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## Pollen morphological variability of Polish native species of *Rosa* L. (Rosaceae)

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**Abstract:** The variability of pollen grains of 16 species from genus *Rosa* L. was studied (i.e. *Rosa agrestis*, *R. canina*, *R. dumalis*, *R. gallica*, *R. inodora*, *R. jundzillii*, *R. kostrakiewiczii*, *R. majalis*, *R. micrantha*, *R. mollis*, *R. pendulina*, *R. rubiginosa*, *R. sherardii*, *R. tomentosa*, *R. villosa*, and *R. zalana*). The material came from 107 native localities of those species in Poland. The measurements are based on at least 30–50 randomly selected mature pollen grains per specimen. In total, 3510 pollen grains were examined. They were analysed for 8 quantitative features, i.e. length of polar axis (P), length of equatorial axis (E), exine thickness on the pole (Exp), exine thickness at the equator plane (Exe), length of ectocolpi (Le), P/E ratio, and relative thickness of exine (Exp/P and Exe/E ratio). Statistically significant differences were found among the examined species with regard to all analysed pollen features. The pollen and ectocolpi dimensions (P, E and Le) were largest in *R. gallica* (35.9, 28.1, and 28.0  $\mu\text{m}$ , respectively) and smallest in *R. majalis* (27.0, 20.2, and 21.2  $\mu\text{m}$ , respectively). The mean coefficients of variability of the pollen features measured can be used to arrange the examined rose species from the least to the most variable as follows: *R. pendulina*, *R. villosa*, *R. jundzillii*, *R. inodora*, *R. canina*, *R. rubiginosa*, *R. dumalis*, *R. gallica*, *R. agrestis*, *R. micrantha*, *R. zalana*, *R. tomentosa*, *R. sherardii*, *R. majalis*, *R. kostrakiewiczii* and *R. mollis*. The obtained data failed to confirm fully both the division of the *Rosa* genus currently in force in taxonomy into sections as well as relationships among the examined species from the *Caninae* section. In addition, values of morphological characters of the same species may differ considerably from one another. The extent of these differences indicated that it was necessary to measure large numbers of pollen grains in order to obtain accurate biometric data.

**Additional key words:** *Rosa*, *Caninae*, *Cinnamomeae*, taxonomy, pollen variability, pollen morphology, quantitative features

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### Introduction

The *Rosa* L. genus belongs to one of 36 European genera in the *Rosaceae* family (Klašttersky 1968). Depending on the adopted approach, the genus contains from 100 to 120 or even up to 250 species and is dis-

tributed in the northern hemisphere in Europe, Asia, Ethiopia, the Middle East and North America (Hutchinson 1964; Zieliński 1987; Nilsson 1997; Henkler 2000). A total of 47 rose species belonging to 5 sections are reported from Europe (Klašttersky 1968). The majority of European and Polish roses belong to

the section of *Caninae* DC. em. Christ (Klašttersky 1968; Zieliński 1985).

Zieliński (1985, 1987), who adopts a broad approach to species, mentions 14 rose species from Poland representing three sections: *Caninae* DC. em. Christ. (11 species), *Cinnamomeae* DC. (2 species), and *Rosa* L. (1 species). On the other hand, Popek (1996) quotes 16 species adding to the *Caninae* DC. em. Christ. section also *Rosa kostrakiewiczii* and *R. mollis*.

Roses belong to a systematically complicated groups of plants. The considerable polymorphism of this genus can be attributed to hybridisation, polyploidy and, especially in the *Caninae* section, to stabilisation mechanisms of cytotypes of uneven numbers of chromosomes resulting from the specific *Caninae* type meiosis and, possibly, apomixis (Gustafsson 1944; Klaštterska 1971; Zieliński 1985; Popek 1996).

The group which poses the greatest classification difficulties is comprised of roses from the *Caninae* section (Zieliński 1987). Taxonomic difficulties in this section are associated with the polyphyletic nature of this relatively young group. Its species are probably hybrids between *Rosa canina* (or its direct ancestor) and some unknown, most probably extinct, representatives of the *Rosa* and *Cinnamomeae* sections. The isolation of the *Caninae* section is of more theoretical than practical significance. Contemporary *Caninae* have the character of a swarm of hybrids with *R. canina* as a link connecting all section taxa (Zieliński 1987).

Palynological investigations on selected species of the *Rosa* genus were carried out by: Teppner (1965), Reitsma (1966), Stachurska et al. (1974-1975, 1976), Kuprianowa and Alyoshina (1978), Eide (1981), Fedoronchuk and Savitsky (1987), Savitsky et al. (1987), Faegri and Iversen (1989), Ueda and Tomita (1989), Hebda and Chinnappa (1990), Moore et al. (1991), Ueda (1992), Ueda and Okada (1994), Popek (1996), Zhou et al. (1999), Beug (2004), Shinwari et al. (2004), Wrońska-Pilarek and Boratyńska (2005), and Wrońska-Pilarek and Lira (2006).

In spite of relatively numerous scientific publications, our knowledge about the morphology of rose pollen grains is still insufficient which can be attributed to descriptions referring to one or several selected taxa or analysis of only one or two basic pollen morphological characters (usually exine sculpture and operculum type). The majority of the quoted investigations dealt with morphology and not pollen variability and were conducted on small numbers of samples. Such comprehensive experiments on pollen variability of species from the *Rosa* genus conducted with the assistance of statistical methods on large numbers of samples as in the current study have not been carried out so far.

The object of this study was to investigate inter-specific and intraspecific morphological variability of quantitative features of pollen grains among sixteen Polish species from the genus *Rosa*, representing three sections, i.e. *Caninae* DC. em. Christ., *Cinnamomeae* DC. and *Rosa* L., with particular consideration of three species, representing the highest number of natural localities in the study, from the mentioned sections, that is *R. canina*, *R. pendulina* and *R. gallica*. Moreover we tested the taxonomical value of the studied morphological features of the pollen grains to assess whether one can use these features to distinguish among the sections and species of genus *Rosa*.

## Material and methods

The pollen grains were collected in Poland, from 107 natural localities (Table 1). Some of the analysed rose species have only a single (*Rosa kostrakiewiczii*) or very few (e.g. *R. micrantha*, *R. villosa*, *R. zalana*) localities and, therefore, they are represented by few individuals in the presented study. From each individual (shrub), seven to twelve randomly selected flowers were collected.

The measurements are based on at least 30–50 randomly selected, mature pollen grains per specimen. In total, 3510 pollen grains were analysed. They were analysed for 8 quantitative features of pollen grains, i.e. length of polar axis (P;  $\mu\text{m}$ ), length of equatorial axis (E;  $\mu\text{m}$ ), exine thickness on the pole (Exp;  $\mu\text{m}$ ), exine thickness at the equator plane (Exe;  $\mu\text{m}$ ), length of ectocolpi (Le;  $\mu\text{m}$ ), P/E ratio, and relative thickness of exine (Exp/P and Exe/E ratio). Relative exine thickness is the ratio of exine thickness on the pole (Exp) to the length of the P axis, and at the equator plane (Exe) to the length of the E axis. All samples were acetolysed according to Erdtman's method (1952), slightly modified by Wrońska-Pilarek (1998). The terminology follows Punt et al. (2007) and Hesse et al. (2009). The observations were carried out both with a light microscope (Biolar 2308, Nikon HFX-DX) and a scanning electron microscope (ISI 60, Zeiss 435 VP).

For each pollen grain feature, one-factor analysis of variance (ANOVA) was used to examine differences in the mean values among taxa overall. When critical differences were noted, multiple comparisons were carried out using Tukey's test for unequal sample sizes. To show similarities and differences among taxa studied, Ward's hierarchical clustering method was used to compute cluster groups based on pollen grain morphological features. Additionally, for three of the studied species, i.e. *Rosa gallica*, *R. canina* and *R. pendulina*, we tested whether there were statistically significant differences in particular pollen grain features among specimens collected from different localities (one-way analysis of variance). Moreover, we

Table 1. List of *Rosa* species analysed in the study

No	Species	Section	No of specimens	No of pollen grains
1.	<i>Rosa agrestis</i> Savi	<i>Caninae</i> DC. em. Christ.	4	120
2.	<i>Rosa canina</i> L.	<i>Caninae</i> DC. em. Christ.	17	510
3.	<i>Rosa dumalis</i> Bechst.	<i>Caninae</i> DC. em. Christ.	9	270
4.	<i>Rosa gallica</i> L.	<i>Rosa</i> L.	15	750
5.	<i>Rosa inodora</i> Fr.	<i>Caninae</i> DC. em. Christ.	6	180
6.	<i>Rosa jundzillii</i> Besser	<i>Caninae</i> DC. em. Christ.	5	150
7.	<i>Rosa kostrakiewiczii</i> Popek	<i>Caninae</i> DC. em. Christ.	1	30
8.	<i>Rosa majalis</i> Herrm.	<i>Cinnamomeae</i> DC.	5	150
9.	<i>Rosa micrantha</i> Borrer ex Sm.	<i>Caninae</i> DC. em. Christ.	2	60
10.	<i>Rosa mollis</i> Sm.	<i>Caninae</i> DC. em. Christ.	4	120
11.	<i>Rosa pendulina</i> L.	<i>Cinnamomeae</i> DC.	10	300
12.	<i>Rosa rubiginosa</i> L.	<i>Caninae</i> DC. em. Christ.	8	240
13.	<i>Rosa sherardii</i> Davies	<i>Caninae</i> DC. em. Christ.	12	360
14.	<i>Rosa tomentosa</i> Sm.	<i>Caninae</i> DC. em. Christ.	5	150
15.	<i>Rosa villosa</i> L.	<i>Caninae</i> DC. em. Christ.	1	30
16.	<i>Rosa zalana</i> Wiesb.	<i>Caninae</i> DC. em. Christ.	3	90

used Ward's hierarchical clustering method to show similarities among studied specimens of those three species. Statistical analyses were performed using JMP 8.0 (SAS Institute Inc., Cary, NC, USA; <http://www.sas.com/>).

### List of localities

**R. agrestis:** Pieprzowe Mts 50°40'N, 21°45'E; Prądnik Korzkiewski 50°10'N, 19°51'E; Las Puławski 51°25'N, 21°58'E; Pieniny Mts 49°26'N, 20°25'E; **R. canina:** Chełmno 53°20'N, 18°25'E; Ojców 50°12'N, 19°49'E; Brzeg 50°51'N, 17°28'E; Całowanie 52°0'N, 21°18'E; Pieniny Mts 49°26'N, 20°25'E; Nowy Sącz 49°37'N, 20°41'E; Smolno 52°04'N, 15°44'E; Zubrzyca Górna 49°33'N, 19°38'E; Kurzętnik 53°23'N, 19°34'E; Przemyśl 49°46'N, 22°46'E; Swarzewo 54°45'N, 18°23'E; Sikory Juskie 53°54'N, 22°16'E; Las Kabacki 52°13'N, 21°00'E; Międzybrodzie 49°36'N, 22°11'E; Zawoja 49°39'N, 19°32'E; Dolina Będkowska 50°12'N, 19°49'E; Biała 49°41', 19°12'E; **R. dumalis:** Smyków 50°51'N, 21°03'E; Tworzymirki Górne 51°35'N, 17°25'E; Ojców 50°12'N, 19°49'E; Kulin 52°39'N, 19°3'E; Zakrzewo 51°40'N, 16°53'E; Pieniny Mts 49°26'N, 20°25'E; Bochońnica 51°20'N, 21°59'E; Złotniki 52°29'N, 16°51'E; Cigacice 52°02'N, 15°37'E; **R. gallica:** Chełmicka Góra 49°38'N, 20°40'E; Gorzyce 50°40'N, 21°50'E; Grzęby Korzeczkowskie 50°48'N 20°25'E; Kobylin 51°43'N, 17°13'E; Kojszków 51°05'N, 16°11'E; Koskowice 51°11'N, 16°14'E; Kosobudy 50°37'N, 23°04'E; Krzywłina Mała 51°21'N, 16°39'E; Lublin 51°14'N, 22°34'E; Raczkowa 51°08'N, 16°11'E; Ryki 51°28'N, 22°01'E; Taczalin 51°10'N, 16°15'E; Teresin 50°58'N, 23°33'E; Ogonowice 51°06'N, 16°14'E; Wrocław 51°06'N, 17°01'E; **R. inodora:** Pieniny Mts, Flaki

49°26'N, 20°25'E; Karczówka 51°41'N, 15°22'E; Małogoszcz 50°48'N, 20°15'E; Chwalim 52°3'N, 15°49'E; Ojcowski National Park 50°12'N, 19°49'E; Pieniny Mts, Ostra Skała 49°26'N, 20°25'E; **R. jundzillii:** Grzęby Korzeczkowskie 50°48'N 20°25'E; Skorków 50°51'N, 20°13'E; Wołów 51°21'N, 16°39'E; Dobrzyca 51°51'N, 17°35'E; Drohiczyń 52°23'N, 22°39'E; **R. kostrakiewiczii:** Pieprzowe Mts 50°40'N, 21°45'E; **R. majalis:** Bocheniec 50°47'N, 20°18'E; Pieruchy 51°58'N, 17°44'E; Opaleń 52°09'N, 20°48'E; Paradyż 51°18'N, 20°06'E; Stopkowa 49°35'N, 19°44'E; **R. micrantha:** Połęcko 52°03'N, 14°54'E; Kamień 50°00'N, 19°35'E; **R. mollis:** Szczerba 53°52'N, 23°05'E; Żelgoszcz 53°50'N, 18°25'E; Leśna 52°51', 23°48'E; Monkinie 53°58'N, 23°05'E; **R. pendulina:** Babia Góra 49°35'N, 19°31'E; Cyrlowa Skałka 49°24'N, 20°23'E; Tatra Mts, Cicha Dolina 49°14'N, 19°58'E; Tatra Mts, Dolina Koprowa, 49°14'N, 19°58'E; Gzawa 49°34'N, 19°44'E; Karkonosze Mts, Mały Staw 50°44'N, 15°44'E; Borowice 50°47'N, 15°41'E; Bieszczady Mts, Połonina Caryńska, 49°04'N, 22°43'E; Tatra Mts, Nosal 49°14'N, 19°58'E; Bieszczady Mts, Tarnica 49°04'N, 22°43'E; **R. rubiginosa:** Prądnik Korzkiewski 50°10'N, 19°51'E; Jaworzycze 52°09', 20°48'E; Niepart 51°42'N, 16°59'E; Golina 51°54'N, 17°28'E; Gorzyce 50°40'N, 21°50'E; Pieniny Mts 49°26'N, 20°25'E; Poznań 52°24'N, 16°54'E; Ostrowąsy 51°36'N, 17°28'E; **R. sherardii:** Puszcza Kampinoska 52°18'N, 20°48'E; Bielawy Pożońskie 51°50'N, 17°11'E; Chwałkowo 51°44'N, 17°01'E; Sikorzyn, 51°50'N, 16°57'E; Puławy 51°25'N, 21°58'E; Pieniny Mts 49°26'N, 20°25'E; Ojców 50°12'N, 19°49'E; Sowiny 51°43'N, 16°50'E; Konarskie 52°13'N, 17°02'E; Jeżewo 51°56'N, 17°13'E; Kąty 49°45'N, 20°50'E; Cigacice 52°02'N,

15°37'E; *R. tomentosa*: Siedlec 52°80'N, 16°00'E; Pieniny Mts 49°26'N, 20°25'E, Tatra Mts 49°14'N, 19°58'E; Besko 49°36'N, 21°57'E; Białe 52°30'N, 19°30'E; *R. villosa*: Małpin 52°01'N, 17°00'E; *R. zalana*: Myszków 51°37'N, 15°18'E; Gryfino 53°15'N, 14°29'E; Rudnica 52°36'N, 15°12'E.

## Results

### Interspecific variability of pollen grains

Statistically significant differences were determined among the examined species with regard to all the analysed pollen features ( $p < 0.0001$ ; Table 2 and 3). The largest length of polar axis (P), equatorial axis (E) and ectocolpi (Le) were found in *Rosa gallica*

(35.9, 28.1, and 28.0  $\mu\text{m}$ , respectively), while the smallest were found in *R. majalis* (27.0, 20.2, and 21.2  $\mu\text{m}$ , respectively) (Table 2). The thickest exine (Exp and Exe) was found in *R. pendulina* (1.94 and 1.94  $\mu\text{m}$ , respectively), while the thinnest was found in *R. mollis* (1.58 and 1.59  $\mu\text{m}$ , respectively). *R. majalis* was found to have the highest values of exine thickness coefficients (Exp/P, Exe/E) (0.064 and 0.088, respectively), whereas in *R. agrestis* and *R. gallica* these values were the lowest (0.053 and 0.068, and 0.053 and 0.060, respectively) (Table 3). Pollen grains characterized by the longest shape (feature P/E) were observed in *R. majalis* (1.35), while in *R. villosa*, pollen grains were the least elongated (1.19).

Mean coefficients of variation (CV, calculated taking into account all the examined rose species) for the

Table 2. Range (min–max), coefficient of variation (CV) and mean values ( $\pm$ SE) of pollen grains morphological features of *Rosa* species studied [e.g. length of polar axis (P), length of equatorial axis (E), exine thickness on the pole (Exp), exine thickness at the equator plane (Exe), length of ectocolpi (Le)]. One way ANOVA's were performed separately for each of the pollen grain feature to determine the differences among *Rosa* species. Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ( $p < 0.05$ )

Species	P [ $\mu\text{m}$ ]			E [ $\mu\text{m}$ ]			Exp [ $\mu\text{m}$ ]			Exe [ $\mu\text{m}$ ]			Le [ $\mu\text{m}$ ]		
	Min–Max	CV [%]	Mean	Min–Max	CV [%]	Mean	Min–Max	CV [%]	Mean	Min–Max	CV [%]	Mean	Min–Max	CV [%]	Mean
<i>R. agrestis</i>	24–40	9.58	31.8cd (0.3)	20–34	13.31	24.7cdef (0.3)	1–2	19.05	1.69fgh (0.03)	1–2	19.12	1.66de (0.03)	20–36	11.51	26.4bc (0.3)
<i>R. canina</i>	22–42	9.95	31.0de (0.1)	16–34	14.28	24.9c (0.2)	1–2	10.71	1.89a (0.01)	1–2	9.40	1.91a (0.01)	18–36	12.27	25.2def (0.1)
<i>R. dumalis</i>	22–40	10.03	30.9de (0.2)	16–34	16.25	24.7cde (0.2)	1–2	13.69	1.79cde (0.01)	1–4	14.09	1.81bc (0.02)	16–34	12.26	24.9efgh (0.2)
<i>R. gallica</i>	25–50	10.89	35.9a (0.1)	20–41	11.43	28.1a (0.1)	1–3	17.09	1.77def (0.01)	0.8–2.4	16.25	1.66e (0.01)	18–38	11.92	28.0a (0.1)
<i>R. inodora</i>	22–40	8.45	30.8def (0.2)	16–32	12.15	24.7cde (0.2)	1.6–2	9.58	1.89ab (0.01)	1.6–2	9.96	1.87ab (0.01)	18–32	10.51	25.0defhi (0.2)
<i>R. jundzillii</i>	28–40	7.71	32.8bc (0.2)	20–36	10.77	27.0b (0.2)	1.6–2	9.54	1.89abc (0.01)	1.6–2	9.19	1.90a (0.01)	20–34	9.80	26.0bcd (0.2)
<i>R. kostrakiewiczii</i>	24–34	9.93	28.7fgh (0.5)	18–28	11.39	21.8ghi (0.5)	1–2	25.43	1.60fgh (0.07)	1–2	24.12	1.62de (0.07)	20–28	11.62	23.3hij (0.5)
<i>R. majalis</i>	20–34	11.68	27.0h (0.3)	16–26	13.05	20.2i (0.2)	1–2	20.98	1.71efg (0.03)	1–2	19.26	1.76cd (0.03)	12–30	13.12	1.2k (0.2)
<i>R. micrantha</i>	26–36	8.44	29.3fg (0.3)	18–32	13.33	23.3defh (0.4)	1–2	18.73	1.70defg (0.04)	0.2–2	20.78	1.73cde (0.05)	20–30	9.78	23.4j (0.3)
<i>R. mollis</i>	24–36	9.65	29.1g (0.3)	18–32	13.49	22.3h (0.3)	1–2	25.97	1.58h (0.04)	1–2	24.88	1.59e (0.04)	18–30	10.44	23.9ij (0.2)
<i>R. pendulina</i>	28–38	6.53	33.1b (0.1)	20–32	6.69	27.6ab (0.1)	1.4–2	6.14	1.94a (0.01)	1.6–2.2	6.04	1.94a (0.01)	18–34	9.00	26.5b (0.1)
<i>R. rubiginosa</i>	24–36	8.70	29.9fg (0.2)	16–32	13.97	23.8dfg (0.2)	1–2	14.35	1.81bcd (0.02)	1–2	10.65	1.87ab (0.01)	18–30	10.60	24.4ghij (0.2)
<i>R. sherardii</i>	26–40	9.02	31.1de (0.1)	16–32	12.48	24.7ce (0.2)	1–2	19.88	1.67gh (0.02)	1–2	19.94	1.66e (0.02)	20–34	11.39	25.7cde (0.2)
<i>R. tomentosa</i>	24–36	7.60	30.8def (0.2)	14–30	11.62	23.5fgh (0.2)	1–2	20.29	1.70efg (0.03)	1–2	21.19	1.68de (0.03)	18–32	9.83	25.4cdef (0.2)
<i>R. villosa</i>	26–32	5.89	29.5efg (0.3)	22–30	7.21	24.8cdef (0.3)	1.6–2	9.69	1.68efgh (0.03)	1.6–2	9.69	1.68cde (0.03)	20–30	9.47	24.1defghij (0.4)
<i>R. zalana</i>	24–36	8.30	30.0efg (0.3)	18–32	12.84	23.9cdefg (0.3)	1–2	19.88	1.66gh (0.03)	1–2	20.51	1.67de (0.04)	20–30	9.42	24.5fghij (0.2)
ANOVA P>F			0.0000			<0.0001			<0.0001			<0.0001			<0.0001

analysed pollen features amounted to: P – 12.1%, E – 14.8%, Exp – 16.4%, Exe – 16.2%, Le – 12.9%, P/E – 12.5%, Exp/P – 18.5%, Exe/E – 21.7%. Exp and Exe features and Exp/P, Exe/E ratios associated with them were more variable than the remaining ones. On the other hand, when analysing mean coefficients of variation of the examined features within individual rose species, it was found that pollen grains of *R. pendulina* (CV=7.4%, n=300), *R. villosa* (CV=9.0%, n=30) and *R. jundzillii* (CV=10.8%, n=150) were characterised by the lowest variability, whereas the highest variability of the examined pollen features was found in *R. mollis* (CV=18.6%, n=120), *R. kostrakiewiczii* (CV=17.1%, n=30) and *R. majalis* (CV=16.6%, n=150). Taking into consideration mean coefficients of variation, the analysed rose spe-

cies can be arranged as follows (from the least to the most variable): *R. pendulina*, *R. villosa*, *R. jundzillii*, *R. inodora*, *R. canina*, *R. rubiginosa*, *R. dumalis*, *R. gallica*, *R. agrestis*, *R. micrantha*, *R. zalana*, *R. tomentosa*, *R. sherardii*, *R. majalis*, *R. kostrakiewiczii* and *R. mollis*.

The agglomeration grouping using the Ward's method yielded a dendrogram (Fig. 1) which was used to divide the examined rose species into two groups. The first group was comprised of: *Rosa agrestis*, *R. sherardii*, *R. tomentosa*, *R. villosa*, *R. micrantha*, *R. zalana*, *R. kostrakiewiczii*, *R. mollis*, and *R. gallica* (the last species with the most distant position on the dendrogram), while the second one includes *R. rubiginosa*, *R. canina*, *R. inodora*, *R. dumalis*, *R. jundzillii*, *R. pendulina* and *R. majalis* (which differs most from the remaining species in the group).

Table 3. Range (min–max), coefficient of variation (CV) and mean values ( $\pm$ SE) of pollen grains morphological features of *Rosa* species studied [e.g. P/E ratio and relative thickness of exine (Exp/P and Exe/E ratio)]. One way ANOVA's were performed separately for each of the pollen grain feature to determine the differences among *Rosa* species. Same letters indicate a lack of statistically significant differences between analyzed species according to Tukey's a posteriori test ( $p < 0.05$ )

Species	P/E			Exp/P			Exe/E		
	Min–Max	CV [%]	Mean	Min–Max	CV [%]	Mean	Min–Max	CV [%]	Mean
<i>R. agrestis</i>	1.00–1.80	10.76	1.298abcd (0.013)	0.03–0.08	18.85	0.053h (0.001)	0.04–0.10	20.19	0.068f (0.001)
<i>R. canina</i>	1.00–2.13	14.32	1.260de (0.008)	0.03–0.09	13.57	0.062ac (0.000)	0.05–0.13	16.27	0.078bc (0.001)
<i>R. dumalis</i>	0.94–1.82	13.19	1.273bcde (0.010)	0.03–0.08	14.83	0.058def (0.001)	0.05–0.14	18.56	0.075cde (0.001)
<i>R. gallica</i>	0.83–1.80	12.74	1.289bcd (0.006)	0.03–0.07	18.55	0.050i (0.000)	0.03–0.10	20.81	0.060g (0.000)
<i>R. inodora</i>	0.93–2.00	12.13	1.259de (0.011)	0.04–0.08	11.81	0.062abc (0.001)	0.05–0.13	16.26	0.077bcd (0.001)
<i>R. jundzillii</i>	1.00–1.73	12.01	1.226ef (0.012)	0.04–0.07	11.95	0.058defg (0.001)	0.05–0.10	15.27	0.071ef (0.001)
<i>R. kostrakiewiczii</i>	1.18–1.60	7.53	1.323abcde (0.018)	0.04–0.08	22.20	0.055bcdefghi (0.002)	0.05–0.11	24.26	0.075bcdef (0.003)
<i>R. majalis</i>	1.08–1.75	10.61	1.345a (0.012)	0.03–0.10	22.66	0.064a (0.001)	0.05–0.13	21.54	0.088a (0.002)
<i>R. micrantha</i>	1.00–1.64	11.40	1.272abcdef (0.019)	0.03–0.08	18.19	0.058bcdefgh (0.001)	0.01–0.11	22.59	0.075bcde (0.002)
<i>R. mollis</i>	0.86–1.70	13.15	1.324abc (0.016)	0.03–0.08	25.76	0.054gh (0.001)	0.03–0.11	25.66	0.072def (0.002)
<i>R. pendulina</i>	1.06–1.65	7.45	1.205f (0.005)	0.04–0.07	8.69	0.059bde (0.000)	0.05–0.10	8.74	0.071ef (0.000)
<i>R. rubiginosa</i>	0.93–1.78	12.36	1.275bcde (0.010)	0.04–0.08	14.23	0.061abcd (0.001)	0.05–0.13	15.80	0.080b (0.001)
<i>R. sherardii</i>	1.00–2.13	12.29	1.271cde (0.008)	0.03–0.08	20.69	0.054h (0.001)	0.03–0.11	24.17	0.068f (0.001)
<i>R. tomentosa</i>	1.07–1.88	11.77	1.324ab (0.013)	0.03–0.08	20.85	0.056fgh (0.001)	0.03–0.13	22.15	0.072def (0.001)
<i>R. villosa</i>	1.00–1.45	8.68	1.193def (0.019)	0.05–0.07	8.74	0.057bcdefgh (0.001)	0.06–0.09	12.72	0.068defg (0.002)
<i>R. zalana</i>	0.88–1.67	11.60	1.269bcde (0.016)	0.03–0.08	19.63	0.056efgh (0.001)	0.04–0.11	22.20	0.070ef (0.002)
ANOVA P>F			<0.0001			<0.0001			<0.0001





Table 6. Mean values ( $\pm$ SE) of pollen grains morphological features of *Rosa pendulina* [e.g. length of polar axis (P), length of equatorial axis (E), exine thickness on the pole (Exp), exine thickness at the equator plane (Exe), length of ectocolpi (Le), P/E ratio and relative thickness of exine (Exp/P and Exe/E ratio)]. One way ANOVA's were performed separately for each of the pollen grain feature to determine the differences among particular localities (=specimens). Same letters indicate a lack of statistically significant differences between analyzed specimens according to Tukey's a posteriori test ( $p < 0.05$ )

Locality	P [ $\mu$ m]	E [ $\mu$ m]	Exp [ $\mu$ m]	Exe [ $\mu$ m]	Le [ $\mu$ m]	P/E	Exp/P	Exe/E
Borowice	34.20ab (0.27)	27.10bcde (0.23)	1.99a (0.01)	1.99a (0.01)	26.73bc (0.30)	1.264a (0.014)	0.058bc (0.000)	0.073ab (0.001)
Tatra Mts, Dolina Koprowa	32.77bcd (0.23)	26.87cde (0.25)	1.97a (0.01)	2.00a (0.00)	26.73bc (0.28)	1.222abc (0.011)	0.060ab (0.001)	0.075a (0.001)
Tatra Mts, Cicha Dolina	31.33d (0.31)	26.53de (0.37)	1.96a (0.01)	1.97a (0.01)	24.47de (0.28)	1.185bcd (0.014)	0.063a (0.001)	0.075a (0.001)
Cyrlowa Skałka	32.00cd (0.24)	27.87abcd (0.29)	1.94a (0.02)	1.93abc (0.02)	26.67bc (0.26)	1.151d (0.012)	0.061ab (0.001)	0.069bc (0.001)
Tatra Mts, Nosal	32.87bc (0.20)	28.40ab (0.25)	1.99a (0.01)	1.99a (0.01)	26.83bc (0.23)	1.159cd (0.009)	0.061ab (0.000)	0.070abc (0.001)
Babia Góra	34.70a (0.43)	28.10abc (0.29)	1.93a (0.03)	1.88bc (0.03)	26.63bc (0.42)	1.237ab (0.016)	0.056c (0.001)	0.067c (0.001)
Bieszczady Mts, Tarnica	32.67cd (0.34)	28.60a (0.32)	1.93a (0.02)	1.93abc (0.02)	25.93cd (0.38)	1.144d (0.012)	0.059bc (0.001)	0.068c (0.001)
Bieszczady Mts, Połonina Caryńska	31.47cd (0.50)	26.40e (0.51)	1.91a (0.03)	1.91abc (0.03)	23.67e (0.51)	1.201abcd (0.024)	0.061ab (0.001)	0.073ab (0.002)
Karkonosze Mts, Mały Staw	34.77a (0.35)	28.00abc (0.28)	1.80b (0.03)	1.85c (0.03)	29.30a (0.32)	1.245ab (0.018)	0.052d (0.001)	0.066c (0.001)
Gzawa	34.17ab (0.30)	27.63abcde (0.24)	1.99a (0.01)	1.95ab (0.02)	27.90ab (0.36)	1.238ab (0.011)	0.058bc (0.001)	0.071abc (0.001)
ANOVA P>F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<.0001

dendrogram (Fig. 2) which revealed a distinct division of the examined localities (bushes) into three groups. With only slight deviations, grouping of localities from which individuals of the same species derived can be observed on the dendrogram.

All the examined localities of *R. gallica* were grouped together. Samples collected in Teresin and Krzywłina Mała as well as in Kojszków and Raczkowa were most similar. The exceptions included a sample derived from Ryki whose pollen grains were characterized by features more similar to *R. pendulina* than to *R. gallica* as well as samples from Kobylin and Kosobudy which were grouped together with *R. canina* samples. All *R. pendulina* samples showed fairly similar features. The most similar pollen grains were those derived from samples collected in Cyrlowa Skałka and Tarnica; pollen derived from samples collected in Cicha Dolina and Połonina Caryńska as well as Gzawa and Borowice were also similar. Material derived from the Tatra Mountains (Nosal), which was characterized by features most similar to samples of *R. canina* from Międzybrodzie, differed somewhat from the above samples. The most conspicuous difference was observed in the case of the sample of *R. pendulina* derived from Mały Staw characterized by features closest to the pollen of *R. canina* from Ojców as well as the majority of *R. gallica* samples. With three exceptions, *R. canina* localities also formed a

compact group. The most different material was that derived from Ojców which showed features closer to the *R. pendulina* sample derived from Mały Staw as well as to several *R. gallica* samples than to the remaining *R. canina* samples. On the other hand, pollen grains from Dolina Będkowska and Międzybrodzie grouped together with *R. pendulina* samples.

## Discussion

*Rosaceae* taxonomy assumes that pollen grain morphological features of individual species are of a conservative nature and as such, they can be of significant importance in investigations of taxonomic relationships, at least on the level of genus (Hebda et al. 1988; Kalkman 1988; Hebda and Chinnappa 1990, 1994; Ueda 1994).

In the case of the *Rosaceae* family and *Rosa* genus, palynologists usually use the exine sculpture and operculum (Fogle 1977; Matsuta et al. 1982; Marcucci et al. 1984; Menge 1985; Hebda et al. 1988; Ueda and Tomita 1989; Hebda and Chinnappa 1990; Ueda and Okada 1994; Popek 1996; Shinwari et al. 2004) as diagnostic features. However, many researchers maintain that features such as pollen shape, equatorial and polar axis of pollen grains and the length of colpi are useful criteria for species delimitation (Eide 1981; Hebda and Chinnappa 1990; Shinwari et al. 2004).



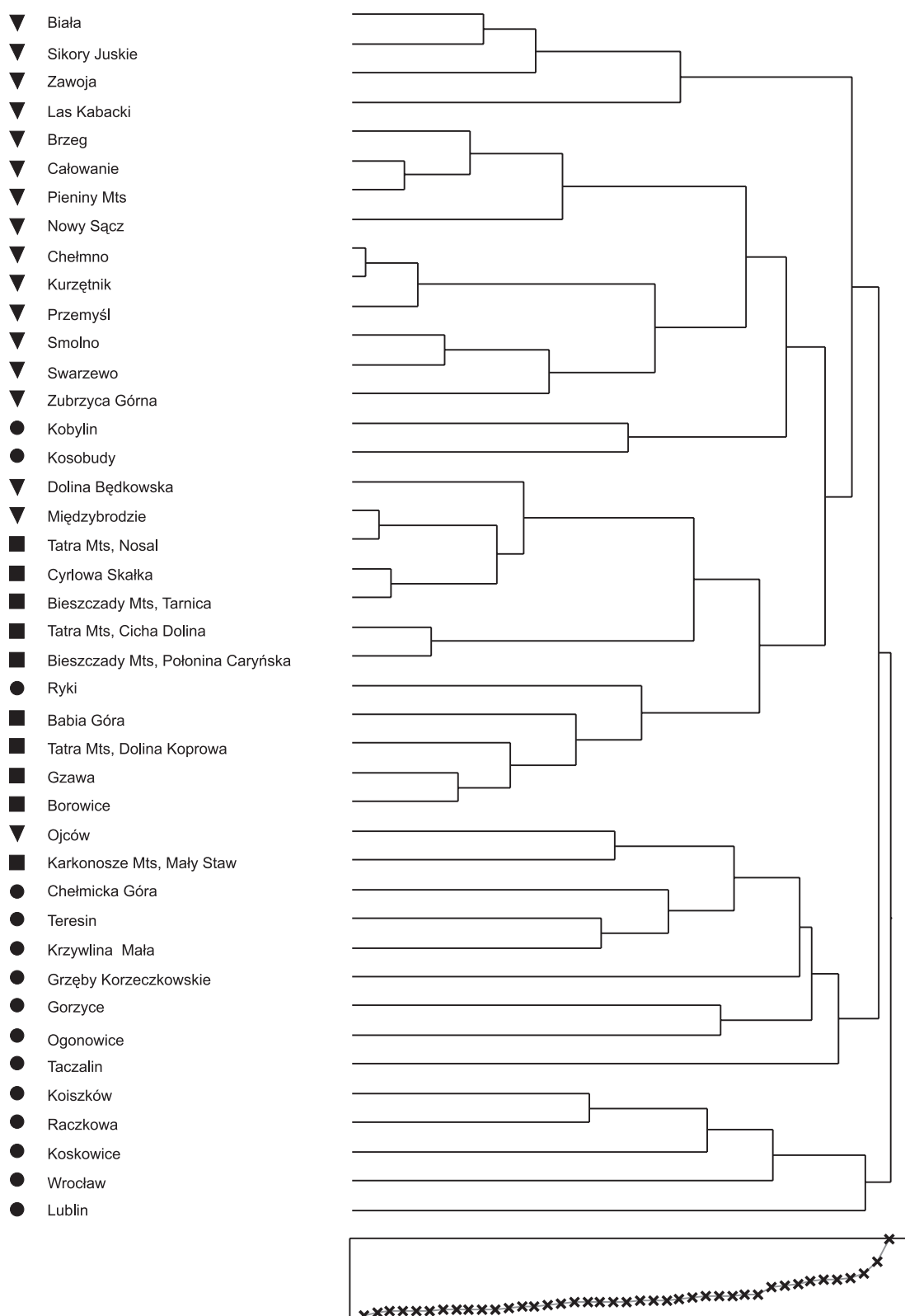


Fig. 2. Dendrogram of cluster groupings of all analyzed specimens of *R. gallica* (circle), *R. canina* (triangle) and *R. pendulina* (square) on the basis of pollen grain morphological features (i.e. P, E, Exp, Exe and Le).

Results obtained in our study confirm the usefulness of the examined pollen features in distinguishing rose sections and species. On the one hand, the authors observed on the obtained dendrogram (Fig. 2) the grouping of *Rosa gallica*, *R. canina*, and *R. pendulina* localities situated in different regions of Poland which showed that pollen features may indicate relationships within species and as such can be treated as auxiliary features in the process of identification of rose species. The obtained research results could have been affected by the selection for analyses of three “good species” as well as by a large number of pollen grains used in the biometric analysis. On the other hand, when all the examined pollen features were compared, it was impossible to identify individual species in accordance with their phylogenetic similarity since these divided only into two large groups which were not closely related taxonomically.

The arrangement of the examined species on the dendrogram (Fig. 1) only partially confirms the division of the *Rosa* genus into sections currently adopted in taxonomy (Henker 2000). Closely related *Rosa pendulina* and *R. majalis* from the *Cinnamomeae* section are grouped together but the same species are characterized by pollen features which are similar to roses from the *Caninae* section (*R. canina* and the remaining species from this section). This appears to confirm Zieliński's (1985, 1987) hypothesis about absence of a strict morphological borderline between the *Caninae* section and groups which contributed to its development, in particular with the *Cinnamomeae* section. On the other hand, *R. gallica* – the only domestic representative of the *Rosa* section, is characterized by features similar to species from the *Caninae* section, although it exhibits a certain peculiarity within this group.

The observed pollen morphological features only partially reflected the relationships between the examined species from the *Caninae* DC. em. Christ section described by Zieliński (1987) and Henker (2000). According to Zieliński (1987), the “initial” species for this section is *Rosa canina*. It is from this species that six developmental lines spread out forming *R. judzillii*, and then *R. micrantha* and *R. rubiginosa*, *R. agrestis* and *R. inodora*, *R. tomentosa*, *R. sherardii* and *R. villosa* as well as two separate species *R. dumalis* and *R. glauca*. Species closely related with one another (e.g. *R. tomentosa*, *R. sherardii* and *R. villosa*) as well as species from other developmental lines (e.g. *R. agrestis* or *R. micrantha*) were found in the same group in the obtained dendrogram. Pollen features of *R. canina* were most similar to those of *R. inodora*, *R. rubiginosa* and *R. dumalis*, and to a lesser degree, to *R. judzillii* (Fig. 1). The obtained ambiguous results were by no means surprising because the *Caninae* section is the most polymorphic group of the *Rosa* genus and contemporary *Caninae* are of the nature of a swarm of

hybrids with *R. canina* as a link connecting all section taxa (Zieliński 1985, 1987).

As mentioned earlier, the examined pollen quantitative features did not allow identification of each species. Using these features, it was only possible to identify two large species groups. This situation can be attributed to many factors and one of them was, undoubtedly, variability of the examined features. The least variable features included: length of polar axis (P) and length of equatorial axis (E), P/E coefficient associated with them and the length of ectocolpi (Le). Exine thickness (Exp and Exe) exhibited slightly higher variability, while the highest values of the mean coefficient of variation were determined in the features of the Exp/P and Exe/E ratio. These results could be attributed to unsatisfactory accuracy of measurements of some pollen features under the light microscope. Length of polar and equatorial axes (P, E) as well as the length of ectocolpi, which are the easiest features to measure, exhibited the least variability, whereas exine thickness – which is much more difficult to measure – and coefficients associated with it showed higher variability. Another result important for biometric investigations of pollen grains is the fact that pollen dimensions can differ from one another both on the intraspecific as well as on interspecific levels. For example, the range of length differences of *Rosa gallica* pollen grains can reach up to 50% and with regard to mean values – about 20%. This confirms doubts expressed by other palynologists concerning the value of *Rosaceae* pollen as a diagnostic feature. These objections stem from their considerable variability and from the method of acetolysis (Reitsma 1969; Moore et al. 1991; Jacob and Pierret 2000). To conclude, in order to obtain precise biometric data necessary to describe pollen of individual species, it is essential to measure large numbers of pollen grains.

The analysis of variability of studied features of individual species revealed that it was the greatest in the pollen grains of *Rosa majalis* and *R. mollis*, and the least in *R. pendulina*. The observed considerable variability of *Rosa mollis* pollen grains could be justified by very high variability of its other morphological features and very few diagnostic features (Henker 2000). That explains why in classical taxonomy *R. mollis* is treated as a poorly distinguished and included in the *R. villosa* L. s.l. (Klaštensky 1968; Zieliński 1987) species, although some researchers classify it as a separate species (Popek 1996; Henker 2000). On the other hand, the results concerning *R. majalis* and *R. pendulina* contradict opinions accepted in taxonomy according to which both these species are closely related (belong to the same *Cinnamomeae* section) with *R. pendulina* considered to be a variable species, while *R. majalis* is considered a taxon of low variability (Popek 1996; Henker 2000).

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