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Pollen morphology of Polish native species of the *Rosa* genus (Rosaceae) and its relation to systematics

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Abstract

The morphology of pollen grains of 16 species from the *Rosa* L. genus were studied (i.e. *R. 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 16 native localities of those species in Poland. The measurements are based on at least 30-50 randomly selected, fully developed pollen grains per specimen. In total, 500 pollen grains were examined. They were analysed for 13 quantitative features of pollen grains and exine sculpturing and the following qualitative traits: outline, shape, “operculum” structure. The diagnostic features of pollen grains of studied species were: length of polar and equatorial axes and length of ectocolpi. The above-mentioned pollen grain morphological features make isolation of one species possible: *R. gallica*. *R. gallica* is distinguished for its highest values of the length of polar and equatorial axes, and the length of ectocolpi. The obtained analytical results of operculum and exine sculpture features, considered as diagnostic, corroborated only slightly their priority significance for the isolation of the examined species and sections. The collected data failed to confirm fully the current taxonomical division of the *Rosa* genus into sections (only section *Gallicanae* from *R. gallica* is isolated) as well as consanguinity relationships between the examined species from the *Caninae* section. On the dendrogram, both species closely related with each other as well as those from other developmental lines were found in the same group. These equivocal results are by no means surprising because the *Caninae* section is the most polymorphic group in the *Rosa* genus, and contemporary *Caninae* are of the nature of a swarm of *R. canina* hybrids as a link combining all taxons of the section.

Keywords: Pollen morphology, *Rosa*, *Caninae*, *Gallicanae*, taxonomy

Introduction

The *Rosa* L. genus constitutes one of 36 European genera in the Rosaceae family [1]. The genus contains, depending on the adopted approach, 100 to 120, or even 250 species and is distributed in the northern hemisphere in Europe, Asia, Ethiopia, the Middle East and North America [2-6].

According to Klaštersky [1], 47 rose species deriving from 5 sections grow currently in Europe. Henker [5] claims that, depending on the approach, 30 to 60 species from the *Rosa* genus occur in Europe, of which 33 species can be found in Central Europe. The majority of European roses, including the studied species, belong to the *Caninae* section [1,5,7].

Zieliński [3,7], who follows the concept of broad approach to species, mentions 14 rose species from Poland which represent the following three sections: *Caninae* (11 species), *Rosa* (2 species), and *Gallicanae* (1 species), whereas Popek [8] enumerates 16 species including the *Caninae* section also *R. kostrakiewiczii* and *R. mollis*. Nearly all (15) of these species occur naturally in Central Europe [5]. The only exception is an endemic *R. kostrakiewiczii* Popek reported by Popek [8] from a single site situated in south-eastern Poland in the Góry Pieprzowe near Sandomierz (50°40'N/21°45'E).

The *Rosa* L. genus belongs to a group of plants which are critical and systematically more complex. Among the most important causes of the polymorphism observed in this genus are: hybridisation, polyploidy and – especially in the *Caninae* section – the mechanism of cytotype stabilisation of odd chromosome numbers resulting from the specific course of the so called *Caninae* type meiosis [7,9,10].

Palynological investigations of the *Rosa* L. genus were carried out by numerous researchers and their beginnings go back to the end of the 19th century [8,11-46].

Despite numerous publications, our knowledge about the structure of rose pollen grains is fragmentary because the available descriptions usually refer to one or several selected taxons or researchers analyse few selected pollen features. At the present time, in their investigations on pollen morphology of genus *Rosa*, palynologists focus on detailed analyses of exine sculpture features considered to be the most important distinguishing characters in *Rosa* pollen grains [8,27,35,36,38].

In the course of this study, pollen grain morphology of 16

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Tab. 1 Location of studied pollen samples of *Rosa*.

Species	Localities	Position	Collector, herbarium
R. agrestis Savi*	Prov. Świętokrzyskie, Pieprzowe Mts, near Sandomierz	50°41'N/21°45'E	Wrońska-Pilarek D; POZNF
R. canina L.*	Prov. Mazowieckie, Kabacki Forest near Warszawa	52°14'N/21°01'E	Dmowska H; UW
R. dumalis Bechst.*	Prov Lubuskie, Cigacice	52°02'N/15°37'E	Wrońska-Pilarek D; POZNF
R. gallica L.***	Prov. Dolnośląskie, Koskowice	51°11'N/16°14'E	Szlachetka A, Wrońska-Pilarek D; POZNF
R. inodora Fr.*	Prov. Małopolskie, near Ostra Skała, Pieniny Mts	49°24'N/20°23'E	Szeląg Z; KRAM
R. jundzillii Besser*	Prov. Dolnośląskie, between Wołów and Krzywlina Mała	51°21'N/16°39'E	Zieliński J; KOR
R. kostrakiewiczii Popek*	Prov. Świętokrzyskie, Pieprzowe Mts, near Sandomierz	50°41'N/21°45'E	Popek R, Wrońska-Pilarek D; POZNF
R. majalis Herrm.**	Prov. Świętokrzyskie, Białogońska Góra, Białogon	50°51'N/20°33'E	Kazanowski K; KOR
R. micrantha Borrer ex Sm.*	Prov. Lubuskie, Połęcko	52°03'N/14°54'E	Boratyński A, Zieliński J; KOR
R. mollis Sm.*	Prov. Podlaskie, Leśna	51°01'N/15°16'E	Sokołowski A; KRAM
R. pendulina L.**	Prov Małopolskie, Nosal, Tatra Mts.	49°14'N/19°58'E	Wrońska-Pilarek D; POZNF
R. rubiginosa L.*	Prov. Dolnośląskie, Ostrowąsy, near Milicz	51°36'N/17°29'E	Kaczmarek C; KRAM
R. sherardii Davies*	Prov Lubuskie, Cigacice	52°02'N/15°37'E	Wrońska-Pilarek D; POZNF
R. tomentosa Sm.*	Prov. Podkarpackie, Besko	49°36'N/21°57'E	Wrońska-Pilarek D; POZNF
R. villosa L.*	Prov. Wielkopolskie, between Mełpin and Kadzewo, near Dolsk	52°01'N/17°00'E	Wrońska-Pilarek D; POZNF
R. zalana Wiesb.*	Prov. Lubuskie, Rudnica	52°36'N/15°12'E	Boratyński A, Zieliński J; KOR

* Species from section *Caninae* DC. em. Christ. ** Species from section *Cinnamomeae* DC. *** Species from section *Rosa* L. PZNF – Herbarium of Department of Forestry Natural Foundations University of Life Sciences in Poznań; UW – Herbarium of Warsaw University; KRAM – Herbarium of Institute of Botany in Krakow; KOR – Herbarium of Institute of Dendrology in Kórnik.

rose species (Tab. 1) – i.e. all species which have their natural sites in Poland (according to Popek [8]) – were investigated. The above roses belong to the following three sections: *Caninae*, *Rosa* and *Gallicanae* [3,5].

The objective of the described investigations was to verify taxonomic usefulness of the examined pollen morphological attributes of 16 domestic rose species, with a special emphasis on exine sculpture and operculum structure features considered as diagnostic. The investigations were conducted using LM and SEM with the aim to check their usefulness for distinguishing sections and individual species from the *Rosa* genus. The interspecific differentiation of the analysed features has been also studied by using statistical methods. Palynological investigations based on such numerous quantitative and qualitative pollen grain features (over 20 features) have not been presented in formerly published literature with reference to the *Rosa* genus.

Material and methods

The pollen grains were collected in Poland, from 16 localities in the wild (Tab. 1). Some to several randomly selected flowers were collected from each individual (rose bush). The measurements are based on at least 30-50 fully developed pollen grains per specimen. In total, 500 pollen grains were analysed. They were analysed for 13 quantitative features of pollen grains and exine sculpturing and the following qualitative ones: outline, shape, "opercula" structure (Tab. 2). The structures described as "opercula" refer to a distinctly delimited sexine/ectexine structure which covers part of an ectoaperture and which is completely isolated from the rest of the sexine [47]; there can be, in some cases, no real opercula, but bulges of the intine at the beginning of pollen tube germination. Despite the above reservations, the term "operculum" is employed in this study as it is used by all palynologists who have described this structure so far [8,18,19,27,32,33,36,37,40].

Exine sculpture elements were measured on the area of 25 μm^2 in accordance with the methods of Ueda and Okada [48] and Ueda and Tomita [35].

All samples were acetolysed according to Erdtman's method [16], slightly modificated by Wrońska-Pilarek [49]. The terminology follows Punt et al. [50] and Hesse et al. [51]. The observations were carried out both with light microscope (Biolar 2308, Nikon HFX-DX) and scanning electron microscope (ISI 60, Zeiss 435 VP).

The empirical data from grain measurements embraced quantitative features (Tab. 2) Their analysis included descriptive statistics and correlation coefficients, univariate analysis of variance and Tukey's test, agglomerative grouping by Ward's

Tab. 2 Pollen grains features analysed.

No.	Features
1	Length of polar axis (P)
2	Length of equatorial axis (E)
3	Thickness of exine along polar axis (Exp)
4	Thickness of exine along equatorial axis (Exe)
5	Length of ectocolpi (Le)
6	P/E ratio
7	Relative thickness of exine (P/Exp ratio)
8	Relative thickness of exine (E/Exe ratio)
9	Number of perforations (Np)
10	Total area of perforations (Ap)
11	Number of muri (Nm)
12	Width of muri (Wm)
13	Width of striae (Ws)

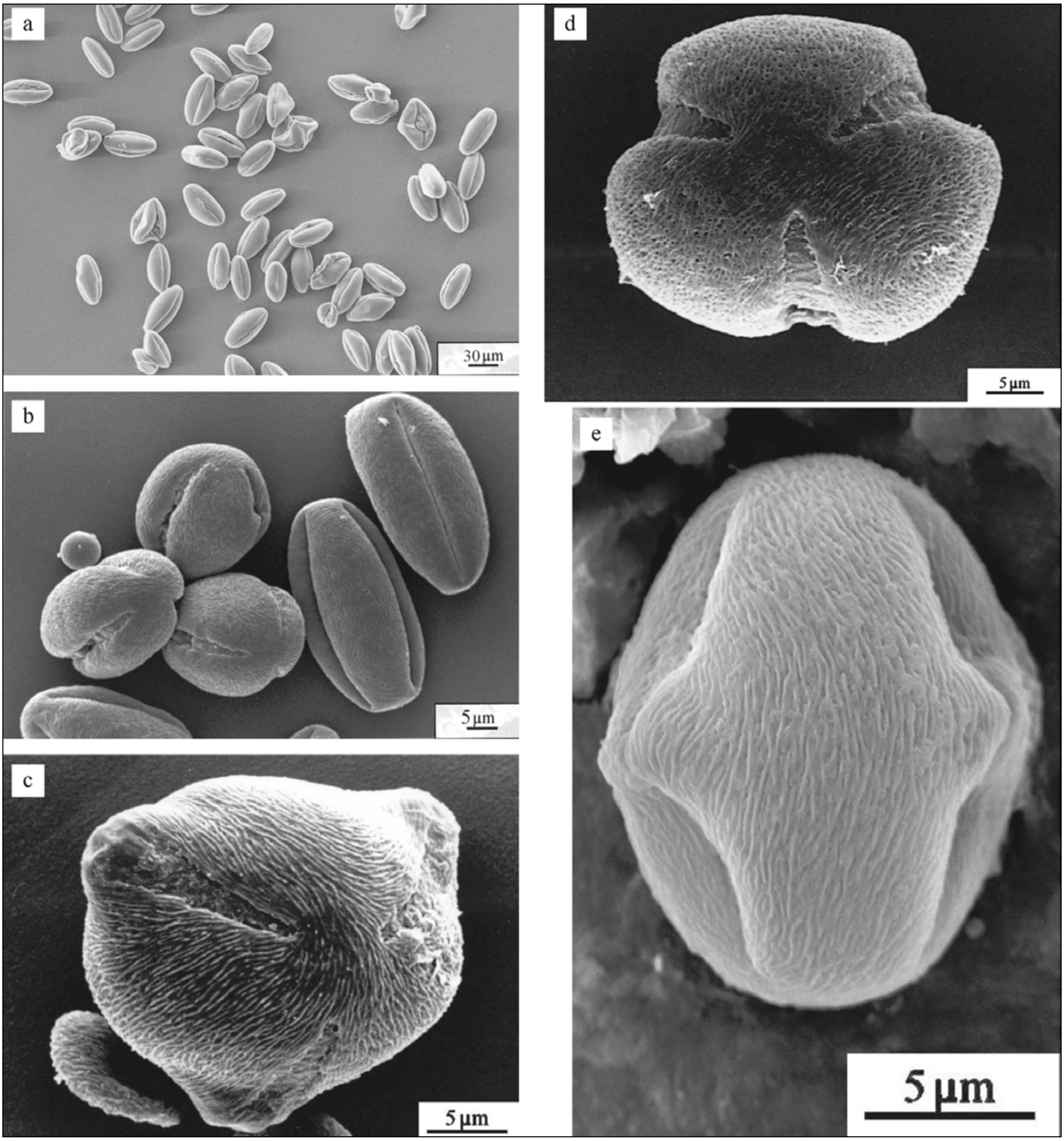

Fig. 1 SEM micrographs of pollen grains of studied *Rosa* species. **a,b** *R. gallica*, *R. pendulina*, respectively. Large, fully developed pollen grains and much smaller, not completely developed pollens in polar and equatorial view. **c** *R. indora*. Pollen grains in polar view – polar area, three ectocolpi and opercula visible. **d** *R. pendulina*. Pollen grains in polar view – polar area and three ectocolpi visible. **e** *R. agrestis*. Pollen grain in equatorial view – two ectocolpi with bridge visible.

method. Statistical analyses were performed using JMP 8.0 (SAS Institute Inc., Cary, NC, USA).

Results

General pollen morphological description

The grains of the studied *Rosa* species are: 3 – zonocolporate (all species), rarely 4 – zonocolporate (e.g. *R. canina*, *R. jundzillii*, *R. micrantha*), isopolar (Fig. 1c,d, Fig. 2a).

Apart from fully developed pollen grains, also much smaller, not completely developed pollen were found in the samples of the examined species (Fig. 1a,b). The majority pollen, according to Erdtman's [16] pollen size classification, is medium (25-50 µm; 92.7%), rarely small (10-25 µm; 7.3%). On average, the largest pollen were found in *R. gallica* (35.5 µm), while the smallest – in *R. majalis* (27 µm). The greatest numbers of small pollen grains were determined in samples of *R. kostrakiewiczii* (36.7% of measured pollen) and in *R. majalis* (20%); in samples of some other species (*R. inodora*, *R. micrantha*, *R. sherardii*) 6.7% to 10% (*R. mollis*, *R. pendulina*, *R.*

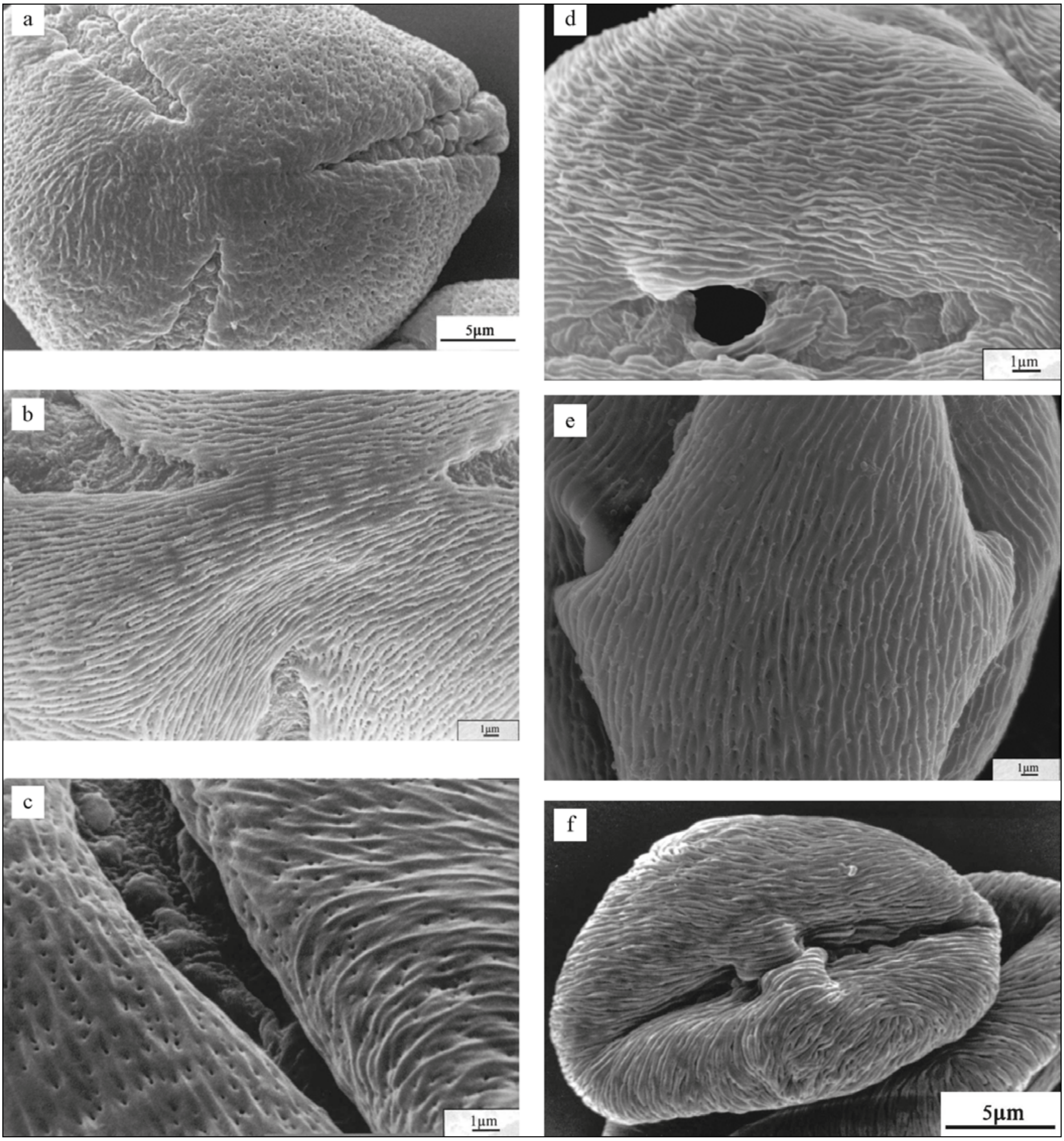

Fig. 2 SEM micrographs of pollen grains of studied *Rosa* species. **a** *R. pendulina*. Pollen grain in polar view – polar area and three ectocolpi visible. **b** *R. gallica*. Polar area and three ectocolpi visible. **c** *R. jundzillii*. Granulate ectocolpus and striate exine sculpture visible. **d** *R. agrestis*. Ectocolpus with opened endoporus visible. **e,f** *R. majalis*, *R. canina*, respectively. The bridge visible.

zalana) small pollen were found.

The average length of polar axes (P) amounted to 29.17 (24-36) µm. The shortest average pollen axes P were found in *R. inodora*, *R. kostrakiewiczii*, *R. majalis* and *R. zalana* (21.6 µm each), while the longest ones – in *R. gallica* (41.4 µm). The mean length of equatorial axes (E) amounted to 22.93 (16.2-34.2) µm. On average, the shortest equatorial axes were determined in *R. inodora*, *R. kostrakiewiczii*, *R. micrantha*, *R. mollis* and *R. zalana* pollen (16.2 µm each), while the longest – in *R. gallica* (34.2 µm).

Outline in polar view – mostly circular or triangular with obtuse apices, more rarely elliptic. In equatorial view – mostly elliptic, rarely circular (Fig. 1a-e, Fig. 3a-c).

The average P/E ratio was 1.24 and ranged from 0.84 in *R. gallica* to 1.77 in the same species. It is evident that *R. gallica* stood out in terms of very significant variability of the pollen shape. The majority of pollen of the examined species were subprolate (55.42%), less frequently – prolate (22.71%) and prolate-spheroidal (17.29%), rarely – spheroidal (3.54%) and oblate-spheroidal (1.04%). Most of the prolate pollen were found in *R. gallica* (53.3%) and *R. majalis* (50%). Prolate-spheroidal and spheroidal pollen were found predominant (66.7%) only in *R. jundzillii*.

Exine is two-layered, well marked in LM (Fig. 3a-c).

Fig. 3 LM micrographs of pollen grains of *R. pendulina*. **a** Three pollen grains in polar and equatorial view; exine visible. **b** Pollen grain in polar view; exine and two ectocolpi visible. **c** Pollen grain in equatorial view; exine and two ectocolpi visible.

Ectexine and endexine were usually of the same thickness, although sometimes ectexine was thicker. Mean exine thickness was found to be 1.61 (0.9-1.98) μm. The thinnest exine was determined in *R. mollis* (on average: Exp-1.36 μm; Exe-1.42 μm), while the thickest - in *R. canina* (on average: Exp-1.8 μm; Exe-1.8 μm). The relative thickness of the exine (Exp/P ratio) averaged 0.06 (0.03-0.08) and (Exe/E ratio) 0.07 (0.04-0.1). The above results are similar indicating more or less equal exine thickness along the entire pollen grain.

Exine sculpture – striate, variable, sometimes less, sometimes more distinct in LM. Muri usually ran parallel to the polar axis but frequently they also formed loops. They were straight or forked of varying length and width (Fig. 4a-f). On average, 11 muri were found on the area of 25 μm^2 and their numbers ranged from 8 (*R. dumalis*, *R. pendulina*) to 13 (*R. jundzillii*, *R. sherardii*). The widths of muri and striae were similar and averaged 0.26 (0.14-0.40) μm for muri and 0.25 (0.09-0.45) μm for striae. Part of the examined species (e.g. *R. gallica*, *R. jundzillii*, *R. zalana*) had muri and striae of similar width, while in others – either muri or striae were wider. The biggest differences between muri and striae widths were observed in *R. mollis* (0.40 μm, 0.09 μm) and *R. inodora* (0.14 μm, 0.45 μm; Tab. 3).

Circular or elliptic perforations of very different diameters (on average – 0.1-0.3 μm; ranging from 0.05 to 0.8 μm) were found at the bottom of striae (Fig. 4a-f). The mean number of those perforations on the area of 25 μm^2 amounted to 69.7 and ranged from 22 (*R. majalis*) to 145 (*R. gallica*), whereas the average area taken up by them amounted to 0.85 μm^2; ranging from 0.42 μm^2 (*R. sherardii*) to 2.16 μm^2 (*R. canina*). The examined species were assigned to 4 types of exine sculpture distinguished by Ueda [38]. Majority of them belong to II A type (*R. canina*, *R. gallica*, *R. jundzillii*, *R. kostrakiewiczii*, *R. majalis*, *R. micrantha*, *R. mollis*, *R. pendulina*, *R. rubiginosa*, *R. tomentosa*, *R. villosa*, *R. zalana*), others to II B type (*R. inodora*, *R. pendulina*, *R. sherardii*, *R. zalana*), III A type (*R. agrestis*, *R. dumalis*) and to III B type (*R. agrestis*, *R. canina*, *R. dumalis*, *R. tomentosa*).

Apertures 3, very rarely 4 ectocolpi and endopores. Usually one, rarely two endopores occurred in ectocolpi. Ectocolpi were arranged meridionally, regularly, more or less evenly spaced (Fig. 2a-d). They were long, with mean length of 22.49 μm ranging from 16.2 μm (*R. inodora*) to 32.4 μm (*R. agrestis*); on average, ectocolpi constituted 79.8% of the length of the polar axis. On average, the longest ectocolpi were determined in *R. gallica* pollen (26.3 μm), while the shortest – in *R. canina* (20.5 μm). Ectocolpi were acute and elliptic in outline. Widths were variable, usually greatest in the equatorial region with sculpturing of ectocolpus membrane approaching regulate. In some species (e.g. *R. agrestis*, *R. canina*, *R. majalis*, *R. rubiginosa*, *R. tomentosa*) ectocolpus crossed at the equator by a bridge dividing into two parts, formed by two intersecting bulges of ectexine (Fig. 2e,f). Bulges were of the same, or unequal length. Ectocolpus margins frequently with small undulations. Costae colpi present. Endopores (1, rarely 2) usually located in the middle of ectocolpi, readily visible after opening, with irregular margins (Fig. 2d). Fastigium present.

Operculum occurred usually in the central part of the ectocolpus, sometimes situated symmetrically; partially covering the ectocolpus. Usually, it was more or less a convex, elongated, exceptionally rosette-form structure (*R. kostrakiewiczii*; Fig. 5a). Operculum may vary from convex, large (about 1/2-1/4

Tab. 3 Exine sculpture features analysed.

Species	Np	Ap (μm^2)	Nm (μm)	Wm (μm)	Ws (μm)
R. agrestis	82	0.44	11	0.30	0.22
R. canina	73	2.16	12	0.18	0.29
R. dumalis	60	1.00	8	0.33	0.39
R. gallica	145	1.19	13	0.20	0.22
R. inodora	62	0.86	9	0.14	0.45
R. jundzillii	63	0.75	13	0.22	0.24
R. kostrakiewiczii	59	0.48	12	0.30	0.14
R. majalis	22	0.66	11	0.29	0.18
R. micrantha	85	0.44	10	0.30	0.12
R. mollis	64	0.75	10	0.40	0.09
R. pendulina	62	0.82	8	0.18	0.36
R. rubiginosa	64	1.47	9	0.23	0.32
R. sherardii	58	0.42	13	0.22	0.28
R. tomentosa	58	0.58	11	0.30	0.18
R. villosa	101	1.08	11	0.31	0.22
R. zalana	57	0.45	11	0.32	0.29

For feature names, see Tab. 2.

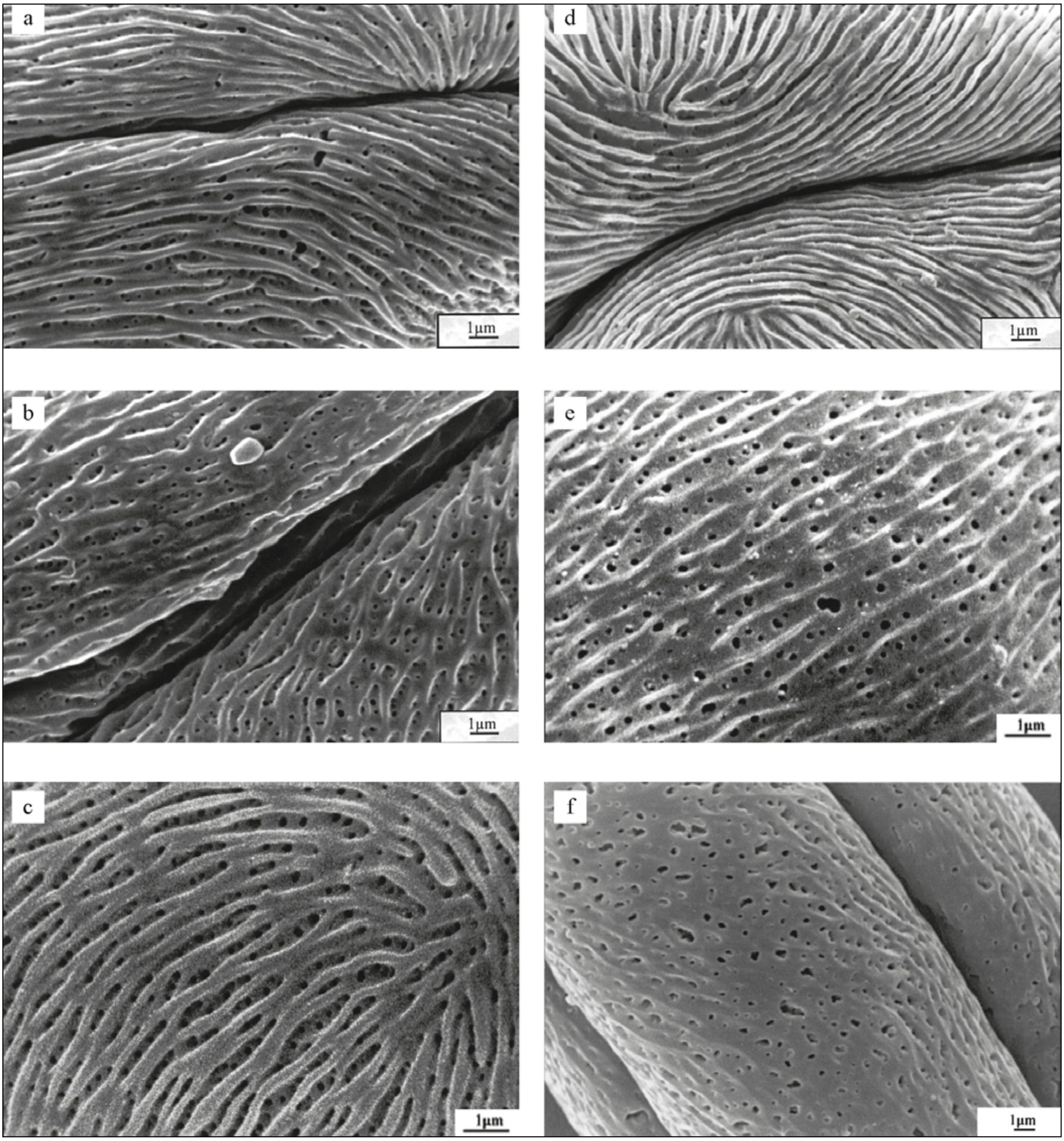

Fig. 4 SEM micrographs of a few striate exine sculpture types of studied *Rosa* species. **a,b** *R. rubiginosa* and *R. dumalis*, respectively. Muri clearly visible, quite high, numerous perforations with very different diameters. **c** *R. gallica*. Muri clearly visible, quite high, slightly flat, perforations with similar diameters. **d** *R. zalana*. Muri clearly visible, quite high, less numerous perforations, smaller with similar diameters. **e,f** *R. inodora* and *R. pendulina*, respectively. Muri lower and narrower, vanishing in some places, numerous perforations with very different and sometimes quite large diameters.

length of ectocolpus), wide (usually equal but sometimes wider than the width of ectocolpus; e.g. *R. canina*, *R. gallica*, *R. inodora*, *R. jundzillii*, *R. kostrakiewiczii*, *R. majalis*, *R. micrantha*, *R. pendulina*, *R. tomentosa*, *R. zalana*) to narrow (equal or narrower than the width of ectocolpus), elongated and flat in *R. pendulina* (Fig. 5b-f). Operculum sculpture usually psilate, less often striate; operculum surface often corrugated.

Despite the fact that the operculum structures of the examined species undergo certain variability, nevertheless all of them should be assigned to a type common for *R. canina*. The author did not decide to classify the *R. kostrakiewiczii* as a separate type. To begin with, a rosette-like, irregularly formed structure was observed only in three out of 60 pollen grains examined specially from the point of view of this trait. In part of the remaining pollen, opercula of the structure similar to the remaining examined species occurred. Secondly, it is also possible that it is not a operculum but a bulging of the intine. Therefore, at the current stage of investigations, the presence of that structure in *R. kostrakiewiczii* cannot be considered as pollen diagnostic feature of this species. If detailed

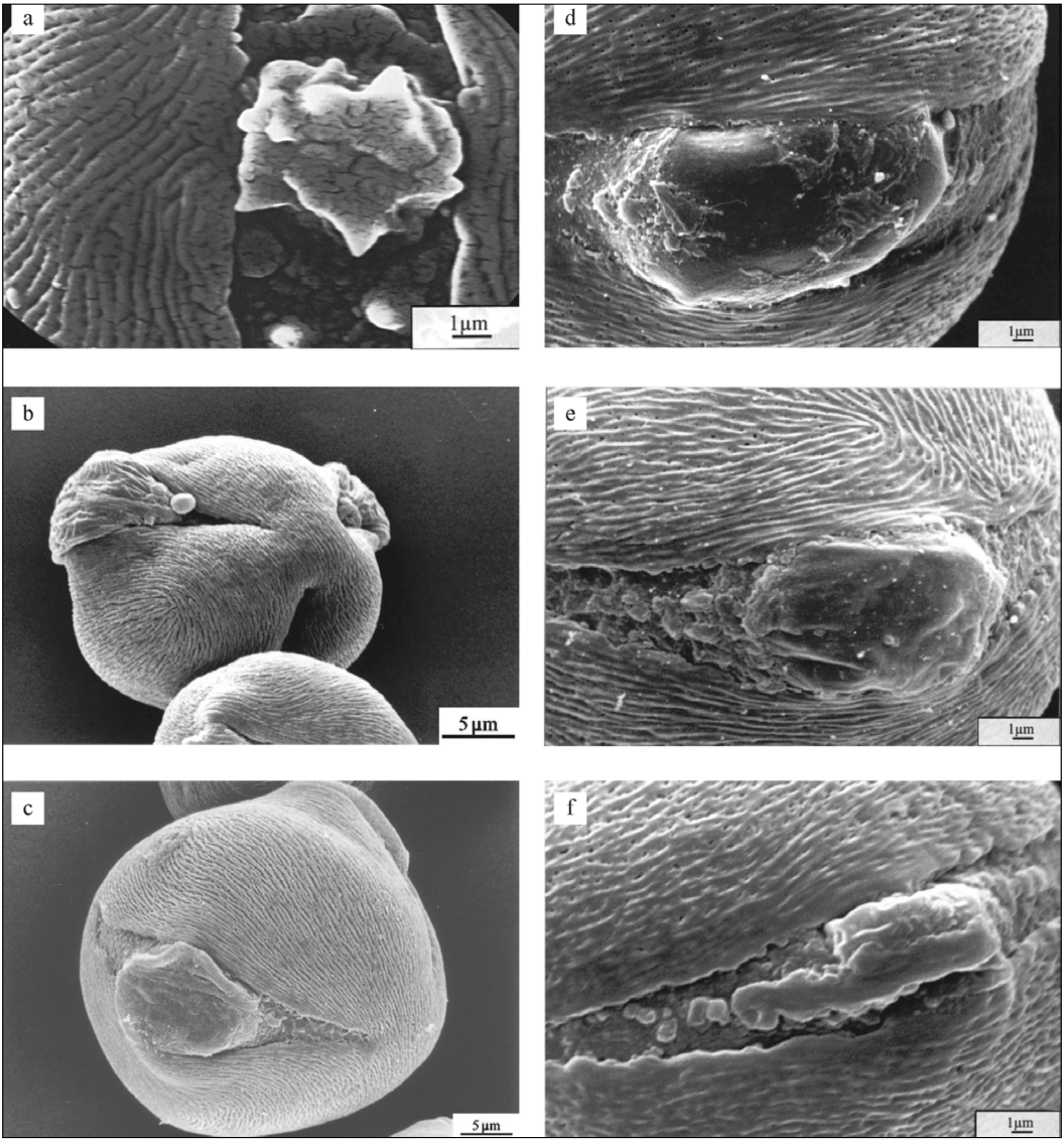

Fig. 5 SEM micrographs of pollen grains of studied *Rosa* species. **a** *R. pendulina*. Pollen grain in polar view – polar area and three ectocolpi visible. **b** *R. gallica*. Polar area and three ectocolpi visible. **c** *R. jundzillii*. Granulate ectocolpus and striate exine sculpture visible. **d** *R. agrestis*. ectocolpus with opened endoporus visible. **e,f** *R. majalis*, *R. canina*, respectively. The bridge visible.

morphological and anatomical studies of this structure confirm its presence, a separate operculum type – *R. kostrakiewiczii* may be established.

Interspecific variability of pollen grains

All the examined features were characterised by moderate variability. Mean coefficients of variability (CV) calculated for the analysed pollen features of all experimental species amounted to: P – 11.3%, E – 13.4%, P/E – 11.9, Exp – 15.1%, Exe – 13.9%, Exp/P – 16.6%, Exe/E – 18.4%, Le – 11.3%. P, E and Le features exhibited lower values of the coefficient of variability than exine thickness features (Exp, Exe, Exp/P, Exe/E). *R. mollis* and *R. kostrakiewiczii* belong to species which show the highest values of the coefficient of variability with regard to all the examined pollen grain features, while e.g. *R. pendulina* and *R. canina* – to those with the lowest values (Tab. S1).

On the basis of the analysis of diagonal elements of the inverse matrix to the correlation matrix R for P, E, P/E, Exp, Exe, Exp/P, Exe/E and Le features, features with low values of the diagonal element which indicated a poor correlation of a given trait with the remaining ones were selected for further investigations. The following trait system of P – 2.56, E – 1.48, Exp – 1.48, Exe – 1.43 and Le – 2.28 diagonal elements was selected.

In order to verify the presumption about dissimilarity of the examined pollen parameters in the 16 examined species, a single factorial analysis of variance was conducted. Assumptions about the normal distribution (Shapiro-Wilk test) and variance uniformity (Levene test) were verified. Due to the lack of homogeneity of the analysed attributes, a logarithmic transformation was employed. Analysing the empirical value of *F*-statistics and probability *p* for each analysed attribute, significant differences at the significance level of 5% were found (feature P – $F_{calc} = 24.70$ and $p = 0.000$, feature E – $F_{calc} = 21.75$ and $p = 0.000$, feature Exp – $F_{calc} = 6.63$ and $p = 0.000$, feature Exe – $F_{calc} = 8.68$ and $p = 0.000$ and feature Le – $F_{calc} = 11.39$ and $p = 0.000$). When analysing individual species (1-16), the absence of significant differences for each attribute was marked by two dots connected by a dashed line (Fig. 6).

It is evident from Fig. 3a that *R. kostrakiewiczii* exhibited the smallest pollen grains (features P, E), not very high mean exine thickness (features Exp, Exe) as well as one of the shortest ectocolpi (feature Le). Also *R. mollis* was characterised by low mean values of the examined characters. On the other hand, *R. gallica* showed the largest pollen (features P, E) and the longest ectocolpi (feature Le) accompanied by low and moderate mean values of the exine thickness (features Exp, Exe).

The above data were confirmed by the dendrogram obtained as a result of agglomeration grouping using Ward method (Fig. 7). *R. gallica* stands apart from all the remaining species which were assigned to one of the following three groups' the first group comprises *R. mollis* and *R. kostrakiewiczii*, the second – *R. majalis*, *R. zalana*, *R. micrantha*, *R. rubiginosa*, *R. inodora* and *R. canina*, and the third – *R. jundzillii*, *R. dumalis*, *R. villosa*, *R. tomentosa*, *R. sherardii*, *R. pendulina* and *R. agrestis*.

Discussion

Morphological descriptions of pollen grains of the following examined species can be found in palynological literature: *R. canina*, *R. gallica*, *R. majalis*, *R. micrantha*, *R. pendulina* and *R. rubiginosa* [19,21,22,26,27,31,33,44,45]. They are similar to the descriptions presented in this article.

Shinwari and Khan [43] maintain that the palynological features of exine thickness, shape, length of equatorial and polar axes and length of ectocolpi were found useful criteria for species of genus *Rosa* delimitation; while sculpturing at LM turned out to be poor criteria. Results presented here corroborate this opinion only partly and indicate that among characters which allowed identification of some of the examined species were: length of polar and equatorial axes, length of ectocolpi and the operculum structure.

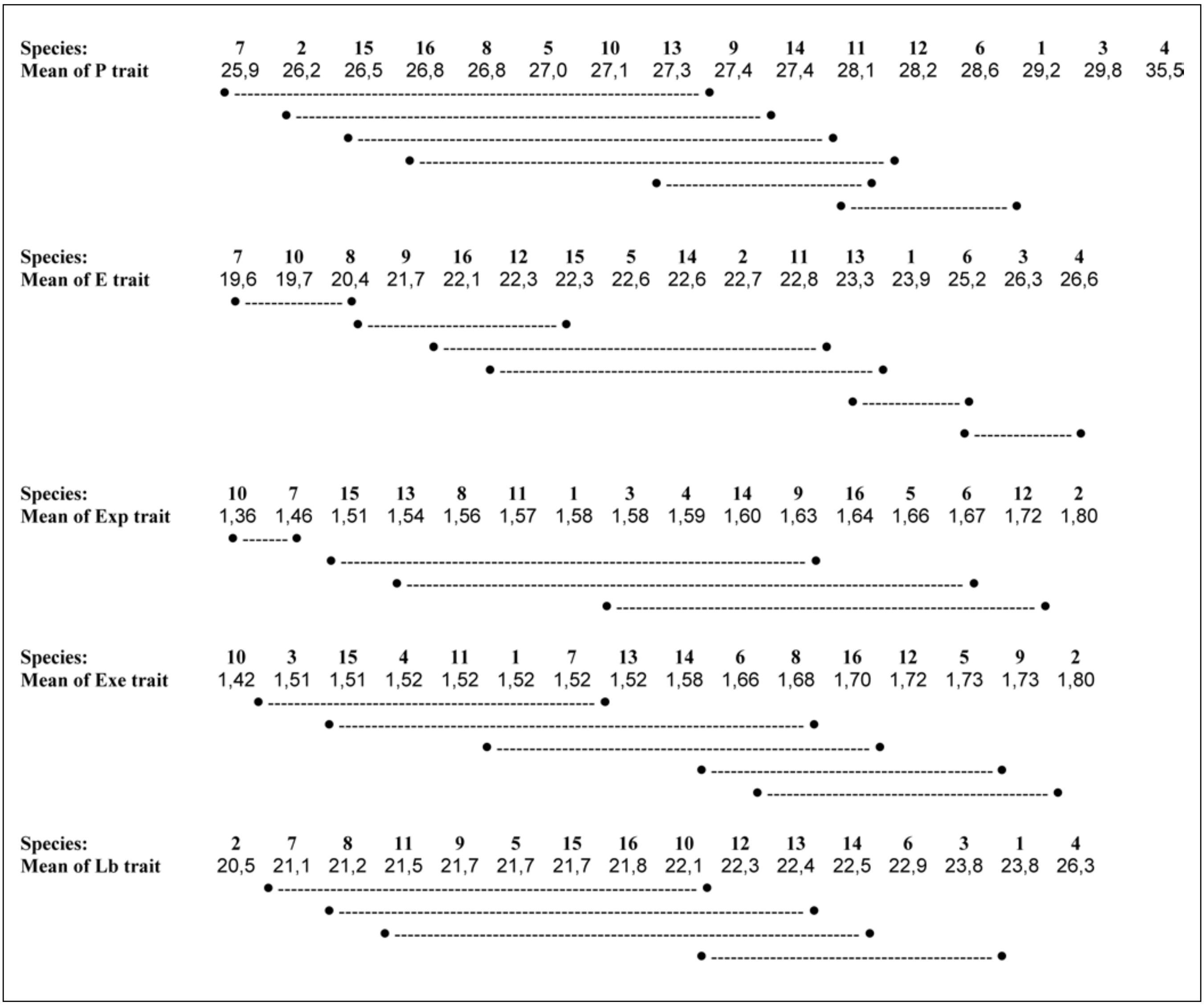

Fig. 6 Multiple comparisons by Tukey's test.

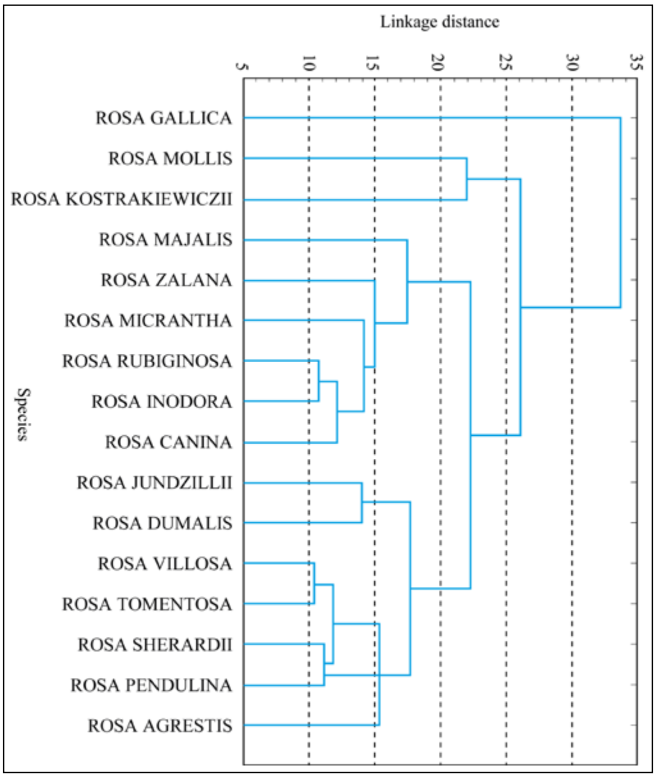

Fig. 7 Dendrogram of cluster groupings of 16 studied species on the basis of pollen grain morphological features.

Reitsma [19] showed that many north-western European rosaceous taxa could be separated on the basis of pollen morphology. He claims that among the most important attributes of *Rosa* pollen grains are: the type of exine sculpture as well as the presence or absence of costae colpi and operculum structure. On their basis, he distinguished the following three pollen grain types: *R. gallica* type, *R. canina* type and *Rubus idaeus* type, to which he also included *R. rubiginosa*, *R. agrestis* and *R. rugosa*. The results of this study corroborate the rightness of separation of *R. gallica*, although they do not justify the distinction of the two remaining types.

Also other palynologists confirm the diagnostic significance of the exine sculpture as a distinguishing attribute of Rosaceae pollen grains both at genus and species levels [8,27,35,36,38]. The most important features of exine sculpture include the number and size of perforations as well as the interval, number and diameter of striae [36,48,52-55]. Hebda and Chinnappa [36,56] distinguished two types of perforations in Rosaceae (striate sculpturing macroperforate and non-striate sculpturing macroperforate, each with 6 subtypes) possibly pointing to different evolutionary lines. Roses were incorporated into the first type of large perforations often extending onto tectal striae. In addition, they also emphasised the fact that in *Rosa*, striae are long and parallel to ectocolpus. This classification was also corroborated by this study. According to the above researchers, pollen of *Rosa* (with *Prunus*, *Rubus* and *Spirea*) belong to the subcategory with striae separated by muri, containing larger perforations (0.1-0.2 μm in diameter). Investigation results presented here confirm this hypothesis with the reservation that, at such variability of perforation diameters as in the case of *Rosa* (ranging from 0.05 to 0.8 μm), it would perhaps be advisable to indicate that "larger" refers to medium diameter values of perforations. Menge [57], on the other hand, emphasises that the number of perforations is also an important trait of exine sculpture. The priority importance of this attribute was not corroborated by this study with regard to the examined species because majority (10) of the 16 examined species exhibited a similar number of perforations (57-64) on the area of 25 μm^2. On the other hand, values of this character varied considerably (from 22 perforations in *R. majalis* to 145 in *R. gallica*). Perforations also exhibited differences in their diameters and therefore, their numbers do not always correspond to the area they occupy (e.g. 22 perforations in *R. majalis* large enough to occupy the area of 0.66 μm^2, whereas in *R. tomentosa* and *R. jundzillii*, a similar area was taken up by 58 and 63 perforations, respectively).

Ueda and Tomita [35] examined pollen grain microstructure of 125 rose taxons and, on this basis, they distinguished 6 types and 3 subtypes of exine structure. Similar values of exine features were obtained in this study. This refers to numbers and area occupied by perforations as well as numbers and diameters of striae. These investigations were continued by Ueda [38] who classified rose species examined in this study into the following types: II A – *R. canina*, *R. gallica*, *R. majalis*, *R. micrantha*, *R. mollis*, *R. pendulina*, *R. rubiginosa*, *R. tomentosa*, II B – *R. inodora*, III A – *R. villosa*, III B – *R. agrestis*. In this study, these rose species were assigned to four types of exine sculpture (II A, II B, III A, III B) distinguished by Ueda [38]. Most of them were included in the same types as in the case of the above-mentioned researcher but two of them were allocated to other classes (*R. villosa* – II A, *R. agrestis* – IIIA), while in several others, two types of exine sculptures (*R. canina*, *R. dumalis*, *R. pendulina*, *R. tomentosa* and *R. zalana*) were found which were not observed by Ueda [38].

Popek [8] described exine sculptures of 54 species and 12 varieties of roses belonging to 4 sub-genera. He distinguished sculpture reticulate and striate, the latter one with 11 subtypes (including *R. gallica* subtype). These investigations of exine sculpture elements failed to corroborate a separate identity of *R. gallica* exine sculpture. It is true that it had the highest number of perforations (145) of all the examined species occupying a relatively extensive area (1.19 μm^2), but similar results were obtained, for example, for *R. villosa* (101, 1.08 μm^2; Tab. 3). The remaining attributes of *R. gallica* exine sculpture were the same as in the remaining species.

Recapitulating, the results of the presented investigations failed to confirm the diagnostic significance of exine sculpture attributes either at the level of species or section. The examined exine features did not allow identification of individual species because, for the majority of them, similar results were obtained. It happens that some species can be distinguished by one attribute (e.g. by a large number of perforations: *R. gallica* – 145 and *R. villosa* – 101 or their small numbers: *R. majalis* – 22; or large area of perforations: *R. canina* – 2.16 μm^2), but the remaining exine sculpture features of these roses are similar to other species. It is only possible to identify groups of species of similar exine sculpture.

The performed analysis of operculum characters confirmed the significance of this trait only for the diagnosis of single species. Majority of the examined roses were characterised by opercula of similar structure. The taxonomic value of this attribute is diminished by the fact that, firstly, it underwent significant variability (e.g. two types of opercula were identified in *R. pendulina*) and, secondly, that pollen grains – with, as well as without – opercula occurred in majority of the examined species. The second of the above *Rosa* pollen features was reported by Reitsma [19] who classified *R. canina* and *R. gallica* as operculate, while *R. rubiginosa*, *R. rugosa* and *R. arvensis* – as deprived of operculum. Also Eide [27] observed that *R. canina*, *R. pimpinellifolia* and *R. rubiginosa* had pollen either with or without opercula and added that in *R. canina*, operculate pollen seem to be less frequent than pollen grains without opercula. Popek [8] distinguished 6 types of opercula in roses (including *R. canina* and *R. kostrakiewiczii* types) and his distinction of *R. kostrakiewiczii* type operculum was later corroborated (the only species with a rosette opercula). On the other hand, all the remaining rose species should be allocated to the *R. canina* operculum type proposed by him. Among interesting results of this study, not found in literature on the subject, was the identification in *R. pendulina* of two operculum types (wide and convex and narrow and flat). However, the diagnostic value of this attribute should be confirmed in further studies. Therefore, also Popek's [8] thesis claiming that two closely related species, namely, *R. pendulina* and *R. majalis* differed regarding operculum structure was not fully confirmed because part of the examined *R. pendulina* pollen grains had opercula with similar structure as in *R. majalis*. On the other hand, however, also narrow and flat opercula were identified in *R. pendulina* differing from those found in *R. majalis*.

Morphological structure of pollen grains by no means resolves the issue of the debaTab. taxonomical position of *R. kostrakiewiczii* and *R. mollis*. Popek [8] described *R. kostrakiewiczii* in the rank of species, whereas Zieliński [3] claims it is a sTab. hybrid between *R. rubiginosa* and *R. agrestis*. The pollen grain morphology, on the one hand, corroborates the first thesis because *R. kostrakiewiczii* differs from the remaining roses by the smallest pollen grains (it has the highest number – 36.7% – of small pollen grains). Popek [8] observed in this rose also rosette-form, irregular structure which was described as a rosette-like operculum. Nevertheless, the results presented here indicate that at the current stage of investigations, this character cannot be considered as a diagnostic feature for pollen grains of that species. However, on the other hand, this species fails to exhibit other distinguishing attributes or common features with *R. rubuginosa* or *R. agrestis* pollen grains. In turn, *R. mollis* is included into *R. villosa* L. s. l. [1,3] or treated as a separate species [5,8]. *R. mollis* and *R. villosa* pollen grains failed to show common features in a dendrogram. In addition, attributes of *R. mollis* pollen grains and its close morphological relative – *R. sherardii* – were not similar. Another interesting result of the performed statistical analyses included a separate identity of *R. kostrakiewiczii* and *R. mollis* visible on the dendrogram (Fig. 7) whose ambiguous taxonomical status is confirmed by the highest variability of pollen grains (Tab. S1). It is worth stressing that among lowest coefficients of variability were those determined in sexually reproduced *R. canina* and *R. pendulina*, often referred to as "good species". *R. gallica* pollen grains, also included in the latter group, revealed moderate variability.

The distribution of the examined species in the dendrogram confirms only slightly the currently adopted taxonomic division of the *Rosa* genus into sections [5]. *R. gallica* separated from the *Gallicanae* section. On the other hand, closely related *R. pendulina* and *R. majalis* from the *Rosa* section characterised by pollen grain features similar to roses from the *Caninae* section stand apart. This confirms Zieliński's [3,7] thesis about the absence of a definite morphological boundary between the *Caninae* section and groups that contributed to its development, especially the *Rosa* section.

Pollen morphological structure reflects only slightly consanguinity relationships between the examined species from the *Caninae* section described by Henker [5] and Zieliński [7]. According to Zieliński [7], *R. canina* was the "initial" species for this section. It is from here that six development lines run formed by *R. judzillii*, then by *R. micrantha* and *R. rubiginosa*, *R. agrestis* as well as *R. inodora*, *R. tomentosa*, *R. sherardii* and *R. villosa* and two single species – *R. dumalis* and *R. glauca*. On the dendrogram, both closely related species (e.g. *R. tomentosa*, *R. sherardii* and *R. villosa*) as well as those from different developmental lines (e.g. *R. agrestis*) can be found. *R. canina* pollen grain attributes are the closest to the features of *R. inodora*, *R. rubiginosa* (Fig. 7). These equivocal results are by no means surprising because the *Caninae* section is the most polymorphic group of the *Rosa* genus and the contemporary Caninae have the nature of a hybrids swarm with *R. canina* as a link connecting all section taxons [3,7].

Conclusions

Pollen grain morphological attributes make it possible to isolate one (*R. gallica*) out of 16 examined rose species. *R. gallica* is distinguished for the highest length values of polar and equatorial axes as well as the length of ectocolpi. The obtained results failed to corroborate diagnostic significance of exine sculpture and operculum both at the level of species and section. The examined features of exine did not allow the identification of individual species, although it was possible to distinguish groups of species of similar exine structure. The examined roses were assigned to four types of exine structure distinguished by Ueda [38]. Majority of the investigated roses had operculum of similar structure. All the examined species were assigned to the *R. canina* operculum type. Bearing in mind the above, it can be said that pollen grain morphology can only be used as an auxiliary attribute for the diagnostication of the examined *Rosa* genus species and sections.

The distribution of the examined species on the dendrogram corroborates only to a limited extent the division of the *Rosa* genus into sections currently adopted in taxonomy [5]. In addition, it only slightly reflects consanguinity relationships between species from the section *Caninae* described by Zieliński [7] and Henker [5].

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Supplementary material

The following supplementary material for this article is available online at https://pbsociety.org.pl/journals/index.php/asbp/rt/suppFiles/asbp.2011.031/0:

1. Tab. S1. Numeral characteristics of features of studied pollen grains.

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