

Article

Pollen Morphological Inter- and Intraspecific Variability in Selected Species of *Rubus* L. (Rosaceae)

Kacper Lechowicz ^{1,*} , Jan Bocianowski ²  and Dorota Wrońska-Pilarek ¹ 

¹ Department of Botany and Forest Habitats, Faculty of Forestry and Wood Technology, Poznań University of Life Sciences, Wojska Polskiego 71 d, 60-625 Poznań, Poland

² Department of Mathematical and Statistical Methods, Faculty of Agronomy, Horticulture and Bioengineering, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland

* Correspondence: kacper.lechowicz@up.poznan.pl

Abstract: Pollen morphology is one of the important vehicles in resolving some taxonomic problems on the family, genera or species level and has become part of the many disciplines and collaborative approaches in plant systematics and evolution. Palynological studies on the large and taxonomically highly complex genus *Rubus* L. have been limited, particularly concerning pollen variability. The aim of current study was to investigate the scope of inter- and intraspecific variability of the studied species based on pollen morphology, as well as verify taxonomic usefulness of pollen traits in distinguishing the studied taxa. The sixteen native Poland and Europe *Rubus* species were studied. Over a dozen quantitative and qualitative features of 2100 pollen (70 samples of 30 pollen each) were analyzed. Exine ornamentation and pollen size proved to be most useful pollen traits in the assessment of variability in the studied *Rubus* species. Pollen traits did not confirm the currently adopted taxonomical division of the genus *Rubus* into subgenera, sections and series. The greatest interspecific variability was found for traits P, E, Le and d, while variability was lowest in the case of Exp, Exe, P/E, Le/P, d/E, Exp/P and Exe/E. Intraspecific variability was determined by the following features ordered from those least to most variable: P, E, d, Le, d/E, Exp/P, Exp, P/E and Le/P.

Keywords: pollen variability; pollen morphology; Polish native species; *Rubus*; SEM



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1. Introduction

The genus *Rubus* L. is one of the most taxonomically complicated groups within the Rosaceae family with a worldwide distribution, including hundreds or even thousands of published species names and infrageneric taxa [1–3]. The number of species in the genus *Rubus* L. may vary from 429 to 750 or up to more than 1000 accepted species worldwide [4–9]. According to the latest data reported by Govaerts et al. [3] this genus comprises 1409 accepted species, of which from 250 to 300 species grow in Central and North-Western Europe [8]. In Poland, the occurrence of 108 native species from the genus *Rubus* has been confirmed so far [10,11]. Many blackberry species are found outside their natural distribution area as a result of their application in horticulture, while some have been introduced accidentally [12].

The difficulty of the genus *Rubus* is due to the large number of species with similar morphological characteristics and often small, local ranges of natural occurrence, as well as polyploid hybridization and apparently frequent facultative apomixis [13,14]. Therefore, among brambles pollen of only 48 European species had been characterized until 2019 [15–24]. These data have been supplemented by the comprehensive palynological studies by Xiong et al. [25], who examined 155 species and 13 varieties representing all the 12 subgenera of this genus, and by Lechowicz et al. [26], who described pollen grains of 58 Polish and European *Rubus* species.

The crucial features of bramble pollen grains include exine ornamentation (ornamentation type, width and orientation of striae and grooves), length of colpi, type of the bridge, costae colpi and the number and size of perforations [16,18–23,25–38]. In the opinion of Tomlik-Wyremblewska [22,32], pollen size and shape prove to be poor criteria in species identification.

The aim of this study was to investigate pollen inter- and intraspecific variability in 16 *Rubus* species native to Poland and Europe, representing three subgenera, two sections and subsections and seven series, based on morphological features of pollen grains. Currently, there are few palynological studies on the intraspecific variability of blackberries. Moreover, we tested the taxonomical value of the studied pollen features to assess whether these features may be used to distinguish among the analyzed taxa of the genus *Rubus*.

2. Material and Methods

The blossoms were collected in the herbarium of the Institute of Dendrology, Polish Academy of Sciences in Kórnik (KOR) and stored in the herbarium of the Department of Botany and Forest Habitats, the Poznań University of Life Sciences (PZNF).

The study was carried out on 70 samples of 16 native *Rubus* species (Table 1). A significant part of the plant material comes from Poland, while single specimens were collected in Austria, Bulgaria and Germany. A list of the samples analyzed with their affiliation to particular taxa is shown in Table 1.

Table 1. The taxonomic classification of the *Rubus* species studied.

No.	Species	Subgenus	Section	Subsection	Series
1	<i>R. bifrons</i> Vest	<i>Rubus</i>	<i>Rubus</i>	<i>Hiemales</i>	<i>Discolores</i>
2	<i>R. caesius</i> L.	<i>Rubus</i>	<i>Caesii</i>	-	-
3	<i>R. constrictus</i> P. J. Müll. & Lefèvre	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>
4	<i>R. divaricatus</i> P. J. Müll.	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>
5	<i>R. gracilis</i> J. Presl & C. Presl	<i>Rubus</i>	<i>Rubus</i>	<i>Hiemales</i>	<i>Rhamnifolii</i>
6	<i>R. henrici-egonis</i> Holub	<i>Rubus</i>	<i>Rubus</i>	<i>Hiemales</i>	<i>Discolores</i>
7	<i>R. idaeus</i> L.	<i>Idaeobatus</i>	-	-	-
8	<i>R. nessensis</i> W. Hall	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Nessenses</i>
9	<i>R. opacus</i> Focke	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>
10	<i>R. plicatus</i> Weihe & Nees	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>
11	<i>R. praecox</i> Bertol.	<i>Rubus</i>	<i>Rubus</i>	<i>Hiemales</i>	<i>Discolores</i>
12	<i>R. radula</i> Weihe	<i>Rubus</i>	<i>Rubus</i>	<i>Hiemales</i>	<i>Radulae</i>
13	<i>R. saxatilis</i> L.	<i>Cylactis</i>	-	-	<i>Saxatiles</i>
14	<i>R. scissus</i> W. C. R. Watson	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Nessenses</i>
15	<i>R. sprengelii</i> Weihe	<i>Rubus</i>	<i>Rubus</i>	<i>Hiemales</i>	<i>Sprengeliani</i>
16	<i>R. sulcatus</i> Vest	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>	<i>Rubus</i>

The taxonomic classification of the studied taxa from the genus *Rubus* was adopted according to Zieliński [10], with further modifications by Kosiński et al. [11]. The verification of the taxa was made by Prof. Jerzy Zieliński (Institute of Dendrology, Polish Academy of Sciences in Kórnik), a batologist–taxonomist specializing in the genus *Rubus*.

Several, randomly selected inflorescences (flowers) were collected from 70 blackberry localities in four European countries (Table 2). Pollen grains were analyzed according to the method of Erdtman [39]. Inflorescences collected from the herbarium were placed in tubes and then centrifuged with glacial acetic acid. Grains were mixed with the acetolysis solution, which consisted of nine parts acetic anhydride and one part concentrated sulfuric acid. The mixture was then heated to the boiling point and kept in the water bath for 2–3 min. Samples were centrifuged in the acetolysis mixture, washed with acetic acid and centrifuged again. The pollen grain samples were then mixed with 96% alcohol and centrifuged four times, with processed grains subsequently divided into two groups. One half of the processed sample was immersed in an alcohol-based solution of glycerin for the

light microscopy (LM), while the other was placed in 96% ethyl alcohol in preparation for scanning electron microscopy (SEM). The SEM observations were carried out using a Zeiss Evo 40 microscope, while the size of the acetolyzed pollen grains were measured at 400x magnification using a Levenhuk D870T microscope equipped with a camera and software enabling accurate grain measurement. Measurements taken from 30 mature, randomly selected, properly developed pollen grains were made using LM, with 2100 pollen grains measured in total.

Table 2. List of proveniences of the *Rubus* species samples studied.

No.	Species	Localities	Geographical Coordinates	Collector, Herbarium
1	<i>R. bifrons</i> 1	Poland, Kietrz	50°04′46.7″ N, 18°00′07.6″ E	Kosiński, Tomaszewski, Zieliński; KOR
2	<i>R. bifrons</i> 2	Poland, Mszana Dolna	49°40′51.9″ N, 20°04′35.4″ E	Białobok; KOR
3	<i>R. bifrons</i> 3	Poland, Braszowice	50°33′15.3″ N, 16°47′53.2″ E	Kosiński; KOR
4	<i>R. bifrons</i> 4	Poland, Faliszówka	49°38′39.5″ N, 21°36′30.1″ E	Oklejewicz; KOR
5	<i>R. bifrons</i> 5	Poland, Pomorzowice	50°17′02.1″ N, 17°45′33.4″ E	Tomlik, Zieliński; KOR
6	<i>R. caesius</i> 1	Poland, Skomętno Wielkie	53°51′00.0″ N, 22°37′29.9″ E	Tomlik; KOR
7	<i>R. caesius</i> 2	Poland, Wiewiórczyn	51°36′24.9″ N, 19°07′23.9″ E	Stefanek; KOR
8	<i>R. caesius</i> 3	Poland, Pilchowice	50°12′57.8″ N, 18°33′49.3″ E	Tomlik, Zieliński; KOR
9	<i>R. caesius</i> 4	Poland, Piła-Młyn	53°30′52.0″ N, 17°53′17.0″ E	Stefanek; KOR
10	<i>R. caesius</i> 5	Poland, Dzierżysław	50°02′51.6″ N, 17°58′09.1″ E	Tomlik, Zieliński; KOR
11	<i>R. constrictus</i> 1	Poland, Zabkowice Śląskie	50°35′21.4″ N, 16°48′40.2″ E	Kosiński; KOR
12	<i>R. constrictus</i> 2	Poland, Staniszków	50°50′24.0″ N, 15°44′13.1″ E	Oklejewicz; KOR
13	<i>R. divaricatus</i> 1	Poland, Lubin	51°23′49.1″ N, 16°12′20.0″ E	Zieliński; KOR
14	<i>R. divaricatus</i> 2	Poland, Moryń	52°48′40.0″ N, 14°21′10.0″ E	Maliński; KOR
15	<i>R. divaricatus</i> 3	Poland, Antonin	51°30′59.1″ N, 17°51′16.9″ E	Zieliński; KOR
16	<i>R. divaricatus</i> 4	Germany, Wiednitz	51°23′14.1″ N, 14°01′45.6″ E	Hans-Werner; KOR
17	<i>R. gracilis</i> 1	Poland, Pątnów	51°08′47.3″ N, 18°37′29.3″ E	Zieliński; KOR
18	<i>R. gracilis</i> 2	Poland, Świdwin	53°46′28.1″ N, 15°46′38.8″ E	Kosiński; KOR
19	<i>R. gracilis</i> 3	Poland, Kutylły	50°34′51.5″ N, 22°15′23.5″ E	Oklejewicz; KOR
20	<i>R. gracilis</i> 4	Poland, Marcinów	50°23′06.7″ N, 16°41′19.9″ E	Kosiński; KOR
21	<i>R. gracilis</i> 5	Poland, Starkowo	51°58′37.8″ N, 16°18′35.7″ E	Zieliński; KOR
22	<i>R. henrici-egonis</i> 1	Poland, Zabkowice Śląskie	50°35′21.4″ N, 16°48′40.2″ E	Kosiński; KOR
23	<i>R. henrici-egonis</i> 2	Poland, Kłodzko	50°26′17.8″ N, 16°39′16.7″ E	Kosiński; KOR
24	<i>R. henrici-egonis</i> 3	Poland, Bardo	50°30′24.3″ N, 16°44′24.9″ E	Kosiński; KOR
25	<i>R. henrici-egonis</i> 4	Poland, Gorzyce	49°57′30.6″ N, 18°23′45.4″ E	Kosiński, Tomaszewski, Zieliński; KOR
26	<i>R. henrici-egonis</i> 5	Poland, Szklary	49°27′58.7″ N, 21°50′28.2″ E	Kosiński, Zieliński; KOR
27	<i>R. idaeus</i> 1	Poland, Pamiątkowo	52°33′15.1″ N, 16°40′56.9″ E	Tomlik, KOR
28	<i>R. idaeus</i> 2	Poland, Kórnik	52°14′57.4″ N, 17°05′24.5″ E	Zieliński, KOR
29	<i>R. idaeus</i> 3	Poland, Lubowidz	53°07′18.0″ N, 19°50′26.9″ E	Anisimowicz; KOR
30	<i>R. idaeus</i> 4	Poland, Pakosław	51°37′00.0″ N, 17°03′26.8″ E	Kortus; KOR
31	<i>R. idaeus</i> 5	Poland, Wąbrzeźno	53°16′46.7″ N, 18°56′52.0″ E	Stempola; KOR
32	<i>R. nessensis</i> 1	Poland, Koźielec	53°15′09.3″ N, 18°13′51.2″ E	Krawiec; KOR
33	<i>R. nessensis</i> 2	Poland, Wolsztyn	52°07′02.1″ N, 16°06′45.6″ E	Paczoski; KOR
34	<i>R. nessensis</i> 3	Poland, Prabuty	53°45′21.6″ N, 19°12′16.0″ E	Boratyński, Zieliński; KOR
35	<i>R. nessensis</i> 4	Poland, Wyspowo	54°33′51.0″ N, 18°18′15.9″ E	Markowski; KOR
36	<i>R. nessensis</i> 5	Poland, Brodnica	53°15′28.9″ N, 19°24′19.6″ E	Boratyński, Zieliński; KOR
37	<i>R. opacus</i> 1	Poland, Poźrzadło	52°18′30.0″ N, 15°15′30.0″ E	Zieliński; KOR
38	<i>R. opacus</i> 2	Poland, Starkowo	51°58′37.8″ N, 16°18′35.7″ E	Zieliński; KOR
39	<i>R. plicatus</i> 1	Poland, Karnocice	53°51′34.5″ N, 14°31′04.3″ E	Piotrowska; KOR
40	<i>R. plicatus</i> 2	Poland, Sarbsk	54°45′07.2″ N, 17°40′02.7″ E	Zieliński; KOR
41	<i>R. plicatus</i> 3	Poland, Karkonosze	50°46′35.2″ N, 15°36′18.9″ E	Boratyrscy; KOR
42	<i>R. plicatus</i> 4	Poland, Jastrzębia Góra	54°49′49.6″ N, 18°18′15.9″ E	Piotrowska; KOR
43	<i>R. plicatus</i> 5	Poland, Kamienna Góra	50°47′00.3″ N, 16°01′59.2″ E	Boratyński, Zieliński; KOR
44	<i>R. praecox</i> 1	Austria, Klausen-Leopoldsdorf	48°06′27.0″ N, 16°01′52.7″ E	Kosiński; KOR
45	<i>R. praecox</i> 2	Germany, Berlin	52°29′03.0″ N, 13°14′08.0″ E	Mackiewicz; KOR
46	<i>R. praecox</i> 3	Poland, Burkatów	50°47′42.1″ N, 16°27′59.6″ E	Boratyrski; KOR
47	<i>R. praecox</i> 4	Bulgaria, Blagoevgrad	42°00′29.9″ N, 23°05′28.5″ E	Boratyrski, Browicz, Zieliński; KOR
48	<i>R. radula</i> 1	Poland, Potakówka	49°42′47.2″ N, 21°36′06.7″ E	Oklejewicz; KOR
49	<i>R. radula</i> 2	Poland, Boszkowo	51°58′28.1″ N, 16°19′41.9″ E	Hantz; KOR
50	<i>R. radula</i> 3	Poland, Psary	51°33′44.2″ N, 21°37′09.9″ E	Danielewicz, Maliński, Zieliński; KOR
51	<i>R. radula</i> 4	Poland, Boguszyn	51°56′36.5″ N, 16°28′16.4″ E	Danielewicz, Maliński; KOR
52	<i>R. radula</i> 5	Poland, Postomino	51°58′28.1″ N, 16°19′41.9″ E	Zieliński; KOR
53	<i>R. saxatilis</i> 1	Germany, Wald	47°43′20.9″ N, 10°33′24.8″ E	Hoffmann, KOR
54	<i>R. saxatilis</i> 2	Poland, Promno	52°27′03.0″ N, 17°14′42.9″ E	Browicz; KOR
55	<i>R. saxatilis</i> 3	Poland, Lublin	51°15′00.8″ N, 22°34′19.3″ E	Fijałkowski; KOR

Table 2. Cont.

No.	Species	Localities	Geographical Coordinates	Collector, Herbarium
56	<i>R. saxatilis</i> 4	Poland, Błazejewo	54°13′45.6″ N, 22°18′19.5″ E	Bugała; KOR
57	<i>R. saxatilis</i> 5	Poland, Bodzewko	51°49′56.0″ N, 17°06′19.9″ E	Kaczmarek; KOR
58	<i>R. scissus</i> 1	Poland, Zielin	54°14′47.9″ N, 17°05′58.9″ E	Zieliński; KOR
59	<i>R. scissus</i> 2	Poland, Słowiński National Park	54°41′57.3″ N, 17°19′10.9″ E	Zieliński; KOR
60	<i>R. scissus</i> 3	Poland, Rudniki	51°02′13.0″ N, 18°36′01.6″ E	Zieliński; KOR
61	<i>R. sprengelii</i> 1	Poland, Wolin	53°50′36.0″ N, 14°36′57.1″ E	Zieliński; KOR
62	<i>R. sprengelii</i> 2	Poland, Ciechnowo	53°51′34.1″ N, 15°44′20.9″ E	Zieliński; KOR
63	<i>R. sprengelii</i> 3	Poland, Słowieńsko	53°51′56.9″ N, 15°38′24.7″ E	Zieliński; KOR
64	<i>R. sprengelii</i> 4	Poland, Kłoczewo	53°57′56.2″ N, 14°36′57.1″ E	Boratyńska, Dolatowska, Zieliński; KOR
65	<i>R. sprengelii</i> 5	Poland, Dobiegniew	52°58′05.5″ N, 15°45′17.4″ E	Boratyńska, Dolatowska, Zieliński; KOR
66	<i>R. sulcatus</i> 1	Poland, Bystrzyca Kłodzka	50°17′48.7″ N, 16°39′07.3″ E	Kosiński; KOR
67	<i>R. sulcatus</i> 2	Poland, Dydnia	49°41′15.6″ N, 22°10′16.8″ E	Oklejewicz; KOR
68	<i>R. sulcatus</i> 3	Poland, Poraż	49°29′05.5″ N, 22°12′55.5″ E	Oklejewicz; KOR
69	<i>R. sulcatus</i> 4	Poland, Jaśliska	49°26′29.9″ N, 21°48′08.5″ E	Kaczmarek; KOR
70	<i>R. sulcatus</i> 5	Poland, Słowik	50°49′54.4″ N, 20°32′20.3″ E	Kaznowski; KOR

The pollen grains were analyzed for nine quantitative characteristics: length of the polar axis (P) and equatorial diameter (E), length of the ectoaperture (Le), the distance between apices of two ectocolpi (d), thickness of the exine along the polar axis (Exp) as well as the P/E, Le/P, d/E and Exp/P ratios. The pollen shape classes (P/E ratio) were adopted according to the classification proposed by Erdtman (1952): oblatespheroidal (0.89–0.99), spheroidal (1.00), prolatespheroidal (1.01–1.14), subprolate (1.15–1.33) and prolate (1.34–2.00). In addition, the following qualitative characteristics were also determined: outline, shape, operculum structure and exine ornamentation.

Exine ornamentation types were identified based on the classification proposed by Ueda and Tomita [40]. The striate exine ornamentation types and subtypes were characterized by the height and width of grooves, width of striae, the number and diameter of perforations.

The palynological terminology adopted by Punt et al. [41] and Halbritter et al. [42].

The normality of distribution of the nine traits, e.g., P, Le, d, E, Exp, P/E, Le/P, d/E and Exp/P, was tested using Shapiro–Wilk’s normality test [43] to verify whether the analysis of variance (ANOVA) met the assumption that the ANOVA model residuals followed a normal distribution. The homogeneity of variance was tested using Bartlett’s test. Box’s M test tested multivariate normality and homogeneity of variance–covariance matrices. All the traits had a normal distribution. Multivariate analysis of variance (MANOVA) was performed based on the following model: $Y = XT + E$, where: Y is the $(n \times p)$ -dimensional matrix of observations, n is the number of all observations, p is the number of traits (in this study $p = 9$), X is the $(n \times k)$ -dimensional matrix of design, k is the number of pollen samples (in this study $k = 70$), T is the $(k \times p)$ -dimensional matrix of unknown effects, and E —the $(n \times p)$ -dimensional matrix of residuals. A one-way (pollen samples) MANOVA was performed. Next, one-way analysis of variance (ANOVA) was carried out to determine the effects of *Rubus* samples on the variability of P, Le, d, E, P/E and Le/P. The minimal, maximal and mean values as well as standard deviations of traits were calculated. Moreover, Fisher’s least significant differences (LSDs), at the 0.001 level, were calculated for individual traits and on this basis homogeneous groups were established. The relationships between the nine observed traits were estimated using Pearson’s linear correlation coefficients based on the means of *Rubus* species. Relationships of six observed traits were presented in a heatmap. The results were also analyzed using multivariate methods. A canonical variance analysis (CVA) was applied to present a multi-trait assessment of the similarity of the tested *Rubus* species in a lower number of dimensions with the least possible loss of information. The Mahalanobis distance was suggested as a measure of “polytrait” *Rubus* species similarity [44], the significance of which was verified by means of critical value D_α called “the least significant distance” [45]. Mahalanobis distances were calculated for all species samples. The differences between the analyzed species were verified by cluster analysis using the nearest neighbor method and Euclidean distances and presented as a

dendrogram. The GenStat v. 18 statistical software package (VSN International) was used for the analyses.

3. Results

3.1. General Morphological Description of Pollen

The results of MANOVA indicated that all studied *Rubus* samples were significantly different when investigated in terms of all nine quantitative traits jointly (Wilk's $\lambda = 0.1687$; $F = 6.42$; $p < 0.0001$). The analysis of variance for the nine quantitative traits [P ($F_{69;2030} = 17.62$), Le ($F_{69;2030} = 12.47$), d ($F_{69;2030} = 13.24$), E ($F_{69;2030} = 15.41$), Exp ($F_{69;2030} = 6.79$), P/E ($F_{69;2030} = 6.70$), Le/P ($F_{69;2030} = 3.51$), d/E ($F_{69;2030} = 10.96$) and Exp/P ($F_{69;2030} = 8.64$)] confirmed variability of the tested species at a significance level $\alpha = 0.001$ (Tables 3–5). The mean values and standard deviations for the observed traits indicated high variability among the tested samples, for which significant differences were found in terms of all the analyzed morphological traits (Tables 3–5). The density plots of P, Exe and P/E by *Rubus* species samples are presented in Figures 1–3, respectively. A description of pollen morphology of the *Rubus* species studied is given below and illustrated in electron micrographs (Figures 4–6). Morphological observations for the other quantitative traits of pollen grains are shown in Tables 3–5. Pollen grains of the *Rubus* species studied were monads, isopolar and tricolporate (Figures 4 and 5). In accordance with the pollen size classification by Erdtman [46] on the basis of the length of the polar axis (P), analyzed pollen grains were small- (10–25 μm ; 81.4%) or medium-sized (25.1–50 μm ; 18.6%). Pollen grains marked a small range of mean values for trait P, ranging from 20.54 to 26.76 μm .

The average length of the polar axis (P) was 23.70 μm (16.56–32.75) μm (Table 3). The smallest mean P was found for the pollen of *R. opacus* (20.54 μm), while the largest—for *R. radula* (26.76 μm) (Table 3). In the *R. opacus* sample, all measured pollen grains were small at a narrow range of polar axis length (17.13–26.54 μm). In turn, the longest pollen grains were found in *R. radula* (20.36–30.87 μm).

The equatorial diameter (E) was 20.57 (12.81–28.32) μm . The shortest mean equatorial diameter was recorded in the pollen of *R. divaricatus* (16.81 μm), while the longest was found in *R. caesius* (22.97 μm , Table 4).

The outline in the polar view was mainly circular with obtuse apices, rarely elliptic, whereas in the equatorial view, the outline was mostly elliptic, less often circular (Figures 4 and 5).

The mean P/E ratio was 1.16, ranging from 0.90 in *R. bifrons* to 1.65 in *R. henrici-egonis* (Table 4). On average the P/E ratio values were always above 1.00, ranging from 1.04 in *R. plicatus* to 1.26 in *R. divaricatus*. Pollen grains of the examined species were most frequently subprolate (42.9%–901 pollen grains) or prolatespheroidal (42.5%–893), rarely prolate (7.9%–166) and oblatespheroidal (6.7%–140).

The mean exine thickness was 1.09 μm (Exp) (with a range of 0.50–2.00 μm). The relative thickness of the exine (Exp/P ratio) averaged 0.05 (Exp/P—from 0.02 to 0.10).

The polar area index (PAI) or apocolpium index, i.e., the d/E ratio, averaged 0.14 (0.04–0.33). The lowest mean value of this ratio (0.04) was recorded in *R. bifrons*, while the highest (0.33)—in *R. caesius*.

Pollen grains usually have three apertures—colpori. The colpori were arranged meridionally, regularly, more or less evenly spaced and they were usually long, with a mean length of 20.04 (12.85–29.08) μm (Table 3). On average, the shortest colpori were found in *R. opacus* and *R. divaricatus* (17.07 μm), while the longest was recorded in *R. radula* (23.15 μm). On average, the length of the colporus (Le) constituted 85% of the polar axis length (P) and ranged from 78 to 89%. The colpori were narrow, linear or fusiform in outline. Their width was variable and usually greatest in the equatorial region. Sculpturing of ectocolpus membranes approached rugulate, rarely partly psilate. Colpus margins frequently had small undulations (Figure 4). In all the studied species a bridge was observed, crossing the colpus at the equator and dividing it into two parts, formed by two bulges of the ectexine that meet in the middle (Figure 5). The bulges were of the same or unequal length.

Table 3. Mean, minimal and maximal values as well as standard deviations (s.d.) for the length of the polar axis (P), length of ectoaperture (Le) and distance between apices of two ectocolpi (d).

Trait		P					Le					d				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
1	<i>R. bifrons_1</i>	25.53	a-e	19.69	30.33	2.206	21.22	c-j	18.28	25.97	2.039	2.36	t-zA	1	4	0.679
2	<i>R. bifrons_2</i>	24.84	b-i	22.14	27.86	1.474	20.89	c-m	17.93	24.58	1.709	2.968	f-w	2	5.2	0.807
3	<i>R. bifrons_3</i>	26.69	a	22.39	32.75	2.495	22.98	ab	18.8	29.08	2.511	2.842	h-x	1.5	4.13	0.702
4	<i>R. bifrons_4</i>	24.78	b-i	20.25	30.5	2.196	21.09	c-k	17.47	26.25	2.083	2.58	p-z	1.4	5.2	0.923
5	<i>R. bifrons_5</i>	25.33	a-f	22.01	30.85	2.185	21.31	c-j	16.67	26.83	2.235	2.531	q-zA	1.7	3.84	0.605
6	<i>R. caesius_1</i>	21.89	w-zABC	19.03	26.36	1.643	18.26	v-y	14.63	22.67	1.736	2.347	u-zA	1.17	3.8	0.672
7	<i>R. caesius_2</i>	26.15	ab	22.53	28.32	1.465	22.38	a-c	19.07	24.27	1.429	3.85	bc	2.35	5.8	0.964
8	<i>R. caesius_3</i>	25.72	a-c	22.02	29.33	1.932	21.75	a-d	17.18	26.52	2.346	3.77	b-d	2	6.3	1.102
9	<i>R. caesius_4</i>	25.58	a-e	23.32	26.91	0.945	21.03	c-k	18.07	24.19	1.474	4.553	a	3.04	6.22	0.829
10	<i>R. caesius_5</i>	23.68	g-v	19.45	26.75	1.85	19.83	g-v	15.87	23.15	2.047	4.075	ab	2.8	5.35	0.664
11	<i>R. constrictus_1</i>	23.89	f-u	19.08	27.58	2.264	20.1	e-s	15.19	24.23	2.401	3.283	c-o	1.9	6	1.079
12	<i>R. constrictus_2</i>	23.46	h-w	19.67	27.28	1.517	19.71	j-v	14.73	22.95	1.657	2.734	k-z	1.4	4.94	0.886
13	<i>R. divaricatus_1</i>	22.34	u-zA	19.17	24.74	1.361	19.15	p-y	15.4	22.08	1.513	1.834	A	1	3	0.464
14	<i>R. divaricatus_2</i>	21.01	BC	17.52	24.21	1.84	17.7	Y	14	20.36	1.803	2.066	zA	1.24	3.6	0.573
15	<i>R. divaricatus_3</i>	21.76	x-zABC	19.04	23.89	1.445	18.17	v-y	13.88	20.5	1.787	2.088	yzA	1.07	3.39	0.594
16	<i>R. divaricatus_4</i>	21.72	x-zABC	18.96	25.26	1.349	18.19	v-y	14.96	21.59	1.49	2.047	zA	1	4.02	0.678
17	<i>R. gracilis_1</i>	25.63	a-d	21.65	31.57	2.563	21.65	a-e	17.47	27.66	2.636	3.098	d-s	1.96	6.3	1.064
18	<i>R. gracilis_2</i>	23.7	g-v	20.69	26.27	1.431	20.33	d-s	17.45	23.08	1.587	2.581	o-z	1.55	5	0.834
19	<i>R. gracilis_3</i>	24.69	b-j	20.74	27.86	1.945	21.41	b-g	17.62	25.49	2.228	3	f-u	1.5	5.35	0.950
20	<i>R. gracilis_4</i>	22.74	o-z	20.28	25.19	1.221	19.29	m-y	16.37	21.85	1.265	2.442	r-zA	1.45	5	0.731
21	<i>R. gracilis_5</i>	25.46	a-f	21.63	29.79	2.163	21.23	c-j	16.47	25.93	2.301	2.72	k-z	1.6	4.9	0.859
22	<i>R. henrici-egonis_1</i>	22.67	r-A	19.41	27.25	1.846	18.84	r-y	14.75	23.28	1.788	2.266	w-zA	1.29	4.6	0.642
23	<i>R. henrici-egonis_2</i>	24.42	c-m	19.27	28.37	2.488	20.87	c-n	15.19	24.51	2.448	3.722	b-e	2.3	5.5	0.930
24	<i>R. henrici-egonis_3</i>	22.42	t-zA	19.31	27.41	2.162	18.91	q-y	15.06	26.36	2.442	2.151	x-zA	1.3	3.2	0.406
25	<i>R. henrici-egonis_4</i>	21.23	zABC	18.97	24.47	1.356	17.92	w-y	15.22	20.85	1.332	2.288	v-zA	0.94	4.8	0.864
26	<i>R. henrici-egonis_5</i>	22.7	q-zA	19.91	27.71	1.614	19.26	n-y	15.8	23.93	1.596	2.614	o-z	1.22	4.02	0.658
27	<i>R. idaeus_1</i>	22.16	v-zA	19.89	25.61	1.518	19.23	o-y	16.34	23.22	1.866	3.444	b-j	2	5.7	1.062
28	<i>R. idaeus_2</i>	24.62	b-l	22.19	26.72	1.288	20.32	d-s	16.14	22.88	1.504	4.053	ab	3.08	5.06	0.534
29	<i>R. idaeus_3</i>	22.81	n-z	18.07	26.18	2.123	19.15	p-y	12.85	21.78	1.992	3.076	d-s	2.05	4.6	0.671
30	<i>R. idaeus_4</i>	21.39	yzABC	16.56	24	2.293	18.39	t-y	14.02	21.23	2.121	3.321	c-n	2.03	4.21	0.669
31	<i>R. idaeus_5</i>	22.52	t-zA	19.07	26.17	1.588	19.12	p-y	14.63	22.89	1.673	3.542	b-h	2.5	4.75	0.569
32	<i>R. nessensis_1</i>	23.1	k-x	19.8	26.69	1.827	19.6	k-v	16.47	23.85	2.135	2.549	p-z	1	4.98	0.828

Table 3. Cont.

Trait		P					Le					d				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
33	<i>R. nessensis_2</i>	24	e-t	19.67	29.93	2.651	20.26	d-s	16.4	25.82	2.546	3.07	d-s	1.6	5.51	1.055
34	<i>R. nessensis_3</i>	25.63	a-d	22.19	31.45	2.488	21.34	c-i	17.74	26.26	2.168	2.597	o-z	1.3	6.13	0.970
35	<i>R. nessensis_4</i>	24.9	b-h	21.62	30.23	2.076	20.82	c-o	16.72	25.64	2.014	2.987	f-v	1.29	5.56	1.064
36	<i>R. nessensis_5</i>	24.31	c-o	21.41	26.82	1.539	20.34	d-s	17.8	24.28	1.769	2.44	r-zA	1.5	4.03	0.632
37	<i>R. opacus_1</i>	20.54	C	17.13	23.8	1.697	17.73	xy	15.58	20.9	1.371	2.147	x-zA	1.5	2.86	0.335
38	<i>R. opacus_2</i>	22.96	m-y	20.73	26.54	1.393	18.46	t-y	13.93	22.66	1.866	3.061	e-t	1.8	5.51	0.915
39	<i>R. plicatus_1</i>	23.11	j-x	18.84	26.5	1.913	19.75	i-v	16.05	23.34	1.837	2.638	m-z	1.56	4.24	0.652
40	<i>R. plicatus_2</i>	21.15	ABC	17	26	2.173	18.36	u-y	14.23	23.12	2.2	2.833	i-x	1.77	4.54	0.758
41	<i>R. plicatus_3</i>	24.29	c-p	21.25	26.62	1.425	19.74	i-v	17.11	21.98	1.23	3.138	d-r	1.5	6	1.206
42	<i>R. plicatus_4</i>	23.46	h-w	20.75	27.9	1.587	19.33	l-x	16.05	22.24	1.392	3.333	c-m	2	6.2	0.931
43	<i>R. plicatus_5</i>	22.7	q-zA	18.85	25.21	1.666	19.3	l-y	16.52	21.58	1.56	3.653	b-f	2	5.65	0.975
44	<i>R. praecox_1</i>	24.29	c-p	19.98	29.78	1.533	20.7	d-p	16.53	24.14	1.459	2.811	j-x	1.24	5.73	1.002
45	<i>R. praecox_2</i>	22.27	v-zAAB	18.68	25.14	1.576	18.87	r-y	16.11	21.4	1.439	3.384	b-l	2	5.52	0.734
46	<i>R. praecox_3</i>	23.04	l-x	21	26.01	1.453	19.78	h-v	17.23	23.14	1.427	2.7	l-z	1.87	4.97	0.673
47	<i>R. praecox_4</i>	25.36	a-f	21.97	29.66	2.275	21.77	a-d	18.56	26.1	2.129	2.979	f-v	2	5.09	0.694
48	<i>R. radula_1</i>	26.76	a	23.43	30.75	2.226	23.15	a	20.4	28.63	2.154	2.428	s-zA	1.32	3.66	0.639
49	<i>R. radula_2</i>	23.63	g-v	20.36	25.98	1.743	20.09	e-s	14.52	22.91	1.943	2.62	n-z	1.94	3.7	0.425
50	<i>R. radula_3</i>	25.63	a-d	21.66	30.87	2.267	21.29	c-j	15.15	26.36	2.525	3.153	c-q	1.5	6.25	1.208
51	<i>R. radula_4</i>	24.27	c-q	20.47	27.72	1.825	19.98	f-u	16.91	22.94	1.586	3.324	c-m	2.05	5.7	0.922
52	<i>R. radula_5</i>	25.05	b-g	22.04	27.21	1.436	21.25	c-j	17.95	24.22	1.838	2.153	x-zA	1.4	5.5	0.844
53	<i>R. saxatilis_1</i>	24.48	c-m	21.81	29.01	1.811	21.57	a-f	18.46	25.6	1.695	3.669	b-f	2.09	5.81	0.913
54	<i>R. saxatilis_2</i>	24.12	d-s	20.96	27.8	1.487	20	f-t	15.46	23.13	1.917	3.421	b-k	2.04	5.44	0.892
55	<i>R. saxatilis_3</i>	22.63	s-zA	18.41	25.3	1.643	19.24	o-y	14.58	21.83	1.659	3.345	c-l	1.99	5.54	0.865
56	<i>R. saxatilis_4</i>	22.22	v-zAAB	16.9	25.8	1.942	18.8	s-y	15.04	21.8	1.664	3.544	b-h	2	5.29	0.954
57	<i>R. saxatilis_5</i>	22.54	s-zAAB	19.63	27.91	1.732	19.15	p-y	15.86	23.4	1.749	3.239	c-p	2.29	4.53	0.590
58	<i>R. scissus_1</i>	22.73	p-zA	20.53	27.89	1.625	19.18	p-y	14.81	22.42	1.695	2.597	o-z	1.6	3.75	0.607
59	<i>R. scissus_2</i>	23.29	i-x	20.72	25.61	1.112	19.8	g-v	17.23	24.11	1.39	3.529	b-i	1.8	6.4	1.190
60	<i>R. scissus_3</i>	24.36	c-n	20.2	28.34	2.183	20.64	d-p	16.73	25.14	1.995	3.576	b-g	2	5.8	1.044
61	<i>R. sprengelii_1</i>	24.8	b-i	21.27	28.72	1.919	21.15	c-k	18.24	25.31	1.961	3.222	c-q	2	5.61	0.867
62	<i>R. sprengelii_2</i>	23.5	g-v	21.2	28.36	1.509	19.75	i-v	16.64	24.38	1.745	2.774	j-y	1.7	5	0.683
63	<i>R. sprengelii_3</i>	24.74	b-i	22	28.65	1.975	20.91	c-l	17.65	25.83	2.192	2.707	l-z	1.76	5.1	0.843
64	<i>R. sprengelii_4</i>	24.22	c-r	21.06	26.2	1.457	20.52	d-q	15.45	23.02	1.58	2.997	f-u	2	4.4	0.652

Table 3. Cont.

Trait		P					Le					d				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
65	<i>R. sprengelii</i> _5	23.58	g-v	19.9	26.43	1.69	20.45	d-r	16.9	23.55	1.83	2.9	g-w	2	5.5	0.863
66	<i>R. sulcatus</i> _1	24.34	c-n	20.38	31.02	2.734	20.41	d-s	16.8	25.21	2.285	3.011	f-u	2	5.73	0.911
67	<i>R. sulcatus</i> _2	22.46	t-zAB	17.25	26.04	1.859	19.31	l-y	14.38	22.76	1.931	2.696	l-z	2	3.99	0.598
68	<i>R. sulcatus</i> _3	23.1	k-x	20	28.15	2.2	19.55	k-v	15	25.37	2.161	2.78	j-y	1.5	4.33	0.799
69	<i>R. sulcatus</i> _4	23.09	k-x	19.07	27.31	2.234	19.38	l-w	15.8	23.36	2.148	2.927	g-w	1.51	4.8	0.979
70	<i>R. sulcatus</i> _5	24.66	b-k	22.02	27.72	1.45	21.38	b-h	17.99	25.2	1.668	2.734	k-z	1.45	4.72	0.786
LSD _{0.001}		1.581					1.618					0.702				
F-ANOVA		17.62 ***					12.47 ***					13.24 ***				

*** $p < 0.001$; in the column means followed by the same letters are not significantly different.

Table 4. Mean, minimal and maximal values as well as standard deviations (s.d.) for equatorial diameter (E), exine thickness along the polar axis (Exp) and P/E.

Trait		E					Exp					P/E				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
1	<i>R. bifrons</i> _1	21.4	a-m	16.21	24.92	2.167	1.058	g-q	0.7	1.5	0.163	1.204	a-l	0.895	1.530	0.150
2	<i>R. bifrons</i> _2	21.54	a-k	18.44	25.2	1.771	1.06	g-q	0.8	1.38	0.141	1.159	d-s	1.025	1.390	0.091
3	<i>R. bifrons</i> _3	22.36	a-e	18.47	25.84	1.956	1.165	a-i	1	1.705	0.167	1.196	a-m	1.058	1.404	0.087
4	<i>R. bifrons</i> _4	21.35	b-n	16.37	26.53	2.419	1.073	f-q	1	1.21	0.082	1.171	a-r	1.009	1.494	0.132
5	<i>R. bifrons</i> _5	21.01	c-q	17	24.23	1.775	0.99	l-q	0.8	1.2	0.109	1.213	a-i	0.979	1.450	0.134
6	<i>R. caesius</i> _1	18.39	x-z	14	20.82	1.498	1.096	e-q	1	1.3	0.093	1.198	a-m	1.006	1.596	0.136
7	<i>R. caesius</i> _2	22.92	ab	19.08	25.05	1.471	1.15	a-j	0.8	1.5	0.250	1.143	g-t	1.044	1.357	0.063
8	<i>R. caesius</i> _3	22.97	a	18.63	27.13	2.007	1.071	f-q	1	1.3	0.105	1.124	i-u	0.980	1.397	0.094
9	<i>R. caesius</i> _4	21.77	a-j	19.52	23.93	1.201	1.067	g-q	0.8	1.44	0.152	1.177	a-q	1.068	1.308	0.061
10	<i>R. caesius</i> _5	22.22	a-f	17.8	24.55	1.469	1.048	h-q	1	1.2	0.064	1.068	tu	0.926	1.208	0.079
11	<i>R. constrictus</i> _1	21.32	b-n	17.2	25.57	2.31	1.068	g-q	1	1.2	0.071	1.128	i-t	0.931	1.433	0.121
12	<i>R. constrictus</i> _2	20.18	j-w	15.98	24.34	1.983	1.131	b-l	1	1.43	0.157	1.168	b-r	1.029	1.370	0.079
13	<i>R. divaricatus</i> _1	18.93	v-y	14.99	22.3	1.674	1.225	a-e	0.8	1.53	0.214	1.187	a-n	1.029	1.500	0.104
14	<i>R. divaricatus</i> _2	16.81	z	12.81	21.19	1.768	1.032	i-q	1	1.2	0.065	1.26	a	1.055	1.527	0.144
15	<i>R. divaricatus</i> _3	19.27	s-y	15.45	24.57	1.9	1.109	c-p	1	1.5	0.138	1.139	g-t	0.955	1.456	0.130

Table 4. Cont.

Trait		E					Exp					P/E				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
16	<i>R. divaricatus_4</i>	19.15	t-y	17.08	23.03	1.546	1.185	a-h	0.8	1.5	0.227	1.138	g-t	0.959	1.369	0.078
17	<i>R. gracilis_1</i>	22.08	a-g	16.57	25.72	2.003	1.08	e-q	0.8	1.3	0.161	1.167	b-s	1.000	1.462	0.127
18	<i>R. gracilis_2</i>	20.7	f-t	18.7	22.81	1.186	1.053	h-q	0.8	2	0.229	1.147	f-t	1.016	1.346	0.084
19	<i>R. gracilis_3</i>	21.43	a-l	17.79	24.5	2.043	1.063	g-q	0.9	1.2	0.103	1.157	d-t	1.003	1.366	0.089
20	<i>R. gracilis_4</i>	18.23	yz	16.39	20.62	1.157	1.098	e-q	0.8	1.5	0.187	1.252	ab	1.074	1.422	0.093
21	<i>R. gracilis_5</i>	21.3	c-n	19.38	25.09	1.34	1.169	a-i	0.99	1.52	0.151	1.196	a-m	1.038	1.372	0.084
22	<i>R. henrici-egonis_1</i>	18.64	w-y	16.33	22.3	1.414	1.057	g-q	0.9	1.23	0.086	1.219	a-h	1.054	1.547	0.098
23	<i>R. henrici-egonis_2</i>	22.53	a-c	16.36	25.71	2.088	1.135	a-l	1	1.4	0.137	1.086	r-u	0.985	1.241	0.074
24	<i>R. henrici-egonis_3</i>	19.13	t-y	14.88	22.56	1.89	1.083	e-q	0.99	1.8	0.150	1.179	a-p	1.001	1.546	0.130
25	<i>R. henrici-egonis_4</i>	18.91	v-y	15.21	23.09	1.832	1.202	a-g	0.69	1.75	0.260	1.128	i-t	0.993	1.260	0.075
26	<i>R. henrici-egonis_5</i>	19.12	t-y	13.8	22.42	1.977	0.993	l-q	0.75	1.2	0.099	1.201	a-l	0.956	1.650	0.161
27	<i>R. idaeus_1</i>	20.13	k-w	13.88	23.47	2.83	0.982	n-q	0.8	1.21	0.118	1.121	k-u	0.970	1.443	0.163
28	<i>R. idaeus_2</i>	22.46	a-d	21.08	24.1	0.86	1.181	a-h	0.9	2	0.287	1.097	n-u	0.953	1.182	0.058
29	<i>R. idaeus_3</i>	19.43	q-y	14.35	22.18	1.847	1.05	h-q	0.7	1.3	0.168	1.183	a-o	0.910	1.477	0.146
30	<i>R. idaeus_4</i>	19.51	p-y	13.87	25.1	2.786	1.141	a-k	0.8	1.35	0.143	1.105	n-u	0.909	1.225	0.080
31	<i>R. idaeus_5</i>	20.77	e-s	16.98	25.1	1.543	1.088	e-q	1	1.36	0.116	1.088	q-u	0.937	1.387	0.095
32	<i>R. nessensis_1</i>	19.00	u-y	14.48	22.35	1.833	1.135	a-l	0.87	1.444	0.171	1.223	a-g	1.010	1.595	0.121
33	<i>R. nessensis_2</i>	20.11	k-w	16.75	25.18	2.066	1.042	h-q	0.9	1.2	0.068	1.198	a-m	0.968	1.451	0.117
34	<i>R. nessensis_3</i>	20.8	e-s	17.39	27.07	2.134	1.052	h-q	0.78	1.23	0.124	1.235	a-f	1.058	1.381	0.076
35	<i>R. nessensis_4</i>	20.63	f-t	16.36	23.66	1.854	1.046	h-q	0.72	1.44	0.175	1.211	a-k	1.104	1.485	0.094
36	<i>R. nessensis_5</i>	19.79	m-y	15.51	23.64	1.93	1.277	a	0.9	1.5	0.210	1.241	a-d	0.988	1.516	0.150
37	<i>R. opacus_1</i>	16.88	z	14.16	19.63	1.697	0.966	pq	0.8	1.2	0.124	1.223	a-g	1.019	1.452	0.111
38	<i>R. opacus_2</i>	18.61	w-y	15.48	21.56	1.489	1.269	ab	0.9	1.5	0.256	1.24	a-e	1.030	1.421	0.118
39	<i>R. plicatus_1</i>	21.3	c-n	19.13	24.98	1.639	1.094	e-q	1	1.5	0.116	1.087	q-u	0.932	1.264	0.075
40	<i>R. plicatus_2</i>	18.97	u-y	15.12	26	2.786	1.222	a-e	0.78	1.75	0.313	1.125	i-u	0.920	1.329	0.099
41	<i>R. plicatus_3</i>	21.79	a-j	18.29	24.5	1.737	1.124	b-n	0.5	1.5	0.295	1.118	l-u	0.970	1.287	0.076
42	<i>R. plicatus_4</i>	21.25	c-n	17.03	25.24	1.973	1.128	b-m	0.5	1.63	0.302	1.109	m-u	1.002	1.483	0.087
43	<i>R. plicatus_5</i>	21.95	a-h	17.02	25.61	1.762	0.963	q	0.6	1.2	0.122	1.036	u	0.908	1.165	0.064
44	<i>R. praecox_1</i>	21.67	a-k	19.49	27.44	1.74	1.236	a-d	0.78	1.62	0.267	1.125	i-u	0.984	1.296	0.085
45	<i>R. praecox_2</i>	19.53	o-y	15.56	23.03	1.973	0.982	n-q	0.8	1.3	0.116	1.15	e-t	0.971	1.458	0.131
46	<i>R. praecox_3</i>	20.44	h-v	15.91	26.05	2.414	1.029	i-q	0.8	1.2	0.111	1.137	g-t	0.994	1.412	0.099
47	<i>R. praecox_4</i>	21.9	a-i	16.07	28.32	2.514	1.027	i-q	0.9	1.2	0.079	1.168	b-r	1.001	1.518	0.132

Table 4. Cont.

Trait		E					Exp					P/E				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
48	<i>R. radula_1</i>	22.14	a-g	19.19	25.07	1.708	1.202	a-g	0.75	1.63	0.281	1.212	a-j	1.006	1.404	0.101
49	<i>R. radula_2</i>	20.58	g-u	16.54	25.56	1.753	0.963	q	0.8	1.2	0.127	1.156	d-t	0.960	1.556	0.133
50	<i>R. radula_3</i>	21.84	a-i	18.16	26.07	1.839	1.071	f-q	1	1.22	0.073	1.177	a-q	1.016	1.390	0.099
51	<i>R. radula_4</i>	21.11	c-p	16.84	24.45	2.024	1.247	a-c	0.9	1.9	0.265	1.155	d-t	1.001	1.385	0.097
52	<i>R. radula_5</i>	21.82	a-i	18.65	25.25	1.834	1.1	d-q	1	1.4	0.124	1.154	d-t	0.968	1.425	0.103
53	<i>R. saxatilis_1</i>	20.31	i-v	17.11	22.31	1.162	0.977	o-q	0.8	1.2	0.107	1.207	a-l	1.049	1.385	0.085
54	<i>R. saxatilis_2</i>	20.86	d-s	18.41	23.34	1.297	1.153	a-i	0.5	1.52	0.266	1.16	c-s	0.998	1.325	0.091
55	<i>R. saxatilis_3</i>	20.83	e-s	16.08	23.15	1.914	1.12	c-o	0.72	1.82	0.228	1.093	o-u	0.915	1.365	0.111
56	<i>R. saxatilis_4</i>	19.77	n-y	16.15	23.44	1.757	1.007	j-q	0.7	1.2	0.148	1.13	h-t	0.952	1.456	0.127
57	<i>R. saxatilis_5</i>	19.37	r-y	16.54	21.7	1.427	1.047	h-q	0.9	1.6	0.144	1.168	b-r	0.997	1.343	0.104
58	<i>R. scissus_1</i>	20.94	c-r	17.99	25.58	2.15	1.033	i-q	0.85	1.25	0.125	1.091	p-u	0.955	1.291	0.081
59	<i>R. scissus_2</i>	21.68	a-k	19.03	25.13	1.49	1.096	e-q	0.9	1.2	0.100	1.077	s-u	0.993	1.215	0.057
60	<i>R. scissus_3</i>	21.13	c-o	17.15	25.9	1.929	1.068	g-q	0.98	1.2	0.074	1.159	d-s	0.989	1.480	0.116
61	<i>R. sprengelii_1</i>	21.08	c-p	17.86	24.54	1.707	1.216	a-f	0.9	1.5	0.223	1.181	a-p	1.003	1.365	0.096
62	<i>R. sprengelii_2</i>	20.72	f-t	16.73	26.34	2.091	0.993	l-q	0.8	1.2	0.129	1.141	g-t	1.009	1.322	0.094
63	<i>R. sprengelii_3</i>	19.92	l-x	16.3	25.34	2.227	1.149	a-j	0.99	1.4	0.119	1.25	a-c	1.116	1.468	0.104
64	<i>R. sprengelii_4</i>	21	c-q	16.84	25.42	2.344	0.997	k-q	0.7	1.3	0.177	1.163	b-s	0.965	1.373	0.108
65	<i>R. sprengelii_5</i>	20.93	c-r	19.1	23.76	1.287	0.985	m-q	0.8	1.11	0.095	1.127	i-t	1.015	1.227	0.066
66	<i>R. sulcatus_1</i>	21.65	a-k	17.38	25.91	2.348	1.104	c-q	0.78	1.333	0.155	1.127	i-t	1.002	1.304	0.082
67	<i>R. sulcatus_2</i>	18.37	x-z	14.57	25.47	2.524	1	k-q	0.8	1.2	0.105	1.239	a-e	1.000	1.614	0.164
68	<i>R. sulcatus_3</i>	21.33	b-n	18.46	24.1	1.474	1.044	h-q	0.78	1.35	0.166	1.085	r-u	0.897	1.229	0.091
69	<i>R. sulcatus_4</i>	20.66	f-t	16.72	24.29	2.096	1.26	ab	0.88	1.75	0.215	1.122	j-u	0.966	1.324	0.094
70	<i>R. sulcatus_5</i>	21.42	a-l	17.79	24.77	1.855	1.098	e-q	1	1.3	0.111	1.157	d-t	1.031	1.429	0.091
LSD _{0.001}		1.61					0.145					0.09				
F-ANOVA		15.41 ***					6.79 ***					6.70 ***				

*** $p < 0.001$; in column means followed by the same letters are not significantly different.

Table 5. Mean, minimal and maximal values as well as standard deviations (s.d.) for Le/P, d/E and Exp/P.

Trait		Le/P					d/E					Exp/P				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	in	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
1	<i>R. bifrons_1</i>	0.832	c-k	0.748	0.928	0.043	0.111	t-x	0.044	0.164	0.031	0.042	n-r	0.030	0.063	0.007
2	<i>R. bifrons_2</i>	0.841	b-j	0.762	0.922	0.042	0.137	h-w	0.091	0.206	0.031	0.043	l-r	0.031	0.058	0.007
3	<i>R. bifrons_3</i>	0.860	a-e	0.811	0.941	0.031	0.127	n-x	0.076	0.181	0.028	0.044	i-r	0.033	0.066	0.009
4	<i>R. bifrons_4</i>	0.851	a-i	0.698	0.949	0.049	0.120	r-x	0.068	0.221	0.037	0.044	k-r	0.035	0.053	0.005
5	<i>R. bifrons_5</i>	0.841	b-j	0.731	0.933	0.043	0.121	r-x	0.073	0.195	0.030	0.039	r	0.032	0.051	0.005
6	<i>R. caesius_1</i>	0.834	b-k	0.743	0.920	0.044	0.129	n-x	0.065	0.234	0.039	0.050	b-k	0.040	0.067	0.006
7	<i>R. caesius_2</i>	0.856	a-i	0.806	0.909	0.026	0.168	b-h	0.098	0.247	0.043	0.044	j-r	0.031	0.067	0.010
8	<i>R. caesius_3</i>	0.845	b-j	0.718	0.919	0.049	0.165	b-k	0.100	0.329	0.051	0.042	n-r	0.034	0.053	0.005
9	<i>R. caesius_4</i>	0.822	i-k	0.704	0.901	0.045	0.210	A	0.148	0.275	0.039	0.042	n-r	0.031	0.062	0.007
10	<i>R. caesius_5</i>	0.836	b-k	0.758	0.899	0.035	0.184	ab	0.121	0.278	0.033	0.045	i-r	0.037	0.058	0.005
11	<i>R. constrictus_1</i>	0.841	b-j	0.751	0.955	0.050	0.155	b-q	0.084	0.265	0.050	0.045	h-r	0.037	0.058	0.005
12	<i>R. constrictus_2</i>	0.840	b-j	0.701	0.907	0.043	0.136	i-w	0.068	0.210	0.041	0.048	d-n	0.040	0.063	0.007
13	<i>R. divaricatus_1</i>	0.857	a-h	0.685	0.916	0.046	0.097	x	0.056	0.151	0.024	0.055	a-d	0.037	0.074	0.011
14	<i>R. divaricatus_2</i>	0.843	b-j	0.692	0.918	0.044	0.123	q-x	0.077	0.229	0.034	0.049	c-l	0.041	0.057	0.005
15	<i>R. divaricatus_3</i>	0.834	b-k	0.713	0.909	0.045	0.109	v-x	0.061	0.210	0.033	0.051	b-i	0.043	0.077	0.007
16	<i>R. divaricatus_4</i>	0.837	b-k	0.789	0.902	0.029	0.107	wx	0.055	0.179	0.032	0.055	a-e	0.036	0.070	0.010
17	<i>R. gracilis_1</i>	0.844	b-j	0.764	0.909	0.041	0.140	f-v	0.079	0.271	0.045	0.042	m-r	0.030	0.055	0.007
18	<i>R. gracilis_2</i>	0.858	a-g	0.769	1.008	0.049	0.125	o-x	0.079	0.237	0.041	0.044	i-r	0.033	0.077	0.009
19	<i>R. gracilis_3</i>	0.866	ab	0.797	0.947	0.042	0.141	f-v	0.062	0.233	0.043	0.043	l-r	0.033	0.053	0.005
20	<i>R. gracilis_4</i>	0.848	a-i	0.807	0.909	0.027	0.134	k-w	0.077	0.267	0.039	0.049	c-n	0.035	0.073	0.009
21	<i>R. gracilis_5</i>	0.833	b-k	0.742	0.905	0.045	0.128	n-x	0.077	0.234	0.039	0.046	g-r	0.035	0.059	0.006
22	<i>R. henrici-egonis_1</i>	0.831	d-k	0.738	0.895	0.039	0.121	r-x	0.069	0.206	0.029	0.047	f-q	0.037	0.058	0.006
23	<i>R. henrici-egonis_2</i>	0.854	a-i	0.770	0.929	0.037	0.167	b-j	0.102	0.245	0.045	0.047	g-q	0.037	0.068	0.007
24	<i>R. henrici-egonis_3</i>	0.842	b-j	0.771	0.991	0.048	0.113	s-x	0.071	0.156	0.019	0.049	c-n	0.036	0.078	0.008
25	<i>R. henrici-egonis_4</i>	0.844	b-j	0.766	0.898	0.033	0.121	r-x	0.053	0.233	0.045	0.057	ab	0.033	0.080	0.012
26	<i>R. henrici-egonis_5</i>	0.848	a-i	0.772	0.914	0.034	0.137	h-w	0.068	0.200	0.031	0.044	j-r	0.032	0.055	0.006
27	<i>R. idaeus_1</i>	0.866	ab	0.796	0.927	0.039	0.170	b-g	0.112	0.247	0.038	0.044	i-r	0.035	0.058	0.005
28	<i>R. idaeus_2</i>	0.825	f-k	0.727	0.886	0.037	0.181	a-c	0.139	0.228	0.023	0.048	e-o	0.037	0.088	0.012
29	<i>R. idaeus_3</i>	0.840	b-j	0.711	0.912	0.038	0.158	b-n	0.105	0.242	0.031	0.046	g-r	0.033	0.057	0.007
30	<i>R. idaeus_4</i>	0.860	a-e	0.750	0.931	0.041	0.171	b-f	0.111	0.221	0.028	0.054	a-f	0.037	0.069	0.009

Table 5. Cont.

Trait		Le/P					d/E					Exp/P				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	in	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
31	<i>R. idaeus_5</i>	0.849	a-i	0.766	0.931	0.040	0.171	b-f	0.127	0.226	0.027	0.049	c-n	0.038	0.069	0.007
32	<i>R. nessensis_1</i>	0.847	b-j	0.744	0.930	0.046	0.134	k-w	0.057	0.268	0.042	0.049	c-m	0.034	0.068	0.007
33	<i>R. nessensis_2</i>	0.844	b-j	0.748	0.919	0.043	0.152	c-r	0.093	0.258	0.046	0.044	k-r	0.033	0.056	0.005
34	<i>R. nessensis_3</i>	0.833	b-k	0.679	0.924	0.046	0.124	p-x	0.058	0.226	0.039	0.041	o-r	0.028	0.054	0.005
35	<i>R. nessensis_4</i>	0.836	b-k	0.759	0.917	0.035	0.144	e-s	0.062	0.244	0.047	0.042	m-r	0.027	0.060	0.008
36	<i>R. nessensis_5</i>	0.836	b-k	0.754	0.922	0.038	0.125	o-x	0.067	0.207	0.036	0.053	a-g	0.034	0.070	0.010
37	<i>R. opacus_1</i>	0.864	a-d	0.800	0.931	0.030	0.128	n-x	0.097	0.163	0.018	0.047	f-p	0.034	0.061	0.007
38	<i>R. opacus_2</i>	0.803	k	0.671	0.878	0.047	0.164	b-l	0.101	0.277	0.045	0.055	a-c	0.037	0.072	0.011
39	<i>R. plicatus_1</i>	0.855	a-i	0.774	0.897	0.030	0.123	q-x	0.081	0.193	0.026	0.048	e-p	0.038	0.064	0.007
40	<i>R. plicatus_2</i>	0.867	ab	0.783	0.946	0.032	0.150	c-r	0.081	0.250	0.038	0.059	a	0.036	0.099	0.019
41	<i>R. plicatus_3</i>	0.814	jk	0.700	0.906	0.046	0.145	e-r	0.082	0.298	0.059	0.046	g-q	0.021	0.071	0.013
42	<i>R. plicatus_4</i>	0.825	g-k	0.755	0.889	0.033	0.157	b-o	0.106	0.255	0.039	0.048	d-n	0.021	0.072	0.013
43	<i>R. plicatus_5</i>	0.851	a-i	0.771	0.918	0.039	0.167	b-j	0.082	0.263	0.043	0.043	l-r	0.027	0.055	0.006
44	<i>R. praecox_1</i>	0.853	a-i	0.784	0.938	0.041	0.128	n-x	0.057	0.210	0.039	0.051	b-j	0.033	0.066	0.011
45	<i>R. praecox_2</i>	0.848	a-i	0.778	0.935	0.039	0.173	b-e	0.106	0.240	0.033	0.044	i-r	0.033	0.067	0.006
46	<i>R. praecox_3</i>	0.859	a-g	0.751	0.905	0.034	0.132	l-w	0.093	0.218	0.028	0.045	h-r	0.035	0.057	0.005
47	<i>R. praecox_4</i>	0.859	a-f	0.753	0.918	0.035	0.136	h-w	0.087	0.205	0.028	0.041	p-r	0.034	0.053	0.005
48	<i>R. radula_1</i>	0.865	a-c	0.788	0.935	0.031	0.110	u-x	0.058	0.170	0.028	0.045	h-r	0.027	0.066	0.011
49	<i>R. radula_2</i>	0.850	a-i	0.696	0.925	0.047	0.128	n-x	0.099	0.178	0.021	0.041	p-r	0.032	0.052	0.006
50	<i>R. radula_3</i>	0.830	e-k	0.699	0.912	0.048	0.143	e-s	0.083	0.283	0.052	0.042	n-r	0.035	0.051	0.004
51	<i>R. radula_4</i>	0.824	h-k	0.760	0.886	0.026	0.156	b-p	0.102	0.233	0.033	0.052	b-h	0.033	0.082	0.011
52	<i>R. radula_5</i>	0.848	a-j	0.750	0.908	0.039	0.098	x	0.061	0.223	0.034	0.044	j-r	0.037	0.059	0.006
53	<i>R. saxatilis_1</i>	0.881	a	0.831	0.939	0.029	0.181	a-c	0.119	0.290	0.044	0.040	qr	0.031	0.048	0.005
54	<i>R. saxatilis_2</i>	0.829	e-k	0.704	0.998	0.057	0.164	b-l	0.087	0.247	0.040	0.048	e-o	0.018	0.068	0.012
55	<i>R. saxatilis_3</i>	0.850	a-i	0.775	0.923	0.034	0.161	b-m	0.105	0.250	0.042	0.050	c-l	0.031	0.077	0.010
56	<i>R. saxatilis_4</i>	0.847	b-j	0.712	0.902	0.040	0.179	a-d	0.093	0.279	0.044	0.045	h-r	0.035	0.058	0.005
57	<i>R. saxatilis_5</i>	0.849	a-i	0.754	0.914	0.037	0.168	b-i	0.118	0.231	0.030	0.047	g-q	0.036	0.069	0.007
58	<i>R. scissus_1</i>	0.844	b-j	0.721	0.960	0.045	0.125	o-x	0.080	0.175	0.029	0.046	h-r	0.032	0.059	0.007
59	<i>R. scissus_2</i>	0.850	a-i	0.753	0.941	0.039	0.164	b-l	0.077	0.305	0.056	0.047	f-p	0.038	0.057	0.005
60	<i>R. scissus_3</i>	0.847	b-j	0.774	0.894	0.032	0.169	b-g	0.089	0.250	0.047	0.044	i-r	0.035	0.059	0.005

Table 5. Cont.

Trait		Le/P					d/E					Exp/P				
No.	Species	Mean	Homogeneous Groups	Min	Max	s.d.	Mean	Homogeneous Groups	in	Max	s.d.	Mean	Homogeneous Groups	Min	Max	s.d.
61	<i>R. sprengelii</i> _1	0.853	a-i	0.780	0.940	0.035	0.152	b-r	0.093	0.250	0.036	0.049	c-m	0.034	0.064	0.009
62	<i>R. sprengelii</i> _2	0.840	b-j	0.695	0.928	0.048	0.135	j-w	0.084	0.230	0.035	0.042	m-r	0.031	0.052	0.005
63	<i>R. sprengelii</i> _3	0.844	b-j	0.765	0.917	0.038	0.136	i-w	0.084	0.253	0.039	0.047	g-q	0.037	0.059	0.006
64	<i>R. sprengelii</i> _4	0.847	a-j	0.734	0.931	0.038	0.143	e-t	0.100	0.220	0.029	0.041	o-r	0.028	0.058	0.007
65	<i>R. sprengelii</i> _5	0.867	ab	0.806	0.915	0.030	0.138	g-w	0.095	0.255	0.038	0.042	n-r	0.033	0.055	0.005
66	<i>R. sulcatus</i> _1	0.840	b-j	0.751	0.917	0.041	0.139	g-w	0.085	0.227	0.035	0.046	h-r	0.031	0.064	0.008
67	<i>R. sulcatus</i> _2	0.859	a-f	0.798	0.916	0.031	0.148	d-r	0.104	0.222	0.030	0.045	h-r	0.038	0.056	0.005
68	<i>R. sulcatus</i> _3	0.846	b-j	0.731	0.917	0.043	0.131	m-w	0.068	0.220	0.037	0.046	h-r	0.032	0.065	0.009
69	<i>R. sulcatus</i> _4	0.839	b-j	0.769	0.905	0.037	0.141	f-u	0.077	0.242	0.043	0.055	a-d	0.038	0.092	0.012
70	<i>R. sulcatus</i> _5	0.867	ab	0.786	0.935	0.034	0.128	n-x	0.072	0.210	0.036	0.045	h-r	0.036	0.056	0.006
LSD _{0.001}		0.034					0.032					0.007				
F-ANOVA		3.51 ***					10.96 ***					8.64 ***				

*** $p < 0.001$; in the column means followed by the same letters are not significantly different.

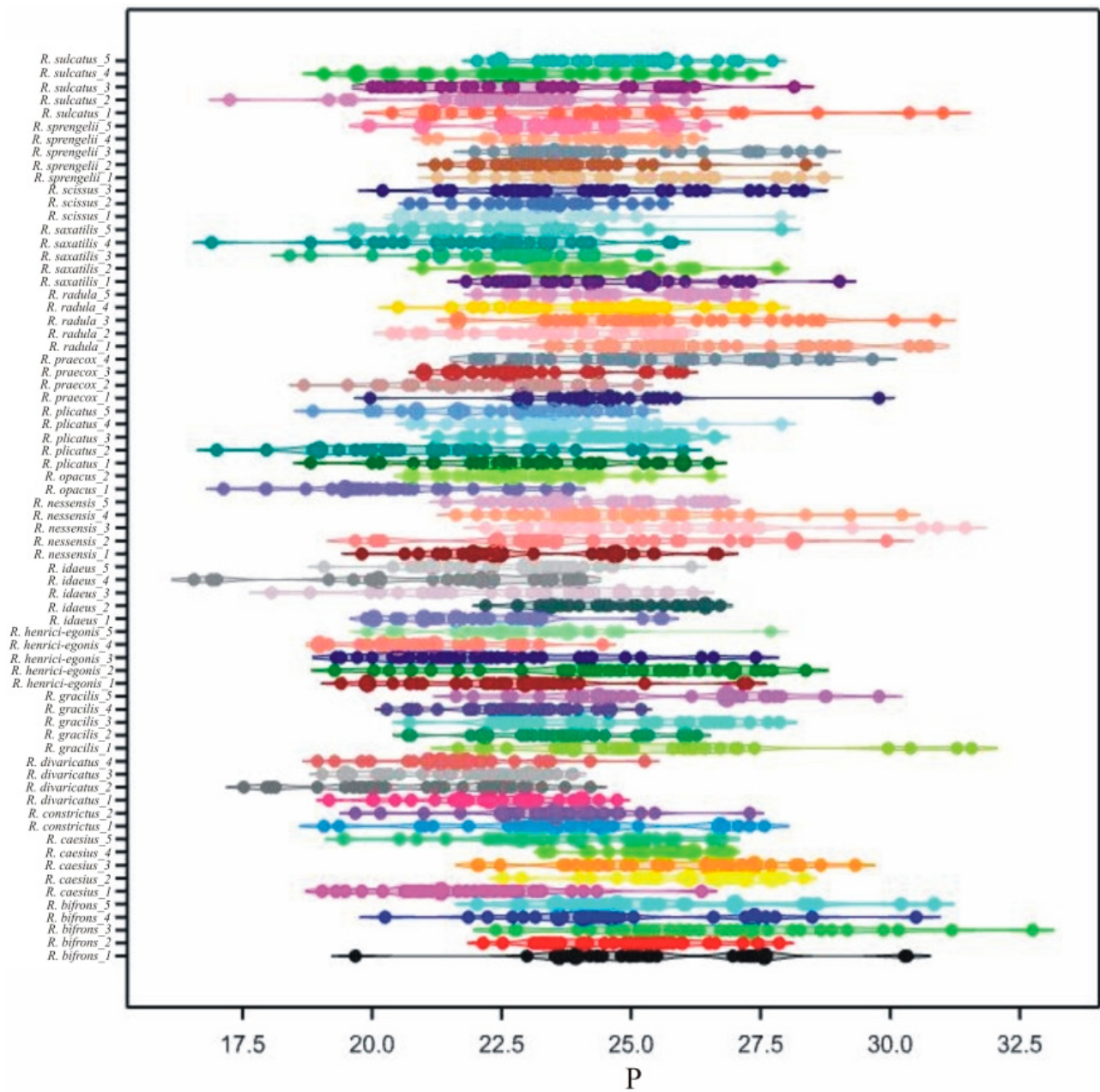


Figure 1. The density plot of P trait for *Rubus* species. The point “●” indicates trait observation for individual species.

Exine ornamentation in all the studied species was striate-perforate (Figure 6). Exine ornamentation elements were variable (Figure 6). Striae and grooves usually ran parallel to colpi and the polar axis, but frequently they also formed fingerprint-like twists. Striae were straight or forked and of varying length, width and height.

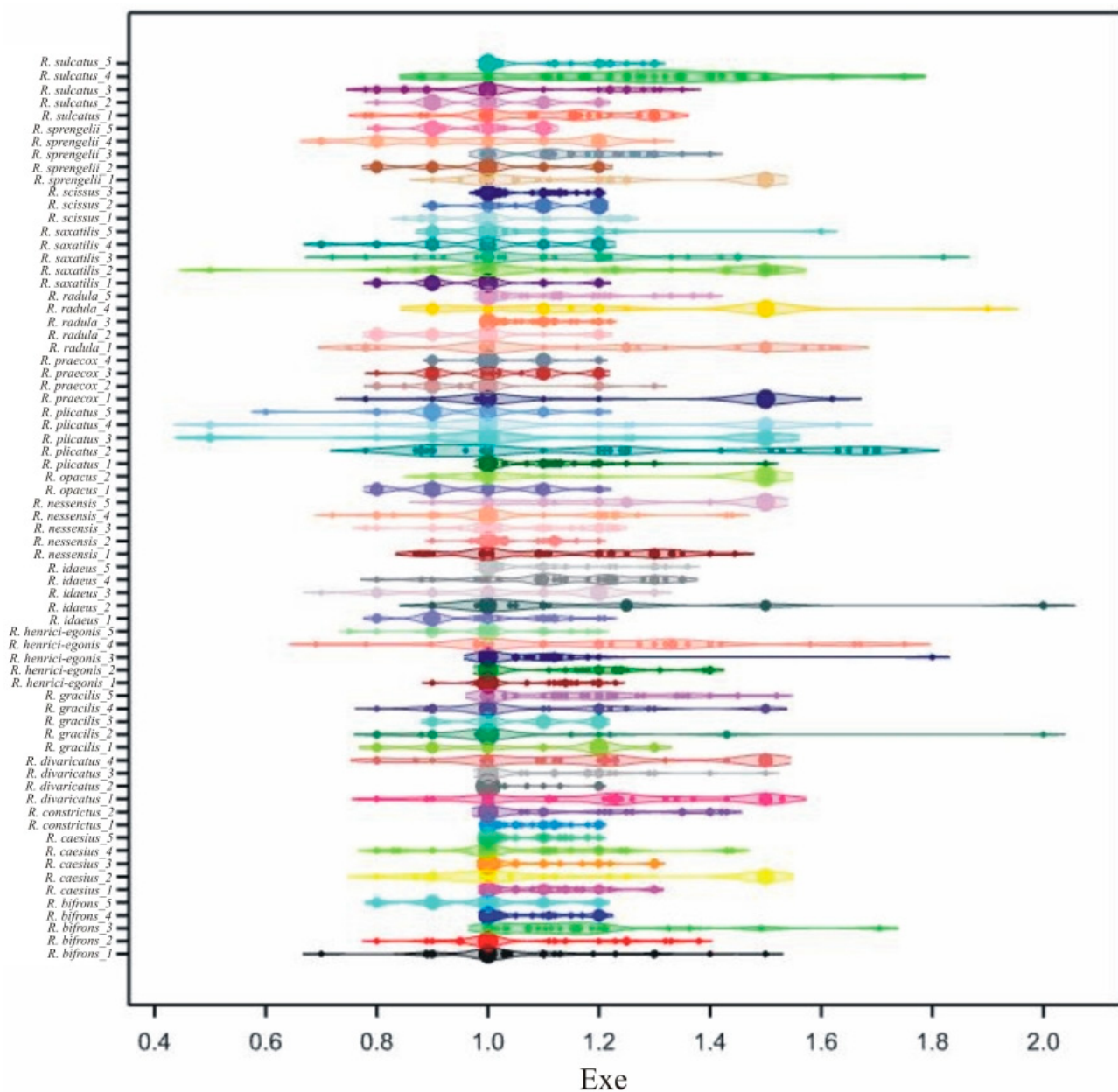


Figure 2. The density plot of Exe trait for *Rubus* species. The point “●” indicates trait observation for individual species.

The investigated pollen of individual *Rubus* species was classified according to the striate exine ornamentation classification proposed by Ueda and Tomita [40]. The cited authors distinguished six types (I–VI) and six subtypes (I–III, each A and B). In our study only subtypes IIA, B and IIIA, B were found (Figure 6, Table 6). Two species (*R. caesius*–Figure 6b, *R. idaeus*–Figure 6c) belonged to the IIB subtype, which was characterized by fairly distinct striae with long gaps and frequently by prominent, numerous perforations. Subtype IIIB was represented by two species (*R. constrictus*, *R. sulcatus*–Figure 6d) which were characterized by having more distinct and long ridges than type II. Four species (*R. bifrons*, *R. nessensis*, *R. opacus*, *R. saxatilis*–Figure 6e) belonged to the IIIA subtype with higher (i.e., more conspicuous than in subtype IIB), fairly narrow striae in comparison with subtype IIB. Subtype IIA was represented by eight species (*R. divaricatus*, *R. gracilis*, *R. henrici-egonis*, *R. plicatus*–Figure 6a, *R. praecox*, *R. radula*, *R. scissus*, *R. sprengelii*) characterized by having prominent perforations and short intervals between ridges.

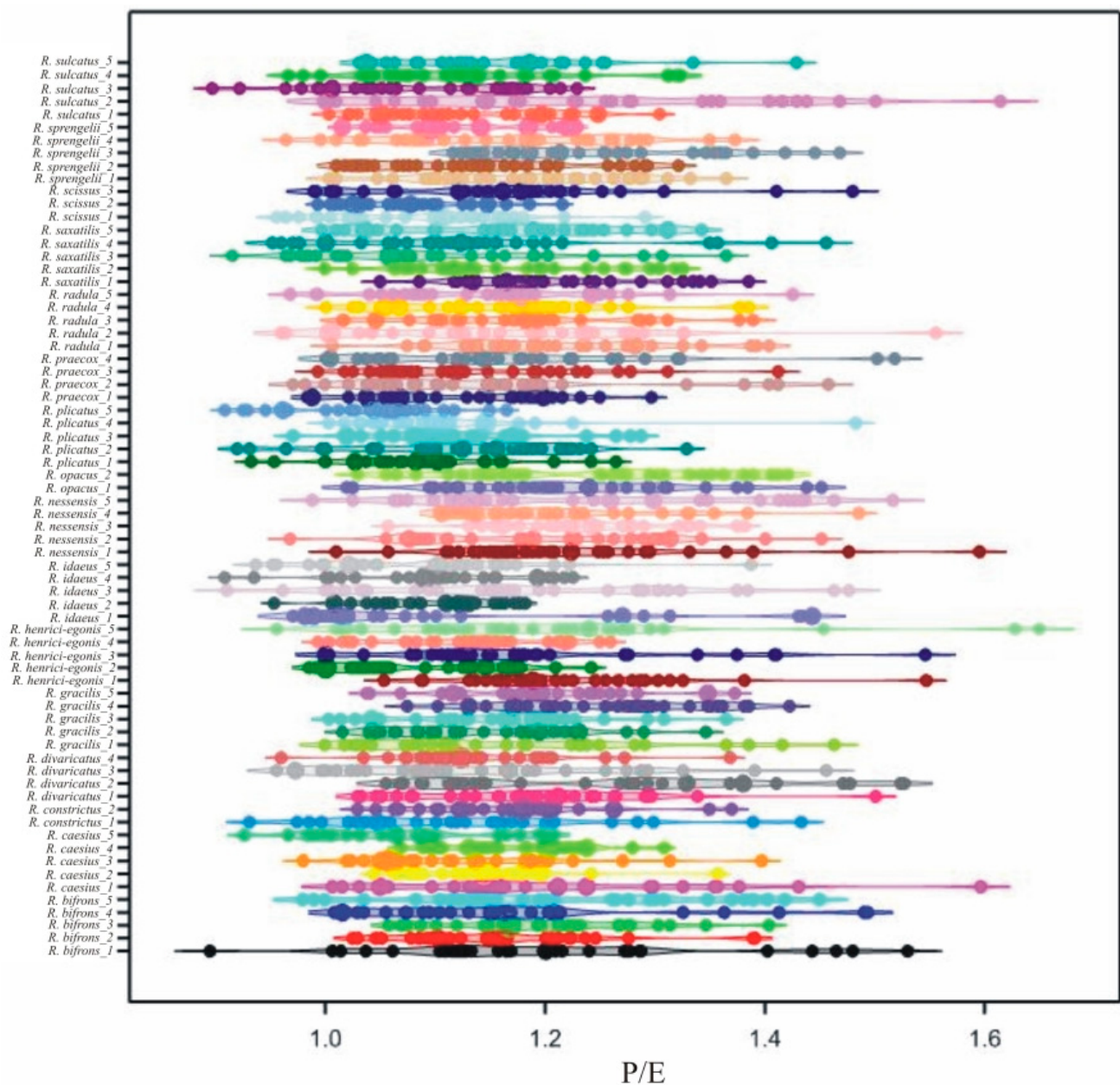


Figure 3. The density plot of P/E trait for *Rubus* species. The point “•” indicates trait observation for individual species.

In all of the species (16), elliptic or circular perforations of different diameters (0.06–0.5 μm) were found at the bottom of the grooves (Figure 6). In the majority of the species studied the perforations were small, with similar diameters (0.1–0.2 μm) and they were more or less numerous.

Significant positive relationships were observed between P and Le (0.964), P and d (0.284), P and E (0.780), Le and d (0.254), Le and E (0.764), d and E (0.546), d and d/E (0.941) as well as Exp and Exp/P (0.749). Negative correlations were observed between d and P/E (−0.464), E and P/E (−0.502), Exp and Le/P (−0.284), P/E and d/E (−0.334), P and Exp/P (−0.575), Le and Exp/P (−0.607), d and Exp/P (−0.268), as well as E and Exp/P (−0.472) (Figure 7).

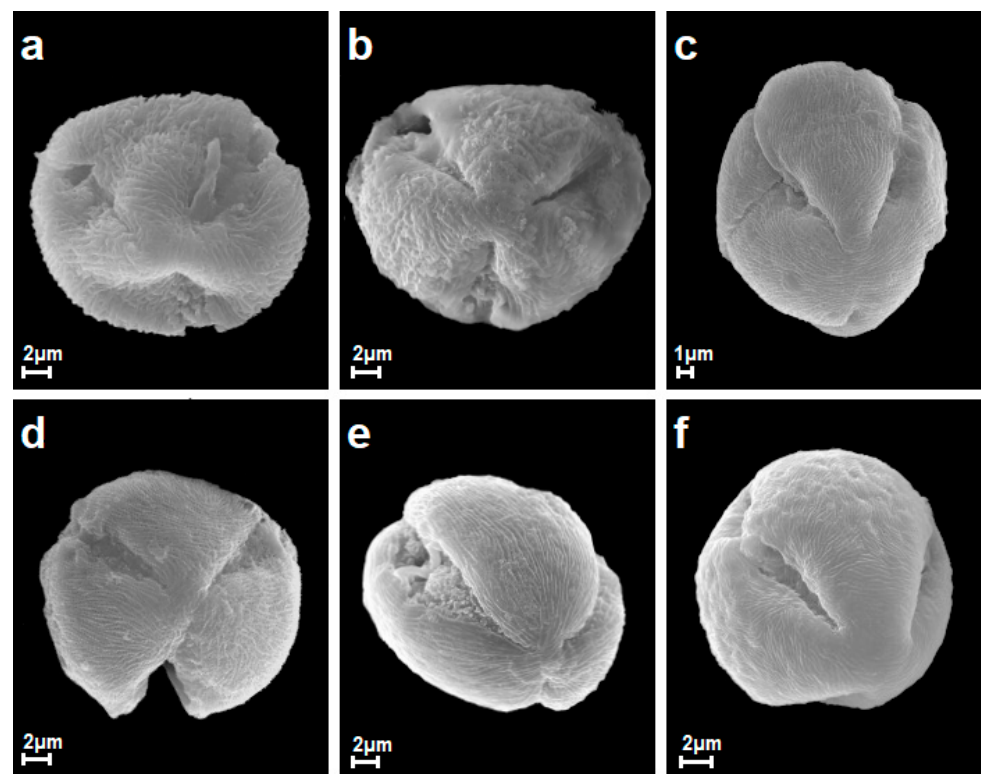


Figure 4. Pollen grains of *Rubus caesius*, *R. idaeus*, *R. henrici-egonis*, *R. plicatus*, *R. radula* and *R. saxatilis* in polar view, (a–f).

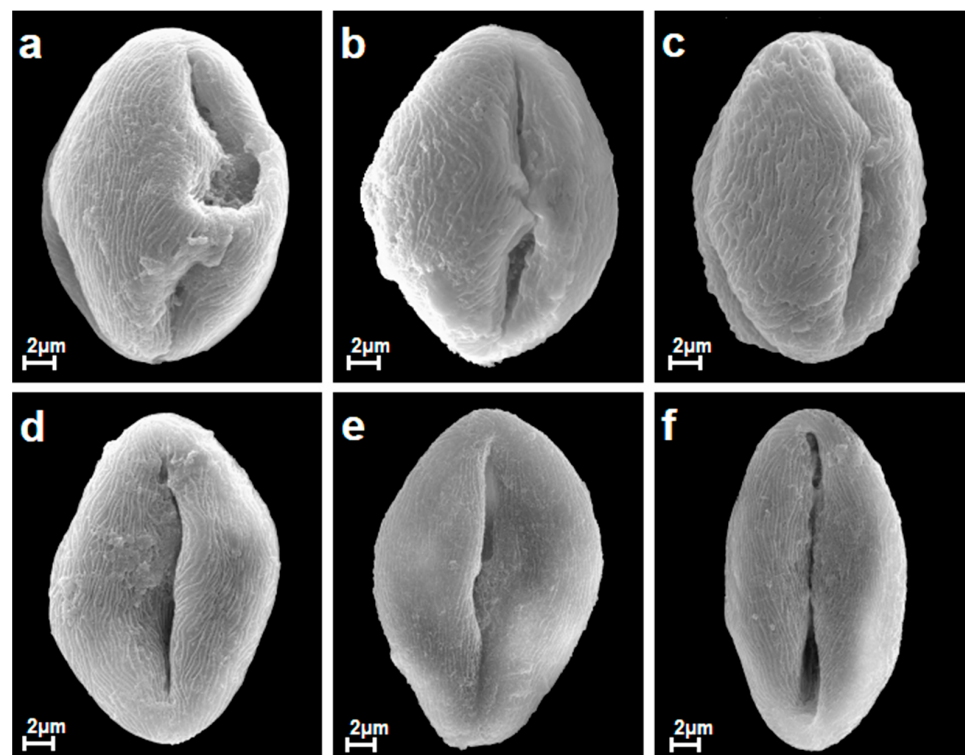


Figure 5. Pollen grains of *Rubus bifrons*, *R. idaeus*, *R. sulcatus*, *R. radula*, *R. nessensis* and *R. gracilis* in equatorial view, (a–f).

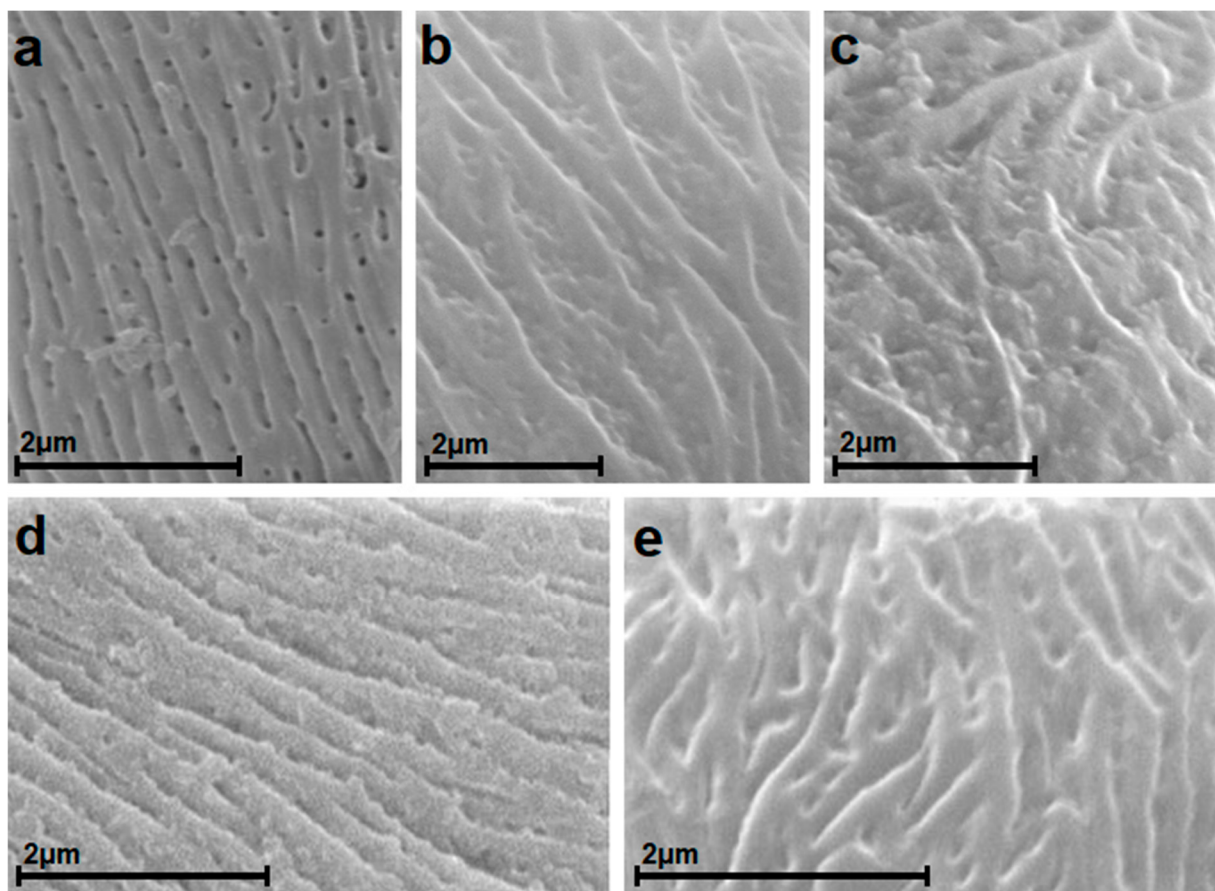


Figure 6. Striate exine ornamentation types according to Ueda and Tomita (1989); see Table 6. *Rubus plicatus*—IIA, *R. caesius*—IIB, *R. idaeus*—IIB, *R. sulcatus*—IIIB, *R. saxatilis*—IIIA, (a–e).

Table 6. Exine ornamentation types and subtypes of *Rubus* species studied (according to Ueda and Tomita [40] classification).

Striate Exine Ornamentation Type or Subtype	Species
IIA	<i>R. divaricatus</i> , <i>R. gracilis</i> , <i>R. henrici-egonis</i> , <i>R. plicatus</i> , <i>R. praecox</i> , <i>R. radula</i> , <i>R. scissus</i> , <i>R. sprengelii</i>
IIB	<i>R. caesius</i> , <i>R. idaeus</i>
IIIA	<i>R. bifrons</i> , <i>R. nessensis</i> , <i>R. opacus</i> , <i>R. saxatilis</i>
IIIB	<i>R. constrictus</i> , <i>R. sulcatus</i>

Individual traits were of varying importance and had different shares in the joint multivariate variation in the studied species. Analysis of the first two canonical variates for 70 *Rubus* species regarding the nine quantitative traits is shown in Figure 8. In the graph the coordinates of the point for a particular genotype were the values for the first and second canonical variate, respectively. The first two canonical variates accounted for 58.40% of the total variability between the individual species (Figure 8, Table 7). The most significant positive, linear relationship with the first canonical variate was found for Exp/P, while the negative—for P, Le, d and E (Table 7). The second canonical variate was significantly positively correlated with d and d/E, whereas it was negatively correlated with P, Exp and P/E (Table 7).

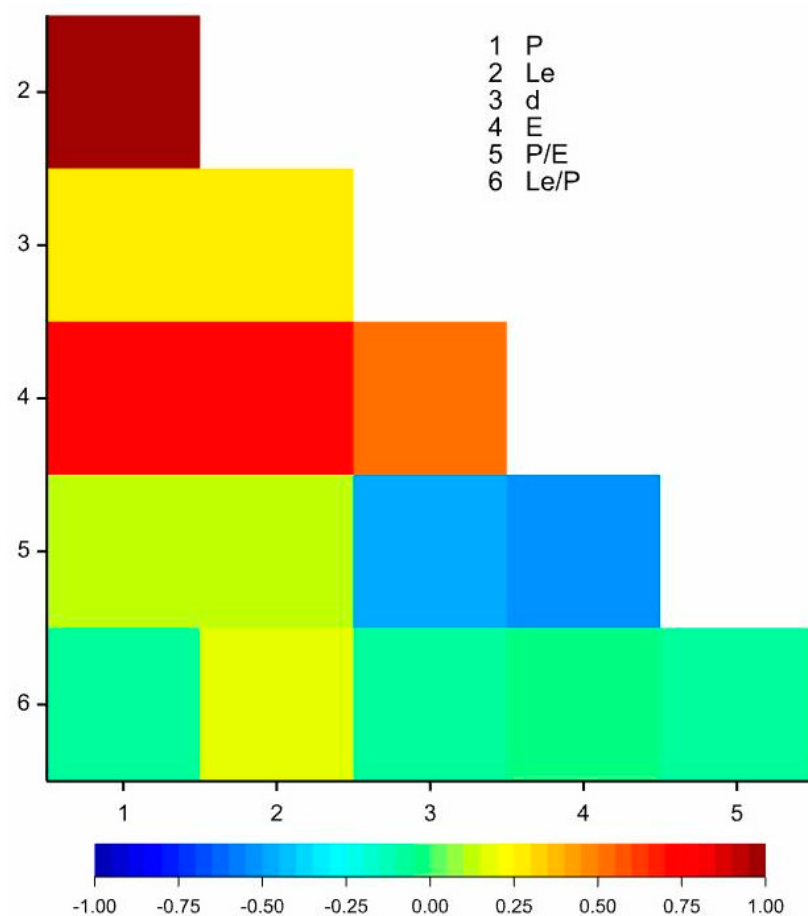


Figure 7. Heatmap for Pearson's correlation coefficients between observed traits ($r_{cr} = 0.232$) for *Rubus* species. The heatmap shows a graphical representation of the correlation matrix between pairs of observed traits. Every element of the correlation matrix is presented by a darkened square indicating the value at that location, using another color or darkening density. P—length of the polar axis, Le—length of the ectoaperture, d—the distance between apices of two ectocolpi, E—equatorial diameter, P/E—length of the polar axis and equatorial diameter ratio, Le/P—the ratio of the length of the ectoaperture and length of the polar axis.

Table 7. Correlation coefficients between the first two canonical variables and original traits.

Trait	First Canonical Variate	Second Canonical Variate
P	−0.951 ***	−0.249 *
Le	−0.914 ***	−0.206
d	−0.485 ***	0.804 ***
E	−0.918 ***	0.189
Exp	−0.062	−0.384 **
P/E	0.141	−0.625 ***
Le/P	0.086	0.162
d/E	−0.201	0.861 ***
Exp/P	0.585 ***	−0.161
Percentage of explained multivariate variability	33.98	24.42

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

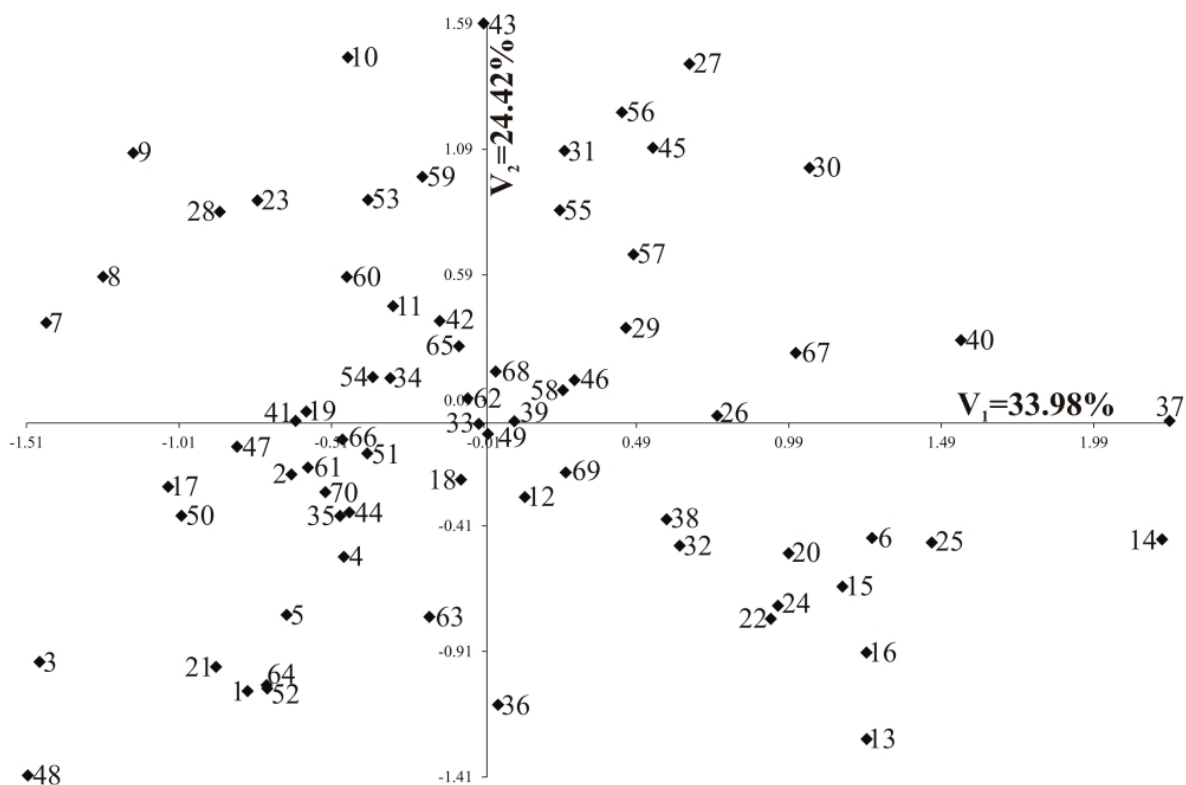


Figure 8. Distribution of 70 *Rubus* samples in the space of the first two canonical variables.

The greatest variation in terms of all the nine traits jointly measured with Mahalanobis distances was found for *R. opacus* 1 and *R. radula* 1 (distance between them amounted to 4.343). The greatest similarity was found between *R. scissus* 1 and *R. sulcatus* 3 (0.395) (data not shown).

In the dendrogram presented in Figure 9 all the examined *Rubus* species were divided into four groups as a result of agglomeration grouping using the Euclidean distance method. The first (I) group comprised 15 *Rubus* samples: *R. caesius* 1, *R. divaricatus* (samples 1–4), *R. gracilis* 4, *R. henrici-egonis* (samples 1, 3–5), *R. idaeus* 4, *R. nessensis* 1, *R. opacus* 1, *R. plicatus* 1 and *R. sulcatus* 2 (Figure 9). The second (II) group comprised two samples: *R. bifrons* 3 and *R. radula* 1; while group III was composed of six samples: *R. nessensis* 5, *R. opacus* 2, *R. praecox* 1, *R. radula* 4, *R. sprengelii* 1 and *R. sulcatus* 4. Group IV comprised the other 47 *Rubus* samples (Figure 9).

3.2. Interspecific Variability of Pollen Grains

Taking into consideration mean coefficients of variation, the analyzed *Rubus* species can be arranged as follows (from the least to the most variable): *R. henrici-egonis*, *R. nessensis*, *R. radula*, *R. sulcatus*, *R. opacus*, *R. plicatus*, *R. gracilis*, *R. idaeus*, *R. caesius*, *R. constrictus*, *R. divaricatus*, *R. praecox*, *R. saxatilis*, *R. bifrons*, *R. scissus* and *R. sprengelii* (Tables 3–5).

The greatest interspecific variability was found for *R. henrici-egonis*, *R. nessensis*, *R. radula*, *R. sulcatus*, *R. opacus*, *R. plicatus*, *R. gracilis* and *R. idaeus*. This variability was determined to the greatest degree by the following traits: P ($F = 33.66$; $p < 0.001$), d ($F = 28.93$; $p < 0.001$), d/E ($F = 26.71$; $p < 0.001$), Le ($F = 25.45$; $p < 0.001$) and E ($F = 25.17$; $p < 0.001$). In contrast, the lowest variability was recorded for *R. sprengelii*, *R. scissus*, *R. bifrons*, *R. saxatilis*, *R. praecox*, *R. divaricatus*, *R. constrictus* and *R. caesius* based on the following features: Exp ($F = 1.79$; $p = 0.03$), Le/P ($F = 2.04$; $p = 0.01$), Exp/P ($F = 11.56$; $p < 0.001$) and P/E ($F = 13.25$; $p < 0.001$) (Tables 3–5).

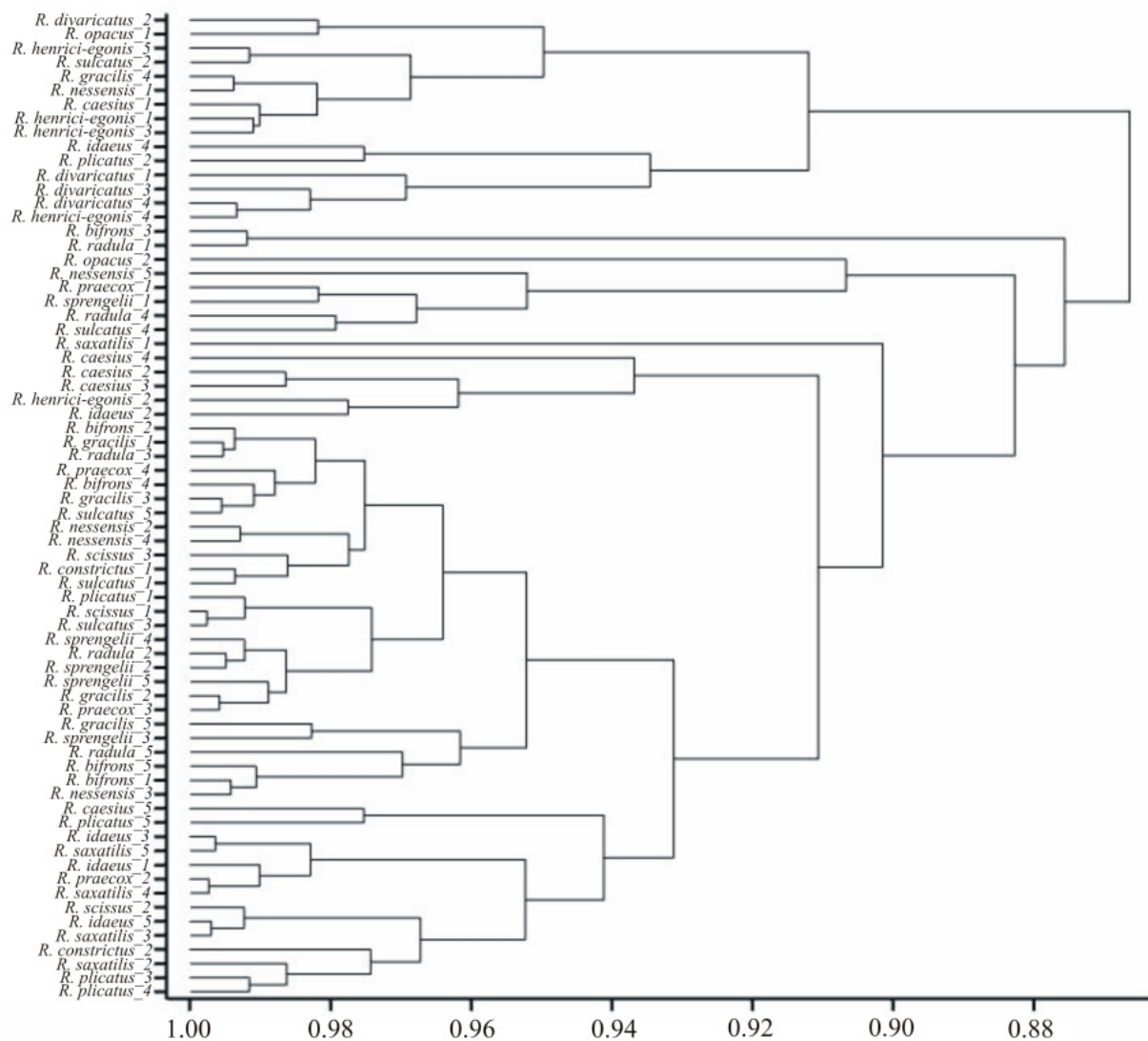


Figure 9. Clustering (neighbor joining method) of *Rubus* species based on nine morphological traits. The length of the lines indicates the similarity/distance between two species or among two groups of species and stated hierarchical clusters.

3.3. Intraspecific Variability of Pollen Grains

The dendrogram (Figure 9) showed clustering of samples of individual species with similar pollen grain characteristics into four groups. Group I is characterized by a considerable intraspecific similarity in relation to *d*, *Le/P*, *Exp/P* and *d/E* in *R. divaricatus* (samples 1–4), while for *Le/P*, *P/E*, *Exp* and *d/E* in *R. henrici-egonis* (samples 1, 3 and 4) (Table 8). No similarities were observed in blackberries of groups II and III. In turn, in group IV the greatest similarity was recorded for *d*, *d/E*, *Exp* and *P/E* in *R. saxatilis* (samples 1–5), followed by *Le/P*, *P/E*, *d* and *E* in *R. praecox* (samples 1–4), *Le/P*, *E*, *Exp/P* and *Exp* in *R. scissus* (samples 1–3) and *Le/P*, *Le*, *P* and *P/E* in *R. constrictus* (samples 1 and 2). The greatest intraspecific variability was found in *R. nessensis* (determined mainly by *Exp/P*, *Exp*, *P*, *E* and *Le*), *R. sulcatus* (*E*, *Exp*, *Exp/P*, *P/E* and *P*), *R. gracilis* (*E*, *P*, *Le*, *P/E* and *Exp/P*), *R. idaeus* (*P*, *E*, *d*, *Exp* and *Exp/P*) and *R. caesius* (*E*, *P*, *d*, *Le* and *d/E*) (Table 8).

Table 8. The ranking of features determining intraspecific variability.

Species	Ranking								
	1 (the Largest)	2	3	4	5	6	7	8	9 (the Smallest)
<i>R. bifrons</i>	Exp ***	Le ***	P **	d **	d/E *	Exp/P *	Le/P	E	P/E
<i>R. caesius</i>	E ***	P ***	d ***	Le ***	d/E ***	P/E ***	Exp/P ***	Le/P *	Exp
<i>R. constrictus</i>	d *	Exp/P *	E *	Exp *	d/E	P/E	P	Le	Le/P
<i>R. divaricatus</i>	E ***	Exp ***	P/E ***	Le **	P *	d/E *	Exp/P *	Le/P	d
<i>R. gracilis</i>	E ***	P ***	Le ***	P/E ***	Exp/P *	d *	Le/P *	Exp	d/E
<i>R. henrici-egonis</i>	d ***	E ***	Exp/P ***	d/E ***	P ***	Le ***	Exp ***	P/E ***	Le/P
<i>R. idaeus</i>	P ***	E ***	d ***	Exp ***	Exp/P ***	Le/P ***	Le **	P/E *	d/E
<i>R. nessensis</i>	Exp/P ***	Exp ***	P ***	E **	Le *	d *	d/E	P/E	Le/P
<i>R. opacus</i>	P ***	Le/P ***	Exp ***	d ***	E ***	d/E ***	Exp/P **	Le	P/E
<i>R. plicatus</i>	P ***	Le/P ***	E ***	Exp/P ***	P/E ***	d ***	d/E **	Exp **	Le *
<i>R. praecox</i>	P ***	Le ***	Exp ***	d/E ***	Exp/P ***	E ***	d **	P/E	Le/P
<i>R. radula</i>	d/E ***	P ***	Le ***	Exp ***	d ***	Exp/P ***	Le/P ***	E **	P/E
<i>R. saxatilis</i>	Le ***	P ***	Le/P ***	Exp/P ***	E ***	P/E ***	Exp ***	d/E	d
<i>R. scissus</i>	d ***	d/E ***	P/E ***	P ***	Le **	Exp	Exp/P	E	Le/P
<i>R. sprengelii</i>	Exp ***	Exp/P ***	P/E ***	P **	Le *	Le/P	d	E	d/E
<i>R. sulcatus</i>	E ***	Exp ***	Exp/P ***	P/E ***	P ***	Le ***	Le/P *	d/E	d

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

4. Discussion

Rosaceae Juss. is a large family consisting of 109 accepted genera [47], which taxonomy is highly complex, particularly in the case of the genus *Rubus* L. [48,49]. Traits of pollen grains due to their conservative character may be of great importance in investigations of taxonomic relationships in the case of such difficult genera as the large genus *Rubus* L. comprising 1409 accepted species [3], of which 108 grow in Poland [10,11].

In relation to studies on pollen morphological features, all palynologists agree that the most important pollen features for the species in the genus *Rubus* are related with exine ornamentation features such as width, number and course of grooves (muri) and the width of striae, as well as the number and diameter of perforations [22,23,25,26,32,33,35,40,50]. In a study by Li et al. [33] the 103 examined *Rubus* species from China belonged to four types of exine ornamentation (rugulate, striate, cerebroid and reticulate–perforate), which were further divided into 11 subtypes. Other palynologists distinguish in blackberries mainly striate or striate–perforate exine ornamentation [16,18,19,21–23,29,30,32,35,37,51]. Except for the typical striate ornamentation, also striate–scabrate, striate–rugulate or rugulate [22,32], echinate or gemmate [16], verrucate [16,29,30], baculate and clavate [18,19] or reticulate ornamentation [51] have been rarely observed. According to current palynological studies, European blackberry species are slightly less variable in terms of this feature than Asian ones. Our results confirm that study, because in the examined pollen grains, only one type of exine ornamentation (striate) was found. Ueda and Tomita [40] and Ueda [31] distinguished six types and six subtypes of striate exine ornamentation in species and other taxa from the genus *Rosa* and the family Rosaceae, including the genus *Rubus*. In our latest research [26] we classified into four types (types IV and VI were not identified) and five subtypes (I A, II A, B, III A, B). Our results were similar to findings of the cited authors, since most of the examined pollen belonged to the IIA and IIIA subtypes and no grains were found in the very rarely represented types IV and VI or subtype IB.

Several authors considered pollen size and shape as potentially important traits in the diagnosis of the analyzed *Rubus* species [21,23,51,52], but others claimed that they have no diagnostic importance [33,34]. Based on our results, we partially agree with the opinion of the former ones, because the length of the polar axis (P) proved to be an important feature, since on its basis pollen grains were divided into small (81.4%) and medium-sized (18.6%).

In our study, the greatest interspecific variability was found in *R. henrici-egonis*, *R. nessensis*, *R. radula*, *R. sulcatus*, *R. opacus*, *R. plicatus*, *R. gracilis* and *R. idaeus*. It turned out that it was determined to the greatest degree by P, and to a slightly lesser extent also by d, d/E as well as Le and E. In turn, the smallest intraspecific variability was recorded

in *R. sprengelii*, *R. scissus*, *R. bifrons*, *R. saxatilis*, *R. praecox*, *R. divaricatus*, *R. constrictus* and *R. caesius*. It was determined by other traits (Exp, Le/P, Exp/P and P/E). Very similar results were also reported by Lechowicz et al. [26], who investigated interspecific variability in 58 blackberry species. The greatest interspecific variability was determined by P, E, Le and d, while the smallest by Exp, Exe, P/E, Le/P, d/E, Exp/P and Exe/E. It needs to be added here that in the cited publication one sample was analyzed for each of the species, whereas in this study our team examined from two to five samples, which may have resulted in slight differences in the recorded results. The range of interspecific variability in the studied blackberries based on pollen traits is not dependent on their natural occurrence ranges. High variability was observed both in such very rare species as those having only several localities in Poland (*R. henrici-egonis*, *R. opacus*), those with larger ranges of occurrence (*R. gracilis*, *R. nessensis*, *R. radula*, *R. sulcatus*), as well as common species with extensive natural occurrence ranges in Poland and in Europe (*R. plicatus*, *R. idaeus*). The situation was similar in the group of species showing limited variability.

The least variable pollen traits in the investigated *Rubus* species included Le/P (1.61%), P/E (4.31%), P (6.01%) and Le (6.12%), while d (18.64%), d/E (15.91%), Exp/P (9.27%) and Exp (7.43%) exhibited the greatest variability. Similar results were reported by Wrońska-Pilarek and Jagodziński [53] when analyzing the same pollen features in 16 species from the genus *Rosa* L. The ordering of the traits from the least to the most variable was as follows: P, E, P/E, Le, Exp and Exp/P.

Analyses of intraspecific variability presented in this paper provided diverse results. The greatest intraspecific variability was found in *R. nessensis*, *R. sulcatus*, *R. gracilis*, *R. idaeus* and *R. caesius* (Table 8), while it was smallest in *R. divaricatus*, *R. saxatilis*, *R. scissus* and *R. constrictus* based on traits ordered here from the least to the most variable: $P > E > d > Le > d/E > Exp/P > Exp > P/E > Le/P$. Identical results were recorded by Singh et al. [54] when analyzing pollen grain of nine species from the genus *Rosa* L.

Within this study it was also investigated whether pollen traits confirm the currently accepted taxonomical division of the genus *Rubus* into subgenera, sections, subsections and series, as presented in Table 1. The distribution of the analyzed species in the dendrogram (Figure 9) did not confirm this division. Group I comprised species from two different subgenera: *Idaeobatus* (*R. idaeus*) and *Rubus* (*R. nessensis*, *R. divaricatus*, *R. opacus*, *R. plicatus*, *R. sulcatus*, *R. henrici-egonis*, *R. gracilis*, *R. caesius*). In turn, group II consisted of species from the same subsection: *Hiemales* (*R. bifrons*, *R. radula*). In the group III, in which *R. nessensis*, *R. opacus* and *R. sulcatus* from subsection *Rubus* are found together with *R. praecox*, *R. sprengelii* and *R. radula* from subsection *Hiemales*. Group IV comprised many species from three different subgenera (*Cylactis*, *Idaeobatus* and *Rubus*), while most species belonged to the largest subgenus *Rubus* (Figure 9). Additionally, it was not observed for different groups to be formed by species belonging to the same series. Identical conclusions were drawn by Xiong et al. [25] when analyzing pollen of species coming from all the 12 subgenera of the genus *Rubus* L. In view of a lack of other studies on the subject it may be assumed that morphological traits of pollen may not be considered useful in the taxonomy of the described genus. It is the opinion of the authors of this study that in pollen of species from the genus *Rubus* the absence of taxonomic dependencies may result from apomixis, defined as asexual reproduction without fertilization replacing sexual reproduction, thus reducing natural variability. Similar results in relation to representatives of the genus *Rubus* L. were reported by Wrońska-Pilarek [23,35] and Lechowicz et al. [26].

5. Conclusions

Pollen features, which proved to be most useful for the assessment of the intra- and interspecific variability of the studied *Rubus* species were the exine ornamentation features (width, number and course of grooves and the width of striae, as well as the number and diameter of perforations) and pollen size.

The range of the interspecific variability of the studied species ordered from the most variable to the least variable was as follows: *R. henrici-egonis*, *R. nessensis*, *R. radula*,

R. sulcatus, *R. opacus*, *R. plicatus*, *R. gracilis*, *R. idaeus*, *R. caesius*, *R. constrictus*, *R. divaricatus*, *R. praecox*, *R. saxatilis*, *R. bifrons*, *R. scissus* and *R. sprengelii*. Such an ordering of species shows no relationships with the taxonomic division of the genus *Rubus* L. or with the ranges of natural occurrence of these species.

The greatest effect on intraspecific variability of the studied species was found for the most variable features, i.e., the distance between apices of two ectocolpi (d, d/E) and exine thickness (Exp/P, Exp), while it was determined to a lesser degree by pollen size (E, P, P/E) and the length of apertures (Le, Le/P).

Grouping of the investigated blackberry species in the dendrogram (Figure 9) based on pollen traits did not confirm the currently adopted taxonomic division of the genus *Rubus* into subgenera, sections and series.

Nevertheless, we believe it is worth expanding the research on the difficulted genus *Rubus* and conducting a full taxonomic review to answer the hypotheses fully.

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