus *Rubus* in Thailand. For all the examined species in this study, the pollen morphology are monad, radial symmetry and isopolar. For almost all species, the pollen morphology will have different characteristics as detected viz., size, equatorial shape, aperture, syncolpate and exine sculpture. The pollen characters of some species are similar in all aspects. The pollen morphology of the genus *Rubus* cannot be used for identification to species level. The data of the anatomy could be used for the identification to species level of the genus *Rubus*. The anatomical characteristics used for the identification key are namely shape of leaf margin, shape of adaxial surface of midrib, pericyclic fibers, idioblast cells, shape of petiole, starch grain, number of vascular bundles, shape of stem and types of trichomes. A key to species is provided based on anatomical characteristics and listed below.

Key to species of *Rubus* in Thailand based on leaf, petiole and stem anatomy.

1. Leaf margins are straight shaped.....2
2. The adaxial surface of midrib is concave shaped.....*R. sumatranus* Miq
2. The adaxial surface of midrib is convex shaped.....*R. elongatus* Smith
1. Leaf margins are curved down.....3
3. The adaxial surface of midrib is concave shaped..... *R. leucanthus* Hance
3. The adaxial surface of midrib is convex shaped.....4
4. Absence of pericyclic fibers in midrib.....5
5. Presence of idioblast cells in spongy layers.....6
6. The outline of petiole are cordate shaped.....*R. niveus* Thunb
6. The outline of petiole are ovate shaped..... *R. malvaceus* Focke
5. Absence of idioblast cells in spongy layers.....7
7. The outline of petiole are ovate shaped..... *R. hasskarlii* Miq
7. The outline of petiole are cordate shaped.....8
8. Presence of pericyclic fibers and starch grains in petiole.....*R. alceifolius* Poir
8. Absence of pericyclic fibers and starch grains in petiole..... *R. lasiotrichos* Focke
4. Presence of pericyclic fibers in midrib.....9
9. The small vascular bundles had more than five separate bundles in petiole.....10
10. The small vascular bundles had six separate bundles in petiole.....11
11. The outline of petiole are ovate shaped..... *R. rufus* Focke
11. The outline of petiole are circular shaped.....12
12. The adaxial surface of petiole alternate convex and concave shapes..... *R. clinocephalus* Focke
12. The adaxial surface of petiole is straight shaped.....13
13. The outline of stem is ovate shaped, presence of pericyclic fibers.....*R. pyrifolius* Smith
13. The outline of stem is circular shaped, absence of pericyclic fibers.....*R. rugosus* Smith
10. The small vascular bundles have seven to eight separate bundles in petiole.....14
14. The outline of petiole is ovate shaped, small vascular bundles had eight separate bundles, absence of starch grains.....*R. ellipticus* var. *obcordatus* Focke
14. The outline of petiole is cordate shaped, small vascular bundles had seven separate bundles, presence of starch grains.....*R. tiliaceus* Smith
9. The small vascular bundles had less than five separate bundles in petiole.....15
15. The small vascular bundles had two separate bundles in petiole.....*R. xanthoneurus* Focke
15 The small vascular bundles had four separate bundles in petiole.....16
16. The outline of petiole is cordate shaped, absence of starch grains.....17

17. The outline of stem is ovate shaped.....*R. blepharoneurus* Cardot
17. The outline of stem is rectangular shaped.....*R. moluccanus* L.
16. The outline of petiole is circular shaped, presence of starch grains.....18
18. Presence of curled hairs on abaxial surface of leaf blade.....*R. chevalieri* var. *angkae* Thunb
18. Absence of curled hairs on abaxial surface of leaf blade.....*R. hastifolius* H.Lév. & Vaniot

Authors' Contributions

Conceptualization: PS and KH; Data curation: KH and PS; Funding acquisition: KH, PS and SS; Investigation: KH and PS; Methodology: KH; Project administration: PS; Resources: KH, PS and SS; Software: KH; Supervision: PS; Validation: KH and PS; Visualization: KH; Writing - original draft: KH; Writing - review and editing: PS and SS.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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